INTERNATIONAL CONFERENCE ON NEW DIRECTIONS IN ENVIRONMENTAL ECOLOGY AND HISTORICAL CLIMATE CHANGES FOR A SUSTAINABLE FUTURE

BIOENVIRON – VII
(2024 - 2025)

13th February 2025

Organized by

SCHOOL OF LIFE SCIENCES
(BIOTECHNOLOGY, BIOCHEMISTRY, MICROBIOLOGY & CHEMISTRY)

MARUTHUPANDIYAR COLLEGE
(Nationally accreted with B** Grade by NAAC)

THANJAVUR, TAMIL NADU, INDIA

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ABOUT INSTITUTION

Maruthupandiyar College, Thanjavur is a highly reputed Arts and Science College, affiliated to Bharathidasan University, Tiruchirappalli. The college was established in the academic year of 1996 under the aegis of Athivetti Karumuthuvalli Educational and Charitable Trust. The trustees started this college with the sole aim of uplifting the educationally downtrodden people of this area. Though the college initially catered to the needs of higher studies, over the years, it has grown into a research institution in various disciplines. The college has well equipped Biotechnology, Biochemistry, Microbiology, Computer Science, Physics and Chemistry laboratories. Our college comprise of 22 under graduates, 13 post graduates and 10 Ph.D. research programme in different field of Arts and Sciences. The institution has a family of enthusiastic students committed teachers and independent thinkers working and learning together to shape the future.

ABOUT THE DEPARTMENTS

The Biotechnology department has played a leading role in molecular techniques based curriculum. The department was established in the year 2002 with a goal of imparting quality education and understands the dynamic nature of science. It was upgraded with Ph.D. research programme in the years 2012. The department places special emphasis on research from its inception more than 30 scholars were successfully completed their research in various disciplines.

The P.G & Research Department of Biochemistry of this College is one of the pioneer Research Department providing facilities to undergo M.Phil. and Ph.D. programmes. Most of research projects are concerned with plant and animal sciences. The department places special emphasis on research from its inception more than 38 scholars were successfully completed. The department has produced two gold medalists and many university rank holders since its inception. So far, the department has conducted advanced research works, class seminars, molecular level workshops and guest lecturers related to the topics like genetic engineering, biochemical techniques and management strategies for food and medical biochemistry and isolation of bioactive compounds from natural materials.

The Department of Microbiology was established in 1996 with the goal of rendering the importance of biosciences to learning aspirers. PG and M.Phil. courses was started in the year 2002 and 2005 respectively. The Doctor of Philosophy was started in the year 2013. The department has produced many university rank holders since its inception. The students are well placed in companies and research institutes. The department also supports research projects and conferences for disemminating information on the latest developments in the field of science towards educating and training students.

The Department of chemistry sits at an intellectual and physical nexus of the basic sciences, engineering and medicine at Stanford. The department was established in 2014. It was upgraded with post graduate programme in the years 2022. We are offering the post graduate programme. The department has qualified faculty members and well equipped laboratory with advanced instruments. Join us as we continue to advance fundamental knowledge in chemistry and open new vistas in the areas of the environment, energy and human health.

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INTERNATIONAL CONFERENCE ON NEW DIRECTIONS IN ENVIRONMENTAL ECOLOGY AND HISTORICAL CLIMATE CHANGES FOR A SUSTAINABLE FUTURE

BIOENVIRON - VII

PROGRAMME SCHEDULE

13.02.2025 (Thursday) 9.00 A.M to 5.00 P.M

9.00 A.M	**: Registration**
10.00 A.M	**: Inauguration**
Welcome Address	**: Dr. R.RAJAKUMAR** Head, Department of Biotechnology, Maruthupandiyar College, Thanjavur
Introduction about the Conferences	**:Dr. G.ARCHUNAN** Dean - Research, Maruthupandiyar College, Thanjavur
Presidential Address	**: Thiru. K. MARUTHUPANDIYAN** Managing Trustee &Secretary,Maruthupandiyar Institutions, Thanjavur
Inaugural Address	**: Dr. M. VIJAYA** Principal, Maruthupandiyar College, Thanjavur
Felicitation	**: Dr. R. THANGARAJ** Vice Principal, Maruthupandiyar College, Thanjavur
Releasing of Proceedings	**: Dr. S. THIRUMENI** **Professor,** Department of Community Medicine, Vinayaka Mission`s Medical College & Hospital, Karaikkal - Pondicherry.
Receiving of Proceedings	**: Thiru. K. MARUTHUPANDIYAN** Managing Trustee &Secretary,Maruthupandiyar Institutions, Thanjavur
11.A.M to 11.30 A.M	**: Refreshment**
11.30 am – 12.20 pm **Topic**	**: Technical Session – I** Chief Guest Introduction **: Dr. L.PRINCE** Head, Department of Microbiology, Maruthupandiyar College, Thanjavur **: Dr. VENKATAJOTHI RAMARAO** Associate Professor in Medical Microbiology, Michael Chilufya Sata School of Medicine, The Copperbelt University, Ndola in Zambia. **:MICROBIAL ECOLOGY EFFECT ON HUMAN HEALTH**

12.20 am – 1.30 pm **Topic**	**: Technical Session – II** Chief Guest Introduction **: Dr. S.NETHAJI** Assistant Professor of Biochemistry, Maruthupandiyar College, Thanjavur **: Dr. S. THIRUMENI** **Professor,** Department of Community Medicine, Vinayaka Mission`s Medical College & Hospital, Karaikkal - Pondicherry. **:BIOTERRORISM: A THREAT TO GLOBAL HEALTH SECURITY**
1.30 pm – 2.10 pm	**: Lunch Break**
2.10 pm – 3.00 pm **Topic**	**: Technical Session – III** Chief Guest Introduction **: Dr. C. KALAIMAGAL** Assistant Professor of Biotechnology, Maruthupandiyar College, Thanjavur **: Dr. T. SIVARAMAN** Dean – Research & Development, Dhanalakshmi Srinivasan Engineering College, Dhanalakshmi Srinivasan Group of Institutions, Perambalur-Tamil Nadu. **: DYNAMIC RELATIONSHIP BETWEEN ORGANISMS AND THEIR ENVIRONMENT**
3.00 pm – 3.15 pm.	**: Refreshment**
3.15 pm – 4.15 pm	**: Oral Presentation – I (Conference Hall)** **Chairperson : Dr. O. Sathick,** Associate Professor of Zoology, Khadir Mohideen College, Adirampattinam and **Dr. G.Sathyaprabha,** Assistant Professor of Microbiology, Maruthupandiyar College, Thanjavur **: Oral Presentation – II (Smart class Room)** **Chairperson : Dr. R. Kadhar Nivas,** Head and Asst. Prof. of Biotechnology, E.G.S.P. College, Nagapattinam and **Dr. K.Durgadevi,** Assistant Professor of Biochemistry, Maruthupandiyar College, Thanjavur. **: Poster Presentation - I (Room No. 101)** **Chairperson : K. Muruganantham,** Head & Assistant Professor of Biotechnology, Bharath College, Thanjavur and **Dr. S. Nethaji,** Assistant Professor of Biochemistry, Maruthupandiyar College, Thanjavur. **: Poster Presentation - II (Room No. 102)** **Chairperson : P. Mathavi,** Head & Assistant Professor of Biochemistry, Enathi Rajappa College, Pattukkottai and **Dr. C.Kalaimagal,** Assistant Professor of Biotechnology, Maruthupandiyar College, Thanjavur
4.15 pm – 4.30 pm	**: Feedback from participants**

4.30 pm – 5.00 pm	**:Valediction**
Valedictory Address	**: Dr. T. SIVARAMAN** Dean – Research & Development, Dhanalakshmi Srinivasan Engineering College, Dhanalakshmi Srinivasan Group of Institutions, Perambalur- Tamil Nadu.
Felicitation	**: Dr. M. VIJAYA,** Principal, Maruthupandiyar College, Thanjavur **:Dr. R. THANGARAJ,** Vice Principal, Maruthupandiyar College,Thanjavur **:Dr. G. ARCHUNAN,** Dean - Research, Maruthupandiyar College,Thanjavur **: Mrs. L. MATHU KRITHIGHA.** Dean - Academic, MPC, Thanjavur
Distribution of Certificates	**: Thiru. K. MARUTHUPANDIYAN** Managing Trustee &Secretary ,Maruthupandiyar Institutions, Thanjavur
Vote of Thanks	**: Dr. V.RAMAMURTHY** Head, Department of Biochemistry& Chemistry, Maruthupandiyar College, Thanjavur

National Anthem

THIRU. K. MARUTHUPANDIYAN

MANAGING TRUSTEE & SECRETARY,
MARUTHUPANDIYAR INSTITUTIONS,
THANJAVUR - TAMILNADU

It gives me a great pleasure to know that the School of Life Sciences of Maruthupandiyar College, Thanjavur. They organize the fifth International Conference on “New Directions in Environmental Ecology and Historical Climate Changes for a Sustainable Future (BIOENVIRON-VII)” during 13thFebruary, 2025. This conference is a significant one and provides a wonderful opportunity for the fellow educators and the research scholars to share and exchange their ideas in the recent advancements conservation of environments. I sincerely hope that the dialog generated at this conference will lead to the implementation of many new ideas in this direction and thus pave wave for further improvements. I am extremely happy that international experts and delegates will be attending the conference to present their papers and also deliver keynote lectures and invited talks. May this event be an insightful and educational experience for all those who participate in this wonderful occasion. This International Conference is a platform encouraging the new direction of environment and its applications leading to a large leap in research activities at our institute.

I congratulate the Organizing Secretaries, Members and students of Life Sciences Departments, Participants from our colleges and other colleges for their efforts in organizing and participating in this conference and wish the conference all the success.

DR. M. VIJAYA
PRINCIPAL,
MARUTHUPANDIYAR COLLEGE,
THANJAVUR - TAMIL NADU

I am extremely happy that the fifth International Conference on "New Directions in Environmental Ecology and Historical Climate Changes for a Sustainable Future (BIOENVIRON-VII)" during 13thFebruary, 2025 is conducted by the School of Life Sciences, MPC. Research is a never ending process; the main inputs to the research are contributed by thorough knowledge in the particular field through immense learning. Immense learning can be brought by attending various forums related to the subject. Hence it becomes essential to conduct conferences of this sort to contribute to the field of environmental research and technology. To keep the knowledge shared and updated its essential to bring the students, faculty members and researchers from various institutes, nationwide into a common forum. I hope this conference brings this to reality by uniting participants from different places to present their research works and exchange their ideas. I wish all the participants to have a good learning experience throughout the conference.

I wish the conference a grand success!

DR. R. THANGARAJ
VICE PRINCIPAL,
MARUTHUPANDIYAR COLLEGE,
THANJAVUR - TAMIL NADU

I am immensely glad that the School of Life Sciences is organizing the Fifth International Conference on fifth International Conference on "New Directions in Environmental Ecology and Historical Climate Changes for a Sustainable Future (BIOENVIRON-VII)" during 13thFebruary, 2025. The field of environment is evergreen and its applications keep increasing to quench the thirst of global needs. Hence there has been a need in today'sdue to increasing pollution to rapid urbanization to keep updated for the latest technologies in the emerging field of environmental conservation. The International Conference focuses on The International Conference targets on imparting the knowledge on high quality advancements in environmental sustainability. This Conference brings together leading researchers, students in the domain of interest nationwide to a common forum helping the delegates to present and share their experience and also to explore new avenues of thoughts. It's my heartfelt gratitude to be a part of this institution and my best wishes for the conference and all the participants of the conference.

DR. G. ARCHUNAN
DEAN - RESEARCH,
MARUTHUPANDIYAR COLLEGE,
THANJAVUR – TAMIL NADU

I am delighted to learn that the School of Biosciences, Maruthupandiyar College, Thanjavur is organizing a International Conference on "New Directions in Environmental Ecology and Historical Climate Changes for a Sustainable Future (BIOENVIRON-VII)" during 13thFebruary, 2025 which will pave the way for exploring the possibility of looking at environmental awareness as a hope for welfare of mankind.

I hope that this seminar will prove to be useful to all participants thereby enhancing exchange of ideas so that it may be possible to develop better understanding of the current developments in biological advancements. It is my sincere hope that this seminar will inform, stimulate and broaden the outlooks of interested specialists and result in recommendations for implementation by concerned agencies.

I wish the whole programme all success.

MRS. L. MATHU KRITHIGHA
DEAN – ACADEMIC
MARUTHUPANDIYAR COLLEGE,
THANJAVUR – TAMIL NADU

I feel immensely happy that School of Life Sciences, MPC is organizing an International Conference on fifth International Conference on "New Directions in Environmental Ecology and Historical Climate Changes for a Sustainable Future (BIOENVIRON-VII)" during 13thFebruary, 2025. In the current context of rapid changes that are taking place in our country, all activities directed towards economic advancements are required to be addressed with highest priority especially research, which is the need of the hour. In this effort, it is imperative to prepare staff and students to meet the expectations and create awareness of the recent trends in the environmental sustainability. This conference aims to provide a multi-disciplinary forum for networking and intellectual exchange among scholars who are indulged in research. It emphasize on igniting the creative young minds and bringing a collaborative platform for learning. The conference also intends to offer a platform for interaction between development researchers, policy makers, practitioners academic and other non-academic actors. I hope this conference fulfills the need and act as common place for the faculties and students to explore and share their views.

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Head, Dept. of Biotechnology

Dr. V.RAMAMURTHY, M.Sc., M.Phil., Ph.D.,
Head, Dept. of Biochemistry & Chemistry

Dr. L. PRINCE, M.Sc., M.Phil., Ph.D.,
Head, Dept. of Microbiology

Greetings!

On behalf of the Organizing Committee, we would like to cordially welcome you to the Fifth International Conference on New Directions in Environmental Ecology and Historical Climate Changes for a Sustainable Future (BIOENVIRON-VII) during 13thFebruary, 2025. The conference provides an excellent research program on the latest research results and the state of practice in ecology, environmental monitoring, hazard waste management, bioremediation and biodiversity. This conference, we received more than 100 research articles from various institutions. Each paper was carefully reviewed after accepted for publish the proceeding. The participants will have the chance to attend to the keynote talks and tutorials. The keynote talks will be delivered by Eminent speakers Dr. Venkatajothi Ramarao, The Copper belt University, Ndola in Zambia, Dr. S. Thirumeni,Vinayaka Missin Medical College and Hospitals, Pondicherry and Dr. T. Sivaraman, Dhanalakshmi Srinivasan Group of Institution, Perambalur. The talks will be covering aspects ranging from Eco-terrorism, Bioprospection and Microbial ecology. BIOENVIRON-VII would not have been possible without the sustained effort and the hard work of many people. Obviously, the authors, the program committee members, and the reviewers are essential to the success of the conference. In particular, we thank Program chief patron, Mr. K. Maruthupandiyan to encourage and motive to conduct this conference successful manner. We also thank conference organizers for their hard work and for attracting a large number of high quality papers to the BIOENVIRON-VII. Last but not least, we would like to express our gratitude to Staff members and students from School of Life Sciences. We hope that you enjoy BIOENVIRON-VII and find the research program interesting and stimulating.

With Regards
Organizing Secretaries

CONTENT

*** *** ***

Dr. VENKATAJOTHI RAMARAO

Department of Medical Microbiology,
Basic Medical Sciences,
Michael Chilufya Sata School of Medicine,
The Copperbelt University,
Ndola, Zambia.

MICROBIAL ECOLOGY EFFECT ON HUMAN HEALTH

Microbial ecology is the study of microbes in environment and their interactions with each other. Microbes are found everywhere, including in humans, plants, animals, and the environment, where they exist in communities called microbiomes. People have their own microbiomes, which help maintain good health and protect them from various infections. Sometimes a microbiome can become unbalanced. The exposure to environmental risk factors is an emerging global problem, and it has led to an increase in the incidence of lung cancer in humans. Air pollution and smoking are synergistic. The second leading cause of lung cancer is air pollution. Air pollution can worsen lung cancer survival. Air pollution is a significant and far-reaching threat to public health, with 99% of the world's population breathing unhealthy air, according to the World Health Organization (WHO). Smoking tobacco and air pollution pose similar risks. Indeed, exposure to air pollution can lead to cancer, stroke, respiratory and cardiovascular diseases, and other health issues. Estimates indicate that air pollution contributes to nearly half of lung cancer cases in individuals who have never smoked. Experts now estimate that air pollution leads to nearly seven million deaths annually. Lung cancer is the leading cause of cancer mortality in both men and women. In 2018, an estimated 18.1 million new cases of cancer worldwide and 9.6 million deaths occurred, as per the WHO report. As per the International Agency for Research on Cancer (IARC),1.8 million people died from lung cancer in 2022. Globally, the five-year survival rate for stage four lung cancer with late diagnosis is 10% to 20%. Surgical removal, followed by radiation, immunotherapy and chemotherapy, is the standard treatment for lung cancer. CimaVax-EGF is a vaccine

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used to treat cancer, specifically non-small-cell lung carcinoma (NSCLC). Treatments such as chemotherapy can put patients under a lot of strain and have various side effects. The use of chemically synthesized drugs has not significantly improved in the past few decades. Therefore, there is a focus on using herbal science in the cancer treatments. Researchers have also shown much interest in focusing herbal medicine therapeutics on microbial ecology and protecting a person's microbiome from infections so that people live longer, healthier lives. A large volume of clinical studies has reported the beneficial effects of herbal medicines on the survival and quality of life of cancer patients. Therefore, there is a focus on using herbal science in the lung cancer treatments. Scientists have to focus on the identification of air pollutants, their measurement, and proposed mechanisms of carcinogenesis. The burden of disease and the underlying epidemiologic evidence linking air pollution to lung cancer in individuals who never or ever smoked also emphasized quantifying the problem, assessing risk prediction models, and developing recommended actions. Although scientists know that microbial ecology plays important roles in maintaining human health, there are more unanswered scientific questions. It is critical to understand the relationships and interactions within microbial communities to prevent infections and their spread, improve antibiotic and antifungal use, and slow the spread of antimicrobial resistance. Continued research on herbal medicine therapeutics will help public health scientists better understand microbial ecology treatment options and save lives.

Keywords: Ecology, Microbes, Air pollution, Lung cancer, Herbal science.

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Dr. S. THIRUMENI

Professor,
Department of Community Medicine, Vinayaka Mission`s Medical College & Hospital, Karaikkal -Pondicherry

BIOTERRORISM: A THREAT TO GLOBAL HEALTH SECURITY

Bioterrorism, the deliberate release or dissemination of biological agents with the intent to harm or kill humans, animals, or plants, is a growing concern for global health security. The threat of bioterrorism is real, and its potential consequences are devastating.

Why is Bioterrorism a Threat?

Several factors contribute to the threat of bioterrorism:

1. Advances in biotechnology: The rapid advancement of biotechnology has made it easier for individuals or groups to access and manipulate biological agents.
2. Global connectivity: The increased connectivity of the world through travel, trade, and communication has created opportunities for the spread of biological agents.
3. Vulnerability of healthcare systems: Healthcare systems around the world are vulnerable to bioterrorism threats, particularly in low- and middle-income countries.
4. Intent and capability of terrorist groups: Some terrorist groups have expressed interest in using biological agents as a means of attack.

Types of Biological Agents

Several types of biological agents could be used for bioterrorism:

1. Bacteria: Anthrax, brucellosis, and plague are examples of bacterial agents that could be used for bioterrorism.
2. Viruses: Smallpox, Ebola, and SARS are examples of viral agents that could be used for bioterrorism.
3. Toxins: Botulinum toxin, ricin, and saxitoxin are examples of toxins that could be used for bioterrorism.

Consequences of Bioterrorism

The consequences of bioterrorism could be severe:

1. High morbidity and mortality: Biological agents could cause widespread illness and death.

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2. Economic disruption: A bioterrorism attack could disrupt economic systems, particularly in the healthcare and tourism industries.
3. Social and psychological impact: A bioterrorism attack could cause significant social and psychological disruption, including fear, anxiety, and mistrust.

Prevention and Response

To prevent and respond to bioterrorism threats, governments, international organizations, and healthcare systems must work together to:

1. Strengthen biosecurity: Implement measures to prevent the theft or loss of biological agents.
2. Improve disease surveillance: Enhance disease surveillance systems to detect and respond to biological threats quickly.
3. Develop and stockpile medical countermeasures: Develop and stockpile vaccines, treatments, and diagnostic tools to respond to biological threats.
4. Conduct research and development: Conduct research and development to improve our understanding of biological agents and to develop new medical countermeasures.

CONCLUSION

Bioterrorism is a real and growing threat to global health security. The consequences of a bioterrorism attack could be severe, including high morbidity and mortality, economic disruption, and social and psychological impact. To prevent and respond to bioterrorism threats, governments, international organizations, and healthcare systems must work together to strengthen biosecurity, improve disease surveillance, develop and stockpile medical countermeasures, and conduct research and development.

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Dr. T. SIVARAMAN
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Perambalur – 621212, Tamil Nadu

DYNAMIC RELATIONSHIP BETWEEN ORGANISMS AND THEIR ENVIRONMENT

Harmony with Nature

Nature, though mysterious, operates on consistent and predictable laws. These laws and forces should be thoroughly analyzed to benefit humanity. While nature is unaffected by appeals or supplications, its benefits can be harnessed by respecting and obeying its principles. Simply put, nature is the whole universe, consisting of organisms and their environment. Organisms can range from single-celled creatures to complex multicellular beings, classified broadly as plants or animals. The environment, on the other hand, includes all the components that surround and influence organisms, such as green fields, forests, rivers, ponds, and the vast blue sky.

Understanding and managing nature falls under the domain of environmental studies, a branch of science that explores the interactions between organisms and their surroundings. In other words, environmental study is the science that deals the influence of organisms on their environments and *vice-versa*. Broadly, the environment is divided into 4 interconnected systems as explained herein:

1. **Atmosphere:** The gaseous layer surrounding the Earth, consisting of elements such as oxygen, hydrogen, nitrogen, carbon dioxide, carbon monoxide, and water vapour.
2. **Hydrosphere:** The water bodies on Earth, including ponds, rivers, lakes, and oceans.
3. **Lithosphere:** The solid, rocky layer of the Earth, comprising minerals, rocks, and soil.
4. **Biosphere:** The biological realm that envelopes the planet, consisting of ecosystems that define the relationships between organisms and their environments.

In the modern era, the environment can be categorized into two main types:

1. **Natural Environment:** This includes the biotic (living organisms like flora and fauna) and abiotic (non-living components such as air, water, and soil) elements of nature.
2. **Man-Made Environment:** This encompasses human-made structures such as buildings, roads, and dams.

The man-made environment often disrupts the natural relationship between organisms and their surroundings, significantly altering the lifestyle of organisms and the quality of the environment, sometimes for the better and other times detrimentally. Environmental studies, therefore, play a vital role in understanding these dynamics and fostering harmony between humanity and nature.

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The Importance of Environmental Awareness for a Sustainable Future

Humankind has exploited the environment - the Atmosphere, Hydrosphere, Lithosphere, and Biosphere - recklessly, without considering the long-term consequences. Natural resources are finite, and if this unsustainable practice continues, we are collectively paving the way toward our own destruction. It is crucial to understand that nature cannot be subdued by force. All living beings are interconnected, forming a delicate balance. Humanity's role is not to dominate nature but to work in harmony with it. By respecting the laws of nature, we can reap its benefits sustainably.

In today's world, rapid industrialization, urbanization, and population growth have disrupted the natural balance between organisms and their environment. Critical natural resources like air, water, and soil are being polluted with harmful contaminants. These unsustainable practices have dire consequences, including fresh water shortages, flooding, soil erosion, depletion of minerals, land degradation, ozone layer depletion, climate change, famine, and widespread diseases.

Although scientists are developing tools and methods to improve environmental quality, success in this endeavour requires that these advancements reach every individual. Public awareness plays a pivotal role in this regard. Governments cannot achieve effective environmental management without the active participation of the public. The concept is simple: when individuals keep their immediate surroundings clean, the collective result is a clean street, a clean district, a clean state, and ultimately, a clean planet. Public cooperation is vital to ensure a sustainable and healthy environment for future generations.

To safeguard our environment, individuals need to be educated about the following:

1. **Proper disposal of waste:** Understanding safe methods for managing garbage.
2. **Impacts of water pollution:** Recognizing the harmful effects of discharging waste into water bodies.
3. **Environmental health factors:** Addressing issues like water quality, sanitation, overcrowded housing, air pollution, and noise pollution.
4. **Sewage management:** The importance of open drains, sewage treatment facilities, and preventing untreated discharge.
5. **Food safety:** Awareness about food manufacturing processes, contamination, and adulteration.
6. **Environmental regulations:** Familiarity with laws governing the disposal of toxic chemicals, wastewater discharge, and industrial effluents.
7. **Resource conservation:** Promoting a positive attitude toward conserving natural resources.

Raising public awareness can be achieved effectively through various communication channels such as newspapers, radio, television, feature films, and more. Engaging society in environmental protection efforts requires a collective approach, including nationwide awareness campaigns, environmental clubs, clinics, and voluntary involvement from local politicians, trade unionists, and other community leaders. In summary, public awareness is essential for equitable use of natural resources and maintaining a healthy environment. It motivates individuals to adopt positive attitudes and actions, fostering a sustainable environment for present and future generations.

The Interconnected Role of Scientific Disciplines in Environmental Studies

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Science is a relentless quest for truth, with each branch or discipline targeting specific phenomena using unique methodologies. For example, chemistry investigates the structure and properties of chemical compounds, while biochemistry explores the structure, dynamics, and functions of biomolecules within biological systems. Similarly, microbiology examines the existence and life cycles of microorganisms like bacteria and viruses.

A multidisciplinary approach arises when two or more scientific disciplines overlap, creating new fields of study. For instance, bioinformatics integrates biology with mathematics and computer science to analyze biological data. Similarly, environmental science is a multidisciplinary field that examines the interactions between organisms and their environments from multiple perspectives.

The multidisciplinary approach offers several advantages:

1. **Comprehensive Understanding:** It enables scientists to examine phenomena from various angles.
2. **Broader Vision:** It encourages research with a wider perspective, allowing for more holistic insights.
3. **Eliminating Narrow Focus:** It avoids the limitations of a segmented approach to problem-solving.
4. **Unbiased Conclusions:** It facilitates objective interpretations of research data.

All disciplines, in some way, contribute to understanding and preserving the environment. Literature, for example, reflects the beauty, dedication, and sacrifice of both biotic and abiotic components of nature, such as mountains, rivers, and trees. Linguistics, by documenting natural creatures and human cultures, serves as a valuable scientific record for fields like geoinformatics and environmental studies. Disciplines such as chemistry, medicinal chemistry, biology, geography, and agriculture play a vital role in exploring and analyzing the environment. Social sciences, including economics, political science, management studies, and commerce, help scrutinize environmental issues and devise ethical solutions. Additionally, engineering fields such as chemical engineering, biochemical engineering, and industrial biochemistry are instrumental in mitigating environmental crises and preventing natural disasters. For a sustainable and healthy future, scientists from all disciplines must collaborate and freely exchange information relevant to environmental studies. This cooperative approach is essential for fostering innovative solutions and creating a healthier environment for future generations.

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WATER BIRD DIVERSITY AND SUBSTRATE QUALITY OF THE BIRD SANCTUARY, UDHAYAMARTHANDAPURAM,TAMIL NADU, INDIA

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ABSTRACT

The diversity of wetlands is intermediary zones between permanently aquatic and dry terrestrial eco-systems. Wetlands require collaborated research involving natural, social and inter disciplinary studied to understanding the various components such as monitoring of water quality, socio-economic dependency, biodiversity and other activities as an indispensable tool for formulating long term conservation strategies. The physico-chemical parameters of one of the major habitats for birds, the wetlands are known to influence congregation of migratory and resident species of birds. The present study deals with the interactions between these abiotic factors and bird diversity of the Udhayamarthandapuram bird sanctuary, Tamil Nadu, India for a period of August 2011 to March 2012. During these study periods the water birds were grouped into five categories namely diving birds, swimming birds, small waders, large waders and aerial foragers. During each visit waterfowl survey was carried out and water samples were also collected to document the seasons. The variations in bird aggregations as well as physico-chemical factors are discussed. Most of the abiotic factors were significantly influenced for the density, diversity and richness of the water bird groups.

Keywords: Diversity, Richness, Density, water birds, abiotic factors.

INTRODUCTION

Wetlands are transitional zones between permanently aquatic and dry terrestrial eco-systems. According to the Ramsar convention of the IUCN at Iran in 1981, wetlands are "submerged or water saturated lands, both natural and man-made, permanent or temporary with water, that is static or flowing, fresh, brackish or salt including areas of marine water", the depth of which at low tides does not exceed six meters. Wetland covers about 6% of the earth's land surface. There are several kinds of wetlands such as Marshes, Lagoons, Bogs, Fens, Open water bodies and Mangroves. India has wealth of wetland ecosystems distributed in different geographical region .Most of the wetlands in India are directly or indirectly linked with major river systems, such as Ganges, Cauvery, Godavari and Tapti. India has totally 27403 wetlands of which 23444 are inland wetlands and remaining 3959 are coastal wetlands (Directory of Asian wetlands, 1989). Wetlands occupy 18.4% of the countries area (excluding river) of which 70% are under paddy cultivation. In India, it has been estimated that 4.1 million hectares are wetlands (excluding paddy fields, rivers, and streams), where as 1.5 million hectares are natural and 2.6 million hectares are manmade. The coastal wetlands occupy 6750 sq.km and are largely determined by mangrove vegetation. In Tamil Nadu it has been estimated that 31 natural wetlands covering an area of 58,068 hectares and 20,030 manmade wetland with an area of 2, 01,132 hectares (Venkatraman, 2005).

The bird assemblages are affected by various factors like the food availability, the size (Paracuellos, 2006) and the abiotic changes in the wetlands (Jaksic, 2004 and Lagos *et al.*, 2008).

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Not only the birds but all the organisms, belonging to the plant and the animal communities, are affected by the physical characteristics of the environment (Euliss *et al*., 2004 and Gillis *et al*., 2008). In a wetland ecosystem these biotic factors are mostly dependent on the season and hydrology (Husain, 1995). Thus wetland being an integrated system is affected by the changes in the physical as well as chemical parameters of hydrosphere at the catchment scale. These in turn, affect the wetland dependent communities as well as ecosystem attributes such as species richness, its distribution and density (Burkett *et al*., 2004).

Wetlands require collaborated research involving natural, social and inter disciplinary study aimed at understanding the various components such as monitoring of water quality, socio economic dependency, bio diversity and other activities as an indispensable tool for formulating long term conservation strategies. A first step toward that is the evaluation of physico-chemical and biological feature of wetlands that influenced their use by water birds. Thus, the physical and chemical properties of water body are characteristics of the climatic, geochemical, geomorphologic as well as pollution conditioned reviling in the drainage basin and the underlying aquifer (Ramachandran *et al.,* 2002). These characteristics with natural and manmade changes determine the quality of water (Anonymous, 2003). Thus wetlands are highly complex ecosystem due to various interactions between the components like water, soil, biosphere and atmosphere (Bodegom *et al*., 2004).

Description of Study Area

Udhayamarthandapuram Lake: Udhayamarthandapuram bird sanctuary is located in Thiruvarur district of Tamil Nadu; it is an important migratory water bird habitat of Tamil Nadu. Southern India. The sanctuary was created in 1991 and has a total area of 45.30 hectares .The area coordination as **$10^0$26''50'' N, $79^0$27''58'**. A large number of resident and migratory water birds visit the sanctuary from October to March every year. They come from places such as North India, Central Asia, Tibet, Ladakh and Northern Russia. The number of birds in the sanctuary is highest during November and December upto 1000 birds congregated during peak season nearly 40 species of water birds have been recorded in the sanctuary that includes species such as the White ibis, Open bill stork, Purple heron, Painted stork, Indian darter, Spoon bill, Cotton teal and Grey pelican. A globally endangered water bird such as Open bill stock up to 4000 gathers in the sanctuary during February and March 2012.

MATERIALS AND METHODS

Population studies: Total counts of water bird densities were made for the entire lake once in a month by following method of Spindler *et al.* (1981) in order to avoid double counting or missing birds a vantage point was used (Nagarajan and Thyagesan, 1996). Field binoculars (7 x 50") were used to observe birds from all side of the lake. The birds were identified with the help of their special features (Ali, 1969 and Grimmett *et al.,* 2001) the checklist was prepared using standardized common and scientific names of the birds following Manakandan and Pittie (2001). The water bird were ecologically grouped into five categories on the basis of their activities as driving birds, swimming birds, small waders, large waders and aerial foragers.

Species density: The individual and total water bird densities for different months, climatic season and regions of the lake were calculated as numbers per hectare (Nagarajan and Thiyagesan, 1996; Sridharan, 2003). The density was also calculated for all ecological groups.

Species richness: Species richness was measured by the number of water bird species recorded on different regions of the lake during weekly consumes (Verner, 1985). The species richness was also enumerated for all ecological groups (Krebs, 1985).

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Species diversity: In order to investigate the variations in diversity of bird species and ecological groups during different month of the study period the species diversity was calculated using Shannon wiener index (Shannon and wiener, 1963)

Physical and chemical analysis

Temperature, pH, temperature and DO were measured in the collection point, using mercury in glass Thermometer. Portable hand pH meter and the aside modification of the Winkler's method respectively. The collected samples are immediately transferred and analyzed in the laboratory. All samples analyzed for various water quality parameters are determine according to standard procedure (APHA, 2005).The metals were analyzed using Elmer Perkin Model 8100c Atomic Absorption Spectrophotometer.

Correlation analysis

The bird density, diversity, richness and physic-chemical factors are correlated with the help of SPSS software. Multiple regression equation model was developed for bird population characteristics feature (density, diversity and richness) and ecological group wise Density, Diversity and Richness to investigate the influence of water quality parameter (Nagarajan, 2002).

RESULTS

Water birds of Udhayamarthadapuram Lake: The present investigation is that the forty species of water birds belonging to 14 families have been identified in the waterways at the study area. Totally 65 percent of the birds identified at the study area belong to the ecological group large and small wader birds (Table 1). These birds were ecologically classified into five groups namely, Divers, Swimming birds, small waders, large waders and aerial foragers.

Density : The results of bird's density characteristics were observed and recorded in theUdhayamarthadapuram Lake are presented in Fig. 1. The density of diving birds swimming birds, small waders, large waders and aerial foragers of the bird observed were slightly higher in the month of November than March. Most of the birds observed and recorded higher in monsoon followed by pre-monsoon and post-monsoon seasons.

Diversity : The diversity of diving birds was highest in the month of February (1.07±0.2) and lowest in August (0.5±0.1). The diversity of diving bird was higher in pre-monsoon (1.06±0.8) than monsoon and post monsoon periods. The diversity of small waders was higher in the month of November (1.67±0.1) and lower in August (1.17±0.1) and same report the diversity of large waders was higher in November (1.88±0.3) and lower in month of March (1.54±0.1). The small waders and large waders were higher in monsoon followed by post monsoon and pre monsoon. In the other hand the diversity of swimming birds and aerial foragers were higher in the month of December and lower in March. Thus the diversity of swimming bird and aerial foragers were very high during monsoon and low in pre monsoon (Fig. 2).

Richness : The species richness of diving bird and swimming bird were higher in the month of January and lower in the month of August in the study periods. The species richness of diving bird and swimming bird were low in pre monsoon than post monsoon (Fig.3). Species richness of small waders was high in the month of February (9±2.6) and lower in the month of September (5±1.5). Thus the post monsoon (8.7 ± 2.2) months of the study periods had higher species richness of small waders. In the other hand richness of large waders was high in the months of

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November (13±1.5) and lower in the month of August (10±1.2). Thus the richness of large waders was higher during monsoon (10±1.2) season of the study periods (Fig.3).

Physico-chemical analysis of water from the Lake

The results of water quality analysis and physico-chemical characteristics of lake water are presented in the table 1. The water was slightly alkaline nature and contained high amounts of pH, dissolved oxygen, electrical conductivity, salinity and turbidity in all the seasons examined. The surface water temperature was recorded highest during the post monsoon season (29.4 ± 0.12° C) than the other seasons. The water depth (115±2.62 cm) was elevated in the monsoon season. The dissolved oxygen (6.4 ± 0.1 mg/l), salinity (54.4 ± 2.9 mg/l) was increased during the post monsoon season. The turbidity (2.5±0.9 NTU) was recorded highest in the pre monsoon season during the study period. The elevated level of electrical conductivity (662.5±15.7 mho/cm) was recorded during the pre monsoon season. High amount of pH was recorded in monsoon seasons during the study periods most of the parameters were slightly higher in the post monsoon than monsoon (Table 2).

Relationship of water quality parameter with water bird population

Density of water birds : The correlation between water bird density and the water quality parameter revealed that the turbidity, Dissolved oxygen, salinity and electrical conductivity were negatively correlated. The pH and water depth were positively correlated. The temperature (-0.803) was negatively correlated at the significant level of $P<0.05$ and water depth (0.722) was positively correlated at the significant level of $P<0.05$ (Table 3).

Diversity water birds : Relationship between the diversity of total water bird and the water quality variables revealed that the electrical conductivity, dissolved oxygen, turbidity and salinity levels were negatively correlated in the study period. The pH level was positively correlated. The temperature (-0.88) level was negatively correlated and its significant level of $P<0.05$. The water depth (0.818) was positively correlated and significant level of $P<0.05$ (Table 3).

Richness of water birds : The correlation between the water bird richness and the water quality variation revealed that the temperature (-0.85) was negatively significant and its significant level of $P<0.01$. The electrical conductivity (-0.709) was negatively correlated at the significantly level of $P<0.01$. The pH (0.7) was positively significant of $P<0.05$. The depth level was positively correlated and the dissolved oxygen, salinity and turbidity levels were negatively correlated in the study period (Table 3).

DISCUSSION

The manmade water bodies constructed by man to satisfy his own needs also form important habitats for several avian species. To study any ecosystem the birds serve as important component as they have the ability to fly away and avoid any obnoxious condition. Hence, they are considered as important health indicators of the ecological conditions and productivity of an ecosystem (Desai and Shanbhag, 2007; Li and Mundkur, 2007). The most important parameters of the bird study are the species richness (Murphy *et al.*, 1984), their density (Nilsson and Nilsson, 1978) and diversity (Krebs, 1985). However among avian communities, the components of diversity are known to differ between locations and seasons (Bethke *et al.*, 1993). Deshkar Sona *et al*., (2010) reported the density and species richness of birds are expected to be highest during winter when migratory population arrive and minimum during monsoon when the migratory populations leave the area and the resident species are engaged in the nesting activities. In the present study the density of diving birds swimming birds, small waders, large waders and aerial foragers of the bird observed were slightly higher in the month of November than March.

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The species richness of diving bird and swimming bird were higher in the month of January and lower in the month of August in the study periods. Species richness of small waders was high in the month of February and lower in September. In the other hand richness of large waders was high in the months of November and lower in August. Thus the richness of large waders was higher during monsoon season of the study periods. Most of the birds observed and recorded higher in monsoon followed by pre-monsoon and post-monsoon seasons.

As anticipated the physico-chemical parameters of water varied according to the seasonal fluctuations. Significant drop in the water cover during the post monsoon is predominantly because of the evaporation, however the water is also utilized for irrigating the neighboring fields. This also results in increasing the solids in water. The bird density was negatively correlated with water cover too. During the monsoon and the post monsoon the water level were high in turn maximum birds were present. The previous reports finding of Deshkar Sona *et al*., (2010) during monsoon the dissolved oxygen and the salinity are high which can be due to vigorous mixing of water because of precipitation. High amount of pH was recorded in monsoon seasons during the study periods most of the parameters were slightly higher in the post monsoon than monsoon

All the water quality factors studies were found to be significantly influence one or more water bird population characteristics. Sampath and Krishnamoorthy, (1990) were reported on the effect of water quality factors which the water bird in a wetland. In the present study the correlation between the water bird richness and the water quality variation revealed that the temperature was negatively significant. The electrical conductivity was negatively correlated at the significantly. The pH was positively significant of $P<0.05$. The depth level was positively correlated and the dissolved oxygen, salinity and turbidity levels were negatively correlated in the study period.

The present study concluded that the importance of the Udhayamarthandapuram Lake as they prove to be the important feeding ground for the migratory and the resident species of the birds. All the groups of birds were with care use the lake in terms of density, diversity and richness. Moreover various abiotic parameters play an important role to make up the density, diversity and richness of the water birds hence indicating a single abiotic factor is unfeasible. Thus it can be concluded that the variation in the water quality and the availability of different prey determined the distribution and diversity of aquatic birds in the Lake during the study period.

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Fig.1 A comparison of season wise variations in the bird density of the study periods

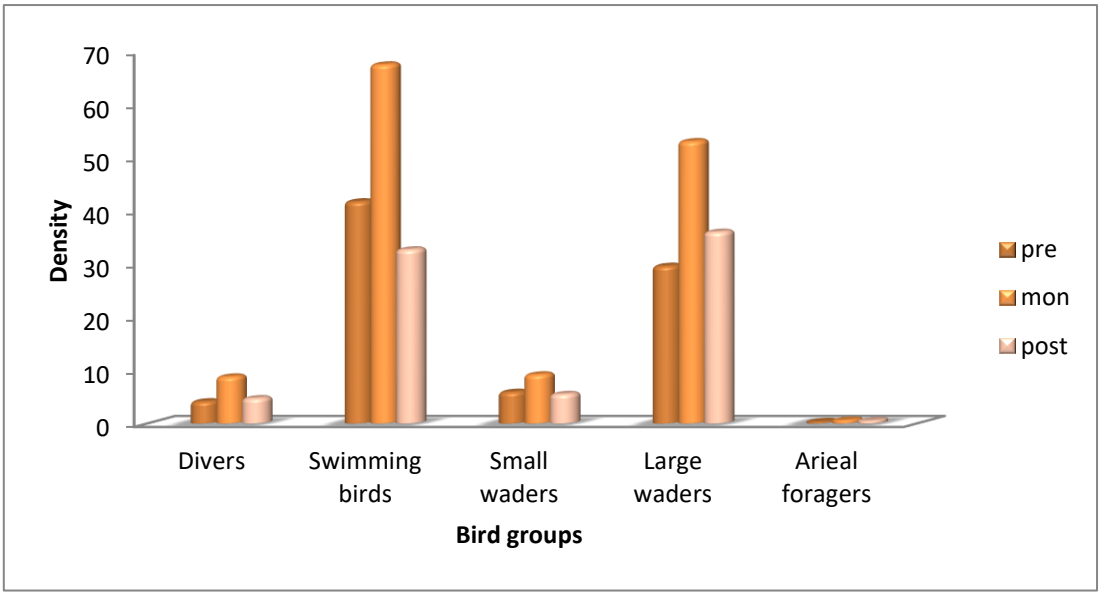

Fig.2 A comparison of season wise variations in the bird diversity of the study periods

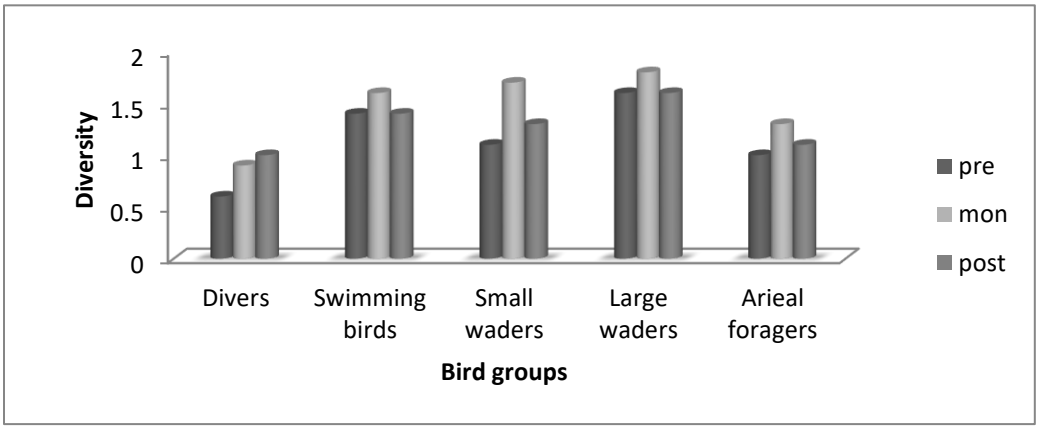

Fig.3 A comparison of season wise variations in the bird richness of the study periods

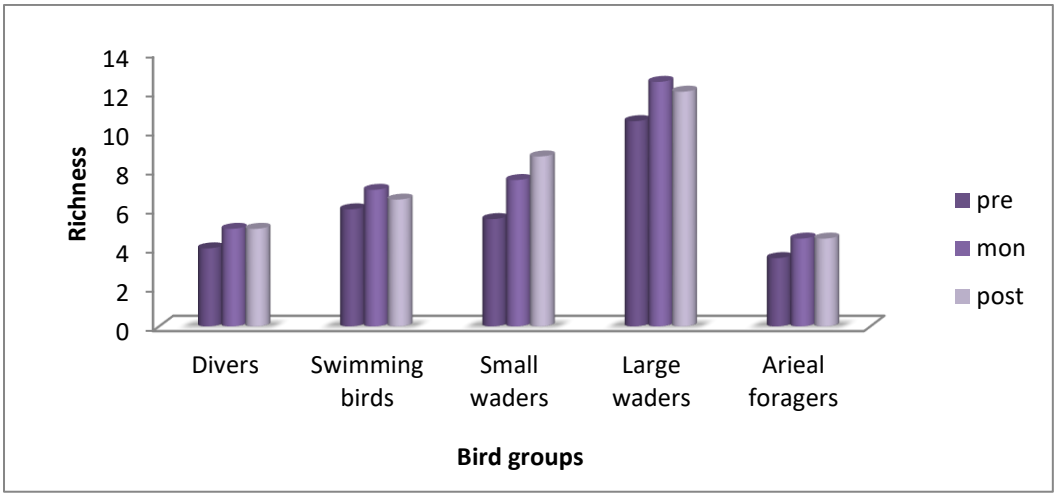

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Table.1 Water birds recorded at the Udhayamarthandapuram Bird sanctuary

S.NO	COMMON NAME	SCIENTIFIC NAME	ORDER	FAMILY	ECOLOGICAL GROUP
1	Little Grebe	*Podiceps ruficollis*	Podicapediformes	Podicipedidae	Diver
2	Little cormorant	*Phalacrocorax niger*	Pelecaniformes	Phalacrocoraxidae	Diver
3	Darter	*Anhinga rufa*	Pelecaniformes	Anhingidae	Diver
4	Common coot	*Fulica atra*	Gruiformes	Rallidae	Diver
5	Grey pelican	*Pelecanus philippensis*	Pelecaniformes	Pelecanidae	Swimming bird
6	Comb duck	*Sarkidiornis melanotos*	Anseriformes	Anatidae	Swimming bird
7	Cotton teal	*Netaphus coromandelianus*	Anseriformes	Anatidae	Swimming bird
8	Spot bill duck	*Anas poecilorhynca*	Anseriformes	Anatidae	Swimming bird
9	Pin tail	*Anas acuta*	Anseriformes	Anatidae	Swimming bird
10	Garganey	*Anas querquedula*	Anseriformes	Anatidae	Swimming bird
11	Common teal	*Anas crecca*	Anseriformes	Anatidae	Swimming bird
12	Purple moorhen	*Porphyrio porpyrio*	Gruiformes	Rallidae	Swimming bird
13	Pheasant tailed jacana	*Hydrophasianus chirurgus*	Charadriiformes	Charadriidae	Small wader
14	Little ringed plover	*Charadrius dubius*	Charadriiformes	Charadriidae	Small wader
15	Black winged stilt	*Himanotopus himanotopus*	Charadriiformes	Charadriidae	Small wader
16	Yellow wattle lapwing	*Vanellus malabaricus*	Charadriiformes	Charadriidae	Small wader
17	Red wattle lapwing	*Vanellus indicus*	Charadriiformes	Scolopacidae	Small wader
18	Green shank	*Tringa nebularia*	Charadriiformes	Scolopacidae	Small wader
19	Green sand piper	*Tringa orchropus*	Charadriiformes	Scolopacidae	Small wader
20	Wood sand piper	*Tringa glareola*	Charadriiformes	Scolopacidae	Small wader
21	Common sand piper	*Actitis hypoleucos*	Charadriiformes	Scolopacidae	Small wader
22	Little stint	*Calidris minuta*	Charadriiformes	Scolopacidae	Small wader
23	Little egret	*Egretta grazetta*	Ciconiiformes	Ardeibae	Large waders
24	Grey heron	*Ardea cinerea*	Ciconiiformes	Ardeibae	Large waders
25	Purple heron	*Ardea purpurea*	Ciconiiformes	Ardeibae	Large waders
26	Large egret	*Ardea alba*	Ciconiiformes	Ardeibae	Large waders
27	Median egret	*Egretta intermedia*	Ciconiiformes	Ardeibae	Large waders
28	Cattle egret	*Bubulcuss ibis*	Ciconiiformes	Ardeibae	Large waders
29	Pond heron	*Ardeola grayii*	Ciconiiformes	Ardeibae	Large waders
30	Night heron	*Nycticorax nyticorax*	Ciconiiformes	Ardeibae	Large waders
31	Painted stork	*Mycteria leucocephala*	Ciconiiformes	Ciconiidae	Large waders
32	Open bill stork	*Anastromus oscitans*	Ciconiiformes	Ciconiidae	Large waders
33	Glossy ibis	*Plegadis falcinellus*	Ciconiiformes	Threskiornithidae	Large waders

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34	White ibis	*Threskiornis melanocephalus*	Ciconiiformes	Threskiornithidae	Large waders
35	Spoon bill	*Platalea leucorodia*	Ciconiiformes	Anatidae	Large waders
36	Little tern	*Sterna albifrons*	Charadriiformes	Laridae	Aerial forager
37	Whisker tern	*Chlidonias hybrida*	Charadriiformes	Laridae	Aerial forager
38	Small blue kingfisher	*Alcedo atthis*	Coraciiformes	Alcedinidae	Aerial forager
39	Pied kingfisher	*Ceryle rudis*	Coraciiformes	Alcedinidae	Aerial forager
40	White breasted kingfisher	*Halcyon smyrnensis*	Coraciiformes	Alcedinidae	Aerial forager

Table. 2 Physico-chemical analysis of water from the Lake Udhayamarthandapuram

S. No	Parameters	Pre monsoon		Monsoon			Postmonsoon		
		Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
1	Water depth	85.5±3.03	73.8±3.53	115±2.62	118±2.72	103.5±5.6	96.1±1.33	58.2±5.8	42.2±1.33
2	Water temperature	29.2±0.38	29.4±0.09	28.2±0.1	26.1±0.22	26.9±0.1	29.5±0.15	31.3±0.11	30.5±0.12
3	Water pH	7.5±0.26	7.6±0.13	8.0±0.2	7.7±0.15	7.6±0.1	7.6±0.1	7.6±0.1	7.5±0.15
4	Electrical conductivity	563.7±10.3	662.5±15	595.4±13.1	540.2±11.2	550.2±16.1	458.1±1.0	588.1±6.6	660±10.5
5	Dissolved oxygen	6.4±0.1	6.4±0.1	6.5±0.14	6.7±0.08	6.8±0.13	6.4±0.1	7.1±0.07	7.1±0.07
6	Turbidity	2.5±0.9	2.2±0.1	2.3±0.9	2.4±0.1	2.3±0.07	2.2±0.15	2.4±0.07	2.4±0.04
7	Salinity	45.5±0.6	45.4±2.9	43.6±2.64	41.5±1.67	45.5±2.16	47.8±1.49	54.4±2.9	46.8±0.96

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	Density	Diversity	Richness	Depth	Temp	pH	DO	Salinity	Turbidity	EC
Density	1									
Diversity	.862**	1								
Richness	.650	.917**	1							
Depth	**.690***	**.354***	.008	1						
Temp	**-.800***	**-.472***	**-.135***	-.821*	1					
pH	.319	.245	**.105***	-.059	.323	1				
DO	-.003	-.380	-.582	-.558	.220	-.174	1			
Salinity	-.653	-.293	-.046	-.871**	.706	-.047	.512	1		
Turbidity	-.271	-.259	-.214	-.198	.095	-.371	.391	.280	1	
EC	-.670	-.528	**-.441***	-.553	.344	.185	.297	.505	.116	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 3. Correlation between water bird density, diversity, richness and water quality parameters at the Udhayamarthandapuram bird sanctuary

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PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF *IN VITRO* ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF *ORTHOSIPHON STAMINEUS*

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ABSTRACT

Orthosiphon stamineus is a widely used folk herb for managing diabetes and other health conditions. Previous research has demonstrated its ability to regulate blood glucose levels in diabetic rat models. However, its precise antidiabetic mechanism remains unclear. Given the economic burden of diabetes, it is crucial to explore common dietary sources as complementary remedies for diabetic patients. This study investigates the antidiabetic potential of ethanol extracts from *O. stamineus* through *in vitro* assays, including α-amylase inhibition, α-glucosidase inhibition, and glucose uptake kinetics. The extract exhibited significant inhibitory effects on α-amylase, α-glucosidase, and glucose uptake, with IC_{50} values of 34.30 ± 0.28 mg/ml, 31.90 ± 0.21 mg/ml, and 37.63 ± 0.26 mg/ml, respectively. Additionally, different extract concentrations significantly enhanced glucose uptake in yeast cells ($P< 0.05$). These findings suggest that α-glucosidase and α-amylase inhibition may be key mechanisms underlying the antidiabetic activity of *O. stamineus*, highlighting its potential role in managing non-insulin-dependent diabetes.

Keywords:*Orthosiphon stamineus*, *in vitro* anti-diabetic activity, phytochemicals.

INTRODUCTION

Diabetes is a chronic condition where the body fails to maintain the blood glucose level or blood sugarduetolackinproductionofsufficientinsulinbythepancreasorthebodyisunabletousethe produced insulineffectively.Diabetesisoneofthemajorlethalepidemicsin Indiaaswellasinthe world(Zhu *et al.,* 2021). According to the International Diabetic Federation (South-East Asia) out of 425 million people sufferingfromdiabetesintheworldinwhich82millionpeoplearelivingin SouthEast Asia andtherewere 72,946,400 cases of diabetes reported in India in the year 2017. It has been estimated that the worldwide diabeticpopulationwillincreasefrom 425millionpeoplein 2017to629 millionby2045, whileIndiaalone will have 98 million diabetic patients by the end of 2030 (Prabhakar *et al.,* 2020). Diabetes is thecollectionofmetabolicillnesses in which increased blood sugar levels persistfora prolongedperiodduetoamalfunctioninginsulinproduction that affects the metabolism ofvariousnutrients such as proteins, lipids, and carbohydrates (Bai *et al.,* 2019). Metabolism is normally altered through congenital and environmental variables (Zhu *et al.,* 2021). The disease pathophysiology suggests that patients mayexperiencefrequent urination, thirst,and hungerwith other symptoms.Serious complications such as kidney,eye,foot, andanother organ failure may beaggravated if properly not managed. In adults, thediseaseaffects4–5%ofpeople,withthenumberanticipatedtoriseto5.4%by2025(Chukwuma *et al.,* 2022). Currently, herbal medicines are getting more importance in the treatment of diabetes (Grover *et al*., 2002; Mukherjee *et al*., 2006).

India is well known for its rich traditional systems of medicine i.e. Siddha, Ayurveda, Unani and Amchi (Tibetan)besidesavastreservoiroflivingtraditionsinethnomedicine. The earliest

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mention of theuseof plants in medicine is found in the Rigveda, which was writtenbetween 4500 and 1600 BC. During British period due to Western culture, our traditional art of natural healing is disappeared. Now it is reappearing due to realization of its importance in curing diseases without any side effects. Plant derived natural products hold great promise for discovery and development of new pharmaceuticals (McChesney *et al.,* 2007). Keeping in view in the present study to investigate the phytochemicals and evaluation of *in vitro* anti-diabetic activity of *Orthosiphon stamineus* leavesextract through alpha amylase, alpha glucosidases assay and glucose uptake in yeast cells methods.

Orthosiphon stamineus is commonly known as *misai kucing* and *kumis kucing*. *O. stamineus* is widely grown in Southeast Asia and the tropical countries. Leaves of this plant are known as "Java tea" and are mainly used for the purpose of making herbal tea commonly in Southeast Asia and European countries (Indubala, 2000). Normally, the leaves and stem tips have medicinal values. Due to this property, this plant has extensively been subjugated traditionally to treat several human ailments and conditions such as diuretic, rheumatism, abdominal pain, kidney and bladder inflammation, edema, gout, and hypertension (Wangner, 1982; Eisai, 1995).The leaves of *O.stamineus* exhibit excellent pharmacological activities such as antioxidant, antibacterial, hepatoprotective, anti-inflammatory, cytotoxic, antihypertensive, and vasodilatation (Chung *et al*., 1998; Masuda *et al*., 1992; Beaux *et al*., 1999; Tezuka *et al*., 2000).

MATERIALSANDMETHODS

Collectionof PlantMaterials

Theleavesof*Orthosiphonstamineus*werecollectedinJanuary2024fromMannargudi,Thiruvarur district, Tamil Nadu, India. Leaves were dried in room temperature and grind using grinder mixture. The powder was stored for further analysis.

PreparationofExtract

Take one gram of *Orthosiphon stamineus* leaves powder and prepared in 50 ml of ethanol and aqueoussolvent, the extract shakeit well for 30 minutes by free hand and wait for 24 hours.After extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

PhytochemicalScreening

Chemicaltestswerecarriedoutontheextractusingstandardprocedurestoidentifytheconstituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984).

Quantitative analysis of phytochemicals

Determinationoftotalphenols byspectrophotometricmethodbyEdeoga*et al.*,(2005) and Flavonoiddetermine by the method ofBohamandKocipai-Abyazan, (1994).

UV-Visiblespectrumanalysis

The extracts were examined under visible UV-Visible spectrum.The extract was scanned in the wavelengthrangingfrom300-800nmusingSystronicSpectrophotometer. Thesesolutionswere scannedin turn at intervals of5 nm and the characteristic peaks were detected.The peak value ofthe UV-Visible was recorded.

*Invitro*anti-diabeticactivity

Invitro α-amylase inhibition assaywascarriedout bythe methodofApostolidis*et al.* (2007), α-glucosidase inhibitory activity was determined according to the method described by Apostolidis

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et al. (2007) and GlucoseuptakeinYeastcells according to the method of Gupta Daksha *et al*. (2013).

RESULTS AND DISCUSSION

Qualitativeandquantitativeanalysisofphytochemicalsin*Orthosiphon stamineus* leaves extract

Natural products such as plant extracts provide unlimited opportunities for new drug discoveries because of unmatched availability of chemical diversity, eitheraspurecompounds orasstandardizedextracts(Sasidharan*et al*.,2011),and recent evidences from the pharmaceutical companies shows that it still represents an extremely valuable source for the production of valuable chemical entities that can be used for the treatmentof some complex diseases (Chin *et al*., 2006).

In the present study was carried out on the *Orthosiphon stamineus* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Orthosiphon stamineus* leaves investigated and summarized in table 1. The phytochemical screening *Orthosiphon stamineus* leaves showed that the presence of tannin, saponin, flavonoids, terpenoids, steroids, polyphenol, antroquinone, glycoside and coumarins in ethanol and aqueous extracts while alkaloids was absentin both extracts. The significantamountof flavonoids (50.00 mg/gm)andtotalphenol(254.00mg/gm)wereestimatedin*Orthosiphonstamineus* leaves represent in table 2.

Table1:QualitativeanalysisofPhytochemicalsin*Orthosiphon stamineus* leavesextract

S. No.	Phytochemicals	Extract	
		Aqueous	Ethanol
1	Tannin	++	++
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	++	++
5	Terpenoids	++	++
6	Alkaloids	-	-
7	Antroquinone	++	++
8	Polyphenol	++	++
9	Glycoside	+	+
10	Coumarins	++	++

(+) Presence, (++) High concentration and (–) Absence

Table2:Quantitativephytochemicalanalysisof*Orthosiphon stamineus* leaves

S. No	Phytochemicals	Results (mg/gm)
1	Flavonoids	50.00±0.35
2	Total phenol	254.00±1.77

Valuesareexpressedasmean± SD fortriplicates

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UV-VisibleSpectralAnalysis

Spectroscopy has become more effective and reliable tools used for phytochemicalanalysis.Ultraviolet-visiblespectrophotometry(UV-Vis)isrelated to photon spectroscopy in the UV-visible region. This technique uses light that is in the visible ranges of the electromagnetic spectrum. The colour of chemicals involvedaffectstheabsorption, and molecules undergoelectrontransitioninthese ranges.The *Orthosiphonstamineus* leaves were examinedundervisibleUV-Visible spectrum. The sample was scanned in the wavelength ranging from 340-800nm usingSystronicspectrophotometer.The UV spectrum profile showed the peaks at 340, 400, 610 and 630 nm and identified phytochemicals are flavonoids and their derivatives, tannins, carotenoids and chlorophyll respectively (Mabasa *et al*., 2021). Table 3 and Figure 1 shows the absorption spectrum of *Orthosiphon stamineus* leaves extract and these are almost transparent in the wavelength region of 300-800 nm.

Figure1:UVvisiblespectrum analysis *Orthosiphonstamineus*leavesextract

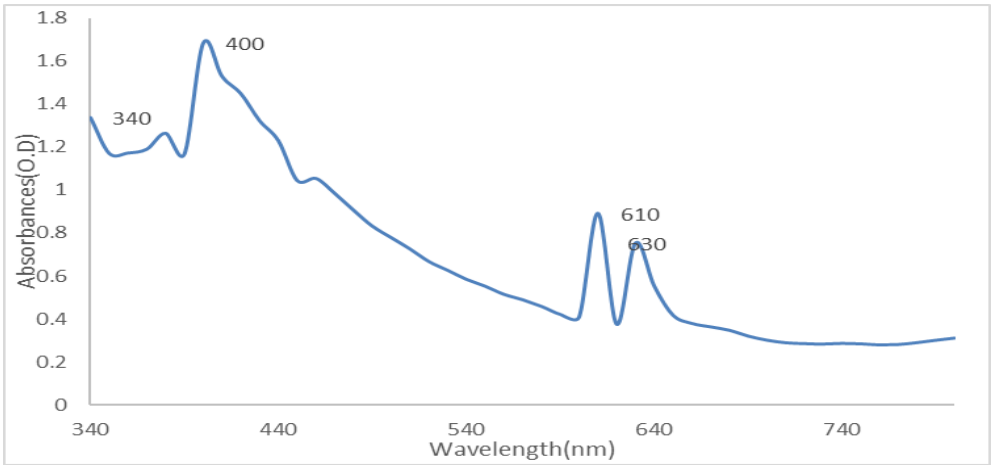

Table3:UVvisiblespectrumanalysis*Orthosiphonstamineus*leaves extract

S.No.	Absorption maxima (Wavelength ranges) nm	Phyto chemical compounds (metabolites)
1	340	Flavonoids and their derivatives
2	400	Tannins, Carotenoids
3	610	Chlorophyll
4	630	Chlorophyll

***Invitro* anti-diabetic activity**

The digestion of starch begins in the mouth with the catalytic fusion of α-amylase secreted in saliva. The process of digestion is continued in the intestine due to the production of α-amylase. The extract of *Orthosiphonstamineus*showed tremendous effect on the inhibition of α-amylase and α-glucosidase enzymes. Therefore, it reduces the digestion of complex carbohydrates and reduces absorption of glucose which further results in the reduction of blood glucose concentration. α-Glucosidase, located in the intestinal lumen and brush border membrane of intestine, is actively engaged in the digestion of polysaccharides and disaccharides before their absorption (Lee, 2002).. Acarbose, a complex oligosaccharide, is a structural analog of starch and competitively inhibits α-glucosidase which delays the digestion of starch and disaccharides. Thus, the inhibition of α-glucosidase is an ideal

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therapeutic approach to decrease the absorption of carbohydrates (Conforti *et al.*, 2005). This study provides an alternative natural product from *Orthosiphonstamineus* with hyperbolic potency and lesser side effects than present synthetic drugs. The inhibition of α-amylase and α-glucosidase may lead to reduction in postprandial hyperglycemia which is an important risk factor for cardiovascular diseases (Cerielloet *al.*, 2004). Thus, the inhibition of α-amylase and α-glucosidase is important to control post-postprandial hyperglycemia in the treatment of diabetes.

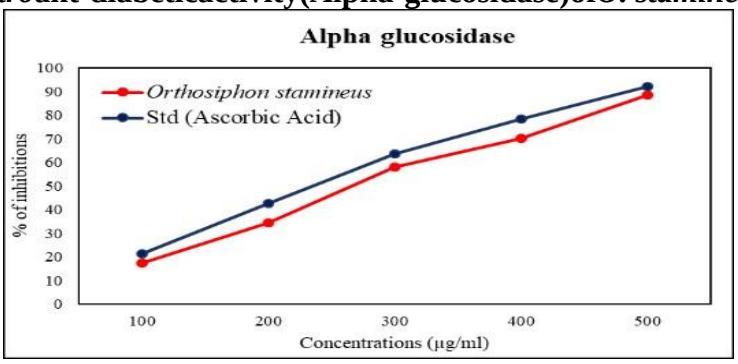
α-glucosidase in the mucosal brush border of the small intestine catalyzes the end step of digestion of starch and disaccharides that are abundant in human diet.Aneffectivemeans of lower ingthelevelsofpostprandialhyperglycemiahave beenofferedby*α*-glucosidase inhibitors (Manohar*et al*.,2002).The mechanism of glucose transport across the yeast cell membrane has been receiving attention as *in vitro* screening method for hypoglycaemic effect of various compounds/ medicinal plants.The *Orthosiphon stamineus* leaves showed inhibitory activity from20.38to86.47%ofinhibitionsat concentration 100and 500µg/mlrespectively and the obtained results were represented in Table4and Figure 2.

Table4:*Invitro* anti-diabeticactivity(Alpha-glucosidase)of*O.stamineus*leavesextract

Concentration(µg/ml)	**% of inhibitions**	
	O.stamineus	**Std. (Acarbose)**
100	17.50±0.11	21.35±0.13
200	34.30±0.28	42.67±0.29
300	58.00±0.43	63.71±0.44
400	70.36±0.50	78.44±0.54
500	88.50±0.62	92.17±0.64
IC_{50}(µg/ml)	278.97	248.92

Valuesareexpressedasmean± SD fortriplicates

Figure2:*Invitro*ant-diabeticactivity(Alpha-glucosidase)of*O. stamineus* leaves extract



The extract showed a percentage inhibition and IC50 value of acarbose was observed. The result was given in Table 5 and Figure 3. Acarbose is a standard antidiabetic drug and it is competitively and reversibly inhibiting the pancreatic α-amylase.

Table5 :*Invitro*anti-diabeticactivity(Alphaamylase)of*O.stamineus*leavesextract

ɔncentration (µg/ml)	**% ofinhibitions**	
	O.stamineus	**Standard(Acarbose)**
100	15.49±0.07	18.32±0.12
200	31.90±0.21	36.28±0.25

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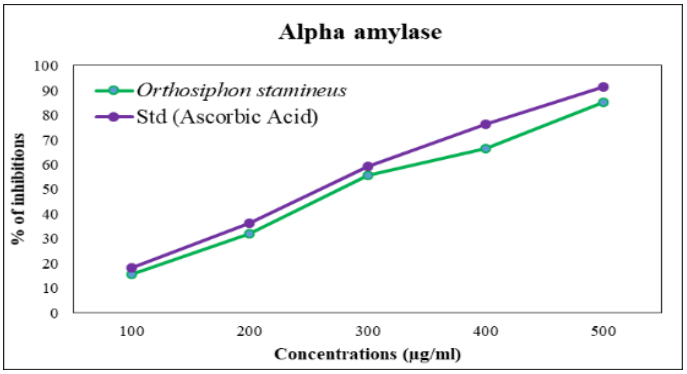
300	55.60±0.37	59.34±0.41
400	66.50±0.47	76.22±0.53
500	85.20±0.61	91.34±0.63
IC_{50}µg/ml)	294.64	266.09

Valuesareexpressedasmean ± SDfor triplicates

Figure3:*Invitro*ant-diabeticactivity(Alphaamylase)of*O. stamineus* leaves extract



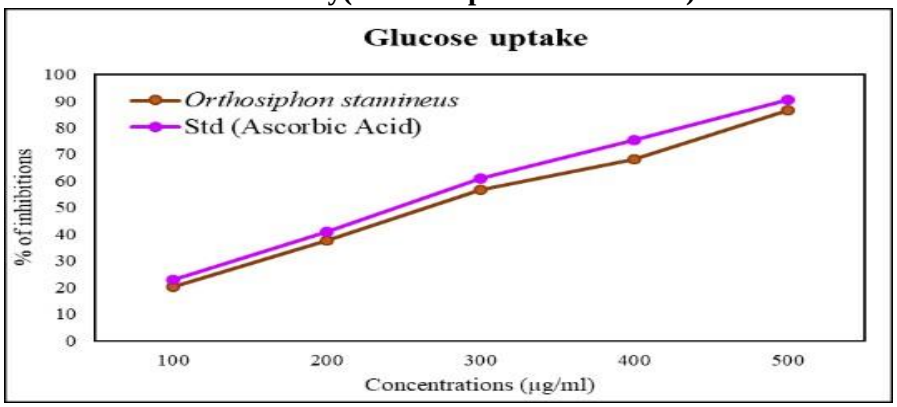
The percentage of glucose uptake by yeast cells increases with increase in the concentration of extract of *O.stamineus*in Table 6 and Figure 4.

Table6 :*Invitro*anti-diabeticactivity(Glucoseuptake inYeastcells)of *O.stamineus* leavesextract

Concentration (µg/ml)	% of inhibitions	
	O.stamineus	**Standard (Metformin)**
100	20.38±0.15	22.75±0.18
200	37.63±0.26	40.89±0.32
300	56.78±0.49	60.78±0.53
400	68.25±0.53	75.36±0.58
500	86.47±0.67	90.57±0.72
IC50(µg/ml)	265.1541	248.925

Valuesareexpressedasmean± SD for triplicates

Figure 4:*Invitro*ant-diabeticactivity(GlucoseuptakeinYeastcells)of *O.stamineus* leavesextract



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For treating diabetes, in the traditional Indian Ayurvedic system, there are several medicinal plants and their formulations are available as well as in ethnomedicinal practices as their principal bioactive components showed good α-amylase inhibitory activity (Kunyanga *et al.,* 2012).

In vitro analysis of the antidiabetic activity of seagrass is in accordance with the previous study of medicinal plants, wherein there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal α-glucosidase and pancreatic α-amylase (Mai *et al.,* 2007; Ramkumar*et al.,* 2010).

CONCLUSION

The present study is the first report on validating the antidiabetic activity of *Orthosiphonstamineus*using appropriate *in vitro* techniques. The study provides the possible mechanism of glucose lowering activity of *Orthosiphonstamineus*which was explained through inhibition of α-amylase, α-glucosidase, and glucose movement, diffusion of glucose across cell membrane and by increasing glucose adsorption.

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DETERMINATION OF THE THERAPEUTIC COMPOUNDS FROM *HELIOTROPIUM INDICUM* BY GC/MS

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ABSTRACT

To study the ethanolic extract of the medicinal plant *Heliotropium indicum*have been investigated by GC-MS to identify compounds responsible for its therapeutical properties. Two kinds of extracts of plant were investigated. The tincture was prepared by mixing all parts of the plant (roots and leaves) with a 50% alcoholic solution for 30 days. The infusion was also prepared by mixing all parts of the plant with hot water for 20 min. These two extraction methods were compared for extraction of the active therapeutic compounds of *Heliotropium indicum*. In parallel, another study was performed to identify the distribution and the concentration of the active compounds in the roots and leaves of this plant. For this purpose we have prepared alcoholic extracts from each part of the plant and we have studied them separately.

Key Words: GC-MS analysis, *Heliotropium indicum,* Fatty acids

INTRODUCTION

Heliotropium indicum, a very toxic herb, member of the Boraginaeceaefamily, popularly named Indian heliotrope can be referenced under the symbol HEIN, is a rare medicinal plant which has a very long history of medicinal use, though it is little used by present-day herbalists. It is an aromatic tonic herb that stimulates the antiulcer, reduces inflammation, controls bacterial infections and promotes healing. The flowering herb, with or without the root, is antiinflammatory, antiulcer, diaphoretic, emmenagogue, febrifuge, oxytonic and stimulant[1&2]. The plant contains a complex of acids so called “organic acids” which stimulate white blood cell activity and speeds the healing of wounds if it is used in correct concentration. Externally it is used in the treatment of slow-healing cuts, eczema, infected toe and fingernails etc., but internal consumption can cause damage to the kidneys and uterine bleeding.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[3]. Over 50% of all modern clinical drugs are of natural product origin[4] and natural products play an important role in drug development programs in the pharmaceutical industry[5].

The objective of this work is to identify and characterized the compounds of therapeutic value extracted from *H. indicum.* The analytical methods chosen are gas chromatography/

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massspectrometry (GC/MS). The methods were applied to characterize the infusion prepared from this plant and to make a comparison between the alcoholic extracts of the roots and leaves.

MATERIALS AND METHODS

Heliotropium indicum L belongs to the family Boraginaeceae was collected from Thanjavur District, Tamilnadu State, India and identified by the special key given in Cambell flora. Voucher sample was prepared and deposited.

The leaf and root of *H. indicum* washed with sterile distilled water, and they were shade dried and powdered by using Pestle and Mortar and for the alcoholic extracts (96% alcoholic solution) roots and leaves. The tincture was prepared by mixing all parts of the plant with a 50% alcoholic solution for 30 days. The infusion was also prepared by mixing parts of the plant with hot water for 20 min and the alcoholic extracts by mixing the fresh parts of the plant with a 96% alcoholic solution for 12 days.

GC-MS ANALYSIS

The dry fractions (20g) were dissolved in 75ml of alcohol and than soaking for 24 hrs. After soaking, collect a filtrate and evaporate under liquid nitrogen. Then concentrate the filtrate for GC-MS analysis.

For the GC-MS analysis a 30m x 0.25mm I.D x 1.0 µm df fused Elite-1 (100% Dimethyl Poly Siloxane) column; GC Clarus 500 Perkin Elmer gas chromatograph with Mass detector-Turbo mass gold- Perkin Elmer, Software- Turbo mass 5.1. The samples (1µl) were introduced *via* an all – glass injector working in the split mode (10:1), with Helium as the carrier gas.

Oven temperature programme: 110 deg-2min hold, upto 280 deg at the rate of 5 deg /9min hold. Injector temperature: 250 deg C. GC time – 45 mins.
MS Programme: Inlet line temperature: 200^{o} C, Source temperature: 200^{o} C, Electron energy: 70eV, Mass scan: (m/z) 45-450. MS time – 46 mins.

The identification of components was accomplished using computer searches in NIST ver 2.1 library.

RESULTS AND DISCUSSION

Use of GC/MS enabled identification of the most components in both samples of *Heliotropium indicum* were analyzed by antimicrobial compounds (Table 1) Fatty acids (Table 2) and anti-inflammatory activity (Table 3). The compounds identified are listed in Tables. In these natural compounds has been a source of medicinal agents for antimicrobial, anti-inflammatory compounds and some essential fatty acids are analyzed in this plant.

The components of the infusion differ from those found in tincture except organic acids derivatives. The concentrations (in %MS) of these derivatives in infusion and tincture are very closed; maybe that is why in traditional medicine are used both types of extracts with success.

The differences between the compounds that we have found in the roots, steams and leaves of *Aristolochia Clematitis* were studied by GC-FID. This study was performed on the alcoholic extracts of the three parts of the plant. From this study we have concluded that the compounds found in the root and steam are very similar. The aristolochic acid derivatives are present in both extracts, but in the leaves these derivatives are in very low concentration[6].

In the present study was the difference between the compounds that we have found in the roots and leaves of *H. indicum* were studied by GC-MS. This study was performed on the

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alcoholic extracts of the two parts of the plant. From this study we have concluded that the compounds found in the root and leaves are very dissimilar. The organic acid derivatives are present in both extracts, but in the leaves these derivatives are in very high concentration.

The analytical methods used GC/MS is suitable for medicinal herb organic compounds determination. The sample preparation method is rapid and precise.There is a difference between the compounds extracted from herb by infusion and tincture but the important thing is that the organic acid and fatty acids derivatives are present in both of them. On the other side the study shows that their concentration is higher in the roots and steams. In the leave extracts organic acid derivatives and vitamin F (polyunsaturated fatty acids) are very higher amount present. In conclusion terpenic compounds, fatty acids, phytol, alkaloids and especially organic acid derivatives are responsible for the therapeutic activity of this plant.

Table 1. ANTIMICROBIAL COMPONENTS IDENTIFIED BY GC-MS STUDY

S.No	Antimicrobial Components	Formula	*H. indicum*	
			Leaf	Root
1	Benzene acetaldehyde	C_8H_8O	+	-
2	5H-1-Pyrindine	C_8H_7N	+	+
3	2-Furan carboxaldehyde, 5-(Hydroxymethyl)-	$C_6H_6O_3$	+	+
4	Benzene acetic acid	$C_8H_8O_2$	+	-
5	Dodecanoic acid	$C_{12}H_{24}O_2$	+	+
6	Phenol, 3-Isopropoxy-5-Methyl-	$C_{10}H_{14}O_2$	+	+
7	3`-Acetyllycopsamine	$C_{17}H_{27}NO_6$	+	-
8	Squalene	$C_{30}H_{50}$	+	-
9	Octanoic acid, Ethyl ester	$C_{10}H_{20}O_2$	-	+
10	Benzaldehyde, 3-Hydroxy-4-Methoxy-	$C_8H_8O_3$	-	+
11	Benzaldehyde, 4-Hydroxy-3, 5-Dimethoxy-	$C_9H_{10}O_4$	-	+
12	4-((1E)-3-Hydroxy-1-propenyl)-2-Methoxy Phenol	$C_{10}H_{12}O_3$	-	+
13	Benzaldehyde, 4-Hydroxy-	$C_7H_6O_2$	-	-
14	Butanoic acid, 2-Methyl-	$C_5H_{10}O_2$	-	-
15	Nonanoic acid	$C_9H_{18}O_2$	-	-
16	Benzene acetic acid, 2,5-Dihydroxy-	$C_8H_8O_4$	-	-
17	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$	-	-
18	Phytol	$C_{20}H_{40}O$	+	-
19	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	-	-
20	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$	-	-

+: Present; ---: Absence

Table. 2. FATTY ACIDS IDENTIFIED BY GC-MS STUDY

S.No	Name of Fatty Acids	Formula	*H. indicum*	
			Leaf	Root
1	Dodecanoic acid	$C_{12}H_{24}O_2$	+	+
2	Tetradecanoic acid	$C_{14}H_{28}O_2$	+	+
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	+	+
4	Hexadecanoic acid, Ethyl Ester	$C_{18}H_{36}O_2$	+	+
5	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	+	+

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6	9,12-Octadecadienoic acid, Ethyl Ester	$C_{20}H_{36}O_2$	+	-
7	9,12,15-Octadecatrienoic acid, Ethyl Ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	+	-
8	Octadecanoic acid, Ethyl Ester	$C_{20}H_{40}O_2$	+	-
9	8,11,14 – Eicosatrienoic acid, (Z, Z, Z) -	$C_{20}H_{34}O_2$	+	-
10	Docosanoic acid, Ethyl Ester	$C_{24}H_{48}O_2$	+	-
11	Hexanoic acid, Ethyl Ester	$C_8H_{16}O_2$	+	+
12	Octanoic acid, Ethyl Ester	$C_{10}H_{20}O_2$	-	+
13	Pentadecanoic acid	$C_{15}H_{30}O_2$	-	+
14	Octadecanoic acid	$C_{18}H_{36}O_2$	-	+
15	Nonanoic acid	$C_9H_{18}O_2$	-	-
16	Undecanoic acid	$C_{11}H_{22}O_2$	-	-

+: Present; ---: Absence

Table. 3. ANTIINFLAMMATORY COMPONENTS IDENTIFIED BY GC-MS

S.No	Antiinflammatory Components	Formula	*H. indicum*	
			Leaf	Root
1	Phytol	$C_{20}H_{40}O$	+	-
2	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$	+	+
3	9,12-Octadecadienoic acid, Ethyl Ester	$C_{20}H_{36}O_2$	+	-
4	Squalene	$C_{30}H_{50}$	+	-
5	Methyl Salicylate	$C_8H_8O_3$	-	+
6	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$	-	-

+: Present; -: Absence

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ISOLATION AND IDENTIFICATION OF AZADIRACHTIN FROM NEEM OIL AND ITS EFFECT ON CONTROL PESTS IN RICE FIELD

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ABSTRACT

Evaluation of the activity of the cold expeller neem oil (*Azadirachta indica* A. Juss.) and the fractions derived through solvent partitioning, against *Rhizoctonia solani* and *Bipolaris oryzae* showed that the active antifungal fraction is a mixture of azadirachtin. Further, testing the azadirachtin mixture derived from the 90% hexane extract of neem oil against two phytopathogenic fungi revealed that various species were inhibited to different degrees. Direct preparative High Performance Liquid Chromatography (HPLC) of the active fractions and subsequent bioassay of the semi pure fractions indicated that the active fractions contained major compounds such as azadiradione. The pure Azadirachtin biopesticides based on fungus *Rhizoctonia solani* and *Bipolaris oryzae* were used on leaf folder of rice, which have reduced the population of these pests effectively both in laboratory and in the field. By using the combination of azadirachtin and solvent formulation the increase in mortality was studied and it was 100% after 96 h. In the field trials a significant effect on leaf folder was observed. On the basis of these results, biopesticides can safely be recommended for the control of rice pests with no harmful effect on its predators as in case with chemical pesticides.

Key words: Biopesticide, HPLC, Azadirachtin, *Rhizoctonia solani* and *Bipolaris oryzae.*

INTRODUCTION

Rice (*Oryza sativa*) is the most important food crop of the world. In India, the yield of rice is very high as compared to other rice producing countries. Several factors contribute for such low yield and among them the most important are loses due to insect attack. The leaf folder (*Cnaphalocrocis medinalis)* is very serious insect pest of rice. Losses due to these insects usually occur 5-10% and sometime reaches to 60% (1). For the control of these pests, chemical spray is most common practice. Concern about environment pollution, resistance to pesticides, residues in food and biodiversity make new and novel strategies for the control of pests like rice leaf folder. Critically important is to secure food for a rapidly growing population. In view of these considerations, biopesticides offers a technically feasible and environmentally acceptable strategy for controlling agronomically important insects. The use of biopesticides in many countries is limited for a variety of reasons most notable among them is the poor efficacy of imported products under local conditions (2).

Use of the crude extractives of seeds of neem for control of plant pathogenic fungi is known and has been amply documented (3). The antifungal activities of neem constituents relate to Azadirachtin (supposedly a mixture of a number of triterpenoids from seed oil) against *Rhizoctonia solani, Bipolaris oryzae* and *Helminthosporium oryzae* (4). Using some phytopathogenic fungi as test organisms, (5) isolated a compound with antifungal activity from neem oil extracts, the identity of which is not known. Herein, we have attempted fractionation, isolation and identification of antifungal triterpenoids from neem oil, the results of which are presented.

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MATERIALS AND METHODS

Extraction of neem compounds

Neem oil was collected in large sterilized container and brought to the laboratory. The collected neem oil was eluted 3:1 ratio with hexane and ether. Then the mixture was filled with a specially designed instrument (for heat extraction) container. After filled the mixture was heated 50^0C to 60^0C for 15 minutes. Then the neem oil extract was collected through Buchner funnel. The extract was purified by Column Chromatography Slurry method. Pure compounds were identified by HPLC analysis. Standard pure compounds were routinely purified in our laboratory through preparative HPLC, which forms the source.

Rice field

The field trial experiment was conducted in premises of Pattukkottai taluk, Thanjavur district, Tamilnadu, during summer season of (March-July) 2011. Total plant area was divided into four treatments and four replicates according to randomized complete block design. The field was sown with supper ADT 43 cultivars of rice. Infestation of rice leaf folder started after 45-50 days of rice plantation. The Azadirachtin was sprayed three times by looking the severity of infestation.

The Azadirachtin was used in the various concentrations. For applying the Azadirachtin the following treatment were employed.

- Test one (T1) = 50g/100lit. / Acre
- Test two (T2) = 100g/100lit. / Acre
- Test three (T3) = 200g/100lit. / Acre
- Test four (T4) = Control

Laboratory bioassay

Laboratory experiments were conducted to check the effect of azadirachtin formulation on the larvae before applying in the field. The data was collected up to 96 hrs. The mortality percentage was calculated after 48, 72 and 96 h and it was recorded.

Pest scouting (6)

Criteria of Azadirachtin evaluation were based on pre and post spray pest scouting. The mortality percentage data was calculated by looking the severity of infestation. Data was recorded after spray on 1, 2, 7 and 9 days.

Antifungal activity (7)

The *A*zadirachtin were tested separately for their fungal toxicity against *Rhizoctonia solani, Helminthosporium oryzae* and *Bipolaris oryzae*in the experiments. The 90% hexane extract of neem oil was also tested against two phytopathogenic fungal such as *R. solani*, *H. oryzae* and *B. oryzae*. The different concentrations of *A*zadirachtin viz., 5, 10, 15, and 20 per cent were added separately to the cooled potato dextrose agar medium. The amended PDA medium was dispersed in Petriplates and allowed to solidify. After solidification 5mm agar blocks cut from the actively growing margin of the pathogen *R. solani, H. oryzae* and *B. oryzae* were inoculated at the center of the plates. The plates were incubated at 30 ± 2°C for five days. The radial growth was measured periodically and the mean growth rate was calculated. Control was maintained in each case without adding *A*zadirachtin. The percentage inhibition of growth was calculated as follows:

$$\text{Percentage of Growth inhibition} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

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RESULTS

Isolation of Azadirachtin: The double refined oil extract from various solvents such as hexane, alcohol and ether. Showed that hexane solvent even effectively coagulated and separation of 4% azadirachtin compound.

Identification of Azadirachtin: Azadirachtin was purified by silica gel using column chromatography. Some fractionated substances were analyzed through HPLC. From the chromatogram, the peak value was calculated by Retention Time (RT) and identified the extract as Azadirachtin.

Effect of Azadirachtin on pest of paddy: The paddy field was separated four plots and sprayed with various concentration of Azadirachtin, the number of larvae was counted in each replicate. The data was collected before spray and after (1, 2, 7 and 9th day). The number of larvae per plant was calculated and presented in Figure 1. High number of larvae was observed in the first treatment (T1), on day 1 and with the passage of time the insect population was reduced due to effect of Azadirachtin and stem borer of the crop. It is usually observed after number of insecticide resistant species. The present study described the uses of Azadirachtin for the control of rice leaf folder and stem borer insect larvae. The experiment was continued up to 96 hours. Mortality due to Azadirachtin was 20% and 49% after 48 and 72 hours respectively in T2. Increased in morality was observed in T3 formulation and it was 100% after 96 hours (Fig. 2). The lower (T1) concentration Azadirachtin was exhibited 89% after 96 hours. In field trials on rice crop, spray data indicates that under natural infestation pest population in the field can never remain same. These plots sprayed Azadirachtin represented decrease in present infestation from 1st to 9th day. These results suggest that Azadirachtin were effective not in laboratory bioassay but also in the field against rice pests. Yield of rice crops in same conditions of attack was also studied. In treated very few insects were recorded on 9th day. Similar results were observed in all other treatments.

Mortality Percentage: The mortality percentage due to the effect of Azadirachtin was calculated. In all the treatments (T1, T2 and T3) higher percentage of mortality was observed than the control (T4). In T1 and T2 formulation, almost equal percentage of mortality was observed and showing the effect of Azadirachtin on insect population (Fig .3).

Effect on Whiteheads: Whitehead was the damage caused by flowering. Stem borer larvae migrate to in between the leaf sheaths. It causes the entire panicle to dry. Average number of whiteheads was counted in each replicates. A significant reduction of whitehead was observed in all treatments due to the effect of spray when it was compared to control. In control group the number of whitehead was very high. This demonstrates, that the effect of biopesticides on stem borer, which ultimately resulted in reduction of whiteheads (Fig. 4).

Antifungal activity of Azadirachtin: The fungal pathogens *R. solani, H. oryzae* and *B. oryzae* were used to study the antifungal activity of azadirachtin. The results showed that the azadirachtin could inhibit the growth of the fungal strains. Among the fungal pathogen *R. solani* showed maximum growth inhibition when compared to *H. oryzae* and *B. oryzae* (Fig. 5).

DISCUSSION

Public concern about chemical residues on fruits, vegetables and other crops has led to a progressive increase of interest in alternative strategies for the control of diseases and pests. The application of biological control is increasing largely because of greater environment awareness and food safety concerns plus the failure of conventional chemicals due to an increasing, preliminary laboratory bioassay gave excellent results on found very effective against lepidopteron insects plots the crop looks healthier and gave better yield than non-treated, which recommends biopesticide as a best biological agent in crop protection.

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Analysis of the 90% hexane extract by analytical HPLC revealed the presence of major triterpenoids (8). The extracted compound is attributed to the triterpenoidal fraction. An attempt was made, therefore, to evaluate the different preparative HPLC fractions of the 90% hexane extract in order to identify the compound that impart Azadirachtin.

Preparative HPLC (on a 5 cm - 25 cm, C18 column with MeOH: Hexane as an eluent in a stepwise gradient) resolved the 90% hexane extract into the peaks (8), after the elution of which the column was washed with 100% methanol in order to remove the non-polar components. Analysis of all the peaks employing analytical HPLC using a C18 column revealed that peaks contained mainly azadirachtins (9). Attempts are being made to collect these fractions in sizeable quantities to investigate further the individual fractions. The Peak was identified as 6-deacetylnimbin of 96% purity (by analytical HPLC). Peak (80% azadiradione as the major component), which resolved into at least three major components in analytical HPLC.

In field trials on rice crop, spray data indicates that under natural infestation pest population in the field can never remain same. Plots sprayed with Azadirachtin represented decrease in percent infestation from 1st to 9th day. These results suggest that azadirachtin were effective not in laboratory bioassay but also in the field against rice pests. Yield of rice crop in same conditions of attack was also studied. In treated plots the crop looks healthier and gave better yield than non-treated, which recommends Azadirachtin as a best biological agent in crop protection.

The effect of azadirachtin on rice leaf borer was also studied which resulted in reduction of whiteheads in all the replicates as compare to control. Azadirachtin based on fungal cry toxin, which binds to a specific receptor on the brush border membrane of insect mid gut epithelial cells, Bt toxin insert rapidly and irreversibly in to plasma membrane of gut cells (10) and either form an ion channel or act on some membrane components to open a pore, which resulted in cell lysis and eventually death of insect larvae (11).

Although neem oil has been used for control of phytopathogenic fungi, such as *Rhizoctonia solani*, *Helminthosporium oryzae* and *Bipolaris oryzae*rust pathogens, concentrations needed for complete field control were shown to be as high as 2% to 10%. High concentrations of neem oil are known to induce phytotoxicity (3). In our experiments the concentration for the antifungal activity assays was kept at 5 to 20 %. Cold expeller neem oil at control either brought about no inhibition (against *R. solani*, *H. oryzae* and *B. oryzae*) or minimal inhibition (5% against *B. oryzae* and 10 % against *R. solani*) against the test sample. While this confirms earlier reports (3), it may, in part, explain the reason for the use of higher concentrations of neem oil for field control.

Assays of the antifungal activities of the mixture against the two test fungi revealed again that maximum inhibition was observed with *H. oryzae* and *B. oryzae* (5 %). It is also not surprising that differences existed in inhibition percentages among the test fungi. Hence, the concentration needed to inhibit each fungal species effectively has to be worked out independently. Both the natural Azadirachtin mixture from neem oil, as well as a mixture made up from pure Azadiradione was similar in their antifungal activity against the test fungi.

Present studies have shown that the 90% hexane fraction contained mainly Azadirachtin. Hence, it was suspected that the Azadirachtin might be antifungal. Loss of activity after purification prompted us to recreate a mixture by combining pure compounds. Retention of

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activity of such mixtures of triterpenoids may indicate that there could be additive/synergistic effects of azadirachtin in 90% hexane extracts.

Fig. 1: Population of rice leaf folder with effect of Azadirachitin

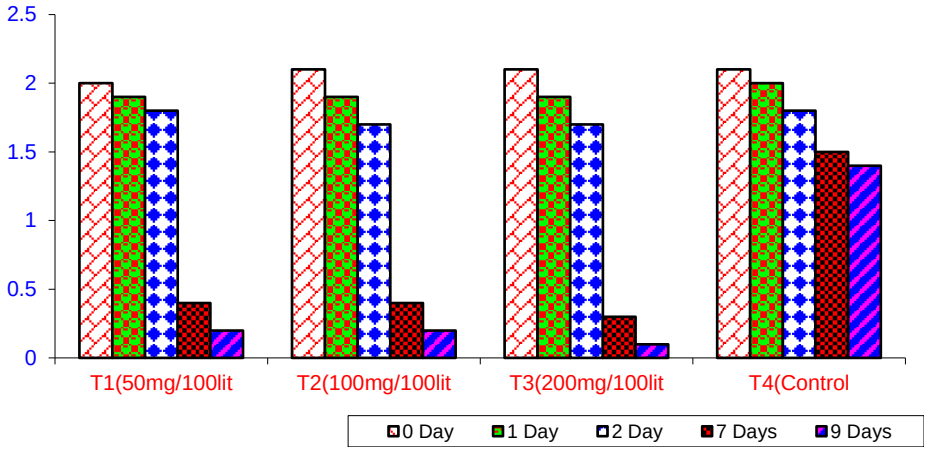

Fig. 2: Mortality persentage whiteheads with effect of different concentrations of Azadirachitin after exposure to different hours of duration

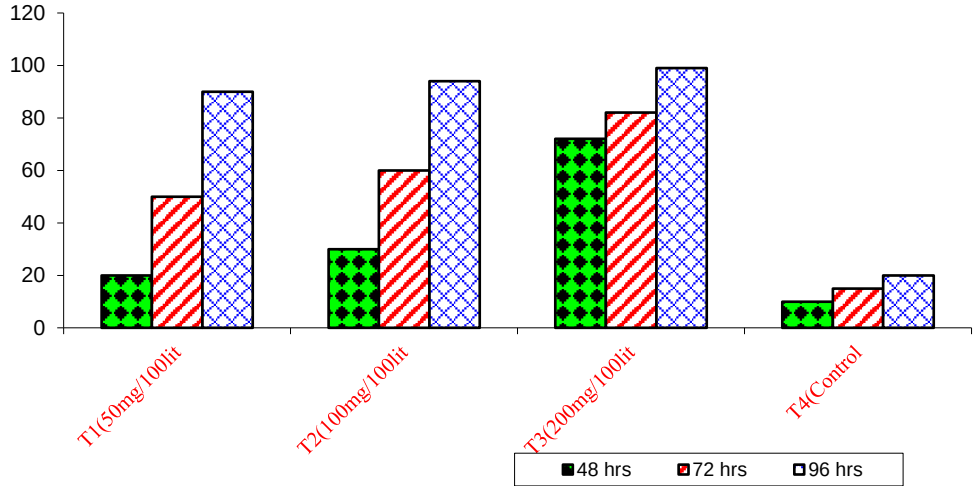

Fig. 3: Mortality percentage whiteheads with effect of different concentrations of Azadirachitin

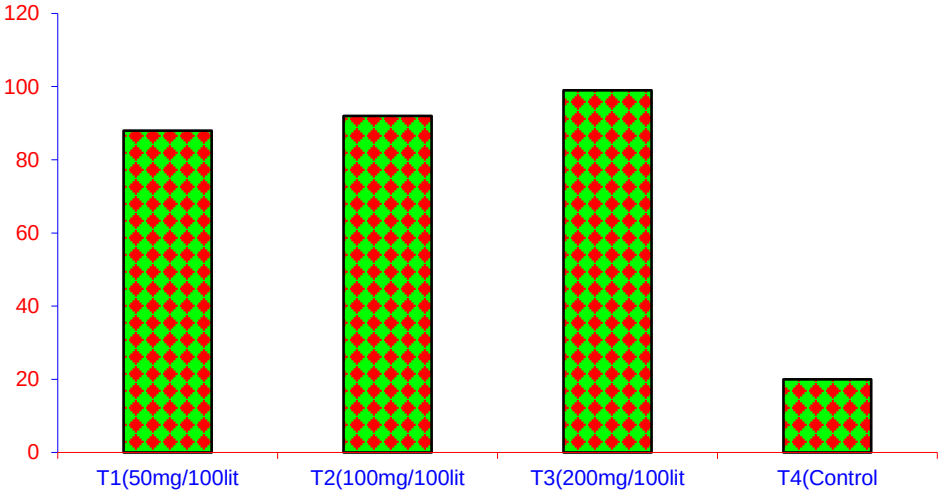

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Fig. 4: The number of dead whiteheads with effect of different concentrations of Azadirachitin paddy leaf folder

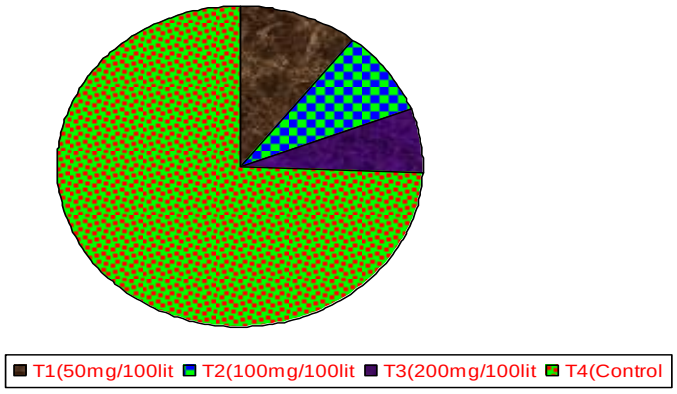
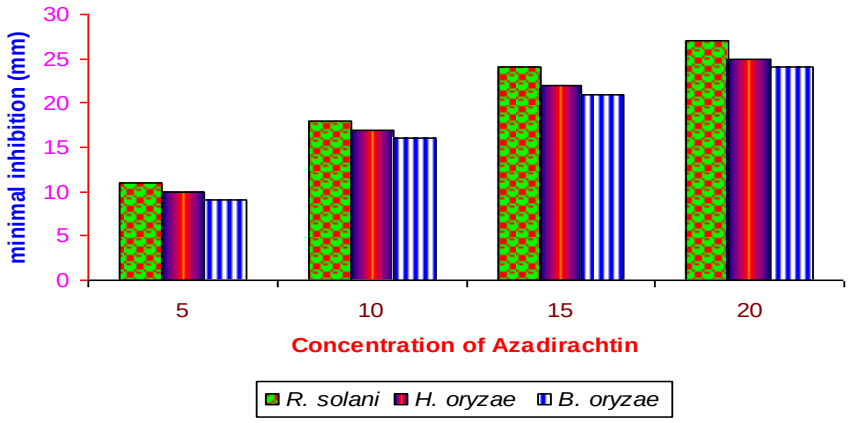


Fig. 5: The antifungal activity of fungal strains with effect of different concentrations of Azadirachitin



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A STUDY ON IMPACT OF INDUSTRY EFFLUENT ON FRESH WATER FISH *CHANNA PUNCTATUS*

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ABSTRACT

The physiological and biochemical changes in *Channa punctatus* after exposure to sublethal concentrations of the textile industry effluent have been investigated for the period of 28 days. Two different concentrations of textile industry effluents were used to carry out the experiment viz 25 cm^3/dm^3 and 50 cm^3/dm^3. The results of physiological and biochemical changes in muscles and blood of *C. punctatus* showed that, fish were under considerable stress during the exposure periods to the sublethal doses. The results obtained in the present study showed that, the industrial effluents from textile caused marked depletion in the biochemical composition of muscles and blood parameters of fish *C. punctatus* after the exposure period. The biochemical changes, protein, lipid and carbohydrate were recorded.

Key Words: Textile industry effluent, toxicity of fishes, *Channa punctatus*, Physiological response, Biochemical changes.

INTRODUCTION

In India, the indiscriminate discharge of raw sewage and industrial effluents has been the major source of pollution of rivers. Uncontrolled discharge of industrial effluent in Alexandria has led to severe impact on ecological balance and appreciable environmental deterioration. Heavy metals are among the pollutants that can cause pollution to aquatic organisms. Their concentrations usually show a considerable rise in the waters receiving industrial wastes.

The textile industry is still regarded as one of the largest industrial sectors, processing cotton and wool as well as synthetic fibers such as polyester, nylon, acrylic etc, and water usage constitutes a major component. The application of water is normally associated with the requirement of large quantities especially within the scouring, bleaching, dyeing and finishing sectors of the industry. Water usage has traditionally been on the basis of abstract, use and discharge with little regard for any long-term environmental impact its continued use may have. Up to 70% of water is being used in process preparation and dyeing with typical requirements for cotton being 100 to 150 m^3 of water per 1000 kg of fabric produced. For wool processing this figure increases to 200 m^3 of water per 1000 kg of fabric produced. In addition to this one needs to consider the chemical constituents of the water at discharge, the flow rate and the volume of effluent all of which can vary immensely from process to process.

Alteration in the chemical composition of a natural aquatic environment by industrial effluents, usually induce changes in the behavioural, biochemical and pathological aspects of the inhabitants, particularly fish (Edwards, 1973). The effluents released from tanneries, pulp factories, paper mills, sugar factories etc., and are having large amounts of toxic chemicals, which cause death of organisms. The level of toxicity of these chemicals may be either increase or decrease, when the effluent is stored.

Although there are several papers available on the effects of the effluent of the Copper to aquatic organisms (Hamza *et al.*, 1985; Mourad, 1995; Aboul-Naga and Allam, 1996), yet no

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information has been published about physiological and histological changes following the exposure to this waste water. The measurement of these changes may provide a sensitive method for predicting the effect of chronic exposure on survival, reproduction and growth. Therefore, the main objective of this study is to assess the physiological and Biochemical changes in *Channa punctatus* after exposure to sublethal concentrations of the effluent of the textile industry.

MATERIALS AND METHODS

The effluents from textiles (at Tirupur, on the bank of Noyyal River) is discharged into a common effluent canal and mixed with the river near Tirupur. The effluent contains chlorides (2050±125mg/l), Sulphate (1053±98 mg/l), Sulfide (35 ± 5 mg/l), Total suspended solids (492 ± 40 mg/l) and total dissolved solids (5580 ± 312 mg/l). About 150 litre of raw effluents from the canal was collected in clean polyethylene containers and stored at room temperatures. The experiment was carried out on the same day of collection.

The fish *Channa punctatus* was collected from fresh water ponds around Thanjavur, Tamil Nadu, South India. They were reared for 15 days in large glass tanks previously washed with potassium permangate to free the walls from fungal infections. The fishes were fed with boiled eggs and fish feed every day. Water in the tank was renewed 2 to 3 times a weak. Fishes ranging in size 10.5±2 cm and weight 15.5 ± 2 gm were brought to the laboratory and acclimated under appropriate experimental conditions for two weeks. The fish were fed on a diet containing 35% of protein. The water temperature ranged from 22 to 24°C. The dissolved oxygen concentration was maintained at, or close to 100% of air Saturation by vigorous aeration.

Acute toxicity lest was carried out according to Standard Methods for the Examination of Water and Wastewater (1975). To study the effects of sublethal concentrations of this wastewater, ten fish were introduced to each aquarium containing 50 litres of different dilutions (2.5; 5.0 cm3/dm3). The time of experiment was 4 weeks. To determine the effects of sublethal concentrations on some haematological parameters of fish, the blood was collected directly from the caudal artery into heparinized capillary tubes. Plasma protein and glucose were measured using Standard kits (Modern Laboratory Chemicals). Plasma ion concentrations of sodium and potassimn were measured using Gallenkamp flame analyser. Hematocrit was determined using microhematocrit tubes. Muscle protein concentration was measured using the method of Biuret (Gornall *et al* 1949). Muscle lipid concentration was measured using the method of Knight *et al* (1972). Moisture was determined by drying at 125°C for 3 hours and ash was measured by heating at 55^0 C for 3 hours.

RESULTS AND DISCUSSION

The results of acute toxicity test for *Channa punctatus* exposed to different concentrations of the textile industry effluent showed that the LC_{50} was 25 cm^3, which means that this wastewater is highly toxic. The toxicity of this waste water is attributed mainly to combination of several synergistic factors e,g. high concentration of heavy metals and solids besides low pH and dissolved oxygen (Mourad, 1995). Table 1 represents the physiological response of *Channa punctatus* after exposure to sublethal concentrations of the effluent of the textiles (2.5; 5.0 cm3/dm3) for 28 days. A significant increase in hematocrit from 25.9% to 30.2 and 31.8% was observed after exposure to this wastewater that may be attributed to gill damage or increased demand for oxygen by certain tissues (Andersson *et al.,* 1988). Several authors also observed hematocrit value increase after exposure to heavy metals of effluents (Mc Kim *et al.,* 1970 amd Hilmy *et al.,* 1987).

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A significant hyperglycaemia was also recorded after exposure to this wastewater e.g. control fish had a mean plasma glucose of 56.8 mg/100 cm^3 while the-treated fish exhibited an increase in the levels of plasma glucose to 65.3 and 81.0 mg/100 cm^3, respectively. This means that the fish were subjected to some sort of hypertoxic stress. It is well known that stressful stimuli elicit rapid secretion of both glucocorticoids (Wedemeyer, 1969) and catecholamines (Nakano and Tomlinson, 1967) from the adrenal tissues of fish and both of these hormones produced hyperglycaemia (Oguri & Nace 1966). The obtained results are in agreement with Dange, (1986) and Benson *et al.,* (1987) who recorded an increase in plasma glucose levels after exposure to heavy metals of effluents.

In this study, tissue and plasma total protein were generally influenced by this wastewater which may be attributed to the relative changes in the mobilization of protein, Changes in the plasma protein concentrations may be a result of increased production of metallothionein which is a sequestering agent (Cousins, 1982). On the other hand, the elevation of plasma glucose that runs parallel to a decrease in muscle protein content may be on indication of a gluconeogenetic response. This additional source of glucose may support the fish with the required energy highly demanded to cope with the presence of a potentially harmful substances such as effluents.

An increase in the levels of plasma sodium and potassium concentrations was also observed after exposure to this wastewater. This may be attributed to the changes in the permeability to sodium and potassium at the branchial site. The obtained results are in accordance with Stagg and Shuttleworth, (1982) who found disturbances in plasma electrolyte concentrations after exposure of the fish to effluents.

The condition of fish exhibited a significant depression after exposure to this wastewater, which might be a result of elevation of the fish metabolic rate and cessation of feeding. Buckley *et al.,* (1982) showed also a decrease in the condition of fish after exposure to effluents. Changes in the muscle lipid, ash, and water content were statistically insignificant.

The textile industry is considered to the oldest manufacturing industry. The textile processing industry is one of the main industry, which uses substantial volume of water and chemicals that causes pollution. About 80,000-1,30,000 cubic meter of fresh water is being; used for treatment plant every day in Tirupur. The wastewater after processing is unfit for use and requires special treatments before discharged. Polluted effluent water has to be treated to save environment from pollution. The effluent from Tirupur is sent to Noyyal River. It has caused great problems to agriculturists around the city. Many studies have been undertaken to treat the effluent water in different method. The most common method used in the industry has been taken for the study to see the efficacy of the treatment.

Table 1. Physiological and Biochemical response of *Channa punctatus* exposed to sublethal concentrations of tannery effluent for 28 days

S. No	Parameter	Concentration (vol./vol.)		
		Control	2.5 cm^3/dm^3	5.0 cm^3/dm^3
1	Hematocrit (%)	26.88 ±1.08	32.23 ±1.19*	33.81 ±0.98*
2	Plasma protein (mg/100 cm^3)	4.83 ±0.1 3	4.95±0.13*	4.99 ±0.21*
3	Plasma glucose (mg/100 cm^3)	59.80 ±4.34	69.30 ±4. 13*	82. 00 ±6.38*
4	Plasma sodium (mmol/dm^3)	140.00 ±3.79	144.50 ±3.08*	146.50 ±4.8*
5	Plasma potassium(mmol/dm^3)	13.50 ±1.10	14.60 ±1.70	15.40±1.80*

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6	Muscle protein (mg/1 00 mg)	25.60 ±1.20	24.20 ±1.08*	22.30 ±1.27*
7	Muscle lipid (mg/100 mg)	1.6810.62	1.72 ±0.48	1.69 ±0.52
8	Water content (%)	80. 20 ±1.84	78.10 ±1.78	76.70 ±1.86
9	Ash content (%)	7.10+0.85	7.30 ±0.94	7.60 ±0.59
10	Fish condition (Kf)	1.62 ±0.03	1.57 ±0.03*	1.58 ±0.02*

* Significant difference in comparison to control group.
Average of 10 fish ± standard deviation.

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A STUDY ON IMPACT OF DISTILLERY EFFLUENTS ON FISH

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ABSTRACT

The physiological, biochemical and histological changes in *Channa punctatus* after exposure to sublethal concentrations of the effluent of the Distillery industry have been investigated. The results of acute toxicity test showed that the LC_{50} was 25 cm3/dm3, which means that this wastewater is highly toxic. The results of physiological, biochemical and histological changes in *Channa punctatus* showed that, fish were under considerable stress during exposure to sublethal doses of this wastewater. Physiological response of fish revealed significant disturbances in respiratory system, fish metabolism, and ionic osmoregulation. Biochemical changes, protein, lipid and carbohydrate were recorded. Pathological changes attributed to effluents were observed in the gills, liver, and kidney.

Key words: Physiochemical, effluent, fish, *Channa punctatus*

INTRODUCTION

In India, the indiscriminate discharge of raw sewage and industrial effluents has been the major source of pollution of rivers. Uncontrolled discharge of industrial effluent in Alexandria has led to severe impact on ecological balance and appreciable environmental deterioration. The effluents concentrations usually show a considerable rise in the waters receiving industrial wastes. Alteration in the chemical composition of a natural aquatic environment by industrial effluents, usually induce changes in the behavioural, biochemical and pathological aspects of the inhabitants, particularly fish (Edwards 1973). The effluents released from distilleries, tanneries, pulp factories, paper mills, sugar factories etc., are having large amounts of toxic chemicals, which cause death of organisms. The level of toxicity of these chemicals may either increase or decrease, when the effluent is stored.

Although there are several works available on the effects of the effluent on the aquatic organisms {Hamza *et al.* 1985, Mourad, 1995, Aboul-Naga and Allam 1996), yet no strong information has been published about physiological and histological changes following the exposure to the distillery waste water. The measurement of these changes may provide a sensitive method for predicting the effect of chronic exposure on survival, reproduction and growth. Therefore, the main objective of this study is to assess the physiological, biochemical and histological changes in *Channa punctatus* after exposure to sublethal concentrations of the effluent of the distillery industry.

MATERIALS AND METHODS

The effluents from distilleries is discharged into a common effluent canal and mixed with the river. The distillery effluents were collected near the town Erode of Tamil Nadu. The effluent contains chlorides (2550±10mg/l), Sulphate (1003±98 mg/l), Sulfide (28 ± 5 mg/l), Chromium (25 ± 2mg/l), Copper (20 ±4 mg/l), Total suspended solids (432 ± 4 mg/l) and total dissolved solids (5320 ± 31 mg/l). About 150 litre of raw effluents from the canal was collected in clean polyethylene containers and stored at room temperatures.

The fish *Channa punctatus* was collected from fresh water ponds around Pattukkottai, Tamil Nadu, South India. They were reared for 15 days in large glass tanks previously washed

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with potassium permangate to free the walls from fungal infections. The fishes were fed with boiled eggs and fish feed every day. Water in the tank was renewed 2 to 3 times a week. Fishes ranging in size from 10±2 to 11±2 cm and weight from 15 ± 2 to 17±2 gm were brought to the laboratory and acclimated under appropriate experimental conditions for two weeks. The water temperature ranged from 24 to 28°C. The dissolved oxygen concentration was maintained at, or close to 100% of air saturation by vigorous aeration.

Acute toxicity test was carried out according to Standard Methods for the Examination of Water and Wastewater (1975). To study the effects of sublethal concentrations of this wastewater, ten fish were introduced to each aquarium containing 50 litres of different dilutions (2.5; 5.0 cm3/dm3). The time of experiment was 4 weeks. To determine the effects of sublethal concentrations on some haematological parameters of fish, the blood was collected directly from the caudal artery into heparinized capillary tubes. Plasma protein and glucose were measured using Standard kits (Modern Laboratory Chemicals). Plasma ion concentrations of sodium and potassimn were measured using Gallenkamp flame analyser. Hematocrit was determined using microhematocrit tubes. Muscle protein concentration was measured using the method of Biuret (Gornall *et al* 1949). Muscle lipid concentration was measured using the method of Knight *et al* (1972). Moisture was determined by drying at 125°C for 3 hours and ash was measured by heating at 55^0 C for 3 hours.

Biopsy specimens were collected for microscopic investigation from the gills, liver and kidney of control and treated fish. Fixed in formalin solution, dehydrated, cleared and embedded in paraffin wax, sectioned at 5-µm-thick sections, stained using Ehrlish haematoxylin and eosin, mounted for microscopically examination.

RESULTS AND DISCUSSION

The results of acute toxicity test for *Channa punctatus* exposed to different concentrations of the tannery effluent showed that the LC_{50} was 25 cm3/dm3, which means that this wastewater is highly toxic. The toxicity of this waste water is attributed mainly to combination of several synergistic factors e,g. high concentration of heavy metals and solids besides low pH and dissolved oxygen (Mourad 1995).

Table 1 represents the physiological response of *C. punctatus* after exposure to sublethal concentrations of the effluent of the distilleries (2.5; 5.0 cm3/dm3) for 28 days. A significant increase in hematocrit from 25.88% to 30.23 and 31.81% was observed after exposure to this wastewater that may be attributed to gill damage or increased demand for oxygen by certain tissues (Andersson *et al.*, 1988). Several authors also observed hematocrit value increase after exposure to heavy metals (McKim *et al.*, 1970 and Hilmy *et al.*, 1987).

A significant hyperglycaemia was also recorded after exposure to this wastewater e.g. control fish had a mean plasma glucose of 56.80 mg/100 cm3 while the-treated fish exhibited an increase in the levels of plasma glucose to 65.30 and 81.00 mg/100 cm3, respectively. This means that the fish were subjected to some sort of hypertoxic stress. It is well known that stressful stimuli elicit rapid secretion of both glucocorticoids (Wedemeyer, 1969) and catecholamines (Nakano and Tomlinson 1967) from the adrenal tissues of fish and both of these hormones produced hyperglycaemia (Oguri and Nace, 1966). The obtained results are in agreement with Dange, (1986) and Benson *et al.*, (1987) who recorded an increase in plasma glucose levels after exposure to heavy metals.

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In this study, tissue and plasma total protein were generally influenced by this wastewater which may be attributed to the relative changes in the mobilization of protein, changes in the plasma protein concentrations may be a result of increased production of metallothionein which is a sequestering agent (Cousins, 1982). On the other hand, the elevation of plasma glucose that runs parallel to a decrease in muscle protein content may be on indication of a gluconeogenetic response. This additional source of glucose may support the fish with the required energy highly demanded to cope with the presence of a potentially harmful substances such as effluents.

An increase in the levels of plasma sodium and potassium concentrations was also observed after exposure to this wastewater. This may be attributed to the changes in the permeability to sodium and potassium at the branchial site. The obtained results are in accordance with Stagg and Shuttleworth (1982) who found disturbances in plasma electrolyte concentrations after exposure of the fish to effluents.

The condition of fish exhibited a significant depression after exposure to this wastewater, which might be a result of elevation of the fish metabolic rate and cessation of feeding. Buckley *et al.*, (1982) showed also a decrease in the condition of fish after exposure to effluents. Changes in the muscle lipid, ash, and water content were statistically insignificant.

Histological examination of the fish after exposure to sublethal concentrations of the effluent of the distilleries revealed serious disturbances in the gills, liver and kidney tissues. As shown in control gills had normal structure of lamellae and wide interlamellar space. The fish exposed to diluted effluent showed hyperplasia of primary lamellar epithelium leading to obstruction of the water passage and reduction of the respiratory surface area. Similar results were recorded in other teleosts exposed to copper (Gardner and Roche, 1973; Daoust 1981; Khadre, 1990).

The liver of control fish group showed the usual polyhedral hepatocytes, homogeneous cytoplasm and rounded nuclei. The exposed fish showed fatty infiltration with nuclear pycnosis. This fatty infiltration may be due to metabolic disturbances in liver tissue that enhanced activity of biotransformation enzymes. Histological lesions involving extensive fatty infiltration due to abnormal lipid accumulation in present work is in agreement with those finding of Backer (1969) and Khadre (1991).

The kidney of control fish showed normal structure of renal tubules (RT) and haemapoietic tissue in between. The exposed fish showed tubular destruction and epithelial ceil oedema. This oedema caused detachment of the epithelial cells from the underling basement membrane, pycnosis of nuclei and swelling of others. Deposition of dark granules inside glomeruli was also observed. This darkly stained granules may be a result of alteration in urinary pH or urinary stasis as a consequence of tubular destruction. Backer (1969) and Wahbi (1998) found the same results in winter flounder and *Solea vulgaris* due to copper and industrial effluent toxicity.

Table 1. Physiological and Biochemical response of *Channa punctatus* exposed to sublethal concentrations of distillery effluent for 28 days

S. No	Parameter	Concentration (vol./vol.)		
		Control	2.5 cm^3/dm^3	5.0 cm^3/dm^3
1	Hematocrit (%)	23.78 ±1.06	32.22 ±1.19*	33.85 ±0.78*
2	Plasma protein (mg/100 cm^3)	4.55 ±0.12	4.68±0.13*	4.99 ±0.29*

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3	Plasma glucose (mg/100 cm^3)	58.08 ±4.34	67.36 ±6. 9*	83.03 ±6.32*
4	Plasma sodium (mmol/dm^3)	138.0 ±3.91	141.5 ±3.8*	144.5 ±4.2*
5	Plasma potassium(mmol/dm^3)	15.80 ±1.10	16.92 ±1.70	17.40±1.80*
6	Muscle protein (mg/1 00 mg)	25.70 ±1.4	24.90 ±1.03*	23.80 ±2.71*
7	Muscle lipid (mg/100 mg)	1.61 ±1.7	1.82 ±0.48	1.75 ±0.52
8	Water content (%)	75. 20 ±1.84	74.10 ±1.78	73.70 ±1.86
9	Ash content (%)	7.10+0.85	7.30 ±0.94	7.60 ±0.59
10	Fish condition (Kf)	1.58 ±0.03	1.49 ±0.03*	1.57 ±0.02*

* Significant difference in comparison to control group.
Average of 10 fish ± standard deviation.

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ANTIMICROBIAL ACTIVITY OF HETEROCYTIC CYANOBACTERIA

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ABSTRACT

Invitro screening of antimicrobial activities of some heterocystous cyanobacteria from agricultural fields in the Pattukkottai, Thanjavur District were studied. The collected cyanobacterial supernatants, ethanolic extract from biomass of 80 strains of cyanobacteria were isolated and screened against five bacteria and five fungi. Ethanolic extracts and culture supernatants of 10 strains of cyanobacteria significant antimicrobial effect. According to these results, it is concluded that strains of *Anabaena* species, seem to be more potential for producing antimicrobial substances than other strains. This is interesting in that the traditional method of treating a microbial infection was by administering a decoction of the cyanobacteria, whereas according to our results a bacterium is better than fungi; hence this may be more beneficial.
Keywords: Antimicrobial, Blue-green algae, cyanobacteria and heterocystous

INTRODUCTION

Cyanobacteria are a very old group of organisms and represent relics of the oldest photoautotrophic vegetation in the world that occur in freshwater, marine and terrestrial habitats (Mundt and Teuscher 1986). Cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising groups of organisms to be able of producing bioactive compounds (Schlegel *et al*. 1999). Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial (Jaki *et al*. 2000), antifungal (Kajiyama *et al*. 1998), antiviral (Patterson *et al*. 1994), anticancer (Luesch *et al*. 2000), antiplasmodial (Papendorf *et al*. 1998), algicide (Papke *et al*. 1997), antiplatelet aggregation (Rho *et al*. 1996) and immunosuppressive (Koehn *et al*. 1992) activities.

One potential commercial application of microalgae derived compounds that have; as yet, received little attention is the area of pharmaceuticals, antibiotics and other biologically active compounds. Both cell extracts and extracts of the growth media of various unicellular algae (*Chlorella vulgaris, Chlamydomonas pyrenoidosa*) have been proved to have antibacterial activity *in vitro* against both Gram-positive and Gram-negative bacteria. It has also been reported that a wide range of *in vitro* active antifungal activities are obtained from extracts of green algae, diatoms and dinoflagellates. Microalgae, such as *Ochromonas* sp., *Prymnesium parvum*, and a number of blue-green algae produce toxins that may have potential pharmaceutical applications (Browitzka and Browitzka 1988).

Biologically active substances were proved to be extracted by microalgae. Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity. Temperature of incubation, pH of the culture medium, incubation period, medium constituents and light intensity are the important factors influencing antimicrobial agent production (Noaman *et al*.

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2004). Screening of cyanobacteria for antibiotics and other pharmacologically active compounds, has received ever-increasing interest as a potential source for new drugs (Ostensvik *et al*. 1998). Cyanobacteria from local habitats seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms (Mundt *et al*. 2001). Cyanobacteria have not yet been studied for antimicrobial activity and little work has been done to screen cyanobacteria isolated from soils with regard to their production of bioactive compounds. In order to find the potential of cyanobacteria for production of antimicrobial compounds in agricultural field. In this study, some heterocystous microalgae were tested wherein we report their efficacy against ten pathogenic microorganisms.

MATERIALS AND METHODS

Isolation and identification of cyanobacteria

Soil samples were collected from different agricultural fields in Thanjavur District, Tamil Nadu, India. Soil samples in laboratory were cultured directly in Nitrogen free BG_{11} media (Browitzka and Browitzka 1988), after colonization, cyanobacteria were transferred to the same medium. Each isolated cyanobacterium was cultured in a 250 ml flask containing 100 ml of BG_{11} medium without shaking, for 30 days. The incubation temperature was 28°C ± 2 and illumination at 3000 lux with a white continuous light. The isolated cyanobacteria and Identification was done using morphological variation studies and taxonomical approaches (Anagnostidis and Komarek 1988; Desikachary 1959; Prescott 1962 and Anand *et al*. 1990).

Preparation of extracts for antimicrobial activity

The cultures were harvested after 30 days by centrifugation at 10000 rpm for 10 min. The aqueous supernatant was collected and the algal pellet was extracted with 15 ml of ethanol followed by 15 ml hexane, with shaking for 20 min. The culture supernatants and solvent extracts were dried under reduced pressure at 40°C and were stored at 10°C for further studies.

The following bacteria and fungi were used as test organisms: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Shigella sonnei*, *Proteus vulgaris*, *Candida krusei*, *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus niger* and *Aspergillus fumigatus*.

Dried extracts and supernatants were dissolved in 4 ml of their extraction solvent and antimicrobial activity was determined by the disc method. Filter paper discs (6.0 mm) were saturated with 50 µl of the test solution, dried under laminar air flow and placed on the Muller-Hinton agar plate for bacteria and potato dextrose agar plate for fungi, which had been inoculated with a lawn of the test microorganisms. Plates were incubated at 37°C, for a period of 18-24 hour for bacteria and at 25°C, for 24-48 hours for fungi. Discs treated with 50µl ethanol was used as negative controls and gentamycine discs were used (10 µg) as positive controls. The extracts and supernatants containing antimicrobial components produced distinct, clear, circular zones of inhibition around the discs and the diameters of clear zones were determined and used as an indication of antimicrobial activity.

RESULTS AND DISCUSSION

The results of culture supernatants and ethanolic extracts of the isolated cyanobacteria that demonstrated antimicrobial activity are shown in Tables 1 and 2. Supernatant and ethanolic extract of 10 strains from the 80 cyanobacteria strains, showed significant antimicrobial activity against bacteria and fungi. Three of them were identified as *Nostoc* species, where 2 *Anabena* species, two *Haplosiphan* species and each one *Westielliopsis, Cylindrospermum, Plectonema* species were also among those showing antimicrobial activity.

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In the present pilot screening of cyanobacterial extracts of 10 species were found to show species specific activity against the 10 human pathogens. The details of activity of aqueous and ethanol extracts of algae along with activity profile with standard commercial antibiotics (gentamycine) was presented in table 1 and 2. The antibacterial activity of cyanobacteria aqueous and ethanolic extracts against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Shigella sonnei* and *Proteus vulgaris* shown in table 1. Maximum antibacterial activity was shown against *Staphylococcus aureus* followed by *Staphylococcus epidermidis* and *Proteus vulgaris*. Neither of the extracts was able to inhibit *Shigella sonnei* and *Staphylococcus haemolyticus*. Generally fungal pathogens have less inhibition than bacterial pathogens. This is interesting in that the traditional method of treating a microbial infection was by administering a decoction of the cyanobacteria, whereas according to our results an organic solvent is better; hence this may be more beneficial. Amongst the 5 bacterial and 5 fungal strains investigated *Staphylococcus aureus* and *Staphylococcus epidermidis* the most resistant and *Aspergillus* less resistant. .

DISCUSSION

A large number of microalgal extracts and extracellular products have been found to have antibacterial activity. However, pH of the medium, incubation period and temperature of incubation were very important for the biosynthesis of antimicrobial agent products as secondary metabolites. Previously reported[12] that temperature 35 °C, pH 8 and 15 days of incubation were the best for growth and antimicrobial agent production. In this study, these were chosen as an optimum pH, incubation periods and temperature.

The cyanobacteria such as *Nostoc commune* (Jaki *et al*., 2000), *Scytonema hofmanni* (Pignatello *et al*., 1983), *Hapalosiphon fontinalis* (Moore *et al*., 1987), *Anabaena* spp (Frankmolle *et al*., 1992), *Nostoc spongiaeforme* (Hirata *et al*., 1996), *Microcystis aeruginosa* (Ishida *et al*., 1997) have been reported as the main cyanobacteria to produce antimicrobial substances. Screening efforts aimed to identify antimicrobial agents in cyanobacteria have revealed several promising lead compounds. Some of these substances identified including Nostocyclyne A (Ploutno *et al*., 1997), Nostofungicidine (Kajiyama *et al*., 1998), Kawaguchipeptin B (Ishida *et al*., 1997), Nostocin A (Hirata *et al*., 1996), Ambigol A and B (Falch *et al*., 1995), Hapalindoles (Moore *et al*., 1987) and Scytophycins (Ishibashi *et al*., 1986).

Most of the studies have only done as *in vitro* assays and, it is likely that most of these compounds will have little or no clinical application, as they are either too toxic or inactive *in vivo* (Browitzka, 1995). They may however serve as useful lead compounds for synthesis of antibiotics or may find application in agriculture. For example Tjipanazoles isolated from the cyanobacterium, *Tolypothrix tjipanensis*, showed appreciable fungicidal activity against rice blast and leaf rust wheat infections.

A few studies have been done to screen cyanobacteria for production of antimicrobial substances from agricultural fields. Possibly the synthesis of highly active toxin is a defense option of cyanobacteria in these environments against other organisms like bacteria, fungi, viruses and eukaryotic microalgae. In one study, the culture media of cyanobacteria belonging to Nostaceae, Microchaetaceae and Scytonematacaea isolated from the Argentinian agricultural fields were found to be active against *S. aureus* and *Candida albicans* (De Caire *et al*., 1993).

In another study, it was shown that cyanobacteria from the agricultural fields of northern Thailand produce bioactive substances with antibiotic activity against *Bacillus subtilis*

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(Chetsumon *et al*., 1993). Cyanobacteria of Pattukkottai have not yet been studied for antimicrobial activity and this screening program is among the first studies done for assessment of antibacterial and antifungal activity of Pattukkottai agricultural field cyanobacteria.

In this investigation, out of 80 strains of cyanobacterial isolates, 10 showed significant *in vitro* antimicrobial effect. The proportion of the isolates with antibacterial and antifungal activities were approximately 13% and 12.5%, respectively, which is comparable with those published earlier in other screening programs 11% (Flores E and Wolk, 1986), 7% (Patterson *et al*., 1993) and 10% (Schlegel *et al*., 1999).

As depicted in Tables 1 and 2 *Nostoc*, *Anabena* and *Hapalosiphon* species produce bioactive substances, which may have potential for antibacterial and antifungal activity. Although some of the cyanobacteria produce active compounds against Gram-negative bacteria such as *S. sonnei* and *P. vulgaris* but less activity was found against*S. haemolyticus*. Different results have been reported by other authors in this case. Falch *et al*., (1995) and Hirata *et al*., (1996) reported active compounds against *E. coli* in the petroleum ether fraction of *Fischerella ambigua* and supernatant of *Nostoc spongiaeforme*, respectively.

Antifungal activity assays showed a good activity against *C. krusei*, *C. albicans* and *C. neoformans* and the minimum against *Aspergillus* sp. Among the isolated cyanobacteria, *Westielliopsis* species had the minimum activity against the test organisms. *Nostoc* and *Anabaena* species from chemical components had the greatest frequency among the species that showing antimicrobial activity and exhibited the most prominent effect. The effect of antimicrobial activity of Stigonemataceae has been reported in other studies (Patterson *et al*., 1994; Falch *et al*., 1995; Smitka *et al*., 1992). Among all of the species studied in this investigation for antibacterial and antifungal activity, it seems *Plectonema* strains are being reported for the first time as producer of antibacterial substances. The results of this work indicate that this group of organisms displays a potential that warrants further investigations.

Table 1. Antibacterial activity of heterocytic cyanobacteria as presented by inhibition zone diameter (in mm)

S. No	Name of Species	*S.epidermidis*	*S.aureus*	*S.haemolyticus*	*P.vulgaris*	*S.sonnei*
1	*Nostoc muscorum*	22	24	16	18	15
2	*N. pauludosum*	19	22	13	15	16
3	*N.spongiaeforme*	21	22	15	17	19
4	*Anabaena sphaerica*	16	17	14	15	16
5	*A. cylindrica*	19	19	16	17	18
6	*Haplosiphon welwitchii*	20	21	17	19	18
7	*H. fontinalis*	18	19	13	16	14
8	*Plectonema* sp	12	15	09	10	10
9	*Westielliopsis prolifira*	15	19	15	16	17
10	*Cylindrospermum* sp	19	22	16	18	17

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Table 2. Antifungal activity of heterocytic cyanobacteria as presented by inhibition zone diameter (in mm)

S. No	Name of Species	*C.krusei*	*C.albicans*	*A.niger*	*A.fumigatus*	*C.neoformans*
1	*Nostoc muscorum*	9	8	9	7	10
2	*N. pauludosum*	10	9	8	8	11
3	*N. spongiaeforme*	9	10	8	6	9
4	*Anabaena sphaerica*	8	7	7	6	9
5	*A. cylindrica*	9	9	6	5	8
6	*Haplosiphon welwitchii*	10	9	6	4	9
7	*H. fontinalis*	9	8	6	7	7
8	*Plectonema* sp	8	7	5	6	7
9	*Westielliopsis prolifira*	6	6	3	4	7
10	*Cylindrospermum* sp	9	10	7	6	11

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TODDALIA ASIATICA: AN UPDATED OVERVIEW OF CURRENT SCIENTIFIC RESEARCH WORK

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ABSTRACT

Toddalia asiatica, commonly known as forest pepper or orange climber, is a medicinal climbing shrub belonging to the Rutaceae family, distributed across tropical and subtropical regions. Rich in bioactive compounds such as alkaloids, flavonoids, coumarins, and essential oils, the plant exhibits diverse pharmacological properties, including antimicrobial, antimalarial, anti-inflammatory, analgesic, antioxidant, anticancer, and antidiabetic activities. Traditionally used in herbal medicine for treating infections, fevers, pain, and inflammatory conditions, its bioactive constituents have also attracted interest in modern allopathic research. Studies highlight its potential applications in the development of antimalarial, anticancer, anti-inflammatory, and antimicrobial drugs. Despite promising preclinical findings, no allopathic drugs incorporating *Toddalia asiatica* have been commercially developed, necessitating further research and clinical trials to validate its efficacy and safety for pharmaceutical use.

INTRODUCTION

Toddalia asiatica, also known as forest pepper or orange climber, is a climbing shrub belonging to the Rutaceae family. It is widely distributed across tropical and subtropical regions, including Africa, India, China, Southeast Asia, and Madagascar. This plant thrives in moist forests, woodlands, and thickets, often growing at elevations of up to 2,500 meters. Preferring well-drained soils with moderate sunlight, it typically climbs on trees or spreads as a sprawling shrub. Known for its medicinal properties, *Toddalia asiatica* has been traditionally used to treat fevers, digestive issues, and respiratory ailments (Lakshmi and Siddiraju, 2022).

Plants are not only considered a food source but also supply an effective bioactive compound such as phytochemicals, which can be used as a drug to combat many disorders of mankind (Upendra and Khandelwal, 2012). Bioactive compounds, known as phytoconstituents, are produced as secondary metabolites in plants that have beneficial effects on health when they are consumed as nutrients (Khandelwal et al., 2012). Phytochemicals play an important role in the development of the color, odor, and taste of the plants (Khandelwal et al., 2013). Traditionally used herbal (or) medicinal plants synthesize a range of phyto compounds of known

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therapeutic values. Presently significant members of researchers worldwide (Prithviraj et al., 2021; Upendra and Ahmed, 2021) report on the pharmacological, healthcare, and antimicrobial activities of herbal plants.

The bioactive phytocompounds originated from plant sources, i.e., alkaloids, flavonoids, phenolic compounds, and tannins have been used to treat various ailments caused by microorganisms (Upendra et al., 2011). *Toddalia asiatica* is rich in bioactive compounds, including alkaloids, flavonoids, coumarins, and essential oils. Among its key alkaloids are toddaline, toddalinine, nitidine, chelerythrine, and skimmianine, which contribute to its medicinal properties. The plant also contains several coumarins, such as toddacoumaquinone, xanthyletin, toddalolactone, and toddaculin. In addition, flavonoids like quercetin, kaempferol, and rutin are present, enhancing its antioxidant activity. The essential oil composition includes limonene, β-caryophyllene, α-pinene, and eugenol, along with other phytochemicals like triterpenoids, β-sitosterol, and lignans. These constituents collectively contribute to the plant's antimicrobial, anti-inflammatory, analgesic, and antimalarial properties, making *Toddalia asiatica* valuable in traditional medicine (Irudayaraj et al., 2022; Jain et al., 2006; Kariuki et al., 2013).

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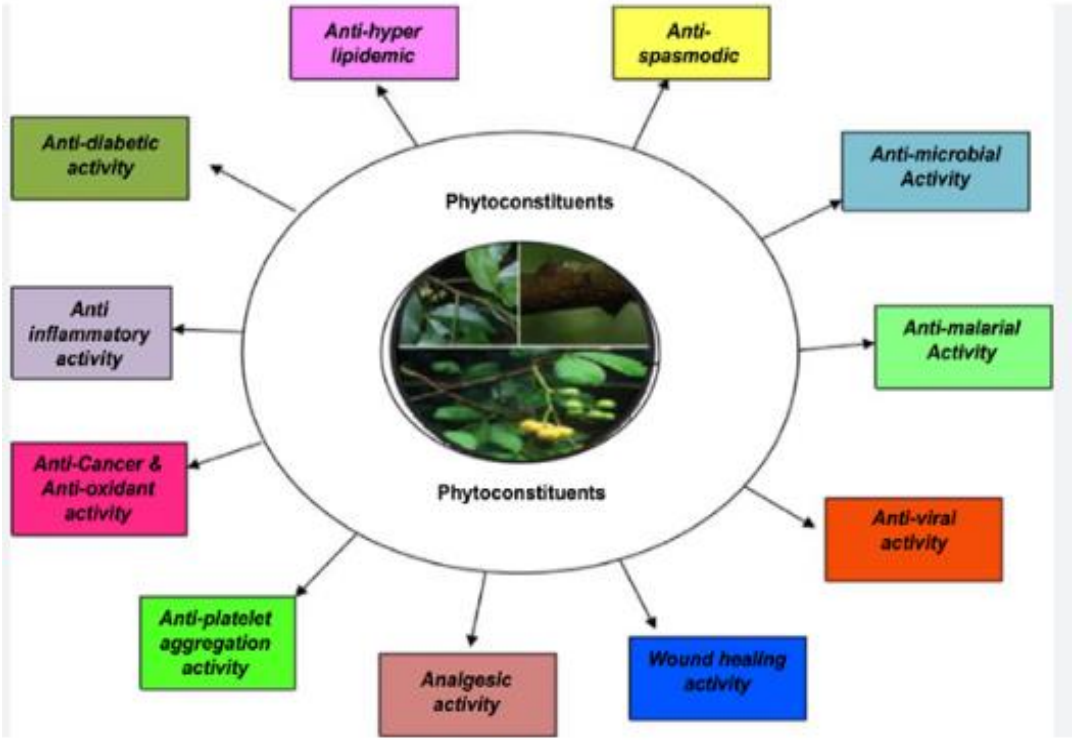

Toddalia asiatica exhibits a wide range of biological activities due to its rich phytochemical composition. Some of its key pharmacological properties include:

1. Antimicrobial Activity

The plant has demonstrated **antibacterial and antifungal** properties, attributed to alkaloids like nitidine and chelerythrine, as well as essential oils such as eugenol and β-caryophyllene. Studies have shown its effectiveness against Staphylococcus aureus, Escherichia coli, and Candida species.

2. Antimalarial Activity

Traditionally used to treat malaria, *Toddalia asiatica* contains alkaloids like skimmianine and coumarins like toddalolactone, which exhibit antiplasmodia**l** properties, inhibiting the growth of *Plasmodium falciparum*.

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3. Anti-inflammatory and Analgesic Activity

The presence of flavonoids and coumarins contributes to anti-inflammatory effects by reducing cytokine levels and oxidative stress. The plant is also known to have pain-relieving properties, making it useful in treating rheumatism and joint pain.

4. Antioxidant Activity

Compounds such as quercetin, kaempferol, and rutin act as powerful antioxidants, helping to neutralize free radicals and protect cells from oxidative damage.

5. Anticancer Activity

Alkaloids like nitidine and chelerythrine have shown cytotoxic effects against cancer cells, particularly in leukemia and breast cancer cell lines, by inducing apoptosis and inhibiting tumor growth.

6. Antidiabetic Activity

Flavonoids and essential oils in *Toddalia asiatica* have been reported to help regulate blood glucose levels, making it potentially beneficial for managing diabetes.

7. Wound Healing Properties

The plant's antibacterial, anti-inflammatory, and antioxidant effects contribute to enhanced wound healing by promoting tissue regeneration and preventing infections.

Due to these diverse biological activities, *Toddalia asiatica* is widely used in traditional medicine for treating various ailments, including infections, fevers, pain, and inflammatory conditions.

Toddalia asiatica used in allopathy medicine

Toddalia asiatica is primarily used in traditional medicine, but its bioactive compounds have attracted interest in modern allopathic research. While it is not yet widely integrated into mainstream allopathic medicine, studies have identified its potential for pharmaceutical applications, particularly in the development of antimalarial, anticancer, anti-inflammatory, and antimicrobial drugs.

Potential Allopathic Uses:

Antimalarial Drugs: Alkaloids like nitidine and skimmianine have shown significant antiplasmodial activity against *Plasmodium falciparum*, the parasite responsible for malaria. These compounds are being investigated as possible lead molecules for developing novel antimalarial drugs.

Cancer Treatment: Nitidine and chelerythrine, two alkaloids from *Toddalia asiatica*, have demonstrated cytotoxic effects on cancer cells, particularly in leukemia and breast cancer studies. Their ability to induce apoptosis (programmed cell death) and inhibit tumor growth has made them potential candidates for chemotherapy drug development.

Anti-inflammatory and Analgesic Medications: Flavonoids and coumarins from the plant exhibit anti-inflammatory effects, which could be beneficial in treating arthritis, chronic pain, and inflammatory diseases. Researchers are studying these compounds for their role in developing new pain-relief and anti-inflammatory drugs.

Antimicrobial Agents: *Toddalia asiatica* contains broad-spectrum antibacterial and antifungal compounds, which may be useful in fighting drug-resistant pathogens. Some of its alkaloids and essential oils have been tested for their effectiveness against Staphylococcus aureus and Escherichia coli, showing potential for new antibiotic development.

Current Status in Allopathy: While the plant's bioactive compounds have shown promising pharmacological properties, there are no widely available allopathic drugs directly derived from *Toddalia asiatica* yet. However, ongoing pharmaceutical research aims to isolate, modify, and synthesize its compounds for future drug formulations. Some of its active constituents have been

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used in laboratory studies and preclinical trials, but further clinical trials are needed to validate their safety and efficacy for human use. As of now, there are no widely recognized allopathic drugs that incorporate *Toddalia asiatica* or its derivatives as active ingredients. However, the plant has been extensively studied for its medicinal properties, and several bioactive compounds have been isolated from it. For instance, aculeatin, a coumarin derived from *Toddalia asiatica*, has been found to enhance differentiation and lipolysis in 3T3-L1 adipocytes. Additionally, the plant's root extract has demonstrated antinociceptive and anti-inflammatory effects in Swiss albino mice. Despite these findings, further research and clinical trials are necessary to develop standardized pharmaceutical formulations for use in allopathic medicine.

CONCLUSION

Although *Toddalia asiatica* is not yet officially part of mainstream allopathic medicine, its bioactive compounds hold great potential for drug development in areas such as malaria treatment, cancer therapy, pain management, and antibiotic research. Further studies and clinical trials could pave the way for its integration into modern pharmaceuticals.

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PHYSICO-CHEMICAL ANALYSIS OF SOIL AND WATER OF VEDARANYAM MANGROVE, TAMIL NADU, INDIA

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ABSTRACT

The present survey was made to study the some physico-chemical parameters in the water and soil of Vedaranyam mangroves during the year 2022-2023 at four-seasonal intervals. The water was slightly alkaline and contained high amounts of pH. The concentration of salinity, total, inorganic and organic phosphate, ammonia, nitrite and nitrate were fairly stable. Other nutrients such as calcium, magnesium, chloride and bicarbonate concentration showed remarkable variations. The mangrove soil was clay in all the seasons. pH and Organic matter level were very low during monsoon and high in summer. The chemical properties of the soils varied considerably among samples particularly in nutrient and iron level. The total amount of N, P, K, Na, Ca and Mg were maximum in the monsoon and minimum in summer season. The micronutrients such as zinc, copper, iron and manganese also present in moderate level in all the season.

Key words: Water analysis, Soil analysis, Vedaranyam mangroves

INTRODUCTION

Vedaranyam is one of the coastal blocks of Nagapattinam district. It is situated on the coast of Bay of Bengal, and is of historical importance, since during ancient days of chola kingdom. It falls within the co-ordinates of 10º15'-10º35'N latitudes and 79º20'-79º55'E longitudes. It has a tropical transitional bioclimate that is characterized by monthly average temperature above 30ºC. Total annual rainfall varies from 1000 to 1500 mm with a dry period of 6 to 7 months. Point Calimere (*Kalli-medu* Tamil) also called Cape Calimere and Kodikkarai, is a low headland on the Coromandel Coast, in the Nagapattinam district of the state of Tamil Nadu, India. It is the apex of the Cauvery River delta and marks a nearly right-angle turn in the coastline. A historic landmark here was the Chola lighthouse, destroyed in the tsunami of 2004.

Mangroves are coastal wetland forests established at the intertidal zones of estuaries, backwaters, deltas, creeks, lagoons, marshes and mudflats of tropical and subtropical latitudes. Approximately one fourth of the world's coastline is dominated by mangroves that are distributed in 112 countries and territories comprising a total area of about 181,000 km^2 (Saravanan, 2005). Among the marine ecosystems, mangroves constitute the second most important ecosystem in productivity and sustained tertiary yield after coral reefs. Productivity in mangrove waters depends on the extent of mangrove canopy cover that supplies carbon, nitrogen and phosphorous. Coastal environment plays a vital role in nation's economy by virtue of the resources, productive habitats and rich biodiversity. India has a coastline of about 7,500 kms. The coastline of Tamil Nadu has a length of about 1076 kms constitutes about 15% of the total coastal length of India and stretches along the Bay of Bengal, Indian Ocean and Arabian Sea. On the char, fishermen do not have any permanent establishment. This fishery is known as the Dubla Char winter fishery, an integrated part of the inshore and offshore marine fishes. Through it is an important forest for its plants, animals and mangrove fishery but there is limited information regarding its soil and water condition. So, the present study has been undertaken to know the some physico-chemical parameters of soil and water of Vedaranyam mangrove forest.

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MATERIAL AND METHODS

The soil and water samples were collected from coastal area of Vedaranyam mangrove forest of Nagappattinam district, Tamil Nadu, India. A sampling programme was consisting of seasonal physico-chemical parameters of water and soil. They survey was conducted for a period of one year from Oct. 2022 to Sep. 2023. The Physico-chemical characteristics of water were done according to the Standard Methods (APHA 1981). The Physico-chemical characteristics of soil were done according to the standard methods Organic matter (Walkley and Black, 1934), Avilable of phosphorus (Olsen *et al.*, 1954), Avilable Nitragen and Potassium (Sankaram, 1966). Sodium was recorded by the method of Aitken, (1984). Some moles like Ca and Mg were determined by according to the International pipette method describe by Piper, (1942) and also Hydrometer method (Bouyoucos, 1962). The temperature and pH of the water and soil were measured at the station itself. All the determinations were replicated thrice and the mean values were used to obtain representation of samples.

RESULTS

The mangrove water was slightly alkaline and contained high amounts of pH, total hardness, calcium, magnesium, chloride, total, inorganic and organic phosphate, ammonia, nitrite and nitrate in all the four seasons examined (Table 1). Most of the parameters tested were slightly higher in summer than the monsoon seasons. Alkalinity and salinity were observed more during the summer. In general, the characteristics of water tested in all the four seasons were not varied much.

The soil nutrients and physico-chemical characteristics of mangrove soil of study sites are given in (Table 2). Edaphic character sties of samples collected from study areas indicated that the soil were Brown to Red brown in colour and the texture was clay was present in the soil of Vedarnyam mangroves. The pH and Organic matter level were very low during monsoon and high in summer. The chemical properties of the soils varied considerably among samples particularly in nutrient and iron level. The total amount of N, P, K, Na, Ca and Mg were maximum in the monsoon and minimum in summer season. The micronutrients such as zinc, copper, iron and manganese also present in moderate level in all the season. Among the soil samples, all the micronutrients were maximum in monsoon and minimum in summer season.

DISCUSSION

The environmental parameters showed variations in different seasons in the study region depending on the topography. Salinity showed the highest values (28 ppt) in summer nearer to the coastal environment associated with low phosphorus (0.925) concentrations. The lowest value of salinity (23 ppt) was noticed in monsoon seasons, accompanying high phosphorus (1.535) concentration due to the freshwater zone of this aquatic environment. This in turn enhances the concentrations of ammonia (10.2 mg/l) and nitrite (8.0 mg/l) at these monsoon periods. High nitrate (22.0 mg/l), Inorganic phosphorus (0.992 mg/l) and Organic phosphorus (0.558 mg/l) concentration observed in the monsoon periods indicates the impact of terrestrial runoff.

Estuarine mangrove waters in general have relatively low stocks of inorganic phosphorus and nitrogen (Alongi *et al.*, 1992). In some cases, the degree of human impact seems to control nutrient profiles (Nedwell, 1975), while in others the degree of upland influence and the hydrology of the system appear to be of greater importance (Boto and Wellington 1988; Ovalle *et al.* 1990). The Muthupet mangrove ecosystem was found to be nutrient rich, and the ratios of N: P (10: 1) as well as TN:TP (8: 1) were low (Ramamurthy *et al.*, 2008). In the present study similar report was survey in Vedaranyam mangrove water. The water pH, temperature and

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salinity fluctuations in the Vedaranyam are consistent with seasonal cycles. However, the influence of the Vedaranyam lagoons on hydrographic conditions was observed at the sampling stations. The spatial and temporal differences in physicochemical variations indicate the diversity of habitats that exist within this lagoon. Monsoon season and post monsoon have a lower temperature and salinity than the premonsoon.

Total alkalinity values ranged from 17.5 to 27.6 mg/l. The higher total alkalinity values recorded in summer irrespective of the season may have been influenced by the presence of domestic waste and the absence of normal tidal action, which would have had flushing and diluting effect on dissolved constituents as well as bicarbonates, which could increase alkalinity levels. Generally, ammonia concentrations were lower in the dry season months than in the rainy season months. Seasonal influence resulting to lower ammonia values during the summer season, over levels in the monsoon season in Muthupet mangrove was also reported by Ramamurthy *et al*. (2008). Total Phosphate concentrations ranged from 0.900 to 1.635 mgl^{-1}. Values were also higher in the rainy months and the lowest concentration of summer was recorded. Phosphate concentrations reported for Muthupet are higher in rainy than other seasons those reported by Ramamurthy *et al*., (2008).

The mangrove soil nutrients and physico-chemical characteristics of mangrove soil of study sites are given in (Table 2). Edaphic character sties of samples collected from study areas indicated that the soil were Brown to Red brown in colour and the texture was clay was present in the soil of Vedarnyam mangroves. Muhibbullah *et al*., (2005) reported the average percentage of clay at Sharankhola, Chandpai, Nalianala and Burigoalini respectively for Sundraban mangrove in Bangladesh. So it is found that the soil texture of the Sundarban is likely silty clay. Choudhury (1968) found that the soil of Sundraban is finely textured and the sub soil is stratified and at greater depth is compacted. Zafer *et al*., (2001) reported that the percentage composition of soil in the chakaria mangrove areas were always found to be sand>clay>silt. Given that these mangroves werenot located in the geographic areas, the variabilityin the properties of the soils underlying these mangroves is notunexpected.

The average pH values were determined by 9.6 to 10.2 in the study periods. Hassan and Razzaque (1981) found that the pH value of soil in Sundarban is neutral to mildly alkaline under field conditions but in some localities the pH value of dried up sub soil samples drops to 6.5. Mohamood and Saikat (1995) reported bthe acidic pH values in the soil of Chakaria mangrove area and consequently, this area have a rich reserve of pyrite in its soil. Muhibbullah *et al*., (2005) reported the average pH values were found 6.3, 6.73, 7.13 and 6.8 in the Sharankhola, Chandpai, Nalianala and Burigoalini respectively for Sundraban mangrove in Bangladesh. Organic mater values in monsoon, post monsoon, pre monsoon and summer were 12.2, 13.7, 14.6 and 13.6 % respectively. Organic matter concentrations were greatest inthe summer in the mangrove (14.6%). Five percent organic matter is ideal for the proper composition of soil. Choudhury (1968) mentioned that the organic matter in mangrove soil belong over 5%. Due to more decomposition of plant and animal residues in mangrove area the percentage of organic matter is higher than other soil tract. For this reson the biological activity in mangrove forest area is highly active. Zafar *et al*., (1999) stated that organic matter varied between 0.86 and 1.9% in the intertidal muddy beach. Escourt (1967); Anderson (1977); Mayer *et al*., (1985) reported that organic carbon is releated to mud percentage in the soil. Mud percentage in the study areas were higher than sand and that is why organic matter was higher in the Sundaraban areas (Muhibbullah *et al*., 2005).

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The chemical properties of the soils varied considerably among samples particularly in nutrient level. Phosphorus concentrations present in the Vedaranyam mangrove ware analyzed in four seasons such as monsoon, post monsoon, pre monsoon and summer were 12.4, 10.1, 11.2 and 9.8 mg kg^{-1} respectively.The extractable phosphorus values reported in Parker *et al*. (1995) were similar to the results presented here; the range from thelower and upper profiles was between 20 and 120 mg kg^{-1}. The total amount of potassium was maximum in the monsoon and minimum in summer season. The potassium content is observed in the range of 250 to 750 µg/g in mangroves. Muhibbullah *et al*. (2005) reported the average potassium values is found 450-750, 250-450, 350-500 and 350-570 µg/g in Sharankhola, Chandpai, Nalianala and Burigoalini, respectively for Sundraban mangrove in Bangladesh. Sodium and potassium content in the soil were varied from soils varied considerably among samples particularly in nutrient level. The total amount of nutrients was maximum in the monsoon and minimum in summer season. Karim (1994) reported the sodium content is varied from 450-1850 µg/g, Calcium 2350 to 3950 µg/g and magnesium content 1000 to 1500 µg/g in the mangrove soils. Muhibbullah *et al*., (2005) reported the average sodium content is varied from 250-750 µg/g, Calcium content 1900 to 4500 µg/g and magnesium content 420 to 1500 µg/g in the Sundraban mangrove in Bangladesh.

Table. 1. Physico-chemical analysis of Vedaranyam mangrove water (October 2022 to September 2023)

S. No	Parameters	Monsoon (Oct-Dec)	Post-monsoon (Jan-Mar)	Summer (Apr-June)	Pre-monsoon (July-Sep)
1	pH	7.6	8.3	8.7	8.2
2	Atmospheric Temp.(^{0}C)	27	28	29	28
3	Surface water Temp.(^{0}C)	26	27	28	27
4	Total dissolved solid	1250	1700	1850	1550
5	Alkalinity	15.5	23.2	25.6	20.4
6	Ammonia	10.2	10.0	9.5	9.8
7	Nitrate	22	19	14	16
8	Nitrite	8	6	4	7
9	Calcium	825	860	951	895
10	Magnesium	295	305	350	332
11	Dissolved chloride	200	220	235	228
12	Salinity (ppt)	23	25	28	24
13	Total Phosphorus	1.535	1.269	0.925	1.299
14	Inorganic phosphorus	0.992	0.865	0.620	0.899
15	Organic phosphorus	0.558	0.454	0.310	0.434

* Except pH and temperature all values expressed in mg^{-1} .

Table. 2. Physico-chemical analysis of Vedaranyam mangrove soil (October 2022 to September 2023)

S. No	Parameters	Monsoon (Oct-Dec)	Post-monsoon (Jan-Mar)	Summer (Apr-June)	Pre-monsoon (July-Sep)
1	Soils texture	Clay	Clay	Clay	Clay
2	pH	9.6	9.8	10.2	10.0
3	Organic matter (%)	12.2	13.7	14.6	13.6
4	Nitrogen	6.9	5.9	5.5	5.7
5	Potassium	6. 7	4.9	3.8	4.1

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6	Phosphorus	12.4	10.1	9.8	11.2
7	Sodium	1.5	1.0	0.85	1.1
8	Calcium	30	25	19	24
9	Magnesium	1.8	1.2	1.0	1.4
10	Manganese	4.81	4.48	4.04	4.54
11	Zinc	1.18	1.16	0.098	1.01
12	Copper	0.59	0.42	0.40	0.43
13	Iron	2.71	2.50	2.02	2.25

* Except pH and organic matter all values expressed in mg/g.

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A STUDY ON BIODIVERSITY OF MICROBES IN TANNERY EFFLUENT

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ABSTRACT

An investigation was carried out to assess the tannery effluent on microbial diversity viz. bacteria, fungi and cyanobacteria. One year study revealed that 8 species of bacteria, 10 fungi and 18 species of cyanobacteria were observed from the effluent stream. Among bacteria, *Pseudomonas* with two species and others with single each were recorded. *Aspergillus* was dominant (4 species) among the fungi with followed by *Penicillium* with two. Cyanobacteria a dominant group, recorded 20 species. *Oscillatoria* with 9 species was the dominant genus followed by *Phormidium* 5, *Plectonema* 2, *Aphanocapsa* and *Chlorogloea* with one each. High amounts of phosphates and nitrates with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and slightly alkaline pH were probably the factors favouring the growth of microbes especially cyanobacteria.

Key Words: Tannery effluent, Biodiversity, Cyanobacteria, Pollution.

INTRODUCTION

Discharge of urban, industrial and agricultural wastes has increased the quantum of various chemicals that enter the receiving waters which in turn alter considerably their physico-chemical characteristics thereby increasing eutrophication. Since effluents are rich in nutrients due to loading of organic wastes, they after ideal habitats for different microorganisms including algae, fungi and bacteria. A variety of algae are growing in these habitats. Algal growth in these habitats significantly influences the ecosystem (Sladeckova 1962). Some algal forms can be useful as indicators on which major water management practices, pollution studies and water quality analysis was carried out by Palmer (1969) and Schubert (1984). The importance of algal dynamics, particularly their response to environmental changes and nutritional fluctuation have been suggested in several studies (Frempong 1981; Tilman *et al.* 1982; Sudhakar *et al.* 1991). In recent years blue green algae have been drawing tremendous attention because of their ability to treat wastewater and improve water quality. Taking the above facts into consideration, a survey was undertaken in tannery effluent to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria and to exploit cyanobacteria as a tool in treating tannery effluent.

MATERIALS AND METHODS

Tannery effluent was collected from Trichy tannery industry situated at Trichy, Tamil Nadu, India. A sampling programme consisting of a series of monthly water quality and microbial survey was conducted for one year (Jan. 2022 to Dec. 2023). Population of bacteria and fungi were identified and isolated from the effluent samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical studies as given by Bergey (1984). Fungi were identified by using standard manuals (Gillman 1957) and (Ellis 1971). Effluent samples were collected in duplicate from the station in pre-sterilized bottles. For cyanobacterial survey, 10 places were selected along the effluent stream. Samples were collected from the places along with effluents in polythene bags. Standard microbiological methods were followed for the isolation and identification of cyanobacteria (Desikachary 1959).

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Physico-chemical characteristics of effluent were done according to the standard methods (APHA 1981). Temperature and pH of the effluent were measured at the station itself.

RESULTS AND DISCUSSION

The effluent was slightly alkaline and contained high amounts of nitrate, nitrite and ammonia; total, inorganic and organic phosphate and calcium in all the three seasons (summer, winter and rainy) examined (Table 1). Values of dissolved oxygen (DO) were very low indicating highly obnoxious conditions. Though BOD and COD levels were high as per IS Standards. In general, the characteristics of effluent tested in all the three seasons were not varied much. Most of the parameters tested were slightly higher in summer than in winter and rainy seasons.

Eight species of bacteria were isolated and identified from the tannery effluent (Table 2) in all the seasons. Jain *et al.* (2001) isolated three different bacterial strains from the distillery sludge, where as ten species of fungi belongings to seven different genera were isolated from the effluent (Table 3). Among the fungal genera, *Aspergillus*was recorded as the dominant genus with four species followed by *Penicillium*with two species and the rest of the genera with single species each. Species of *Verticillium* were recorded only during summer. Similarly *Neurospora crussa* was not observed during rainy season. Kousar *et al.* (2000) isolated 23 species of fungi from dye effluent polluted habitat with *Aspergillus*as the dominant genus.

Altogether 18 species of cyanobacteria belonging to five genera were identified from the effluent stream (Table 4). Among the genera, *Oscillatoria* dominated the effluent with 9 species (Table 4). *O. acuminata* and*O. pseudogeminata* were not recorded in winter. Similarly *O. terebriformis* was not observed in rainy season. The rest of the species of *Oscillatoria* were noted in all the seasons. *Phormidium* with five species were identified in all the seasons. Unicellular form such as *Aphanocapsa pulchra* was also isolated in all the seasons whereas *Chlorogloea fritschii* was identified in winter and rainy seasons but not in summer. Of the algal species recorded, 17 species were observed in summer with 16 species in winter and 15 species in rainy season. The cyanobacteria have not showed much variation in their distribution in different seasons. Species of *Plectonema* recorded during summer and winter were not observed in rainy season. Among the species of *Oscillatoria, O. animalis* was found to be the dominant species as it was recorded at all the sites in all the seasons of our observations. Many authors emphasize the importance of light, temperature, pH, carbon-di-oxide, organic matter, alkalinity, nitrates and phosphates as factors important in determining the distribution of cyanobacteria (Singh 1960; Philipose 1960; Venkateswaralu 1969b; Munawar 1970). In the present study, as a whole, conditions in the effluent appeared to be favourable for the cyanophycean members. The effluent had high oxidizable organic matter, nutrients such as nitrates and phosphates with high calcium content. Observations of Munawar (1970) suggest that Cyanophyceae grow luxuriantly with great variety and abundance in ponds rich in calcium. The present data also shows that the calcium is one of the factors for the growth of cyanophycear members. Dense cyanobacterial population was observed during summer in all the sites under study when the oxygen content was very low (Table 1). Similar observations were made by Rao (1953) and Venkateswarlu (1969b). They noted that oxygen deficiency favoured cyanobacterial growth. Dominant and persistent occurrence most of the species of *Oscillatoria* and *Phormidium* indicate their capacity to thrive in the type of man-made habitat. Moreover, these findings show that there are certain species of cyanobacteria which are tolerant to organic pollution and resist environmental stress caused by the pollutant.

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The cheapest sources of nutrients for the mass culturing of cyanobacteria are undoubtedly sewage and other organic industrial wastes. The cyanobacteria that are isolated from an effluent stream could be grown on large scale in controlled waste stabilization ponds and thus pollution is taken care of to certain extent. Several investigators (Whitton 1975; Palmer 1980) have pointed out that the indicator species could be used to monitor pollution in controlled waste stabilization ponds. In this investigation, *O. animalis*was observed in all the months of all seasons (Table 4) and hence it could be treated as indicator species of tannery effluent. On the basis of this fact it is suggested that the indicator species could be used for pollution abatement programmes.

Table. 1. Physico-chemical characteristics of tanneryeffluent (mean ± SD values of 4 observations in each season)

S. No	Parameters	Summer (Feb.-May)	Winter (Oct.-Jan.)	Rainy (June-Sep.)
1.	Temperature (^{0}C)	19.86 ± 1.31	16.30 ± 0.49	19.64 ± 0.72
2.	pH	7.54 ± 0.21	7.40 ± 0.23	7.38 ± 0.18
3.	Total solids	1062.50 ± 130.25	1033.33 ± 250.33	1137.50 ± 219.98
4.	Total dissolved solids	450 ± 119.52	400 ± 154.92	437.50 ± 130.25
5.	Free carbon dioxide	20 ± 3.55	20.33 ± 6.62	13.50 ± 6.82
6.	Carbonate	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.49
7.	Bicarbonate	95.50 ± 10.89	98.33 ± 18.95	96.75 ± 10.36
8.	Nitrate	107.13 ± 19.49	104.33 ± 43.90	104.88 ± 19.80
9.	Nitrite	88.50 ± 12.20	81.50 ± 10.27	79.88 ± 15.68
10.	Ammonia	45.50 ± 4.34	48.83 ± 4.02	48.75 ± 6.04
11.	Total phosphate	77.25 ± 7.55	71.17 ± 4.96	80.13 ± 5.38
12.	Inorganic phosphate	38.25 ± 3.49	38.17 ± 3.60	38.13 ± 3.48
13.	Organic phosphate	40.25 ± 7.19	33.00 ± 5.33	42.00 ± 6.89
14.	Total hardness	189 ± 13.98	167.33 ± 17.37	187.25 ± 11.26
15.	Calcium	55.17 ± 4.79	52.45 ± 4.37	55.30 ± 7.81
16.	Magnesium	32.64 ± 2.72	28.03 ± 3.31	32.19 ± 1.65
17.	Chloride	31.02 ± 3.38	30.36 ± 5.35	30.53 ± 3.66
18.	Dissolved oxygen	2.09 ± 0.76	2.90 ± 0.55	2.26 ± 0.71
19.	BOD	161.25 ± 38.71	105 ± 35.07	108.63 ± 38.67
20.	COD	400.00 ± 37.03	463.33 ± 29.44	350.00 ± 38.54

Except pH and temperature, all values are expressed in mg/l.

Table. 2. Seasonal Variations of Bacterial Flora in Tannery Effluent

S.No.	Name of bacteria	Summer (Feb.-May)	Winter (Oct.-Jan.)	Rainy (June-Sep.)
1.	*Escherichia coli*	+	+	+
2.	*Enterobacter aerogens*	+	+	+
3.	*Klebsiella pneumoniae*	+	+	+
4.	*Lactobacillus* sp.	+	+	+
5.	*Proteus vulgaris*	+	+	+
6.	*Pseudomonas aeruginosa*	+	+	+
7.	*P. fluorescens*	+	+	+
8.	*Salmonella* sp.	+	+	+

Note : + : Observed in all the four months

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Table. 3. Seasonal variations of fungal flora in tannery effluent

S.No.	Name of fungi	Summer (Feb.-May)	Winter (Oct.-Jan.)	Rainy (June-Sep.)
1.	*Aspergillus flavus*	++++	+++	+++
2.	*A. fumigatus*	++	++	+
3.	*A. luchensis*	++	+	+++
4.	*A. niger*	++++	++++	++++
5.	*Helminthosporium oryzae*	++	+	+
6.	*Neurospora crussa*	+	+	-
7.	*Penicillium janthinellum*	+++	++	++
8.	*P. javanicum*	++	+	+
9.	*Trichoderma viride*	+++	++	+++
10.	*Verticillium* sp.	+	-	-

Note :
++++ : Observed in all the four months, +++ : Observed in three months only
++ : Observed in two months only, + : Observed in one month only
- : Not observed

Table. 4. Seasonal variations of cyanobacterial flora in tannery effluent

S.No.	Name of cyanobacteria	Summer (Feb.-May)	Winter (Oct.-Jan.)	Rainy (June-Sep.)
1.	*Aphanocapsa pulchra*	+++	++	+
2.	*Chlorogloea fritschii*	-	+	+++
3.	*Oscillatoria acuminata*	+++	-	+++
4.	*O. animalis*	++++	++++	++++
5.	*O. brevis*	+++	+	++++
6.	*O. claricentrosa*	+++	+	++
7.	*O. pseudogeminata*	++++	-	+
8.	*O. salina*	++++	++	+++
9.	*O. subbrevis*	+++	+	+++
10.	*O. terebriformis*	+++	++	-
11.	*O. willei*	+	+++	+
12.	*Phormidium anomala*	+++	+	++++
13.	*P. incrustatum*	++++	+++	+
14.	*P. papyraceum*	++	++	+
15.	*P. submembranaceum*	+++	++	+
16.	*P. tenue*	+++	+	+
17.	*Plectonema* sp.	+	++++	-
18.	*Plectonema* sp.	+	++++	-

Note :
++++ : Observed in all the four months +++ : Observed in three months only
+++ : Observed in two months only + : Observed in one month only
- : Not observed

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ANTIMICROBIAL ACTIVITY OF *OSCILLATORIA PRINCEPS* AND *PHORMIDIUM AMBIGUAM* AGANIST FISH PATHOGENS

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ABSTRACT

Invitro antibacterial and antifungal activity of aqueous and ethanol extracts of *Oscillatoria princeps* and *Phormidium ambiguam* species specific activity in inhibiting the growth of six virulent strains of bacteria and four fungal pathogenic to fish viz., *Edwardsiella tarda, Vibrio alginolyticus, Pseudomonas fluorescens, Pseudomonas aeruginosa, Aeromonas hydrophila, Aeromonas salmonicida, Aspergillus niger, Penicillium sp, Candida albicans* and *Trichoderma viride*.

Key Words: Fish pathogen, antimicrobial activity, *Oscillatoria princeps* and *Phormidium ambiguam*.

INTRODUCTION

Microbial diseases are responsible for heavy mortality in wild and cultured fish. The problems in the farms are usually tackled by preventing disease outbreaks or by treating the actual disease with herbal drugs or chemicals. The use of antimicrobial agents has increased significantly in aquaculture practices (Alderman and Michel, 1992). Antibiotics used in both human as well as veterinary medicines have been tried experimentally to treat bacterial infections of fish. Problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in food fish culture. Decreased efficacy and resistance of pathogens to antibiotics has necessitated development of new alternatives (Smith *et al*., 1994).

Many bioactive and pharmacologically active substances have been isolated from algae. For instance, extracts of algae were reported to exhibit antibacterial activity (Siddhanta *et al*., 1997; Mahasneh *et al*., 1995; Sachithananthan and Sivapalan, 1975). Many authors had found antibacterial activities of microalgae due to fatty acids (Cooper *et al*., 1983; Findlay and Patil, 1984; Viso *et al*., 1987; Kellam *et al*., 1988). Changyi *et al*., (1997) opined that the fatty acids (PUFA) in litter fall of mangroves might have positive role on the growth of fishes and shrimps.

Srinivasa Rao and Parekh (1981) showed that crude extracts of Indian seaweeds were active only against Gram positive bacteria. Ethanol extracts from 56 Southern African seaweeds from the divisions Chlorophyta (green), Phaeophyta (brown) and Rhodophyta (red) scored highest antibacterial activity for Phaeophyta (Vlachos *et al*., 1997). Similar results were reported by (Caccamese and Azzolina, 1979; Pesando and Caram, 1984) for screening studies on seaweeds of Mediterranean and Eastern Sicily coast respectively. In this study, *Oscillatoria princeps and Phormidium ambiguam* were tested where in we report their efficacy against six bacteria and four fungal pathogenic to fish.

MATERIALS AND METHODS

Oscillatoria princeps and *Phormidium ambiguam* sampleswere collected from coastal area of lagoons at Vedaranyam mangrove forest, Nagappattinam district, Tamil Nadu, South

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India in low tide. Epiphytic and extraneous matters were removed by washing first in mangrove water and then in fresh water. The algae were transported to the laboratory in polyethylene bags at ice temperature. Specimens were preserved in 5% formalin. Experts in the respective fields identified both *Oscillatoria princeps* and *Phormidium ambiguam* samples.

To test the antimicrobial activity of cyanobacteria, species such as *O. princeps* and *P. ambiguam* were used and were mass cultured using ASN III medium. After 15 days the cyanobacterial cultures were harvested by centrifugation. For extraction of cyanobacteria distilled water was used as the solvents. Then the pellets were washed with distilled water. From the pellets a known amount was taken in a pestle and mortar and grind with distilled water.

The extraction of solvents was carried out individually on samples. The extracts from two consecutive soakings were pooled and freed from solvent by evaporation under reduced pressure. The residues (crude extracts) obtained were finally dried under vacuum and were tested for their inhibitory effects on ten species of fish pathogenic microbes isolated from mangrove environment namely; *Vibrio alginolyticus, Pseudomonas aeruginosa*. *Aeromonas hydrophila, Edwardsiella tarda, Pseudomonas fluorescens, Aeromonas hydrophila, Aspergillus niger, Penicillium sp, Candida albicans* and *Trichoderma viride*. The test microbial pathogen cultures were obtained from the stock cultures maintained in nutrient agar medium in bacteria and potato dextrose agar medium in fungi.

Antimicrobial assay

A loop full of the strain was inoculated in 30 ml of nutrient and PDA broth in a conical flask and incubated on a rotary shaker for 24 hrs to activate the strain. Mueller Hinton Agar No. 2 was prepared for the study. The assay was performed using 2 methods. Agar disc diffusion (Salie *et al*., 1996) for aqueous extract and Agar ditch diffusion (Perez *et al*., 1990) for solvent extract. The media and the test microbial cultures were poured into Petri dishes (Hi-Media). The test strain (0.2 ml) was inoculated into the media when the temperature reached 40-42°C. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions.

For the Agar disc diffusion method, the test compound (0.1 ml) was introduced onto the disc (0.7 cm) and then allowed to dry. Thus the disc was completely saturated with the test compound. Then the disc was introduced onto the upper layer of the medium with the bacteria and fungi. The plates were incubated overnight at 37°C. For the agar ditch diffusion method, after the medium was solidified, a ditch was made in the plates with the help of a cup-borer (0.85 cm). The test compound was introduced into the well and the plates were incubated overnight at 37°C. Microbial growth was determined by measuring the diameter of the zone of inhibition. Ethanol and distilled water were used as the control. The control activity was deducted from the test and the result obtained was plotted.

RESULTS AND DISCUSSION

In the present pilot screening of *Oscillatoria princeps* and *Phormidium ambiguam*extracts of two species were found to show species specific activity against the ten fish pathogens. The details of activity of aqueous and ethanol extracts of algae along with activity profile with standard commercial antibiotics are tested.

Hornsey and Hide (1974) tested 151 species of British marine algae and found that, although antibacterial activity was more evident in some taxonomic groups, it also varied

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seasonally. They found Gracilaria marked no activity sp., *Enteromorpha* sp. and *Cladophora dalmatica*. But, in our case the alcoholic extract of *Oscillatoria princeps* and *Phormidium ambiguam*showed good antimicrobial activity. Our results clearly showed that the ethanol solvent system was efficient in extracting the active compounds. The antimicrobial activity found in two extracts showed the success of the non-polar hydrophobic extracts independent of diffusion parameters in the assay method employed.

Padmakumar and Ayyakkannu (1986) reported toluene-methanol (1:3) extracts of species belonging to Rhodophyceae exhibited broad-spectrum activity when compared to Chlorophyceae and Phaeophyceae. Vidyavathi and Sridhar (1991) reported chloroform-methanol extract of fully grown *G. corticata* showed maximum activity against *S. aureus* compared to medium and young stages of growth. Srinivasa Rao and Parekh (1981) analyzed *Enteromorpha intestinalis* and *G. corticata* collected from Gujarat coast of India for antibacterial activity and found that the algae were active throughout the year with a peak during the winter season. The antibacterial activity of *O. princeps* and *P. ambiguam*extracts of both solvents (aqueous and ethanolic) against *Vibrio alginolyticus*, *Pseudomonas aeruginosa*. *Aeromonas hydrophila*, *Edwardsiella tarda*, *Pseudomonas fluorescens* and *Aeromonas salmonicida*. The antifungal activity of *O. princeps* and *P. ambiguam*extract of both solvents (aqueous and ethanolic) against *Aspergillus niger, Penicillium sp, Candida albicans* and *Trichoderma viride*.

Aqueous and ethanol extracts of microalgae *O. princeps* and *P. ambiguam*collected from coastal area of lagoons at Muthupet mangroves in the seasons showed good inhibitory activity against ten fish pathogens. The results differ from the findings of (20) who had recorded significantly different inhibitory activity from season to season. Our results also showed that the sensitivity of pathogens is more to *O. princeps* extracts compared to *P. ambiguam*extracts with *E. tarda* and *A. hydrophila* showing maximum sensitivity. *P. aeruginosa*, *A. salmonicidaV. alginolyticus*, *P. fluorescens, A. niger, Penicillium sp* and *C. albicans* were moderately sensitive to the algal extracts and *T. viride* were low sensitive to the other organisms. In their studies with algae, (Padmakumar and Ayyakkannu, 1997) found that *S. aureus* was the most susceptible bacterial pathogen followed by *Vibrio* sp. whereas *P. aeruginosa* was most resistant.

The aqueous extract appears to have less antimicrobial activity than the ethanolic extract. Generally fungal pathogens have less inhibition than bacterial pathogens. This is interesting in that the traditional method of treating a microbial infection was by administering a decoction of the cyanobacteria, whereas according to our results an organic solvent is better; hence this may be more beneficial. Amongst the 6 bacterial and 4 fungal strains investigated *E. tarda* and *A. hydrophilia* the most resistant and *T. viride* less resistant. . This might be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract as observed by Sastry and Rao, (1994). The variation of antibacterial activity of our extracts might be due to distribution of antimicrobial substances, which varied from species to species as suggested by Lustigman and Brown, (1991). Sastry and Rao, (1994) found the benzene extract of *G. corticata* showed antibacterial activity only against *Salmonella typhi* and *Escherichia coli* whereas the methanol and chloroform extracts had activity against *P. aeruginosa*. Overall, the present study provides enough data to show the potential of microalgae extracts for development of anti-pathogenic agents for use in aquaculture.

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Table.1.Antimicrobial activity of *O. princeps* and *P. ambiguam*

S. No	Name of organism	Zone of inhibition (in mm)			
		P. ambiguam		O. princeps	
		Aqueous	Ethanol	Aqueous	Ethanol
1	*P. aeruginosa*	05	17	08	19
2	*P. fluorescens*	05	15	07	17
3	*V. alginolyticus*	04	12	05	15
4	*E. tarda*	07	19	10	22
5	*A. hydrophilia*	06	17	08	20
6	*A. salmonicida*	05	16	09	18
7	*Aspergillus niger*	05	12	06	15
8	*Penicillium sp*	04	10	05	14
9	*Candida albicans*	04	09	06	12
10	*Trichoderma viride*	03	07	04	10

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PHARMACOLOGICAL PROPERTIES OF *PLUMBAGOZEYLANICA*: A COMPREHENSIVE REVIEW

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ABSTRACT

Plumbago zeylanica, a member of the Plumbaginaceae family, is widely known for its diverse pharmacological properties, which have been utilized in traditional medicine for centuries. The plant has gained attention for its bioactive compounds, particularly plumbagin, which are responsible for its therapeutic effects. This review synthesizes current research on the pharmacological activities of *Plumbago zeylanica*, including its antioxidant, antimicrobial, anti-inflammatory, anticancer, analgesic, neuroprotective, hepatoprotectiveand anti-diabetic effects. In vitro and in vivo studies have confirmed the significant biological activities of the plant, supporting its potential in clinical applications. However, further research, particularly human clinical trials, is needed to validate its efficacy and safety. This paper provides an overview of the mechanisms underlying the pharmacological effects of *Plumbagozeylanica* and highlights its potential in modern therapeutics.

Keywords:*Plumbagozeylanica*, plumbagin, pharmacological activities, therapeutic effects.

INTRODUCTION

Plumbago zeylanica (commonly known as Ceylon leadwort or wild leadwort) is a medicinal plant native to tropical and subtropical regions. It is known for its wide range of therapeutic applications in traditional medicine, particularly in Ayurveda and Chinese medicine, where it has been used to treat conditions such as fever, wounds, gastrointestinal issues, and inflammatory disorders. Its bioactive compounds, especially plumbagin, have been extensively studied for their diverse pharmacological effects (Gupta and Sharma, 2016).Plumbagozeylanica is classified under the family Plumbaginaceae, and its therapeutic value is mainly attributed to the presence of naphthoquinone derivatives, primarily plumbagin (C15H10O4). These compounds exhibit a range of biological activities, making *Plumbago zeylanica* a promising candidate for modern pharmacological research (Sharma and Sharma, 2019).

This review aims to compile and evaluate the pharmacological properties of *Plumbagozeylanica*, emphasizing its antioxidant, antimicrobial, anti-inflammatory, anticancer, analgesic, and neuroprotective effects. The review also discusses the mechanisms of action and highlights the current state of research, gaps in knowledge, and the potential for clinical application.

METHODOLOGY

This review is based on an extensive analysis of scientific literature sourced from multiple databases, including PubMed, Google Scholar, and Scopus, using keywords such as "*Plumbago zeylanica* pharmacology", "plumbagin", and "medicinal properties". We focused on articles published from 2000 to 2025, including both in vitro and in vivo studies, as well as clinical trials, that investigated the pharmacological properties of *Plumbago zeylanica*. Studies were selected based on their relevance to the plant's biological effects and mechanisms of action.

Inclusion Criteria:

- Peer-reviewed journal articles.

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- Studies reporting on the pharmacological properties of *Plumbago zeylanica*.
- Research on the active compounds, especially plumbagin.
- Studies conducted in animal models or human trials.

Exclusion Criteria:

- Non-peer-reviewed articles.
- Studies not related to *Plumbago zeylanica*.
- Research on other species of Plumbago.

PHARMACOLOGICAL ACTIVITIES OF *Plumbago zeylanica*

1. **Antioxidant Activity**

The antioxidant properties of *Plumbagozeylanica* are mainly attributed to plumbagin, which has been shown to scavenge free radicals, reducing oxidative stress in cells. Several studies have reported that plumbagin's antioxidant effects protect against damage from reactive oxygen species (ROS) and nitrogen species (RNS), playing a critical role in preventing degenerative diseases such as Alzheimer's and cardiovascular diseases (Gupta and Sharma, 2016). In addition to its well-known antioxidant properties, *Plumbagozeylanica* has been shown to exhibit significant free radical scavenging activity, which is crucial for protecting cells against oxidative damage caused by environmental stressors. A study by Jain *et al*. (2020) demonstrated that the methanolic extract of *Plumbagozeylanica* significantly reduced lipid peroxidation and increased the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase in rats exposed to oxidative stress.

2. **Antimicrobial Activity**

Plumbagozeylanica extracts exhibit significant antimicrobial activity against various pathogens, including both Gram-positive and Gram-negative bacteria, as well as fungi. This is largely due to the presence of plumbagin, which disrupts microbial cell walls and inhibits enzyme systems essential for microbial survival. The antimicrobial properties of the plant have been validated in several studies, suggesting its potential as a natural antimicrobial agent. Further studies have demonstrated the broad-spectrum antimicrobial activity of *Plumbagozeylanica* (Sharma and Sharma, 2019). In a study by Chaudhary *et al.* (2022), the ethanolic extract of *Plumbagozeylanica* showed potent activity against multi-drug resistant strains of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The study attributed this activity to the presence of plumbagin, which exhibited a strong inhibitory effect on bacterial cell wall synthesis and fungal cell membrane integrity.

3. **Anti-inflammatory Activity**

Khan and Zafar, 2020 have demonstrated that *Plumbagozeylanica* exerts potent anti-inflammatory effects, which have been attributed to its ability to inhibit pro-inflammatory cytokines and enzymes such as cyclooxygenase-2 (COX-2). These effects make the plant a potential treatment for inflammatory disorders such as rheumatoid arthritis, asthma, and inflammatory bowel disease (IBD). A study by Chowdhury *et al*. (2018) examined the effects of *Plumbagozeylanica* in a rat model of IBD and found that its administration significantly reduced histopathological damage in the colon, reduced levels of inflammatory cytokines, and inhibited the activity of cyclooxygenase-2 (COX-2). These results reinforce the traditional use of the plant in managing gastrointestinal inflammation.

4. **Anticancer Activity**

Plumbagin is a well-documented anticancer agent in *Plumbagozeylanica*. In various studies, plumbagin has shown cytotoxicity against different cancer cell lines, including breast, lung, and colon cancers. The mechanisms underlying its anticancer effects include induction of apoptosis, inhibition of cell proliferation, and suppression of tumor metastasis. The anticancer properties of *Plumbagozeylanica* have also been extensively investigated (Vermaand Singh,

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2018). A recent study by Singh *et al*. (2021) evaluated the cytotoxic effects of plumbagin on human liver carcinoma cells (*HepG2*). The study found that plumbagin induced cell cycle arrest at the G1 phase and promoted apoptosis by activating the caspase-3 pathway. Furthermore, plumbagin demonstrated strong tumor-suppressive effects in in vivo models, suggesting its potential as an adjuvant in cancer therapy.

5. **Analgesic Activity**

Kumar and Tiwari, (2016) have reported the analgesic effects of *Plumbagozeylanica*, suggesting its potential use in pain management using animal model. The plant's analgesic activity is believed to be mediated by inhibition of prostaglandin synthesis, similar to the mechanism of action of non-steroidal anti-inflammatory drugs (NSAIDs).Mehta*et al.* (2020) studied that the *Plumbagozeylanica* has been shown to exert analgesic effects in various pain models. In a study, the administration of *Plumbagozeylanica* extracts significantly reduced pain responses in mice subjected to formalin-induced pain. The analgesic effects were comparable to those of morphine, suggesting that the plant's compounds, including plumbagin, might act through similar pathways to those of conventional painkillers.

6. **Neuroprotective Activity**

Plumbagozeylanica has been studied for its neuroprotective properties, particularly in the context of neurodegenerative diseases like Alzheimer's and Parkinson's. Plumbagin has shown promise in protecting neuronal cells from oxidative damage, as well as inhibiting the aggregation of amyloid-beta plaques, a hallmark of Alzheimer's disease.Recent research has demonstrated the neuroprotective effects of *Plumbagozeylanica* in models of neurodegeneration (Ahmed & Rahman, 2017). A study by Agarwal *et al*. (2021) showed that plumbagin exhibited significant neuroprotective effects against the neurotoxin-induced degeneration in *Drosophila melanogaster* models. The study concluded that plumbagin's antioxidant and anti-inflammatory properties were responsible for its neuroprotective effects, highlighting its potential as a therapeutic agent for diseases such as Parkinson's and Alzheimer's.

7. **Hepatoprotective Activity**

Several studies have also highlighted the hepatoprotective potential of *Plumbagozeylanica*. Reddy *et al*. (2017) showed that oral administration of *Plumbagozeylanica* extract in rats protected against liver damage induced by alcohol consumption. The extract reduced serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, indicating liver protection, and normalized histopathological changes in liver tissue.

8. **Antidiabetic Activity**

Recent pharmacological studies have also explored the antidiabetic effects of *Plumbagozeylanica*. A study by Pandey *et al*. (2021) investigated the effect of *Plumbagozeylanica* extract on blood glucose levels in diabetic rats. The study found that *Plumbagozeylanica* significantly reduced fasting blood glucose levels and improved insulin sensitivity, suggesting potential use in managing Type 2 diabetes mellitus.

SUMMARY AND CONCLUSION

The pharmacological properties of *Plumbagozeylanica* have been well-documented in recent studies, revealing its diverse therapeutic potential. With its wide range of biological activities—ranging from antioxidant, antimicrobial, and anti-inflammatory to anticancer, analgesic, neuroprotective, hepatoprotective, and antidiabetic effects—this plant holds substantial promise as a source of novel therapeutic agents. The pharmacological activities have been primarily attributed to the bioactive compound plumbagin. These findings support its traditional use in treating various diseases and indicate its potential for modern therapeutic applications. However, despite the promising preclinical results, human clinical trials are still needed to further evaluate its efficacy and safety.

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BIODEGRADATION OF HEAVY METAL ON FISH *CATLA CATLA* BY *PSEUDOMONAS PUTIDA*

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ABSTRACT

In the present investigation the efficiency of *Pseudomonas* sp. in the degradation of cadmium residues of a fresh water fish *Catla catla*has been studied. It has been found out that survival of *Catla catla*in various concentration of cadmium as a function of different medium period indicates that mortality of fish increased with increasing concentration of heavy metals and exposure periods. Though there was drop in both experimental fishes the drop significantly reduced in the experimental ones treated with *Pseudomonas sp*. than that of the control treated without *Pseudomonas* sp. Results showed significant fall in all the biochemical constituents in all the tissues except glucose prompting to suggest that the fish cultured in the aquatic systems closer to the industrial locations would not have the expected nutritive value. The elevated levels of glucose are apparently indicative of the organism's response to the toxicant stress. Also, such fish when consumed as food leads to the deposition of the heavy metal in the soft tissues of the human body leading to exposure to a health effects.

Key Words: Heavy metal, *Catla catla, Pseudomonas* sp, Biodegradation.

INTRODUCTION

The microbial removal of heavy metals from soil and water by selected or genetically engineered strains has been described by Mac Rae (1986). This technique known as bio-remediation first used in 1970 to treat oil spill on U.S. tank farms, is now tackling more challenging clean up. Due to the efforts of a number of researchers, it has been possible to establish a collection of microoganisms able to degrade volatile toxic pollutants like toluene, isomeric-xylenes, styrene, chlorobenzoid acids, isomeric aryldicarboxylic acids, aromatic nitrocompounds and many more toxic chemicals. The active destructor strains are mainly representive of the *Pseudomonas* and *Rhodococcus* (Golovleva *et al*., 1992).

Cadmium (Cd) is a well-known heavy metal toxicant with a specific gravity 8.65 times greater than water (Lide, 1992). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones (Roberts, 1999). The reported symptoms of acute Cd toxicity in human beings include nausea, vomiting, abdominal pain and breathing difficulty. Chronic exposure to Cd can result in obstructive lung disease, renal disease and fragile bones. Symptoms of chronic exposure may also include alopecia, anaemia, arthritis, learning disorders, migraines, growth impairment, osteoporosis, emphysema and cardiovascular diseases. If the laboratory testing procedures indicate blood levels of cadmium above 5 mcg/dL and creatinine levels in urine above 10 mcg/dl, then it can be considered to be suggestive of Cd toxicity (Dupler, 2001).

Catla catla is one of the major fresh water carps native to India, Bangladesh, Myanmar, Nepal and Pakistan introduced in many other countries as exotic species. *C. catla* is a very rich source of proteins and is reported to attain a maximum size of 182 cm and weight of about 50 Kilograms (these figures vary). It is a surface and mid-water feeder, mainly omnivorous with

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juveniles feeding on aquatic and terrestrial insects, detritus and phytoplankton. It has a characteristically large, upturned mouth with a prominent protruding jaw. Because of its high nutritive value, it is a highly priced food fish and there is great demand in the market.

MATERIALS AND METHODS

Catla catla fingerlings (average weight: 6-7 grams) used in the present study were collected from the local fish farm located at Thanjavur Dt, Tamil Nadu, India and acclimatized to the laboratory for about a week in plastic pools of 50 liters capacity. Ground water used for maintaining the fish in the fish tanks had a pH 7.2±0.1, dissolved oxygen 8.0±0.3 mg/L and bicarbonates 95.0±5.0mg/L. LC_{50} was determined by following Renewal bioassay and was calculated by Finney (1971) probit analysis method.

The period of experiment was conducted at 24 hrs, 48 hrs, 72 hrs and 96 hrs. Fish were exposed in cylindrical aquarium (60 x 60 cm) containing 20 liter of water. Control, *Pseudomonas*and heavy metals medium were exposed separately. During the period of rearing, fish were fed on *adlibitum* diet of chopped at normal pellet diet. The water was renewed daily to give constant effect of heavy metals and *Pseudomonas* on tank. After 20^{th} day the analysis of biochemical, enzymes and heavy metals was estimated in muscle of fish. The estimation of Glucose by Dubois *et al*., (1956), Proteins by Lowry *et al*., (1951), free aminoacids by Jayaraman, (1981), lipids by Folch *et al*., (1957), Alanine transaminase and Aspartate transaminase by Mohun and Cook, (1957), Lipase by Oser, (1965), Lipid Peroxidation by Ohkawa *et al*., (1979), GSH content measurement by Sedlak and Lindsay (1968) and cadmium (Cd) (IS 3025, 1992) were estimated.

RESULTS AND DISCUSSION

The results of toxicity test in the present study indicated that the ionic form of Cd to *Catla catla* fingerlings are more sensitive to cadmium toxicity. The behaviour and mortality rate of *C. catla* during our experimentation was found to depend on both duration of exposure and concentration of the toxicant. *C. catla* are very sensitive than some of the other fish and crabs. This is evident from the reported values of 96 hr LC50 for *Poecili reticulata*, which is 30.4 mg/l in a static bioassay test system, 43 mg/kl for *Uca rapax* (Zanders and Rojas, 1996), 25 mg/l for scorpion fish, *Scorpaena guttata* (Brown *et al*., 1984). The effect of the metal also depends on the size of the animal, salinity of water, temperature and the type of the animal. Though the organisms survive the initial attack of toxins/pollutants because of their protective adaptations, the injuries caused by the progressive exposure even in small doses will get manifested at later stages when the organism's resistance weakens due to ageing. Also, the condition and response of the test organism to the amount of metal penetrating into its body, the degree of retention and the rate of excretion influence the toxic effect of heavy metal.

In the present investigation the efficiency of *Pseudomonas* sp. in the degradation of cadmium residues of a fresh water fish *Catla catla*has been studied. It has been found out that survival of *Catla catla*in various concentration of cadmium as a function of different medium period indicates that mortality of fish increased with increasing concentration of exposure periods. It has been found out that 100% mortality of the fish occurred at a concentration of 2-ppm cadmium. At a concentration of 2ppm, 50% of fishes died within 96 hours of an exposure (LC). It has been observed that the fish *Catla catla* is comfortable at a concentration of 2-ppm cadmium in the medium, without any mortality. It may be that this concentration is nearer to the sublethal concentration so that the fishes do not suffer any mortality

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Catla catla on in introduction to lethal concentration of cadmium showed abnormal behaviours such as excitation; attempt to jump out of water, heavy mucus secretion, rapid opercular movement, such above behavioural changes may be due to osmatic imbalance which affects nervous system. The above symptoms of poisoning have also been investigated by Sharma *et al*., (1983) in *Clarias batrachus* when treated with Malathion and *B. stigma* treated with carbaryl.

In the present experimental conditions, the results presented here have clearly demonstrated that the elevated metal ion concentrations for cadmium are associated with high production of the TBARS (Table 2). We observed a strong link between concentration effect of metal ions used and extent of TBARS. Here, cadmium acted as potent oxidants to biological membranes, both plasma and intracellular membranes. Therefore, these results indicate that in spite of physiological role of metal ions in maintenance of cell functions, could also function as toxic agents when present in excess.

The metal ions as transition metals cause cellular damages via formation of highly reactive oxygen free radical viz .OH. LPO initiation phase results in the formation of lipid hydroperoxides (Lipid – OOH) in the presence of OH (Sharma and Agarwal 1996), which is derived from $.O_2$- and hydrogen peroxide (H_2O_2), generally metabolically generated in medium. Neither $.O_2$- nor H_2O_2 is energetic enough to initiate LPO directly, but in presence of catalytic amounts of metal ions, they can react and form .OH radicals under a net equation, Harber – Weiss reaction (Halliwel and Gutteridge 1984).

We found that GSH was marginally affected by the elevated metal ion concentration effect (Table 2) to a lesser content. GSH can react with peroxyl radicals to achieve a steady state for them selves and itself converts to thiyl radical (GS.) which is not rapid as oxidative attack at polyunsaturated membrane lipids. It seems that the metal ion extrusion from cells presumably involves movement of diffusible complexes such as Cd – GSH, it also gives rise to alleviating in the level of thiol groups pool including GSH in medium.

Due to more reactivity of cadmium with GSH molecules than cadmium could present more oxidative stress on living organism making its cells being more susceptible to damages. Despite the extensive evidence implicating the depletion and / or oxidation of GSH in a wide variety of experimental toxicities (Smith *et al*., 1996), here it has been shown that GSH content was not much altered under the influence of metal ion stress. An explanation here is that relative inhibition following metal ion impact on some enzymatic defense systems such as GPx (Radi *et al*., 1988), converts lipid – OOH to respective alcohol through oxidation of GSH to GSSG, causes no remarkable alteration in GSH content due to less GSH consumption by inhibited enzyme.

It may be suggested that this reduction may be due to the tissue protein metabolized to produce glucose by the process of gluconeogenesis and the glucose is utilized for energy production during stress condition (Kabeer Ahamed, 1979). However in the experimental fish (treated with heavy metal and *Pseudomonas*) the sliding down of protein content is minimized to 19.7%. This may be due to the degrading activity of *Pseudomonas* in the experimental medium, which lessens the toxic stress on the fish. Shanthi (1991) made a similar observation in degradation of insecticide carbaryl in *Catla catla*by *Pseudomonas* sp.

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Another observation made in this experiment is that cadmium residues steadily increased in the tissue (Muscle and total homogenate) of the control fish from the first week to the fourth week while it is decreased in the experimental ones (treated with *Pseudomonas*) towards the end of experiments. Microbial processes play an important role in being about the biological transformation of insecticide (Matsumara, 1974). Due to their ability to adopt and proliferate in diverse situation, their total biomass and surface area, the microorganisms are considered to represent the principle force of environmental alteration of heavy metals residue. Insecticide residue may also act as a carbon source to the microorganism in natural environments (Gupta, 1984; Munnecke and Hsieh, 1974).

Table 1: Effect of different concentration of cadmium on mortality percentage of *Catla catla*as a function of different exposure periods

S. No	Concentration of cadmium (ppm)	Exposure periods 24 hrs	48 hrs	72 hrs	96 hrs
1	5	60	80	100	100
2	4	50	70	80	90
3	3	30	50	60	80
4	2	10	10	20	50
5	1	-	-	10	20

Table 2: Analysis of muscle tissues of *Catla catla* treated different groups

S. No	Parameters (mg/g)	Control	Cadmium treated fish	*Pseudomonas* treated fish	Cadmium and *Pseudomonas*tre ated fish
1	Total protein	24.7	12.8	15.9	22.3
2	Carbohydrate	10.5	9.2	9.4	10.4
3	Phosphatase	18.7	15.5	18.0	16.5
4	Protease	6.8	4.5	6.5	5.9
5	Lipase	1.08	2.05	1.0	1.5
6	AST	4.52	2.36	4.2	3.8
7	ALT	0.44	0.69	0.4	0.5
8	LPO	1.05	0.75	0.95	0.8
9	GSH	0.78	0.52	0.69	0.6
10	Copper (ppm)	Nil	1.8	Nil	1.0

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EFFECT OF *LYNGBYA MAJUSCULA* ON THE IMMUNITY AND SURVIVAL OF *EDWARDSIELLA TARDA* INFECTED *MUGIL CEPHALUS*

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ABSTRACT

The aim of this study was to evaluate dietary dosages of *Lyngbya majuscula* on the immune response and disease resistance against infections due to the opportunistic pathogen *Edwardsiella tarda*, in fish fingerlings. Cyanobacteria, *Lyngbya majuscula* was incorporated into the diets of fish, *Mugil cephalus* (Mangrove snapper) fingerlings. Every 20 days, different biochemical, haematological and immunological parameters were analyzed. Superoxide anion production, lysozyme, serum bactericidal, serum protein and albumin were enhanced in cyanobacteria treated groups compared with the control group. After 60 days, fish were survival with *Edwardsiella tarda*, and mortality percentage was recorded up to day 10 post-challenge. Survival decreased in control group (57%) up to day 10 after infection. However, this was increased in the cyanobacteria treatment group, i.e. 85% survivability in the 1 g cyanobacteria/kg, 5 g cyanobacteria/kg and 71% survivability in the 10 g cyanobacteria/kg, respectively. These results indicate that *Lyngbya majuscula* stimulates the immunity and makes *Mugill cephalus* more resistant to infection by *E. tarda*.

Key wards:*Lyngbya majuscula, Edwardsiella tarda, Mugil cephalus,* Fish, cyanobacteria, Biochemical, Haematological and immunological properties of fish

INTRODUCTION

The use of immunostimulants in aquaculture is becoming popular for enhancing the activity of non-specific defense mechanisms and increasing disease resistance in mangrove fish. The use of antibiotics and other chemotherapeutics has several drawbacks such as risk of generating resistant pathogens, problems of drug residues accumulating in treated fish and detrimental effect on the environment. Commercial vaccines are expensive for fish producers and may not be available for all species and against emerging diseases. Therefore, uses of immunostimulants seem to be an alternative way of reducing disease risk in fish culture (Dalmo and Seljelid, 1995; Raa, 1996).

Cyanobacteria are a very old group of organisms and represent relics of the oldest photoautotrophic vegetation in the world that occur in freshwater, marine and terrestrial habitats (Mundt and Teuscher, 1988). Cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising groups of organisms to be able of producing bioactive compounds (Fish and Codd, 1994; Schlegel *et al.,* 1997). Cyanobacteria are known to produce metabolites with diverse biological activities such as antibacterial (Jaki *et al.,* 2000), antifungal (Kajiyama *et al.,* 1998), antiviral (Patterson *et al.,* 1994), anticancer (Luesch *et al.,* 2000), antiplasmodial, (Papendorf *et al.,* 1998), algicide (Papke *et al.,* 1997), antiplatelet aggregation (Rho *et al.,* 1996) and immunosuppressive (Koehn *et al.,* 1992) activities.

Cyanobacteria have not yet been studied for immunostimulants in aquaculture activity and little work has been done to screen cyanobacteria isolated from mangrove with regard to their

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production of bioactive compounds. The present study was determined the immunostimulating effect of cyanobacteria in mangrove major carp, *Mugil cephalus*, which is an important species in mangrove water aquaculture. The study was undertaken to evaluate the effects on biochemical, immunological and haematological parameters of the serum/blood of fishes for the first time.

MATERIALS AND METHODS

Experimental fish and husbandry

Fingerlings of fish, *Mugil cephalus* (20 ± 2 g) were collected from, Muthupet lagoon, Tiruvarur district, Tamil Nadu. Fishes were stocked in a 50 litre tank and kept for quarantine and health check. After quarantine, fish were acclimatized for 30 days in 40 litre chlorine-free lagoon water and fed with commercial diet. Water exchange (50%) was done daily and water quality was monitored throughout the experiment at three days intervals. Temperature was 29 ± 2^0C, pH, 7.8 ± 4, salinity 26 ppt, dissolved oxygen concentration 6.0 ± 0.4 mg/l, ammonia-nitrogen concentration 9.4 ± 0.08 mg/l and nitrite-nitrogen 5 ± 0.02 mg/l. Fishes were fed their respective diet at the rate of 4% of body weight per day throughout the experiment.

Cyanobacteria

One kilogram of powdered cyanobacteria (*Lyngbya majuscula*) was collected from the mangrove environs and purified at laboratory in ASN III medium (Desikachary, 1959) and oven-dried at 50^0C, powdered by mortar and pestle and sieved. For each experiment, the required percentage (0.1%, 0.5%, 1.0% dry weight basis) was included in the feed. These represent diets B, C and D, respectively. Diet A (no cyanobacteria) served as control.

Experimental design and feeding diet

Mugil cephalus fingerlings was selected for the study and divided into 4 groups (A, B, C and D). Each group of 10 fingerlings was again divided into two equal duplicate subgroups. Group A was fed with basal diet and acted as the control. The remaining groups were fed with 1 g cyanobacteria/kg of feed (Group B), 5 g cyanobacteria/kg (Group C) and 10 g cyanobacteria/kg of feed (Group D) for 50 days. Blood and serum samples were collected from fish in each subgroup and examined for the following parameters, total protein, albumin, globulin, albumin/globulin ratio, blood glucose, haemoglobin, serum bactericidal activity, serum lysozyme activity and superoxide anion production, WBC and RBC.

Collection of blood

Feed was withheld from fish for 24 hour before blood samples were collected. From randomly picked fish at 20 day intervals, after anaesthetizing with 0.2 ppm MS-222, blood was collected from the caudal vein with a 1 ml plastic syringe ringed with heparin and stored at 4^0C and used the same day. Blood samples were also collected without heparin, allowed to clot, centrifuged at 3000 rpm for 20 minutes from this supernatant was collected and stored in refrigerated, for estimation of immunological and biochemical parameters.

Determination of immunological parameters

The superoxide anion production of blood phagocytes challenged with *Edwardsiella tarda* was measured according to methods of Chung and Secombes (1988). The turbidimetric assay for lysozyme was carried out according to methods of Parry *et al*. (1965). Serum bactericidal activity was done following the methods of Kajita *et al*. (1990).

Determination of blood haematological parameters

Blood haemoglobin content was determined following the cyanomethemoglobin method (Van Kampen and Zijlstra, 1961). Total erythrocyte count was performed following the method of Hendricks (1952) using a haemocynometer where a total leucocyte count was determined following the method of Shaw (1930).

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Determination of serum/blood biochemical parameters

Serum samples were analyzed for total protein following the method of Lowry *et al*., (1951), albumin content by Doumas *et al*., (1971), globulin content (subtracting albumin from total protein) and albumin/ globulin ratio. Blood glucose content was estimated following the procedure of Schmidt (1974).

Survival of fish

After 60 days of feeding, 5 fish from each subgroup were challenged intraperitoneally with a lethal dose of *Edwardsiella tarda* and observed for a 20day period for mortality. Biochemical, immunological and enzymatic parameters were assayed in post-challenged groups as per the methods described earlier.

RESULTS

The result of different dosages of cyanobacteria on production of serum immunological parameters was shown in Table 1. These results indicated that superoxide anion production in the three experimental groups was significantly higher than the control at all the assay periods, except group B in the post-challenge period. Highest superoxide anion production (0.18 ± 0.08) was found in group D fish on day 60. Lysozyme activity in the serum of cyanobacteria fed groups was significantly higher at all sampling times including post-challenge, when compared with the control group. A significant difference was also observed between the treated groups at all sampling times. Highest lysozyme activity (165 ± 0.6) was observed in group C fish on day 40. Serum bactericidal activity in different cyanobacteria fed groups was significantly higher when compared with control at all sampling times, including post-challenge. Highest bactericidal activity (70 ± 0.08) was found in group C fish on day 60.

Blood haematological parameters were shown in Table 1. Haemoglobin content was significantly higher as compared to control only in group D on day 20 and groups C and D on day 40. There was no significant impact of different doses on day 60 and post-challenge. WBC count in different treatments did not show a significant difference on day 20, however, a significantly higher WBC count was found with 5 g cyanobacteria/kg feed and 10 g cyanobacteria/kg feed on day 40 as well as post-challenge and on entire groups B, C and D on day 60. RBC count was significantly higher in all fish fed the different doses of cyanobacteria compared with the control group on all assay days.

Serum biochemical parameters are shown in Table 2. Serum protein content was significantly different in groups B and D when compared with group A on day 20. Groups B and C fishes fed for 60 days had a significantly higher serum protein content than group A fish. Post-challenge, a significantly higher serum protein content was found in groups C and D when compared with control. Serum albumin content in all fish fed the cyanobacteria doses was significantly higher than the control on day 20, whereas only group D on day 40 and group C on day 60 had significantly higher albumin content than the control fish. Post-challenge, more of the treated fish had elevated serum albumin content compared with control fish. The serum globulin level was significantly higher in group D on day 40 and group C on day 60 in comparison with the control. However, a significantly higher globulin content was found in groups C and D when compared with control group A after the post-challenge period. Albumin/globulin ratio was significantly higher in groups B and C fish on day 20, there was no significant elevation in the treated groups thereafter. Blood glucose level at all sampling times was significantly lower in fish fed with different doses of cyanobacteria compared with the control.

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After challenging fish with *Edwardsiella tarda*, the mortality was recorded for 10 days. There was no mortality of fish up to 12 hours. The group of fish fed with different percentages of cyanobacteria showed higher survival percentage when compared with control. The highest survival (85%) was shown in groups B and C (Fig. 1).

DISCUSSION

It has been shown that the protective effect of spices may be associated with its antioxidant properties (Pedraza-Chaverri *et al.,* 2000; Rahman, 2003). Neutrophil activity can also be an indicator of the non-specific response. Cells become more adherent to tissue cell surfaces by the production of adhesion protein, which facilitates their migration from the capillaries to the site of injury (Kishimoto *et al.*, 1989; Magnuson *et al.*, 1989). They also exhibit an increased production of oxygen radicals, which are potentially capable of destroying invading pathogens (Hassett and Cohen, 1989). In the present experiment, all diets containing various percentages of *Lyngbya majuscula* showed significant increases in superoxide activity over the control diets, thereby indicating that the level of formation of reactive oxygen species was enhanced. Immunostimulants can increase the non-specific immunity by either increasing the number of phagocytes or activating phagocytosis and respiratory burst (Shoemaker *et al.,* 1997). Post-challenge with *E. tarda* showed reduced superoxide activity when compared with the 60-day pre-challenged specimens, but the reduction was not significant. Many things might happen following challenge but it is necessary to speculate that production of superoxide anions by the *Lyngbya majuscula* in fish act against *E. tarda* infection. It has been found that aqueous extract of raw garlic and dried powder scavenges hydroxyl radicals (Yang *et al.*, 1993; Kim *et al.*, 2001) and superoxide anion (Kim *et al.,* 2001). Similar types of activities might have occurred in the present work.

Immunostimulants can increase serum lysozyme activity, due to either an increase in the number of phagocytes secreting lysozyme or to an increase in the amount of lysozyme synthesized per cell (Engstad *et al.*, 1992). Changes in lysozyme activity are greatly influenced by the potency and type of immunostimulants to which fish are exposed. Elevation of lysozyme following immunostimulation has been demonstrated in a number of fish species (Paulsen *et al.*, 2003). Lysozyme activity was elevated significantly in the groups of fish fed all three levels of cyanobacteria when compared with the control. Serum bactericidal activity was also enhanced in all treated groups when compared with the control group. Many investigators have reported enhanced bactericidal activity by the phagocytic cells of different fish species treated with immunostimulants (Jorgensen *et al.,* 1993).

The serum total protein after long-term feeding with cyanobacteria increased in comparison to the control diet. Siwicki, (1989) observed an increase in total protein content after feeding of b-glucan (0.2%) and chitosan (0.5%) in the diet. Serum albumin and globulin values in fish fed with cyanobacteria were higher than the control. Increases in serum protein, albumin and globulin levels are thought to be associated with a stronger innate immune response of fish (Wiegertjes *et al.*, 1996).

Dietary garlic decreases blood glucose by increasing the level of serum insulin (Ahmed and Sharma, 1997). According to Sheela and Augusti (1992), the s-allyl cysteine sulfoxide present in garlic is responsible for its hypoglycaemic activity. Results of the present study indicate that continuous feeding of raw cyanobacterial powder fights against stressors, as was evident from the low glucose value in fishes of groups B, C and D during the experiment.

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Reduced mortalities against pathogenic challenges at lower dosages of herbal principals were also reported by Kim *et al*. (2001) and Jain and Wu (2003). Citarasu *et al*. (2002) developed an Artemia-enriched herbal diet for *Penaeus monodon* with a combination of five herbs, which significantly increased the growth and survival during stress conditions. Several herbs were tested for their growth-promoting activities in aquatic animals (Citarasu *et al*., 2002; Sivaram *et al*., 2004). It is evident from the present work that *Lyngbya majuscula* could enhance fish immunity after incorporation in feed, even at a lower dose, i.e. 1 g/kg of feed. The present results suggest that inclusion of cyanobacteria in the diet would improve the non-specific immunity of fish and prevent bacterial infections in culture systems. Field trials incorporating these doses merit investigation. Further purification of the active compounds and their evaluation may substantially improve quality as well as their usage in the culture system.

Fig. 1. Effect of cyanobacteria on survivability of fish after bacteria challenge

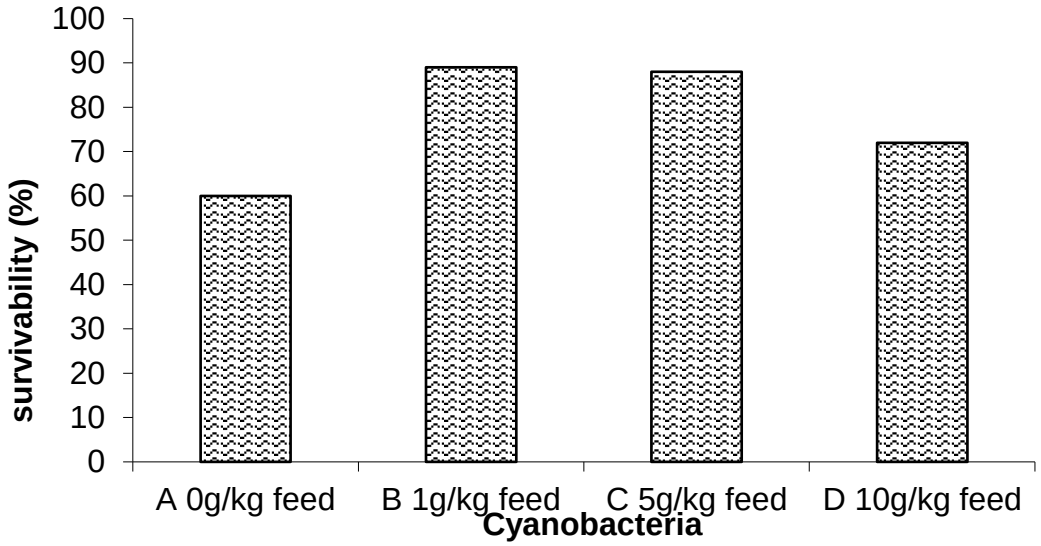

Table 1. Cyanobacteria powder feeding effect on Immunological and Haemtological parameters of *Mugil cephalus* followed by challenge of *Edwardsiella tarda* after 60 days

Parameters	Group	Before challenge			After challenge
		20 days	40 days	60 days	70 days
Superoxide anion production (O.D.)	A	0.05 ± 0.01	0.06 ± 0.04	0.08± 0.05	0.07 ± 0.06
	B	0.15 ± 0.12	0.12 ± 0.06	0.12 ± 0.05	0.09 ± 0.04
	C	0.08 ± 0.20	0.13 ± 0.02	0.15 ± 0.08	0.11 ± 0.05
	D	0.1 ± 0.36	0.11 ± 0.62	0.18 ± 0.08	0.14 ± 0.01
Lysozyme activity (U/ml)	A	110 ± 0.2	110 ± 0.5	130 ± 0.3	105 ± 0.2
	B	130 ± 0.6	135 ± 0.5	150 ± 0.9	130 ± 0.5
	C	150 ± 0.6	165 ± 0.6	160 ± 0.5	140 ± 0.8
	D	145± 0.4	146 ± 0.2	150 ± 0.1	155 ± 0.6
Bactericidal activity	A	25 ± 0.02	28 ± 0.36	30 ± 0.01	23 ± 0.06
	B	35 ± 0.12	38 ± 0.42	45 ± 0.05	40 ± 0.02

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(cfu/control)	C	38 ± 0.56	45 ± 0.50	70 ± 0.08	42 ± 0.01
	D	40 ± 0.32	52 ± 0.36	50 ± 0.04	52 ± 0.52
Haemoglobin (g%)	A	6.5 ± 0.2	8.2 ± 0.4	7.0 ± 0.6	9.0 ± 0.6
	B	7.5 ± 0.6	10.2 ± 0.2	8.0 ± 0.2	10.5 ± 0.1
	C	7.5 ± 0.1	10.5 ± 0.8	9.5 ± 0.8	6.5 ± 0.12
	D	9.0 ± 0.6	10.2 ± 0.2	10.1 ± 0.5	7.0 ± 0.62
WBC count (1000 cells mm^3)	A	14.0 ± 0.2	15.0 ± 0.6	15.5 ± 0.3	14.0 ± 0.5
	B	15.0 ± 0.5	17.0 ± 0.8	18.1 ± 0.3	15.5 ± 0.2
	C	15.5 ± 0.3	21.8 ± 0.6	24.0 ± 0.9	20.3 ± 0.6
	D	16.0 ± 0.8	22.2 ± 0.5	23.1 ± 0.6	21.2 ± 0.3
RBC count (10 00 000 cells mm^3)	A	0.8 ± 0.6	0.9 ± 0.9	0.95 ± 0.4	0.91 ± 0.25
	B	0.9 ± 0.6	1.2 ± 0.5	1.05 ± 0.6	1.00 ± 0.31
	C	1.0 ± 0.3	1.1 ± 0.21	1.30± 0.3	1.05 ± 0.40
	D	1.1 ± 0.1	1.3 ± 0.65	1.45 ± 0.6	1.20 ± 0.98

Table 2. Cyanobacteria powder feeding effect on biochemical parameters of *Mugil cephalus* followed by challenge of *Edwardsiella tarda* after 60 days

Parameters	Group	Before challenge			After challenge
		20 days	40 days	60 days	70 days
Blood glucose (g/dl)	A	159.4 ± 0.5	144.9 ± 0.88	143.3 ± 0.17	125.25 ± 1.79
	B	136.6 ± 0.4	135.2 ± 0.63	123.5 ± 0.08	123.63 ± 4.37
	C	135.8 ± 0.7	125.2 ± 0.61	119.2 ± 0.45	116.92 ± 0.50
	D	106.9 ± 2.2	99.96 ± 1.34	95.16 ± 0.29	89.36 ± 4.15
Total protein (g/dl)	A	1.17 ± 0.05	1.77 ± 0.06	2.82 ± 0.05	2.15 ± 0.10
	B	1.68 ± 0.07	1.96 ± 0.08	2.31 ± 0.08	1.81 ± 0.24
	C	1.00 ± 0.02	1.88 ± 0.1	3.94 ± 0.03	3.39 ± 0.21
	D	1.73 ± 0.12	2.81 ± 0.12	2.74 ± 0.04	2.93 ± 0.07
Albumin (g/dl)	A	0.39 ± 0.02	0.97 ± 0.02	1.31 ± 0.03	0.89 ± 0.05
	B	0.82 ± 0.01	0.96 ± 0.01	1.10 ± 0.04	0.74 ± 0.12
	C	0.61 ± 0.01	0.99 ± 0.01	1.53 ± 0.09	0.95 ± 0.02
	D	0.69 ± 0.13	1.24 ± 0.01	1.30 ± 0.01	1.01 ± 0.00
Globulin (g/dl)	A	0.77 ± 0.03	0.79 ± 0.09	1.39 ± 0.10	1.19 ± 0.04
	B	0.88 ± 0.11	0.99 ± 0.10	1.35 ± 0.07	0.60 ± 0.23
	C	0.39 ± 0.03	0.94 ± 0.04	2.48 ± 0.06	2.33 ± 0.22
	D	1.03 ± 0.13	1.54 ± 0.11	1.43 ± 0.05	1.91 ± 0.07
Albumin/ globulin	A	0.50 ± 0.01	1.26 ± 0.18	0.86 ± 0.04	0.72 ± 0.03
	B	0.96 ± 0.12	0.95 ± 0.09	0.85 ± 0.03	0.68 ± 0.03
	C	1.58 ± 0.16	1.04 ± 0.05	0.58 ± 0.05	0.41 ± 0.04
	D	0.69 ± 0.10	0.81 ± 0.04	0.90 ± 0.04	0.52 ± 0.02

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SYNTHESIS OF SILVER NANOPARTICLES USING *CTENOLEPIS GARCINII* PLANT EXTRACT AND THEIR ANTIPROLIFERATIVE ACTIVITY AGAINST HUMAN BREAST CANCER MDA-MB-231 CELL LINES

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ABSTRACT

Cancer is a disease in which there is an uncontrolled multiplication and spread, within the body, of abnormal forms of the body's own cells. Assessment of in-vitro cytotoxicity has been recently become popular as a primary screening method for evaluating anticancer activities of various natural sources. This study was carried out to characterize anti- cancer activity of aqueous extracts of *Ctenolepis garcinii*. Medicinal plants have been in use from time immemorial and their utility has been increasing day by day in the present world. Naturally, obtained compounds considered safer and easily biodegradable than synthetic compounds and the problem of drug resistance observed in synthetic drugs is to reduced. *Ctenolepsis garcinii* is one of the traditional remedies used for the treatment of infectious diseases. The phytochemical screening was carried out. Those activities were due to the presence of secondary metabolites. The anticancer activity was performing against human breast cancer MDA-MB-231 CELL LINES by MTT assay was used to analyze the cell growth inhibition. IC_{50} values were also observed. *Ctenolepis garcinii* has a significant anticancer activity. However, further detailed studies are required to determine the active components responsible for these effects and mechanism pathway.

Key words: *Ctenolepis garcinii*, Phytochemical Screening, MTT assay, anticancer activity.

INTRODUCTION

Plants have been used as medicines for thousands of years. People depend on plants for several purposes like for wood, timber, non-timber forest products, food, medicine etc. (Jain *et al*., 2005). They have always been used as a rich source of biologically active drugs and have numerous traditional uses to serve mankind for many thousand years (Kirtikar *et al*., 1999). Now a day, they are used widely because of growing awareness of people towards unwanted side effects and high cost of the allopathic medicines which makes them beyond the reach of common people.

Cancer-Global and Indian scenario

Cancer is a leading cause of death in India and other countries. Cancer can affect any part of the human body and people at all ages, but risk for most types cancer increases with age. An estimated 12.7 million new cancer cases and 7.6 million deaths occurred in 2008. Around 25 million persons are living with cancer around the world (Dikshit *et al*., 2012). Lung, stomach, liver, colon and breast cancer cause the most cancer deaths each year. The most commonly diagnosed cancers worldwide are lung (1.61 million, 12.7% of the total), breast (1.38 million, 10.9%) and colorectal cancers (1.23 million, 9.7%). The most common causes of cancer death are lung (1.38 million, 18.2% of the total), stomach (0.74 million, 9.7%) and liver cancers (0.69 million, 9.2%). Cancer is a major public health burden in both developed and developing countries. About 72% of all cancer deaths in 2007 occurred in low and middle-income countries. Every year about 8,50,000 new cancer cases being diagnosed and about 5,80,000 cancer related death occurs every year in India (Ali *et al*., 2011; Dhanamani *et al*., 2011).India had the highest

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number of the oral and throat cancer cases in the world. Deaths from cancer worldwide are projected to continue rising, with an estimated 12 million deaths in 2030.

Methods of cancer treatment

Conventionally, surgical procedures, chemotherapy and radiotherapy are the major means of treatment for cancer patients (Baskar *et al*., 2012). These treatments suffer from the major drawback of non specific effect on normal cells (Moran, 2000), and cost intensive. Depending upon the type and stage of cancer involved, treatments include chemotherapy, radiotherapy, surgery, hormonal therapy, or a combination of these. Chemotherapy involves the use of chemical drugs that directly or indirectly interfere with or inhibit the proliferation of cancer cells. Examples of drugs commonly used in cancer treatment are abraxane, altretamine, docetaxel, doxorubicin, herceptin, melphalan, methotrexate, mitomycin, nipent, novadol (cisplatin), novantrone, tamoxifen (for breast cancer), taxol, temodar (temozolomide) and zoladex (NCI reports, 2013).

Ctenolepis garcini

Ctenolepis garcini is a climber. It is have three to five lobed palmate leaves. It contains fruits and leaves. It is a monoecious plant. Leaves deeply 3-5 lobed to 5ˣ6 cm in ovate shaped. It is two types of flowers. They are female and male flowers. Female flowers are borne in singly and solitary. Male flowers are small in size and borne in cymes. Fruits are born in December to January. Fruits contain one to two seeds. It is kidney shape or hammer shape. The roots are used in colic, fever, indigestion, anorexia and rheumatism and leaves are used in gonorrhea and antioxidant property. The fruit pulp is useful in foot sole to treat the temperature in body. The plant already proved these activities are hepatoprotective activity, anticancer activity, antibacterial activity, antifungal activity and anti-inflammatory activity.

In the present era of drug development and discovery of newer drug molecules many plant products are evaluated on the basis of their traditional uses. One of the many plants which are being evaluated for their therapeutic efficacies is *Ctenolepis garcinii* which is commonly known as Latjeera (Hindi) & Rough Chaff tree (English). The present study was to evaluate the antioxidant activity of aqueous extract of *Ctenolepis garcinii* in *in vitro* models.

MATERIALS AND METHODS

Collection, Identification and Authentication of plant materials

The plant species namely *Ctenolepis garcinii* plant was collected. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's college, Tiruchirappalli (Voucher number of the specimen, AMTA 001) (Gamble, 1997). The plant was air dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used for further analysis.

Preparation of the extract

The extracts were prepared according to the methodology of |Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210ml ethanol and 90ml distilled water. These extract were concentrated to dryness in flash evaporator under reduced pressure controlled at a temperature (400C – 500C). The paste form of the extracts was put in an air tight container stored in refrigerator.

RESULT AND DISCUSSION

Anticancer activity

The plant kingdom represents an enormous reservoir of biologically active molecules and so far, only small fractions of plants with medicinal activity have been assayed. Nearly 50% of drugs used in medicine are of plant origin.

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The whole plant of *Ctenolepsis garcinii* is evident from the results in table 17, table 18. The cytotoxic effect of aqueous and ethanolic extracts of *Ctenolepsis garcinii* against Breast cancer MDA-MB-231 cell lines *in vitro* by method increases with the increase in concentration of the extracts. The aqueous extract at 15.62, 31.25, 62.50, 125, 250µg/ml caused mortalities of 6.11, 16.24, 26.37, 40.50 and 59.07 in EAC. Similarly, the extract at 15.62, 31.25, 62.50, 125, 250µg/ml caused mortalities of 7.80, 19.62, 33.33, 48.10 and 68.77 respectively in EAC.

In this study, cytotoxicity data obtained from EAC cultures the aqueous and ethanolic extracts of whole plant of *Achyranthes aspera* evaluated were able to bind to EAC membrane and readily penetrate within the cells. These findings suggest that, in terms of cellular injury, the above extracts evaluated were potent cytotoxicants, and presented more pronounced effects. It is important to stress that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia and ultimately tumor development (Mally *et al*., 2002).

Table 1: *In vitro* cytotoxicity effect of aqueous extract of *Ctenolepsis garcinii* against Breast cancer MDA-MB-231 cell lines (MTT Assay)

Concentration	OD-1	OD-2	OD-3	Average	% of Inhibition	IC_{50} Value (µg/ml)
Control	0.480	0.471	0.473	0.474	-	192.64
15.62	0.451	0.445	0.441	0.445	6.11	
31.25	0.401	0.399	0.393	0.397	16.24	
62.50	0.353	0.349	0.345	0.349	26.37	
125	0.287	0.281	0.279	0.282	40.50	
250	0.201	0.197	0.185	0.194	59.07	

Figure 1: *In vitro* cytotoxicity effect of aqueous extract of *Ctenolepsis garcinii* against Breast cancer MDA-MB-231 cell lines (MTT Assay)

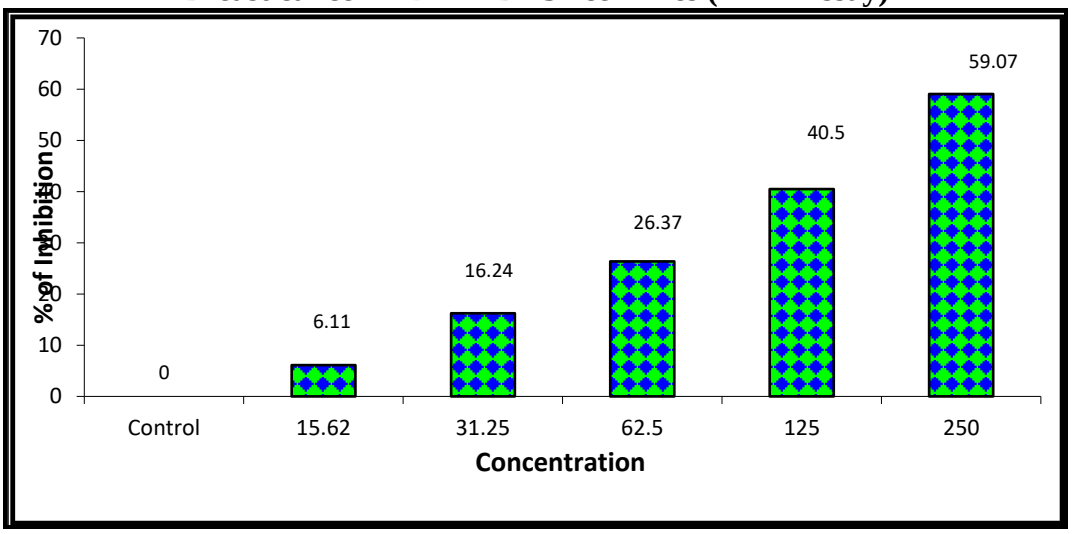

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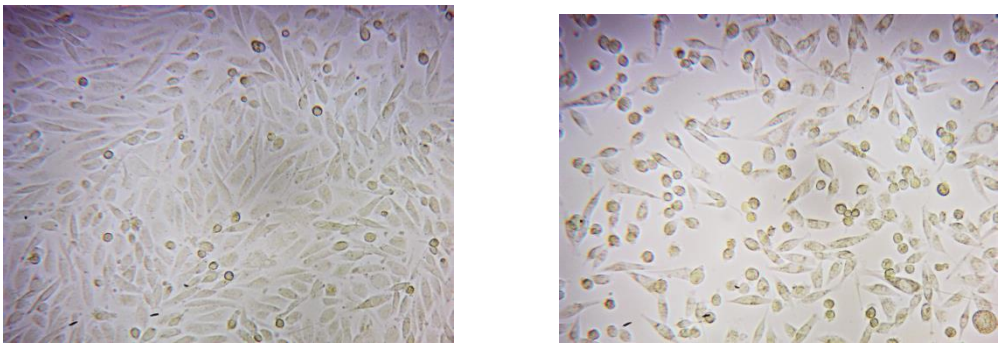
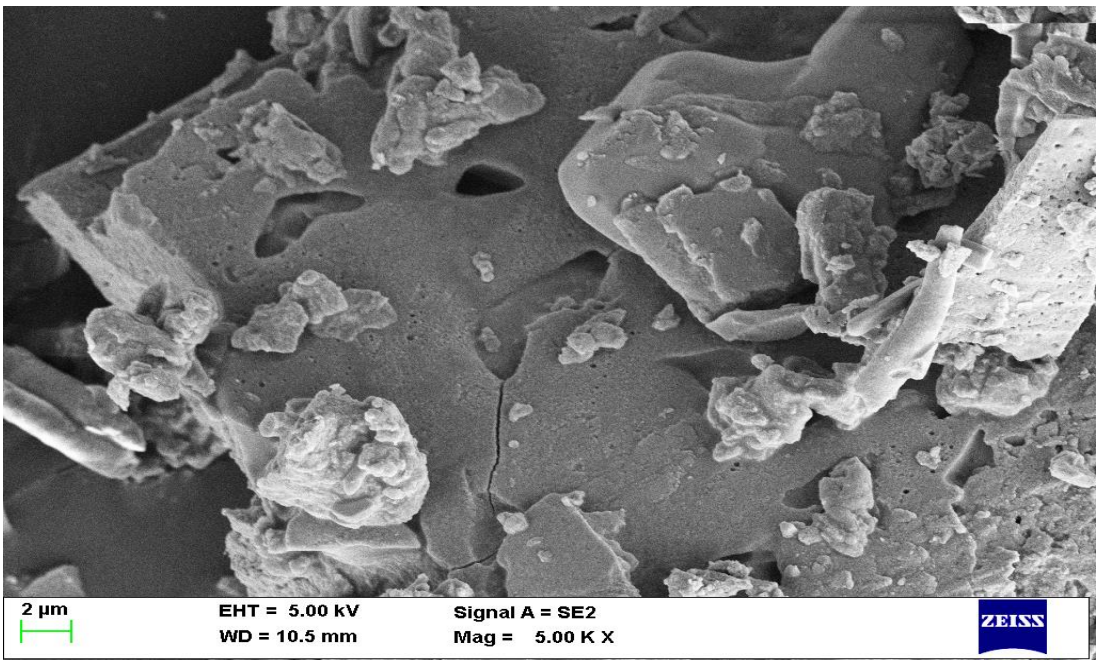
Figure 2: Breast cancer MDA-MB-231 cells for 24 h.

Control **10 µg**



Photomicrograph (20x) represents morphological changes in MDA-MB-231 cells such as shrinkage, detachment, membrane blabbing and distorted shape induced by the plant extract treatment (10 µg/ml for 24 h) as compared with control. Control showed normal intact cell morphology and their images were captured by light microscope.



CONCLUSION

Ctenolepsis garcinii has been evaluated for its *in vitro* anticancer activity against EAC cell lines by MTT assay method. Both the aqueous and ethanolic extracts showed remarkable cytotoxic activity against EAC cell lines, as judged from cellular death in a dose–related fashion, the strongest effect being observed at higher concentrations. The results suggested that the anticancer effects of aqueous and ethanolic extracts of whole plant of *Ctenolepsis garcinii* may be related to their content of flavonoids. This study validates the traditional use of the plant in management of Cancer. From the results of the above investigation, it was concluded that both the aqueous extracts of whole plants of *Ctenolepsis garcinii* possess good *invitro* cytotoxic activity against human breast cancer MDA-MB-231 cell lines. On the basis of the results obtained in the present study, it is concluded that the aqueous and ethanolic extract of *Ctenolepsis garcinii* has potent anticancer activity. However, further detailed studies are required to determine the active components responsible for these effects and mechanism pathway.

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A STUDY OF BIODEGRADATION OF CADMIUM ON FISH BY *PSEUDOMONAS AERUOGINOSA*

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ABSTRACT

Efficiency of *Pseudomonasaeruginosa* in the degradation of cadmium residues of a freshwater fish *Catla catla*has been studied. It has been found out that survival of *Catla catla*in various concentrations of cadmium as a function of different exposure period indicates that mortality of fish increased with increasing concentration of heavy metals uptake and exposure periods. The four-treatment groups of fishes were control, cadmium treated, without cadmium treated (*Pseudomonas*) and cadmium and *Pseudomonas* treated. Results showed significant fall in all the biochemical constituents of fish tissues in heavy metals induced groups. Of these, *Pseudomonas aeruginosa* strain has become a reference strain and has been used to elucidate sequences of the catabolic enzymes despite this, until now unknown or novel microorganisms, with unique sequence and different enzyme-mediated operative pathways, warrant continued investigations for effective biodegradation. The extract of capitula of muscle biochemical functions as a biodegradation agent and this microbial activity of fish may be due to normalization of impaired membrane function activity. Also, such fish when consumed as food leads to the deposition of the heavy metal in the soft tissues of the human body leading to exposure to a health effects.

Key wards:*Catla catla, Pseudomonasaeruginosa*, cadmium, biodegradation.

INTRODUCTION

The microbial removal of heavy metals from soil and water by selected or genetically engineered strains has been described by Mac Rae (1986). This technique known as bio-remediation first used in 1970 to treat oil spill on U.S. tank firms, is now tackling more challenging clean up. Due to efforts of a number of researchers, it has been possible to establish a collection of microoganisms able to degrade volatile toxic pollutants like toluene, isomeric-xylenes, styrene, chlorobenzoid acids, isomeric aryldicarboxylic acids, aromatic nitrocompounds and many more toxic chemicals. The active destructor strains are mainly representive of the *Pseudomonas* and *Rhodococcus*(Golovleva *et al*., 1992).

Cadmium (Cd) is a well-known heavy metal toxicant with a specific gravity 8.65 times greater than water (Lide 1992). Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones (Roberts 1999). The reported symptoms of acute Cd toxicity in human beings include nausea, vomiting, abdominal pain and breathing difficulty. Chronic exposure to Cd can result in obstructive lung disease, renal disease and fragile bones. Symptoms of chronic exposure may also include alopecia, anaemia, arthritis, learning disorders, migraines, growth impairment, osteoporosis, emphysema and cardiovascular diseases. If the laboratory testing procedures indicate blood levels of cadmium above 5 mcg/dL and creatinine levels in urine above 10 mcg/dl, then it can be considered to be suggestive of Cd toxicity (Dupler 2001).

Catla catla is one of the major freshwater carps native to India, Bangladesh, Myanmar, Nepal and Pakistan introduced in many other countries as exotic species. *C. catla* is a very rich source of proteins and is reported to attain a maximum size of 182 cm and weight of about 50 Kilograms. It is a surface and mid-water feeder, mainly omnivorous with juveniles feeding on aquatic and terrestrial insects, detritus and phytoplankton. It has a characteristically large, upturned mouth with a prominent protruding jaw. Because of its high nutritive value, it is a highly priced food fish and there is great demand in the market. Therefore, the main objective of this study is to assess the biochemical changes in *Catla catla* after exposure to sublethal concentrations of the heavy metal and different treated groups.

MATERIALS AND METHODS

Catla catla fingerlings (average weight: 6 to 9 grams) used in this study were collected from the local fish farm located at Thanjavur and acclimated to the laboratory for about a week in plastic pools of 50 liters capacity. Ground water used for maintaining the fish in the fish tanks had a pH 7.1, dissolved oxygen 8.0 mg/l and bicarbonates 95.0 mg/l. LC_{50} was determined by following Renewal bioassay and was calculated by Finney (1971) profit analysis method.

The period of experiment was conducted at 24, 48, 72 and 96 h. Fish were kept in cylindrical aquarium (60 x 60 cm) containing 20 liter of water. Control, media control, *Pseudomonas*and heavy metals were exposed separately. During the period of rearing, fish were fed *ad libitum* of on normal pellet diet. The water media were renewed daily to give constant effect of heavy metals and *Pseudomonas* in aquaria. After 20^{th} day analysis of biochemical, enzymes and heavy metals was estimated in muscle of fish. The estimation of glucose (Dubois *et al* 1956), protein (Lowry *et al* 1951), free aminoacids (Jayaraman 1981), lipids (Folch *et al* 1957), Alanine transaminase and Aspartate transaminase (Mohun & Cook 1957), Lipase (Oser 1965), Lipid peroxidation (Ohkawa *et al* 1979), GSH content measurement (Sedlak & Lindsay 1968) and cadmium (Cd) (IS 3025, 1992) were estimated.

RESULTS AND DISCUSSION

To *Catla catla* fingerlings are more sensitive to ionic forms of cadmium. The behaviour and mortality rate of *C. catla* during the experiment was dependent on both duration of exposure and concentration of the toxicant. *C. catla* are more sensitive than some of the other fish and crabs. This is evident from the reported values of 96 h LC_{50} for *Poecili reticulata*, which is 30.4 mg/l in a static bioassay test system, 43 mg/kl for *Uca rapax* (Zanders & Rojas 1996), 25 mg/l for scorpion fish, *Scorpaena guttata* (Brown *et al* 1984). The effect of the metal also depends on the size of the animal, salinity of water, temperature and the type of the species. Though the organisms survive initial attack of toxins/pollutants because of their protective adaptations, the injuries caused by the progressive exposure even in small doses will get manifested at later stages when the organism's resistance weakens due to ageing. Also, the condition and response of the test organism to the amount of metal penetrating into its body, the degree of retention and the rate of excretion influence the toxic effect of heavy metal.

Efficiency of *Pseudomonasputida* in the degradation of cadmium residues of a freshwater fish *Catla catla*has been studied. It has been found out that survival of *Catla catla*in various concentrations of cadmium as a function of different periods indicates that mortality of fish increased with increasing concentrations of exposure periods. It has been found out that 100% mortality of the fish occurred at a concentration of 2-ppm cadmium. At a concentration of 2-ppm, 50% of fishes died within 96 hours of exposure (LC). It has been observed that *Catla catla* was comfortable at a concentration of 2-ppm cadmium free medium without any mortality. It may be

that this concentration is nearer to the sublethal concentration so that the fishes do not suffer any mortality

Catla catla on introduction to sublethal concentration of cadmium showed abnormal behaviours such as excitation; attempt to jump out of water, heavy mucus secretion, rapid opercular movements. Such above behavioural changes may be due to osmatic imbalance which affects nervous system. The above symptoms of poisoning have also been investigated by Sharma *et al* (1983) in *Clarias batrachus* when treated with Malathion and bacteria treated with carbaryl.

The results presented here have clearly demonstrated that the elevated metal ion concentrations for cadmium are associated with high production of the Thiobarbutiric acid reactive substances (TBARS) (Table 2). Ohkawa *et al.* (1979) observed a strong link between concentration effect of metal ions used and extent of TBARS. Here, cadmium acted as potent oxidants to biological membranes, both plasma and intracellular membranes. Therefore, these results indicate that in spite of physiological role of metal ions in maintenance of cell functions, could also function as toxic agents when present in excess.

The metal ions as transition metals cause cellular damages via formation of highly reactive oxygen free radical viz .OH. LPO initiation phase results in the formation of lipid hydroperoxides (Lipid – OOH) in the presence of OH (Sharma & Agarwal 1996), which is derived from $.O_2$- and hydrogen peroxide (H_2O_2), generally metabolically generated in the medium. Neither $.O_2$- nor H_2O_2 is energetic enough to initiate LPO directly, but in the presence of catalytic amounts of metal ions, they can react and form .OH radicals under a net equation, Harber – Weiss reaction (Halliwel & Gutteridge 1984).

GSH was marginally affected by the elevated metal ion concentration effect (Table 2) to a lesser content in this study. GSH can react with peroxyl radicals to achieve a steady state for themselves and itself converts to thiyl radical (GS.) which is not rapid as oxidative attack at polyunsaturated membrane lipids. It seems that the metal ion extrusion from cells presumably involves movement of diffusible complexes such as Cd – GSH and it also gives rise to alleviating in the level of thiol groups pool including GSH in the medium.

Due to more reactivity of cadmium with GSH molecules than cadmium could present more oxidative stress on living organism making its cells being more susceptible to damages. Despite the extensive evidence implicating the depletion and/or oxidation of GSH in a wide variety of experimental toxicities (Smith *et al* 1996), it has been shown that GSH content was not much altered under the influence of metal ion stress. An explanation here is that relative inhibition following metal ion impact on some enzymatic defense systems such as GPx (Radi *et al* 1988), converts lipid – OOH to respective alcohol through oxidation of GSH to GSSG, causes no remarkable alteration in GSH content due to less GSH consumption by inhibited enzyme.

It may be suggested that this reduction may be due to tissue protein metabolized to produce glucose by the process of gluconeogenesis and the glucose is utilized for energy production during stress condition (Kabeer Ahamed 1979). However in the experimental fish (treated with heavy metal &*Pseudomonas*) the sliding down of protein content is minimized to 19.7%. This may be due to the degrading activity of *Pseudomonas* in the experimental medium, which lessens the toxic stress on the fish. Shanthi (1991) made a similar observation in degradation of insecticide carbaryl in *Catla catla*by *Pseudomonas* sp.

Another observation made in this experiment is that cadmium residues steadily increased in the tissue (Muscle & total homogenate) of the control fish from the first week to the fourth week, while it is decreased in the experimental ones (treated with *Pseudomonas*) towards the end of experiments. Microbial processes play an important role in being about the biological transformation of insecticide (Matsumara 1974). Due to their ability to adopt and proliferate in diverse situation, their total biomass and surface area, the microorganisms are considered to represent the principal force of environmental alteration of heavy metal residues. Insecticide residue may also act as a carbon source to the microorganism in natural environments (Gupta 1984; Munnecke & Hsieh 1974).

Table 1: Effect of different concentrations of cadmium on mortality (%) of *C.catla*as a function of different exposure periods

S. No	Concentration of cadmium (ppm)	Exposure periods (h) 24	48	72	96
1	5	60	80	100	100
2	4	50	70	80	90
3	3	30	50	60	80
4	2	10	10	20	50
5	1	-	-	10	20

Table 2: Analysis of muscle tissues of *Catla catla* treated different groups

S. No	Parameters (mg/g)	Control	Cadmium treated fish	*Pseudomonas* treated fish (Without Cd)	Cadmium and *Pseudomonas*tre ated fish
1	Total protein	24.7	12.8	15.9	22.3
2	Carbohydrate	10.5	9.2	9.4	10.4
3	Phosphatase	18.7	15.5	18.0	16.5
4	Protease	6.8	4.5	6.5	5.9
5	Lipase	1.08	2.05	1.0	1.5
6	AST	4.52	2.36	4.2	3.8
7	ALT	0.44	0.69	0.4	0.5
8	lpo	1.05	0.75	0.95	0.8
9	GSH	0.78	0.52	0.69	0.6
10	Copper (ppm)	Nil	1.8	Nil	1.0

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ACCUMULATION OF HEAVY METAL AND ANTIOXIDANT RESPONSES IN *AMARANTHS DUBIUS*

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ABSTRACT

In the present study was explored that the effects of metal contaminated soil on the antioxidant response of *Amaranths dubius.* The soil pH and electrical conductivity were noted as 6.77±0.52 and 1.82±0.37 dSm-1 respectively and the moister content (23.26 ±0.87%) and organic matter (2.82± 0.21%) were noted in the metal contaminated soil. The heavy metal concentration of *A.dubius*leaf was analysed. The highest heavy metal concentration was found in a Fe (162.3±1.3 mg/kg dw), the moderate accumulation was noted in the Zn (45.3±1.2 mg/kg dw), and followed by Ni (23±0.5 mg/kg dw) and lowest concentration was observed in the Cd (18±0.2 dw), Cu (17.1±0.7 dw) and Pb (12.2±0.5 dw). The effect of heavy metal stress on antioxidant activity in *A.dubius*was investigated. The enzymatic activity such as Superoxide dismutase, catalase, polyphenol oxidase, peoxidase and phenylalanine ammonia lyase were estimated. Among this, the CAT activity was more 27.5±1.4 Ug^{-1}in the stress conditions when it compared to other activities. The non enzymatic antioxidant like proline was showed the 12.3±1.1 (mg g^{-1}) and 19.2±1.3 (mg g^{-1}) in untreated and treated conditions of *A.dubius*.In the present study, all the enzyme activities were much higher in the metal treated *Amaranths dubious*when compared to control.

Key words: *Amaranths dubius,* Phytoremediation, Heavy metals, Antioxidant and Proline.

INTRODUCTION

The agricultural and industrial revolutions in the last few decadeshave resulted in increased concentration of toxins in our environment thatare the major causes of toxicity in plants and animals. Among differenttoxins, increasing levels of salts, heavy metals, pesticides and otherchemicals are posing a threat to agricultural as well as natural ecosystems ofthe world. Human activities have dramatically been changing thecomposition and organisation of the soil on earth. Industrial and urbanwastes, in particular the uncontrolled disposal of waste and the applicationof various substances to agricultural soils, have resulted in the contaminationof our ecosystem.

Heavy metals of soil and water are ofserious concern to the environment due to their non-degradable state. All plants have the ability to accumulate essential metal from the soilsolution. Plants need different concentration for growth and development.This ability allows the plants to accumulate other non-essential metals (Al,As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, Ti and U) which have no knownbiological function (Djingova and Kuleff, 2000). The capacity of plants to concentrate metals has usually beenconsidered a detrimental trait since some plants are directly or indirectlyresponsible for a proportion of the dietary uptake of toxic heavy metals byhuman (Chaney *et al*., 1997).Plants often accumulate heavy metals to concentrations exceedingtheir levels in soil by several folds, where from they enter the food chain.The capacity of plants to accumulate such metals and tolerate their highconcentrations is a species-specific trait. Plants ideal for phytoremediationshould grow fast, have high biomass and tolerate or accumulate a wide rangeof heavy metals in their harvestable parts.

Plants contain high concentrations of numerous redox-activeantioxidants such as polyphenols, flavanoids, carotenoids, tocopherols,glutathione, ascorbic acid and enzymes with antioxidant activity, which fightagainst hazardous oxidative damage of plant cell components. Phenolics are antioxidants, which allow them to act as reducingagents, hydrogen donators and singlet oxygen quenchers (Pietta 2000). Theyalso have metal chelation properties. To overcome heavy metal toxicity, plant cells are equipped with enzymatic mechanisms to eliminate or reduce their damaging effects. The anti-oxidant enzymes system, mainly including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) has the ability to scavenge reactive oxygen species and, thereby, prevent oxidative damage.

The plant used in the phytoremediation technique must have aconsiderable capacity of metal absorption, its accumulation and strength todecrease the treatment time. Many families of vascular plants have beenidentified as metal hyperaccumulator (Reeves and Baker, 2000; Prasad andFreitas 2003), and many of them belong to Brassicaceae andAmaranthaceae. These hyperaccumulators are metal selective, having slowgrowth rate, produce small amounts of biomass and can be used in theirnatural habitats only (Kamnev and van der Lelie, 2000).*Amaranthus*belongs to the family *Amaranthaceae*with approximately 60 species that are recognized (Anjali *etal.*, 2013). Amaranths have been domesticated as leaf vegetables, fodder, potherbs or asornamentals. Amaranth species are characterized by a high level of diversity and wide spectrum of adaptabilityto diverse environmental conditions. Information on genetic as well as nutritional diversity among thespecies and their wild relatives is essential for efficient utilization of plant genetic resources such as cropimprovement. In the present study, it is aimed to analyse the impact of metals on the antioxidative response in *Amaranthsdubius*.

MATERIALS AND METHODS

Preparation of Soil

Top soils (5-10cm) were collected from Garden of Marudupandiyar College, Thanjavur, Tamil Nadu, India. The soils were thoroughly mixed by a mechanical mixer and passed through 4 mm sieve to remove fibre and non soil particulate in the sample (Spirochova *et al.*, 2003). The following physio chemical properties of experimental soils were assessed.

pH and Conductivity

A soil suspension was prepared with soil and deionized water in 1:5 ratio (20 g of soil and100 mL of water) and allowed to stand for one hour. Soil pH and electrical conductivity weremeasured using a portable combination probe (Hanna Instruments, United Kingdom) calibrated in accordance with the manufacturer's instructions.

Moisture content

About 10 g ± 0.001 g soil was weighed into a clean pre-weighed tarred porcelain crucible andplaced in an oven at 105°C overnight. The sample was then placed in a desiccator using tongs, allowed to cool and then weighed to a constant weight. The moisture content is expressed as apercentage via the following algorithm.

Moisture content = [(mass of air-dried soil – mass of oven-dried soil)/mass of air-dried soil] x100 (Watts and Lyndsay, 1996).

Organic matter content by loss on ignition analysis

A clean dry porcelain crucible was placed in an oven at 100 °C for an hour then allowed tocool before taking the weight of the crucible (W1). About 5 g of 2 mm sieved soil wasweighed in the pre-weighed crucible (W2) and dried in an oven at 105 °C for 24 hours. Thepre-ignition

weight after oven drying at 105 °C was measured and calculated (DW105). Thecrucible was placed in the oven at 550 °C for 4 hours. The post ignition weight was taken andcalculated as DW550 after heating the soil at 550°C (Heiri *et al*., 2001 and Ribeiro *et al*., 2011).

Cultivation conditions

This soil sample was uniformly saturated with the concentration of 50 mg/kg of Cd, 100 mg/kg of Ni, 150 mg/kg of Pb, 300 mg/kg of Cu, 500 mg/kg of Fe and 1000 mg/kg of Zn were added. *Amaranthsdubius*was grownin pot filled with 2 kg of soil samples saturated with corresponding concentration of metals. Deionised water (300ml) was added twice a week during the first month. All the pots to place on 500 µmols of photo synthetically active radiation at the plant top with a 12:12 hr photoperiod at 22± 2° C for 30 days. After 30 days of acclimatization, the heavy metal application was performed three times with an interval of 2 days and one time a day in the early hours of the day.

Analysis of metal accumulation in the soil and plants samples

The concentrations of Cu, Cd, Ni, Pb, Zn and Fe were analysed in the leaves of *Amaranths dubius* after experimental periods.The leaves of *Amaranths dubius* was washed in a tap with distilled water and dried at 105°C. 0.15-g portion of dried plant material was treated with 5 ml of concentrated nitric acid and left for 24 h. Next, the samples were digested at 110°C until complete mineralization was achieved. After mineralization, the samples were diluted with deionized water to a volume of 10 ml. Concentration of Cu, Cd, Ni, Pb, Zn and Fe were measured using inductively coupled plasma-atomic emission spectroscopy. The concentration of various heavy metals were computed and expressed as mg Kg^{-1}

Antioxidant activity

Enzymatic antioxidant

Crushed plant parts were homogenized in a 100 mM phosphate buffer (pH6.8) and centrifuged at 12,000×g for 20 min. The supernatants were used to determine the enzyme activity levels. The whole procedure was carried out at 4 °C. The activity of Superoxide dismutase (SOD) was assayed spectrophotometrically by measuring its ability to inhibit the photochemical reduction of Nitro blue Tetrazoilum (Beauchamp and Fridovich, 1971). One unit of SOD is the amount of extracts that gives 50% inhibition in the rate of NBT reduction. Catalase activity (CAT) was determined by consumption of H_2O_2 and was monitored spectrophotometrically at 240 nm for 3 min (Luck, 1974). For Polyphenol oxidase activity, catechol was used and the activity was expressed as changes in absorbance at 495 nm min-1/g fresh weight of tissue (Esterbauer *et al*., 1977), For Peroxidase assay (POD) the increase in absorbance due to oxidation of guaiacol (extinction coefficient 26.6 mM^{-1} cm^{-1}) was monitored at 470 nm (Putter, 1974). Phenylalanine ammonia lyase activity was estimated by the method of Brueske (1980).

Non- enzymatic antioxidants

Prolinewas analysed spectrophotometerically at 520 nm using toluene for a blank as per Bates *et al* (1973). The acid–ninhydrin method was used to determine the proline content. The plant material (0.5 g) was homogenized in 10 mL of sulfosalicylic acid (3 g per 100 mL) and the homogenate was filtered through Whatman No. 2 filter paper. The reaction mixture containing 2 mL of homogenate, 2 mL of acid ninhydrin and 2 mL of glacial acetic acid was incubated at 100 °C for 1 h. The reaction mixture was placed on ice and extracted with 4 mL of toluene. The absorbance was read at 520 nm using toluene as the blank. The proline content expressed in micromoles proline per gram fresh weight was calculated.

RESULS AND DISCUSSION

Physico-chemical analysis of soil

Soil quality can be monitored by a set of measurable attributes termed indicators. These indicators can be broadly grouped as physical and chemical indicators and one can assess overall soil quality by measuring changes in these indicators (Dalal and Moloney 2000). In the present study various physico-chemical characteristics of the metal contaminated soil was analysed. The soil pH and electrical conductivity were noted as 6.77±0.52 and 1.82±0.37 dSm-1 respectively. This low pH enhances solubility and mobility of heavy metals (Akan *et al.,* 2013) and the presence of humic acid which is the major acid in soil organic matter. Heavy metal mobility decreases with increasing soil pH, hence most of the sites with low pH had relatively high concentration of selected heavy metals. The moister content (23.26 ±0.87%) and organic matter (2.82± 0.21%) were noted in the metal contaminated soil (Table.1). presences of many organic waste residues which add more organic matter after their decay. Values of soil organic matter and organic carbon content obtained were higher than the control sites. This observation corroborated Oyedele*et al.,* (2008) who reported that polluted sites had significant higher soil organic matter and organic carbon as compared to the control site.Apart from this accumulation and subsequent decomposition of plant residues also result in building organic matter.

Heavy metal analysis

Soil was contaminated with mixture of heavy metals such as Cd, Cu, Pb, Ni, Fe and Zn. After experimental periods, the leaves of *Amaranths dubius*were extracted with concentrated nitric acidand analysed the heavy metal concentration from this plant leaves. The highest heavy metal concentration in leaves of *Amaranths dubius*was found in a Fe (162.3±1.3 mg/kg dw), the moderate accumulation was noted in the Zn (45.3±1.2 mg/kg dw), and followed by Ni (23±0.5 mg/kg dw) and lowest concentration was observed in the Cd (18±0.2 dw), Cu (17.1±0.7 dw) and Pb (12.2±0.5 dw) (Table.2). Thevariable accumulation in plant tissues at different sites may beinterpreted in terms of decrease in soil pH, increased solubility ofmetals in spoils and their mobility in the plant tissue (Kumar *et al.,* 2009).Not only the vegetative parts but also the ultimate seeds followed thesame sequence of the extent of accumulation of metal in reference.The reason of high uptake of Fe may be due to presence of Fe inthe form of iron pyrites lowering pH of the spoil and thus renderingsoluble metals more available for plant uptake (Pandey*et al.,* 2008).Another reason for increased metal accumulation at low pH may be the metal binding properties of the organic matter (Pandey*et al.*,2006b).

Antioxidant activity

The antioxidant enzyme and non enzyme activity of the *Amaranths dubious* were analyzed under the heavy metal accumulated conditions (Fig.1). The activity of SOD wasrecorded as 10.3±0.2 Ug^{-1} in control plants and 16.1±0.5 Ug^{-1} in metal stressed plants. The increase Superoxide dismutase activity can be considered as an indirect evidence for enhanced production of free radicals. In earlier study reported that the metal stressed plants showed the higher SOD during oxidative damage (Chongpraditnum*et al.,* 1992).Catalase activity in *Amaranths dubious* leaf was 16.2±1.2 Ug^{-1} in untreated conditions. The leaf of metal treated plant showed higher catalase activity (27.5±1.4 Ug^{-1}) when compared to control. In the previous study *Sinapisarvensis*L. at the highest concentration of heavy metals, the activity of CAT was higher in Cd treatment (Mostafa and Semin, 2001).

The activity of the enzymes PPO and POD were higher in the leaf of *Amaranths dubious.* Generally, these activities were higher in metal stresses plants than in the control. The PPO activity was 0.5±0.1 Ug^{-1} and 1.8±0.1 Ug^{-1} respectively in control and treated plant. The POD

activity (14.2±1.5 Ug^{-1}) was also higher in the metal treated plants when compared to untreated plants. Saffar (2009) reported that in *Arabidopsis thaliana* PPO activity might be the result from prolonged heavy metal stress. The highest Phenylalanine Ammonia Lyase (PAL) activity (0.04±0.2 Ug^{-1})was recorded in metal stressed Amaranths *dubious* leaf when compared to control plants (0.01±0.1 Ug^{-1}). Phenylalanine ammonia lyase (PAL) is the first committed enzyme involved in the plant phenylpropanoid pathway. The PAL activity increased in *Jatrophacurcas*L. under heavy metal stress in all bioparts (Devi Chinmayee*et al*., 2014).

Proline can play an important protective role against heavy metal stress. The proline content of Amaranths *dubious* leaf was 12.3±1.1 (mg g^{-1}) and 19.2±1.3 (mg g^{-1}) in untreated and treated conditions. Free proline accumulation under heavy metal exposure seems to be widespread among plants (Costa and More, 1994). Among this, the CAT activity was more 27.5±1.4 Ug^{-1}in the stress conditions when it compared to other activities.

CONCLUSION

The metalaccumulations in plant tissues increasedwith increasing metal concentration as well as the plant growthperiod. Application of Fe and Zn led to a significant increase in these metals concentration detected in plants. They are enhanced the antioxidant defence. In the present study concluded that the *Amaranths dubious*had ability in metal stress depends on oxidative stress defense mechanisms. The enzymatic activities showed the depending on the concentration of heavy metals. In the present study, all the enzyme activities were much higher in the metal treated *Amaranths dubious*than in the control plants. *Amaranths dubious* was able to protect against multi-metal stress that serves as an important component in antioxidant defense mechanisms.

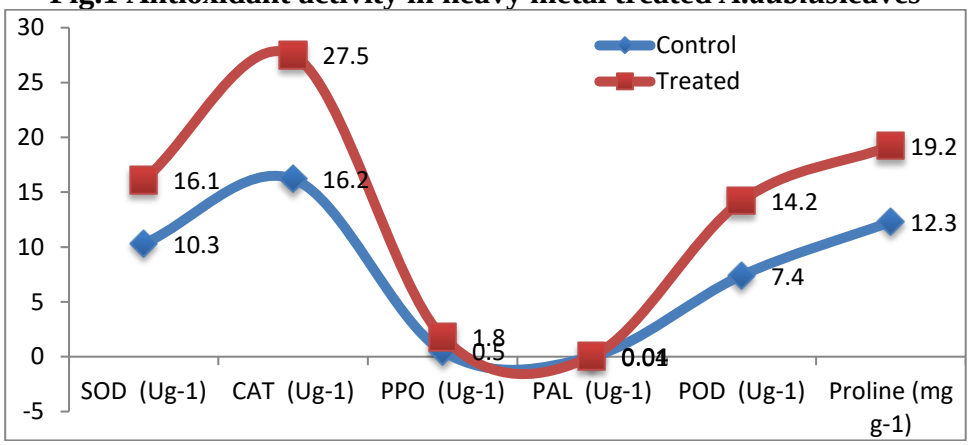
Table. 1 Physio chemical properties of soil

S.No	Physico-chemical properties	Values
1	pH	6.77±0.52
2	Electrical conductivity (dSm-1)	1.82±0.37
3	Moister content (%)	23.26 ±0.87
4	Organic matter (%)	2.82± 0.21

Table.2 Heavy metal accumulation of *A.dubius*leaves

S.No	**Heavy metal**	**Concentrations mg/kg**	***A.dubius* leaves mg/kgdw**
1	Cd	50	18±0.2
2	Ni	100	23±0.5
3	Pb	150	12.2±0.5
4	Cu	300	17.1±0.7
5	Fe	500	162.3±1.3
6	Zn	1000	45.3±1.2

Fig.1 Antioxidant activity in heavy metal treated *A.dubius*leaves



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ANTI-ULCER ACTIVITY OF CRUDE ETHANOLIC EXTRACT OF *HELIOTROPIUM INDICUM* L.

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ABSTRACT

The ethanolic extract of *Heliotropium indicum* leaf and root was evaluated for its anti-ulcer activity against pylorous ligation induced gastric ulcer (antisecretory) in rats.We found that *H. indicum* extract at a dose of 100 mg/kg p.o. markedly decrease the incidence of ulcers. Ethanol extract of *H. indicum* showed significant reduction in gastric volume, free acidity, total acidity and ulcer index. The plant extract also showed gastro protective activity (55.37%), whereas standard drug Famotidine showed 70.34%.

Key words: Anti-ulcer; *Heliotropium indicum*; Pylorous ligation ulceration; Boraginaeceae.

INTRODUCTION

Ulcers are thought to be a result of an imbalance between “aggressive and defensive factors”. Acid and pepsin components form the aggressive factors and mucin layer (mucin – bicarbonate secretion, phospholipid layer, tight junction), cell proliferation, prostoglandins, the urogastrone epidermal healing factors form the defensive factors. It is widely accepted that pathogenesis of ulcer is complex and increased acid secretion, pepsin activity and reduced mucus and bicarbonate secretion, enhanced contractility of the gastric wall and reduced gastric mucosal blood flow represent some of the established pathogenic factors of gastric ulceration[1].

Most of the commonly used drugs namely antacid, anticholinergic selective M_1- blockers (pirenzepine, telenpine etc.) H_2 – blockers (ranitidine, famotidine, etc.) act by reducing the aggressive factors while carbon oxolone, sucralfate, sodium-bimuth subcitrate and prostoglandins by strengthening the mucosal resistance. Considering the several side effects (arrythmias, impotance, gynaecomastia and haematopoietic changes) associated with the use of modern medicine. Because of the wide geographical distribution of ulcer diseases in India, attempts were and are being made to look for suitable anti ulcerogenic plant products as a better alternative for the treatment of ulcer.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[2].Over 50% of all modern clinical drugs are of natural product origin[3] and natural products play an important role in drug development programs in the pharmaceutical industry[4].

Traditionally the flowers used as emmenagogue, used in menstrual disorders. Bark is powerful astringent, and in various forms of ulceration, anti periodic in fever, heumatism, tonic, beneficial in chronic dysentery, powdered bark externally applied to ulcer, leprosy, cures fever, headache, blood complaints (Ayurveda), cardiotonic, aphrodisiac, anthelmentic; good for scabies and expectorant[5,6].

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large number of researchers in different parts of the world[7]. Much work has been done on ethanomedical plants in India[8]. Interest in a large number of traditional natural products has been increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents[9]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[10].The current study was undertaken to evaluate the anti-ulcer activity of *Heliotropium indicum* Lextract by pylorous ligation induced gastric ulcer, in rats.

MATERIALS AND METHODS

Collection of plants

Heliotropium indicum L belongs to the family Boraginaeceae was collected from Thanjavur District, Tamilnadu State, India and identified by the special key given `The Flora of Presidency of Madras'. Voucher sample was prepared and deposited in the Department of Botany and Microbiology, A.V.V.M Sri Pushpam College, Poondi.

Preparation of leaf and root powder

The leaf and root of *Heliotropium indicum* L washed with sterile distilled water. After, the leaves were shade dried and powdered by using Pestle and Mortar.

Preparation of extracts

25g of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 hrs. All the extracts were concentrated using rotary flash evaporator and preserved at 5^{o}C in airtight bottle until further use. All the extracts were subjected to anti-ulcer activity.

Antiulcer Activity

The ulcer study was done at Periyar College of Pharmaceutical Sciences, Tiruchirappalli District, Tamilnadu, India by using their animal house facility under the guidance of Committee for the Purpose of Control and Supervision of Expeiments on Animals (CPCSEA/265).

Albino rats of either sex weighing about 100 – 130gms were divided into groups of six each. Pregnancy was excluded. The animals were deprived of food for 24 hours before the commencement of the experiment, but water was allowed adlibitum. The drugs were given orally 2 hours prior to pylorus legation, which was carried out according to the technique [11].

Testing drug:

* Group I	Untreated control	---
* GroupII	Ulcer control	5 ml/kg (5% w/v Acacia)
* Group III	*H. indicum*leaf	100mg/kg

* Group IV	*H. indicum*root	100mg/kg
* Group V	Famotidine	20 mg/kg

5% w/v acacia mucilage was used as a vehicle at a dose of 5 ml/kg. The solvent control received equal volume of acacia mucilage. The animals were sacrificed 6 hours after pylorus ligation. The gastric juice was collected, centrifuged and its pH and volume were measured. Free and total acidity were estimated titrimatically with 0.01N NaoH using methyl orange and phenolphthalein as indicators.

Pipette 1 ml of filtered gastric contents into a small beaker, add 2-3 drops of methyl orange and titrate with 0.01N NaoH, until all trace of the red color disappears and the color is yellowish orange. Note the volume of alkali added. Then add 2-3 drops of phenolphthalein and continue titration until a definite red tinge reappears. If a yellow color is obtained on adding methyl orange no free acid is present. Add the phenolphthalein and titrate the combined acid. This then equals the total acid. The data concerning the pH, volume, acid secretion of gastric juice and ulcer index were analyzed by student `t' test.

Total Acidity: A volume of 2 ml diluted gastric juice was titrated with 0.01N NaoH run from a micro burette using phenolphthalein as indicator and the acidity was expressed as mg. HCL/100g. body weight of rat[12].

Free Acidity: It is determined in similar manner using topfer`s reagent as indicator and sodium hydroxide was run until canary yellow color was observed[12].

Ulcer index: Ulceration in rats was induced as described[13]. On the fourth day pylorus part was ligated following 36 h fasting[11]. Four hours after the pyloric ligation the animals were sacrificed by decapitation. The stomach was opened and the percentage inhibition of ulcer was determined[14]. A score for the ulcer was made as follows:

0: normal colored stomach.
0.5: red coloration.
1: spot ulcers.
1.5: haemorrhagic streak.
2: ulcers.
3: perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows:

$$\text{Inhibition of ulcer (\%)} \quad \frac{\text{Control mean ulcer index-test mean ulcer index}}{\text{Control mean ulcer index} \times 100}$$

RESULT AND DISCUSSION

The effect of alcoholic extract of *H.indicum* leaf and *H.indicum* root on pylorus ligated induced ulcer model is presented Table 1.It was observed that increase the ulcer index (35.4 ± 3.2) in ulcer control rats. Significant reduction in ulcer index was observed in *H.indicum* leaf (100mg/ body weight) and *H.indicum* root (100mg/body weight). Therefore, the decrease in the ulcer index in the *H.indicum* leaf and *H.indicum* root extract treated groups are an ulcer

indication of the ulcer curative nature of *H.indicum* leaf and *H.indicum* root. Our results concordant with earlier report[15].

It is significant to note that increase the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer control rats compared to untreated control rats. The severity in terms of volume of volume, total acidity and free acidity showed decreased in-group V animals when compared to those in groups III and IV. The increased pH of gastric juice was also observed in group III and IV animals when compared to that in-group V.

The increase in volume in the ulcer control rats undoubtedly due to increased production of hydrochloric acid as is evident from the total acidity and decrease pH value of gastric juice. Inauen *et al.*,[16] have reported that inhibition of acid secretion accelerated ulcer healing. The decrease in volume of the gastric juice and concomitant decrease in the acidity and increase in pH, proving the anti-ulcer activity of *H.indicum* leaf and *H.indicum* root.

Preliminary phytochemical investigations of the ethanol extract of *H.indicum* showed positive test for Liberman burchard test (sterols) and the extract treated with tin and thionyl chloride appears pink color indicates the presence of terpenoids, hence the anti-ulcer activity of *H.indicum* in this experimental model may be due to the terpenoids and steroids. The results demonstrated that *H.indicum* extract produced antiulcerogenic effects possessing antisecretory, cytoprotective and proton pump inhibition mechanism. This interesting observation indicates that *H.indicum* extract can be a potential source for the treatment of ulcer. However, detailed study like isolation of active molecule and characterization is required to confirm the phytochemicals responsible for the activity.

Recent reports have indicated that many flavonoids possess antiulcerogenic activity[17]. So the anti-ulcer activity of alcoholic extract of may due to its flavonoids content.In this study we observed that alcoholic extract of both *H.indicum* leaf and *H.indicum* root provides significant anti-ulcer activity.

Table 1: The effect of *H. indicum*leaf and root (100 mg/kg) and Famotidine (20ml/kg) on gastric ulcers

S.No	Groups	Volume of Gastric juice (ml/100g)	pH	Total Acidity (mEq/l)	Free Acidity (mEq/l)	Ulcer Index (mm in length)
1	Untreated control	0.4±0.03	4.2±0.16	30.1±1.0	15.9±0.9	10.2±0.8
2	Ulcer control	2.8 ± 0.04	1.3 ± 0.07	98 ± 7.3	77 ± 6.3	35.4 ± 3.2
3	*H. indicum* leaf (100 mg/kg)	0.61** ± 0.03	3.5* ± 0.25	38.3** ± 2.8	33.8** ± 1.8	15.8* ± 1.8
4	*H. indicum* root (100 mg/kg)	0.78* ± 0.03	3.8** ± 0.10	47.2* ± 0.0	33.2* ± 0.1	21.5* ± 1.0
5	Famotidine (20mg/kg)	0.50**± 0.02	4.10** ± 0.18	29.3** ± 1.0	16.2** ± 0.9	10.5** ± 0.8

*****P< 0.01 vs ulcer control
**P< 0.001vs ulcer control by student ' t' test
Values are ± S.E.M

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THE TRANSFORMATIVE ROLE OF VACCINES IN CONTROLLING RARE INFECTIOUS DISEASES -A REVIEW

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ABSTRACT

Vaccines are vital tools in modern medicine, offering effective prevention and control of infectious diseases, including rare and neglected infections such as rabies, Japanese encephalitis, meningococcal infections, and Ebola. These diseases present unique public health challenges due to their sporadic nature, high mortality rates, and limited treatment options. Vaccination significantly reduces morbidity and mortality, prevents outbreaks, and safeguards vulnerable populations. Recent advancements in vaccine development, including mRNA, vector-based and conjugate vaccine platforms, have enhanced the ability to address rare diseases more effectively. Successful campaigns, such as those against rabies and meningococcal disease, highlight the profound impact of immunization programs in reducing disease burden. However, challenges persist in developing and deploying vaccines for rare diseases, particularly in low-resource settings. Financial constraints, limited market incentives, and logistical barriers hinder accessibility. Global initiatives like the World Health Organization's Expanded Programme on Immunization (EPI) and Gavi, the Vaccine Alliance, play a critical role in improving vaccine equity and reaching underserved populations. Sustained investment in vaccine research and robust public health infrastructure is essential to combat emerging rare diseases and potential pandemics. By addressing these challenges and fostering innovation, vaccines can eliminate rare infectious diseases as significant public health threats, advancing global health equity and safeguarding future generations.

KEY WORDS: Encephalitis, Meningococcal infections, Ebola, Expanded Programme on Immunization

INTRODUCTION

Vaccines have played a transformative role in global health by preventing, controlling, and, in some cases, eradicating infectious diseases. While vaccines have been instrumental in tackling widespread diseases such as measles, influenza, and polio, their impact on rare infectious diseases is equally significant but often underappreciated. Rare infectious diseases, though less prevalent, pose serious public health threats, especially in vulnerable populations and regions with limited healthcare infrastructure. The development and deployment of vaccines targeting these diseases have not only reduced morbidity and mortality but have also contributed to strengthening global disease surveillance and outbreak preparedness.

Historically, vaccines have led to remarkable success stories, such as the eradication of smallpox and the near-elimination of polio. More recently, advances in vaccinology, including the development of recombinant, vector-based, and mRNA vaccines, have revolutionized the fight against emerging and re-emerging rare infectious diseases. These innovations have enabled rapid vaccine development, particularly in response to outbreaks of diseases such as Ebola, rabies, and meningococcal infections. Additionally, vaccines against human papillomavirus

(HPV) and hepatitis B have significantly reduced the burden of virus-induced cancers, demonstrating their role beyond direct infection prevention.

Despite these successes, challenges persist in vaccine development and distribution. Limited commercial interest, logistical constraints, and vaccine hesitancy hinder efforts to control rare infectious diseases effectively. Moreover, the emergence of novel pathogens, as seen with the COVID-19 pandemic, underscores the need for continued investment in vaccine research, global cooperation, and equitable access to immunization programs.

Vaccination is one of the most effective public health interventions, drastically reducing morbidity and mortality from infectious diseases. While much attention has been given to vaccines targeting widespread pathogens, those aimed at rare infectious diseases have also contributed immensely to global health. This review examines how vaccines have controlled rare infectious diseases, emphasizing case studies and emerging vaccine technologies.

HISTORICAL SUCCESSES

1**. Smallpox**: The First Eradicated Disease: Smallpox, caused by the Variola virus, was eradicated in 1980 through a global vaccination campaign led by the World Health Organization (WHO). The disease had a case fatality rate of 30%, but the introduction of the smallpox vaccine significantly reduced transmission, ultimately leading to its eradication (Fenner, *et al* ., 1988).

2. **Polio: Near Elimination:** Poliovirus, which caused widespread paralysis and death, has been nearly eradicated due to extensive immunization campaigns with oral and inactivated polio vaccines. As of 2021, wild poliovirus remains endemic in only a few countries, with vaccines preventing millions of cases of paralysis globally (Global Polio Eradication Initiative , 2021).

3. **Rabies:** Preventing Fatal Outcomes: Rabies remains a rare infectious disease in many developed countries due to successful vaccination programs for both humans and animals. Post-exposure prophylaxis (PEP) using rabies vaccines has a nearly 100% success rate in preventing disease progression (World Health Organization, 2020).

4. **Meningococcal Disease Reduction in Africa** – The introduction of the MenAfriVac vaccine in 2010 has dramatically decreased cases of meningitis A in the African meningitis belt. This vaccine has prevented tens of thousands of deaths and showcased the power of region-specific immunization campaigns.

5. **HPV Vaccination and Cervical Cancer Prevention** – The development of the human papillomavirus (HPV) vaccine has been a milestone in preventing cervical cancer. Widespread vaccination programs have led to significant reductions in HPV infections and precancerous lesions, paving the way for the potential elimination of HPV-related cancers in the future.

6. **Ebola Vaccine Development** – The outbreak of the Ebola virus in West Africa (2014–2016) led to the accelerated development of the rVSV-ZEBOV vaccine, which has since been used to contain subsequent outbreaks. This marked a turning point in rapid vaccine deployment during epidemics and demonstrated the power of modern vaccine research.

7. **mRNA Technology and COVID-19 Response** – While not a rare disease, the rapid development and global distribution of mRNA-based COVID-19 vaccines revolutionized vaccinology. This breakthrough has opened new possibilities for vaccine development against other rare infectious diseases, enabling faster responses to emerging threats.

IMPACT ON RARE EMERGING DISEASES

Vaccines have played a critical role in controlling rare and emerging infectious diseases, many of which pose significant public health threats due to their high mortality rates and

potential for outbreaks. The rapid development and deployment of vaccines have been instrumental in mitigating the impact of these diseases.

1. **Ebola Virus Disease (EVD) Control** – The development of the **rVSV-ZEBOV** vaccine has been a milestone in combating Ebola outbreaks. Clinical trials demonstrated its efficacy in protecting high-risk populations, and it has been successfully deployed in outbreak-prone regions of Africa, reducing transmission and mortality rates (Henao-Restrepo *et al*., 2017).
2. **Rabies Prevention in Endemic Regions** – Rabies remains a fatal yet vaccine-preventable disease. WHO has recommended widespread vaccination campaigns targeting both human and animal populations to achieve "Zero by 30," a goal to eliminate human deaths caused by dog-mediated rabies by 2030 (WHO, 2020).
3. **Meningococcal Disease in the African Meningitis Belt** – The introduction of MenAfriVac**, a** low-cost vaccine tailored to African populations, has nearly eliminated meningitis A outbreaks in the region. This success underscores the importance of developing vaccines for region-specific rare infectious diseases (Trotter *et al.,* 2017).
4. **Lassa Fever Vaccine Development** – Lassa fever, a viral hemorrhagic disease endemic in West Africa, has no widely available vaccine. However, ongoing efforts in recombinant and mRNA vaccine technologies are showing promise. Clinical trials for IAVI Lassa fever vaccines have been initiated, offering hope for future prevention strategies (Pardi *et al*., 2018).
5. **Nipah Virus Vaccine Research** – Nipah virus, a zoonotic pathogen with high fatality rates, has caused sporadic outbreaks in South Asia. Currently, vaccine candidates using recombinant and vector-based platforms, such as ChAdOx1 Nipah, are being developed to prevent future epidemics (Dolgin, 2021).
6. **mRNA Technology and Future Rare Disease Vaccines** – The success of mRNA-based COVID-19 vaccineshas accelerated research into their application for rare infectious diseases, including Crimean-Congo hemorrhagic fever, Marburg virus, and Zika virus. This breakthrough offers a new pathway for rapid vaccine development against previously neglected diseases (Pardi *et al*., 2018; Dolgin, 2021).
7. **Human Papillomavirus (HPV) and Cervical Cancer:** Though HPV is not an infectious disease in the traditional sense, it causes rare cancers such as cervical cancer. Vaccines like Gardasil and Cervarix have led to a drastic reduction in HPV-related malignancies (Frazer, 2006).

CHALLENGES AND FUTURE DIRECTIONS

Despite these successes, several challenges remain in the development and distribution of vaccines for rare diseases:

Vaccine Hesitancy: Public skepticism regarding vaccine safety can impede immunization efforts (Dubé, *et al*., 2013).Economic Barriers: The high cost of research and development for vaccines targeting rare diseases limits their accessibility (Plotkin, 2014).Emerging Pathogens: New infectious threats, such as zoonotic viruses, necessitate rapid vaccine development using novel technologies like mRNA platforms (Dolgin, 2021).

Advances in Vaccine Technology

New approaches in vaccine development, including mRNA technology, vector-based vaccines, and nanoparticle formulations, have accelerated the creation of vaccines against rare infectious diseases. For example, mRNA vaccines, which proved highly effective against COVID-19, are being explored for diseases like Lassa fever and Nipah virus (Pardi, et al., 2018).

GLOBAL EFFORTS AND POLICY INITIATIVES

- The fight against rare infectious diseases relies not only on scientific advancements but also on coordinated global efforts and policy initiatives that drive vaccine development, distribution, and accessibility. Key initiatives and strategies include:
- **Gavi, The Vaccine Alliance** – Gavi plays a critical role in ensuring vaccine equity, particularly in low-income countries. It has facilitated the introduction of vaccines against rare diseases such as meningococcal A, HPV, and Ebola, protecting vulnerable populations (Gavi, 2021).
- **The Coalition for Epidemic Preparedness Innovations (CEPI)** – CEPI was established in response to the **2014–2016 Ebola outbreak** to accelerate the development of vaccines for emerging infectious diseases, including Nipah virus, Lassa fever, and Chikungunya (Dolgin, 2021).
- **WHO's Blueprint for Epidemic Preparedness** – The WHO developed a Research and Development (R&D) Blueprint to fast-track vaccine development for emerging pathogens like Marburg virus, Crimean-Congo hemorrhagic fever, and Rift Valley fever (WHO, 2020).
- **The Global Polio Eradication Initiative (GPEI)** – Launched in 1988, GPEI has led to a 99% reduction in polio cases, serving as a model for future eradication efforts (Global Polio Eradication Initiative, 2021).
- **Regulatory Frameworks for Emergency Vaccine Deployment** – Organizations like the U.S. FDA (Emergency Use Authorization) and the European Medicines Agency (EMA) accelerated pathways enable rapid vaccine approvals during outbreaks, as seen with Ebola and COVID-19 vaccines (Pardi *et al*., 2018).
- **mRNA Vaccine Platforms and Pandemic Preparedness** – The rapid development of mRNA vaccines has set a precedent for tackling future rare infectious diseases, with ongoing initiatives investing in mRNA-based vaccines for Zika, Lassa fever, and tuberculosis (Dolgin, 2021).

CONCLUSION

Vaccines have played an essential role in controlling, preventing, and even eradicating rare infectious diseases. Historical successes such as smallpox eradication,polio near-elimination, and meningococcal disease control in Africa underscore their transformative impact. Advances in vaccine technologies, including mRNA platforms, viral vectors, and recombinant approaches, offer new hope for tackling emerging infectious threats. Despite scientific progress, challenges remain in ensuring equitable access, overcoming vaccine hesitancy, and addressing logistical hurdlesin vaccine distribution. Global initiatives such as Gavi, CEPI, and WHO's R&D Blueprint continue to drive innovation and policy frameworks that support the development and deployment of vaccines for rare diseases. As the world faces new and evolving infectious disease threats, sustained investment in research, public health infrastructure, and international cooperation will be crucial in leveraging vaccines as a tool forglobal disease control and epidemic preparedness**.** By learning from past successes and strengthening global health policies, vaccines will continue to shape the future of infectious disease prevention and public health security.

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COMPARATIVE STUDIES ON VARIOUS PHYSICO-CHEMICAL PARAMETERS OF FOUR DIFFERENT INDUSTRIAL EFFLUENTS

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ABSTRACT

Physico-chemical characteristics of four different industrial effluents such as tannery, paper mill, textile and sugarcane collected from various parts of Tamil Nadu were analyzed. The effluents were collected at discharge site after pre-treatment indicated different parameters of alkalinity and salinity. pH, chloride, organic and inorganic constituents. Tannery (220 mg/l), textile mill (156 mg/l) and paper mill (185 mg/l) effluent contained high amount of chloride, whereas sugarcane mill effluent contained high value of nitrate (130 mg/l). Due to the discharge of these effluents, the ground water and crops adjacent to these industries were cruelly affected. The tannery effluent was found to be highly toxic for the growth of crop plants compared to other industrial effluents.

Key wards: Tannery, Paper mill, Textile, Sugarcane effluent, Physico-chemical characteristics.

INTRODUCTION

A major environmental problem facing the chemical dyeing and manufacturing industry is that the industry produces large volumes of high strength aqueous waste continuously. The discharge of wastewater containing recalcitrant residues into rivers and lakes lead to higher biological oxygen demand (BOD) causing serious threat to native aquatic life. The modern civilization, industrialisation, urbanisation and increased population have led to fast degradation of our environment. The environment pollution espeacially water pollution is a major problem in India. In the modern context of environmental pollution, not only the industries are playing spoil spot but also the domestic sewages. The water after it is used for industrial purpose is often let free without any treatment. In many places in India, the town authorities let the sewage into streams or brooks or rivers without any effluent treatment. In the present study, some important water quality parameters were analysed for effluents and it has been correlated with ICMR values.

MATERIALS AND METHODS

The samples of effluents were collected immediately outside the boundary of the industry. The physico-chemical analyses of the effluents were done following the method of APHA (1995). The important parameters considered were total solids, total dissolved solids, chloride, carbonate, calcium, magnesium, sodium, potassium, phosphate, salinity, sodium absorption ratio, dissolved oxygen, dissolved bcarbon dioxide, COD, BOD, oil and grease. The water quality index (WQI) values were calculated from these physico-chemical parameters (Tiwari & Ali 1987).

RESULTS AND DISCUSSION

The results of physico-chemical characteristics of effluent are presented in Table 1. The effluent was slightly alkaline and contained high amounts of nutrients. Though BOD and COD levels were high as per IS Standards, their levels were not so much high as compared to other types of effluents. The pH of the textile mill effluent was very high, while in the other effluents

the pH was very low. Electrical conductivity was very high in tannery effluent (10,000 micromhos/cm). The values of four industrial effluents were exceeded the permissible limits. Total dissolved solids were responsible for the higher EC values. The treated tannery effluent was having high total dissolved solids. The values are lower than that of WHO value. Total hardness was very similar in both tannery effluent and sugarcane mill effluent. The four industrial effluents contained four cations such as Na, K, Ca and Mg. Mg content were high in treated tannery effluent. Calcium content was higher in sugar cane mill effluent than other effluents. Chloride content was an indicator of salinity, which was analysed in four effluents. Textile mill, paper mill and tannery effluent contained high amount of chloride content. The effluent contained high amounts of ammonia, which affects the soil as well as quality (Satry 1981). Tannery wastewater when applied in fields resulted in land infertility and poor germination of seeds (Rajagopalan & Davis 1967).

The effluents discharged from the tanneries have high values of pH (alkaline), EC, Chlorides, Sulfides, BOD, COD etc. The values are much higher than the tolerance limits for an industrial effluent discharged into land surface or into public swears as prescribed by ISI standards (Sastry 1981).

Jayabalan *et al* (1994) reported that the higher level of these factors indicated that the tannery effluent is more toxic than other effluents. But the amounts of calcium, magnesium, nitrogen and phosphate were low in the tannery effluent. The high amount of organic matter in the effluent inhibits the enzymatic activity of the plant thereby causing the decay of the plant resulting in bad smell (Vilmmen 1972). The biological activity in the soil is also thus severely affected (Rao *et al* 1993), Dutta (1999) reported that paper mill wastewater at Nagon Paper Mill of Assam, had toxic effect on growth of paddy plants. It can be concluded from the presented investigation that the effluents of sugar cane mill effluent is sufficiently polluted as they produce toxic effects on the growth and physico-chemical parameters of plantsand hence it is not suitable for irrigation unless it is pretreated. Water quality index (WQI) calculated for wastewater sample from textile mill effluent showed a very high value than other effluents. Hence, the effluent is not suitable for any use by human activities.

Table 1.Physico-chemcial parameters of selected industrial effluents

S. No	Parameters	Effluent			
		Tannery Mill	Textile Mill	Sugar Mill	Paper Mill
1	Tamperature (°C)	29.2	30.1	30.0	8.6
2	pH	7.69	9.5	4.2	6.8
3	Colour	Brownish	White	Brownish	White
4	Total solids	1062	1080	1033	1137
5	TDS	450	430	400	437
6	Calcium	25.8	12.6	32.4	16.0
7	Magnesium	28.2	11.5	18.5	13.1
8	Chloride	220	156	68	185
9	Carbonate	6.0	9.0	4.0	2.5
10	Bicarbonate	10.3	11.0	5.40	4.4
11	Dissolved O_2	1.5	3.0	4.4	5.2
12	BOD	190	185	225	210

13	COD	210	180	452	320
14	Free CO_2	20	33	13	25
15	Nitrate	107	101	130	109
16	Nitrite	88	84	90	88
17	Ammonia	45	41	44	42
18	Total phosphate	77.25	71.17	80	76
19	Inorganic P	38.25	38.17	38	41
20	Organic P	40.25	33.2	42.5	45

Except pH and temperature, all values are expressed in mg/l.

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BIODEGRADATION OF PESTICIDES BY USING *PHENEROCHETE CHRYSOSPORIUM* AND *TREMETES VERSICOLOR*

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ABSTRACT

Soils are to some extent contaminated by naturally occurring harmful or toxic elements. Among these contaminants, pesticides are of primary importance due to their continuous entry into the soil environment. Pesticides are used in a number of different activities especially agriculture. However, in un-sterilized and untreated soils, the degradation rate was at the medium where compared to the sterilized soil of the rates mentioned-above, indicating that the soil microorganisms played an important role in pesticide degradation. The pesticides were reduced in their level after the treatment of fungal isolates. Among the pesticides Simazine was reduced maximum when compared to Trifluralin and Dieldrin. Similarly *P.chrysosporium* was removed maximum amount of pesticides than the *T.versicolor*

Key Words:*Biodegradation of soil, Pesticides analysis, HPLC, Phenerochete chrysosporium and Tremetes versicolor*

INTRODUCTION

Currently more number of possible mechanisms is available to clean up of pesticides in soil, viz chemical treatment, volatilization and incineration. Chemical treatment and volatilization are feasible and are problematic as large volumes of acids and alkalis are produced and subsequently must be disposed of by incineration, which is a very reliable method for destruction of these compounds, has met serious public opposition, because of its potentially toxic emissions, and its elevated economic costs (Kearney, 1998; Zhang and Quiao, 2002). Overall most of these physico-chemical cleaning technologies are expensive and rather inefficient (Kearney, 1998; Nerud *et al*. 2003) because the contaminated soil has to be excavated at a site and moved to a storage area where it can be processed. Due to environmental concerns associated with the accumulation of pesticides in food products and water supplies there is a great need to develop safe, convenient and economically feasible methods for pesticide remediation (Zhang and Quiao, 2002). For this reason several biological techniques involving biodegradation of organic compounds by microorganisms have been developed (Schoefs *et al*. 2004).

The use of microorganisms (fungi or bacteria), either naturally occurring or introduced, to degrade pollutants is called bioremediation (Pointing, 2001). Microbial metabolism is probably the most important pesticide degradative process in soils (Kearney, 1998) and is the basis for bioremediation, as the degrading microorganisms obtain carbon, nitrogen or energy from the pesticide molecules (Gan and Koskinen, 1998). The goal of bioremediation is to at least reduce pollutant levels to undetectable, nontoxic or acceptable levels, i.e. within limits set by regulatory agencies (Pointing, 2001) or ideally completely mineralize organ is pollutants to carbon dioxide. From an environmental point of view this total mineralization is desirable as it represents complete detoxification (Gan and Koskinen, 1998). The use of bioremediation to remove pollutants is typically less expensive than the equivalent physico-chemical methods. This technology offers the potential to treat contaminated soil and groundwater on site without the need for excavation (Balba *et al*. 1998; Kearney, 1998), it requires little energy input and

preserves the soil structure (Hohener *et al*. 1998). Perhaps the most attractive feature of bioremediation is the reduced impact on the natural ecosystems, which should be more acceptable to the public (Zhang and Quiao, 2002).

Application of fungal technology for the cleanup of contaminants has shown promise since 1985 when the white rot species *Phanerochaete chrysosporium* was found to be able to metabolize a number of important environmental pollutants. This ability is generally attributed to the lignin degrading enzymatic system of the fungus and a similar degrading capacity was later described for other white rot fungal species (Sasek, 2003).

An environmental factor that may well have a crucial effect on bioremediation is soil water availability, as it varies naturally throughout the year. Nevertheless, very few studies look at the effects of water availability on bioremediation. This study was aimed to isolate the fungi from pesticide-contaminated soil and to determine pesticide degradation rates in soil extract liquid broth after the incubation with fungal inoculation.

MATERIALS AND METHODS

Collection of soil samples

For this present study the soil samples were collected from agricultural field of Pattukkottai, Thanjavur District, Tamilnadu, India. The soil samples were collected from the study site at a depth within 15 cm from the surface of the soil at random during the study period. The collected soil samples were brought to the laboratory in sterilized polythene bags, hand picked, air dried and stored in containers for future use.

Isolation of fungi

One gram of the soil sample was suspended in 100 ml of sterilized Minimal media, without sample considered as control. Both were incubated at 37^0C for 24 to 48 hrs. 0.1 ml of inoculum was transferred to petriplate having PDA medium by spread plate technique. Population of fungi was isolated from the soil samples by serial dilution technique. Fungi were identified by using standard manuals, viz Mannual of Soil Fungi (Gillman, 1957), Dematiaceous Hyphomycetes (Ellis, 1971).

Physico-chemical properties of the soil

Shallow, duplicate soil cores were collected from the study site with a piston corer (54.3 cm^3, 12 cm long x 1.2 cm radius) and stored in watertight plastic bags until analysis of pH, nutrients and organic content. The soil sample were air dried and powdered to pass through a sieve (2 mm). They were collected in a plastic container, sealed and stored at 4°C for further use. Determination of pH was done by a digital pH meter, electrical conductivity by a conductivity meter (Elico). The moisture content was determined after drying at 105°C for 24 hrs. Organic matter and organic carbon was determined titrimetrically (Walkley and Black, 1934) and expressed as percentage (%). The phosphorus content was estimated by Olsen *et al*. (1954). The nitrogen and potassium was estimated according to the method of Sankaram, (1966). Micronutrients viz Zinc, Copper, Manganese and Iron were estimated by Lindsay and Norwell, (1978).

Inoculation with fungi, sampling and dry weight determination

A soil extract liquid broth was used in this study. 100 ml of soil extract was taken in Erlenmeyer flasks (250 ml) to which four plugs of actively growing mycelium of *Tremetes versicolor* and *Phanerochaete chrysosporium* were inoculated in each flask. It was maintained at

27±1°C, for 25 days with constant agitation at 150 rpm. After the incubation period the mycelium was filtered through whatman No. 1 filter paper and biomass determined by drying the mycelium for 48 hours at 80°C. The fresh filtrate was frozen at 20°C and used later for pesticide quantification determination.

Pesticide analysis

The HPLC was used to determine the amount of simazine, trifluralin and dieldrin (Eliassy, 1997).

RESULTS

The present study was undertaken to assess the distribution and occurrence of fungi from pesticides polluted soil, which were collected from agricultural field of Kurichi village, Pattukkottai, Thanjavur District, Tamilnadu, India. Totally 8 species of fungi belongs to 5 genus were isolated from the soil samples. Among them the genus Aspergillus was recorded with 3 species viz., *A. niger, A. terreus*, and *A.flavus* followed by Tremetes with 2 species viz., *T. socotrana* and *T.versicolor*. The genus *Phenerocheate*, *Cladosporium* and *Fusarium* were recorded with single species each (Table 1).

The physico-chemical characteristics of soil samples of the study sites were given in the Table 2. The texture and colour of the soil was sandy and brown respectively in the soil samples. The pH and electrical conductivity values were reported viz., 7.8 and 0.32 respectively in the soil samples. The percentage of organic matter was 0.56%. The macronutrients content in the study site were given in the Table 2.

The impact of the two fungal inoculants such as *T.versicolor* and *P.chrysosporium* on degradation of pesticides were observed and recorded for soil sample. Initially 3 different types of pesticides such as Simazine (5.1ppm), Trifluralin (3.34 ppm) and Dieldrin (1.76 ppm) were observed through HPLC. These pesticides were reduced in their level after the treatment of fungal isolates. Among the pesticides content the Simazine was reduced the maximum levels when compared to Trifluralin and Dieldrin. The fungi *P.chrysosporium* removed maximum amount of all pesticides than the T.*versicolor* in the present soil sample analysis (Table. 3).

DISCUSSION

Successful bioremediation is dependent on an interdisciplinary approach involving such disciplines as microbiology, engineering, ecology, geology and chemistry (Boopathy, 2000). To evaluate the outcome of bioremediation it is critical to assess some microbiological and biochemical parameters all giving information on soil quality. It is difficult to choose which parameters are more reliable, as the relationship between an individual biochemical property and the total microbial activity is not always clear, in complex systems like soils where the microorganisms and processes involved in the degradation of organic compounds are highly diverse (Nannipieri *et al*. 1990).

White rot fungi possess a number of advantages that can be exploited in bioremediation systems. Because key components of their lignin-degrading system are extracellular, these fungi can degrade insoluble chemicals such as lignin or an extremely diverse range of very persistent or toxic environmental pollutants (Barr and Aust, 1994). The mycelial growth habit is also advantageous as it allows rapid colonisation of substrates, and hyphal extension enables penetration of soil reaching pollutants in ways that other organisms cannot do (Reddy and Mathew, 2001). This can maximise physical, mechanical and enzymatic contact with the

surrounding environment (Maloney, 2001). In addition, these fungi use inexpensive and abundant lignocellulosic materials as a nutrient source. They can tolerate a wide range of environmental conditions, such as temperature, pH and moisture levels (Maloney, 2001) and do not require pre-conditioning to a particular pollutant, because their degradative system is induced by nutrient deprivation (Barr and Aust, 1994).

In a more recent study by biological degradation of benzene and toluene by *T.versicolor* was analyzed and the biomass were determined by Demir (2004). Within an incubation period of 48 hrs, it was observed that, removal was completed after 4 hours when initial toluene concentration was 50 mg l-1 and was completed in 36 hrs when this was 300 mgl^{-1}. Biodegradation was completed by the end of the 4th hr at benzene concentrations of 50 mgl^{-1} while it continued for 42 hrs at 300 mgl^{-1}. With the addition of veratryl alcohol, a laccase inducers, to the basic feed medium, the operation of the enzyme system was enhanced and biodegradation completed in a shorter time period.

The microbial population of a site contaminated with pesticides may be eliminated, significantly reduced or altered; but alternatively, microbes may adapt to the presence of toxic compounds and can survive by degrading them as some microorganisms can utilize pesticides as a nutrient source. As soil microorganisms are not equal resistant to xenobiotics, some of them are very sensitive and do not grow when toxic compounds are present in high concentrations or constitute a low carbon and energy source, while other are able to adapt (Guirard *et al*. 2003) and grow well. For this reason the microbial communities within contaminated ecosystems tend to be dominated by those organisms capable of utilizing and/or surviving toxic contamination. Han *et al*., (2004) studied the degradation of phenanthrene by *T.versicolor* and its laccase was purified. After 36 hrs incubation, about 46 and 65% of 100 mg l^{-1} of phenanthrene added in shaken and static fungal cultures were removed, respectively. Although the removal percentage was highest (76.7%) at 10 mgl^{-1} of phenanthrene, the transformation rate was maximal (0.82 mgh^{-1}) at 100 mgl^{-1} of phenanthrene in the fungal culture. When the purified laccase of *T.versicolor* reacted with phenanthrene, the compound was not transformed. Another interesting example of contaminant degradation and enzyme activity was in the study described by Barr and Aust (1994). They described cyanide to be quite toxic to spores of *P.chrysosporium* (50% inhibition of glucose metabolism at 2.6 mgl^{-1}).

Previous studies have also correlated pesticide degradation with dehydrogenase activity. For example Min *et al*. (2001) reported that increasing concentrations of butachlor in soil enhanced the activity of dehydrogenase with the highest activity on the 16th day after application of 22-mg/kg soil of butachlor. Baran *et al*. (2004) reported high dehydrogenase activity in soil contaminated with PAH. Previously, Felsot and Dzantor (1995) described the effect of alachlor and organic amendment on soil dehydrogenase activity and on pesticide degradation rates. Alachlor initially inhibited soil dehydrogenase in soil. Amendment of soil with corn meal caused fasterdegradation of alachlor. At very high concentrations of alachlor (750 mg/kg soil) dehydrogenase activities in amended soils surpassed levels in corresponding nopesticide controls after 21days with coincident alachlor degradation >50% during the same period. They suggested that stimulation of microbial activity by addition of organic amendments might enhance co-metabolism of high concentrations of pesticides in soil. Totally three different pesticide such as simazine, trifluralin and dieldrin was recorded in soil extract through HPLC. Among the pesticide simazine was recorded maximum than trifluralin and dieldrin. The results obtained in this study provide valuable knowledge on the abilities of *T.versicolor* and *P.chrysosporium* might serve as a

sound basis for the further exploitation of these species as fungal inoculants in biological remediation processes.

Table 1. Fungal flora in the soil sample

S. No	Name of the organism
1	*Aspergillus niger*
2	*A. terreus*
3	*A. flavus*
4	*Phenerochete chrysosporium*
5	*Tremetes socotrana*
6	*T.versicolor*
7	*Fusarium sp*
8	*Cladosporium sp*

Table 2. Physico-chemical characteristics of soil sample

S. No	Parameters	Values
1	Soils texture	Sandy
2	Colour	Brown
3	pH	7.8
4	Ecdcm^{-1}	0.32
5	Organic carbon %	0. 56%
6	Nitrogen (mg/g)	91.2
7	Potassium (mg/g)	78.1
8	Phosphorus (mg/g)	9.5
9	Zinc (ppm)	0.93
10	Copper (ppm)	0.36
11	Iron (ppm)	3.23
12	Manganese (ppm)	8.83

Table.3. Analysis Pesticide level in soil extract by HPLC

S. No	Pesticide	Initial (ppm)	Control (ppm)	Treated	
				P.chrysosporium (ppm)	*T.versicolor* (ppm)
1	Simazine	5.1	5.1	2.48	2.96
2	Trifluralin	3.34	3.31	2.63	2.77
3	Dieldrin	1.76	1.73	0.98	1.21

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EVALUATION OF ANTIOXIDANT ACTIVITYOF *CAMELLIA SINENSIS* L

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ABSTRACT

Medicinal plants are widely used in management of diseases all overthe world. Historically, the use of medicinal plants is as old as mankind andmedicine. Thepresentstudyinvestigated that the antioxidant activity of *C.sinensis*, was studied in albino rats by using methanolic extracts of these plants. The phytochemical studies in *Camellia sinensis*showed the presence of alkaloids, flavonoids, carbohydrates, protein, phenols, saponins, tannins, phytosterols, Phlobatannins and terpenoids. In the present GC-MS analysis, totally 20 compounds were identified from the methanol extract of *C.sinensi*. Among the 20 compounds, the highest peak percentage was noted in the 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl (86.5030 %). From this study, methanolic leaves extract of *C.sinensi* showed good ABTS (86.44±6.1 µM TEAC/mg DW) followed by DPPH(78.72±6.2 %) and FRAPP (68.24±7.5 µM TEAC/mg DW) radical scavenging activities. In future, stability studies are required to ensure the usage of this plant for formulations and invivo pharmacokinetic evaluation

Key words: *Camellia sinensis*, DPPH, Phytochemical, Pawedema, Anti-inflammation

INTRODUCTION

Plants are the basic source of information of modern medicine. Nature hasbeen asourceof medicinal agentsforthousands of years andanimpressivenumber of modern drugs have been isolated from them, many based on their usein traditional medicine. Medicinal plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity that is identified as secondary metabolites. Some of these secondary metabolites are produced for self-defense. Herbal medicines represent one of the most importantfields of traditional medicine all over the world. To stimulate the use of herbalmedicine and to determine their potential as a source for novel drugs, it is essentialto study medicinal plants which have folklore reputation in a more intensified way[8]. Humanbeingshaveusedplantsformedicinalpurposesforcenturies.Traditional forms of medicine have existed and still exist in many countries of theworld including countries in the Indian sub-continent like India, Pakistan andBangladesh[8]. The writings indicate that therapeutic use ofplants is as old as 4000– 5000 B.C. and Chinese were the first to use the natural herbal preparations as medicines[12].

The natural products form an integral part of human life from ancientcivilizations to the current century and more than half of the drugs in the market are natural products or derivatives of them. India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard togenetic resources of medicinal plants. The agro-climatic conditions are favorable for introducing new exotic plant varieties[11]. Secondary metabolites have been extensively investigated as sources of medicinal agents[10]. On a global basis, at least 130 drugs,all single chemical entities extracted from higher plant or modified further synthetically are currently in use[13]. Awide range of medicinal plant parts is used for extract as raw drugs and theypossess varied medicinal properties. The different parts used include, root, stem, flower, leaves. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have

not been adequately evaluated. Systematic investigation was undertaken to screen the antimicrobial activity of selected medicinal plants against oral bacterial infection. To overcome this problem, bioactive compounds with no side effects have to be identified from themedicinalplants.

Inflammation is a severe response by living tissue to any kind of injury.There can befourprimary indicators of inflammation:pain,redness,heatorwarmness and swelling. When there is injury to any part of the human body, thearteriolesin the encircling tissue dilate. This gives a raised blood circulationtowards the area[3].Inflammation is either acute orchronic inflammation. Acute inflammation may be an initial response of the bodyto harmful stimuli. In chronic inflammation, the inflammatory response is out ofproportion resulting in damage to the body. Cyclooxygenase (COX) is the key enzymes in the synthesis of prostaglandins, prostacyclins and thromboxanes which are involved in inflammation, pain and platelet aggregation[15]. Inflammation, which was recognized as a simple allergic reaction for decades, is currently being considered to underline pathophysiology of a muchbroader spectrum of diseases than previously expected. The complex interplay of cellular and humoral mediators during inflammation is unfolding but our understanding of the inflammatory reaction is still incomplete all inflammatoryprocessesdevelopalongaknownsequence:locallyincreasedbloodsupply,leakage of fluid, small molecules and proteins, and infiltration of cells. Based on visual observation, the ancients characterized inflammation byfive cardinal signs, viz. redness, swelling, heat, pain and loss of function. Hence the present study is aimed to investigate the antioxidant and anti-inflammatory activities of methanolic leaves extract of *Camellia sinensis*(L).Kuntze.

MATERIALS AND METHODS

Plantcollection

Young leaves of *Camellia sinensis*(L).Kuntzewere poised distinctly from Thanjavur District, Tamil Nadu. The leaves were separated from stems, washed in clean water, and dried at room temperature. The shaded dried leaves were weighted and ground in a sterile mortar.

Extract preparation

The collected leaves were cleaned and dried in shade for 7 days, then ground well to fine powder. About 500 g of plant powder was extracted with methanol (80%) at 70°C by continuous hot percolation using soxhlet apparatus. The extraction was continued for 24 hrs. The methanolic extract was then filtered and kept in hot air oven at 40°C for 24 hrs to evaporate the methanol from it. A dark brown residue was obtained. The residue was kept in air tight containers and stored in a deep freezer for further uses[6].

Qualitative Analysis

Phytochemical analysis of the plant extracts was undertaken using standard qualitativemethods as described by various authors[6,7], [14.] Theplant extracts were screened for the presence of biologically active compounds such as alkaloids,flavonoids, carbohydrates, phytosterols, proteins, phenolics, tannins and saponins.

GC-MS analysis

30 g pulverized sample of *C.sinensis* was soaked and dissolved in 75 ml of methanol for 24 h. Then the filtrates were collected by vaporized under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µmdf capillary column. The apparatus was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and preserved for 9 min. Injection port temperature was

ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samplewas injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Antioxidant activity

DPPH Radical Scavenging Activity

Antioxidant scavenging activity on DPPH was estimated according to the method of Blois[2] with modification involving the use of high-throughput micro plate system. 50 μL of Sample (1.0mg/mL) was added to 50 μL of DPPH (FG: 384.32) (1mM in methanolic solution) and 150 μL of methanol (absolute) in a 96-well microtiter plate in triplicates. The plate was shaken (15 seconds, 500 rpm) and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution was measured spectrophotometric ally at 520 nm. Ascorbic acid was used as positive control.

ABTS scavenging activity

The technique is based on the scavenging of ABTS•+ [(3-ethyl benzothiazoline 6-sulfonic acid) diammonium salt] radical cation which was generated by mixing solutions of ABTS (7 mmol/L) and potassium persulfate (2.45 mmol/L) [16]. The mixture was then incubated in the dark at room temperature for 16h. The product was diluted for optimal absorbance of 0.7 at 734 nm. The decolorization of the ABTS•+ solution by 100 µg/mL of the test samples or reference compound (Trolox) was monitored by a decrease in absorption at 734 nm during 30 min. The antioxidant activity expressed in µMtrolox equivalent antioxidant capacity (TEAC)/ mg dry weight.

FRAP assay

The method is based on reduction of ferric tripyridyltriazine (Fe3 – TPTZ) to ferrous complex tripyridyltriazine (Fe2 – TPTZ) by an antioxidant in acidic pH. The ferrous Fe (II) complex -TPTZ develops a blue color with maximal absorbance at 593 nm[1]. FRAP (Ferric reducing antioxidant power) mixture consists of 10 parts of acetate buffer solution (300 mM) at pH 3.6, 1 volume of 10 mmol/l 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/ l HCl and 1 volumes of a solution of $FeCl_3$ 6H2O (20 mM). To 2 ml of the FRAP mixture were added 10 µl of the plant extract. After incubation of 15 min at room temperature, the absorbance was measured at 593 nm. The calibration range was prepared with Trolox. Results are expressed as µmolTrolox equivalent antioxidant capacities (TEAC)/mg dry weight.

RESULTS AND DISCUSSION

Phytochemicalanalysis

Phytochemical screening was performed to *Camellia sinensis*. The qualitative phytochemical analysis of methanolic extracts revealed the presence of majority of the phytochemicals such as, alkaloids, carbohydrates, protein, saponins, phenols, terpenoids, phytosterols, flavonoids, phlobatannins and tannins.

GC-MSanalysis

In the present GC-MS analysis, totally 20 compounds were identified from the methanol extract of *C.sinensis*is presented in Table.1. The plantsamplesrevealed thesynthesis of biologically active compounds such as,1,2,5,6-Tetrahydropyridin-2-one,5-methy l,2,3-

Pentanedione, 4-methyl-, 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl,1,2,5,6-Tetrahydropyridin-2-one,5-methyl, 3-Amino-2-oxazolidinone, 4H-Pyran-4-one, 2,3-dihydro-2-[1-(benzyloxy)ethyl], 2-Methoxyresorcinol, Eugenol, Naphthalene,6-ethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethy l-7- (1-methylethenyl) 1,2,3-Benzenetriol, Sucrose, D-Allose, Oxalicacid, 2-ethylhexylhexylester, Bicyclo[3.1.1]heptan-3-ol,2,6,6-trimethyl,3,7,11,15-Tetramethyl-2-hexadecen-1-ol,1HPurine-2,6-dione,3, 7-dihydro-1, 3, 7-trimethyl, 1H-Purine-2,6-dione,3,7-dihydro-1,3-dimethyl, Phytol, Myristoyl chloride and Squalene.Among the 20 compounds, the highest peak percentage was noted in the 1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl (86.5030 %). The highest retention time was recorded in the Squalene(30.24)(Fig.1).This was supported by an earlier study was identified the Squalene from leaves of *Terminaliacatappa*L. through GC-MS[9]. However, the seed extracts only exhibited potent scavenging activity. In an ethno pharmacological approach, extracts of the stem bark of *Terminalia spinosa* were investigated for antibacterial and antifungal activity due to the presence of poly phenol and terpenoids[22].

Table 1. Biologically active compounds of *C.sinensis*by GC-MSanalysis

S.No.	Nameofthecompounds	Molecularformula	Retentiontime	%Peak Area
1	1,2,5,6-Tetrahydropyridin-2-one,5-ethyl-MW: 111	C_6H_9NO	6.26	0.4019
2	2,3-Pentanedione,4-methyl-MW:114	$C_6H_{10}O_2$	6.74	0.1258
3	3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl-MW:126	$C_6H_{10}N_2O$	7.39	0.2896
4	1,2,5,6-Tetrahydropyridin-2-one, 5-methyl-MW:111	C_6H_9NO	7.90	0.3882
5	3-Amino-2-oxazolidinoneMW:102	$C_3H_6N_2O_2$	8.50	0.0517
6	4H-Pyran-4-one, 2,3-dihydro-2-[1-(benzyloxy)ethyl]-MW:232	$C_{14}H_{16}O_3$	8.60	0.3107
7	2-MethoxyresorcinolMW:140	$C_7H_8O_3$	10.72	0.2252
8	EugenolMW:164	$C_{10}H_{12}O_2$	12.17	0.1297
9	Naphthalene, 6-ethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-7-(1-methylethenyl)-MW:256	$C_{19}H_{28}$	12.91	0.1881
10	1,2,3-BenzenetriolMW: 126	$C_6H_6O_3$	13.36	1.4953
11	SucroseMW: 342	$C_{12}H_{22}O_{11}$	13.99	1.3970
12	D-AlloseMW:180	$C_6H_{12}O_6$	15.30	0.1463
13	Oxalicacid,2-ethylhexylhexylesterMW: 286	$C_{16}H_{30}O_4$	17.70	2.9581
14	Bicyclo[3.1.1]heptan-3-ol,2,6,6-trimethyl-MW: 154	$C_{10}H_{18}O$	17.91	0.8308
15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol MW: 296	$C_{20}H_{40}O$	20.08	0.7611
16	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-MW:194	$C_8H_{10}N_4O_2$	20.79	86.5030

17	1H-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-MW:180	$C_7H_8N_4O_2$	21.18	2.1275
18	PhytolMW:296	$C_{20}H_{40}O$	23.23	0.5934
19	MyristoylchlorideMW:246	$C_{14}H_{27}ClO$	25.01	0.1816
20	SqualeneMW:410	$C_{30}H_{50}$	30.24	0.8951

Antioxidant activity

The antioxidant activity of DPPH free radical scavenging assay showed the methanolic leaves extract of *C. sinensis.* The scavenging activity of DPPH was increased at a concentration of 500 µg/ml, the scavenging ability on DPPH was 78.72±6.2% in methanolic leaves extract of *C. sinensis*(Table.2). DPPH assay is widely used to determine the antioxidant activity ofplant extract. This assay is based on the ability of antioxidant compound to decolorize thepurple color of DPPH free radical in alcoholic solution to yellow color[15]. The assay is based on the reduction of alcoholic DPPH solution in the presenceof a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H bythe reaction[18].

The ABTS radical assay is also one of the most commonly used methods to evaluatethe antioxidant activity (Table.2). ABTS scavenging activity at various concentrations of the methanolic leaves extract of *C. sinensis* showed the highest percentage inhibition (86.44±6.1 µM TEAC/mg DW) in the concentration of500µg/ml. These results showedthat varying solvent polarities differ significantly in their extraction capacity of antioxidantcompounds and therefore, their antioxidant activities. The ABTS+scavenging assay, whichemploys a specific absorbance (734 nm) at a wavelength remote from the visible region andrequires a short reaction time, can be used as an index that reflects the antioxidant activity ofthe test samples[21]. The present result also agreed that themethanol fraction of a medicinal species *Eriobotrya japonica* showed the most interestingantiradical power compared to other one[23].

In the present study, the trends for ferric ion reducing activities of plant extracts wereshown in Table. 2. The absorbance of methanolic leaves extract of *C. sinensis*was clearly increased, due to the formation of the Fe2+-TPTZcomplex with increasing concentration. FRAP assay measures the reducing potential of anantioxidant reacting with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and producing acolored ferrous tripyridyltriazine[1] (Fe2+-TPTZ). In this study alsoreported that the methanol leaves extract of *C. sinensis*68.24±7.5 µM TEAC/mg DW was showed increased ferric reducing power withthe increased concentration (500µg/ml) and as compared with standard antioxidants(Table.2). In earlier report, the water and ethanol extracts of sumac (*Rhus.coriaria*L.)showed increased ferric reducing power with the increased concentration as standardantioxidants[5].

Table.2Antioxidant activities of methanolic leaves extracts ofselectedplants

Antioxidant activity	**MethanolicPlants extracts(µg/ml)**				
	100	**200**	**300**	**400**	**500**
DPPH (%)	21.05±1.5	42.45±2.4	68.15±2.6	72.20±4.5	78.72±6.2
ABTS (µM TEAC/mg DW)	19.05±0.5	42.45±2.1	68.15±3.3	72.20±7.5	86.44±6.1
FRAP (µM TEAC/mg DW)	46.22±3.1	52.33±4.2	59.42±5.3	62.63±4.5	68.24±7.5

CONCLUSION

Several Indian medicinal plants have been extensively usedin the traditional system of medicine; the managementof liver disorder by athoroughly standardized and evaluated natural, simple and precise herbal drug isstillanintriguingproblem. In the presentstudy reported that the good sources of phytocompoundfrom these experimental plants. In this study also resulted that the experimentalplants are involved protective role of liver damages. In future, stability studies are required to ensure the usage of this plant for formulations and *invivo* pharmacokinetic evaluation is also required to assess the bioavailability.

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PHYTOCHEMICAL AND ANTIBACTERIAL SCREENING OF DIFFERENT SOLVENT EXTRACTS OF *CARDIA SEBESTENA*

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ABSTRACT

The aim of the present study was to investigate antibacterial activity of the leaf of *Cardia Sebestena* plant against common pathogenic bacteria. The agar diffusion method was used to examine the antibacterial activity of different solvent extracts of *C.Sebestena* tested against *Escherichia coli*, *Pseudomonas aeruginosa*, Bacillus subtilis and *Staphylococcus aureus*. For qualitative phytochemical investigation, several solvent extracts of the complete *C. Sebestena*plant were investigated. The minimum inhibitory concentration (MIC) of *C.Sebestena*ethanol extract had significant antimicrobial effectiveness against *Escherichia coli* (489 µg/ml), and *Pseudomonas aeruginosa* (449 µg/ml). The ethanolic extract showed (437 µg/ml and 410 µg/ml) antimicrobial activity against *Bacillus subtilis* and *Staphylococcus aureus* respectively. The lowest antibacterial activity was found in acetone extract with MIC (216µg/ml) of *C.Sebestena*Various parts of the plant have the presence of variety of phytoconstituents such as flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin.

Key words:*Cardia Sebestena*, antimicrobial, agar diffusion method, Phytochemical analysis

INTRODUCTION

Due to increasing consumers concerns regarding processed and ready-to-eat foods containing antibiotics, pesticides, hormones, and synthetic additives and also increasing demand to replace artificial antimicrobial agents with natural alternatives, the usage of natural and organic foods has been experiencing explosive market growth [1 – 3]. However, the untreated products and natural foods may be more susceptible to growth of food-borne pathogens than the conventional food version [4]. The most important food-borne pathogenic bacteria that have survived and grow in these products include *Staphylococcus aureus, Bacillus spp., Listeria monocytogenes, Salmonella spp. Escherichia coli, Yersinia spp.,* and *Clostridium* spp. [5 – 6]. These bacteria cause a great proportion of food-borne outbreaks in different foods such as dairy products, vegetables, and meat and fish products [7]. In this context, plant essential oils are attracting interest as natural food preservatives in order to ensure the safety of food [8].

Cardia Sebestena belongs to the Boraginaecea family, Boraginaceae is commonly known as the Forget me-not family. It includes about 100 genera and 2000 species widely distributed throughout temperature, tropical regions and more abundant in Mediterranean region. The interest in cultivating is mainly related to the commercial importance of its essential oil, which is is used in many industries, including pharmaceuticals, cosmetics, food, and chemicals. Spearmint is also known for its ability to improve memory. Besides being a stimulant, it has several biological uses, such as in insecticides, antimicrobials, antioxidants, antispasmodics, and anti-platelets [9].

Cordia sebestena grows to a maximum height of 8–9 m (25–30 ft) at maturity, with a nearly equal spread. The crown is round to vase-shaped. Branches tend to be somewhat drooping, and the tree is naturally multitrunked. When only a single trunk is allowed to develop, it can

attain a diameter of 30 cm (12 in).The dense, evergreen foliage consists of dark green, leathery, alternate, ovate leaves, 18 cm (7 in) long, with wavy margins. These leaves are covered with small hairs, lending them a rough, "sandpapery" texture[9].

Flowers are produced in clusters at branch ends throughout the year, particularly in the spring and summer. Flowers are 5 cm (2 in) wide, red-orange in color, tubular, flaring (salverform) with 5–7 lobes, bearing 5–7 stamens of similar height. The species is heterostylous and presumably self-incompatible.[7] Pear-shaped fruits follow the flowers, averaging 5 cm (2 in) in length. Fruits are fragrant and edible, but not flavorful[9].

Numerous medications have caused microbes to become resistant, which presents a substantial therapeutic issue in the management of infectious diseases. The overuse of commercially available antimicrobials, which are routinely used to treat illnesses, led to the development of the bacteria' tolerance [14]. In order to find new antimicrobial chemicals, researchers were motivated to investigate in other sources, particularly herbal resources.

MATERIALS AND METHODS

Collection and Identification of plant material: For the study, the whole plant of *Cardia Sebestena* belongs to Boraginaecea family was collected from in and around Thanjavur, Tamilnadu. The whole plant were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Preparation of plant materials and extract: The leaves were carefully cleaned, shade dried and powdered. The powdered material was stored in a closed air-tight plastic container at low temperature. The powdered plant material (50 g) was extracted with 300 mL of each solvent ethanol by maceration (3×24 h) at room temperature. The collected solvents were concentrated by rotary vacuum evaporator at 45°C and then dried using a freeze dryer. All extracts and acyclovir (extracted from commercial tablet) were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was 0.1% v/v in cell culture environment.

Phytochemical Analysis: The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The resultant extracts were evaporated to dryness under vacuum. These extracts were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described [15-16]. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Test microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* were used as test organisms in the current study. For the current experiment, the obtained cultures were repeatedly sub cultured.

Antimicrobial activity by agar diffusion method: The antibacterial efficacy of several *C. Sebestena* solvent extracts was assessed using the agar diffusion method. For spreading agar media, a subcultured microbial suspension (100 µl) was prepared. Various concentrated varied extracts were used to measure antimicrobial activity [17]. The plates were filled with the sample and then left to allow for an hour to enable the extract to disperse. The plates were maintained in

an incubator for 24 hours at 37°C, and the inhibitory zone was measured in millimeters (mm). Results are compared with those of conventional antibacterial drugs.

RESULTS AND DISCUSSION

Phytochemical studies of *C. Sebestena* showed that it contains a number of phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides and pectin. The main bioactive constituents found are bioflavonoid, amentoflavone with minute amount of cupressoflavone [18]. All of the extracts from the *C. Sebestena* contained saponin, phenols, tannins, glycosides, terpenoids, flavonoids, alkaloids, and coumarins, according to a preliminary phytochemical examination. With the exception of the chloroform extract's lack of saponins, glycosides, and coumarins and the extract from ethyl acetate's absence of saponin. The results of the phytochemical analysis are displayed in Table 1. A higher degree of biological activity derives from the presence of a high concentration of phytochemicals in the plant.

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized [19]. In the present study, it was clearly understood that the ethanolic extracted maximum amount of the different type of metabolites present in the *C. Sebestena* Boominathan and Ramamurthy [20] reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti- cancer activity [21]. It also suggests that the plant might have diuretic properties [22]. The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns [23]. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions [24]. Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties [25]. Volatile oils are used in the industries for various purposes, both as a pharmaceutical/ cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections.

The antibacterial properties of various solvent-based extracts of *C. Sebestena* are displayed in Table 2. The ethanol extract of *C. Sebestena* had the highest antimicrobial activity with MIC (459 µg/ml) against the *Escherichia coli*, (409 µg/ml) against the *Pseudomonas aeruginosa*, (401 µg/ml) against the *Bacillus subtilis* and (391 µg/ml) against the *Staphylococcus aureus*. The various extracts of *Mentha spicata* tested against *Escherichia coli* and showed considerable MIC results in water extract (352 µg/ml), chloroform extract (301µg/ml), ethyl acetate extract (292 µg/ml), acetone extract (211 µg/ml), hexane extract (257 µg/ml). The results were compared with standard Cephalosporins as reference compounds with MIC (55 µg/ml). The different extracts of *C. Sebestena* were checked against the *Pseudomonas aeruginosa* and exhibited significant MIC values in water extract (386 µg/ml), chloroform extract (287 µg/ml), ethyl acetate extract (302 µg/ml), acetone extract (227 µg/ml), hexane extract (263 µg/ml). The obtained results were compared with Cephalosporins with MIC (49 µg/ml).

The individual extract of *C. Sebestena* was checked against *Bacillus subtilis* and found impressive MIC values in water extract (385 µg/ml), chloroform extract (294 µg/ml), ethyl

acetate extract (274 µg/ml), acetone extract (209 µg/ml), hexane extract (242 µg/ml). The different solvent extract of *C. Sebestena* was evaluated against *Staphylococcus aureus* and found impressive MIC values in water extract (336 µg/ml), chloroform extract (258 µg/ml), ethyl acetate extract (255 µg/ml), acetone extract (216 µg/ml), hexane extract (224 µg/ml). The gentamicin (35 µg/ml) was used as a standard compound.

A considerable inhibitory zone may also be caused by the variety of phytochemicals present in the extract. The presence of different flavonoids, alkaloids, terpenoids, phenols, saponins, and coumarins has bactericidal properties [26]. According to various scientific studies, high concentrations of phytochemicals and bioactive compounds are thought to have a stronger potential for treating a variety of pathogenic bacteria. Numerous plants and their various portions of them have historically been used to treat a variety of chronic illnesses, such as gastrointestinal problems, urinary tract infections, skin conditions, and various respiratory issues etc. [27]. Several chronic illnesses caused by various bacteria may be prevented and managed with the use of plant-based remedies. Many societies still employ ethnomedicines to treat illnesses and overcome obstacles without creating negative side effects. The inclusion of several phytoconstituents, including alkaloids, flavonoids, coumarins, saponins, polyphenols, tannins, and terpenoids, is what gives herbal preparations their therapeutic effects [28]. The presence of secondary metabolites prevents the growth of harmful microorganisms causing serious diseases [29]. The microorganisms are resistant to many antibiotics that is very harmful to humans. The researchers are finding an alternative to commercial antibiotics to prevent harmful infections against a variety of microorganisms using plant-based medicines [30]. The higher concentration of crude extracts sometimes may cause cytotoxicity in humans hence the dose-dependent values are determined using in vitro cell cytotoxicity assay [29]. As compared to commercial antibiotics, plant-based medicines have very small side effects if they are consumed in excess quantity [31]. In the world, 80 % of different pharmaceuticals are prepared from plant-based medicines and which are effective to cure any chronic disease.

CONCLUSION

C. Sebestena used for its wide therapeutic potential of antimicrobial agents. According to the studies, ethanol extract has the most potential, which may be because it includes the majority of the phytochemical compounds and bioactive compounds that have antibacterial activity. The complete plant extract of *C. Sebestena* has to be further studied in order to identify and purify chemicals that might be used as natural medicinal alternatives to synthetic commercial ones. The future aspects of the plant can be anti microbial as it contains many of the phytochemicals and work has not been performed yet.

Table 1: Qualitative Phytochemical screening on extracts of *C. Sebestena*

S. No	Name of Test	Test applied / Reagent used	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]Dragndorff's test	+++ +++ +++ ++	++ ++ ++ ++	++ ++ ++ ++	++ ++ +++ ++	+++ +++ +++ ++	++ ++ ++ +
2	Flavonoids	HCl and magnesium turnings	+++	++	+	++	+	++

3	Carbohydrate	Molisch's test	+	+	+	+	+	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+++ +++	+ +	++ ++	++ ++	++ ++	++ ++
5	Test for Steroids	A]Salkowski's Test B] Libermann-Burchard's Test	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
6	Gums & Mucilages	Alcoholic Precipitation	-	-	-	-	-	-
7	Fixed oil & Fats	Spot test	+	-	+	+	-	-
8	Saponins	Foam test	+	+	+	+	+	+
9	Phytosterols	LB test	+	+	+	+	+	+
10	Volatile oils	Hydro distillation method	+	+	+	+	+	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C]Xanthoprotein test	++ +++ +++	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++

Table 2. Antimicrobial activity of the plant extracts *C. Sebestena*

Microorganism	**Minimum inhibitory concentration (MIC)**							
	Plant extract of *Mentha spicata* (µg/ml)							
	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate	Gentamicin (µg/ml)	Cephalosporins (µg/ml)
P.aeruginosa	459	386	287	212	211	292	ND	49
B. subtilis	391	385	294	242	209	274	ND	47
E. coli	401	352	301	257	230	302	ND	55
S. aureus	410	336	258	224	216	255	35	ND

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A REVIEW ON TRADITIONAL USES, PHYTOCHEMISTRY AND PHARMACOLOGY *OF DESMODIUM TRIFLORUM* (L.)

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ABSTRACT

Desmodiumtriflorum is a small creeping leguminous herb widely distributed in tropical and subtropical regions. It has been traditionally used in ethnomedicine for its anti-inflammatory, antimicrobial, hepatoprotective, and antioxidant properties. Phytochemical studies reveal the presence of flavonoids, alkaloids, tannins, and triterpenoids, which contribute to its pharmacological activities. Scientific research supports its role in treating respiratory ailments, gastrointestinal disorders, and liver dysfunctions. This review provides an overview of the botanical characteristics, phytochemistry, pharmacological properties, and potential therapeutic applications of Desmodiumtriflorum, highlighting its significance in modern herbal medicine. Further studies are needed to explore its bioactive compounds and mechanisms of action for potential drug development.

Keywords:*Desmodiumtriflorum*, Phytoconstituents,pharmacological properties

INTRODUCTION

Desmodiumtriflorum (L.) DC., a member of the Fabaceae family, is commonly found in pastures, roadsides, and open lands in tropical and subtropical regions (Smith et al., 2020). Traditionally, it has been used in folk medicine to treat respiratory disorders, fever, diarrhea, and inflammatory conditions (Kumar & Sharma, 2019). With increasing scientific interest, numerous studies have validated its medicinal potential, revealing its bioactive constituents and pharmacological activities.

In many indigenous cultures, *D. triflorum* has been utilized for its medicinal properties. Traditional practitioners have employed various parts of the plant to treat ailments such as respiratory disorders, fever, diarrhea, dysentery, rheumatism, and wounds. The plant is also used in ethnoveterinary medicine, particularly for treating livestock ailments. Over the years, increasing scientific interest has led to the identification of various bioactive compounds within *D. triflorum*, which may contribute to its therapeutic potential. Phytochemical investigations have revealed the presence of flavonoids, alkaloids, tannins, terpenoids, and saponins, suggesting a broad spectrum of pharmacological activities.

Recent research has demonstrated that *D. triflorum* possesses significant anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and analgesic properties. These findings align with its traditional uses and indicate its potential for pharmaceutical applications. Studies have also highlighted its role in modulating immune responses and promoting gastrointestinal health. Furthermore, *D. triflorum* has been explored for its potential in managing metabolic disorders such as diabetes and hyperlipidemia, making it a subject of interest in modern pharmacological research.

Apart from its medicinal uses, *Desmodiumtriflorum* plays a crucial role in agriculture and ecological sustainability. It serves as an important forage plant for livestock due to its nitrogen-fixing ability, which enhances soil fertility and supports pasture productivity. The plant's creeping growth habit and dense foliage make it useful for erosion control and ground cover, preventing soil degradation in vulnerable areas. Moreover, its ability to grow in nutrient-deficient soils contributes to land restoration efforts in degraded ecosystems.

Despite its widespread distribution and ethnobotanical significance, *D. triflorum* remains underexplored in terms of its full pharmacological and therapeutic potential. There is a need for more comprehensive phytochemical and pharmacological studies to validate its traditional uses and discover new bioactive compounds that may be beneficial in modern medicine. Additionally, further research on its ecological benefits and sustainable utilization can contribute to its conservation and commercial applications.

Botanical Description and Distribution

Desmodiumtriflorum is a small, creeping, perennial herb with trifoliate leaves, pink to purple flowers, and slender, branched stems (Jones & Miller, 2021). It thrives in warm climates with well-drained soils and is often found in disturbed lands. The plant's ability to fix nitrogen contributes to soil fertility, making it ecologically significant (Lee et al., 2020).

Phytochemical Composition

Phytochemical analysis of *Desmodiumtriflorum* has identified flavonoids, alkaloids, terpenoids, tannins, and saponins as key bioactive compounds (Wang et al., 2021). Among these, flavonoids such as quercetin and kaempferol exhibit antioxidant and anti-inflammatory properties (Chen et al., 2020). Alkaloids contribute to antimicrobial activity, while saponins have been reported to possess immune-boosting effects (Patel & Gupta, 2019).

Traditional and Ethnobotanical Uses

In traditional medicine, *Desmodiumtriflorum* has been employed to treat various ailments. In Ayurveda and traditional Chinese medicine, it is used to alleviate respiratory ailments, digestive disorders, and skin infections (Gopal et al., 2018). Indigenous communities in Southeast Asia utilize the plant for wound healing and as an anthelmintic (Singh et al., 2021). Decoctions and poultices made from its leaves are applied for pain relief and inflammation reduction (Dutta& Roy, 2020).

Pharmacological Properties

Antimicrobial Activity

Desmodium triflorum exhibits broad-spectrum antimicrobial activity against bacteria and fungi. Methanolic extracts have demonstrated significant inhibition of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (Rahman et al., 2022). This suggests potential applications in developing natural antimicrobial agents.

Anti-inflammatory and Analgesic Effects

Studies indicate that *Desmodiumtrif*lorum possesses anti-inflammatory and analgesic properties. Flavonoids and terpenoids contribute to its ability to suppress inflammatory cytokines, reducing pain and swelling in experimental models (Nair & Thomas, 2021).

Antioxidant Properties

Antioxidants play a crucial role in preventing oxidative stress-related disorders. Ethanolic extracts of *Desmodiumtriflor*um exhibit high free radical scavenging activity, attributed to its flavonoid and phenolic content (Chakraborty& Banerjee, 2019). These findings suggest its potential in combating oxidative stress-related diseases.

Hepatoprotective Potential

Preclinical studies have demonstrated that *Desmodiumtriflorum*extracts protect liver cells against toxic damage induced by carbon tetrachloride and acetaminophen. The hepatoprotective effects are linked to its antioxidant and anti-inflammatory properties (Mehta et al., 2020).

Antidiabetic Activity

Recent investigations indicate that *Desmodiumtriflorum* exhibits hypoglycemic effects by enhancing insulin secretion and glucose uptake in diabetic animal models (Prasad et al., 2021). This supports its traditional use in managing diabetes.

Toxicological Considerations

Toxicity studies on *Desmodiumtriflorum* have reported minimal adverse effects at therapeutic doses. However, excessive consumption may cause gastrointestinal discomfort in some individuals (Sharma et al., 2021). Further toxicological evaluations are required to establish its safety profile.

Future Perspectives and Research Directions

Despite promising pharmacological activities, clinical trials on *Desmodiumtriflorum* remain limited. Future research should focus on isolating and characterizing its bioactive compounds, elucidating mechanisms of action, and assessing its clinical efficacy (Kumar et al., 2022). Additionally, its potential for drug development warrants further exploration.

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PHYSICO-CHEMICAL CHARACTERIZATION AND IDENTIFICATION OF MICROBES IN CHEMICAL FACTORY EFFLUENT

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ABSTRACT

An investigation was carried out to assess the impact of chemical factory effluent on the microbial diversity viz. bacteria, fungi and cyanobacteria. Results of one year ecological study revealed that altogether 7 species of bacteria, 9 species of fungi and 15 species of cyanobacteria were isolated from the effluent stream. Among bacteria, *Pseudomonas* with three species and others with single each were recorded. *Aspergillus* was dominant among fungi with four species followed by *Penicillium* with two. Cyanobacteria one of the dominant group of algae, inhabiting all kinds of water (effluents), recorded 15 species. *Oscillatoria* with 8 species was the dominant genus followed by *Phormidium* (3), *Plectonema* (2), *Aphanocapsa* and *Chlorogloea* with single each. Higher amounts of phosphates and nitrates with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and slightly alkaline pH were probably the factors favouring the growth of microbes especially cyanobacteria. The utilization of dominant species of cyanobacteria to monitor pollution in chemical effluent has been discussed.

Key words: Cyanobacteria, bacteria, fungi, Physiochemical, effluent

INTRODUCTION

A major environmental problem facing the chemical dyeing and manufacturing industry is that the industry produces large volumes of high strength aqueous waste continuously. The discharge of wastewater containing recalcitrant residues into rivers and lakes lead to higher biological oxygen demand (BOD) causing serious threat to native aquatic life. Discharge of urban, industrial and agricultural wastes has increased the quantum of various chemicals that enter the receiving waters, which alter considerably their physicochemical characteristics and thereby increasing eutrophication. Since effluents are rich in nutrients due to loading of organic wastes, they afford ideal habitats for different microorganisms including algae, fungi and bacteria. A variety of algae are growing in these habitats. Algal growth in these habitats significantly influences the ecosystem (Sladeckova, 1962). Some algal forms can be useful as indicators on which major water management practices, pollution studies and water quality analysis can be carried out (Palmer, 1969; Schubert, 1984). The importance of algal dynamics, particularly their response to environmental changes and nutritional fluctuation have been suggested in several studies (Frempong, 1981; Tilman *et al.*, 1982; Sudhakar *et al.*, 1991). In recent years blue green algae have been drawing tremendous attention because of their ability to treat wastewater and thereby improving water quality. Taking the above facts into consideration, a survey was undertaken in chemical factory effluent to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria in order to exploit cyanobacteria as a tool in treating chemical factory effluent.

MATERIALS AND METHODS

Chemical effluent was collected from Chemical Factory at Vadasery, Tiruvarur, Tamil Nadu, India. A sampling programme consisting of series of monthly water quality and microbial survey was conducted for a one-year. Population of bacteria and fungi were isolated from the effluent samples by serial dilution technique. Bacteria were identified based on colony

characteristics, Gram staining methods and by various biochemical studies as given by Bergey's (1984) Manual of Determinative Bacteriology. Fungi were identified by using standard manuals, such as Mannual of Soil Fungi (Gillman, 1957), Dematiaceous Hyphomycetes (Ellis, 1971). Effluent samples were collected in duplicate from station in pre-sterilized bottles. Samples were collected from the places along with effluents in polythene bags. Standard microbiological methods were followed for the isolation and identification of cyanobacteria (Desikachary, 1959). Physico-chemical characteristics of effluent were done according to the Standard Methods (APHA, 1995). Temperature and pH of the effluent were measured at the station itself.

RESULTS AND DISCUSSION

The results of physico-chemical characteristics of effluent are presented in Table 1. The effluent was slightly alkaline and contained high amounts of nutrients like nitrate, nitrite and ammonia; total, inorganic and organic phosphate and calcium in all the seasons examined. Values of DO were very low indicating highly obnoxious conditions. Though BOD and COD levels were high as per IS Standards, their levels were not so much high as compared to other types of effluents. In general, the characteristics of effluent tested in .all the three seasons were not varied much. Most of the parameters tested were slightly higher in summer than in winter and rainy seasons.

In all, seven species of bacteria were isolated and identified from the chemical industry effluent (Table 2). All the species were recorded in all the seasons. There was not been much work regarding the isolation and identification of bacteria from chemical industry and other related effluent samples. Jain *et al.* (2001) isolated three different bacterial strains from the distillery sludge to treat predigested distillery wastewater. In the present study totally, nine species of fungi belonging to five different genera were isolated from the effluent (Table 3). Among the fungal genera, *Aspergillus*was recorded as the dominant genus with four species followed by *Penicillium*with two species and the rest of the genera with single species each. Species of *Curvularia* and *Verticillium* were recorded only during summer. Similarly *Neurospora crussa* was not observed during rainy season. Kousar *et al.* (2000) isolated 23 species of fungi from dye effluent polluted habitat with *Aspergillus*as the dominant genus.

Altogether 15 species of cyanobacteria belonging to five genera were collected from the effluent stream (Table 4). Among the genera, *Oscillatoria* dominated the effluent with 8 species. *O. acuminata* and*O. pseudogeminata* were not recorded in winter. Similarly *O. terebriformis* was not observed in rainy season. The rest of the species of *Oscillatoria* were noted in all the seasons. *Phormidium* with three species were reported in all the seasons. Unicellular form such as *Aphanocapsa pulchra* was also isolated in all the seasons whereas *Chlorogloea fritschii* was reported in winter and rainy seasons but not in summer. Many publications emphasize the importance of light, temperature, pH, carbon dioxide, organic matter, alkalinity, nitrates and phosphates as factors important in determining the distribution of cyanobacteria (Singh, 1960; Philipose, 1960; Venkateswaralu, 1969, Munawar, 1970). In the present study, as a whole, conditions in the effluent appeared to be favourable for the cyanophycean members. The effluent had high oxidizable organic matter, nutrients such as nitrates and phosphates with high calcium content. Observations of Munawar (1970) suggest that Cyanophyceae grow luxuriantly with great variety and abundance in ponds rich in calcium. The present data also shows the calcium is possibly one of the factors for the growth of cyanophycear members. Dense cyanobacterial population was observed during summer in all the sites under study when the oxygen content was very low (Table 1). Similar observations were made by Rao (1953) and Venkateswarlu (1969b). They noted that oxygen deficiency favoured cyanobacterial growth. Dominant and persistent

occurrence of most of the species of *Oscillatoria* and *Phormidium* indicate their capacity to thrive in the type of man-made habitat. Moreover these findings show that there are certain species of cyanobacteria which are tolerant to organic pollution and resist environmental stress caused by the pollutant.

Table 1. Characteristics of effluent for the period from April 2007 to March 2008

S.No.	Parameters	Summer	Winter	Rainy
1.	Temperature	19.86 ± 1.31	16.30 ± 0.49	19.64 ± 0.72
2.	pH	7.54 ± 0.21	7.40 ± 0.23	7.38 ± 0.18
3.	Total solids	1062.50 ± 130.25	1033.33 ± 250.33	1137.50 ± 219.98
4.	Total dissolved solids	450 ± 119.52	400 ± 154.92	437.50 ± 130.25
5.	Free carbon-di-oxide	20 ± 3.55	20.33 ± 6.62	13.50 ± 6.82
6.	Carbonate	0.00 ± 0.00	0.00 ± 0.00	3.00 ± 0.49
7.	Bicarbonate	95.50 ± 10.89	98.33 ± 18.95	96.75 ± 10.36
8.	Nitrate	107.13 ± 19.49	104.33 ± 43.90	104.88 ± 19.80
9.	Nitrite	88.50 ± 12.20	81.50 ± 10.27	79.88 ± 15.68
10.	Ammonia	45.50 ± 4.34	48.83 ± 4.02	48.75 ± 6.04
11.	Total phosphate	77.25 ± 7.55	71.17 ± 4.96	80.13 ± 5.38
12.	Inorganic phosphate	38.25 ± 3.49	38.17 ± 3.60	38.13 ± 3.48
13.	Organic phosphate	40.25 ± 7.19	33.00 ± 5.33	42.00 ± 6.89
14.	Total hardness	189 ± 13.98	167.33 ± 17.37	187.25 ± 11.26
15.	Calcium	55.17 ± 4.79	52.45 ± 4.37	55.30 ± 7.81
16.	Magnesium	32.64 ± 2.72	28.03 ± 3.31	32.19 ± 1.65
17.	Chloride	31.02 ± 3.38	30.36 ± 5.35	30.53 ± 3.66
18.	Dissolved oxygen	2.09 ± 0.76	2.90 ± 0.55	2.26 ± 0.71
19.	BOD	161.25 ± 38.71	105 ± 35.07	108.63 ± 38.67
20.	COD	400.00 ± 37.03	463.33 ± 29.44	350.00 ± 38.54

Except pH and temperature, all values are expressed in mg/l.

Summer (April-July), Rainy (August -November), Winter (December - March)

Table 2. Seasonal variations of bacterial flora in chemical effluent

S.No.	Name of bacteria	Summer	Winter	Rainy
1.	**Escherichia coli**	+	+	+
2.	*Enterobacter aerogens*	+	+	+
3.	*Lactobacillus* sp.	+	+	+
4.	*Pseudomonas aeruginosa*	+	+	+
5.	*P. fluorescens*	+	+	+
6.	*Salmonella* sp.	+	+	+
7.	*Shigella sonnei*	+	+	+

Table 3. Seasonal variations of fungal flora in chemical effluent

S.No.	Name of fungi	Summer	Winter	Rainy
1.	**Aspergillus flavus**	++++	+++	+++
2.	*A. fumigatus*	++	++	+
3.	*A. luchensis*	++	+	+++
4.	*A. niger*	++++	++++	++++
5.	*Curvularia* sp.	+	-	-
6.	*Penicillium sp*	+++	++	++
7.	*P. javanicum*	++	+	+
8.	*Trichoderma viride*	+++	++	+++
9.	*Verticillium* sp.	+	-	-

Table 4. Seasonal variations of cyanobacterial flora in chemical effluent

S.No.	Name of cyanobacteria	Summer	Winter	Rainy
1.	*Aphanocapsa pulchra*	+++	++	+
2.	*Chlorogloea fritschii*	-	+	+++
3.	*Oscillatoria acuminata*	+++	-	+++
4.	*O. animalis*	++++	++++	++++
5.	*O. brevis*	+++	+	++++
6	*O. curviceps*	++	+++	+++
7	*O. guttulata*	+	++	++
8	*O. pseudogeminata*	++++	-	+
9	*O. terebriformis*	+++	++	-
10	*O. willei*	+	+++	+
11	*Phormidium tenue*	+++	+	++++
12	*P. incrustatum*	++++	+++	+
13	*P. submembranaceum*	+++	++	+
14	*Plectonema* sp.	+	++++	-
15	*Plectonema* sp.	+	++++	-

Note :

++++ : Observed in all the four months ; +++ : Observed in three months only
+++ : Observed in two months only ; + : Observed in one month only
- : Not observed

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BIOCHEMICAL CHARACTERISTICS OF *AMARANTHS DUBIUS*IN THE METAL CONTAMINATED SOIL

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ABSTRACT

Phytoremediation uses plants to clean up pollution in the environment. Plants can help clean up many kinds of pollution including metals, pesticides, explosives, and oil. The plants also help prevent wind, rain, and groundwater from carrying pollutants away from sites to other areas. In the present study focused on the growth and biochemical characteristics of metal exposed *Amaranths dubius.* The result of the present study, the dry (7.85 g) and fresh weight (12.73 g) of these plants were decreased as compared to control (without metal mix) 9.59 g and 17.86 g respectively. Further investigation, *Amaranths dubius* showed the maximum of total chlorophyll of 0.536 (mg/g-1) in control and followed by 0.487 (mg/g-1) in after treatment of heavy metal contaminated soil. Total chlorophyll content declined progressively with increasing concentrations of the heavy metal. The protein and lipid content were reduced and phenol content was increased the heavy metal exposed *Amaranths dubius.* The mineral analysis like, Fe (34.3 mg/100g), Zn (3.36 mg/100g), Ca (292.3 mg/100g), K (346.3 mg/100g) and Mg (64.23 mg/100g) content were present higher in the metal exposed *Amaranths dubius*when it compared to control.
Key words:*Amaranths dubius,* Phytoremediation, Chlorophyll, Protein and Heavy metal.

INTRODUCTION

The contamination of soil by heavy metals is one of the most serious environmental problems and has significant implications for human health. Some industrial activities and agricultural practices increase their level in the substrate, and the possible introduction of the elements in the food chain is an increasing human health concern (Cakmak*et al.*,2000). Phytoremediation is a process, which uses green plants for reduction or removal of contaminants from contaminated soil, water, sediments and air. Specially selected or engineered plants are used for this process. The health hazards associated with soil contamination with trace elements having toxic effects together with high cost of removal and replacement of polluted soil have prompted to develop alternative and cheaper technologies to recover the degraded land. Current research in this area includes plants to remediate polluted soils and to facilitate improvement of soil structure and this innovative technique is known as phytoremediation.

Plant-based technologies are applicable for removing metals from areas of low concentrations with shallow soils and water, although longer treatment times may be required. Use of plants that have constitutive and adaptive mechanisms for tolerating or accumulating high metal contents in their rhizosphere and tissues, is the emerging *in situ* remediation technology employed in the cleanup of soils, sediments and water that have been polluted by organics, salinity and heavy metals (Qadir*et al.*, 2003) or stabilize various organic and inorganic pollutants present in soils, muds or wastewaters (Otte and Jacob 2006).Preliminary surveys of domesticated plants species as candidates for phytoremediation resulted in identification of plants of the genus *Amaranthus*belonging to the family Amaranthaceae. It contains about 60 genera and more than 800 species of herbaceous plants and a few shrubs, trees, and vines, native to tropical India, America, central Asia and Africa. In the present study *Amaranths dubius*was chosen the heavy metal analysis from contaminated soil.

MATERIALS AND METHODS

Collection of seeds

The healthy *Amaranths dubius* seeds were collected from local market in Thanjavur, and brought to the laboratory under sterile condition.

Soil preparation

Top soils (5-10cm) were collected from Garden of Marudupandiyar College, Thanjavur, Tamil Nadu, India. The soils were thoroughly mixed by a mechanical mixer and passed through 4 mm sieve to remove fibre and non soil particulate in the sample (Spirochova*et al.*, 2003). To take two plastic pots were filled with 1 kg of soil samples in each that passed through a 4mm sieve. The first pot labelled as control because of doesn't add metal concentration. 5mg/kg of each Cu, Cd, Ni, Pb, Fe and Zn were added to second pot of sample. The soils in the pots were thoroughly mixed for even distribution of the contaminant and watered to field capacity. Three seeds of *Amaranths* were planted in each pot. Deionised water (300ml) was added twice a week during the first month. All the pots to place on 500 µmols of photo synthetically active radiation at the plant top with a 12:12 hr photoperiod at 22± 2° C for 30 days.

Growth characteristics of *Amaranths dubius*

After 30 days, the grown plants were removed carefully from the soil without any damage to the root. The lengths of the root, shoots were measured and fresh and dry weight was weighed (Navari-Izzo and Quartacci, 2001).

Biochemical characteristics of *Amaranths dubius*

Chlorophyll determination

The chlorophyll content of plant leaves was estimated by the method of Arnon, (1949). 100mg of leaf samples were ground with 80% of acetone followed by centrifugation at 5000 rpm for10 min. The supernatant was collected and its optical densities at 645 nm were measured using UV-visible spectrophotometer.

Estimation of total protein

500 mg of *Amaranths dubius*leaves were homogenized in 5 mL of phosphate buffer. The extract was centrifuged at 10000 rpm for 10 min and the supernatant was collected. The extract was precipitated by adding in equal volume of ice cold TCA. Then it was centrifuged at 12000 rpm for 10 min. The pellet was collected and dissolved in 2 mL of NaOH (Bradford 1976). To 1 mL of the sample and 5 mL of the reagent were added and mixed thoroughly. The absorbance was read at 595 nm against the reagentblank. The amount of total protein in the sample was determined using astandard graph prepared from bovine serum albumin ranging from 10to100 µg/mL.

Estimation of total lipids

The total lipid content extracted from the sample was determined by Folch*et al* (1957). Briefly, 500 mg of the Amaranths leaves were taken and homogenized with 6 mL of chloroform: methanol. It was then transferred to a separating funnel and the organic phase was separated. To this, 2 mL of physiological saline was added and mixed well. The mixture was left undisturbed for overnight and about 0.5 mL of the lower chloroform phase containing lipid was collected in test tubes. The solvent was then allowed to evaporate at room temperature and the pellet was collected. 0.5 mL of concentrated sulphuric acid was addedto the pellet and mixed well. The sample was closed and kept in a boiling water bath for10 min and allowed to cool at room temperature. The resulting sample wastaken and 5 mL of phosphovanillin reagent was added and

mixed well thanallowed to stand for 30 min and its measured at 520nm. Standard graph was prepared using cholesterol ranging from 10 to 100µg/mL.

Estimation of Phenol

Phenol content was calculated following folin-ciocalteau method (Slinkard and Singleton, 1977). The blue colour developed in solution was read at 650 nm against a blank reagent. The concentrations of phenols were expressed as mg phenol g-1 tissue.

Mineral Analysis

Mineral elements comprising calcium, potassium, magnesium, iron and zinc were determined following the method by Shahidi*et al* (1999) with minor modifications. Sample preparation was done through drying to ash and then mineral content in diluted acid was determined by Atomic Absorption Spectrophotometer (AAS) (Horwitz, 2000). Porcelain crucibles were pre-heated at 550°C for 2 hours and then put in a desiccator to cool down to room temperature. Approximately 2 g of each sample was weighed and subjected to drying ash in porcelain crucibles at 550°C for 2 hours after which samples were dissolved in 5.0 ml of HNO3/HCL/H2O in a 1:2:3 ratio and heated on hot plate till disappearance of brown fumes. Contents of the crucibles were filtered using Whatman No.1 filter paper into 100ml volumetric flasks. Solutions in 100 ml volumetric flasks were then filled to the mark and used for mineral analyses using Atomic Absorption Spectrophotometer (AAS). The standards, blank and sample solutions were then read on AAS at the following wavelengths: 248.3 nm for iron, 213.9 for zinc, 422.7 nm for calcium, 285.2 nm for magnesium and 766.5 nm for potassium.

RESULTS AND DISCUSSIONS

Growth characteristics

The dry and fresh weight content of *Amaranths dubius*was analysed after treatment in artificially metal contaminated soil. The dry (7.85 g) and fresh weight (12.73 g) of these plants weredecreased as compared to control (without metal mix) 9.59 g and 17.86 g respectively. Similarly,the shoot (21.6 cm) and root height (15.5 cm) were decreased when compared to control (27.5 and 18.7 cm) (Table.1).The results of the present study coincided with the report of Vilar*et al*., (2007) in plant *Thalaspicaerulescens*against Zn and Hg. The heavy metals penetrate the plant cells and contact with living protoplasm in plant. It inhibits great variety of biochemical reactions in plant and reduces the endogenous respiration and photosynthetic capacity. Inhibition of photosynthesis retards nitrogen stimulatory as a result plant growth was affected.

Biochemical characteristics

Amaranths dubius showed the maximum total chlorophyll of 0.536 (mg/g-1) in control and followed by 0.487 (mg/g-1) in after treatment of heavy metal contaminated soil (Table.2). Total chlorophyll content declined progressively with increasing concentrations of the heavy metal. Rao*et al.*, (1994) reported the relationship between the total chlorophyll content in heavy metal exposed and bacterial treated plants. Due to exposure of heavy metals the chlorophyll content was affected due to less photosynthetic activity. Many researchers have reported decreased chlorophyll in several different plant species under the impact of heavy metals (Chaurasia*et al.*,2012). Chlorophyll content is often measured in plants in order to assess the impact of environmental stress, as changes in pigment content are linked to visual symptoms of plant illness and photosynthetic productivity.

The protein content was increased in the control plants (10.21 g/100g) when compared to the metal exposed plants (8.37 (g/100g). Whereas, the metal contaminated soil was observed the maximum decline in leaf protein (Table.2).Plant protein synthesis in general has been known to be sensitive to heavy metals and the inhibition in the amount of protein can also be due to the inhibitory effects of spoil during the period of comparison (Davis, 1992). It seems that during

transport heavy metals act at different sites to inhibit a large number of enzymes having functional sulphydryl group. It results deleterious effects in the normal protein form (Yurela, 2005). The basic mechanism starts with formation of complexes between metals and bio molecules leading to conformational changes in nucleotides and polypeptides (Sharma and Talukder, 1989).

Phenol content (14.37 (mg/g-1) was increased the heavy metal exposed amaranths as compared to control plants (10.21 (mg/g-1) (Table.2).In the previous research *Jatrophacurcas* L increased phenolic content was visible under all heavy metal stress but *Phaseolus vulgaris* when exposed to Cd, reported to accumulate soluble and insoluble phenolics (Smeets*et al.*, 2005) and *Phyllantustenellus* leaves reported to have more phenolics than control plants.

The lipid content (5.8 g/100g) was significantly decreased in metal exposed amaranths species when it's compared to the control (7.0 g/100g). The heavy metals were induced the stress and also influence the reduction of lipid content in plants (Table.2). Previous published results indicated that copper and cadmium stress decreased the total lipids, phospholipids, glycolipids and sterols in pea (Quartacci*et al.*, 2000) pepper (Jemal*et al.*, 2000) and cucumber (Janicka*et al.*, 2008).

Mineral analysis

The Fe (34.3 mg/100g), Zn (3.36 mg/100g), Ca (292.3 mg/100g), K (346.3 mg/100g) and Mg (64.23 mg/100g) content were present higher in the metal exposed *Amaranths dubius*(Table.3). The control plant showed the slightly difference between the metal exposed plants. Among this, Potassium level was higher and followed by Calcium. The metal exposed plants were uptake the mineral elements and enhanced the plant physiology. An increase the accumulation of heavy metals is further linked to the mechanisms of plant physiology facilitating the uptake of elements under conditions of their deficiency in the soil. These compounds increased the biological assimilation of nutrient elements, unfortunately also increasing the bioavailability of heavy metals (Gherardi and Rengel 2004). In the previous study, the mineral contents were increased the biogas digested and mining waste soil when it's compared to the control soil. All the parameters were slightly increased with the increasing heavy metal concentrations (Rozylo*et al.*, 2017).

CONCLUSION

In the present study investigated that the inhibition in the plants growth noticed through reduction chlorophyll content, protein and lipid content in the heavy metal exposed *Amaranths dubius*. In the same plants the larger heavy metals concentrations were also acuminated in plant tissue. The heavy metals were induced the stress and also influence the reduction of biochemical content in plants. The significant reduction in shoot and root length in plant may be due to arrested growth under stressed conditions as water contains heavy metals. Further research is needed to investigate mechanisms involved in heavy metal degradation, various chemical aspects of metal accumulation, and transfer of metals to plants parts.

Table.1 Growth characteristics of *Amaranths dubius*in different experimental condition

S. No	Growth characteristics	Experimental conditions	
		Control Without metal mix	Heavy metals Exposed *A.dubius*
1	Dry weight (g)	9.59	7.85
2	Fresh weight (g)	17.86	12.73
3	Shoot length (cm)	27.5	21.6
4	Root length (cm)	18.7	15.5

Table.2Biochemical characteristics of *Amaranths dubius*in different experimental condition

S. No	Biochemical characteristics	Experimental conditions	
		Control Without metal mix	Heavy metals Exposed *A. dubius*
1	Total chlorophyll (mg/g-1)	0.536	0.487
2	Phenol (mg/g-1)	10.21	14.37
3	Protein (g/100 g)	17.2	15.3
4	Lipids (g/100 g)	7.0	5.8

Table.3Mineral content of *Amaranths dubius*in different experimental condition

S. No	Mineral content (mg/100g)	Experimental conditions	
		Control Without metal mix	Heavy metals Exposed *A.dubius*
1	Iron	23.5	34.3
2	Zinc	1.05	3.36
3	Calcium	227.4	292.3
4	Potassium	315.6	346.3
5	Magnesium	42.67	64.23

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COMPARATIVE PHYTOCHEMICAL PROFILING AND *IN-VITRO* ANTIHYPERGLYCEMIC EFFECTS OF *COLOCASIA ESCULENTA* AND *AMORPHOPHALLUS PAEONIIFOLIUS* PEEL EXTRACTS

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ABSTRACT

Diabetes mellitus is defined as increase of glucose level in blood, which is one among the autoimmune disorders. It has become a great threat to the people suffering from the kidney failure, kidney failure, nerve impairment, cardiovascular disease and loss of vision. In order to prevent the diabetes, medicinal plant extracts serve as the therapeutic supplements. In the current study, the corm peel of AP and CE were used. The phytochemicals of the corm peel of AP and CE were extracted using methanol by cold percolation method. Extract yield % for AP and CE was found to have 0.355g/7g and 0.35 g/7g. The qualitative analysis of phytochemicals was analyzed for the corm peel extracts of AP and CE in TLC plate. The R_f values for spot was similar in both. The antioxidant activity of CE showed good result than AP. The total flavonoid content for the corm peel extracts of CE was 1177.08 µg and AP was found to have 809.275 µg. The % of alkaloid for the corm peel of AP and CE extract possess 1.519 g/ 2g and 0.244 g/ 2g. Anti-diabetic activity (invitro) was carried out for the corm peel extracts of AP and CE was 31.523 % and 33.317 % of inhibition, when comparing with the positive control (26.24 %).

Keywords: Diabetes mellitus, *Amorphophallus paeoniifolius* (AP), *Colacasia esculenta* (CE), Thin layer chromatography (TLC), flavonoid, alkaloid,

INTRODUCTION

Diabetes' is a metabolic syndrome of multiple etiologies characterized by chronic hyperglycemia with abnormalities in carbohydrate, fat and protein metabolism due to defect in insulin secretions. Diabetes is associated with long term damage such as malfunction of eyes, kidneys, nerves, heart and blood vessels. It is associated with health complications including renal failure with risk of foot ulcers, including sexual dysfunction, heart disease, stroke and blindness.

Diabetes is epidemic underway. Due to sedentary habits, urbanization more peopleis suffering from diabetes. A decade later, the global burden of diabetes was estimated to be 135 million(Azubike, 2015). Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, the latest WHO estimate – for the number of people with diabetes, worldwide, in 2000 among adults ≥ 20 years of age was estimated to be ~ 171 million (Wild *et al*., 2004). This is likely to increase to at least 366 million by 2030 where the global cost of treating diabetes and its complication could reach US $ 1 trillion annually. Plant derivatives with anti-diabetic potentials have been used in traditional healing systems around the world (Yeh*et al*., 2003).

Amorphophallus paeoniifolius(Dennst.) Nicolson (family - Araceae) or Elephant foot yam is a crop of South East Asian origin(Thomas *et al*.,2005). In India, it is commonly known as suran or jimikand. The tuber of this plant has high medicinal value and consumed by many people as food. It is an important constituent of many Ayurvedic preparations. The phytoconstituents present in the corms are quercetin, rutin, sitosterol, etc (Sharstry*et al*., 2010). A

watersoluble polysaccharide containing galactose, glucose, 4-O-acylmethyl galacturonate, and arabinose was isolated from the aqueous extract of tuber (Das *et al*., 2009).

Colocasia esculenta (CE) Linn. (Family: Araceae) is an annual herbaceous plant witha long history of usage in traditional medicine in several countries across the world, especially in the tropical and subtropical regions (Holt, 2004). The herb has been known sinceancient times for its curative properties and has been utilized for treatment of variousailments such as asthma, arthritis, diarrhea, internal hemorrhage, neurologicaldisorders, and skin disorders. The juice of CE corm is widely used for treatment ofbody ache and baldness. A wide range of chemical compounds including flavonoids,β-sitosterol, and steroids have been isolated from this species. Extracts from this planthave been found to possess various pharmacological activities (Linderberg *et al*., 2004). This contributionprovides a comprehensive review of its ethnomedical uses, chemical constituents, andthe pharmacological profi le as a medicinal plant. Particular attention has been givento analgesic, anti-inflammatory, anti-cancer, and hypolipidemic effects presented inthis review in order to evaluate the potential use of this plant in pharmaceuticals (Rakesh P *et al.,* 2011).Thus,objective of the present study is to compare the corm peel of *Colocasia esculenta* and *Amorphophallus paeoniifolius*for its evaluation of anti-diabetic activity (invitro)

MATERIALS AND METHODS

Sample Collection

Amorphophallus paeoniifolius **(AP)(Elephant foot yam) and** ***Colocasia esculenta*** **(CE)(Taro root)** were purchased from the local market, Puducherry. The corm peel of the AP and CE was taken and dried in hot air oven at 40°C, to remove the moisture. Further, both the corm peel of AP and CE were powdered using blender. These corm peel powder of AP and CE were used for the following experimentations. The extraction was carried out by hot percolation method. The solvent used was methanol. About 7 gm of powder was extracted with 90 ml of methanol. The extract was concentrated to dryness under controlled temperature 40-60°C.

TLC analysis for the extracts using phytochemicals standards

TLC silica gel plates of 60 F_{254}Aluminium sheets (20 x 20 cm) were used for extracting phytochemicals from the corm peel extracts of AP and CE. Chloroform: Methanol was used as a mobile phase. A space of 1cm between each spotted sample was retained to spot the sample. The peel extracts of AP and CE were allowed to run. At the end of the run, the TLC plate was viewed under UV chamber and dipped in the solution of vanillin-sulphuric acid R_f value was calculated. The samples were compared with different phytochemical standards standards using Chloroform, ethyl acetate, formic acid (5:4:1) as mobile phase.

R_f = Distance travel by the sample/Distance travel by the solvent

Antioxidant assay by TLC analysis for the extracts using DPPH:

The peel extracts of AP and CE were spotted and eluted in mobile phase using Chloroform: Methanol. The spots obtained were sprayed with DPPH reagent. The fade yellowish spots with purple background was considered as antioxidant compounds. The R_f were calculated.

Quantitative determination of anti- diabetic effective compounds:

Flavonoid

To a series of test tubes, respective volumes of standard and the corm peel extracts of AP and CE sample was added. Ethanol of 1.5mlwas added to all the tubes and mixed well. 1ml of 10 % Aluminium chloride was added and followed by 0.1ml of 1M sodium acetate. The volume up

to 2.8ml was diluted with distilled water and mixed well. The tubes were wrapped with foil and the tubes were incubated at room temperature at 30 minutes. The absorbance was read at 415nm and the quantity of total flavonoid was determinedwith mg equivalent of quercetin using standard graph.

Dried sample of corm peel (powdered) (2g) was dispensed into 20ml of 10% acetic acid solution.The mixture was shaken well and allowed to stand for 4 hours at room temperature.The solution was filtered using filter paper and the filtrate was collected.The filtrate was evaporated to one quarter of its original volume by keeping it in a water bath.Ammonium hydroxide was added in drop wise to precipitate the alkaloids.The precipitate was filtered using a pre weighed filter paper.It was washed with 1% ammonium hydroxide solution.The precipitate was dried (i.e., along with filter paper) in oven for 30 minutes at 60°C. The dried precipitate was weighed again.The weight of the alkaloid was determined by weight difference of the filter paper and expressed as a percentage of the sample weight analyzed

Invitro methodology to determine the anti-diabetic activity for the extract
Non-enzymatic glycosylation of haemoglobin assay

Antidiabetic activity of corm peel extract of C E and AP were studied by quantitatively by the degree of non-enzymatic haemoglobin glycosylationmeasured colorimetrically at 520nm. Glucose (2%), haemoglobin (0.06%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4. 1 ml each of above solution was mixed. The corm peel extract of 100 µl and 150 µl of AP and CE were added to the respective test tubes. The mixture was incubated in dark at room temperature for 72 hrs. The degree of glycosylation of haemoglobin was measured colorimetrically at 520nm. Alpha-Tocopherol (Trolax) was used as a standard drug for assay.

RESULTS

. The Extract yield % for both the corm peel extracts of AP and CE was found to have 0.355 g/7 g, which was higher than the corm peel extract of CE with 0.35g/7g.The corm peel extracts of AP and CE was estimated for flavonoid and alkaloid. Flavonoid content was higher for the corm peel extract of CE with 1177.08 µg and lower for the corm peel extract of AP with 809.275 µg. The % of alkaloid was evaluated for the corm peel extract of AP with 1.519 g/ 2g (highest) and the corm peel extract of CE with 0.244 g/ 2g (lowest). Anti-diabetic activity (invitro) was compared for both the corm peel extracts of AP and CE. When comparing the corm peel extracts of AP and CE, α- tocopherol (standard) results with the lowest % of inhibition.

Table-1: Rf value of AP & CE in Chloroform: Methanol (8:2, v/v) in TLC

The qualitative analysis of phytochemicals was analyzed for the corm peel extracts of CE and AP in TLC. The R_f values of spot was similar in both. The antioxidant activity for the corm peel extracts of CE showed good results than AP.

Sample name	Spot	Rf value	Visualization
CE	Spot1 Spot2	0.70 0.55	UV (254nm)
AP	Spot1 Spot2	0.96 (yellow) 0.62	UV (254nm)

Table-2: Rf values of Antioxidant capacity compounds analysed by TLC using DPPH

Sample Name	Spot	Rf value	Visualization
CE	Spot1 Spot2	0.80 0.71	DPPH Reagent

	Spot3 Spot4 Spot5	0.61 0.38 0.09	
AP	Spot1 Spot2 Spot3	0.67 0.44 0.09	DPPH Reagent

The total flavonoid content for the corm peel extracts of AP and CE was found to have 809.275 µg (minimum) and 1177.084 µg (maximum)

Table-3: OD values and Inhibition % for the corm peel of AP and CE extracts

The corm peel extracts of CE was found to have higher % of inhibition with 33.317% and AP was found to have lower % of inhibition with 31.523%.

Samples	OD(520 nm)	% of Inhibition
Control	1.425	-
PC T1	1.932	26.24%
PC T2	1.209	-
APT1	1.298	-
AP T2	2.081	31.523%
CE TI	1.309	-
CE T2	2.137	33.317%

DISCUSSION

Generally, the corm of AP and CE is consumed by many people as food for its high medicinal importance.These types of corms consist of more phytoconstituents, which gain in health benefits. But its peel, which is thrown as waste were not taken as edible. Even these corm peels has the capacity to cure many diseases for its bioactive compounds. In this study, the corm peel of AP and CE were compared for its invitro anti-diabetic activity.

The present study was performed by the corm peel of AP and CE. These corm peels were dried and powdered by the help of the blender. To extract the phytochemicals, the corm peel powder of 7 g of each of AP and CE were followed by cold percolation method by using methanol as a solvent for 6hrs at 40°C. Then both the methanolic corm peel extracts of AP and CE were filtered to collect the filtrate. Further the filtrates of AP and CE were dried in hot air oven at 60°C. When the samples of AP and CE were at viscous state, it was transferred to the desiccator. Finally the extractyield % was calculated. The yield % for the corm peel of AP extract was 0.355 g/ 7 g (maximum) than the corm peel extract of CE, which was found to have 0.35 g/ 7 g (minimum).Thin layer chromatography is a very cost effective method. The qualitative analysis of phytochemicals wereanalysed for CE and AP, the Rf values of spot was similar in both. Comparatively CE contains more antioxidant capacity compounds than AP.

The total flavonoid content was found for both the corm peel extracts of AP and CE. The highest flavonoid content for the corm peel extract of CE was 1177.08 µg. The lowest flavonoid content was found in the corm peel extract of AP, which results in 809.275 µg. The % of alkaloid was increased for the corm peel of AP extract with 1.519 g/ 2g and the reduced % of alkaloid for

the corm peel of CE extract was found to have 0.244 g/ 2g. Anti-diabetic activity (invitro) was carried out for the corm peel of AP and CE extracts.Compare to the positive control (α-tocopherol) (26%), the corm peel of AP and CE extracts was found to have higher % of inhibition with 31.523% and 33.317 %. From this, we can conclude that, the corm peel of CE revealed higher % of inhibition than the AP extracts.

CONCLUSION

Thus the corm of AP and CE was already proven for its remedial and digestive properties. The current studies verified that the corm peel of AP and CE was revealed for its phytochemicals, which may serve as a therapeutic and nutraceutical supplement for diabetics.In future studies, the antioxidant studies for the corm peel extracts of AP and CE will be carried out. The antioxidant compound will be isolated and separated by column chromatography.

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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *KIGELIA PINNATA*

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ABSTRACT

World is rich in knowledge with a wide diversity of medicinal plants which provide people with traditional healing methods for diseases in general healthcare area. The agar diffusion method was used to examine the antibacterial activity of different solvent extracts of *kigelia pinnata*tested against *Staphylococcus aureus, Bacillus subtilis,Streptococcus pyogens Pseudomonas aeruginosa* and *Klebsiella pneumonia*, and also tested fungal species like Aspergillus niger, *Trichoderma Viride* and *Candida albicans* For qualitative phytochemical investigation, several solvent extracts of the complete *k. pinnata*plant were investigated. The *minimum* inhibitory concentration of *k. pinnata*ethanol extract had significant antimicrobial effectiveness against Staphylococcus *aureus* (19 mm). The ethanolic extract antibacterial activity against *Klebsiella pneumonia* (12 mm)and *Streptococcus pyogens* (11 mm) respectively. The ethanolic extract of *K. pinnata* antifungal activity maximum against *Candida albicans* (19) and *Aspergillus niger*(11 mm)Various parts of the plant has the presence of variety of phytoconstituents such as flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin.

Key words:*kigelia pinnata*, antimicrobial, agar diffusion method, Phytochemical analysis

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural source, many based on their use in traditional medicine. Over 50% of all modern clinical drugs one of natural product origin[1] and natural product play an important role in drug development. Programs in the pharmaceutical industry[2]. Various medicinal plants have been used for years in daily life to treat disease all over the world. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[3].

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effect of plant extract on bacteria have been studied by a very large number of researchers in different parts of the world[4]. Much work has been done on ethanomedical plants in India[5]. Interest in a large number of traditional natural products has been increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents[6]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[7].

K.africana (Lam) Benth, *(K.pinnata)* belongs to the family Bignoniaceae. Its common names include sausage tree (Eng.); worsboom (Afr,); um vunguta, umfongothi (zulu); modukguhlu (North Sotho); muvevha (Venda)[8,9] saucissonnier; Faux baobab (Fr) mvungunya, mwegea, mwicha, mranaa (Sw)[10]. It is a tree growing up to 20m tall or more. The bark is grey and smooth at first, peeling on older trees. The bark it can be as thick as 6mm on a 15cm branch.

The wood is pale brown or yellowish, undifferentiated and not prone to cracking[11]. The tree is evergreen where rainfull occurs throughout the year, but deciduous where there is a long dry season. The tree is leaves are opposite or in whorls of three, 30 – 50 cm long, pinnate, with six to ten oval leaflets up to 20 cm long and 6 cm broad; the terminal leaflet can be ether present or absent. The flowers (and later the fruit) hang down from branches on long flexible stems (2-6 m long).

Flowers are produced in panicles; they are bell shaped (Similar to those of the African tulip tree but darker and more waxy), orange to reddish or purplish green and about 10 cm wide. Individual flowers do not hang down but one oriented horizon - tally[12] some birds one attracted to these flowers and the strong stems of each flower make idea footholds. Their scent is most notable at night indicating their reliance on pollination by bats, which visit them for pollen and nectar. Flowers are bisexual, very large; pedicel up to 11(-13.5) cm long up curved at tip; calyx shortly tubular to campanulate, 2-45 cm long, suddenly widening and incurving upwards, limp 2-lipped, with the super or lip 2-lobed, the lower one 3-lobed and recurved[10].

The fruit is a woody berry from 30-10cm long and up to 18 cm broad; weighs between 51- Kg hangs down on a long rope-like peducles[12]. The fruit is indehiscent, with woody wall and heavily marked with lenticels at the surface. It is grey-brown and many seeded when matured. Seeds one abovoid, co. 10mm x 7 mm with leathery testa, embedded in a fibrous pulp[10]. The fruit is eaten by several species of mammals, including Baboons, bush pigs, savannah Elephants, Giraffes, Hippopotami, monkeys and porcupines. The seeds are dispersed in their dung. The seeds one also eaten by brown parrots and Brown – headed parrots, and the foliage by elephants and Greater kudu[11,12,13].The tree is found on riverbanks, along streams and on floodplains, also in open woodland, from kwazulu-Natal to Tanzania. The plant is widely distributed in the south, central and west Africa[14].

MATERIALS AND METHODS

Collection of plants

K.pinnata belongs to the family Bignoniaceae was collected from Thanjavur district, Tamilnadu State, India and identified by the special key given Gamble flora of the presidency of madras[15].Voucher sample was prepared and deposited in the Department of Bio-Chemistry, Maruthupandiyar College, Thanjavur.

Preparation of leaf powder

The leaves of *Kigelia pinnata* was washed with sterile distilled water. After, the leaves were shade dried and powdered by using mortar and pestle.

Preparation of extract

25g of powder was filled in the thimble and extracted successively with ethanol using a soxhlet extractor for 12 hrs[16]. The extract was concentrated using rotary flash evaporator and preserved at 5º in airtight bottle until further use. The extract was subjected to phytochemical analysis and antimicrobial activity assay.

Phytochemical analysis

A small portion of the dry extract used for phytochemical screening test[17,18]. Dragendorffs reagents were used to test for alkaloids, ferric chloride for tannins, while benedict's solution was used to test for saponins.

Test Organisms

The following organisms were employed for this study as test organisms:

Bacteria

Staphylococcus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas auroginosa and *Klebsiella preumonia.*

Fungi

Aspergillus niger, Trichoderma viride and Candida albicans.

Pure cultures of these organisms were obtained from Sea Horse Hospital, Trichy and A.V.V.M. Sri Pushpam College culture collection center, Poondi. Then they were sub cultured and maintained in a laboratory for further use.

Antimicrobial activity[19]

Antimicrobial activity was conducted against *staphylococcus aureus, Bacillus subtilis, streptococcus pyogenes, pseudomonas aeroginosa, klebsiella pnemoniae* and *three fungus, Aspergillus niger, Trichoderma viride and Candida alblcans.* Sterile nutrient ager (NA) medium (peptone 5g; Beef extract 3g; Nacl 2g and Agar 15g/litre; pH7) and Potato Dextrose Agar (PDA) medium (200g potato extract, Dextrose 20g, Agar 16g/ (litre; pH6) were used as basal media for growing these pathogenic bacteria and fungus respectively, Inoculums of the pathogen for the ssay were prepared in liquid media of the respective composition. One ml of the broth inoculums was mixed with medium poured into the petri plates and allowed for solidification. After solidification 6mm diameter duplicates well was made with the help of a sterile cork borer in the medium. In each well 100ml of the filtrate was poured. All the plates were incubated at room temperature and the zone of inhibition was recorded. For bacteria, the plates were incubated for 24 hours and fungi 48 hours. Solvents used for extraction served as control.

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical analysis of the extract revealed that the presence of alkaloids, carbohydrates and glycosides, phytosterols, Fixed oils and fats, Phenolic compounds and tannins, favonoids, proteins and amino acid and absence of saponin,gums and mucilage and volatile oils in ethanolic extracts of *K.Pinnata* (Table-I). Further Phytochemical analysis of ethanolic extract of the plant[20] revealed that the antimicrobial activity is due to the presence of phenolic compounds.

The Phytochemical analysis of the *K.Pinnata* extracts showed the presence of tannins, alkaloids, flavonoids and Phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis[21]. This activity was exhibited against test organisms with the plant extract.

Antimicrobial assay

Ethanolic extract was tested against bacteria and fungi. The leaf extract of *K.Pinnata* was effective against bacteria and fungi (Table: 2). A part from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect. Medicinally, this is important for the treatment of inflamed or ulcerated tissues[22]. Tannins have important role such as stable and potent antioxidants[23]. Herbs that have tannins as their main component are astringent in nature and used for treating intestinal disorders such as diarrhoea and dysentery[24], thus exhibiting antimicrobial activity. One of the largest group of chemical produced by plant are the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications.

K.Pinnata one used for the treatment of inflammation, wound healing, antitumor and antianelgesic, hence different formulations could be prepared for clinical trials. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural orgin. It is hoped that this report will serve as a basis of information fortune project to be embark on in order to evaluate the potentials of *K.Pinnata* as a strong medicinal plant in improving human health status.

Table 1 PHYTOCHEMICAL ANALYSIS OF *K.pinnata*

Test for	Leaf extract of *K.pinnata*
Alkaloids	+
Carbohydrates and Glycosides	+
Phytosterols	+
Fixed oils and Fats	+
Phenlic Compounds	+
Compounds and	+
Tannins	+
Saponins	-
Flavonoids	+
Proteins and Amino acids	+
Gums and Mucilage	-
Volatile oils	-

+:Present-:Absent

Table 2. Antimicrobial efficacy of *K.pinnata*

S.No.	Pathogens	Leaf extract of *K.pinnata*
	Bacterial species	
1	*Staphyloccus aureus*	19
2	*Bacillus subtilis*	8
3	*Streptococcus pyogens*	11
4	*Pseudomonas aurogonosa*	9
5	*Klebsiella Pnemonia*	12
	Fungal Species	
7	*Aspergillus niger*	11
8	*Trichoderma Viride*	6
9	*Candida albicans*	19

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PHYSICO-CHEMICAL CHARACTERIZATION AND DIVERSITY OF MICROBES IN MUTHUPET MANGROVE ENVIRONS

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ABSTRACT

The diversity and load of Microorganisms, cyanobacteria, bacteria and fungi associated with the Muthupet mangrove water were determined by using the standard method from Oct-2022 to Sep. 2023. One year study revealed that 12 species of bacteria, 18 fungi and 24 species of cyanobacteria were observed from the water stream. Among bacteria, *Pseudomonas, Aeromonas and Vibrio* with two species and others with single each were recorded. *Aspergillus* was dominant (6 species) among the fungi followed by *Penicillium* with three. Cyanobacteria were a dominant group, recorded 24 species. *Spirulina* and *Lyngbya* with each two species were the dominant genus and other with single one each. More hardness and alkalinity with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and alkaline pH were probably the factors favouring the growth of microbes especially cyanobacteria.

Key Words: Mangrove, Biodiversity, Cyanobacteria, Bacteria, Fungi.

INTRODUCTION

Mangroves are coastal wetland forests established at the intertidal zones of estuaries, backwaters, deltas, creeks, lagoons, marshes and mudflats of tropical and subtropical latitudes. Approximately one fourth of the world's coastline is dominated by mangroves that are distributed in 112 countries and territories comprising a total area of about 181,000 km^2. Among the marine ecosystems, mangroves constitute the second most important ecosystem in productivity and sustained tertiary yield after coral reefs. Mangrove plants have morphologically and physiologically adapted to habitats with high salinity, tidal inundation, high wind velocity, high temperature and anaerobic clayey soils. These forests are of great ecological importance, social significance and economic value. Mangrove forests generate considerable amount of detritus such as leaf litter, woody debris and inflorescence and hence constitute an ideal environment for many detritus dependent fauna and microbes. Productivity in mangrove waters depends on the extent of mangrove canopy cover that supplies carbon, nitrogen and phosphorus. The Indian peninsula comprises approximately 7000 km^2 of mangroves, out of which 70, 18 and 12% exist at the east coast, Andaman and Nicobar Islands, and west coast respectively (Krishnamurthy *et al* 1987).

Microorganisms can be defined as life forms that cannot be seen with the unaided eye. This broad definition encompasses an extensive and diverse assemblage of organisms, which exhibit widely different morphological, ecological and physiological characteristics. They are represented by groups like viruses, bacteria, fungi, diatoms, algae, protozoans etc; Of these the first three groups require not only microscopic examination but also in many cases growth in pure culture is essential for identification and hence, they can be strictly treated as microorganisms. As cyanobacteria is a unicellular and autotrophic photosynthesis and they do not take part either in biogeochemical cycles or biological interactions usually the bacteria and fungi are considered important microbial groups. The microbial diversity encompasses vast number of species and the number of species of these groups known to science is only a tip of the iceberg. The total number of known species of bacteria (including Cyanobacteria) in the world is only 4000 whereas; the estimated species number is 3,000,000. A scanty of 0.1 percent alone has been

described. In the case of fungi (including yeasts forming fungi, slime moulds, oomycetes) the described species were 70,000 but the estimated number is 1,500,000. Only 5 percent of the species were described (Groombridge 1992). Considerable difficulty arises in the estimation of those, which remain undescribed. This may be because they are unculturable by the conventional methods and lack of suitable methods of culture of all groups or because we have not explored enough. There are evidences for the occurrences of large numbers of bacterial species, which are 'unculturable' forms. In recent years blue green algae have been drawing tremendous attention because of their ability to treat biomedicine and improve disease quality. Taking the above facts into consideration, a survey was undertaken in mangrove environs to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria.

MATERIALS AND METHODS

Lagoon water was collected from Muthupet mangroves situated at Muthupet, Tamil Nadu, India. A sampling programme consisting of a series of monthly water quality and microbial survey was conducted for one year (Oct. 2022 to Sep. 2023). Population of bacteria and fungi were identified and isolated from the water samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical studies as given by Bergey (1984). Fungi were identified by using standard manuals (Gillman 1957; Ellis 1971). Water samples were collected in duplicate from the same place in pre-sterilized bottles. For cyanobacterial survey, 10 places were selected along the water stream. Samples were collected from the places along with water in polythene bags. Standard microbiological methods were followed for the isolation and identification of cyanobacteria (Desikachary 1959). Physico-chemical characteristics of mangrove water were done according to the standard methods (APHA 1981). Temperature and pH of the effluent were measured at the station itself.

RESULTS AND DISCUSSION

The environmental parameters showed variations in different seasons in the study region depending on the topography. Salinity showed the highest values (29 ppt) in summer nearer to the coastal environment associated with low phosphorus (0.925 mg/l) concentrations. The lowest value of salinity (24 ppt) was noticed in monsoon seasons, accompanying high phosphorus (1.535 mg/l) concentration due to the freshwater zone of this aquatic environment. Low DO (5.2 mg/l) values in summer season may be due to the stagnant not conditions of the water with increasing waste load in the mangrove environment. This in turn enhances the concentrations of ammonia (11.5 mg/l) and nitrite (9.0 mg/l) at these monsoon periods. High nitrate (25.0 mg/l), Inorganic phosphorus (0.992 mg/l) and Organic phosphorus (0.558 mg/l) concentration observed in the monsoon periods indicates the impact of terrestrial runoff.

Estuarine mangrove waters in general have relatively low stocks of inorganic phosphorus and nitrogen (Alongi *et al* 1992). In some cases, the degree of human impact seems to control nutrient profiles (Nedwell 1975), while in others the degree of upland influence and the hydrology of the system appear to be of greater importance (Boto & Wellington 1988; Ovalle *et al* 1990). In the present study, the ecosystem was found to be nutrient rich, and the ratios of N: P (10: 1) as well as TN:TP (8: 1) were low. DO and nutrients; and among the nutrients themselves (Table 1). The water pH, temperature and salinity fluctuations in the Muthupet lagoons are consistent with seasonal cycles. However, the influence of the Muthupet lagoons on hydrographic conditions was observed at the sampling stations. The spatial and temporal differences in physicochemical variations indicate the diversity of habitats that exist within this lagoon. Monsoon season and post monsoon have a lower temperature and salinity than the premonsoon.

Bacteria isolated from the water, soil and infected fish were identified based on colony morphology, Gram staining, and various biochemical characteristics. Totally 12 different bacteria were isolated from the mangrove environs (Table 2). Mangroves provide a unique ecological environment for diverse bacterial communities. The bacteria fill a number of niches and are fundamental to the functioning of these habitats. They are particularly important in controlling the chemical environment of the mangal. For example, sulfate-reducing bacteria Chandrika *et al* (1990) are the primary decomposers in anoxic mangrove sediments. These bacteria largely control iron, phosphorus, and sulfur dynamics and contribute to soil and vegetation patterns (Sherman *et al* 1998). Subsurface bacterial communities may sequester nutrients and hold them within nutrient-limited mangrove muds (Alongi *et al* 1993; Rivera-Monroy & Twilley 1996).

In addition to processing nutrients, mangrove bacteria may also help process industrial wastes. Iron-reducing bacteria are common in mangrove habitats in some mining areas (Panchanadikar 1993). Eighteen bacterial isolates that metabolize waste drilling fluid have been collected from a mangrove swamp in Nigeria. Interestingly, four additional bacterial strains isolated from the same swamp depress growth rates of *Staphylococcus* and *Pseudomonas* species and could, therefore, decrease normal rates of organic decomposition (Benka-Coker & Olumagin 1996).

Other mangrove bacteria are parasitic or pathogenic. Vibrios capable of parasitizing *Vibrio* sp. are common in an Australian mangrove habitat. Their abundance there (36.6 ml-1) is much higher than in nearby Great Barrier Reef habitats (9.5 ml^{-1}) Sutton and Besant (1994). Also in Australia, *Bacillus thuringiensis*, which shows insecticidal activity against mosquito larvae of *Anopheles maculatus*, *Aedes aegypti* and *Culex quinquefasciatus*, has been isolated from mangrove sediments (Lee *et al* 1990a; Lee & Seleena 1990). In the present studies the pathogenic bacteria were isolated from infected mangrove fish and Muthupet lagoon water and soils. The isolated pathogenic bacteria were identified by various biochemical tests.

Fungi, from the mangrove sample were isolated based on serial dilution technique. Totally, eighteen different species of fungi belonging to eleven genera were isolated from the mangrove samples (Table 3). Among the fungi recorded, *Aspergillus* was found to be dominant with six species followed by *Penicillium* three species. Mangals are home to a group of fungi called “manglicolous fungi.” These organisms are vitally important to nutrient cycling in these habitats (Hyde & Lee 1995; Kohlmeyer *et al* 1995). They recognized 43 species of higher fungi, including 23 Ascomycetes, 17 Deuteromycetes, and 3 Basidiomycetes. Hyde (1990a) listed 120 species from 29 mangrove forests around the world. These included 87 Ascomycetes, 31 Deuteromycetes, and 2 Basidiomycetes.

Work in individual habitats has revealed surprisingly diverse fungal communities (Hyde 1990b; Hyde 1996). Chinnaraj (1993a) identified 63 species of higher fungi in mangrove samples from Andaman and Nicobar Islands alone. Similar samples from Lakshadweep Island yielded 32 species (Chinnaraj 1992) and 39 species were found in mangrove samples from the Maldives (Chinnaraj 1993a). Ravikumar & Vittal (1996) found 48 fungal species in decomposing *Rhizophora* debris in Pichavaram, south India. Table 3 lists some of the fungal species identified in these studies.

Altogether twenty-four species of cyanobacteria belonging to twenty-two genera were collected from the water stream (Table 4). Among the genera cyanobacteria *Lyngbya* and

Spirulina with each two species were the dominant genus and other with single one each. Phytoplankton and benthic microalgal communities make important contributions to the functioning of mangrove environments. However, their contribution to total estuarine production is relatively small in most regions of Southeast Asia, Australia, Central America and tropical South America. Robertson and Blaber (1992) suggested that the contribution of plankton to total net production in mangrove habitats ranges from 20 to 50%. Careful measurements are verifying that predication for large systems. Phytoplanktons are responsible for 20% of the total production in mangrove estuaries in the Fly River Delta in Papua New Guinea (Robertson *et al* 1991) and 20-22% of the total production in the Pichavaram mangroves of south India (Kawabata *et al* 1993). In the present studies the 24 species of cyanobacteria were isolated from Muthupet lagoons.

Of the total estimated area of 150 million sq km of the earth, about 70.7% is occupied by oceans. However, of all the total photosynthetic productivity of 555.2 billion tons of dry weight/year on earth, only 34.4% is contributed by the oceans (Bassham 1975). India has a vast coastline of over 7500 km; in addition it has many lakes, ponds, puddles, backwater areas and a tropical climate that results in abundance of natural populations of varied organisms. Cyanobacteria are widespread and abundant in most marine habitats. Their ability to grow in seawater is presumably related to a preference for alkaline conditions and an ability to tolerate high salt concentrations. The resistance, which many species show towards osmotic shock, extremes of temperature and reducing conditions, suits their existence in a variety of intertidal habitats. Desikachary (1959) suggested that probably 20% of all known cyanobacteria occur in saline conditions and a majority of them are truly marine. However, little work has been done to understand the cyanobacterial biodiversity of marine environments of India (Thajuddin *et al* 2000 & 2002).

Table 1. Physico-chemical water analysis of muthupet mangrove

S. No	Parameters	Monsoon	Post-monsoon	Summer	Pre-monsoon
1	pH	7.8	8.2	8.5	8.0
2	Atmospheric Temp. (^{0}C)	30	32	36	33
3	Surface water Temp. (^{0}C)	29	30	34	31
4	Alkalinity	17.5	25.2	27.6	22.4
5	Free carbon dioxide	1.2	1.5	1.8	1.6
6	Dissolved Oxygen	7.9	8.3	5.2	5.4
7	Ammonia	11.5	10.7	10.0	10.5
8	Nitrate	25	20	15	17
9	Nitrite	9	7	5	8
10	Total hardness	1155	1200	1340	1250
11	Calcium	845	880	971	905
12	Magnesium	310	320	369	345
13	Chloride	89	96	98	95
14	Salinity (ppt)	24	25	29	26
15	Total Phosphorus	1.535	1.269	0.925	1.299
16	Inorganic phosphorus	0.992	0.865	0.620	0.899
17	Organic phosphorus	0.558	0.454	0.310	0.434
18	BOD	06	09	10	07
19	COD	54	61	66	60

* Except pH and temperature, all values expressed in mg^{-1}

Table 2. Bacterial flora in the mangroves

S. No	Name of bacteria	Monsoon	Post-monsoon	Summer	Pre-monsoon
1	*Streptococcus sp.*	+	+	-	+
2	*Escherichia coli*	+	+	+	+
3	*Vibrioanguillarum*	+	+	+	+
4	*V.alginolyticus*	+	+	-	+
5	*Pseudomonas aeruginosa*	+	+	-	+
6	*P. fluorescens*	+	+	+	+
7	*Aeromonas salmonicida*	-	+	+	+
8	*A. hydrophilia*	+	+	+	+
9	*Lactobacillus sp.*	+	+	+	+
10	*Edwardsiella tarda*	+	-	+	+
11	*Bacillus sp*	-	+	+	+
12	*Enterobacter aerogenes*	+	+	-	+

Monsoon (Oct-Dec); Post-monsoon (Jan-Mar); Summer (Apr-June); Pre-monsoon (July-Sep)

Table 3. Fungal Flora in the Mangroves

S. No	Name of Fungi	Monsoon	Post-monsoon	Summer	Pre-monsoon
1	*Aspergillus niger*	+	+	+	+
2	*A. flavus*	+	+	+	+
3	*A. fumigatus*	-	-	+	-
4	*A.terreus*	+	+	+	+
5	*A. nidulans*	-	-	+	-
6	*A. luchensis*	+	+	+	+
7	*Fusarium sp*	+	-	-	-
8	*Curvularia sp*	-	-	+	-
9	*Candida albicans*	+	+	+	+
10	*Xylaria sp.*	+	-	-	-
11	*Verticillium sp*	-	-	+	-
12	*Geotrichum sp*	-	-	+	-
13	*Penicillium sp*	+	+	+	+
14	*P. javanicum.*	+	+	+	+
15	*P. citrinum*	+	+	+	+
16	*Trichoderma viride*	+	+	+	+
17	*Rhizobus sp.*	-	-	+	-
18	*Helminthosporium oryzae*	-	-	+	-

Monsoon (Oct-Dec); Post-monsoon (Jan-Mar); Summer (Apr-June); Pre-monsoon (July-Sep)

Table 4. Cyanobacterial Flora in the Mangroves

S. No	Name of cyanobacteria	Monsoon	Postmonsoon	Summer	Premonsoon
1	*Oscillatoria formosa*	+	+	+	+
2	*Anabaena sphaerica*	-	+	+	+
3	*Nostoc muscorum*	+	+	+	+
4	*Chamaesiphon sideriphilus*	+	+	+	+
5	*Xenococcus acervatus*	+	+	+	+
6	*Phormidium valderianum*	+	+	+	+
7	*Trichodesmium erythraeum*	+	+	+	+
8	*Richelia intracellularis*	+	+	+	+
9	*Hapalosiphon welwitchii*	+	+	+	+
10	*Dichothrix bauriana*	-	+	+	+
11	*Spirulina subsalsa*	+	+	+	+
12	*S. platensis*	+	+	-	+
13	*Lyngbya majuscula*	+	+	-	+
14	*L. hieronymusii*	+	+	+	+
15	*Stichosiphon sansibaricus*	+	+	+	+
16	*Nodularia spumigena*	+	+	+	+
17	*Microcoleus chthonoplasts*	+	+	+	+
18	*Myxosarcina concinna*	+	+	+	+
19	*Merismopedia glauca*	+	+	+	+
20	*Chroococcus turgidus*	+	+	+	+
21	*Gomphospaeria aponina*	+	+	+	+
22	*Microcystis pulverea*	-	+	+	+
23	*Synechocystis pevalekii*	+	+	+	+
24	*Gloeothece rupestris*	+	+	+	+

Monsoon (Oct-Dec); Post-monsoon (Jan-Mar); Summer (Apr-June); Pre-monsoon (July-Sep)

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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF *OXALIS DEBILIS*

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ABSTRACT

World is rich in knowledge with a wide diversity of medicinal plants which provide people with traditional healing methods for diseases in general healthcare area. The agar diffusion method was used to examine the antibacterial activity of different solvent extracts of *Oxalis debilis* tested against *Escherichia coli, Pseudomonas aeruginosa,* Klebsiella pneumonia, and *Staphylococcus aureus*. For qualitative phytochemical investigation, several solvent extracts of the complete *O.debilis* plant were investigated. The minimum inhibitory concentration (MIC) of *O.debilis* ethanol extract had significant antimicrobial effectiveness against *Escherichia coli* (456 µg/ml), and *Pseudomonas aeruginosa* (419 µg/ml). The ethanolic extract showed (419 µg/ml and 398 µg/ml) antimicrobial activity against *Klebsiella pneumonia* and *Staphylococcus aureus* respectively. The lowest antibacterial activity was found in acetone extract with MIC (198µg/ml) of *O.debilis*. Various parts of the plant has the presence of variety of phytoconstituents such as flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin.

Key words:*Oxalis debilis*, antimicrobial activity, agar diffusion method, phytochemical analysis

INTRODUCTION

In recent years, herbal products have gained popularity in industrialized countries as well as a number of other nations. According to the World Health Organization, 80 percent of people worldwide currently utilize herbal medicine for various kinds of primary healthcare[1]. In ethnomedicine, a lot of the plants are utilized to treat a variety of illnesses. Antimicrobial drugs either eradicate or prevent the growth of microorganisms. Disinfectants are antimicrobial chemicals used on no-living items or outside the body parts.

The genus Biophytum is a member of the family Oxalidaceae and distributed in tropical Asia, Africa, America and the Philippines. In India, nine species of *Oxalis debilis* are prominently found and out of these, three species viz. *O. debilis*. Syn The plant is distributed up to an altitude of 1,800 m and is available during the rainy season in moist shady places [2]. The plant is traditionally used for centuries in the treatment of various health aliments. It is an important medicinal plant in the Indian traditional system of medicine like Ayurveda [3].

Microorganisms play an important role in the manufacture of bioactive small molecules from natural resources for the prevention of several diseases and the creation of effective medications. The acetone extract of leaves of *O. debilis* had significant antifungal activity. The leaf extract of *O. debilis* inhibited the growth of fungal pathogens *A. fumigatus, A. niger, C. neoformans* and Norcardia sp. in disc method [4].

Numerous medications have caused microbes to become resistant, which presents a substantial therapeutic issue in the management of infectious diseases. The overuse of commercially available antimicrobials, which are routinely used to treat illnesses, led to the

development of the bacteria' tolerance [5]. In order to find new antimicrobial chemicals, researchers were motivated to investigate in other sources, particularly herbal resources

MATERIALS AND METHODS

Collection and Identification of plant material: For the study, the whole plant of *O. debilis* belongs to Oxalidaceae family was collected from Wayanad, Kerala, South India. The whole plant were identified taxonomically and authenticated according to various literatures, Flora of Madras Presidency and Wealth of India including other pertinent taxonomic literature.

Preparation of plant materials and extract: The leaves were carefully cleaned, shade dried and powdered. The powdered material was stored in a closed air-tight plastic container at low temperature. The powdered plant material (50 g) was extracted with 300 mL of each solvent ethanol by maceration (3×24 h) at room temperature. The collected solvents were concentrated by rotary vacuum evaporator at 45°C and then dried using a freeze dryer. All extracts and acyclovir (extracted from commercial tablet) were dissolved in dimethyl sulphoxide (DMSO). The final concentration of DMSO was 0.1% v/v in cell culture environment.

Phytochemical Analysis : The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described [6-7]. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Test microorganisms: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Staphylococcus aureus* were used as test organisms in the current study. For the current experiment, the obtained cultures were repeatedly subcultured.

Antimicrobial activity by agar diffusion method: The antibacterial efficacy of several *O. debilis* solvent extracts was assessed using the agar diffusion method. For spreading agar media, a subcultured microbial suspension (100 µl) was prepared. Various concentrated varied extracts were used to measure antimicrobial activity [8]. The plates were filled with the sample and then left to allow for an hour to enable the extract to disperse. The plates were maintained in an incubator for 24 hours at 37°C, and the inhibitory zone was measured in millimeters (mm). Results are compared with those of conventional antibacterial drugs.

RESULTS AND DISCUSSION

Phytochemical studies of *O. debilis* showed that it contains a number of phenolic and polyphenolic compounds, saponin, essential oil, polysaccharides and pectin. The main bioactive constituents found are bioflavonoid, amentoflavone with minute amount of cupressoflavone [9]. All of the extracts from the *O. debilis* contained saponin, phenols, tannins, glycosides, terpenoids, flavonoids, alkaloids, and coumarins, according to a preliminary phytochemical examination. With the exception of the chloroform extract's lack of saponins, glycosides, and coumarins and the extract from ethyl acetate's absence of saponin. The results of the phytochemical analysis are displayed in Table 1. A higher degree of biological activity derives from the presence of a high concentration of phytochemicals in the plant.

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized [10]. In the present study, it was clearly understood that the ethanolic extracted maximum amount of the different type of metabolites present in the *O. debilis*. Boominathan and Ramamurthy [11] reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

For instance, the presence of flavonoids suggest that the plant have been reported to exert multiple biological effects including, anti-allergic, anti-inflammatory, anti- microbial antioxidant, anti- cancer activity [12]. It also suggests that the plant might have diuretic properties [13]. The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and anti-bacterial activities and can relief in wound healing and burns [14]. Saponins and glycoside are also very important classes of secondary metabolites as some are cardio-active and used in treatment of heart conditions [15]. Some researchers have also investigated that some saponins have anti-cancer and immune modulatory properties [16]. Volatile oils are used in the industries for various purposes, both as a pharmaceutical/ cosmetic raw material for production of emollients and active ingredient for the respiratory tract infections.

The antibacterial properties of various solvent-based extracts of *O. debilis* are displayed in Table 2. The ethanol extract of *O. debilis* had the highest antimicrobial activity with MIC (423 µg/ml) against the *Escherichia coli*, (456 µg/ml) against the *Pseudomonas aeruginosa*, (419 µg/ml) against the *Klebsiella pneumonia* and (398 µg/ml) against the *Staphylococcus aureus*. The various extracts of *O. debilis* tested against *Escherichia coli* and showed considerable MIC results in water extract (352 µg/ml), chloroform extract (311 µg/ml), ethyl acetate extract (312 µg/ml), acetone extract (217 µg/ml), hexane extract (236 µg/ml). The results were compared with standard Cephalosporins as reference compounds with MIC (56 µg/ml). The different extracts of *O. debilis* were checked against the *Pseudomonas aeruginosa* and exhibited significant MIC values in water extract (368 µg/ml), chloroform extract (279 µg/ml), ethyl acetate extract (298 µg/ml), acetone extract (206 µg/ml), hexane extract (245 µg/ml). The obtained results were compared with Cephalosporins with MIC (48 µg/ml).

The individual extract of *O. debilis* was checked against *Klebsiella pneumonia* and found impressive MIC values in water extract (348 µg/ml), chloroform extract (267 µg/ml), ethyl acetate extract (256 µg/ml), acetone extract (198 µg/ml), hexane extract (228 µg/ml). The different solvent extract of *O. debilis* was evaluated against *Staphylococcus aureus* and found impressive MIC values in water extract (325 µg/ml), chloroform extract (246 µg/ml), ethyl acetate extract (248 µg/ml), acetone extract (201 µg/ml), hexane extract (217 µg/ml). The gentamicin (34 µg/ml) was used as a standard compound.

A considerable inhibitory zone may also be caused by the variety of phytochemicals present in the extract. The presence of different flavonoids, alkaloids, terpenoids, phenols, saponins, and coumarins has bactericidal properties [17]. According to various scientific studies, high concentrations of phytochemicals and bioactive compounds are thought to have a stronger potential for treating a variety of pathogenic bacteria. Numerous plants and their various portions of them have historically been used to treat a variety of chronic illnesses, such as gastrointestinal problems, urinary tract infections, skin conditions, and various respiratory issues., etc. [18].

Several chronic illnesses caused by various bacteria may be prevented and managed with the use of plant-based remedies. Many societies still employ ethnomedicines to treat illnesses and overcome obstacles without creating negative side effects. The inclusion of several phytoconstituents, including alkaloids, flavonoids, coumarins, saponins, polyphenols, tannins, and terpenoids, is what gives herbal preparations their therapeutic effects [19]. The presence of secondary metabolites prevents the growth of harmful microorganisms causing serious diseases [20]. The microorganisms are resistant to many antibiotics that is very harmful to humans. The researchers are finding an alternative to commercial antibiotics to prevent harmful infections against a variety of microorganisms using plant-based medicines [21]. The higher concentration of crude extracts sometimes may cause cytotoxicity in humans hence the dose-dependent values are determined using in vitro cell cytotoxicity assay [20]. As compared to commercial antibiotics, plant-based medicines have very small side effects if they are consumed in excess quantity [22]. In the world, 80 % of different pharmaceuticals are prepared from plant-based medicines and which are effective to cure any chronic disease.

CONCLUSION

O. debilis used for its wide therapeutic potential of antimicrobial agents. According to the studies, ethanol extract has the most potential, which may be because it includes the majority of the phytochemical compounds and bioactive compounds that have antibacterial activity. The complete plant extract of *O. debilis* has to be further studied in order to identify and purify chemicals that might be used as natural medicinal alternatives to synthetic commercial ones. The future aspects of the plant can be anti microbial as it contains many of the phytochemicals and work has not been performed yet.

Table 1: Qualitative Phytochemical screening on extracts of *Biophytum sensitivum*

S. No	Name of Test	Test applied / Reagent used	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]Dragndorff's test	+++ +++ +++ ++	++ ++ ++ ++	++ ++ ++ ++	++ ++ +++ ++	+++ +++ +++ ++	++ ++ ++ +
2	Flavonoids	HCl and magnesium turnings	+++	++	+	++	+	++
3	Carbohydrate	Molisch's test	+	+	+	+	+	+
4	Tannins & Phenols	A] 10% Lead acetate B] $Fecl_3$	+++ +++	+ +	++ ++	++ ++	++ ++	++ ++
5	Test for Steroids	A]Salkowski's Test B] Libermann-Burchard's Test	++ ++	++ ++	++ ++	++ ++	++ ++	++ ++
6	Gums & Mucilages	Alcoholic Precipitation	-	-	-	-	-	-
7	Fixed oil &	Spot test	+	-	+	+	-	-

	Fats							
8	Saponins	Foam test	+	+	+	+	+	+
9	Phytosterols	LB test	+	+	+	+	+	+
10	Volatile oils	Hydro distillation method	+	+	+	+	+	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C]Xanthoprotein test	++ +++ +++	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++	++ ++ ++

Table 2. Antimicrobial activity of the extracts *Biophytum sensitivum*

Microorganism	**Minimum inhibitory concentration (MIC)**							
	Extract of *Biophytum sensitivum* (µg/ml)							
	Ethanol	Water	Chloroform	Hexane	Acetone	Ethyl acetate	Gentamicin (µg/ml)	Cephalosporins (µg/ml)
P.aeruginosa	456	368	279	245	206	298	ND	48
K. pneumonia	419	348	267	228	198	256	ND	46
E. coli	423	352	311	236	217	312	ND	56
S. aureus	398	325	246	217	201	248	34	ND

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BIODEGRADATION OF SYNTHETIC DYES BY FUNGAL INOCULATED ON SOLID MEDIA

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ABSTRACT

Water pollution caused by industrial waste discharges has become an alarming trend worldwide, while dye industries are considered as the most polluting among all others. In recent years, bio-treatment took attraction in removing the unwanted colour and toxicity of dyes than other conventional treatment processes. The release of dyes in to environment is of great concerned due to color, toxicity, mutagenicity and carcinogenicity of the dye, considerable attention has been given in evaluating the capability of microorganism in decolourisation and degradation diazo dye. The present study fungi were inoculated on different solid media to attain biodegradability of a diazo dye used for the coloration of paper products. For the solid, to be employed as media, special characteristics are needed with regards to adsorption capacity for concentrating substrate within the cell environment and an adequate particle size and surface texture for assuring fungal colonization. These factors were in different pH, temperature, incubation period, inoculum sizes, carbon sources, nitrogen sources and different concentrations of yeast extract. In the present investigation the ability of heat killed fungus *Aspergillus terreus* to adsorb the dyes Methylene blue and Congo red was investigated. The removal of the dyes Congo red and Methylene Blue from aqueous solutions on the fungal *A. terreus* was demonstrated by a series of batch experiments.

Keywords:Decolorization, Biodegradation, Congo red, Methylene Blue and *Aspergillus terreus*.

INTRODUCTION

Environmental pollution has been recognized as one of the major problems of the modern world. The increasing demand for water and dwindling supply has made the treatment and reuse of industrial effluents an attractive option. One of the most important environmental pollution problems is the color in water courses, although some of this color is normally present and of "natural" origins (e.g. the color originates from the activity of some microorganisms in ponds), a considerable proportion, especially in the lower reaches of rivers draining large industrial conurbations, originates from industrial effluents. Some colored effluents are associated with the production and use of dyestuff.

Dye wastewaters are highly visible even at very low concentrations of dyes (less than 1.0 mg/l for some dyes). When they are discharged into receiving water bodies, they make the water aesthetically unpleasing, affect water transparency and gas solubility in water bodies which cause adverse impacts on aquatic life and may be toxic to aquatic life[1]. Wastewaters have been known to be detrimental to the microorganisms involved in biological wastewater treatment; thus dye wastewaters cause low removal efficiency or failure of the treatment plants[2]. To comply with environmental legislation restricting the discharge of wastewater, the textile industry is attempting to develop technologies for wastewater remediation. Dye removal is of particular concern because it is largely unaffected by conventional treatment systems[3].

Synthetic dyes are extensively used in the textile industry. Due to inefficiencies of the industrial dyeing process, 10–15% of the dyes are lost in the effluents of textile units, rendering

them highly colored[4-5]. It is estimated that 280,000 tons of textile dyes are discharged in such industrial effluents every year worldwide[6]. Direct discharge of these effluents causes formation of toxic aromatic amines under anaerobic conditions in receiving media. In addition to their visual effect and their adverse impact in terms of chemical oxygen demand, many synthetic dyes are toxic, mutagenic and carcinogenic[7]. The efficient removal of dyes from textile industry effluents is still a major environmental challenge[8]. The frequently high volumetric rate of industrial effluent discharge in combination with increasingly stringent legislation, make the search for appropriate treatment technologies.

Degradation of dyes, especially azo dyes, which comprise about 70% of all dyes used, is difficult due to their complex structure and synthetic nature[6,9]. Currently, various chemical, physical and biological treatment methods are used to remove colour[10-11]. Because of the high cost and disposal problems, most of the chemical and physical methods for treating dye wastewater were not widely applied in the textile industries[12-13]. Because synthetic dyestuffs are resistant to biological degradation, color removal by bioprocesses is also difficult[14-15]. Decolorization generally occurs by the adsorption of dyestuffs on bacteria, rather than oxidation in aerobic systems. Some bacteria can biodegrade dyestuffs by azoreductase activity. However, the effluent at the end of biotransformation of dyestuffs could be toxic[7]. These problems limit large-scale application of bacterial decolorization.

There are a number of methods to treat dye waste waters. These can be classified into two general categories: biological treatment and physical/chemical treatment. Since dyes are designed to be bio-resistant, conventional aerobic biological processes generally are not efficient to biodegrade dyes. These processes remove dyes primarily through adsorption to the biomass[16]. An anaerobic biological pretreatment followed by an aerobic treatment may represent a significant advancement in biological treatment for dye wastewaters[17]. Loyd[18] studied anaerobic /aerobic processes for a dye wastewater treatment and produced much better color reduction than aerobic treatment alone. However, this innovative process is in a developmental stage.

Due to the low removal efficiency of biological treatment process, dye wastewaters are usually treated by physical/ chemical processes. There are six distinct groups: coagulation, adsorption, membrane techniques, electrochemical technology, reduction and oxidation[17,19].

Biosorption has been studied since 1980s in removing heavy metals and dyes as well as other organic pollutants from wastewaters. It is a promising alternative to replace or supplement present treatment processes. Biosorption may be defined as the removal of metal and metalloid species, compounds and particulates from solution by biological materials, called biosorbents[20]. Various mechanisms of biosorption range from physico-chemical interactions, such as adsorption, deposition, ion-exchange, to processes dependant on cell metabolism. Living and dead cells as well as derived products, such as cell wall constituents, are able to function as biosorbents[20]. Compared with the living cells, dead cells posses various advantages. They may be stored or used for extended periods. Their biosorptive capacities may be greater, equal, or less than those of living cells. Their operation is easier and they can be regenerated by certain methods[21]. Therefore, dead cells are preferred than living cells as biosorbents.

Fungus *Aspergillus terreus* is used in industrial processes to produce citric acid, kojic acid, cellulases, lipases and glucanases[22]. These industries can serve as an economical and constant source of fungal biomass, which means that *A. terreus* could be used in practice to remove color from dye wastewater in the future. At the same time, the amount of available

information on a systematic evaluation of the potential of *A. terreus* to remove dyes from dye wastewaters and the explanations for the mechanisms are limited. By keeping all the above facts in mind, the present investigation was carried out to know the biosorption efficiency of *Aspergillus terreus*on dyes. In this paper, we studied the location of the dye degrading enzyme, decolorization of other direct and basic dyes, and the ability of the isolates to decolorize Azo dye in microcosm.

MATERIALS AND METHODS

Sources of Study Materials

*Aspergillus terreus r*eceived from National Center for Industrial Microorganisms (NCIM), Pune, India. The strain was maintained on PDA (potato, dextrose and agar) medium slant. The slant was inoculated and incubated at 30°C for 7–8 days and then stored at 4°C and periodically sub-cultured.

Dyes Used For Experiments

Congo red (Merck) and Methylene blue (Sigma) were used.

Solid Cultures

Solid media are used for the growth of fungi and for storage of cultures *Aspergillus terreus* was grown in potato dextrose agar (PDA) petridishes and was incubated for 7 to 10 days at room temperature (28° C). The strain was routinely transferred every 7 to 10 days to fresh PDA petridishes by streaking. *A.terreus* was always stored in sealed plastic bags to prevent the loss of moisture from agar.

Preparation of Dye Solutions

The two dyes used in this study were Basic blue or methylene blue ($C_{16}H_{18}ClN_3S.3H_2O$) is commonly available as a chloride salt and soluble in water. It is widely used in textile industry. It is often used as a biological strain and as antidote for cyanide poisoning in humans and animals. Congo red ($C_{32}H_{22}N_6Na_2O_6S_2$) is soluble in water. It is a pH indicator which is blue and red at pH 3.0 and 5.2 respectively. Congo red has an affinity for both proteinaceous and cellulosic substrates. It can dye cotton directly and wool out of a neutral bath.

Name	Classification	Color index number	Molecular Weight	Solubility in water (mg/L)
Congo Red	Anionic direct diazo	22120	696.7	40
Methylene blue	Cationic thiazine	52015	373.9	50

In this study the initial concentration of each dye solution was fixed at 50.0 mg/l. This concentration is in the range of concentrations usually observed in actual dye wastewaters. Dye solution (50 mg/l) was prepared by dissolving accurately 50.0 mg dye in 1 L distilled water.

Calibration of Dye Solution

The dye solutions were initially calibrated for concentration in terms of absorbance units. Each of the standard dye solution (50.0 mg) was diluted with distilled water to concentrations of 0, 0.5, 1.0, 5.0, 10.0, 15.0, 20.0, 30.0, 40.0, 50.0 mg/l, respectively. The pH of the dye solutions with different concentrations was adjusted to 7.6 by using dilute HCl or NaOH solution. Each concentration of solutions was measured for its absorbance value at its corresponding λ_{max}. The absorbance values versus concentrations were then plotted. All calibration plots were straight lines passing through the origin (0, 0).

Concentration Calculation

In order to compare removal of dye on the same basis, the pH of all samples was adjusted to 7.6 before measurement. The pH value of 7.6 was selected based on the Standard Methods for examination of water and wastewater[23]. Dilute HCl or NaOH was used for pH adjustment. The absorbance values of the samples were determined by a spectrophotometer operating at the corresponding maximum absorbance wavelength (λ_{max}) on absorbance mode.

The dye concentration was obtained by interpolating the measured absorbance values on the linear portion of its calibration plot. If the absorbance values of the samples were outside of the linear range of the calibration plot, the samples were diluted before absorbance measurements.

RESULT AND DISCUSSION

Growth on Solid Medium

The growth of the fungus *Aspergillus terreus* on petri dishes was observed after four days. In six days, the surface of PDA solid medium in the petri dishes was covered by brown colored spores. In eight to ten days, a brown colored mat covered the whole surface of PDA solid medium in petri dishes. Figure 1 shows a picture of a PDA petridish covered by *A. terreus*. Previous reports on azo dye degradation by bacteria investigated a certain enzyme which is the azo reductase as responsible for the reduction of azo dye. According to Russ et al. [(24)], enzymatic reduction can occur both intracellularly and extracellularly. In a study of the anaerobic reduction by whole cells, cell extracts and cell membranes of *Sphingomona*s sp. strain BN6, enzymatic azo dye reduction activity was found to be located in the cytoplasm as well as in the membrane fraction but it was suggested that azo dye reduction by whole cells is mainly related to the membrane fraction[25].

Dyes Maximum Absorbance Wavelengths (λ_{max})

Figure 2 shows the absorbance of Methylene Blue solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations. It was observed that the values of λ_{max} varied slightly with concentrations. The solution with a high concentration had a slightly higher value of λ_{max}. The differences were in a range of 10- 20 nm when the dye concentrations were changed from 10 mg/L to 50 mg/L. In this study, λ_{max} of Methylene Blue solution with a concentration of 10 mg/l was used. So the value of λ_{max} for Methylene Blue was 660 nm.

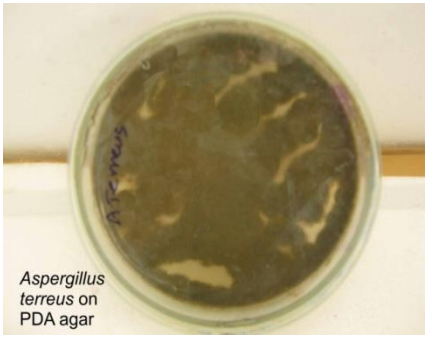

Fig. 1. The growth of the fungus *Aspergillus terreus* on PDA Agar

Figure 3 shows the absorbance of Congo red solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations. It was observed that the values of λ_{max} were same at different concentrations. The value of λ_{max} for Congo Red was 500 nm. Culture medium and culture conditions employed in Congo red decolorization studies were utilized. Aerobic and anaerobic conditions were provided. Absorbance of Methylene Blue solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different

concentrations. Absorbance of Congo red solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations. No decolorization was observed in the aerobic set-up with six months of incubation. All the isolates and consortia showed positive growth in all the dyes as evidenced by the presence of gas in the Durham tubes except for methyl violet in which no gas was produced.

Figures 4 and 5 show the calibration plots for the dyes Methylene Blue and Congo Red. The calibration equations based on linear regression. Only direct dyes were decolorized by the isolates and consortia. The isolates and consortia were enriched in medium with Congo red which is a direct dye. This could explain why the isolates were only able to decolorize the direct dyes. *Aspergillus* strain was able to decolorize a wide range of direct azo dyes at different rates but was not able to decolorize basic dyes and acid dyes[26]. Direct dyes have different components than basic and acid dyes. The ability of microorganisms to decolorize different dyes varies depending on the structure and complexity of the dye[27]. In the study of Paszczynski et al. [(28)] on the mechanism of azo dye oxidation by lignin peroxidase produced by the white rot fungi *P. chrysosporium*, it was concluded that the susceptibility of a particular compound to degradation depends on both structure and culture conditions. Under different culture conditions, the fungus may secrete different isozymes of its peroxidases.

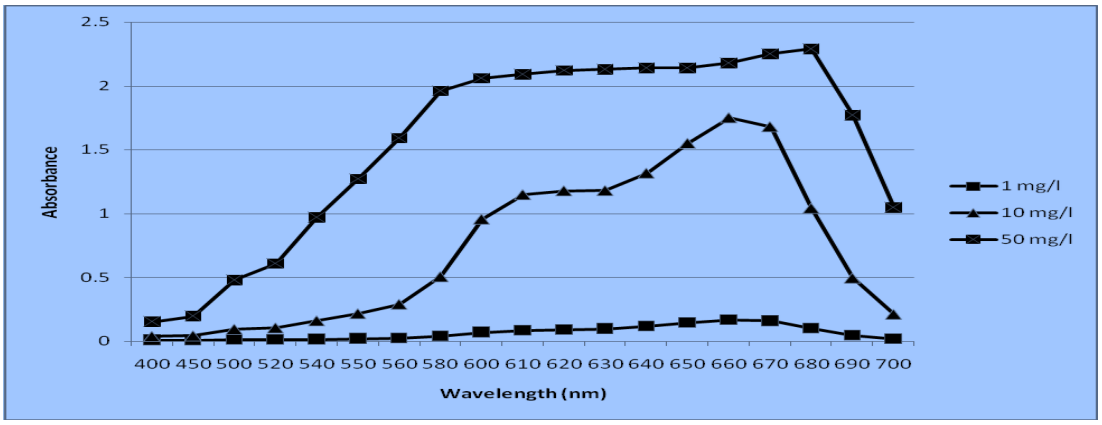

Fig. 2. Absorbance of Methylene Blue solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations

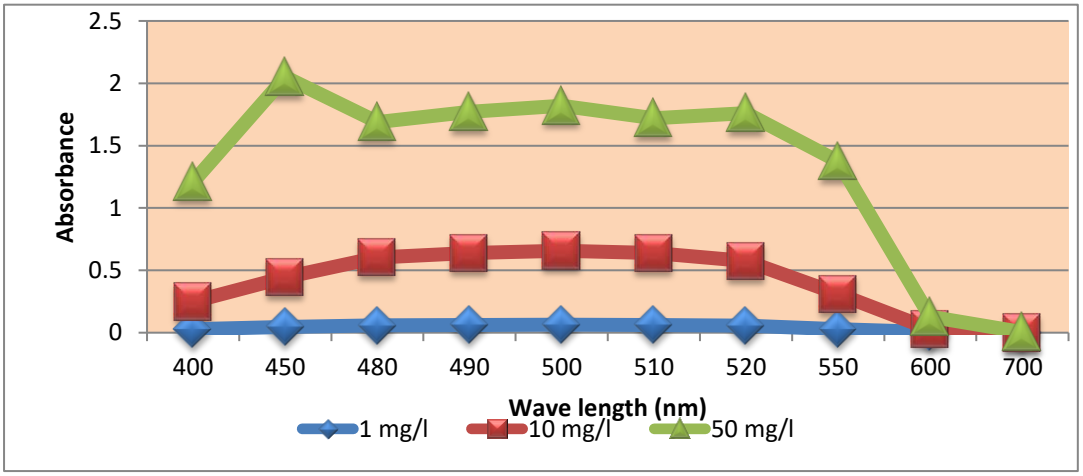

Fig. 3. Absorbance of Congo red solution versus the wavelength in the visible range (400 – 700 nm) at a pH of 7.6 for different concentrations

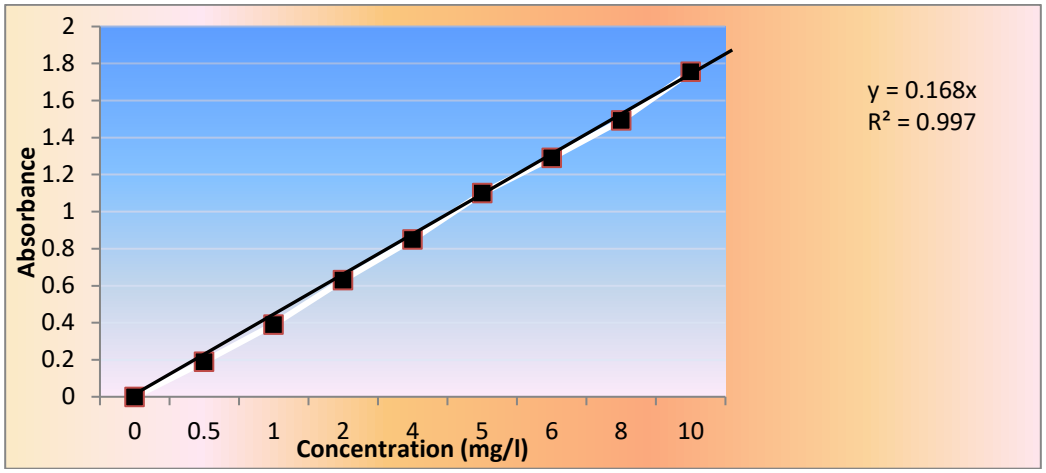

Fig. 4. Calibration plot for Methylene Blue at 660 nm

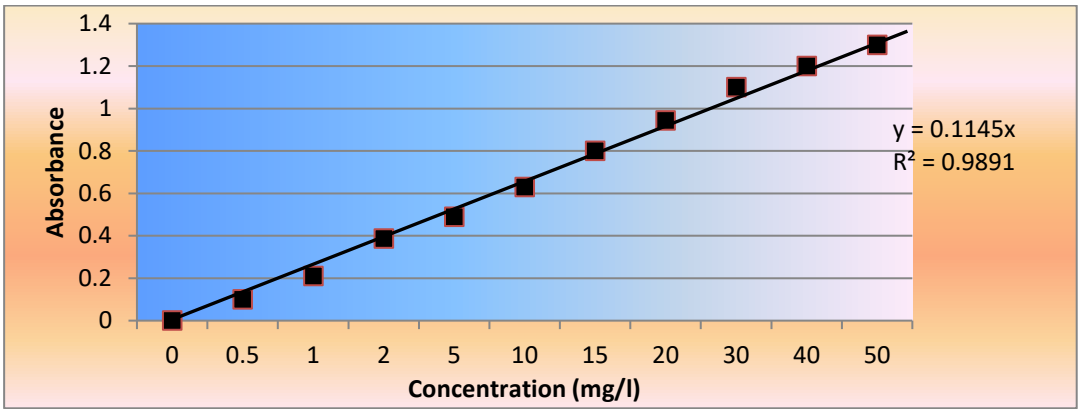

Fig. 5. Calibration plot for Congo Red at 500 nm

For the removal of dyes from wastewaters, a combination of the anaerobic cleavage with an aerobic zone to degrade the amines formed was generally accepted. Two strategies have been developed: sequential and simultaneous processes. Sequential processes combine the anaerobic and the aerobic steps either in the same reaction vessel or alternatively in a continuous system in separate vessels. The simultaneous processes utilize anaerobic zones within basically aerobic bulk phases, such as observed in biofilms[29], granular sludge or biomass immobilized in other matrices[30-31]. In the sequential and simultaneous processes, auxiliary substrates are required, for supplying bacteria with a source of reduction equivalents for the cleavage of the azo bonds.

In this research we demonstrate that the addition of a fungal culture on the surface of solid media produces dye decolorization. Particle size and surface texture have played an important role on dye biodegradation because substrate has to be concentrated in the proximity of the cells (adsorption capacity of the solid) and an irregular solid surface and a proper particle size are required. In conclusion, biodegradation of recalcitrant compounds as azo dyes can be facilitated by the presence of surfaces that have convenient pores to promote formation of microaerophilic niches and permit microbial growth. Furthermore, they should have adsorptive capacity of the pollutant and nutrients to assure fungal feeding. In this sense, some agroindustrial residues could be used as convenient supports. Fungal decolorization was found to be promising alternative to replace or supplement present treatment process. However, using fungal biomass to remove color in a dye wastewater is still in an early stage. More studies are needed to develop a practical application.

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PHYSICO-CHEMICAL CHARACTERIZATION OF VEDARANYAM MANGROVE AND SEASONAL VARIATIONS OF FISH FAUNA

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ABSTRACT

The present survey was made to study the diversity of fish fauna and physico-chemical parameters in the Vedaranyam mangrove during the year 2007-2008 at four-seasonal intervals. The water was slightly alkaline and contained high amounts of pH. The concentration of salinity, total, inorganic and organic phosphate, ammonia, nitrite and nitrate were fairly stable. Other nutrients such as calcium, magnesium, chloride and bicarbonate concentration showed remarkable variations. The diversity of fish fauna total number of 28 species of fishes belonging to 16 family under Perciformes orders have been identified in the waterways at the study area. The presence of species like *Tenulosa toli*, *Lutjanus madras* and *Scatophagus argus*is an indicator of the productive well being of mangrove ecosystem especially with *Lutjanus madras* which occurs only in mangrove waters.

Key words: Diversity of Perciformes fish, Water analysis, Vedaranyam mangrove

INTRODUCTION

Coastal lagoons are shallow water bodies lying parallel to the coastline and separated from the open sea by a narrow strip of land or sand bank. They are often highly productive and serve as a habitat for variety of plants and animals. They also function as nurseries for fishes and act as ideal sites for aquaculture. They, however, generally experience widely fluctuating physicochemical regimes that induce immense stress on the organisms inhabiting them. Therefore, an adequate knowledge of the prevailing physicochemical regime in a coastal lagoon is important for the understanding of its ecology and for its management.

Odum and Heald, (1972) collected a large number of fishes, crabs and insect larvae from mangroves in southern Florida and identified fifty-three species of fishes, five species of Decapoda, five species of Amphipoda and 3–80 species each of Isopod, Cumacea, Mysidecca, Copepoda, Ostracoda, Mollusca, Ciliata and Chironomid larvae. About 400 species of fishes are reported to depend on mangrove habitat (World Resources, 1996-1997). A more comprehensive data produced by Rao, (1987) indicates that mangrove ecosystems of the world have 193 plant species, 397 fishes, 259 crab species, 256 molluscan species, 450 insect species and more than 250 species of mammals and other associated species of plants and animals.

Indian mangrove areas are excellent nursery grounds for a variety of commercially important prawns, crabs and finfishes, as they provide abundant food and shelter for these organisms. These ecosystems provide food, roosting and nesting site and shelter to a large variety of birds. Several insects, reptiles, birds and mammals inhabit the evergreen canopies of mangroves. The mangroves support many trophic levels of aquatic and terrestrial organisms, by enriching the fertility of estuarine waters for production of planktons. Local increase of fish populations and community diversity in his classical study of tropical rainforests and coral reefs rather emphatically brought out the role of moderate levels of disturbances in increasing the species diversity in biological communities by preventing one or a few species from dominating resources (Mc Guinness, 1987b).

There may be other such impacts on fish communities that have not yet been noticed. Accumulated debris of boulders and broken coral can simulate 'reefs' in shallow coastal waters that can lead to a temporary increase in local fish diversity. Such a diverse fish community is however unstable and may soon succumb to predation. Some of the newer fish habitats and communities that the tsunami created in mangrove in particular are likely to adapt locally and diversify. Such mangroves are 'natural experiments' and would offer a lot of scope for the scientific study of succession in marine and coastal fish communities (Ramamurthy *et al.,* 2008). Unfortunately however, the mangroves are already being fished rather intensely as the fishermen have not been going to the sea. Elsewhere, the mangroves are being used as local garbage disposal pits. In this study, Vedaranyam mangrove fish and water quality parameters are reported.

MATERIALS AND METHODS

The fishes and water samples were collected from coastal area of mangroves at Vedaranyam mangrove forest of Nagappattinam district, Tamil Nadu, India. A sampling programme was consisting of seasonal physico-chemical parameters of water and diversity of perciformes fishes. They survey was conducted for a period of one year from Oct. 2022 to Sep. 2023. The fishes were identified with the help of fish identification manuals of FAO fish identification sheets (1994). Physico-chemical characteristics of water were done according to the Standard Methods (APHA, 1981). Temperature and pH of the water were measured at the station itself.

RESULTS

The water was slightly alkaline and contained high amounts of pH, total hardness, calcium, magnesium, chloride, total, inorganic and organic phosphate, ammonia, nitrite and nitrate in all the four seasons examined (Table 1). Value of dissolved oxygen level was very low during summer and high in postmonsoon. Most of the parameters tested were slightly higher in summer than the monsoon seasons. Alkalinity and salinity were observed more during the summer. In general, the characteristics of water tested in all the four seasons were not varied much.

Twenty-eight species of fishes belonging to 16 families under Perciformes orders have been identified in the waterways at the study area. *Gerrus filamentosus*, *Leiognathus bindus*, *L. brevirostris*, *L. splendens*, *Mugil cephalus*, *Siganus canaliculatus*, *S. javus*, *Sillago sihama*, *Terapon jarbua* and *Terapon puta* were observed in abundance throughout the study period. The presence of species like *Boleophthalmus boddarti*, *Lutjanus madras* and *Scatophagus argus*is an indicator of the productive well being of mangrove ecosystem especially with *Lutjanus madras* (Mangrove snapper) which occurs only in mangrove waters. Commercially important species such as *Ambasis commersoni*, *Atule mate*, *Johnius carutta*, *Kathala axilaris*, *Lutjanus russelli*, *Monodactylus argenteus* and *Lutjanus argentimaculatus*occur seasonally and are caught by fishermen in this mangrove waters. The rest of the species *Carangoides chrysophrys*, *Epinephelus suratensis*, *Gazza minuta*, *Platycephalus vittatus*, *Platycephalus gibbosus*, *Siganus lineatus*, *Nibea maculata*, *Upeneux vittatus* were recorded in one or two months (Table 2).

DISCUSSION

The environmental parameters showed variations in different seasons in the study region depending on the topography. Salinity showed the highest values (27 ppt) in summer nearer to the coastal environment associated with low phosphorus (0.925) concentrations. The lowest value of salinity (22 ppt) was noticed in monsoon seasons, accompanying high phosphorus (1.535) concentration due to the freshwater zone of this aquatic environment. Low DO (4.5 mg/l) values

in summer season may be due to the stagnant not conditions of the water with increasing waste load in the mangrove water. This in turn enhances the concentrations of ammonia (10.5 mg/l) and nitrite (8.4 mg/l) at these monsoon periods. High nitrate (23.0 mg/l), Inorganic phosphorus (0.992 mg/l) and Organic phosphorus (0.558 mg/l) concentration observed in the monsoon periods indicates the impact of terrestrial runoff.

Ramamurthy et al., (2008) reported that Muthupet mangrove water was slightly alkaline and contained high amounts of pH, total hardness, calcium, magnesium, chloride, total, inorganic and organic phosphate, ammonia, nitrite and nitrate in all the four seasons recorded. Estuarine mangrove waters in general have relatively low stocks of inorganic phosphorus and nitrogen (Alongi *et al.*, 1992). In some cases, the degree of human impact seems to control nutrient profiles (Nedwell, 1975), while in others the degree of upland influence and the hydrology of the system appear to be of greater importance (Boto and Wellington, 1988; Ovalle *et al.*, 1990). In the present study, the ecosystem was found to be nutrient rich, and the ratios of N: P (10: 1) as well as TN:TP (8: 1) were low (Table 1).

The water pH, temperature and salinity fluctuations in the Muthupet mangrove are consistent with seasonal cycles. However, the influence of the Muthupet mangrove on hydrographic conditions was observed at the sampling stations (Ramamurthy *et al*., 2008). In the present investigation of Vedaranyam mangrove water value of dissolved oxygen level was very low during summer and high in postmonsoon. Most of the parameters tested were slightly higher in summer than the monsoon seasons. Alkalinity and salinity were observed more during the summer. In general, the characteristics of water tested in all the four seasons were not varied much. The spatial and temporal differences in physicochemical variations indicate the diversity of habitats that exist within this mangrove. Monsoon season and post monsoon have a lower temperature and salinity than the premonsoon.

All together 30 species of Perciformes fishes were identified from this pristine Muthupet mangrove ecosystem (Ramamurthy *et al.*, 2008). However, the figure is too small when compared to that of the other nearby mangrove ecosystems like Pitchavaram and Pondicherry. It is quite considerable as rich in biological diversity if Muthupet is concerned. Previous records are of reports on fishes and true microbial species and not on all the macrofauna of this mangrove ecosystem. About 52 finfish species were previously reported by Rajendiran and Kathiresan, (1998). The present investigation is the first kind recording all the diverse fish and microbes including existing microfauna of Vedaranyam mangrove ecosystem. The presence of such a biologically diverse life forms especially in this recently emerged ecosystem indicates the productive well being of this pristine Vedaranyam mangrove ecosystem. However, this report lags behind in filling the species index of soil and water invertebrates. In addition to these, more biologically diverse microorganisms present there are yet to be identified and documented.

The highest species richness was recorded in monsoon. Together with pre and post monsoon, the mangrove least influenced these seasons. This finding agrees with earlier findings that estuaries and lagoons have a smaller number of species than the surrounding regions (Allen, 1982). Fish assemblages it seasonal monsoons were similar. These seasons were also similar with respect to hydrographic conditions and the number of species.

The high number of species recorded in spring shows that the Vedaranyam mangroves were an important spawning and nursery area. The nursery function of estuaries, lagoons and inshore waters has been well documented throughout the world (Lenanton, 1982; Robertson and

Duke, 1987; Blaber and Milton, 1990; Tzeng and Wang, 1992; Biagi *et al.*, 1998). The more number of individuals in monsoon was primarily attributed to *Mugil cephalus* and *Siganus lineatus*, which are euryhaline and tolerate a wide range of salinity (Fouda, 1995). After the spring summer spawning of *Lutjanus madras* (Jardas, 1996), the lagoon fills with juveniles. A similar seasonal migration of *Mugil cephalus* was observed in the water system of the Camarque in southern France (Rosecchi and Crivelli, 1995). A high contribution of *A. boyeri* to fish assemblages was also noted by Guidetti and Bussotti, (2002). The great number of individuals in autumn and winter can also be attributed to the abundance of species of the Mugilidae family that peaks in October (Claridge and Potter, 1985). Other species were more abundant in spring and early summer, when juveniles migrate to deeper waters to avoid extremely high or low temperatures in shallow coves (Kraljeviae and Pallaoro, 1991).

The Vedaranyam mangrove is an essential biotope for some jeopardized populations (*Ambasis commersoni*, *Atule mate*, *Johnius carutta*, *Kathala axilaris*, *Lutjanus russelli*, *Monodactylus argenteus&Sillago sihama*) that require conditions exclusive to mangroves. Such locations are devastated mainly by anthropogenic influences. *Mugil cephalus* is already an alarmingly thinned and imperiled mangrove species (Ramamurthy *et al*., 2008). The other above-mentioned species are similarly, though not as critically, imperiled due to the rarity of estuary and lagoon biotopes along the eastern coast. Their populations are thinned, unconnected, and widely separated. *A. boyeri* population of the Mesolongi and Etolikon lagoons in western Greece is slightly overexploited (Leonardos and Sinis, 2000).

Table. 1. Physico-chemical analysis of Vedaranyam mangrove water

S. No	Parameters	Monsoon	Postmonsoon	Summer	Premonsoon
1	pH	7.8	8.2	8.5	8.0
2	Atmospheric Temp. (^{0}C)	30	32	36	33
3	Surface water Temp. (^{0}C)	29	30	34	31
4	Alkalinity	17.5	25.2	27.6	22.4
5	Free carbon dioxide	1.2	1.5	1.8	1.6
6	Dissolved Oxygen	7.9	8.3	5.2	5.4
7	Ammonia	11.5	10.7	10.0	10.5
8	Nitrate	25	20	15	17
9	Nitrite	9	7	5	8
10	Total hardness	1155	1200	1340	1250
11	Calcium	845	880	971	905
12	Magnesium	310	320	369	345
13	Chloride	89	96	98	95
14	Salinity (ppt)	24	25	29	26
15	Total Phosphorus	1.535	1.269	0.925	1.299
16	Inorganic phosphorus	0.992	0.865	0.620	0.899
17	Organic phosphorus	0.558	0.454	0.310	0.434
18	BOD	06	09	10	07
19	COD	54	61	66	60

Monsoon (Oct-Dec); Post Monsoon (Jan-Mar); Summer (Apr-June); Pre Monsoon (July-Sep)
* Except pH and temperature all values expressed in mg^{-1}

Table. 2. Classified list of fish species of the class osteichthyes and order perciformes identified at Vedaranyam mangroves

S. No	Family	Species
1	Ambassidae	**Ambasis commersoni**
2	Carangidae	*Atule mate*
3	Gobidae	*Boleophthalmus boddarti*
4	Carangidae	*Carangoides chrysophrys*
5	Serranidae	*Epinephelus suratensis*
6	Leiognathidae	*Gazza minuta*
7	Gerridae	*Gerrus filamentosus*
8	Sciaeridae	*Johnius carutta*
9	Sciaeridae	*Kathala axilaris*
10	Leiognathidae	*Leiognathus bindus*
11	Leiognathidae	*Leiognathus brevirostris*
12	Leiognathidae	*Leiognathus splendens*
13	Lutjanidae	*Lutjanus argentimaculatus*
14	Lutjanidae	*Lutjanus madras*
15	Lutjanidae	*Lutjanus russelli*
16	Monodactylidae	*Monodactylus argenteus*
17	Mugilidae	*Mugil cephalus*
18	Sciaenidae	*Nibea maculata*
19	Haemulidae	*Platycephalus gibbosus*
20	Haemulidae	*Platycephalus vittatus*
21	Scatophagidae	*Scatophagus argus*
22	Siganidae	*Siganus canaliculatus*
23	Siganidae	*Siganus juvus*
24	Siganidae	*Siganus lineatus*
25	Sillaginidae	*Sillago sihama*
26	Terapontidae	*Terapon jarbua*
27	Terapontidae	*Terapon puta*
28	Mullidae	*Upeneux vittatus*

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COMPARATIVE PHYTOCHEMICAL SCREENING OF *BOERHAAVIA DIFFUSA* LEAVES AND ROOTS

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ABSTRACT

Today's world is full of huge numbers of different diseases because of our hasty schedule, and to treatment of those diseases, a huge number of medicines are taken every day to control and cure those diseases. Therefore, willingly or unwillingly, we are required to accumulate a large amount of chemicals which are lethal and unsafe for our body. Consequently, these stored chemicals produce another disease in our body, and to alleviate it, another medicine is needed which adds up more poisonous chemicals which were previously stored in our body. For this reason, the uses of natural or herbal medicines are increasing day by day due to their biodegradable nature. *Boerhaavia diffusa* commonly known as punarnava is a perennial herbaceous plant belonging to family Nyctaginaceae widely studied and has a long history of therapeutic uses by the indigenous and tribal people and also in Ayurvedic and Unani medicines. The chemical presents in the leaves and root of *Boerhaavia diffusa* L. which makes it as outstandingly useful plant is now been traced out. The present paper provides a detailed account of phytochemical analysis of leaves and root of *Boerhaavia diffusa* linn.

Keywords:*Boerhaavia diffusa* L., Phytochemicals, Pharmacological properties, Ayurveda.

INTRODUCTION

Plant materials are a rich source of biologically active metabolites. The active secondary metabolites produced by some of these plants have potential bioactive compounds of interest in the pharmaceutical industry. Plant-derived substances have recently become of great interest due to their applications as drugs, as model compounds for drug synthesis or as intermediates for synthetic drugs [Farnsworth, 1998, Ncube *et al*., 2008]. The WHO (World Health Organization) [WHO,2001] defined medicines from plants as herbal preparations produced by extraction, fractionation, purification, concentration or other physical or biological processes which may produce nutritional or medical compounds for immediate consumption or as a basis for herbal products.

Some authors defined Medicinal plants as plants containing active ingredients used to cure disease or relieve pain [Okigbo, 2008]. Nearly 50,000 [Schippmann, 2002] species of higher plants have been used for medicinal purposes. Plants offer a large number of natural compounds belonging to different molecular families which have various biological activity in humans. The plant-based, traditional medicines continue to play an important role in health care, with about 80% of the world's inhabitants depending mainly on traditional medicines for their primary health care [Owolabi *et al*., 2006]. Eleven percent of 552 drugs are considered as basic and essential by the WHO originate from plants [Rates, 2001]. Plants contain a variety of valuable substances useful as food additives, cosmetics, perfumes, and for medical treatment of various diseases [Mukherjee and Wahile, 2007]. In view of the increasing development of resistant microorganisms, treatment of various diseases caused by microorganisms has become a major challenge in the human medical field and it needs to offer continuously new compounds with potential activities. Modern pharmacopoeia contains at least 25% drugs that are derived from plants, which are synthetic, and built on compounds isolated from plants [Lucy and Edgar, 1999].

Modern medicine has evolved from folk and traditional medicines through chemical and pharmaceutical screening. Synthetic medicine can cause side effects and as a result people are more favorable to use natural compounds obtained from plants. Thus, plants remain a major source of medicinal compounds. Phytochemical analysis of plants used in traditional medicines has yielded a number of compounds with various pharmacological activities. Seventy four percent of 119 plant derived drugs were discovered as a result of chemical studies to isolate the active substances [Farnsworth, 1985].

The use of plant-based natural products in the treatment and prevention of diseases and health enhancement has led to the significant attention of the scientific community and the public nowadays. The availability of these medicinal plants provides a cost-effective source with lesser side effects to develop new drugs has drawn much attention among the researchers. Plant-based traditional medicine has a long history since ancient civilization and uses plant materials as a major ingredient in synthesizing drugs [Chanda and Dave 2009]. It is a widely accepted fact that the rapid development of deriving pharmacologically active drugs from medicinal herbs has a tremendous impact on current medicinal practices.

Boerhaavia diffusa

Boerhaavia diffusa is the most important herb for the life, commonly known as Punarnava in Sanskrit, is an herbaceous plant of the family Nyctaginaceae. The whole plant of *Boerhaavia diffusa* and its specific parts i.e. leaves, stem, and roots are known to have medicinal properties and have a long history of traditional use by indigenous and tribal people in India and is used throughout India. The curative value of *Boerhaavia diffusa* linn or punarnava in the management of a various number of clinical diseases is proved in Charaka Samhita, Sushruta Samhita and Ayurveda. *Boerhaavia diffusa* linn has several ethanobotanical uses such as the leaves are used as vegetable and the roots juice is used to cure asthma, urinary disorders, leukorrhea, rheumatism, and encephalitis. *Boerhaavia diffusa* is a chief medicinal plant of Kumaun Himalaya, have different pharmacological activities and used as a medicine in Ayurvedic, Unani, Siddha and Homoeopathy Systems. *Boerhaavia diffusa* plant was named in honor of Hermann Boerhaave, a well-known Dutch physician of the 18th century. The plant of *Boerhaavia diffusa* is mentioned in the Atharvaveda with the name ‘Punarnava’, because the top of the plant dries up for the period of the summer season and regenerates again during the rainy season. It is called as Punarnava (Punar + nava) because of Punar means - once again, Nava means - becoming new. This is also known as spiderlings as this plant grows low and spreads like spider. [Mayur *et al*., 2017]

Figure 1- Leaves of *Boerhaavia diffusa*

Figure 2- Roots of *Boerhaavia diffusa*

Scope of the Research Work

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 20,000 plants have been studied during the last 5 year period. Two important fact to focus on medicinal plant research is one on nutraceutics, in which research on plants that form a part of our normal diet has been compiled irrespective of activity and the second on phytochemical studies which are associated with pharmacological activity (Dahanukar *et al*., 2000) Keeping in view, present study was to investigate the phytochemical analysis of *Boerhaavia diffusa* extract in relation to biological activities.

The following are main objectives

- To investigate the qualitative and quantitative phytochemical analysis in *Boerhaavia diffusa* leaf extract.
- To investigate the qualitative and quantitative phytochemical analysis in *Boerhaavia diffusa* root extract.

MATERIALS AND METHODS

Boerhaavia diffusa were collected in Mannargudi, Tiruvarur District, Tamil Nadu, India. The *Boerhaavia diffusa* were first washed well and dust was removed from the Plant. Plant was washed several times with distilled water to remove the traces of impurities from the Plant. The plant was dried at room temperature and coarsely powdered. The powder was extracted with aqueous and ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Phytochemical screening

The powdered samples were analysed for the presence of various phytochemicals using the following standard methods (Debiyi and Sofowora, 1978; Trease and Evans, 1989; Sofowora, 1993 and Roopashree *et al*., 2008).

RESULTS AND DISCUSSION

Plants have basic organic process importance by their content of macromolecule, sugar, fats and oils, minerals, vitamins and water for growth and development in man and animals.

Phytochemical simply means plant chemicals. Secondary metabolites are reported to have many biological and therapeutic properties. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah *et al*., 2012).

Phytochemical screening of Leaves of *Boerhaavia diffusa*

Eleven phytochemicals were investigated in aqueous and ethanol extract of *Boerhaavia diffusa*. Results showed the presence of tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, anthroquinone, polyphenol, and glycoside in both extract. Alkaloids and anthocyanins were present only in ethanol extract. Among the two extracts, ethanol extract has rich content in phytochemicals and used for subsequent studies. The phytochemical characters of the *Boerhaavia diffusa*investigated and summarized in Table 1.

Identify and isolate novel phytocompounds from B. diffusa has led many researchers to discover various compounds such as flavonoids, alkaloids, glycosides, steroids, triterpenoids, lipids, lignans, carbohydrates, proteins, and glycoproteins from its leaves, stems, seeds and roots. Ghosal (1910) was the pioneer to study and characterize the phytochemical properties of B. diffua. Later on a number of researchers isolated and characterized the active principles of B. diffusa extracts. Selectively Carbohydrate. flavonoids, tannins, glycosides, terpenoids and saponins were estimated quantitatively and given in (Table 2). The plant is rich in the saponin content when compared to other phytochemicals.

Table.1: Preliminary Qualitative phytochemical screening of Leaves of *Boerhaavia diffusa*.

S. No	Test analysis	Aqueous	Ethanol
1	Tannin	+	++
2	Saponin	++	++
3	Flavonoids	+	+
4	Steroids	+	++
5	Terpenoids	++	++
6	Triterpenoids	+	++
7	Alkaloids	-	+
8	Anthroquinone	+	++
9	Polyphenol	++	++
10	Glycoside	+	++
11	Anthocyanins	-	+

(-) Absent, (+) Present and (++) High concentration

Table.2: Quantitative analysis of phytochemicals

S. No	Phytochemicals	Results (mg/gm)
1	Carbohydrate	43.1 ± 0.3
2	Flavanoid	38 ± 0.23
3	Saponin	50 ± 0.9
4	Glycoside	33.1 ± 0.3
5	Terpenoid	19 ± 0.4
6	Tannin	47 ± 0.43

Values expressed as Mean ± SD for triplicates

Phytochemical screening of Roots of *Boerhaavia diffusa*

Eleven phytochemicals were investigated in aqueous and ethanol extract of *Boerhaavia diffusa* roots. Results showed the presence of High amount of tannin, saponin, flavonoids, steroids, terpenoids, triterpenoids, anthroquinone, polyphenol, and glycoside in both extract. Alkaloids and anthocyanins were present only in ethanol extract. Among the two extracts, ethanol extract has rich content in phytochemicals and used for subsequent studies. The phytochemical characters of the *Boerhaavia diffusa*investigated and summarized in Table 3. Selectively Carbohydrate, flavonoids, tannins, glycosides, terpenoids and saponins were estimated quantitatively and given in (Table 2). The root is rich in the Flavonoid content when compared to other phytochemicals.

Table.3: Preliminary Qualitative phytochemical screening of Roots of *Boerhaavia diffusa*.

S. No	Test analysis	Aqueous	Ethanol
1	Tannin	+	++
2	Saponin	++	++
3	Flavonoids	++	++
4	Steroids	+	++
5	Terpenoids	++	++
6	Triterpenoids	+	++
7	Alkaloids	-	++
8	Anthroquinone	+	++
9	Polyphenol	++	++
10	Glycoside	+	++
11	Anthocyanins	-	++

Table.4: Quantitative analysis of phytochemicals

S. No	Phytochemicals	Results (mg/gm)
1	Carbohydrate	36 ± 0.3
2	Flavanoid	65 ± 0.23
3	Saponin	50 ± 0.9
4	Glycoside	43.1 ± 0.6
5	Terpenoid	29 ± 0.5
6	Tannin	57 ± 0.43

SUMMARY AND CONCLUSION

The bioactive compounds like flavonoids, alkaloids, phenolic compounds, saponins, tannins, terpenoids, glycosides, steroids etc. are present in the extracts of *Boerhaavia diffusa* or its individual parts like leaves, and roots. These bioactive compounds are responsible for the healing effects of *Boerhaavia diffusa* against a number of human ailments like cancer, diabetes, inflammation, harmful radiations induced damage, gastrointestinal problems, microbial infections, prostatic hyperplasia, liver disorders, cardiac problems, anxiety problem etc. So, *Boerhaavia diffusa* which is used in traditional medicines, now its pharmacological potential is validated by a number of scientific experiments. But, further extensive research is needed to increase the acceptance and inclusion of *Boerhaavia diffusa* in mainstream medicines.

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TANNERY EFFLUENT TREATMENT BY FREE AND IMMOBILIZATION BACTERIA

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ABSTRACT

In our study the bacterial diversity and treatment of effluent by using free and immobilized bacteria. For treating the Tannery industry effluent,Pseudomonas putidawas selected based on the screening process. For immobilizing the bacteria sodium alginate was used as the carrier material .From the present investigation the following observation were made. Totally 6 species of bacteria were isolated from the effluent. Among the isolated bacteria Pseudomonas was the dominant in the effluent. Except dissolved oxygen, all other parameters showed decreased level when compare to control.DO level was increased to free and immobilized cell when compared to control. The BOD and COD level were reduced nearly 75% and 65% respectively on 15^{th} day with immobilized cells. Nitrate and nitrite level were reduced nearly 50% with free cell. Ammonia level was reduced 50% with immobilized cell.The total organic and inorganic phosphates were decreased on 15^{th} day incubation.

Key words: effluent, Immobilization, Pseudomonas, dissolved oxygen and Ammonia

INTRODUCTION

Water pollution is prime cause of unavailability of the suitable water for irrigation purpose. Since many industries discharge their effluent on to open lands because of high cost of dilution and inadequate treatment facilities effective and profitable utilization of the effluent of the industries needs greater attention. The rapid industrialization is accompanied by both direct and indirect adverse effect on environment. Industrial development results in the generation of industrial effluents and if untreated, results in water sediment and soil pollution. It has been observed that a wide majority of industries discharge untreated effluent into river and only 10 % industries surveyed had primary treatment plants ranging from oxidation tanks, sedimentation tanks in developing countries.

In the waste water industries uncertainty exists regarding the bacteria involved in denitrification as well as the extent to which these bacteria contribute to nitrate and nitrite reduction under anoxic conditions. It is generally presumed that *Pseudomonas sp*., as well as being involved in EBPR are the predominant microorganisms through which dentrification is achieved. According to Otlanabo (1993) various species of *Achromobacter, Agro bacterium, Alcaligens, Bacillus, Chromo bacterium, Flavobacterium, Hyphomicrobium, Pseudomonas, Vibrio* and others are responsible for denitrification in soil. It therefore seems unlikely that only *Pseudomonas sp.*, are responsible for denitrification occurring in such an incredibly diverse microbial consortia as that of activated sludge.

High level of pollutants mainly organic matter in river water causes an increase in biological oxygen demand, chemical oxygen demand, total dissolved solids, total suspended solids and faecal coliforms. They make water unsuitable for drinking, irrigation or any other use. When the effluent is released into the environment without proper treatment, it alters the characters of ecosystem. Farmers are using these raw effluents for irrigation and found that the growth, yield and soil health are reduced (Nandy and Kaul, 1994).

In the waste water industries uncertainty exists regarding the bacteria involved in denitrification as well as the extent to which these bacteria contribute to nitrate and nitrite reduction under anoxic conditions. It is generally presumed that *Pseudomonas sp*., as well as being involved in EBPR are the predominant microorganisms through which dentrification is achieved. According to Otlanabo (1993) various species of *Achromobacter, Agro bacterium, Alcaligens, Bacillus, Chromo bacterium, Flavobacterium, Hyphomicrobium, Pseudomonas, Vibrio* and others are responsible for denitrification in soil. *Pseudomonas sp.*, are responsible for denitrification occurring in such an incredibly diverse microbial consortia as that of activated sludge.

MATERIALS AND METHODS

Source of effluent

For the present study the effluent samples were collected from tannery Industry, Trichy, Tamil Nadu, India. Samples were collected in large sterilized bottles and bought to the laboratory. Physico-chemical characteristics were done on the same day when the samples were brought to the laboratory. The effluent samples were filtered through cotton to remove suspended solids.

Isolated of bacteria

Ten ml of the effluent sample was taken in a 250ml conical flask containing 90ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and transferring serially 10ml of the effluent suspension to 90ml of sterile distilled water made different dilutions viz., 10-1, 10-3, 10-4 and 10-5. One ml of 10(-5) dilution was plated in petridishes containing nutrient agar medium.

Composition of nutrient agar medium

Peptone	-	5g
Beef extract	-	3g
NaCl	-	5g
Agar	-	15g
Distilled water	-	1000ml

The pH of the medium was adjusted to 7.The inoculated plates were incubated at 25 ± 2°C for one or two days and bacteria appearing over the medium were picked up and mounted on a clean slide, stained with crystal violet, Grams iodine and safranine and observed under the microscope. The bacteria were identified based on colony characteristics. Gram staining methods and by various biochemical tests as given by Bergey's (1984) manual of Determinative Bacteriology.

Biochemical tests

The physiological and biochemical tests were conducted following the methods of Somasegaran and respectively, as described by Cappuccino and Sherman (1999) to identify the bacteria.

Immobilization of Bacteria in Alginate beads (Fig.1)

Sodium alginate was used as the immobilization agent for bead preparation. Exponentially growing cells were harvested by centrifugation (5000x g for 10 min) and resuspended in 50ml of sterile water. To this 50ml of 4% alginate solution was mixed thoroughly to get a final alginate concentration of 2%. The alginate-bacteria mixture was then added drop

use into CaCl2 (0.1 M) solution. The beads were kept in the same solution for 30 mins at 4c for hardening.

Experimental condition (Fig2)

For the present study the following treatments were employed

I. Effluent sample (100ml) without inoculation-control
II. Effluent sample (100ml) inoculated with free bacteria-treatment
III. Effluent sample (100ml) inoculated with immobilized bacteria-treatment
IV. The experiment was conducted in batch cultures in duplicates for a total period of 15 days in 250ml Erlenmeyer flasks. Effluent samples (control and treated) were periodically (every five days) analyzed for various physico-chemical parameters and recorded.

Physico-chemical analysis of effluent (APHA, 1991)

The water pH, alkalinity, ammonia, dissolved oxygen, nitrate, nitrite, calcium, magnesium, free CO2, COD, total phosphorus, organic phosphate, inorganic phosphate and chloride was estimated with standard methods (APHA, 1991).

RESULTS

For the present study, the bacterial flora and physic-chemical analysis of effluent were carried out. For effluent treatment by free and immobilized bacterial cell of *Pseudomonas pudita* was selected based on screening process.

Bacterial flora in the effluent:

Bacteria were isolated from the effluent by serial dilution techniques. Then the isolated bacteria were identified through number of various biochemical tests (Table.1). totally 6 species of bacteria such as *Pseudomonas putida, P. fluourescens, Klebsiella pnemoniae, Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* were identified from the effluent sample. Among this genus *Pseudomonas sp.,* dominated with two species (Table. 2).

Physico-chemical characteristics of effluent

The physic-chemical characteristics of Tannery industries effluent were recorded from different experimental condition. Among the observation except dissolved oxygen, all other parameters showed decreased level when compare to control. Except nitrates and nitrite all the parameters were reduced in immobilizes condition (Table.3).

Table 1. Biochemical characteristics of isolated bacteria

S.No	Biochemical Test	*P.pudita*	*P.flurescens*	*K.pnemoniae*	*E.coli*	*S.aureus*	*B.subtilis*
1	Mac Conkey agar test	+	+	+	-	-	-
2	Indole test	-	-	-	+	-	+
3	Methyl red test	+	+	-	+	+	+
4	Voges proskauer test	+	+	+	-	+	-
5	Citrate utilization test	-	-	-	-	-	-
6	Starch hydrolysis test	+	+	+	-	+	-
7	Urea hydrolysis test	-	-	+	-	-	-
8	Nitrate reduction test	-	-	-	+	+	+
9	Hydrogen sulphide	+	+	-	-	+	-
10	production	+	+	+	+	-	+

11	Cytochrome oxidatase	-	-	-	+	-	+

Table.2 Bacterial flora from the sample

Sl.No	Name of the Bacterial species
1	*Pseudomonas putida*
2	*Pseudomonas florescence*
3	*Klebsiella pneumonia*
4	*Escherichia coli*
5	*Bacillus subtilis*
6	*Staphylococcus aureus*

Table.3 Physico-chemical characteristics of effluent

Parameters	Initial	5th Day			15th Day		
		Control	Treated		Control	Treated	
			Free cell	Immobilized cell		Free cell	Immobilized cell
pH	6.8	6.7	6.7	6.7	6.7	6.7	6.7
Free CO2	31	31	20	17	30	10	6
Alkalinity	108	108	97	75	107	25	18
DO	1.3	1.3	1.3	1.4	1.3	1.4	1.7
Nitrate	90	90	77	73	88	42	46
Nitrite	48	48	38	31	46	21	29
Ammonia	42	42	35	32	40	28	22
Phosphate	120	116	101	95	114	78	64
Inorganic	65	55	49	46	54	39	32
Organic	55	51	48	40	50	28	21
Calcium	83	79	59	62	76	56	42
Magnesium	64	62	51	47	61	30	24
Chloride	57	54	52	49	52	45	41
BOD	340	340	300	260	340	120	90
COD	610	610	530	480	610	380	220

The pH was recorded in the effluent initially 6.8 and it was brought down to 6.7 in treated effluent. The initial free CO2 was recorded 31 mg l-1 and it was reduced slightly from 5th day onwards. The maximum reduction was observed in 15th day (6 mg l-1) in treated effluent. The alkalinity was recorded 108 mg l-1 initially. It was brought down from 5th onwards. The maximum (45%) percentage reduction was observed in 15th day in effluent treated with immobilized cell (Fig.3). The DO level was increased in control and treated effluent. The DO level was increase 1.3 to 1.7 mg l-1 on 15th day (Table.3) in immobilized bacteria inoculated effluent (Fig. 4).The Nitrate, Nitrite and Ammonia were recorded initially 90, 48 and 42 mgl-1 respectively. On 15th day Nitrate and Nitrites were reduced nearly 50% in free bacterial cell and 45% in immobilized bacterial cell and Ammonia was reduced 50% in immobilized cell and 43% in free cell when compared to control (Fig. 5).Total inorganic and organic phosphate levels observed and recorded in both control and treated effluent. All the forms of phosphate were reduced from 5th day onwards. The maximum amount of reduction was observed in 15th day of immobilized cell when compared to control (Fig. 6).The calcium level was recorded initially 83 mgg l-1. It was reduced to 42 mg l-1 on 15th day. The percentage reduction was nearly 50% than

control .The similar trend was noticed in magnesium levels as in calcium. The 48% reduction was observed on 15th day onwards. When compared to control .The Chloride level was reduced in 41 mg l-1 on immobilized cell and 45 mg l-1on free cell at 15th day of incubation (Fig.7).The BOD and COD levels were recorded 340 mg l-1 and 610 mg l-1 initially it was reduced to 90 mg l-1 and 220 mg l-1 respectively on 15th day (Fig. 8).

Fig. 3 Level of pH, CO2 and Alkalinity of effluent on different experimental periods

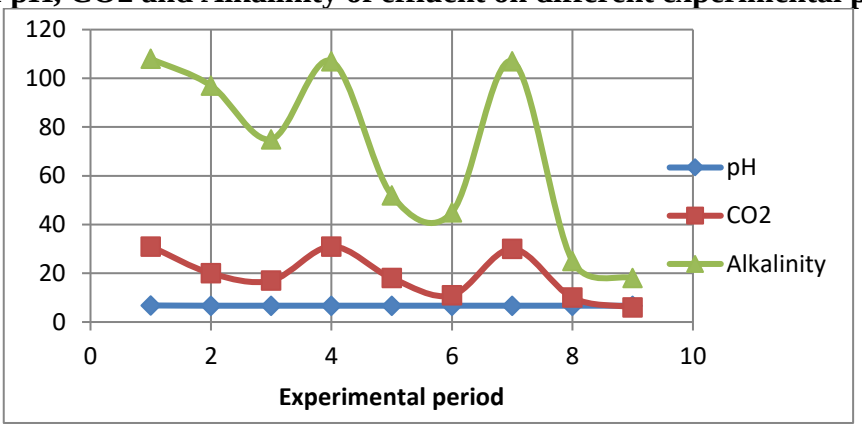


Fig. 4 Level of DO in effluent on different experimental periods

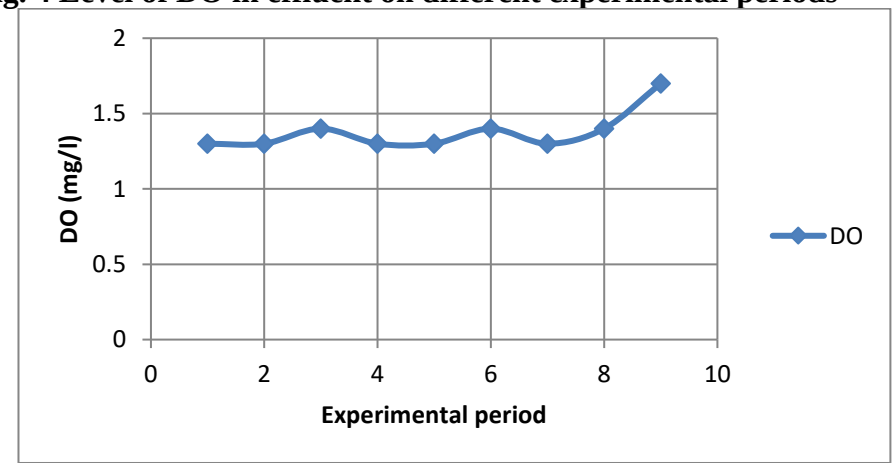


Fig. 5. Level of NO2, NO3 and NH4 of effluent on different experimental periods

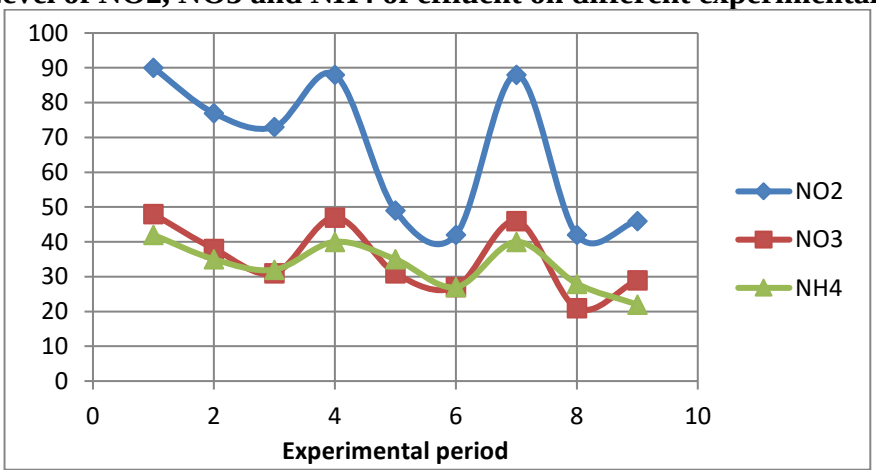


Fig. 6. Level of Total PO4, Organic and Inorganic PO4 of effluent on different experimental periods

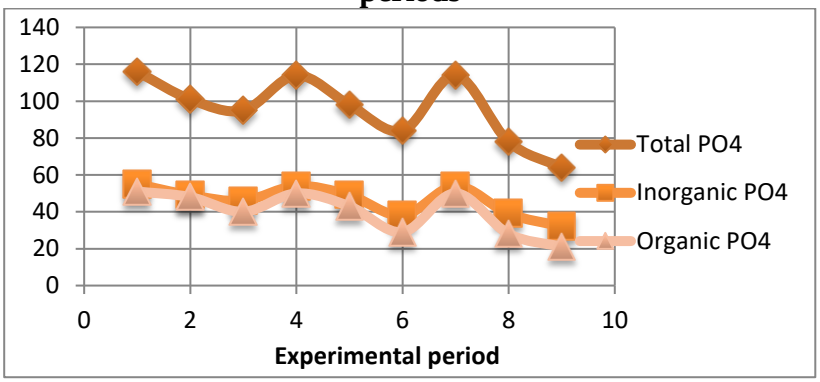


Fig. 7 Level of Ca, Mg and Cl of effluent on different experimental periods

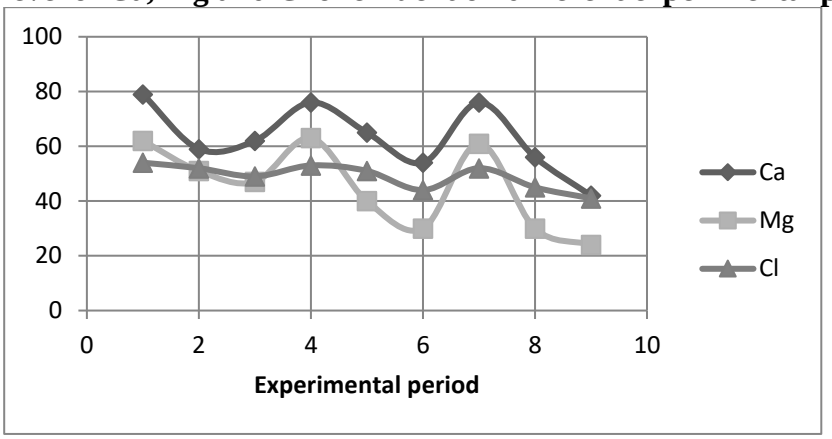
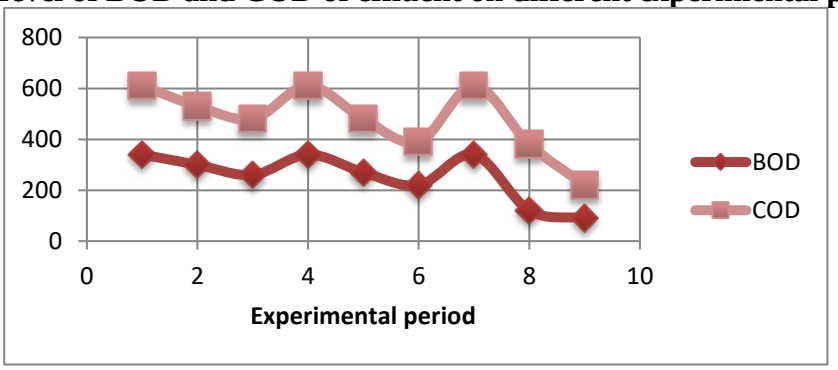


Fig. 8 Level of BOD and COD of effluent on different experimental periods



DISCUSSION

The primary purpose of wastewater treatment is to remove the suspended and soluble organic constituents measured as chemical oxygen demand (COD) or biochemical oxygen demand (BOD). Biological treatment processes are used to degrade the organics in the wastewater before it is discharged. The most common biological process for wastewater treatment, the microbes is suspended with the wastewater. In order for this process to work effectively, the biomass must be separated from the water and this is accomplished by gravity setting in a 'final clarifier'. To effectively settle, the microbes must flocculate, and then aggregate into units large enough and dense enough to settle out of solution. If the biomass does not flocculate well, some microbes will end up in the effluent (supernatant turbidity). Furthermore,

the characteristics of the flocculated biomass will have important impacts on the biomass (sludge) disposal process.

In order know the bacterial diversity in effluent, effluent sample was collected from tannery industry. From this totally 6 species of bacteria such as *Pseudomonas putida, P. fleorescens, Klebsiella pneumoniae, Escherichia coil, Staphylococcus aureus* and *Baccillus subtilis* were isolated. Among the genus *Pseudomonas* was found dominant group of bacteria. It is confirmed and supported by earlier finding of Ramlake and Bhattachrjer (1992). They also suggested the polluted habitats found mostly *Pseudomonas* because it having ability to degrade various pollutants from water samples.

Bacterial growth is highly dependent on the amount of inoculation added and it was very difficult to remove from the water sample. Hence the present investigation was carried out by using free and immobilized bacteria. For immobilization sodium alginate was used. Remesh and Singh (1993) reported the immobilized bacteria having more efficiency to remove the suspended particles than free cells.

For the evaluation of the pollution load of industrial or domestic wastewaters, a measure of oxygen requirement of pollution matter has been developed as standard parameters, which is known as biochemical Oxygen Demand (BOD). For the present investigation the BOD level was recorded 340 mg/l initially. Before discharging any industrial effluent should be removed the BOD because it adversely affect the aquatic organisms (Gurjar, 1994). In this present study the BOD level was reduced on 15^{th} day nearly 75% it is inconformity the previous reports of Gurjar (1994). He reported the bacterial culture removed BOD almost completely on two weeks from the date of inoculation in the water samples. The BOD removal was higher than free cell to immobilized cell.

Mythili, *et al.*, (2011) reported that the effluent treatment, heavy metalresistant bacteria were isolated from tannery effluent. Among them, *Bacillus, Pseudomonas* and *Micrococcus* were selectedfor the further studies. The selected isolates were tested for their efficiency on the bioremediation of tannery effluent. Thephysico-chemical properties such as colour, odour, pH, electrical conductivity, total solids, suspended solids, dissolved solids,BOD, COD, chromium, zinc, iron, nickel were found decreased in effluent after 72 hrs. The present report suggested that the BOD and COD level were reduced nearly 75% and 65% respectively on 15^{th} day with immobilized cells

In the present report revealed that the Biological oxygen demand which indicated the high pollution level have decreased significantly indicating that pollutants level have been reduced. The phenolic content was found to be decreased after treatment; it indicates the utilization of phenol as a carbon source by the isolate. The decrease in level of COD indicates the reduction of biologically oxidisable and inert organic materials as result of the degradation by the *Pseudomonas sp.* The result revealed that the Pseudomonas sp. Isolated from the effluent is efficient enough to degrade the tannic components and it is useful to make the effluent non toxic after treatment, and these waste waters can be reused and certainly this biodegradation study will be helpful to some extent for making a pollution free environment.

CONCLUSION

From the above observation it is conclude that the compare with immobilized bacterium *Pseudomonas putida* than free cell having good efficiency of treated various industrial effluent at

different time. In the present investigation all the parameters were reduce the 15th day of incubation .In the concluded that above report the long time exposure was giving more efficient on effluent treatment.

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ANTI-ULCER ACTIVITY OF CRUDE ETHANOLIC EXTRACT OF *COLDENIA PROCUMBANS*

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ABSTRACT

The ethanolic extract of *Coldenia procumbans* leaf and root was evaluated for its anti-ulcer activity against pylorous ligation induced gastric ulcer (antisecretory) in rats.We found that *C. procumbans* extract at a dose of 100 mg/kg p.o. markedly decrease the incidence of ulcers. Ethanol extract of *C. procumbans*showed significant reduction in gastric volume, free acidity, total acidity and ulcer index. The plant extract also showed gastro protective activity (55.37%), whereas standard drug Famotidine showed 70.34%.

Key words: Anti-ulcer; *Coldenia procumbans*; Pylorous ligation ulceration; Boraginaeceae.

INTRODUCTION

Ulcers are thought to be a result of an imbalance between “aggressive and defensive factors”. Acid and pepsin components form the aggressive factors and mucin layer (mucin – bicarbonate secretion, phospholipid layer, tight junction), cell proliferation, prostoglandins, the urogastrone epidermal healing factors form the defensive factors. It is widely accepted that pathogenesis of ulcer is complex and increased acid secretion, pepsin activity and reduced mucus and bicarbonate secretion, enhanced contractility of the gastric wall and reduced gastric mucosal blood flow represent some of the established pathogenic factors of gastric ulceration[1].

Most of the commonly used drugs namely antacid, anticholinergic selective M_1- blockers (pirenzepine, telenpine etc.) H_2 – blockers (ranitidine, famotidine, etc.) act by reducing the aggressive factors while carbon oxolone, sucralfate, sodium-bimuth subcitrate and prostoglandins by strengthening the mucosal resistance. Considering the several side effects (arrythmias, impotance, gynaecomastia and haematopoietic changes) associated with the use of modern medicine. Because of the wide geographical distribution of ulcer diseases in India, attempts were and are being made to look for suitable anti ulcerogenic plant products as a better alternative for the treatment of ulcer.

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[2].Over 50% of all modern clinical drugs are of natural product origin[3] and natural products play an important role in drug development programs in the pharmaceutical industry[4].

Traditionally the flowers used as emmenagogue, used in menstrual disorders. Bark is powerful astringent, and in various forms of ulceration, anti periodic in fever, heumatism, tonic,

beneficial in chronic dysentery, powdered bark externally applied to ulcer, leprosy, cures fever, headache, blood complaints (Ayurveda), cardiotonic, aphrodisiac, anthelmentic; good for scabies and expectorant[5,6].

Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extract on bacteria have been studied by a very large number of researchers in different parts of the world[7]. Much work has been done on ethanomedical plants in India[8]. Interest in a large number of traditional natural products has been increased. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents[9]. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[10].The current study was undertaken to evaluate the anti-ulcer activity of *C. procumbans*extract by pylorous ligation induced gastric ulcer, in rats.

MATERIALS AND METHODS

Collection of plants

C. procumbans belongs to the family Boraginaeceae was collected from Thanjavur District, Tamilnadu State, India and identified by the special key given `The Flora of Presidency of Madras'. Voucher sample was prepared and deposited in the Department of Botany and Microbiology, A.V.V.M Sri Pushpam College, Poondi.

Preparation of leaf and root powder

The leaf and root of *C. procumbans* washed with sterile distilled water. After, the leaves were shade dried and powdered by using Pestle and Mortar.

Preparation of extracts

25g of powder was filled in the thimble and extracted successively with ethanol using a Soxhlet extractor for 48 hrs. All the extracts were concentrated using rotary flash evaporator and preserved at 5^{o}C in airtight bottle until further use. All the extracts were subjected to anti-ulcer activity.

ANTIULCER ACTIVITY

The ulcer study was done at Periyar College of Pharmaceutical Sciences, Tiruchirappalli District, Tamilnadu, India by using their animal house facility under the guidance of Committee for the Purpose of Control and Supervision of Expeiments on Animals (CPCSEA/265).

Albino rats of either sex weighing about 100 – 130gms were divided into groups of six each. Pregnancy was excluded. The animals were deprived of food for 24 hours before the commencement of the experiment, but water was allowed adlibitum. The drugs were given orally 2 hours prior to pylorus legation, which was carried out according to the technique[11].

Testing drug:

* Group I	Untreated control	---
* GroupII	Ulcer control	5 ml/kg (5% w/v Acacia)
* Group III	*C. procumbans*leaf	100mg/kg
* Group IV	*C. procumbans*root	100mg/kg
* Group V	Famotidine	20 mg/kg

5% w/v acacia mucilage was used as a vehicle at a dose of 5 ml/kg. The solvent control received equal volume of acacia mucilage. The animals were sacrificed 6 hours after pylorus ligation. The gastric juice was collected, centrifuged and its pH and volume were measured. Free and total acidity were estimated titrimatically with 0.01N NaoH using methyl orange and phenolphthalein as indicators.

Pipette 1 ml of filtered gastric contents into a small beaker, add 2-3 drops of methyl orange and titrate with 0.01N NaoH, until all trace of the red color disappears and the color is yellowish orange. Note the volume of alkali added. Then add 2-3 drops of phenolphthalein and continue titration until a definite red tinge reappears. If a yellow color is obtained on adding methyl orange no free acid is present. Add the phenolphthalein and titrate the combined acid. This then equals the total acid. The data concerning the pH, volume, acid secretion of gastric juice and ulcer index were analyzed by student `t' test.

Total Acidity: A volume of 2 ml diluted gastric juice was titrated with 0.01N NaoH run from a micro burette using phenolphthalein as indicator and the acidity was expressed as mg. HCL/100g. body weight of rat[12].

Free Acidity: It is determined in similar manner using topfer`s reagent as indicator and sodium hydroxide was run until canary yellow color was observed[12]..

Ulcer index: Ulceration in rats was induced as described[13]. On the fourth day pylorus part was ligated following 36 h fasting[11]. Four hours after the pyloric ligation the animals were sacrificed by decapitation. The stomach was opened and the percentage inhibition of ulcer was determined[14]. A score for the ulcer was made as follows:

0: normal colored stomach.
0.5: red coloration.
1: spot ulcers.
1.5: haemorrhagic streak.
2: ulcers.
3: perforation.

Mean ulcer score for each animal was expressed as ulcer index. The percentage of ulcer inhibition was determined as follows:

Inhibition of ulcer (%)

$$\frac{\text{Control mean ulcer index-test mean ulcer index}}{\text{Control mean ulcer index}} \times 100$$

RESULT AND DISCUSSION

The effect of alcoholic extract of *C. procumbans* leaf and *C. procumbans* root on pylorus ligated induced ulcer model is presented Table 1.It was observed that increase the ulcer index (35.4 ± 3.2) in ulcer control rats. Significant reduction in ulcer index was observed in *C. procumbans* leaf (100mg/ body weight) and *C. procumbans* root (100mg/body weight). Therefore, the decrease in the ulcer index in the *C. procumbans* leaf and *C. procumbans* root extract treated groups are an ulcer indication of the ulcer curative nature of *C. procumbans* leaf and *C. procumbans* root. Our results concordant with earlier report[15].

It is significant to note that increase the volume, total acidity and free acidity and decreased pH of gastric juice were observed in ulcer control rats compared to untreated control

rats. The severity in terms of volume of volume, total acidity and free acidity showed decreased in-group V animals when compared to those in groups III and IV. The increased pH of gastric juice was also observed in group III and IV animals when compared to that in-group V.

The increase in volume in the ulcer control rats undoubtedly due to increased production of hydrochloric acid as is evident from the total acidity and decrease pH value of gastric juice. Inauen *et al.*,[16] have reported that inhibition of acid secretion accelerated ulcer healing. The decrease in volume of the gastric juice and concomitant decrease in the acidity and increase in pH, proving the anti-ulcer activity of *C. procumbans* leaf and *C. procumbans*root.

Preliminary phytochemical investigations of the ethanol extract of *C. procumbans* showed positive test for Liberman burchard test (sterols) and the extract treated with tin and thionyl chloride appears pink color indicates the presence of terpenoids, hence the anti-ulcer activity of *C. procumbans* in this experimental model may be due to the terpenoids and steroids. The results demonstrated that *C. procumbans* extract produced antiulcerogenic effects possessing antisecretory, cytoprotective and proton pump inhibition mechanism. This interesting observation indicates that *C. procumbans* extract can be a potential source for the treatment of ulcer. However, detailed study like isolation of active molecule and characterization is required to confirm the phytochemicals responsible for the activity.

Recent reports have indicated that many flavonoids possess antiulcerogenic activity[17]. So the anti-ulcer activity of alcoholic extract of may due to its flavonoids content.In this study we observed that alcoholic extract of both *C. procumbans*leaf and *C. procumbans*root provides significant anti-ulcer activity.

Table 1: The effect of *H. indicum*leaf and root (100 mg/kg) and Famotidine (20ml/kg) on gastric ulcers

S. No	Groups	Volume of Gastric juice (ml/100g)	pH	Total Acidity (mEq/l)	Free Acidity (mEq/l)	Ulcer Index (mm in length)
1	Untreated control	0.4±0.03	4.2±0.16	30.1±1.0	15.9±0.9	10.2±0.8
2	Ulcer control	2.8 ± 0.04	1.3 ± 0.07	98 ± 7.3	77 ± 6.3	35.4 ± 3.2
3	*H. indicum* leaf (100 mg/kg)	0.51** ± 0.03	3.4* ± 0.25	36.3** ± 2.8	31.8** ± 1.8	15.1* ± 1.8
4	*H. indicum* root (100 mg/kg)	0.68* ± 0.03	3.1** ± 0.10	44.2* ± 0.0	31.2* ± 0.1	20.5* ± 1.0
5	Famotidine (20mg/kg)	0.40**± 0.02	3.80** ± 0.18	27.3** ± 1.0	15.2** ± 0.9	10.1** ± 0.8

*****P< 0.01 vs ulcer control
**P< 0.001vs ulcer control by student ' t' test
Values are ± S.E.M

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BIO SORPTION OF HEXAVALENT CHROMIUM FROM AQUEOUS SOLUTION BY BIOMASS OF *SPIROGYRA SPECIES*

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ABSTRACT

For the present study the filamentous algae Spirogyra sp., was obtained from local pond near Thanjavur, Tamil Nadu. This dry and wet biomass used as the removal of Cr (VI) from synthetic samples. From the present study the following observation were made. Various concentration of algal biomass, different level of pH and temperature was important for the adsorption of Cr (VI).The removal of Cr (VI) ranges from 9.5 to 53.3 % in wet condition of biomass with different concentration. The highest adsorption was recorded 54.1% in dry biomass concentration. The percent adsorption of Cr (VI) increase in 15g and decrease in 1g of biomass concentration in both experimental condition.Increase level of Cr (VI) removal was observed by dry biomass at the pH level 2.0.

Key word: *Spirogyra sp*, biomass, temperature, pH and Biosorption

INTRODUCTION

Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material (Gadd, 1993). Large quantities of metals can be accumulated by a variety of processes dependent and independent on metabolism. Both living and dead biomass as well as cellular products such as polysaccharides can be used for metal removal. Heavy metal pollution is one of the most important environmental problems today. Various industries produce and discharge wastes containing different heavy metals into the environment, such as mining and smelting of metalliferous, surface finishing industry, energy and fuel production, fertilizer and pesticide industry and application, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electric appliance manufacturing, metal surface treating, aerospace and atomic energy installation etc. Thus, metal as a kind of resource is becoming shortage and also brings about serious environmental pollution, threatening human health and ecosystem.

In biosorption, various algae were used and investigated as biosorbents for metal removal. The nature of the chlorophyll(s), the cell wall chemistry, flagellation, form in which food or assimilatory products of photosynthesis are stored, cell morphology, habitat; reproductive structures; life history patterns, etc., these characteristics can be used for the classification of algae. The important differences between brown algae and other algae are in the storage products they utilize as well as in their cell wall chemistry.

Algae are of special interest in search for and the development of new biosorbents materials due to their high sorption capacity and their ready availability in practically unlimited quantities in the seas and oceans. Algae present a high affinity for Pb, followed by chromium, cadmium, copper, nickel and zinc, all of which present very similar values. The best performer for metal biosorption by brown algae is lead (Romera *et al.*, 2006). The aim of this work is to present the state of the art of biosorbent investigation of the capability of dried and wet biomass of *Spirogyra hyalina* to remove heavy metal from aqueous solution at initial concentrations of the

heavy metals and at different exposure period (time) of wet and dry biomass and different concentration.

MATERIALS AND METHODS

Collection of biomass

The filamentous algae *Spirogyra sp*., was obtained from local pond near Thanjavur, Tamil Nadu. The algae were washed twice with tap water and thereafter with double distilled water thoroughly to eliminate adhering foreign particles like sand and debris. The 75g of washed biomass was transfer in to the plastic container and stored into the refrigerator for further studies and remaining are air dried for 24 hrs and then in an oven at 80°C to constant weight. The dried biomass was then ground in an analytical mill and then sieved through a 2 mm mesh size sieve and stored in polyethylene bottles.

Preparation of synthetic sample

A working solution of hexavalent chromium (Cr VI) (50mg/l) was prepared in distilled water with potassium dichromate.

Instrumentation

pH measurement were made using a pH meter. The temperature was measured by portable thermometer and the chromium was determined after acid digestion and was analysed using an atomic absorption spectrophotometer at a wave length of 360nm.

Experimental procedure:

pH , temperature and biomass concentrations are the factors which affects the bio sorption process. Particularly, pH (Gourdon *et al.,* 1990), temperature and biomass concentration (Gong *et al.*, 2005) on bio sorption experiments was investigated by optimization process.

Optimization for heavy metal removal

Effect of pH

The pH was varied from 2 and 5 by adjusting the medium amended with 50 mg/L of Cr. The biomass concentration was varied from 1 to 15g (1, 3, 5, 10 and 15) in the medium containing 50 mg/L of Cr. Sorption studies were conducted in 150ml conical flask at natural solution pH 2.0 and 5.0. Wet and Dry *spirogyra* species was added in different concentration (1, 3,5,10 and 15g) to separate conical flask with 100ml of chromium solution and suspension were shaken at 20 °C and 39°C. Chromium concentration was analysed in before incubation and after 7 days time interval by atomic absorption spectrophotometer.

Effect of temperature

Sorption equilibrium experiments were carried out using 500 mL shake flasks containing 100 mL Cr (VI) solution of desired concentration. After the addition of the biomass, the flasks were shaken in an overhead shaker at room temperature (20-39°C). In case of the equilibrium experiments, the flasks were shaken until adsorption equilibrium has been achieved (minimum 24h). The metal concentration in the bulk solution was determined by atomic absorption spectroscopy (Klimmek *et al.* 2001). With the help of the mass balance the adsorption capacity was calculated by the equilibrium concentration in the liquid phase.

RESULTS

The results of present chromium adsorption as a function of time interval at different concentration of *Spirogyra species* have been shown in fig.1 to 4. The sorption of chromium

increases (at varying initial concentration with various algal doses of 1 – 15g) with time from 5 to 7 days and after that become almost constant up to the end of the experiment.

Biomass concentration

The removal chromium (VI) ranges from 9.5 to 53.3 % in wet biomass with various concentrations. Than 9.5 to 54.1% of Cr (VI) was removal in dry biomass of various concentrations (Table 1 and 2). The percent adsorption of Cr (VI) increases in 15g of biomass concentration and decreases in 1g of biomass concentration in both experiments.

Effect of pH

Cr (VI) removal was studied as a function of pH at various algal doses and the result shown in fig.1 and 2. The percent adsorption of Cr (VI) increases with increase in pH 2.0 and thereafter decreases with further increase in pH 5.0 (Table 1 and 2).

Increase level of Cr (VI) removal was observed by the dry biomass (54.1 %) at the pH level of 2.0 and decreases level was observed by the wet biomass (9.5 %) at the pH range was 5.0. It is important to mention that the maximum adsorption at all the concentration takes place at pH 2.0.

Table.1 Effect of different level pH on chromium removal by various concentration of wet *Spirogyra sp.,*

S.No	Biomass concentration (g)	pH 2.0		% of chromium removal	pH 5.0		% of chromium removal
		Initial	Final		Initial	Final	
1	1	2.40	2.12	11.6	2.40	2.17	9.5
2	3	2.40	2.10	12.5	2.40	2.12	11.6
3	5	2.40	1.40	41.6	2.40	2.10	12.5
4	10	2.40	1.31	45.4	2.40	1.40	41.6
5	15	2.40	1.12	53.3	2.40	1.29	46.2

Table.2 Effect of different level pH on chromium removal by various concentration of dry *Spirogyra sp.,*

S.No	Biomass concentration (g)	pH 2.0		% of chromium removal	pH 5.0		% of chromium removal
		Initial	Final		Initial	Final	
1	1	2.40	2.10	12.5	2.40	2.18	9.8
2	3	2.40	2.05	14.5	2.40	2.12	11.6
3	5	2.40	1.30	45.8	2.40	2.09	12.9
4	10	2.40	1.25	47.9	2.40	1.40	41.6
5	15	2.40	1.10	54.1	2.40	1.15	52.0

Effect of temperature

Cr (VI) removal was observed as an effect of temperature at various concentrations of algal doses and the result shown in fig.3 and 4. Highest Cr (VI) removal was observed by the 15 g of dry biomass concentration in temperature 20 °C. The percent adsorption of Cr (VI) was reducing in 1 g of wet biomass concentration at 20 to 39 °C (Table 3 and 4).

Table.3 Effect of different level Temperature on chromium removal by various concentration of wet *Spirogyra sp.*,

S.No	Biomass concentration (g)	Temperature (20°C)		% of chromium removal	Temperature (39°C)		% of chromium removal
		Initial	Final		Initial	Final	
1	1	2.30	2.25	2.17	2.30	2.25	2.1
2	3	2.30	2.15	6.5	2.30	2.20	4.3
3	5	2.30	2.10	8.6	2.30	2.15	6.5
4	10	2.30	1.45	36.9	2.30	1.50	34.7
5	15	2.30	1.30	43.4	2.30	1.40	39.1

Table.4 Effect of different level Temperature on chromium removal by various concentration of dry *Spirogyra sp.*,

S.No	Biomass concentration (g)	Temperature (20°C)		% of chromium removal	Temperature (39°C)		% of chromium removal
		Initial	Final		Initial	Final	
1	1	2.30	2.20	4.3	2.30	2.18	3.5
2	3	2.30	2.10	8.6	2.30	2.12	7.8
3	5	2.30	2.06	10.4	2.30	2.08	11.4
4	10	2.30	1.30	43.4	2.30	1.45	36.9
5	15	2.30	1.25	45.6	2.30	1.30	43.4

DISCUSSION

The presence of heavy metal ions in surface water continues to be the most pervasive environmental issues of present time (Hotton and Symon, 1986; Nriagu, 1988). Chromium is one of the contaminants, which exists in hexavalant and trivalent forms. Hexavalent form is more toxic (Smith and Lee, 1972) than trivalent and requires more concern. It is therefore essential to remove Cr (VI) from waste water before disposal. In the present research work indicate that the biomass of *Spirogyra species* is suitable for the development of efficient bio sorbent for the removal and recovery of Cr (VI).

Earlier studies have indicated that solution pH is an important affecting biosorption of heavy metals ions (Matheikal *et al.*, 1991 and Fourest *et al.*, 1994). In the present studies stated that the percent adsorption of Cr (VI) increases with increase in pH from pH 2.0 to 5.0 and thereafter decreases with further increases in pH. It is important to mention that the maximum adsorption at all the concentration takes place at pH 2.0.

Tobin *et al.*, (1984) demonstrated a linear relationship between the maximum loading and the ion radius of the used heavy metals. These results were not confirmed by this study. Ni2+ has a smaller ionic radius (78 pm) as compared to Cd2+ (103 pm), but it was bound preferentially by the biosorbents based on immobilized *L. taylorii*. Further indications can be found for the selective uptake of the heavy metals by the biosorbents regarding the chemical and physical characteristics of the examined heavy metals. Due to the small solubility constants Ksp of the product (Lide, 2003), the precipitation concentration for lead hydroxide at a constant pH is reached first (compared with the three other metal hydroxides) and micro precipitation of lead hydroxide takes place on the surface of the algae.

The cell wall of *Spirogyra* species contains a large number of surface functional groups. The pH dependence of metal adsorbtion can largely be related to type and ionic state of these functional groups and also on the metal chemistry in solution (Matheickal *et al.*, 1999).

Adsorption of Cr (VI) below pH 2.0 suggests that the negatively charged chromium species bind through electrostatic attraction to positively charged functional groups on the surface of algal cell wall because at this pH more functional groups carrying positive charges would be exposed.

CONCLUSION

From the above results it is conclude that the rate of Cr (VI) binding with algal biomass is less at initial stages, which gradually increases an optimum period of incubation. The data of the bio sorption of Cr (VI) on algae *Spirogyra species* provided fundamental information in terms of optimum pH, temperature and algal dose for maximum removal of chromium from the solution. The study also indicated that *Spirogyra species* biomass can be used to develop high capacity bio sorbent materials for the removal and recovery of heavy metal ions from the dilute industrial waste water. Among this study investigated that the dry biomass was having more efficiency for removal of heavy metals when compared to wet biomass. Further studies are needed in understanding the interaction behaviour between the activated biomass and heavy metal ions in the contaminated sites.

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FREE RADICAL SCAVENGING ACTIVITY OF PHYTOCHEMICAL COMPOUNDS DETECTED IN *TABERNAEMONTANA DIVARICATA* (L.) STEM

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ABSTRACT

Plants have therapeutic value because of the presence of active compounds in it. So there is a need to identify the antioxidant capacity of those compounds for the eradication of infectious and chronic illnesses. Free radical scavenging assays (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, Superoxide anion radical, Nitric oxide radical, hydrogen peroxide radical) were performed in an ethanolic stem extract of *Tabernaemontana divaricata* (L.). In this study, significant free radical scavenging activity was observed in a concentration reliant way. In a higher concentration of extract (1000µg/ml), superoxide anion radicals were inhibited at 14.7% followed by DPPH radicals at 14.3%, hydrogen peroxide radicals at 7.3% and nitric oxide radicals at 4.1%. From these results, the present work confirmed that stem of *T. divaricata* (L.) can be used to treat vaious disease caused by free radicals, owing to the existence of various phytochemical constituents to exhibit antioxidant activity.

Keywords:*Tabernaemontana divaricata* (L.), Phytochemical, GC-MS analysis, Free radical scavenging

INTRODUCTION

During food storage, lipid peroxidation is the main cause for the descentation of food as well as instigates cancer and aging. Free radicals contain unpaired electrons in an atomic orbital. Two types of free radicals are formed through metabolism in numerous ways. They are Reactive Oxygen Species (ROS) as well as Reactive Nitrogen Species (RNS). Some external agents induce the production of free radicals like radiation, smoking, environmental pollutants, pesticides, etc[1,2]. The accumulation of reactive oxygen species generates immense reactive condition known as oxidative stress.

The above mentioned stress results in the destruction of various biomolecules lead to several chronic illnesses for instance obliteration of DNA, atherosclerosis, diabetes mellitus, inflammation, cancers, cardiac, and nerve problems[3-5]. So diminishing these health problems causing molecules is imperative. There is a need to reduce the formation of free radicals with the help of neutralizing compounds that are called antioxidants.

Antioxidants hold the capacity to interrupt oxidation process. It has been divided into two types. They are synthetic and natural antioxidants. BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) are exploited as synthetic antioxidants in industrially modified foods. Some investigations stated that these types of components have a number of side effects. Hence research focus is attracted to natural antioxidants owing to its availability, low cost, and fewer side effects[6-8].

Natural antioxidants are originated from an exogenous resource such as diet[9]. Search on medicinal plants has been considered because of the presence of various phytochemical constituents as natural antioxidants. The inefficiency of these natural antioxidant mechanisms

leads to the intake of dietary supplements with antioxidant compounds. So herbs are widely used to treat many health-related complaints[10,11].

Tabernaemontana divaricata (L.) is a medicinal shrub or small tree. It bestows pretty double stratum white colored aromatic flowers and emerges periodically all over the year. Leaves are looking like glossy and deep green in color. *T. divaricata* (L.) is disseminated as an ornamental plant in India. Also, it is situated in Bangladesh as well as other areas of South East Asia. It holds more beneficial actions like anti-inflammation, antioxidant and anticancer properties[12-14].

MATERIALS AND METHODS

Plant material collection:

Tabernaemontana divaricata (L.) plant was found through the book as well as literature references[16,17]. *T. divaricata* (L.) stem (Double layered flower type) were gathered from Thanjavur. The collected stem was washed and dried in gloom for 14 days. Then it was pulverized with the use of mixer into fine powder. Exracts were prepared from that powder for free radical scavenging assays.

Preparation of extract for scavenging assays:

30g stem powder had been mingled with ethanol (200 ml) and soxhlet extraction was done for 12 hours. After extraction, extracts were concentrated by evaporation, and further, it was dispersed under vacuum. The dehydrated extract was hoarded in sealed vials for scavenging assays.

DPPH radical scavenging assay[15]:

Ethanolic extract of the stem with various concentrations from 200 - 1000 µg/ml was used. Different concentrations of extract (2.5 ml) was added in 1ml ethanolic solution of DPPH (0.3 mM) and kept at room temperature. Later than 30minutes, optial density (OD) was measured at 518 nm. Blank was prepared using ethanol without extract. DPPH with 1 mM morin was taken as standard.

Scavenging capacity (%) = $[(A_0 – A_1) /A_0] \times 100$

Where, A_0 and A_1 are Control and Sample absorbance respectively

Superoxide anion radical scavenging assay[16]:

In this assay, each 3ml reaction mixture comprised 0.05 M of Phosphate Buffered Saline (PBS, pH 7.8), Methionine (13 mM), Riboflavin (2 µM), 100 µM of EDTA, Nitro Blue Tetrazolium (NBT, 75 µM) and 1 ml ethanolic extract of stem with different concentrations from 200 – 1000 µg/ml and 1 mM rutin were used as standard. All tubes were placed facing towards a fluorescent light (725 lumens, 34 W). After 20min of reaction, optical density values were taken at 560 nm. These assembled tubes were covered in an aluminium foil coated box.

Superoxide anion radical inhibition was estimated through the expression given below,

Inhibition (%) = $[(A_0 – A_1)/A_0] \times 100$

Nitric oxide (NO) radical scavenging assay[17]:

Stem extract was taken with various concentrations ranging from 200 – 1000 µg/ml. PBS with 0.5 ml of sodium nitroprusside (10 mM) was added in the extract (1 ml) tubes then kept for 180 min at 25˚C opposite to visible illumination. NO react with O_2 and produe nitrite ion, whih was identified after 3 hours when extract was mingled in same amount of freshly made Griess reagent with the composition of sulphanilamide (1%) in phosphoric acid (2.5%) in addition with naphthyl ethylene diamine dihydrochloride (0.1%). Gallic acid (1 mM) was used as a standard. Optical density was calculated at 546 nm. Scavenging percentage of radical was evaluated using given equation,

Nitric oxide radical scavenging (%) = $[(A_0 – A_1)/A_0] \times 100$

Hydrogen peroxide (H_2O_2)scavenging assay[18]:

The different volumes of stem extracts (200 – 1000 µg/ml) were combined with the solution of phosphate buffer (pH 7.4) comprising hydrogen peroxide (0.6 ml, 40 mM). Later than 10 minutes, Optical density values were obtained at 230 nm. Phosphate buffer was used as blank. Ascorbic acid was exploited as standard.

The percentage of hydrogen peroxide quenched was measured by means of given expression,

$$H_2O_2 \text{scavenging } (\%) = [(A_0 - A_1)/A_0] \times 100$$

Statistical analysis:

All assays were executed at thrice. Values are given as mean ± standard deviation. Graphs were plotted for results of above assays and discussed in the results section.

RESULTS AND DISCUSSION

Cancer threat can be diminished by 20% through the usage of diets rich in phytochemicals. Under *in vitro* and *in vivo* state, triterpenes restrain tumor cell growth and persuade tumor cell apoptosis[19,20].

Free radical scavenging activity:

In plant secondary metabolites, phenolic compounds play an essential role in several biological actions like antibacterial, anti-allergic, anti-inflammatory, and anticarcinogenic nature. Flavonoids are capable of terminating chain reactions formed by free radicals through the scavenging process[21,22]. Table 1 denotes various free radicals scavenging activity of ethanolic stem extract of *T. divaricata* (L.).

Table 1: Neutralizing capacity in stem of *Tabernaemontan divaricata* (L.) against different types of radicals

Scavenging activity				
Concentration of extract (µg/ml)	**DPPH radical inhibition**	**Super oxide anion radical inhibition**	**Nitric oxide radical inhibition**	**Hydrogen peroxide radical inhibition**
200	2.3 ± 0.1	1.2 ± 0.09	0.7 ± 0.02	0.7 ± 0.04
400	4.2 ± 0.5	2.8 ± 0.18	1.2 ± 0.03	1.6 ± 0.09
600	7.9 ± 0.9	5.2 ± 0.38	1.9 ± 0.06	2.9 ± 0.11
800	11.2 ± 1.3	8.9 ± 0.78	2.8 ± 0.09	4.3 ± 0.23
1000	14.3 ± 1.5	14.7 ± 1.25	4.1 ± 0.12	7.3 ± 0.47

All values are expressed as mean±SD

In DPPH assay, antioxidants present in the stem extract decreases the stability of DPPH radical, through the conversion of radical (DPPH) to the non-radical form (DPPH-H). After the generation of non-radicals, absorption is decreased and color of the DPPH solution varies from purple to yellow. Ethanol extracts of *Caralluma flava* also have a higher neutralizing ability of DPPH radical in a concentration reliant response[23]. DPPH radicals are neutralized by the capacity of extract and and it is given in Table 1.

Superoxide anion radical (O^{2-}) is a very destructive molecule to the cells because it can act as a precursor of ROS and result in the formation of H_2O_2*in vivo*[24]. Then H_2O_2 produces OH^-

radicals that can cause cell damage. Therefore, the removal of H_2O_2 from a cell is imperative. In this assay, O^{2-} generated from flavin reduces NBT, as a consequence of blue formazan formation. But the ethanolic stem extract of *T. divaricata* (L.) has the capacity to neutralize O^{2-} radicalsthrough the inhibition of blue formazan.Quenching ability of the extract against superoxide anion radicals is depicted in Table 1.

Macrophages liberate nitric oxide as an essential inflammatory negotiator[25]. It is responsible for several processes, for example, relaxation of muscles, neuro signalling, and inhibition of platelet accumulation. In severe diseased conditions with the presence of nitric oxide reacts with superoxide anion leads to the formation of harmful molecule, peroxynitrite.So there is a necessity to reduce the encumber of NO levels in human being using plant extract. Nitric oxide radical reduction through the activity of extract was observed and its similarity was matched with standard and it is denoted in Table 1.

Once H_2O_2 enters into the cells, it can react with Fe^{2+} and Cu^{2+} ions. Then it produces toxic hydroxyl radicals. This process can be terminated through the elimination of H_2O_2 radical species. Figure 5 shows the potential of an ethanolic stem extract of *T. divaricata* (L.) inhibits H_2O_2 mediated damage and displayed more radical scavenging activity when a higher concentration of extract used (1000 µg/ml).

In all free radical scavenging assays, the percentage of radical inhibition was increased in a dose reliant mode. From the results of above scavenging assays, the order of higher inhibition was observed against superoxide anion radicals, DPPH radicals, hydrogen peroxide radicals, and nitric oxide radicals.

More attention have been given to plant polyphenols due to their functions in human body, including neutralization of free radicals, anti-inflammatory in addition to its anticarcinogenic action leads to the prevention of several diseases (Diabetes mellitus, neurodegenerative syndromes, cancer, and cardiovascular diseases)[26]. Moreover, phenolic constituents are responsible for the action of prospective metal chelators, hydrogen suppliers along with reductones, because it holdsredox status[27,28].

CONCLUSION

Free radicals are the main source for the generation of several inveterate disorders. The phytochemical compounds present in the stem extract can compete against different types of free radicals. Further investigation will be required, for the separation and characterization of the specific phytochemical compound in opposition to a particular illness that can be helpful for the preparation of drugs in pharmaceutical industries.

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PRODUCTIVE EFFECT OF ANTIHYPERGLYCEMIC ACTIVITY OF ETHANOLIC *EXTRACT OF MADHUCA LONGIFOLIA* BARK POWDER

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ABSTRACT

Madhuca longifolia is a widely used traditional medicine in many parts of the world for the treatment of various diseases viz. tonic, aphrodisiac, rheumatism, ulcers, and tonsillitis pharyngitis as well as bronchitis It is claimed in traditional medicine that the barks of the plant are used in the treatment of Diabetes mellitus, which are more common in Madhya Pradesh and Chattisgarh states of India. In the present study, the aqueous extract of b*arks* of *Madhuca longifolia* was screened for its Antihyperglycemic activity using alloxan induced hyperglycemia model. The aqueous extract at the dose of 500 mg/kg exhibited significant Anti-hyperglycemic activity in alloxan induced hyperglycemia model ($p<0.01$) indicating that test sample significantly lowered the blood glucose level in Diabetic albino rats, but no effect on normal healthy albino rats. These results have established pharmacological evidence for the folklore claim of the drug to be used as an anti-hyperglycemic agent.

Keywords: Anti-hyperglycemic, diabeties mellitus and Traditional medicinal uses

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolism, associated with absolute or relative deficiencies in insulin secretion and/or insulin action. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, there is a growing interest in herbal remedies, due to the side effects associated with these therapeutic agents. Because of perceived effectiveness, minimal side effects in clinical experience and relatively low-cost herbal drugs are widely prescribed when their biologically active compounds are unknown. [1]So, the diabetes mellitus can be commonly treated with herbal extracts. Such treatment may be of considerable benefit especially during the early stages of the illness.The plant *Madhuca longifolia* Koeng. (Mahua) which belongs to Sapotaceae family [(1)] and its bark is traditionally used to treat diabetes mellitus [(2)]. It is a deciduous tree with dark coloured, cracked bark and rusty-tomentose, branch lets and elliptic leaves, flowers are white in colour and fascicled. It is widely distributed in throughout the India. Its seedsare the source of mahuaoil, usedin inflammation,skin infections and as a laxative. Mahua flowers have been traditionally usedascoolingagent, tonic, aphrodisiac, astringent,anddemulcent.Mahucabarksarealsoused in the treatment of rheumatism, ulcers, and tonsillitis. *Madhuca longifolia* Koeng bark contains lupeol acetate, β-amyrin acetate, α-spinasterol, erthrodiolmonocaprylate, betulinic and oleanolic acids, caprylates, xylose, rhamnose, glucose and galactose. Leaves contains β-sitosterols, myricetin. Seeds containsaponins, Mi-saponin A,Mi-saponinB.Seedkernelcontains protobassic acid, prosapogenol, Mi-saponin C [3,4,5,6].

MATERIALS AND METHODS

Plant Material: The Barks of *Madhuca longifolia* were collected from the local market in Tiruchirapalli, Tamilnadu, India.

Preparation of plant extract: The bark was cut or sliced into small pieces and air-dried in the shade. The dried samples were then ground into powder.

Extractionofcrudedrug: One hundred grams of barks powder were mixed with 400 ml of distilled water and stirred magnetically overnight(12hours) atroom temperature. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolvedin saline and usedin the study. Theyield of the extract was 2.3% w:w.[7]

Experimental Animals: Male albino rats, weighing 150–200 g, were obtained from the Department of Animal Science, Bharathidasan University, Trichy. They were housed in clean polypropylene cages under standard conditions of humidity (45±4%), temperature (25±20℃), and light (12 h light/12 h dark cycle).and fed a standard diet and water ad libitum. This study was approved by the Institutional Animal Ethics Committee (IAEC) (1416/PO/a/11/CPCSEA).

Preparation of sample: The aqueous extract was suspendedin Tween 80 (0.5%) in normal saline (vehicle) and was usedfor anti hyperglycemic activity studies. Tween 80 (0.5%) was used as it is the commonly used suspending agent in earlier reported studies.

Acute toxicity study: Acute toxicity studies were carried out as per fixed dose OECD guidelines No: 420 using albino Mice. In brief, albino mice of either sex weighing between20-30gwereused for acute toxicity study. The animals were fasted overnight prior to the experimental procedure. The animals were kept for fasting overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kgbody weightand observed for 7 days. If mortality was observed in2 outof3animalstheoral dose administered was assigned as toxic dose. If mortality was observedin1animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 50,300 and 2000 mg/kg body weight[8].

Evaluation of hyperglycemia activity: The antihyperglycemic activity was evaluated using alloxan induced hyperglycemia model. The ethical clearance was obtained by the Institutional Animal Ethics Committee (Registration No:126/1999/CPCSEA) before carrying out the experiment.

Alloxaninducedhyperglycemia: Diabetes was induced in rats by intraperitoneal administration of 150 mg/kg of alloxan monohydrate. After 2 weeks, animals with moderate diabetes having glycosuria (indicated by Benedict'stestforurine) andhyperglycaemia,i.e.withbloodglucoselevelsof 200–280mgper100mlweretakenfortheinvestigation. Blood was collected from eyes (venouspool). They were divided into four groups of six rats each: group I (normal rats); group II (normal rats treated with 0.5 g/kg of aqueous extract of *Madhucalongifolia* bark); group III (diabetic untreated rats); and group IV (diabetic rats treated with 0.5 g/kg of aqueous extract of *Madhuca longifolia* bark) Fasting blood was collected for blood glucose estimation before starting the treatment on the first day.TheratsingroupsIIandIVweregivendaily0.5g/kgof aqueous sextractofMadhuca longifoliabarkby gastricgavageforaperiodof 6,weeks,whiletheratsingroups IandIIIwere fed distilled water alone.On the last day of the treatmentbloodsamples were collected from tail veinofallthegroupsofratsorbloodsugarestimations. Bodyweightsofalltheanimalswere recorded prior to the treatment [9].

Statistical Analysis: Theexperimental datawereexpressedasthemean±SE. The standard error of the mean(SEM) is the standard deviation of the sample mean estimate of a population mean. SEM is estimated by the sampleestimate of the population standard deviation (sample standarddeviation) dividedby the square root of the sample size. Statistical analysis was carried out using one- way analysis of variance followed by Dunnett's Multiple Comparison Test and p values implied significance($p<0.001$).

RESULTS

Acute toxicity studies: Acute toxicity studies revealed the non-toxic nature of ethanolic extract at the two dose levels i.e. 1000mg/kg and 2000mg/kg were tested. There were no morphological changes like distress, hair loss, restlessness, convulsions, laxative effect, coma, wloss, etc. At the end of the treatment period, there was no lethality or toxic reaction at any of the doses selected.

The Table1rats were grouped randomly into four groups, each containing six animals. Group I, the untreatednormalgroup, received the vehicle (normal saline). Group II served as Treatednormalsgroup and received the vehicle (normal saline). Group III was treated with standard drug alloxan at 100 mg /kg body weight. Group III and IV treated with *Madhuca longifolia* bark powder extract at the dose levels of 200 and 400 mg/kg body weight respectively for five days [10].

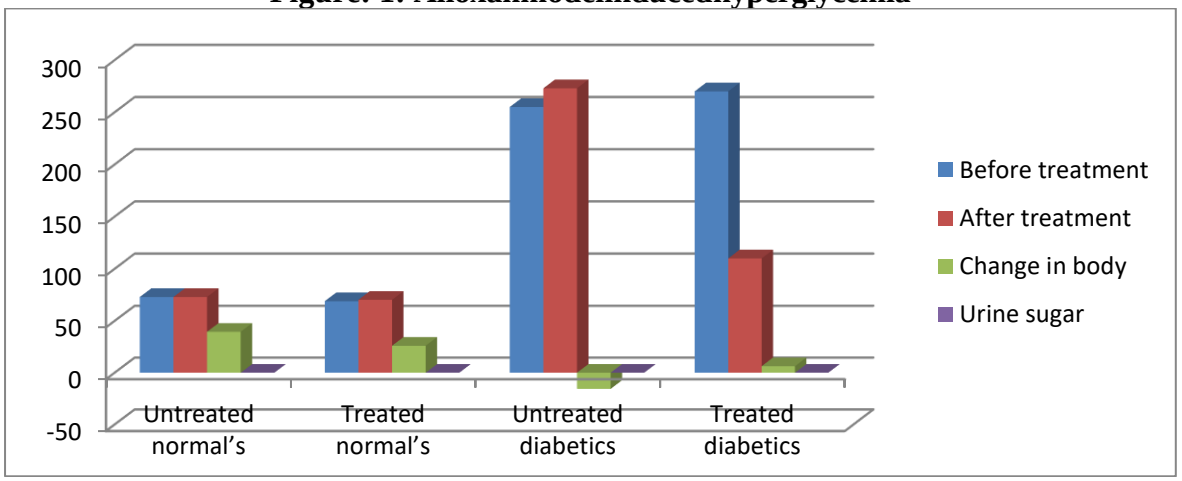
Group-I: Untreated normal; **Group-II:** Treatednormal
Group-III: Untreated diabetics; **Group-IV:** Treateddiabetics

Table:1: Alloxanmodelinducedhyperglycemia

Group of rats	Fastingbloodglucose(mg/dl)		Change in body After treatment	Urine sugar
	Before treatment	After treatment		
Untreated normal's	73.5±7.2	73±6.6	39.5±3.4	-
Treated normal's	69.5±10.5	70.3±10.6	26±2.8	-
Untreated diabetics	255.6±7.5*	273.8±5.03	-15.6±3.2*	+++
Treated diabetics	270±10.9*	110.3±14.26**	6.3±1.4***	+

**P*-0.001comparedtothebloodglucoseinnormal.
***P*-0.001comparedtotheinitialbloodglucoseintreateddiabeticsandalsofinalbloodglucosein untreated diabetics.

Figure: 1: Alloxanmodelinducedhyperglycemia



DISCUSSION

A wide range of synthetic oral antidiabetic drugs such as sulfonylureas and biguanides have been used for 50 years now in the treatment of diabetes. However, they have not been of much benefit in controlling the complications of the disease. In the present study, the antihyperglycemic activity of ethanolic bark extract of *Madhuca longifolia* was assessed in normal and STZ induced diabetic rats. Oral administration of a single dose of ethanolic bark

extract of *Madhuca longifolia* caused a significant decrease in serum glucose level in normal rats. A dose of 200 mg/kg of ethanolic extract produced maximum glucose lowering effect, whereas 100 mg/kg of ethanolic extract showed a significant hypoglycemic effect throughout the study period. In the oral glucose tolerance test, the *Madhuca longifolia* bark extract showed significant reduction of serum glucose levels and these effects were dose dependent. The extract of *Madhuca longifolia* bark displayed a significant hypoglycemic effect in normal rats. The main mechanism by which the extracts bring the hypoglycemic effects most probably involves stimulation of peripheral glucose consumption.

CONCLUSION

Based on the present study, it can be concluded that ethanolic extract of *Madhuca longifolia* bark powder have potent hyperglycemiaactivity in a dose dependent manner. Further isolation of active principles will be advantageous to produce novel bioactive constituents from these extracts, which may possess more significance in the treatment of diabetes mellitus diseases, and to elucidate its exact mechanism of action. Attempts are being made to isolate and characterize the active principle to which the hyperglycemia activity can be attributed.This research provides information which could trigger further research in the direction of partial or full isolation and characterization of the constituents of bark extract of *Madhuca longifolia* in order to decipher the specific phytochemical constituent(s) responsible for the hypoglycemic activity and free radical scavenging activity of the plant. When this is done, extracts of *Madhuca longifolia* could find important application in phytotherapy.

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GAS CHROMATOGRAPHY – MASS SPECTROMETRY ANALYSIS FOR STEM OF *TABERNAEMONTANA DIVARICATA* (L.)

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ABSTRACT

Nature have many plants with various medicinal poperties Plants have therapeutic value because of the presence of active compounds in it. So there is a need to identify the antioxidant capacity of those compounds for the eradication of infectious and chronic illnesses. Gas Chromatography-Mass Spectrometry (GC-MS) analysis and free radical scavenging assays (2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, Superoxide anion radical, Nitric oxide radical, hydrogen peroxide radical) were performed in an ethanolic stem extract of *Tabernaemontana divaricata* (L.). In GC-MS analysis, twelve phytochemical compounds were identified with maximum peak area percentage such as lupeol (50.16%), 9,12-Octadecadienoic acid (Z,Z)- (16.46%), and n-Hexadecanoic acid (10.98%). The molecular weight and molecular formula of these compounds were matched with known spectrum of NIST library. Based on the results, this study concluded that stem of *T. divaricata* (L.) have the capacity to act against microbial infections.

Keywords:*Tabernaemontana divaricata* (L.), Phytochemical constituents, GC-MS analysis, Free radical scavenging

INTRODUCTION

Plants are used as medicines in various cultures and serve as a source of many potent drugs due to the presence of certain bioactive compounds for pharmaceutical industries[1]. Plants contain different phytochemicals useful for the treatment of certain disorders by their individual, additive, or synergic actions to improve health[2,3]. Phytochemicals are vital in pharmaceutical industry for development of new drugs and preparation of therapeutic agents[4].

The development of new drugs starts with identification of active principles from the natural sources. GC-MS is the most commonly used method to find out the chemical constituents of alkaloids, steroids, long chain hydrocarbons, alcohols, acids, esters, etc.,[5]. This technique is employed to detect the chemical compounds present in herbal extracts.

Tabernaemontana divaricata (L.) is a medicinal shrub or small tree. it blooms in autumn season heavily. The leaves are shiny and deep green in colour. *T. divaricata* is found in Tropical Asia, Australia and Polynesia. In India, it occurs in upper Gangetic plain, Garhwal, East Bengal, Khasia Hills, Assam, Burma, hills of Vishakapatnam. It is cultivated as an ornamental plant, grows wild in hedges and shady forests It holds more beneficial actions like anti-inflammation, antioxidant, anticancer, and antidiabetic properties[6-9].

MATERIALS AND METHODS

Plant material collection:

Tabernaemontana divaricata (L.) plant was found through the book as well as literature references[10,11]. *T. divaricata* (L.) stem (Double layered flower type) were

gathered from Thanjavur. The collected stem was washed and dried in gloom for 14 days. Then it was pulverized with the use of mixer into fine powder. Exracts were prepared from that powder for GC-MS and free radical scavenging assays.

Extract Preparation for GC-MS analysis:

25 g stem powder was mixed with ethanol (50 ml) and leave it for 12 h then sieved utilizing Whatmann No.41 filter paper along with Na_2SO_4 (2g). Before this separation, filter paper with Na_2SO_4 was wetted with ethanol (95%). Sediments and water can be removed in this filteration. Nitrogen gas was used to condense the filtrate. Extract (2 µl) was injected into GC-MS apparatus[12].

GC-MS Technique:

GC Clarus 500 Perkin Elmer system was utilized to perform GC-MS technique. GC-MS apparatus worked using subsequent states: Elite-5MS column (30 x 0.25 mm x 0.25 m df), made up of 95% Dimethyl polysiloxane. Helium - carter gas (persistent flow of 1ml/min). Injection volume was 10:1 ratio (2 µl). Injector temperature - 250°C. Inlet and source temperature - 200°C. The oven temperature - without clasp (up to 200°C from 10°C/min) and 9 min clasp (up to 280°C from 5°C/min). 70 eV was used to take mass spectra. The fragments of mass spectra in the range of m/z were obtained from 45 to 450 Da and overall process taking time for GC and MS was 36 min [13].

Identification of components:

62,000 paradigms were stored in NIST (National Institute Standard and Technology) database. Unknown component spectrum identified from GC-MS method was correlated with spectrum of familiar compounds accumulated in the NIST library and. After comparison, names, structure, and molecular weight were ascertained for the identified components from stem extract[14].

RESULTS AND DISCUSSION

In this present examination, GC-MS method analysis was employed to detect the existence of various phytochemical compounds in an ethanolic stem extract of *T. divaricata* (L.). It revealed the occurrence of 12 compounds. From this analysis, higher peak area percentage was noted in specific compounds of stem namely lupeol (50.16%), 9, 12-octadecadienoic acid (Z,Z)- (16.46%), n-hexadecanoic acid (10.98%) and Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)- (10.33%). Table 1 listed molecular formula, molecular weight, retention time as well as biological functions of various constituents ascertained and its respective chromatogram is also exhibited in Figure 1.

Figure 1: GC-MS chromatogram for stem of *T. divaricata* (L.)

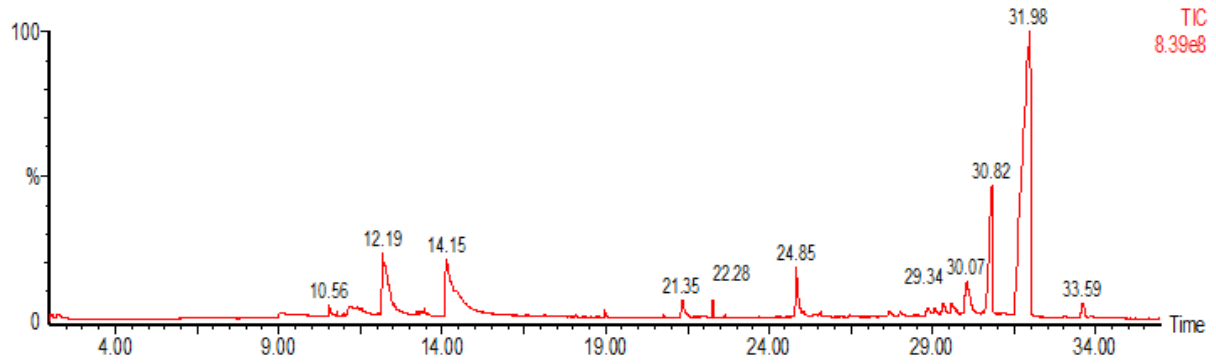

Table 1: Compounds identified through GC - MS analysis in *Tabernaemontan divaricata* (L.) stem

S. No	RT	Name of the Compound	MF	MW	Peak area %	Biological activities
1.	10.56	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.61	Antimicrobial, Antiinflammatory Anticancer
2.	12.19	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.98	Antioxidant, Antibacterial, Hypocholesterolemic Pesticide, Antiandrogenic
3.	13.45	Phytol	$C_{20}H_{40}O$	296	0.43	Antimicrobial, Antiinflammatory, Anticancer, Diuretic
4.	14.15	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	16.46	Antiinflammatory, Antiarthritic hypocholesterolemic, Cancer preventive Hepatoprotective activity
5.	21.35	Aspidospermidine-3-carboxylic acid, 2,3-didehydro-, methyl ester, (5à,12á,19à)- (Vincadifformine)	$C_{21}H_{26}N_2O_2$	338	1.31	Antiplasmodial, cytotoxic activity
6.	22.28	Squalene	$C_{30}H_{50}$	410	0.55	Anticancer, Antimicrobial, Antioxidant, Pesticide
7.	24.85	Ibogamine-18-carboxylic acid, 12-methoxy-, methyl ester (Voacangine)	$C_{22}H_{28}N_2O_3$	368	3.22	Anti-angiogenic, Antiaddictive
8.	29.61	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8ahexahydro-1H-naphthalen-2-one	$C_{15}H_{22}O$	218	0.71	No activity reported
9.	30.07	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	3.85	Antimicrobial, Antiinflammatory Antioxidant
10.	30.82	Urs-12-en-24-oic acid, 3-oxo-, methyl ester, (+)-	$C_{31}H_{48}O_3$	468	10.33	No activity reported
11.	31.98	Lupeol	$C_{30}H_{50}O$	426	50.16	Antiasthma, Antiinflammatory Diuretic, Anticancer, Antiarthritic
12.	33.59	Spiro[5.5]undec-2-ene, 3,7,7-trimethyl-11-methylene-, (-)-	$C_{15}H_{24}$	204	1.39	Antibacterial

Molecular weight – MW, Molecular formula - MF, Retention time - RT

Lupeol is a triterpene, used to treat inflammation in arthritic mice[15] and a mouse model of bronchial asthma leads to the modulation of immune system in addition to the formation of inflammatory molecules[16]. Lupeol supplementation was effective against cardiac oxidative injury caused by an anticancer drug, Cyclophosphamide. Its esters showed promising activity in stimulating skin repairing processes and can be applied as topical formulations for the treatment of skin burns[17] as well as wound healing[18].

9,12-octadecadienoic acid (Z,Z)- possess antiarthritic and anti-inflammatory properties[19,20]. Earlier reports suggested that n-hexadecanoic acid has antioxidant and antibacterial[21], cytotoxic activity against leukemia, MOLT-4[22], and *in vivo* anticancer properties[23].2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro- is a flavonoid, possess antimicrobial, anti-inflammatory, and antioxidant activities[24].

Vincadifformine and Voacangine are indole alkaloids. Vincadifformine and its some derivatives showed in vitro antiplasmodial activity and cytotoxic effect on HeLa cells[25]. Voacangine has anti-angiogenic property in both *in vitro* and *in vivo* conditions[26] likewise, it has antiaddictive property[27].

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QUALITATIVE PHYTOCHEMICAL SCREENING IN VARIOUS LEAF EXTRACTS OF *VITEX NEGUNDO*LINN.

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ABSTRACT

Plants are widely distributed throughout the world with several therapeutic values. They have various phytochemicals with different biological actions. In this study, leaf of *Vitex negundo* was taken for qualitative phytochemical analysis. Preliminary phytochemical screnning was carried out in aqueous, hot water and acetone leaf extracts of *V. negundo*. Phytochemicals like carbohydrates, protein, alkaloids, flavonoids, steroids, terpenoids, glycosides, cardiac glycosides, quinones, anthraquinones, tannins, phenols, coumarins and phlobatannins were identified qualitatively. Most of the phytochemical constituents were present in aqueous leaf extracts of *V. negundo* when compared to hot water and acetone extracts. Through findings of this work, it is concluded that leaf extracts can serve bioactive compounds responsible for fighting capacity opposed to a number of microbial infections.

Keywords: Qualitative phytochemical analysis, *Vitex negundo*, Bioactive compounds

INTRODUCTION

Herbs retain the capacity to perform imperative biological actions by producing more number of chemical compounds for their routine metabolism is referred as phytochemicals. Medicinal plants are important species of plants that according to the traditional medicinal practices and also from modern scientific studies are useful for medicinal purposes to alleviate diseases, make human health more invigorating.

These plants are contemplated as rich sources of ingredients that can be used in the synthesis and production of drugs[1]. Plants consist of various kinds of chemical constituents known as phytoconstituents[2]. Phytoconstituents save the plants by contributing some secondary functions like; helps in plant growth, safeguarding the plants by activating defence mechanism, imparting colour, odour, and flavour to the plants[3]. Natural products and their derivatives exhibit minimal side effects and improved efficacy than other synthetic counterparts[4].

Therapeutic significance of the plants depends upon the occurrence of these plant derived chemical constituents. Fundamentally they are classified into primary and secondary phytochemicals. Carbohydrates, proteins, fats and chlorophyll are comes under the category of primary metabolites. Secondary metabolites protect plant as well as human through the assault against a range of infectious agents.

Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals that have protected human from various diseases. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body.

Diverse types of medicinal plants are disseminated throughout the world. Currently 80% of people have hope about traditional medicines prepared from plants for their basic and essential health needs. It can be utilized to heal several disorders owing to its least or no side effects in the human being. Therefore, the major objective of the present study was to carry out qualitative phytochemical screening in leaf extracts of *Vitex negundo*.

MATERIALS AND METHODS

Collection of Plant materials

Leaves of the plant *Vitex negundo* were collected from in and around area of Thanjavur, Tamilnadu. The plant was identified with the help of reference[5]. (Alka Bameta *et al*., 2019)

Preparation of plant powder

Collected leaves were separately washed and shade dried for 15 days. Dried plant materials were grounded into fine powder and then utilized to prepare various extracts.

Extract preparation

10g of leaf powder had been extracted with 30ml of acetone, distilled water and hot water separately using cold maceration method for 5 days. After extraction, the extracts were filtered and stored in sealed vials at 4˚C until used for further analysis.

Hot water extract was prepared using the following step that is leaf powder was packed and then immersed in boiling water and the temperature was maintained at 60°C for 1 hour. Above all extracts were used for qualitative, quantitative analysis, antimicrobial activity as well as for identification of total antioxidant activity and reducing power assay.

Qualitative Phytochemical analysis:

Phytochemical analysis was performed using the method given by the standard procedure[6].

Test for carbohydrate:

In 2ml of stem extracts, 1ml of molisch reagent and a few drops of concentrated sulphuric acid were added. After addition, appearance of purple or reddish shading demonstrates the presence of carbohydrate.

Test for protein:

Few ml of extracts was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observation of violet or pink colour indicates the occurrence of protein.

Test for tannins:

To 1ml of stem extracts, 2ml of 5% ferric chloride ($Fecl_3$). It leads to the appearance of dark greenish or dull blue confirms the existence of tannins.

Test for alkaloids:

With 2ml of stem extracts, 2ml of concentrated hydrochloric acid (Hcl) was mixed. At that point, few drops of Mayer's reagent were added, the appearance of green or white precipitate reveals the presence of alkaloids.

Test for flavonoids:

In 2ml of stem extracts, 1ml of 2N sodium hydroxide (NaoH) was added. Yellow shading appearance indicates the existence of flavonoids.

Test for glycosides:

Addition of 3ml chloroform and 10% ammonia solution to 2ml of stem extracts contributes to the formation of pink colour shows the existence of glycosides.

Test for terpenoids:

To 0.5ml of stem extracts, 2ml chloroform and then concentrated sulphuric acid was closely added into the sides of the test tubes. Red brown color formation at the interface indicates terpenoid existence.

Test for quinones:

1ml of concentrated sulphuric acid was blend with 1ml of stem extracts. The formation of red color indicates the occurrence of quinones.

Test for anthraquinones:

With 1ml of stem extracts, few drops of 10% ammonia solution were combined. The pink color precipitate shows the presence of anthraquinones.

Test for phenol:

With 1ml of stem extracts, few drops of phenol Ciocalteau reagent followed by a few drops of 15% sodium carbonate were mixed. Blue (or) green colour appearance reveals the incidence of phenols.

Test for saponins:

In a graduated flask, 2ml of distilled water was added to 2ml of stem extracts and shaken for 15 minutes. Then the appearance of 1cm layer of lather shows the incidence of saponins.

Test for coumarins:

1ml of 10% of sodium hydroxide was mixed with 1ml of stem extract and leads to yellow colour formation suggest the occurrence of coumarins.

Test for steroids:

To 1ml of stem extracts, equal volume of chloroform and then exposed with few drops of concentrated sulphuric acid leading to brown ring formation shows the existence of steroids and bluish brown ring appearance indicates the incidence of phytosteroids.

RESULTS AND DISCUSSION

Distilled water, hot water and acetone extracts of leaf were chemically tested for the presence of different phytochemical constituents such as carbohydrates, protein, alkaloids, flavonoids, phenols, glycosides, cardiac glycosides, steroids, terpenoids, saponins, tannins, quinines, anthraquinones, coumarins and phlobatannins.

Distilled water, hot water and acetone leaf extracts showed the presence and absence of some phytoconstituents. The results are summarized in Table 1. Phytochemical constituent's variation present in different solvents as shown in the result of phytochemical screening might be experienced to the ability of the solvents to dissolve into solution for specific type

of phytochemicals. Among the three solvents, aqueous extract showed the presence of alkaloids, flavonoids, phenols, cardiac glycosides, steroids, triterpenoids, saponins, tannins, carbohydrates, quinone, anthraquinone, coumarins, phlobatannins and protein.

Table 1: Preliminary phytochemical screening in various leaf extracts of *V. negundo* Linn.

Phytochemicals	Water extract	Hot Water extract	Acetone extract
Carbohydrates	++	+	+
Protein	++	+	+
Alkaloids	+	+	+
Flavonoids	++	++	+
Steroids	+	+	-
Terpenoids	+	+	-
Quinones	++	+	-
Glycosides	++	+	-
Cardiac glycosides	++	-	-
Saponins	++	+	-
Tannins	+	+	+
Phenols	+	+	+
Coumarins	+	+	+
Phlobatannins	+	+	-
Anthraquinones	+	-	+

Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals[7] implicated in several diseases. Flavonoids have anti-oxidative and mucosal protective effect[8,9]. Flavonoid-rich vegetables are widely used functional foods since they can be used to treat cardiovascular diseases[10]. They are characterized by their good bioavailability and, hence, constant dietary consumption of flavonoids has been reported to give pharmacologically relevant plasma concentrations inhumans[11]. In addition, several studies have reported possible cardioprotective effects of flavonoids against ischemia reperfusion12,[13].

Saponins may activate mucous membrane protective factors, while tannins reduce the permeability of mucosa to chemical irritation. Consequently, they reduce inflammation, exert astringent and protective action on the stomach mucosa, and curb excess acidity. In addition, terpenoids and alkaloid compounds have also been reported to have potent activity against gastric ulcers[14,15]. Terpenoids have been reported to relax cardiovascular smooth muscle by inhibition of Ca^{2+}influx in vascular smooth muscle or via quenching of reactive oxygen species (ROS) and stimulation of nitric oxide (NO) synthesis[16].

CONCLUSION

Plants are the major source of highly effective conventional drugs. Natural products from medicinal plants serve as alternate source of combating infections in human beings because of lower cost and lesser toxicity. Medicinal plants provide active constituents which act directly against various ailments or indirectly by providing leads for the development of potential novel agents. Further studies will be required which compound is responsible for the particular action of disease resistant capacity.

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EFFECT PESTICIDES ON GROWTH TOLERENCE AND BIOCHEMICAL CHARECTERIZATION OF *OSCILLATORIA PRINCEPS*

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ABSTRACT

Cyanobacteria have been proved to resist the action of some pesticides. Such cyanobacteria are also employed to monitor pesticide pollution as it posse s major threat to the paddy field in recent years. Present investigation to study the probable effects of the pesticide Chlorpyriphos on the marine cyanobacterium Oscillatoria princeps occurring very commonly in South East coast of India. Growth was measured in terms of chlorophyll'a' in all the treatments. Carotenoid pigments were also found to be steep rise up to10 days, and then there was a slight increase up to 15th day followed by a steady decline in growth till the end of the experimental period. The increasing concentration of phycophilin up to 15th days at 100 ppm of pesticide concentration. As the concentration of pesticide increases, the total carbohydrate level decreased considerably. The maximum reduction (71%) was found at 1000 ppm and minimum (22%) at 100 ppm were also decreased gradually with increasing concentration of pesticide.

Key words: Cyanobacteria, Chlorpyriphos, *Oscillatoria princeps*, phycophilin and chlorophyll

INTRODUCTION

Cyanobacteria are one of the major components of the nitrogen fixing biomass in paddy fields and it's provide a potential source of nitrogen fixation at no cost. Due to the important characteristic of nitrogen fixation, cyanobacteria have a unique potential to contribute to enhance productivity in a variety of agricultural and ecological situations. Cyanobacteria play an important role to build-up soil fertility consequently increasing the yield. Biofertilizer being essential components of organic farming play vital role in maintaining long term soil fertility and sustainability by fixing atmospheric dinitrogen (N=N), mobilizing fixed macro and micro nutrients or convert insoluble phosphorus in the soil into forms available to plants, thereby increases their efficiency and availability.

Some cyanobacteria are able to reduce atmospheric nitrogen to ammonia, a process where oxygen evolved by photosynthetic activity in the same cell is detrimental to nitrogen fixation. Strategies to avoid oxygen range from temporal separation of nitrogen fixation and oxygen evolution (in many unicellular and filamentous, non heterocysts strains) to spatial separation of heterocysts (in filamentous cyanobacteria).Heterocysts are terminally differentiated cells whose interior becomes anaerobic, mainly as a consequence of respiration, allowing the oxygen-sensitive process of nitrogen fixation to continue. The regulation of dinitrogen fixation has been extensively studied in the heterocyst system (Bhme, 1998). The maintenance of a healthy aquatic ecosystem depends on the abiotic properties of water and the biological diversity of the ecosystem (Harikrishnan *et al.*, 1999). Such cyanobacteria are also employed to monitor pesticide pollution as it posse s major threat to the marine environment in recent years. The occurrence of pesticides in the marine environments (Selvaraj *et al.*, 1985) prompted the present investigation to study the probable effects of the pesticide on the marine cyanobacterium Oscillatoria *princeps*occurring very commonly in paddy field.

MATERIALS AND METHODS

Samples collection

Samples were collected from Paddy filed in Orathanadu, Thanjavur district, Tamil Nadu and transported to the laboratory under the sterile condition for further use.

Isolation of Cyanobacteria (Rippka, 1988)

The paddy field soil samples were spread in to Petridis with ASN III medium (NaCl-25, $MgCl_2.6H_2O$-2, KCl-0.5, $NaNO_3$-0.75,$K_2HPO_4.3H_2O$-0.02,$MgSO_4.7H_2O$,$CaCl_2.2H_2O$-0.5,Citric acid-0.003,Ferric ammonium citrate-0.003,EDTA-.00055,Na_2CO_3-0.02,Trace metal mix-1ml,Distilled water-1000ml and pH-7.5.). Streak agar plates. After 7days the unialgal cultures were isolated. At this stage microscopic examination was made to identify that the unialgae isolated were single species. A portion of cyanobacterial mat was taken in an eppendorf tube and crushed with the help of sterile micro pestle, and 0.1 ml of this suspension was spread over the plate. It containing ASN III medium. Then the plates were incubated at 2000 – 3000 lux light intensity for 10 – 15 days.

Identification of cyanobacteria

Small pieces of microbial colonies of sea water cyanobacterial isolates were picked out from the ASN – III medium. The semi-permanent slides were prepared and observed under the Nikon microscope at oil immersion (400X and 1000X) objective. The organism thus chosen was the cyanobacterium *Oscillatoria pseudogeminata*. The above organism was identified with the help of standard works of Desikachary (1959).

Effect of pesticide Chlorpyriphos

To test the effect of pesticide on growth of *Oscillatoria princeps* was treated with different concentration of pesticide which was used as Chlorpyriphos. The insecticide used was of high quality Chlorpyriphos manufactured by Bayer Crop science Limited, Gujarat. The composition of Chlorpyriphos as follows: Chlorpyriphos- 1.620 %, Xylem-2.730 %, Diethylene glycol- 2.000 %, Silica- 3.000 % and Filter material- 90.650 %.

Preparation of Stock solution

Prepared by dissolving in 1 g carbaryl in100ml of sterile media = 10,000 ppm Stock solution of the pesticide Chlorpyriphos was prepared in sterile media (ASN-III-Nmedium) from which different concentrations of Chlorpyriphos (ppm) were prepared viz. 100,200,300,400,500 and 1000 ppm respectively (PLATE-II). Exponentially growing Oscillatoria pseudo geminate was inoculated into 100 ml of each test solution taken in 250 ml conical flasks and experiments were conducted. This experiment was conducted for 24 days in a culture room illuminated with white fluorescent light (2,000 lux) by maintaining a 10/14 light dark cycle at 22 10C. Duplicates were run in all the experiments. The test organism treated with different concentrations of Chlorpyriphos is observed under microscope and care was taken to record the morphological characteristics including abnormalities.

Growth measurements

Growth rate was measured in terms of chlorophyll-a as biomass components (McKinney s method, 1941) , carotenoids (Davis, 1976) and phycobilins (Moreno *et al.*, 1994) at day 10^{th}, 15^{th}, 20^{th} and 25^{th} days after inoculation. All the biochemical content viz., carbohydrates, proteins, total lipid and free amino acids content calculated at 25^{th} day after the inoculation. Estimation of carbohydrates followed by Dubois *et al.*, 1956, estimation of total proteins followed by Lowery *et al.*, 1951, estimation of total lipid followed by Sato and Murata, 1988, estimation of free amino acids followed by Jayaraman 1981.

Estimation of Chlorophyll 'a' (Mackinney, 1941)

The sample was centrifuged at 5,000 xg for 10 minutes. The pellets were washed with distilled water; suspended in 4 ml of 80 per cent methanol and vortexed thoroughly. Then the tubes were covered with aluminium foil to prevent solvent evaporation and incubated in a water bath set at 60^{o}C for 1 hour in dark with occasional shaking. After 1 hour the contents were cooled and centrifuged at 5,000 xg for 5 minutes. The supernatant was saved and the above procedure was repeated twice with pellets to ensure complete extraction of the pigment. The pooled supernatant was made up to a known volume with 80 per cent methanol (To compensate the solvent loss during heating). The absorbency was measured at 663 nm in Spectronic 20 against methanol as blank.

Estimation of Carotenoids (Davis, 1976)

The sample was centrifuged at 5,000 xg for 10 minutes. The pellets were washed with distilled water; suspended in 3 ml of 85 per cent acetone and homogenized. The contents were centrifuged at 5,000 xg for 5 minutes and the supernatant was stored in refrigerator. The above procedure was repeated until the acetone colourless. The pooled supernatant was made up to a known volume with 85 per cent acetone. The absorbency was measured at 450 nm in Spectronic 20 against acetone as blank.

Estimation of Phycobilins (Phycobiliproteins) (Moreno *et al.*, 1994)

The sample was centrifuged at 5,000 xg for 10 minutes. The pellets were washed with distilled water; suspended in 3 ml of phosphate buffer (0.05 M) and homogenized. The contents were freezing thawed repeatedly and centrifuged at 5,000 xg for 5 minutes. The supernatant was stored in refrigerator. The above procedure was repeated to ensure complete extraction. The absorbency of the pooled supernatant was measured at 565, 615 and 652 nm against phosphate buffer as blank.

Estimation of Carbohydrate (Dubois *et al.*, 1956)

The sample was centrifuged at 5,000 xg for 10 minutes. From the pellet 100 mg was taken in a test tube and hydrolysed with 2 ml of concentrated H_2SO_4 for 30 minutes at 100^{o}C. To 0.5 ml of hydrolysate, 1 ml of 5 per cent phenol and 5 ml of conc. H_2SO_4 were added and mixed thoroughly. The colour developed was measured at 490 nm in Spectronic 20 against the reagent blank. The amount of carbohydrate was calculated using a standard graph prepared from glucose and expressed as mg g^{-1} dry weight.

Estimation of Total Protein (Lowry *et al.*, 1951)

The samples were centrifuged at 5,000 xg for 10 minutes. From the pellet 100 mg was treated with reagent A and centrifuged at 10,000 xg for 10 minutes. The resulting pellet was resuspended in reagent B and boiled for 30 minutes; cooled and then recentrifuged to eliminate light scattering materials. The supernatant was made upto a known volume. To 0.1 ml of the supernatant 0.9 ml of distilled water and 5 ml of reagent E were added and allowed to stand for 10 minutes. Finally 0.5 ml of reagent F was added. The absorbancy was measured after 30 minutes at 750 nm in Spectronic 20 against the reagent blank. The amount of protein was calculated using a standard graph prepared from Bovine Serum Albumin (BSA) and expressed as mg g^{-1} dry weight.

Estimation of free Amino acids (Jayaraman, 1981)

10 ml of samples was homogenized with 80 percent ethanol in a pestle and mortar. The homogenate was centrifuged at 5000 rpm. The clear supernatant was made up to a known

volume. From this 1 ml was pipetted out in to a test tube and diluted to 4ml with distilled water. To this, 1 ml of ninhydrin reagent was added and kept in boiling water bath for 15 minutes. The test tubes were then cooled and 1 ml of 50 percent ethanol was added. The purple colour developed was measured in spectronic 20 at 540nm.

Lipid Analysis (Sato and Murata, 1988)

Cultures were centrifuged at 5,000 xg for 10 min. Equal amount of samples on dry weight basis was homogenized in a mortar and pestle with extraction solvent (Chloroform : Methanol 2:1 v/v) and filtered through filter paper. The filtrate was vortexed with sodium sulphate to remove moisture. Then it was taken in a pre-weighed bottle and dried by a steam of nitrogen.

Estimation of total lipids

The dried extracts were weighed and the total lipids were estimated by subtracting the initial from the final weight. The amount of total lipid was expressed as mg g^{-1} dry weight.

RESULTS AND DISCUSSION

Effect of different concentrations of pesticide Chlorpyriphoson the growth, carotenoids, phycobilin pigments and biochemical constituents such as carbohydrate, protein, lipid and amino acid of *Oscillatoria princeps* are shown in Table 1 and 2.

Chlorophyll' a' content:

Growth was measured in terms of chlorophyll'a' in all the treatments. In control, growth was well pronounced up to 15 days from the day of inoculation, and then there was a gradual decline in growth till the end of experimental period (Table.1). in other treatment there was slight enhancement up to 10 days . Then gradual increase up to the 15th day and showed a gradually decrease up to 25th day. Maximum growth was observed in control and minimum was noticed at 1000 ppm on the 25th day. Muruganantham and Manoharan (1998) observed the inhibition of chlorophyll a in *Westiellopsis prolifica* treated with different concentrations of 2, 4-D. Similar type of differential effect of various pesticdes on growth and nitrogen fixation of different algae has earlier been reported (Da Silva *et al.*, 1975; Kapoor and Sharma, 1980; Sardeshpande and Goyal, 1982).

Carotenoid content:

Carotenoid pigments were also found to be steep rise up to10 days, and then there was a slight increase up to 15th day followed by a steady decline in growth till the end of the experimental period (Table.1). In control, maximum carotenoid content (0.080 µg/ml) was noticed in 15th day and minimum (0.038 µg/ml) on 25th day. In all other concentrations there was a gradual increase in carotenoid pigment up to 15th day followed by a gradual decline up to 25th day. The reduction was observed at 1000 ppm throughout the experimental period when compared to control. Influence of pesticide on the carotenoid and phycobilin pigments of the test alga was similar to that observed for growth. This in in agreement with the findings of Kobbia *et al.*, (1991) who has investigated the effects of pesticde trifluralin suppressed the total carotenoid accumlation in *Anabena and Nastoc.*

Phycophilin content:

A Phycophilins pigment was recorded in all the concentrations including control. The increasing concentration of phycophilin up to 15th days at 100 ppm of pesticide concentration. The least value was recorded at 1000 ppm for 25th days of incubation period. In this regarding, the phycophilin content was gradually decreased up to 25th day. Similarly the repressed effect of

phycobilin pigments has been reported by Chen (1986) who observed the algae *Anabaena* exposed to Butachlor, reduced 51% allophycocyanin and 58% of Cphycocyanin and Bromocil at 50 ppm inhibited 25% of allophycocyanin and 45% C-phycocyanin pigments. The carbohydrate, protein and lipid contents were decreased with increased concentration of pesticide.

Table. 1 Effect of different concentration of pesticide Chlorpyriphos on Chlorophyll'a, Carotenoid and Phycobilins content of *Oscillatoria princeps* in various incubation periods

Incubation periods	Pesticides concentration	Chlorophyll'a content	Carotenoid content	Phycobilins content
10 days	Control	0.6	0.072	7
	100 ppm	0.57	0.065	5
	200 ppm	0.45	0.064	4.8
	400 ppm	0.37	0.058	3
	500 ppm	0.35	0.055	2
	1000 ppm	0.3	0.045	1.5
15 days	Control	0.6	0.080	7
	100 ppm	0.54	0.072	6
	200 ppm	0.5	0.067	5.5
	400 ppm	0.42	0.062	4.7
	500 ppm	0.37	0.060	4.5
	1000 ppm	0.32	0.050	3.5
20 days	Control	0.55	0.065	6
	100 ppm	0.5	0.060	5.5
	200 ppm	0.49	0.058	5
	400 ppm	0.38	0.052	4
	500 ppm	0.35	0.050	3.4
	1000 ppm	0.3	0.045	3
25 days	Control	0.52	0.063	5.5
	100 ppm	0.48	0.057	5
	200 ppm	0.45	0.055	4.5
	400 ppm	0.35	0.050	3.5
	500 ppm	0.3	0.045	3
	1000 ppm	0.25	0.038	2.5

Carbohydrate content:

The greater reduction in the level of carbohydrate (50 µg/ml) noticed at the highest concentration (1000 ppm) of pesticide on the 25th day and the lesser reduction (125 µg/ml) at 100 ppm as compared to control. As the concentration of pesticide increases, the total carbohydrate level decreased considerably (Table.2). The present findings agree well with those of Megharaj *et al.* (1991) who proved that pesticide exposed algae *Nostac Linckia* and *N.muscorum* inhibited the total carbohydrate level even at the lowest concentration. Similar other observations were also made by many workers (Anand and Veerappan, 1980; Shabana, 1985; Mansour *et al.*, 1994).

Protein content:

The protein content was maximum (38µg/ml) in control, Apart from control, all the concentration showed gradual reduction in protein content. Nearly 40% reduction of protein was observed in *Oscillatoria princeps* treated with 1000 ppm, but in the lowest concentration (100

ppm) only 11% reduction was noticed as compared to control (Table. 2). Likewise *Westiellopsis prolific* treated with 2,4-D showed maximum reduction of carbohydrate, protein and lipid content in all concentration (Muruganantham and Manoharan, 1998). The report made earlier by Kashyap and Pandey (1982), Kent and Currie (1995) also confirm this. The amino acid content of the test algae was decreased at the increased concentration of pesticide.

Lipid content:

Lipid level was decreased drastically in treated increasing pesticide concentration except control (250 µg/ml). The highest reduction of 50 µg/ml and the lowest of 220 µg/ml of lipids were observed at 1000 ppm and 100 ppm respectively.

Amino acid content:

The maximum reduction (71%) was found at 1000 ppm and minimum (22%) at 100 ppm were also decreased gradually with increasing concentration of pesticide (Table.2).Further research in essential to find out the indicator algae for removing the pesticide from the aquatic environment. The present study Effect of pesticide Chlorpyriphos on the Cyanobacterium *Oscillatoria princeps* was conducted in the laboratory to test the effect of Chlorpyriphos on growth and biochemical components. Growth was measured in terms of chlorophyll a content and it was decreased in all the concentrations (100,200,300,400,500 and 1000 ppm) of pesticide. Carotenoids and phycobilins were significantly reduced. Carbohydrate, protein, amino acids contents were also decreased gradually with increasing concentration of pesticides. Lipid level was decreased drastically in all treated concentration except control. Further research on this organism with different herbicides, pesticides and fungicides is needed to consider and use this organism as a successful feed and biofertilizer in future

Table. 2 Effect of different concentration of pesticides Chlorpyriphos on Carbohydrate, Protein, Lipid and Amino acid content of *Oscillatoria princeps*

Pesticides concentration	Carbohydrate (µg/ml)	Protein (µg/ml)	Lipid (µg/ml)	Amino acid(µg/ml)
Control	140	38	250	50
100 ppm	125	34	220	40
200 ppm	120	30	198	35
400 ppm	100	20	120	20
500 ppm	80	18	80	15
1000 ppm	50	15	50	10

CONCLUSION

The present study concluded that the effect of pesticide Chlorpyriphos on the Cyanobacterium *Oscillatoria princeps* was conducted in the laboratory to test the effect of Chlorpyriphos on growth and biochemical components. Growth was measured in terms of chlorophyll a content and it was decreased in all the concentrations (100,200,300,400,500 and 1000 ppm) of pesticide. Carotenoids and phycobilins were significantly reduced. Carbohydrate, protein, amino acids contents were also decreased gradually with increasing concentration of pesticides. Lipid level was decreased drastically in all treated concentration except control. Further research on this organism with different herbicides, pesticides and fungicides is needed to consider and use this organism as a successful feed and biofertilizer in future.

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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF COCONUT WATER

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ABSTRACT

The phytochemical composition and antimicrobial activity of coconut water against selected microbial pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*e, *streptococcus mutans* and fugus *candida albicans*, *Aspergillus niger*. Phytochemical screening revealed the presence of flavonoids, tannins, phenols, anthocyanins, terpenoids, and alkaloids. Quantitative analysis showed 0.822±0.11 mg of total phenols and 25.44±3.68 mg of total flavonoids in coconut water. Antimicrobial activity was assessed using the agar disc diffusion method. At 30 mg/ml, coconut water exhibited a lower inhibitory effect compared to ciprofloxacin, with zones of inhibition ranging from 20–23 mm. However, at 60 mg/ml, coconut water demonstrated significant antimicrobial efficacy, with Staphylococcus aureus showing the highest inhibition zone of 28 mm and Pseudomonas aeruginosa the least at 21 mm. and fungus antifungal activity for Aspergillus nigar 14 mm and candida albicans the laest at10mm. These findings indicate that coconut water contains bioactive compounds with potential antimicrobial properties, warranting further research for its application in medical and pharmaceutical fields.

Key words : phytochemical screening, antioxidant properties, antimicrobial activity coconut water microbial pathogens.

INTRODUCTON

Coconut (*Cocos nucifera*) is a common fruit in the tropics cultivated in nearly 90 different countries (Pires et al.,2004). The endosperm contains water referred to as coconut water, which contains carbohydrates (glucose, fructose, and sucrose), vitamin C, minerals, amino acids, enzymes, hormones, and phytochemicals (Bandalam et al.,2016). Coconut is cultivated for its multipurpose values (nutritional and medicinal). It is a unique source of various natural products for the development of drugs and industrial products (Floriana et al., 2015). It was reported to have antioxidant properties (Manisha et al., 2010). Coconut water has been used to treat various ailments, it is used as an antibacterial, antifungal, antiviral, and anti-dermatophyte agents (Fawole et al., 2007). The antimicrobial property of coconut water is because of its high lauric acid content which has been used as a medication for certain oral infections. Several studies reported that sucrose monolaurate and glycolipid compound present in coconut water have anti-caries properties (Demandal et al., 2011) Phytochemicals are bioactive compounds produced by plants as a result of primary or secondary metabolism (Molyneux et al.,2007). Some phytochemicals have been used as poisons and others as traditional medicines. Phytochemicals present in plants such as alkaloids, phenols, steroids, saponins, cardiac glycosides, flavonoids, etc, have played several roles in disease prevention (Babosaa et al., 2013) Chemical compounds derived from plants play an imporant role in preventing activities such as anti-inflammatory, anti-diabetic, anti-aging, antimicrobial, anti-parasitic, antidepressant, anticancer, antioxidant, and wound healing (Bharamsoltant et al., 2014). Due to the antimicrobial resistance of microorganisms to antibiotics as a result of broad-spectrum antibiotics and immunosuppressive agents and other factors, the use of plants as an alternative is being studied globally, especially in developing countries like Nigeria, since plants are considered nutritionally safe, biodegradable, and possess antimicrobial

phytochemicals (Berahuo et al.,2007) And (Chika et al.,2007). Plants have also been reported to be an excellent source of secondary metabolites that can be used in the production of modern medicines (Chandra et al.,2017). Many of the secondary metabolites such as tannins, flavonoids, and alkaloids have been demonstrated in many studies to have antimicrobial activities (Akinpelu et al.,2017). This study is therefore aimed at evaluating the phytochemicals and antibacterial activity of coconut water on some pathogenic microorganisms.

METERIAL AND METHODS

Sample Collection

Fresh coconut water derived from coconut was collected from Radhanasimmapuram village in Mannargudi fresh tender coconut water was extracted and collected in its natural from in a sterile conical flack.

Sample preparation

The coconut shells ware removed and the water in the nut harvested and aseptic container. A substrate of fermentation was prepared by adding filtered and pasteurised molasses to the filtered mature coconut water in 1.3 ratio and kept in a sterile plastic container. The substrate was incubated under the anaerobic at room temperature of 25c for 15-20 days. The fermented mature coconut water was preserved in a sterilised bottle by maintaining of ph 4-5 under the aseptic condition for further investigation.

Collection of isolates

The microbial isolates were collected from the medical KMC hospital at trichy stock culture. They were cultured on nutrient agar and incubated at 37c for 24 hours before usage.

Confirmation of isolate bacteria

The identification of isolate of Escherichia coli was on Eosin Methylene blue agar, Staphylococcus aureus on Mannitol salt agar, Klebsiella pneumonia and Pseudomonas aeruginosa on MacConkey agar and Streptococcus on mitis-salivarius agar was confirmed after incubation using morphological, cultural, and biochemical characteristics which include Gram staining, catalase, citrate, oxidase, indole and coagulase tests

Fungus isolate

The identification of isolate of Candida albicans on CHROM agar and Aspergillus nigar Potato dextrose agar. Was confirmed test for lactophenol cotton blue test.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was done using agar disc diffusion method (Abbas et al.,2017). They were measured and recorded in millimetre

Qualitative phytochemical analysis

Tests for flavonoids, tannins, carbohydrates, glycosides, saponins, resins, terpenoids and alkaloids were carried out using standard methods (Harborne et al., 1973) and (Trease et al., 1989).

Quantitative phytochemical analysis

Total phenolics were determined using Folin-Ciocalteau Reagent (FCR) as described by (Velioglu et al.,1998), with slight modifications.Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as described by Makkar

et al.,(1993).The flavonoids and flavonols content were determined according to the method described by Kumaranet et al.,2006) with slight modifications Monomeric.

Quantitative DPPH radical-scavenging assay

Scavenging activity on DPPH free radicals by the extract was assessed according to the method reported by (Gyamfi et al.,1999) with slight modifications.

Hydroxyl radical (OH)-scavenging assay

The 2-deoxyribose assay was used to determine the scavenging effect of the extract on the hydroxyl (.OH) radical, as reported by (Halliwell et al.,1987).

Superoxide radical (O2.-)-scavenging assay

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) (and the method used by Martinez et al.,2001)to determine superoxide dismutase with slight modifications.

Lipid peroxidation assay

A modified thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid-rich media (Ruberto et al.,2000) .

Nitric oxide radical (NO.) scavenging assay

Nitric oxide (NO.) generated from Sodium Nitroprusside (SNP) was measured according to the method of (Marcocci et al.,1994).

Statistical analysis

Data was expressed as mean standard deviation. The data obtained was subjected to Analysis of Variance (ANOVA) test to determine the significant difference at 95% confidence limit.

RESULTS

The results of the present study showed that coconut water had a growth inhibitory effect on *Klebsiella peumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus mutans, Escherichia coli* and fungus *Candida albicans, Aspergillus nigar* There was no zones of inhibition recorded against all the four isolates when tested with the negative control 2.5 % of dimethylsofloxide (DMSO). The positive control is as shown in Table 1. Using ciprofloxacin, *Klebsiella peumoniae* exhibited the highest zone of inhibition of 28mm while the least zone of inhibition was exhibited by *Pseudomonas aeruginosa* and Escherichia coli at 21mm respectively.

Table 1 Antibacterial Activity of coconut water in(mm) daimeter

S.NO	Organism Name	Acetone	Distilled Water	Ethyl Acetate
1	*Klebsiella peumoniae*	25mm	10mm	9mm
2	*Escherichia coli*	21mm	15mm	5mm
3	*Staphylococcus aureus*	28mm	20mm	10mm
4	*Pseudomonas aeruginosa*	21mm	10mm	5mm
5	*Streptococcus mutans*	22mm	12mm	7mm

The results in the antimicrobial efficacy of coconut water was significantly lower when compared to the positive control at 28mm 30mg/ml which was used as a standard against the test bacteria but higher at an increased concentration of 60mg/ml with *Staphylococcus aureus* having the highest zone of inhibition of 28mm while *Pseudomonas aeruginosa* showed the least zone of inhibition at 21mm.

Table 2. Positive control antibacterial activity of (mm) antibiotics disc

S.NO	Bacteria Name	Streptomycin	Kanamycin	Ampicillin
1	*Klebsiella peumoniae*	10mm	7mm	9mm
2	*Escherichia coli*	11mm	5mm	6mm
3	*Staphylococcus aureus*	15mm	10mm	20mm
4	*Pseudomonas aeruginosa*	10mm	5mm	5mm
5	*Streptococcus mutans*	15mm	10mm	10mm

The antibacterial activity of antibiotics disc positive control result in the coconut water was compared in the antibiotic in higher at an increased in *Staphylococcus aureus* 20mm of ampicillin. The lowest zone of inhibition in the 5mm *Pseudomonas aeruginosa*.

Table 3. Negative control antibacterial activity of organic solvent

S.NO	Bacteria Name	Acetone	Distilled Water	Ethyl Acetate
1	*Klebsiella peumoniae*	-	-	-
2	*Escherichia coli*	-	-	-
3	*Staphylococcus aureus*	-	-	-
4	*Pseudomonas aeruginosa*	-	-	-
5	*Streptococcus mutans*	-	-	-

Table 4. Antifungal activity of coconut water(mm) diameter

S.NO	Fungus	Acetone	Distilled Water	Ethyl Acetate	Glysonazole
1	*Candida albicans*	-	-	10	13
2	*Aspergillus nigar*	-	-	12	14

Table 5 Antifungal activity of coconut water in organic solvent

S.NO	Fungus	Acetone	Distilled Water	Ethyl Acetate
1	*Candida albicans*	-	-	-
2	*Aspergillus nigar*	-	-	-

Table 1 Zone of inhibition (mm)of coconut water against test bactreia

Organism	Concentration (Mg/Ml)		
	75	**50**	**25**
Klebsiella peumoniae	24	18	10
Escherichia coli	21	20	11
Staphylococcus aureus	29	25	12
Pseudomonas aeruginosa	22	16	8
Streptococcus mutans	23	17	9

Table 6. Zone of inhibition (mm) of coconut water against test fungus

S.NO	**Fungus**	**Concentration (Mg/Ml)**		
		75	**50**	**25**
1	*Candida albicans*	14	10	8
2	*Aspergillus nigar*	12	10	6

Qualitative analysis on coconut water revealed the presence of important phytochemical constituents including phenolic compounds (tannins and flavonoids)saponins,alkaline, glycosides as bioactive compounds as shows on table.

Table 7. Phytochemical constituents in coconut water

S.NO	Phytochemicals	Results
1	Flavonoids	++
2	Tannins	++
3	Phenols	+
4	Anthocyanins	+
5	Alkaloids	+
6	carbohydrates	+
7	Terpenoids	-
8	Glycosides	+
9	Saponins	+
10	steroids	-

Phenolic compounds were found to be the major class of bioactive comoponents in the coconut water. The amount of total phenolics was 0.865±0.008mgGAE/mg of coconut water extract as shows in table 8

Table 8. Quantitative phytochemical constitutent

Extra Ct	Phenolic Contents			Total Anthocyanin	Total Flavanols	Total Flavonoids
	Total Phenols	Non-Tannins	Tannins			
Coconut Water	0.865± 0.008	0.411± 0.004	0.365± 0.088	0.45±0.02	37.75± 0.32	25.45±3.65

DISCUSSION

The results of the present study showed that coconut water had a growth inhibitory effect on *Klebsilla pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus mutans* and fungus *Candida albicans, Sspergillus nigar*.There were no zones of inhibition recorded against all the five tested with the negative control and positive control, exhibited the highest zone of inhibition of *Staphylococcus aureus* 20mm. The least zone of inhibition was exhibited by pseudomonas aeruginosa and *Escherichia coli* at 5mm.and fungus highest zone incubation *Aspergillus nigar* 14mm. least zone of in *Candida albicans* in 10mm. respectively. Staphylococcus aureus was slightly higher at 28 mm. The coconut water exerted significant antimicrobial effect on the test isolates at a concentration of 60mg/ml in our study as compared with previous work done by (Baharvand et al.,2021) which observed inhibition at 100% concentration. The inhibition efficacy of coconut water can be attributed to the presence of phytochemical, such saponins cardiac glycosides,and alkaloids. This is in line with the report of (Shiny et al.,2014) that fermented tender coconut water has a antimicrobial effect on E.coli and the report of (Fowoyo et al., 2016) that tender coconut water exhibited antimicrobial effect on *staphylococcus aureus, Escherichia coli* and *p.mirabilis*. Nasimuddin et al. (2016) investigated the antimicrobial activity of coconut t water and oil on gram-positive and gram-negative bacteria including *staphylococcus aureus,Escherichia coli, klebsiella pneumonia, pseudomonas aeruginosa, streptococcus mutans* and fungus positive and negative control of in *candida albicans ,aspergillus nigar*. The results that coconut water had antibacterial properties. The quantitative analysis on phytochemical concstituents revealed the phenolic compounds found to be the major class of bioactive compounds in the coconut water. The amount of total phenolics was 0.865±o.088mgGAE/mg of aliquot of coconut water extract and total flavonoid of 25.44±3.65 rutin equivalents /g dry extract of coconut water. Over the years, coconut has been extensively explored for its use in different fields. Result from this study also revealed high phenol and flavonoid contents contributes to inhibitory effect of the coconut water, consistent with a study on the phytochemical analysis of *Cocos nucifera* endosperm by (offor et al., 2017) and (Anyiam et l.,2020) conferring its antioxidant ability to significantly lower cellular oxidative strees (Tan etal.,2018). Flavonoids also function as signaling molecular and exerting a positive effect on cognitive function (Ferrari et al.,2003). The phenolic compounds derived mostly from plants possess an antioxidant potential to mange oxidative stress-associated disease like Alzheimers and other neurodegenerative disease (Barreira et ai., 2008). This study showed that the phenol rich aliquots of coconut water in SNP solution decreased levels of nitrite, a stable oxidation product of NO. liberated from SNP. The aliquots exhibited strong NO. Radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The NO. Scavenging capacity was concentration dependent with 250µg/ml of the extracts scavenging most efficiently compared to α-tocopherol.

CONCLUSION

Coconut water was proved to be a valuable antibacterial agent against *Klebsiella pneumoniae Pseudomonas aeruginosa , Staphylococcus aureus, streptococcus mutans, Escherichia coli*. And antifungal against in *candida albicans, aspergillus nigar*. Therefore the antioxidant activities of coconut water are of interest as a potential natural food antioxidant additive, nutraceutical, and require further evaluation. *C. nucifera* has significant inhibitory action against microbial pathogens indicating the presence of highly effective active compounds, which can be identified and incorporated into modern medical systems for controlling various diseases. Further studies should be done to isolate and characterize bioactive compounds present in coconut water.

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ASSESSMENT OF *ULVA RETICULATE* EXTRACT ON ANTIINFLAMMATORY MARKERS IN FRUCTOSE INDUCED EXPERIMENTAL RATS

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ABSTRACT

Adipose tissue is a crucial organ that regulates energy homeostasis. It is well-accepted that adipocytes not only store excessive fat but also have endocrine functions by secreting green hormones and inflammation-related adipokines. Seaweeds, especially green seaweeds, are a common food ingredient in Eastern Asian countries such as India and are a rich source of proteins, vitamins, essential minerals, fibre, polyunsaturated fatty acids, and various bioactive components. The aim of the study was to investigate the effect of Ulva reticulata extract on hormones-and-anti-inflammatory markers in fructose-induced experimental rats. High fructose-induced rats showed significant elevation in the serum leptin, IL-6, serum TNF-a and a decrease in adiponectin and ghrelin in HFD fed rats as compared with control. On supplementation of Ulva lactuca extract to obese rats showed restoration of altered parameters such as serum leptin, IL-6, serum TNF-a, adiponectin and ghrelin. The restoration of adipose tissue activity by Lactuca Ulva reticulata may be due to the presence of phytochemicals such as flavonoids, reticalata terpenoids and saponins.

Keywords: *Ulva reticulata* extract, Hormones, cytokines, Bioactive substances, TL-6, INF-, Adiponectin Ghrelin.

INTRODUCTION

Inflammation is a physiological response necessary to restore homeostasis altered by diverse stimuli and inflammation state chronically established or an excessive response can involve deleterious effects (Wang and Trayhurn, 2006). In overweight and obesity, there exists low-grade chronic inflammation. Recent studies have unveiled some of the intracellular pathways of inflammation associated with these conditions; studies in mice and humans evidence that consumption of nutrients may acutely evoke inflammatory responses. It is thought that the starting signal of inflammation is overfeeding and the pathway origins in tissues involved in metabolism, that is, adipose tissue, liver, and muscle, which in response of this stimulus triggers the inflammatory response (Faloia et al., 2012). The main source of proinflammatory cytokines in obesity is the adipose tissue and they are mainly produced by infiltrating macrophages, although adipocytes play a role. Many studies also implicate chronic low-grade inflammation in the interplay between obesity and metabolic complications, as many chronic degenerative disorders, including atherosclerosis are also commonly associated with hypertension, which itself has also been linked recently to inflammation (Hotamisligil, 2006).

In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs and health foods. Marine algae are one of the richest sources of structurally diverse natural products. Indeed, an increasing number of novel compounds have been isolated from marine algae as bioactive ingredients including polyunsaturated fatty acids, polyphenols, flavonoids, sterols, proteins, sulfated polysaccharides, and vitamins (Manikkam et al., 2016; Ruocco et al., 2016; Suleria et al., 2016). Many of these compounds possess different biological properties such as antidiabetic, antioxidant, anticancer, antiobesity, and anti-inflammatory activities (Zhao et al., 2015; Fernando et al., 2016). Marine algae have been

identified as an under-exploited plant resource and a source of functional food (Senni et al., 0 2011). Therefore, the aim of our study was to investigate the effect of the green algae *Ulva reticulata* Inflammation in fructose-induced experimental rats.

MATERIALS AND METHOD

Animals

Male albino rats of Wistar strain approximately weighing 180-220g were used in this study. They were healthy animals procured from Sri Venkateswara enterprises, Bangalore, India. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2°C and 12 hours light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water ad libitum. They were acclimatized to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control **(687/PO/Re/S/02/CPCSEA (05-12-2018))** and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Collection and preparation of extract

Fresh *Ulva reticulata* was collected from South coast areas, Rameswaram mandapam, Tamil Nadu. The Collected *Ulva reticulata* was first thoroughly washed with distilled water and allowed to dry *Ulva reticulata* the shade with optimal ventilation. The dried *Ulva reticulata* was then chopped to a coarse powder. Maceration extraction was carried out in a closed conical flask for 72 h. 10 grams of the coarse powdered material was macerated in ethanol and then the extract was filtered using Whatman filter paper No. 1 and dried in hot air oven at 40 degrees centigrade. The solvent-free ethanol extract was obtained. The dried extract was then kept in a desiccator to maintain dryness till used in the experiment.

Preparation of control and high fructose diet

The control and high fructose diet were prepared by the method of Nandhini et al., (2002). Table 1 represents the composition of the experimental diets.

Table 1 Composition of the experimental diets (g/kg diet)

Ingredients	Control diet (%)	High-Fructose(HF) diet(%)
Corn starch	61	-
Fructose	-	61
Casein	20	20
Methionine Groundnut	0.7	0.7
Oil	5	5
Wheat bran	9.6	9.6
Mineral mixture	3.5	3.5
Vitamin mixture	0.2ml	0.2ml

Experimental design

Body weight of the animals were recorded and they were divided into 4 groups of 6 animals each as follows. **Group 1:** Normal rats fed with control diet. **Group 2:** High fructose diet (HFD) fed animals received fructose enriched diet for a period of 8 weeks. **Group 3:** HFD fed animals co-administrated with Ethanolic Extract of *Ulva reticulata* by oral gavage daily at a dose of 500 mg/kg body weight (based on effective dosage fixation studies) for 8 weeks. **Group**

4: HFD fed animals treated with standard drug Orlistat at a dose of 9 mg/kg bodyweight for 8 weeks.

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with and without EDTA as anticoagulant. Serum was separated for the estimation of various parameters.

Determination of adiponectin, leptin and ghrelin

Serum adiponectin, leptin and ghrelin were measured by Radio Immuno Assay (RIA) using Linco Research kits (Linco Research Inc., St Charles, Missouri, USA) with the following characteristics: Adiponectin assay detection limit was 2 ng/ml. Leptin assay's detection limit was 0.8 ng/ml while ghrelin detection limit was 0.8 ng/ml.

Quantification of cytokines (TNF-a and IL-6 serum determination)

TNF-a and IL-6 were tletermined in serum of mice by enzyme-linked immunosorbent assay (ELISA) with Quantikine ELISA Kit (Bender Med Systems, Australia). Cytokines such as IL-6 (BMS634) and TN-a (BMS622) were quantified by ELISA procedure using kits from Bender Med system, according to manufactures protocols. 50µl of ELISA diluents were pipetted into antibody coated wells (anti IL-6 and TNF-a) followed by 100µl of each standard and 50µl of test samples (serum) for 5 seconds to mix the contents in the wells, covered with plate sealed and incubated for 2 hrs at room temperature. After incubation, contents of the wells were aspirated and washed three times with wash buffer. After complete removal of the wash buffer in the final wash, 100µl of detection solution was added, covered with plate sealer and incubated for 1 hr.

The wells were washed three times with wash solution and 100µl of substrate reagent was added and incubated for 30 minutes in dark. The color development was arrested by adding 100µl of stop solution and the absorbance was recorded at 450nm with a reference wavelength of 570 nm. Values were expressed as pg/ml

Statistical Analysis

Values were expressed as Mean SD for six rats in each group and statistically significant differences between Mean values were determined by one way analysis of variance (ANOVA) followed by the DMRT test for multiple comparisons (Harvey and Paige, 1998). The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 and $P< 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

The diet-regulating hormones ghrelin and leptin show a major influence on energy balance (Popovic and Duntas, 2005), and therefore measuring their plasma levels may indicate the sensitivity of an animal to weight gain when exposed to a HFD. Leptin, the adipocyte hormone, is a mediator of long-term regulation of energy balance, suppressing food intake and thereby accounting for weight loss. It is believed to tonically act as an afferent signal from adipose tissue to the hypothalamus, as a part of a negative feedback loop. On the other hand, ghrelin secreted from the stomach is a fast-acting brain gut peptide with growth hormone-releasing and appetite-stimulating activities, acting as an afferent signal to the hypothalamus as a part of a positive feedback loop. In the present study, rat fed an HFD for 8 weeks showed decrease in the circulating level of the orexigenic hormone ghrelin (Table 1 and Figure 1) and an increase of the anorexigenic hormone leptin, a finding consistent with earlier reports (Dogan er

al., 2007; Klok et al., 2007). Possibly, the ghrelin and leptin secretions are dysregulated with high fructose diets, impairing the homeostasis and eventually promoting obesity. The *Ulva reticulata* treatment restores the level re Healata de Melo et al., (2009) study.

Adipose tissue is a potential source of another bioactive substance, adiponectin. In the present study, decline levels of plasma adiponectin in HFD fed rats were observed as compared with control rats. Present finding is in agreement with Hajer et al., (2008) studies, which have been reported that adiponectin synthesis is reduced in obesity, insulin resistance, metabolic syndrome, and type-2 diabetes. Earlier studies also have shown its inverse relationship with body weight, especially abdominal visceral fat accumulation. Adiponectin has an insulin-sensitizing property and low plasma adiponectin levels are associated with insulin resistance as found in obesity (Yamauchi et al., 2001) and the peroxisome proliferator- activated receptor gamma (PPARA), a nuclear factor that regulates the expression of key genes involved in lipid and glucose metabolism and adipocyte differentiation. Agonists such as rosiglitazone used widely in the treatment of diabetes mellitus seem to promote adiponectin secretion, accounting for its insulin sensitizing property (Aprahamian et al., 2009).

Table 1: Effect of *Ulva reticulata* on Hormones and inflammatory markers in fructose-induced experimental rats

Parameters	Group I	Group II	Group III	Group IV
Leptin (ng/ml)	0.53 0.09a	1.76 0.07b	0.57 0.05a	0.55 0.05a
Adiponectin ng/ml)	1.24 0.12a	0.43 0.05b	1.19 0.11a	0.89 0.06a
Ghretin (ng/ml)	0.92 0.09a	0.25 0.03b	0.85 0.07a	0.89 0.06a
TNF (pg/ml)	3.56 0.39a	23.74 1.28b	3.49 0.41a	3.49 0.38a
IL-6 (pg/ml)	33.29 2.18a	90.74 2.93b	35.89 2.54a	34.81 2.85a

Values are expressed as Mean ± SD for 6 rat. Data were analyzed by one-way ANOVA followed by post-hoc DMRT test using SPSS ver. 20. Mean values are within the row followed by different letters Superscript (homogeneous subsets) are statistically significant (l ^ 2 < 0.05) and same letter was statistically non-significant (P>0.05) from each other groups, significant level alpha 0.05. Group 1, Group III and Group IV were non-significant compare with between the groups while Group II was significant compare with Group 1, Group III and Group IV. Group I: Normal control; Group II: Negative control (High fructose diet only); Group III: Negative + *Ulva reticulata* extract 500mg/Kg; Group IV: Negative + Orlistat 9mg/Kg

Effect of *Ulva reticulata* on Hormones and inflammatory markers in fructose induced experimental rats

The origin of inflammation during obesity and the underlying molecular mechanisms that explain its occurrence are not yet fully understood, but pro-inflammatory cytokines play a central role. In obesity, there are higher circulating concentrations of inflammatory cytokines than in lean beings, and it is believed that they play a role in causing insulin resistance. The main source of pro-inflammatory cytokines in obesity is the adipose tissue; they are mainly produced by infiltrating macrophages, although adipocytes play a role. In this way, blood concentrations these cytokines are lowered following weight loss (Faloia et al., 2012). The main cytokines responsible of chronic inflammation are tumour necrosis factor-a (TNFa) and interleukin-6 (IL-6) mentioned earlier (Gregor et al., 2011).

TNF-a is a pleiotropic molecule that plays a central role in inflammation, immune system development, apoptosis, and lipid metabolism, with numerous effects in adipose tissue, including

lipid metabolism and insulin signaling. Circulating TNF-a is increased in obesity and decreased with weight loss. TNF-a promotes the secretion of other powerful pro-inflammatory cytokine, IL-6, and reduces anti-inflammatory cytokines like adiponectin. TNF-a induces adipocytes apoptosis and promotes insulin resistance by the inhibition of the insulin receptor substrate 1 signalling pathway (Wang and Trayhurn et al., 2006).

IL-6 is a cytokine that plays important roles in acute phase reactions, inflammation, hematopoiesis, bone metabolism, and cancer progression. IL-6 regulates energy homeostasis and inflammation; it is capable of suppressing lipoprotein lipase activity, and it controls appetite and energy intake at hypothalamic level. IL-6 is important in the transition from acute inflammation to chronic inflammatory disease. It contributes to chronic inflammation in conditions such as obesity, insulin resistance, inflammatory bowel disease, inflammatory arthritis, and sepsis when deregulated (Naugler and Karin, 2008).

The high consumption of sugary beverages rich in fructose is directly related to the development of obesity and its consequences, such as metabolic syndrome (Johnson et al, 2007). Leptin, the adipocyte hormone, is a mediator of long-term regulation of energy balance, suppressing food intake and thereby accounting for weight loss. Table 1 and Fig. I represent a significant elevation in the serum leptin, IL-6, serum TNF-a and a decrease in adiponectin, and ghrelin in HFD fed rats as compared with control. In Orilstat treated animals significant restoration of hormones was observed. Our result agrees with the earlier report (Lira et al., 2011; Ashok Kumar and Sharunetha, 2018).

The inflammatory markers levels were significantly increased in HFD fed rats as compared to control. Supplementation of *Ulva reticulata* to HFD fed rats decline the level of TNF-a and IL-6 suggesting that it regulate the secretion of proinflammatory cytokines in adipose tissue. It was shown more than two decades ago that insulin resistance in obesity was closely related to adipose tissue inflammation (Khan et al., 2020), when increased tumour necrosis factor a (TNFa) expression in the adipose tissue of obese rodents and humans was identified (Hotamisligil et al., 1995). Further animal and human studies confirmed the increased expression and/or secretion of several proinflammatory cytokines, such as TNFa, interleukin 1B (IL-1B) and interleukin 6 (IL-6) in the adipose tissue from obese subjects (Madan et al., 2006).

CONCLUSION

HFD fed rat shows the deregulation of adipose tissue hormones and cytokines. Supplementation of *Ulva reticulata* to to HFD HFD fed rats regulates the hormones and cytokines and thereby maintained the adipocyte functions. The restoration of adipose tissue activity by *Ulva reticulata* may be due to the presence of phytochemicals such as flavonoids terpenoids and saponins.

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ANTIBACTERIAL ACTIVITY AND BIOACTIVE COMPOUNDS ANALYSIS FROMMARINE ACTINOMYCETES

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ABSTRACT

Actinomycetesare one of the major sources of identification bioactive compounds that are used against antibacterial, antifungal, antiviral, anticancer and anti-inflammatory based drug discovery in pharmaceutical industry. Streptomyces is the largest genus of actinomycetes which is known for the production of most antibiotics. Present study to isolate the novel actinomycetes from marine sediment and find the bioactive compounds for selected strains. Streptomyces griseus DR38 has identified through 16S rRNA sequencing and extract the extracellular product to has found against the significant activity of E.coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus sp. The active compounds have identified using GCMS studies. The extracellular compound of D-xylose, Rutinose, Diethyl Phthalate, Phthalic acid, 1,2 Benzenedicarboxylic acid has a major active compound for found. This compound has usage of pharmaceutical, cosmetic and food industry for the medical application and preservation process.

Keywords: Actinomycetes, Bioactive compounds, Antibiotics, Streptomyces.

INTRODUCTION

Actinomycetes, particularly marine actinomycetes are prolific source of bioactive compounds with diverse biological activities such as antibacterial, antifungal, antiviral, and anticancer properties. Studies have highlighted the isolation of numerous bioactive compounds from actinomycetes, including terpenes, terpenoids, meroterpenoids, polyketides, non-ribosomal peptides and other cytotoxic compounds, showcasing their potential in drug discovery and antibiotic development (Mondal & Thomas, 2022; Tarasovaet al., 2023).Actinomycetes from various environments like marine sediments, hot springs and terrestrial ecosystems have been explored for their ability to produce novel bioactive compounds, emphasizing the importance of these microorganisms in generating new sources of pharmaceutical agents. The bioactive compounds isolated from actinomycetes have shown promising activities against drug resistant pathogens, making them valuable candidates for the development of new antibiotics and therapeutic agents (ZiyanQiua., 2022;KumariAparana and Rao K V Bhaskara 2023). Streptomyces strains have been identified as valuable sources of specialized metabolites, including pigments, which have various bioactivities such as antioxidant, antibiofilm and antibiotic activities. Present study has isolated streptomyces griseus from marine sediment soil and finding the active compounds through GCMS. These studies highlight the diverse range of bioactive compounds for potential pharmaceutical and nutritional applications.

MATERIALS AND METHODS

Sample Collection:

Mangrove soil originating from a specific site in India was procured for research purposes. The soil sample was collected from Pichavaram Mangrove National Forest in Killai, Tamil Nadu India. The collection process involved obtaining soil specimens from a depth of 15 centimetres beneath the soil's surface, which were subsequently placed in aseptic containers for transportation to the laboratory. The process of isolating Actinomycetes from the Mangrove soil

entailed utilizing a Starch Casein Agar (SCA) medium comprising of a mixture of 50% seawater and 50% distilled water.

Screening for Actinomycetes that Produce Bioactive Compounds:

The strains that were isolated underwent screening to assess their capability in producing bioactive compounds. These isolated actinomycetes strains were introduced into a medium containing 50% seawater known as (SCA) medium. The flask containing the inoculated strains was placed in an incubator at room temperature for duration of 5 days while being agitated on a rotary shaker operating at 120 rpm and a temperature of 28oC. Following the completion of the incubation period, the liquid broth was filtered, and the resulting filtrate was utilized to evaluate antimicrobial activity.

Antimicrobial activity

Antimicrobial efficacy was evaluated using well susceptibility methods, with crude ethyl acetate extracts of active producing strains tested for minimum inhibitory concentration (MIC) against various microorganisms including E.coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus and Streptococcus sp. using Muller Hiton Agar Medium (Himedia, India) (Ban AI Jouboria et al., 2023). We select the maximum inhibitory concentration of strain has used for further studies.

Molecular Characterization

The genomic DNA was isolated using HiPureA Bacterial Genomic DNA Purification kit (Himedia). Primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 R (5'-TACGGCTACCTTGTTACGACTT -3') were used to amplify the 1480 bp 16S rRNA. The following were the PCR (Prime96 – Himedia) conditions: Initial denaturation 94oC for 3min, followed by 30 cycle of denaturation at 94oC for 1min, annealing at 55oC for 1min, extension at 72oC for 1 min, and final extension at 72oC for 3 min. The amplified product were confirmed with 1.2% of agarose gel. The PCR products were sequenced with Sanger Sequencing 3730xl DNA Analyzer (Barcode BioSciences – Bengaluru). The 16S rRNA sequence was entered into the NCBI nucleotide blast to determine the identity and similarity of the sequence with other species in the database. Mega 11 software was used for sequence alignment and generation of phylogenetic trees.

Identification of Bioactive compounds with GCMS

The analysis of the active ethyl acetate extract was executed by Gas Chromatography Mass Spectrometry (GCMS). Shimadzu QP2010 ultra was used for identification of compounds and gas chromatography interfaced to a mass spectrometer. The appliance was built with Elite-1 fused silica capillary. Helium gas (99.99%) was the carrier gas with a constant flow rate of 1.21 ml/min and split ratio:10. Temperature of Injector was set on 160oC; Ion-source temperature was 200oC. The oven temperature was intended from 60oC with an increment of 280oC for around 22 min. Mass spectra were taken at 70eV; a scan interval of 0.5 sc. The chemical composition of the extract was determined by measuring the peak area and the retention time by comparing the NIST 14 library (Kumari et al., 2020 &2021).

RESULT AND DISCUSSION

Primary and secondary screening of strain was performed against bacterial pathogens in well diffusion method was applied on Muller Hinton Agar Plates (Kumariet al., 2020).The maximum zone of inhibition in Pseudomonas aeruginosa for 13.7mm and minimum of Streptococcus sp. 8.9mm other organism have a significance of zone of inhibition occur from the

selected strain Figure1. The selected strain has amplified and sequenced 1532bp of 16S rRNA and searched by NCBI nucleotide Blast to confirmed as Streptomyces griseus DR38, sequence has aligned and deposited to NCBI GenBank Accession No:PP329837. The phylogenetic tree was constructed using MEGA 11.0 (Tamura et al., 2021) software using various streptomyces species. The evolutionary history was inferred using the Neighbor joining method (Saitou N. and Nei M; 1987). The optimal tree is shown figure 2. The evolutionary distance were computed using Maximum composite Likelihood method (Tamura., et al., 2004) and are in the units of the number of base substitutions per site. This analysis involved 13 nucleotide sequences. All ambiguous positions were removed for each sequence pair . There were a total of 1460bp position in the final dataset. The ethyl acetate extract was analysed by GCMS and found the Bioactive compounds are listed in table1. The presence of D-xylose is a sugar widely used as a diabetic sweetener in food and beverage process. Rutinose disaccharide sugar is attached at the 3-hydroxyl position of quercetin an interesting biological active compound, rutin is formed due to their numerous pharmacological activities ranging from antioxidant action to chronic diseases such as cancer, diabetes and inflammation (Habtemariam, 2000 & 2011; Habtemariam and Varghese, 2015).Diethyl Phthalate used for personal care products, plasticizers, cosmetics etc (Huang et al., 2021).Secondary metabolites produced from marine actinomycets have distinct chemical structures, which may form the basis for the synthesis of new drugs. Streptomyces griseus DR38 alone produces a wide range of metabolites having different biological activities. Enrichment and selective isolation methods can also be used to isolate rare actinomycetes from marine ecological niches having the potential to biosynthesize novel bioactive compounds. Continues findings of novel organisms possible to isolate the novel bioactive compounds.

Figure 1: Inhibition activity of *Streptomyces griseus* DR38 extract of selected organisms.

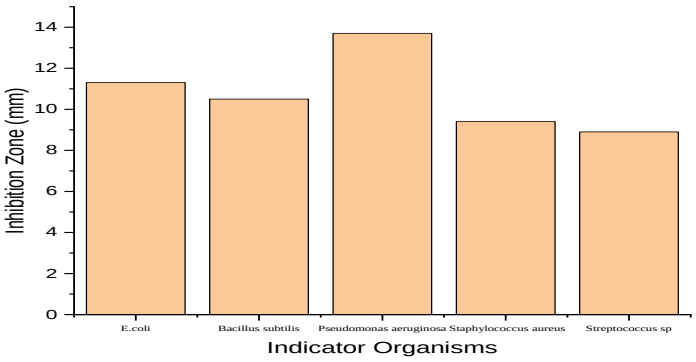

Figure2: Phylogenetic study of *Streptomyces griseus* DR38 with Streptomyces species.

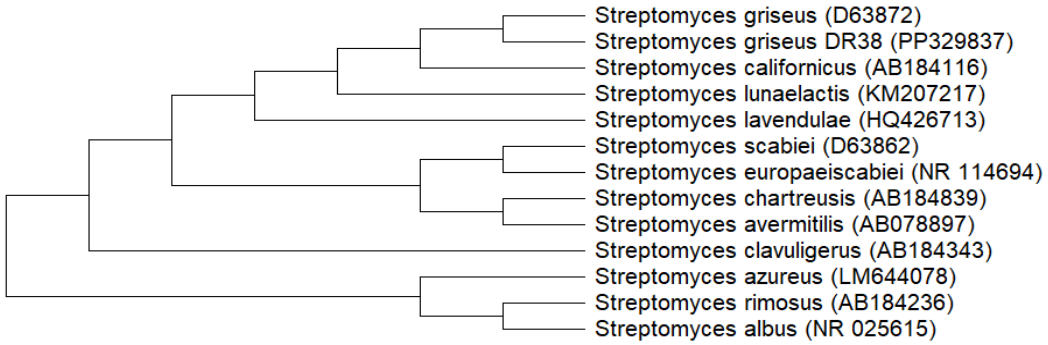

Table1: Identification of Bioactive compounds from *Streptomyces griseus* DR38 through GCMS Analysis.

S.No	Compound Name	Rev. Score	Prob. %	molecular formula	molecular weight
1	D-Xylose	842	36.92	C5H10O5	150.13
2	Rutinose	838	31.19	C27H30O15	594.5
3	D-Xylose	787	36.92	C5H10O5	150.13
4	Diethyl Phthalate	864	36.22	C12H14O4	222.24
5	Phthalic acid, ethyl 2- pentyl ester	825	5.71	C15H20O4	264.32
6	Monoethyl phthalate	868	5.71	C10H10O4	194.18
7	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	664	7.22	C24H38O4	390.6
8	Phthalic acid, 2-methoxyethylhexadecyl ester	671	6.66	C27H44O5	448.6
9	Phthalic acid, 2-methoxyethyltetradecyl ester	670	6.4	C25H40O5	420.6

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EFFECT OF ACID VALUE ON OIL ABSORPTION OF PIGMENT BY USING SUNFLOWER OIL

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ABSTRACT

The aim of the project is to analyse the effect of acid value on oil absorption of pigment using sunflower oil. Sunflower oil is primarily composed of linoleic acid, a polysaturated fat, and an oleic acid, a monosaturated fat. Through selective breeding and manufacturing process, oils of differing proportions of the fatty acids are produced. The expressed oil has a neutral taste profile. Experiments were conducted using sunflower oil with varying acid values, and the oil absorption of pigment was measured using standardised methods. This research investigates how the acid value of sunflower oil influences its interaction with pigments, particularly interms of oil absorption capacity.

INTRODUCTION

Pigments play a crucial role in various industries, including paints, coatings, plastics, and cosmetics. One of the key factors influencing their performance is oil absorption, which determines how much oil a pigment requires to form a workable paste. The oil absorption capacity of a pigment is influenced by several factors, including particle size, surface area, and chemical composition.

This study focuses on the effect of acid value on the oil absorption of pigments using sunflower oil. The acid value of an oil is a measure of its free fatty acid content, which can affect its interaction with pigment particles. Oils with higher acid values may influence the dispersion, wet ability, and binding properties of pigments, potentially altering their oil absorption characteristics.

By analyzing the relationship between acid value and oil absorption, this study aims to provide insights into optimizing pigment formulations for various industrial applications. The findings can help in selecting suitable oils for pigment dispersion, improving product performance, and enhancing cost efficiency in manufacturing processes.

Acid value : Acid value is the mass of potassium hydroxide that is required to neutralise one gram of chemical substance**.**

Oil absorption: Oil absorption of a pigment is a practical measure of the surface particle shape of pigment**.**

Mechanism: When the oil absorption test is performed the surface of each pigment particle is wet and saturated to a certain extent with oil and thus is encased in an oil shell that has been estimated to be as thick as eight molecules. The amount of oil needed depends on specific pigment area which is a function of particle size roughness and porosity. With temperatures and humidity constant the oil absorption value depends on the duration and vigor of the rubbing operation and on the affinity of the oil for pigment. The amounts of oil required for this stage of the test is a function of packing of the packing taken on by the particles.

METHODS FOR DETERMINING OIL ABSORPTION

1. Gardner – Coleman method

In this test soft paste is formed from the pigment by drop wise addition of sunflower oilwith an acid numberfrom a burette to the gently stirred pigment. As the oil is slowlyadded the ,mixture is continuously stirred and folded so the oil strikes dry pigment as long as anyremains. The mixture is not vigorously rubbed as in the rub out nethod, eventually the mixturecollects in small lumps that gradually coalesce. The rate of oil addition is reduced as the endpoint is reached. The amount of oil required to from a paste is used to calculate the OA value.The end point for paste formation is taken to be the point when the lumps of wet pigment form asingle ball or when a excess of oil smears the walls of the container. This will takes place within one or two drops of oil. It is important that there is no rubbing or grinding when this test iscarried out.

2. Spatula-Rub-out method.

In this test, a stiff, putty-like paste is formed by adding linseed oil in a drop wise manner to thepigment while it is being thoroughly rubbed with a spatula. The rub-out test required a thoroughrubbing action of the pigment and oil with a steel spatula.Raw linseed oil that means ASTM D234 but with an acid numbe is slowly added in adrop wise manner to known amount of pigment. After the addition of cach drop of oil, the oil isincorporated into the pigment by working or rubbing the two together with the spatula. The endpoint is taken so be the point where sufficient oil has been incorporated into the pigment toproduce a very stiff, putty like paste that does not breakup. The weight of oil is determined, andthe oil absorption value is calculated as the grams of oil used per 100 gm of pigment.

3. Azam method

The Azam method is essentially the same test as ASTM D 281 with the main difference being amore rigorously defined end point. The end point is defined as the point where the paste justadhers to the spatula and the paste was turned a "complete" paste. Azam found that a completepaste absorbed no more oil when immerse in oil, but an "incomplete" paste would absorb oil inan amount sufficient to make it a "complete" paste. The end point check was made byimmersing the rubout mass in a known amount of oil and then determining the change after 2 or3 days.

Determination of Acid Value of Sunflower Oil

Reagents

1. Phenolphthalein : 1 gm in 100 mm alcohol
2. Alcoholic KOH : 6.6 gm of KOH in 1000 ml water.
3. Reagent in (solvent) mix: equal parts of isopropanol (1PA) or ethanol and tolene
4. Potassium Phosphate

Procedure

Standardization of alcoholic KOH with KHP About 1.65 gm of KOH is out accurately into a 250 ml standard flask. The crystals are dissolved in water and made up to the mark.

Principle: Potassium hydroxide (KOH) reacts with potassium hydrogen phthalate (KHP) solution to titrate water.

$$HKC_8H_4O_4 + KOH \; K_2C_8H_4O_4 + H_2O$$

Standardization: About 0.5 gm of KHP is accurately weighed and transferred in to a 250 ml comical flask. It is dissolved in water and 1 ml of phenolphthalein indicator is added to the KHP solution. It is titrated against the KOH solution taken from the burette. The titration is continued till the permanent pink colour in the end point. Note the volume. The experiment is repeated till concordant values are obtained.

Calculations:

Standardization of KOH :

I. Weight of the sample = 0.5014 g
Titrate volume = 25.9
Molecular weight of KHP =204.23
Normality of KOH = 0.094N

II. Normality of KOH = 0.0958N
Average of Normality = 0.094N

1) Determination of acid value of an oil : Weighed out 20 gm of oil in to an Erlenmeyer flask. Add 100 ml solvent mix and 1 ml of phenolphthalein indicator. Titrate against the standard KOH from the burette permanent pink colour note the volume. Neutralize the solvent mixture with alcoholic KOH to pink color

Determination of Acid Value of Sunflower Oil

Trial No	Weight of sample[ml]	Burette reading Initial	final	Volume of KOH[ml]
1	19.958	0	1.9	1.9
2	19.958	0	2	2

Acid value of sunflower oil = $\frac{[\text{mg of KOH} \times \text{Titration volume}]}{\text{Weight of sample}}$ mg of KOH = N × 56.11= 5.3248

1.Acid value of sunflower oil =[5.3248 ×1.9]÷19.9558 = 0.5069
2.Acid value of sunflower oil =[5.3248×2] ÷ 19.9558 = 0.5296
Average value = 0.5182

Result

Acid value of sunflower oil = 0.3679

Determination of Oil Absorption

Determination of oil absorption by pigment using sunflower oil

1.Initial weight of dropping bottle = 52.217
Final weight of dropping bottle = 50.851
Weight of oil consumed = 1.366
Oil absorption of pigment = weight of oil consumed x 20= 27.32

2.Initial weight of dropping bottle = 50.851
Final weight of dropping bottle = 49.453
Weight of oil consumed = 1.398
Oil absorption of pigment = weight of oil consumed x 20= 27.96

Average value of oil absorption of pigment = 27.64

CONCLUSION

The acidity of oil dependent on the amount of free fatly acids present. This is turn is depend on the dependent of hydrolysis of the oil or the nature of the processing which the oil may have undergone. Refined oils are often referred to as neutralized, ie, they have a low acidity. Raw and crude oils are naturally hydrolyzed and consequently have a higher acid value. From the table it is clear that acid value it is clear that of an oils (fatty acids) increases with decreasing oil absorption of oils. Acid value and oil absorption of oils are inversely related. The oil absorption value helps the paint manufacture to formulate the paint composition.

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PRELIMINARY STUDIES ON THE ANTIBACTERIAL EFFECT OF MEDICINAL PLANTS AGAINST URINARY TRACT PATHOGENS

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ABSTRACT

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, with increasing antibiotic resistance posing a significant global health concern. Medicinal plants offer a promising alternative for developing new antibacterial agents. This study evaluates the antibacterial potential of *Capparis sepiaria* L. against UTI-causing pathogens. Leaves of *Capparis sepiaria* were extracted using distilled water, ethanol, and methanol, and their antimicrobial efficacy was tested using the well-diffusion method. A total of 58 bacterial isolates were obtained from 25 urine samples, with dominant pathogens including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus, Providencia stuartii,* and *Klebsiella aerogenes*. The ethanol and methanol extracts exhibited significant antibacterial activity, with the highest inhibition zones recorded against *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Providencia stuartii*. These findings highlight the potential of *Capparis sepiaria* as a natural antimicrobial agent. Further research is needed to isolate and characterize the bioactive compounds responsible for its antibacterial properties, supporting its potential use in pharmaceutical applications.
Keywords: *Capparis sepiaria* L., Urinary tract infection, Microbes

INTRODUCTION

Medicinal plants are widely accepted and used to prevent and manage many ailments. Urinary tract infections (UTIs) are the most common form of bacterial infections, affecting people throughout their lifespan. The emergence and spread of antibiotic resistance among UTI patients, as well as the evolution of new strains of disease-causing agents, are of great concern to the global health community.

Commonly used medicinal plants such as *Capparis sepiaria* L in our locality could be an excellent source of medicine to fight against this problem. The leaves of *Capparis sepiaria* contain a variety of biologically active compounds, including reducing sugars, flavonoids, steroids, tannins, glycosides, alkaloids, gums, resins, amino acids, proteins, and anthraquinones. These compounds suggest the plant's potential as a source of crude drugs (1). Also, *Capparis sepiaria* has demonstrated significant antimicrobial activity, particularly against Gram-negative bacteria such as Klebsiella pneumoniae and Escherichia coli (2). The ethanol extract of the stem also shows potent anti-inflammatory effects, comparable to standard drugs like indomethacin and penicillin (3,4). Hence the present study evaluates the antibacterial potential of folk medicine against UTI-causing pathogens.

METHODOLOGY

In the present study, 25 urine samples were collected and from the participants, a total of 58 bacterial colonies were isolated from the urine samples. Among these 58 colonies, six dominant UTI bacterial pathogens were identified by using biochemical and morphological characteristics. The identified pathogens were *Escherichia coli, Klebsiella pneumoniae,*

Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus, Providencia stuartii and *Klebsiella aerogenes*.

Leaves of *Capparis sepiaria* L. were sorted, rinsed, air-dried, and milled using a mechanical blender. The powder was then sieved to obtain the sample. The flour was packed in a plastic container and stored at room temperature until required. About 20 g of the sample was soaked in 100 ml of three different organic solvents, distilled water (Sample A), ethanol (Sample B), and methanol (Sample C), for 72 hours. The pale-yellow supernatant was passed through Whatman filter paper for filtration.

The antimicrobial testing was done using a well-diffusion technique. We dissolved the crude extracts in Dimethyl sulfoxide (DMSO) and applied different amounts (50, 75, and 100 µl) onto pre-inoculated Mueller Hinton Agar (MHA) plates with the respective cultures, then incubated them at 37℃ for 24 hours. For bacteria, we used Streptomycin (20 µg) as a positive control, while for fungi, we used amphotericin-B (50 µg). DMSO served as the negative control. After incubation, the zone inhibition around the well (diameter in mm) was measured using the Hiantibiotic zone scale (HiMedia, PW096).

After 24 hours, the three sterile Petri dishes containing solidified agar were inoculated with bacterial subcultures using sterile swabs inside the laminar flow chamber. Three wells were created in each Petri dish using a cork borer, and a volume of 20µl from each sample (A, B, and C) was introduced into the wells with a micropipette and incubated for 24 hours. This procedure was conducted to evaluate the antimicrobial activity of the test samples in comparison to the standard samples.

The area of inhibition was noted for each of the petri dishes. The area of inhibition was a distinct clear space surrounding the wells where the sample was applied. The clear region is an indication of the absence of bacterial growth and it reveals the potency of the sample as an antibacterial agent. The diameter of the inhibition zone was measured using a calibrated ruler, and the measurement was reported in millimetres.

RESULTS

The antimicrobial effectiveness of *Capparis sepiaria L*. is evaluated against Samples A, B, and C. **Figure 1** shows the antimicrobial activity of Samples Control (A), Sample A (B), Sample B (C) and Sample C (D) against UTI-causing pathogens The measurement of the zone of inhibition is tabulated in **Table 1.**

From **Table 11,** it is evident that the Zone of inhibition of Sample A against *Escherichia coli* is 9.0 mm and Sample B and C exhibit a zone of inhibition of 16.0 mm against *Escherichia coli*. From this, it is inferred that ethanol and methanol extract of the plant act better than aqueous medium.

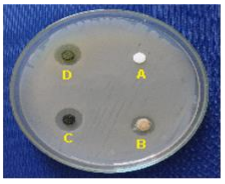

Figure 1A: *Escherichia coli*

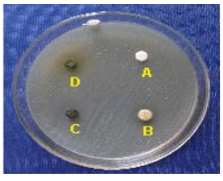

Figure 1B: *Klebsiella pneumoniae*

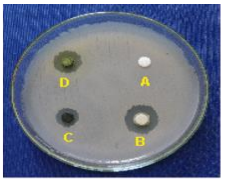

Figure 1C: *Pseudomonas aeruginosa*

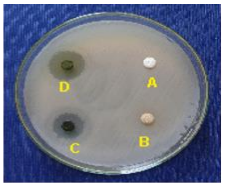

Figure 1D: *Staphylococcus aureus*

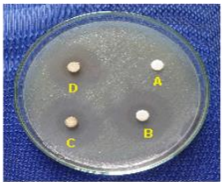

Figure 1F: *Providencia stuartii*

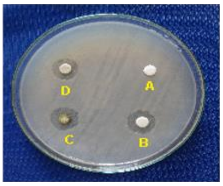

Figure 1E: *Staphylococcus saprophyticus*

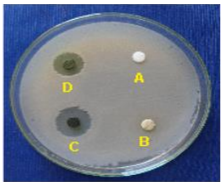

Figure 1G: *Klebsiella aerogenes*

Likewise, Sample C possesses the highest antimicrobial activity against *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticu* and *Providencia stuartii*. However, the zone of inhibition of *Staphylococcus aureus* and *Klebsiella aerogenes* against sample A found to be nil. The combined results highlight the antimicrobial properties of *Capparis sepiaria*.

Table 1: Zone of Inhibition (mm in diameter) of UTI-causing pathogens against Control, Samples A, B and C

Sn.No	Isolated Bacteria	Zone of Inhibition (mm in diameter) (Mean ± SD)		
		Sample A	Sample B	Sample C)
1	*Escherichia coli*	9.0 ± 1.6	16.0 ± 1.0	16.0 ± 1.3
2	*Klebsiella pneumoniae*	13.0 ± 0.8	18.0 ± 1.6	20.0 ± 1.6
3	*Pseudomonas aeroginosa*	18.0 ± 1.4	16.0 ± 1.4	22.0 ± 1.4
4	*Staphylococcus aureus*	-	18.0 ± 0.7	24.0 ± 1.2
5	*Staphylococcus saprophyticu*	16.0 ± 1.2	21.0 ± 1.3	22.0 ± 0.8
6	*Providencia stuartii*	18.0 ± 0.9	20.0 ± 0.8	23.0 ± 0.4
7	*Klebsiella aerogenes*	-	21.0 ± 1.0	19.0 ± 0.8

DISCUSSION

Capparis sepiaria has exhibited notable antimicrobial properties against a range of pathogenic bacteria. Extracts derived from the leaves and stems of *Capparis sepiaria* have effectively inhibited gram-negative bacteria, with ethanol extracts demonstrating particularly potent effects (M & L, 2023). Furthermore, the fruit extracts of this plant showed greater antibacterial activity than the leaf extracts, with the most significant inhibition zones recorded against *Pseudomonas aeruginosa* and *Escherichia coli* at a concentration of 1000 ppm (Kalpana & Prakash, 2016). The ethanol-soluble fraction of the stem extract from Capparis sepiaria revealed strong anti-inflammatory and antibacterial effects against gastrointestinal pathogens (Satyanarayana et al., 2010). Comparable antimicrobial activities were noted in other species of Capparis, such as *Capparis cartilaginea and Capparis mucronifolia*, where their methanolic fractions exhibited the highest antibacterial efficacy and flavonoid concentrations (Rahimifard et al., 2015).

Additionally, the ethanol-soluble fraction of the stem extract of *Capparis sepiaria* displayed significant anti-inflammatory and antibacterial activities against gastrointestinal pathogens (6). Extracts from both leaves and stems, prepared in various solvents, also exhibited antimicrobial effects against clinically relevant pathogens (Kumar & Prince, 2023). Although not directly related to *Capparis sepiaria*, research on the closely related species *Capparis spinosa* revealed that its aqueous extract effectively inhibited the growth of P. aeruginosa (MIC 12.50%) and diminished biofilm formation in both single- and dual-species biofilms involving Staphylococcus aureus (8). These findings indicate that *Capparis sepiaria*, hold significant potential as an antimicrobial agent against various bacterial strains and Capparis genus may serve as a promising natural antimicrobial agent.

CONCLUSION

The effective biomolecules which act as antibacterial have to be identified isolated and subjected to extensive scientific and pharmacological screening that can be used as sources for

new drugs. This study also encourages the cultivation of the highly valuable plant on a large scale to increase the economic status of the cultivators in the country.

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IN VITRO STUDY OF ANTI-DIABETIC ACTIVITY AND ANTI-OXIDANT ACTIVITY OF *OCIMUM BASILICUM* SEED EXTRACT

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ABSTRACT

Many modern pharmaceutical researchers continue to focus on the discovery and evaluation of natural compounds for possible therapies for diabetes, infections, and oxidative stress. Extraction of *Ocimum basilicum* seed and evaluation of its phytochemicals, antioxidant and antidiabetic activities were the goals of the current study. *O. basilicum* seed was extracted and evaluated for its antioxidant and anti-diabetic properties utilizing standard biomedical assays. *O. basilicum* seed showed good pharmacological activities. Moreover, as for the anti-amylase test, IC50 was 72.20% ± 50 µl, a potent effect compared with the IC50 of acarbose, which was 32% ± 10 µl. And the anti-glycosidase test, the IC50 was 76.50% ± 50 µl a moderate effect compared with the IC50 of acarbose, which was 30% ± 10 µl. Finally, the seed had a potent antioxidant effect with an IC50 of 74% ± 50 µl compared with ascorbic acid (IC50 was 32% ± 10 µl). This study has provided initial data that supports the importance of *O. basilcum* seed in traditional medicine. The extracted seed not only exhibited significant antioxidant properties but also antidiabetic effects, which provided a foundation for future research.

KEYWORDS:*Ocimum basilicum*, Phytochemical, Antioxidant, Anti-diabetic, ethanol extract.

INTRODUCTION

One of the most significant sources of medications is plants. Many of the medications used today are made from plants. The use of plants for therapeutic and medicinal purposes to treat illnesses and enhance human health is known as herbal medicine or phytomedicine. Phytochemicals are secondary metabolites found in plants. he medicinal plants are abundant in essential oils with therapeutic value and secondary metabolites, which could be used to make medications. Pharmacologically active chemicals are more likely to be found in plants used for food and traditional medicine. Recent scientific advancements worldwide have examined the medicinal qualities of plants because of their strong therapeutic efficacy, antioxidant activity, lack of adverse effects, and economic feasibility.

Great basil, also called Saint-Joseph's wort or Ocimumbasilicum (O. basilicum), is a major member of the Lamiaceae family, also referred to as the mint family (Miraj S, et al., 2016 &Antora R A, et al., 2017). Numerous pharmacological effects in curing various health issues were revealed by previous scientific studies; this plant showed potent antioxidant, anticancer, antiviral, anti-aging, and antimicrobial properties (Ch M A, et al, 2015 &Rahayu S, et al., 2017). The seeds of O. basilicum are tiny, ellipsoid seeds, as seen (Nazir S, et al., 2021). The seeds of O. basilicum are used extensively in traditional medicine to treat colic ulcers, dyspepsia, and diarrhea (Poddar S, et al, 2020).

Numerous pharmacological actions, including as anti-cancer, antibacterial, radioprotective, anti-microbial, anti-inflammatory, immunomodulatory, antistress, anti-diabetic, anti-pyretic, anti-arthritic, and antioxidant qualities, are among the many medical advantages of

basil. According to Naji-Tabasi and Razavi et al. (2017), traditional uses include the treatment of kidney problems, warts, worms, coughs, headaches, and constipation.

MATERIALS AND METHODS

Collection of Plant Materials

The basil seeds used in this study were obtained from my college Garden, in Sri Akilandeswari women's college, wandiwash. The seeds were cleaned manually to remove all foreign matter such as dust, dirt, stones and chaff. The moisture content of the seed was determined by the vacuum oven method (temperature 70C and pressure 250 mbar) until a constant mass was obtained.

Preparation F Plant Extract

5g of powdered seeds were extracted successively with 100ml of water and ethanol at 40-50 degree C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 40 degree C for future use.

Anti-Diabetic:

a. Alpha-amylase enzyme inhibition assay

Added 390 ml of 0.02M Phosphate buffer pH 7/ Positive control/ Different concentration of test samples with 10 µ L of Alfa Amylase Pre-incubated at 37^0C for 10 mins Added 10 ml of Starch Re-Incubated at 37^0C for 1h Added 0.1 ml 1% Iodine solution+5ml of distilled water Measured OD at 565 nm.

We used the formula: amylase inhibition% = (Ab - As)/ Ab * 100%.

Ab: blank absorbance, as: sample absorbance.

b. Alpha-glycosidase enzyme inhibition assay

Added 225 ml of 80mM Phosphate buffer pH 7.0/ Positive control/ Different concentration of test samples with 75 ml of alpha- glucosidase pre-incubated at 37C for 30 mins Kept in boiling water bath for 2 mins, cooled and added 250 ml of glucose reagent, Incubated at RT for 10 mins, Measured OD at 510 nm.

We used the formula: glycosidase inhibition% = (Ab - As)/ Ab * 100%.

Ab: blank absorbance, as: sample absorbance.

Antioxidant Activity

DPPH Radical Scavenging Activity

The free radical scavenging activity of *OcimumBasillicum* was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) The scavenging activity for DPPH free radicals was measured according to the procedure described by (Braca*et al.*, 2001). An aliquot of 3 ml of 0.004% DPPH solution in methanol and 10 to 50 µl of *OcimumBasillicum* / ascorbic acid at various concentrations were mixed. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. Decolorization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of DPPH radicals by the extract/compound was determined by comparing the absorbance values of the control and the experimental tubes.

$$\text{Scavenging activity \%} = \frac{\text{A518 (control) - A518 (sample)=045}}{\text{A518 (control)}} \times 100$$

RESULTS

Anti- Diabetic Activity

A. Alpha-Amylase Enzyme Inhibition Assay

The α-amylase inhibitory activity of the extracted *O. basilicum* seeds was evaluated against α-amylase. The result is shown in Fig. 1& table 1. When compared to the standard a carbose against *O. Basilicum* seeds extract demonstrated strong α -amylase inhibitory activity.

Table 1: Alpha Amylase Enzyme Inhibition Assay of *O. Basilicum* Seed Extracts

Concentration	Standard (Acarbose)	*Ocimum basillicum*
10 µl	32 %	29.00 %
20 µl	48 %	42.50 %
30 µl	55 %	50.30 %
40 µl	72 %	65.00 %
50 µl	87 %	72.20

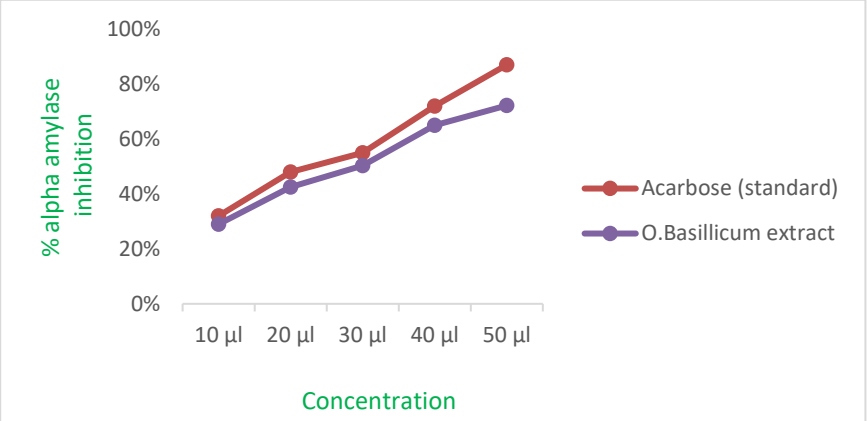

Figure 1: Alpha Amylase Enzyme Inhibition Assay of *O. Basilicum* Seed Extracts

B. Alpha-Glycosidase Enzyme Inhibition Assay

The α-glycosidase inhibitory activity of the extracted *O. basilicum*seeds was evaluated against α-amylase. The result is shown in Fig. 2 & table 2. When compared to the standard acarbose against*O. basilicum*seeds extract demonstrated strong α -amylase inhibitory activity.

TABLE 2: Alpha Glycosidase Enzyme Inhibition Assay of *O. basilicum* Seed Extracts

Concentration	Standard (Acarbose)	*Ocimum Basillicum*
10 µl	30 %	25.20 %
20 µl	46 %	39.00 %
30 µl	58 %	51.00 %
40 µl	73 %	68.30 %
50 µl	81 %	76.50 %

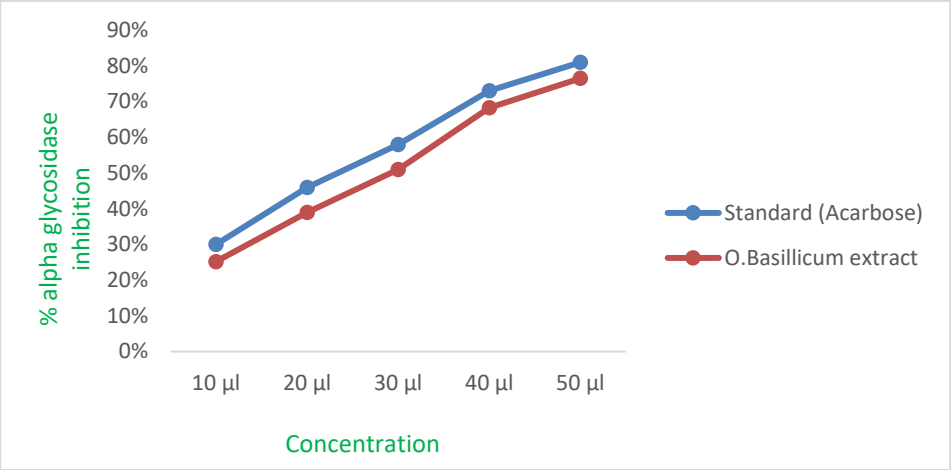

FIGURE 2: Alpha Glycosidase Enzyme Inhibition Assay of *O. basilicum* Seed Extracts

Antioxidant Activity

a. DPPH Assay

The antioxidant activity of the extracts was evaluated by DPPH radical scavenging assay. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a synthetic free radical with deep violet color when is in form of solution which has a λ max at 517 nm. Petroleum ether extract of *O.basilicum*seeds has shown higher antioxidant activity than methanol extract (Table 3 & figure 3).

TABLE 3: DPPH Assay of *O. basilicum* Seed Extracts

Concentration	Standard (Ascorbic acid)	*Ocimum basillicum*
10 µl	32	18.05
20 µl	48	27.00
30 µl	55	45.75
40 µl	72	62.30
50 µl	87	74.00

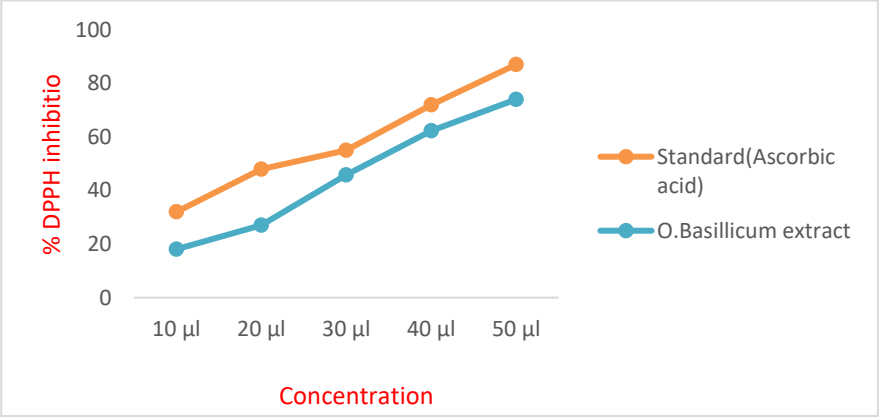

FIGURE 3: DPPH Assay of *O. basilicum* Seed Extracts

DISCUSSION AND CONCLUSION

According to previous research findings, *O. basilicum* inhibits human pancreatic α-amylase in the small intestine, suppressing carbohydrate digestion and thus controlling the entry of glucose into the human body to maintain a reasonable postprandial glucose level (Noor Z.I, *et al*, 2019). Malapermal*et al.* examined the α-amylase inhibitory activity of aqueous, 60% ethanolic, and 70% ethanolic extracts of the leaves of *O. basilicum*, and the extracts exhibited nearly equivalent efficacy to acarbose (Malapermal V, *et al*, 2017). Noor *et al.* evaluated the anti-diabetic potential of *O.basilicum* using porcine pancreatic α-amylase inhibitory activities. The results revealed that *O. basilicum* exhibited α-amylase inhibitory activity and α-glycosidase inhibitory activity almost equivalent to the drug acarbose. As we obtained in this study, the anti-amylase test confirmed these previous results as the IC50 was 72.20 ± 50µl which meant a potent effect compared with the IC50 of acarbose (the reference compound), which was 32% ± 10µl. And the anti-glycosidase test confirmed these results as the IC50 was 76.50 ± 50µl which meant a potent effect compared with the IC50 of acarbose (the reference compound), which was 30% ± 10µl. When compared both enzyme inhibitory activity was observed this study more potential in α-glycosidase enzyme inhibition other than α-amylase enzyme inhibitory.

For the first time, numerous biological investigations of the *O. basilicum*seed were carried out in this study. The findings demonstrated that it has potent antioxidant, anti-α-glucosidase, and anti-α-amylase. These results showed that the seeds of *O. basilicum*might be a significant source of natural antioxidants that are potentially effective in the detoxification mechanisms of living organisms, particularly against illnesses caused by oxidative stress.

Our results showed that variations in chemical composition ofseeds of *O. basilicum* obtained from India. The basil extracts contained appreciable levels of exhibited good DPPHradical scavenging capacity higher than that of seeds. Highlevel of antioxidant activity of basil extracts was observed. High variation in free radicalscavenging activity of seeds was found. The results ofthe present investigation demonstrated significant variations in theantioxidant activities of sweet basil seeds.

The results revealed that *O. basilicum* exhibited α-amylase inhibitory activity and α-glycosidase inhibitory activity almost equivalent to the drug acarbose. When compared both enzyme inhibitory activity was observed this study more potential in α-glycosidase enzyme inhibition other than α-amylase enzyme inhibitory.

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FORMULATION AND EVALUATION OF ANTI-FUNGAL CREAM CONTAINING *AZADIRACHTA INDICA* EXTRACTS AGAINST *CANDIDA ALBICANS* AND *TRICHOPHYTON RUBRUM*

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ABSTRACT

Fungal infections pose a significant challenge to public health, necessitating the development of effective and safe antifungal agents. Herbal remedies have garnered attention for their potential antimicrobial properties, including antifungal activity. In this study, we formulated antifungal cream containing *Azadirachta indica extracts.* The cream was prepared using a standardized method and evaluated for its antifungal efficacy against *Candida albicans* and *Trichophyton rubrum* using agar well diffusion and broth dilution methods. Additionally, physicochemical properties such as pH, homogeneity, consistency, and washability were assessed to ensure product quality and consistency. Preliminary results demonstrated promising antifungal activity of the cream against common fungal pathogens, including Candida albicans and dermatophytes. Overall, our findings suggest that the antifungal cream containing *Azadirachta indica* holds great potential as a natural alternative for the management of fungal infections
Keywords: Antifungal cream, *Azadirachta indica extracts, Candida albicans, Trichophyton rubrum*

INTRODUCTION

Fungal infections cause about 1.7 million deaths worldwide perC year mainly in immunocompromised individuals with two or more pathological conditions. The incidence of infections caused by the genus *Candida* has steadily increased since the 1970s perhaps due to an increased risk of opportunistic infections, the improvement in clinical procedures that identify fungi causing nosocomial infections, as well as the development of antifungal resistance due to prolonged exposure to treatment **(**V.K. Chin *et al.*, .,2016**).**

There are currently more than 150 species of *Candida*, and approximately 20 are known to cause infections in humans. *Candida albicans* is the main causative agent of candidiasis and the primary fungal infection in adults and pediatric patients. In INDIA, it was reported that sepsis caused by *C. albicans* has a mortality rate of approximately 40%, which is higher than any other sepsis caused by bacteria or fungi Infections caused by the genus *Candida* are the main cause of nosocomial fungal infections especially in tertiary care hospitals (M. Dadar et al., .,2018). In particular, *C. auris* is considered an emerging serious global health threat by the Centers for Disease and Control Prevention (CDC) because of its multidrug resistance. However, *C. albicans* stands as the major fungal pathogen of humans.(J.C.O. Sardi *et al.*, ., 2013).

The neem tree *(Azadirachta indica)* has been thought to have miraculous health-promoting qualities. Truth be told, there is evidence that neem was used for medicinal purposes as far back as 4,500 years ago. Its use dates back to ancient India and surrounding countries, where it has long been revered as one of the most useful plants on the planet. The neem tree is still renowned as the "Village Dispensary" since all parts of it are recognized to have unique medicinal potential. Neem tree belongs to the family Meliaceae which is found in abundance in tropical and semitropical regions like India, Bangladesh, Pakistan, and Nepal. It is a fast-growing tree with 20–23 m tall and trunk is straight and has a diameter around 4-5 ft. The leaves are

compound, imparipinnate, with each comprising 5–15 leaflets. Its fruits are green drupes which turn golden yellow on ripening in the months of June–August. (Lee *et al* 2015).

It has therapeutics implication in diseases cure and formulation based on the fact that neem is also used to treat various diseases. The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Leaves contain ingredients such as nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol Quercetin and ß-sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties (Mohit Solanki et al.,2019). The exact molecular mechanism in the prevention of pathogenesis is not understood entirely. It is considered that *Azadirachta indica* shows therapeutic role due to the rich source of antioxidant and other valuable active compounds such as azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol, salannin, and quercetin (Govindachari T. R et al., 1998).

Topical treatment of fungal infections has several superiorities including, targeting the site of infection, reduction of the risk of systemic side effects, enhancement of the efficacy of treatment and, high patient compliance. Different type of topical effective herbal antifungal compounds has been used in the treatment of a variety of dermatological skin infections and candidiasis. Currently, these antifungal drugs are commercially available in conventional dosage forms such as creams, gels and lotions however, oral antifungals are associated with adverse effects that can cause patients to discontinue treatment, which may be complicated by the presence of comorbid conditions Lim (EH et al., 2014). Therefore, In the present study we formulate new antifungal cream using neem extracts for the treatment of dermatophyte and candida infections.

MATERIALS AND METHODS

Collection, authentication and preparation of the extract

Fresh Neem leaves (*Azadirachta indica*) (4Kg) were collected from vallam, Thanjavur. The plant materials were authenticated by Asso Prof Dr Anbu jeba sunilson, Department of Siddha medicine, Tamil University. Then leaves were shade dried for few days at room temperature and powdered with a grinder. Dried powder (150 gm) of *A. indica* leaves was mixed with 70% ethyl alcohol and kept at room temperature for 36 hr. The slurry was stirred intermittently for 2 hr and left overnight using mechanical stirrer. The mixture was then filtered and the filtrate was concentrated using water bath at 50°C and finally dried to form the extract which is kept for phytochemical screening (Akpuaka, A, 2013).

Preliminary Qualitative Phytochemical Analysis

Quality analysis done on the 75% ethanol extract of the leaf extract of *Azadirachta indica* and the presence of various phytochemical constituent such as alkaloids, glycosides, flavonoids, steroids, , terpenoids, tannis, saponins, was detected by using standard methods.

Collection of Fungal Strain

The fungal strains, *Candida albicans* and *Trichophyton rubrum* were obtained from Microbiology laboratory, Kavuery Hospital, Tiruchirappalli.

Maintenance of Inoculum

The stock culture of each strain was stored in Potato dextrose agar at 4˚C. To maintain the stock culture, the fungal were sub-cultured separately on prepared Potato dextrose agar media and maintained at 4˚C.

Standard drug

Fluconazole (150mg) have been used as standard antibiotic for fungal infections. It disrupts fungal cell membrane by inhibiting the synthesis of ergosterol.

Determination of Invitro Anti-fungal of *Azadirachta indica* Leaf Extract against *Candida albicans* and *Trichophyton rubrum* by using Well Diffusion Technique Agar well diffusion technique was used to identify the antifungal activity of plant extract of *Azadirachta indica.*

The Muller Hinton agar media plate was labelled with the 75% ethanol extract standard and negative control. Firstly, the Mc Farland solution was prepared. A loop full of organism was taken from the stock culture and was diluted into the prepared Mc Farland solution. The cloudiness of mixture was compared with the normal saline. This was done to ensure the accuracy of the concentration of the organism was prepared to be used for the streaking on the Muller Hinton agar plate in well plate method. Inoculation was done by spreading a volume of *Candida albicans* and *Trichophyton rubrum* over the entire agar surface by using a cotton swab. A well of 8mm diameter was made with a sterile cork aseptically on the agar plate. Then, A volume of standard volume in concentration of 150mg/ml and ethanol extract (500mg) was introduced into the well. The agar plate was incubated under 30˚C for5 to 7 days. The antifungal agent diffuses in the agar medium and inhibits the development of the fungal strain. The zone of inhibition was measured in millimetre. The experiment was done in triplicates (Kumar Yadav. et al.2022).

Determination of minimum inhibitory concentration (MIC) of *Azadirachta indica* against *Candida albicans* and *Trichophyton rubrum.*

Broth dilution method was used to determine the value of minimum inhibitory concentration. The extract was serially diluted with 1ml of Muller Hinton broth to give concentration of (1000mg/ml, 500mg/ml, 200mg/ml, 62.5 mg/ml, and 31.25 mg/ml). Each test tube containing plant extract was inoculated with 1ml of fungal strains. The test tubes were incubated at 30˚C for 5 to 7 days and the results were observed. The minimum inhibitory concentration that does not have any fungal growth was selected as MIC.

Formulation of the herbal antifungal cream

The formulation trails were done as per formula given in the Table 1. The formulation containing and *A. indica* was formulated by the following method: Different amount of ingredients was incorporated together in 2 phases i.e. oil phase and aqueous phase separately. The oil phase consists of liquid paraffin, bees wax, stearyl alcohol, tween-80 and stearic acid while the aqueous phase was composed of methyl paraben, sorbitol solution and potassium hydroxide. Both aqueous and oil phases were heated to 75 °C on a water bath separately. The aqueous phase was then added drop wise to the oil phase with continuous stirring and finally the herbal extracts of *A. indica* were incorporated in the emulsion. Gradually temperature was decreased with continuous stirring and emulsion was formed which was then stored in the air tight wide-mouth container.

Physical Evaluation of formulation

The physical properties of the cream formulation were evaluated by measuring pH, assessing homogeneity, consistency, and washability. pH was measured using a digital pH meter after dissolving the cream in distilled water. Homogeneity was assessed visually and by touch, while consistency was determined visually. Washability was checked manually after application to the skin. Finally, antifungal activity was evaluated by measuring the zone of inhibition.

Statistical Analysis

The data was expressed as mean ± S.E.M. The assessment for MIC study was performed in triplicate and the data was subjected to one way analysis of variance (ANOVA) using Dunnett 'T' test and p values < 0.05 was considered as significant

RESULTS

The colour, consistency and percentage of yield of ethnolic extract of *Azadirachta indica* were shown in the Table 1 and Fig 1

Table No 1 The nature and yield percentage

No	Extract	Colour	Consistency	% Of Yield
1	Ethanol	Dark green	Semisolid	57.84

Phytoconstituents Present in the Leaf Extract of *Azadirachta indica*

Qualitative phytochemical screening of Azadirachta indica (neem) leaf extract revealed the presence of several bioactive compounds. The ethanol extract tested positive for alkaloids, flavonoids, steroids, tannins, and saponins. However, terpenoids were not detected in the extract. These findings suggest that neem leaves contain a variety of potentially beneficial phytochemicals.

Invitro antifungal activity of ethanol extract of *Azadirachta indica* against *Candida albicans*, and *Trichophyton rubrum.The results were shown in Table 2and Fig 2*

Test Organism	Standard Drug (Fluconazole 150 mg/ml)	Ethanol Extract (500 mg/ml)
Candida Albicans	26±0.88	24±0.88*
Trichophyton Rubrum	17±1.45	25±0.58**

n= 3; ***p< 0.001 = high significant, **p< 0.01 = significant and *p< 0.05 = less significant

Minimum Inhibitory Concentration (MIC) Activity of ethanol extract of *Azadirachta indica*

The MIC values of ethanol extracts of ethanol extract of *Azadirachta indica* were determined by using dilution method. The ethanol extract showed inhibitory effect against *Candida albicans* the concentation of 125mg/ml..The results were shown in Table 3.

Test organism	Different concentration of ethanol extract of *Azadirachta indica* (mg/ml)					Standard drug Fluconazole (150mg/ml)
	500	250	125	62.5	31.25	
C.albicans	0.52±0.15	0.45±0.01	0.67±0.02**	1.28±0.02	1.68±0.06	0.38±0.01
T.rubrum	0.13±0.01	0.44±0.01	0.63±0.01**	1.25±0.01	1.35±0.02	0.32±0.02

n= 3; ***p< 0.001 = high significant, **p< 0.01 = significant and *p< 0.05 = less significant

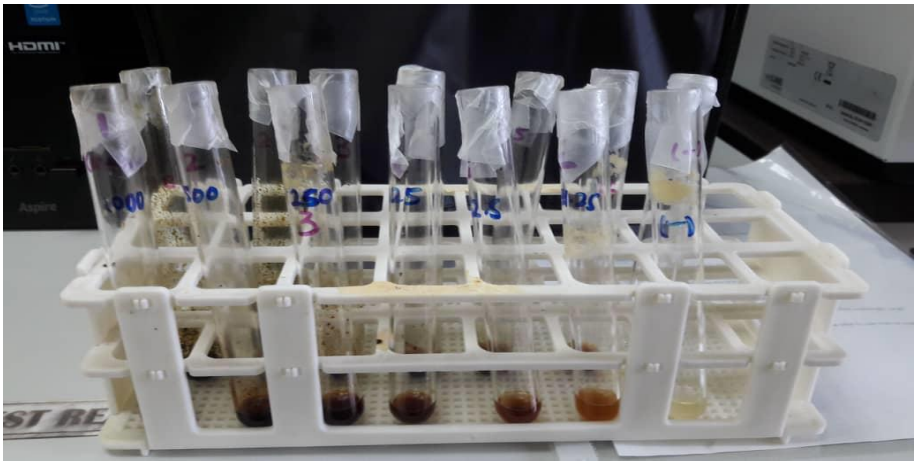

Fig 2 MIC test of different concentration of ethanol extract of ***Azadirachta indica*** against *Candida albicans and Trichophyton rubrum*

Evaluation of Formulated cream

The formulated cream was evaluated using various physicochemical parameters.The results were shown in Table: 4and Fig 3

Table 4 Physical evaluation of the cream

S NO	TEST	FORMULATED CREAM
1	Ph	6.57
2	Colour	Creamy Green
3	Consistency	Semi solid
4	Homogeneity	Homogenous
5	Washability	Good

Antifungal activity of the formulation containing ethanol extract of *Azadirachta indica* leaves

The results of antifungal activity revealed that the formulation of ethanolic extract of *Azadirachta indica* leaves exhibited significant antifungal activity. Both the standard sample and test sample were compared on the antifungal testing. The result showed good antifungal activity of formulated cream as shown in table 6 and fig 6.

Test organisms	Zone of inhibition (mm)	
Candida albicans	Standard Flucanozole (150mg/ml)	Formulated cream containing *Azadirachta indica leaves*
	24±0.333***	20±0.333***
Trichophyton rubrum	22±0.333***	18±0.333***

Results presented the mean ± SEM value of n = 3; ***p<0.001=highly significant, **p<0.01 = significant *p<0.05=less significant

DISCUSSION

Invasive fungal infections, which include candidiasis, have improved in incidence international over the past two decades, and consequently, the use of antifungal drugs inclusive of azoles has extended (Borgers M et.al 2015). Antifungal drugs such as fluconazole and ketoconazole, have significant roles within the treatment of candidiasis ,dermatophytosis and other invasive fungal infections but from time to time with using these marketers, clinically

essential toxic effects including skin rash, nausea, pores, increased liver enzyme (for fluconazole) gynecomastia, adrenal insufficiency and hepatotoxicity (for ketoconazole) are visible. Overtime, beneath a few medical settings, the efficacy of azoles has decreased due to accelerated resistance to the antifungals (Ajello, L et.,al 2019).

Cold maceration was used as the method of extraction in the study. Ethanol used as the extraction solvents. These solvents were incorporated to extract the bioactive compounds from the leaf of the Azadirachta indica. Different solvents have different polarities which have ability to extract different hydrophobic and hydrophilic compounds in the samples. Ethanol is chosen because it is used for extraction various polar compounds and also certain group of non-polar compounds are fairly soluble in Ethanol. Other than that, it easily evaporates so it can be separated from the extract. Ethanol also brings out trace amounts of various substances from the plant (Aditi, G et.,al 2011).

The phytochemical qualitative analysis revealed that alkaloid, protein, terpenoid, tannin, phytosterol, and saponin, were present in the ethanol extracts of of *Azadirachta indica* extract. In this study, the presence of alkaloid ,flavonoid, saponin, polyphenol and tannin in the extract exhibit the antifungal activity. Much of the protective effects of herbal plants have been attributed to their phytochemicals constituents alkaloids, flavonoids, glycosides, saponins exert multiple biological effects like anti-inflammatory, anti-allergic, antioxidant, anti-diabetic, anti-viral and anti-cancer activities, anti-leprosy activities, antimicrobial activity (Singh V et al., 2014).

These research were conducted on antifungal activity of *Azadirachta indica* against *Candida albicans*, and *Trichophyton rubrum*. In the antifungal activity, the ethanol extract of *Azadirachta indica* showed efficient antifungal activity against *Candida albicans* (24±0.88 mm) and *Trichophyton rubrum* (25±0.58 mm) From this study we can conclude that, the ethanol extract of *Azadirachta indica* against *Candida albicans* are significant with p value of <0.05 while ethanol extract for *Trichophyton rubrum* are high significant with p value of <0.01. *Tricophyton rubrum* is known as dermatophyte, which are responsible for the superficial fungal infection while *Candida albicans* are yeast that causes opportunistic fungal infection that effects the immune system. The leaves of *Azadirachta indica* are used traditionally for the treatment of fungal infection. Quercetin and ß-sitosterol, polyphenolic flavonoids purified from neem fresh leaves and were known to have antibacterial and antifungal properties (Govindachari T.R et al., 1998). Therefore, the leaves of *Azadirachta indica* can used against *Trichophyton rubrum and Candida* species.

The minimum inhibitory concentration (MIC) is regarded as the lowest concentration that needed to inhibit the fungal growth . In the present research, there is no growth for *Candida albicans* in methanol extract for the following concentration of 500mg/ml, 250mg/ml and 125mg/ml and it was identified by the absence of turbidity in Mueller Hilton broth. While no growth is observed at 500mg/ml, 250mg/ml, and 125mg/ml of methanol extract of *Trichophyton rubrum*. It was observed that the lower the extract concentration, the higher the visibility of fungal growth. The MIC should be done carefully as it can easily get affected by the environment, incubation condition and time of incubation (Al-Haj et al., 2009) The MIC value for methanol extract for both *Candida albicans* and *Trichophyton rubrum* is 125mg/ml. Thus, it revealed that the ethanol extract of plant *Azadirachta indica* exhibit inhibitory activity which can be detected by the absence of turbidity in the test tubes. From this study we can conclude that, the ethanol extract of *Azadirachta indica* against both *Candida albicans* (0.67±0.02) and *Trichophyton rubrum* (0.63±0.01) are highly significant with a p value of <0.01. This study also

revealed that ethanol extract of *Azadirachta indica* shows significant antifungal activity against *Candida albicans* and *Trichophyton rubru* A herbal formulation was prepared using *Azadirachta indica* extracts against *Candida albicans* and *Trichophyton rubrum.* A study of physical parameters were carried out and the results obtained were satisfactory. The optimized formulation has pH 6.5, creamy green in colour, semi solid consistency and homogenous. From the above compiled data the study clearly shows that the formulation is showing good in-vitro anti-fungal activity against *Trichophyton rubrum* and *candida albicans.* The formulation of antifungal along with Neem extract exhibited enhanced rate of diffusion and antifungal activity (Mei X. Chen et al., (2016).

CONCLUSION

In conclusion, the ethanol extracts of *Azadirachta indica* leaves extract exhibited good antifungal activities and were capable of reducing growth of *Candida albicans* and *Trichophyton rubrum*. The in-vitro assessment of the plant extracts against the test organism and the phytochemical compounds present in the plants shows good inhibitory activity. Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects. The herbal cream formulation of ethanol leaf extract of *Azadirachta indica* appeared to have more antifungal performance. Therefore, it is considered in future for clinical trials as a potential antifungal agent product for the treatment of fungal infection.

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ASSESSMENT OF VERMICOMPOST QUALITY AND ITS IMPACT ON PLANT GROWTH USING DIFFERENT ORGANIC WASTE SUBSTRATES

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ABSTRACT

The conversion of organic waste into nutrient-rich compost using earthwarms is an eco-friendly and sustainable method to enhance soil fertility and crop productivity. The chemical composition of vermicompost varies depending on the substrate used, making it essential to analyze its nutrient content for optimal agricultural application. In this study, vermicompost was prepared using the earthwarm *Eisenia fetida* and various organic waste materials, including Cow dung, neem leaves, and other agricultural residues. The nutrient composition-including carbon(C), hydrogen(H), nitrogen(N), sulfer(S), phosphorus(P), pottasium(K), and humic acid-was analysed for each compost type. The impact of these compost on plant growth was also assessed. The findings revealed that vermicompost consistently had higher nutrient content than untreated organic waste. Among the different substrates, Organic waste and Cow dung produced the most nutrient–rich compost. Plant treated with these vermicompost samples exhibited significantly better growth compared to the control group without vermicompost application. A substantial decrease in the C/N ratio was observed, indicating increased nitrogen availability for plants. Additionally, this process effectively transformed agricultural waste into a valuable soil amendment, reducing environmental pollution caused by waste dumping or burning.

Key Words : Agriculture, Cow dung, Organic waste, Vermicompost, Eiseniafetida, Soil fertility, Plant growth.

INTRODUCTION

Vermicomposting is an efficient and eco-friendly process in which earthworms and associated microorganisms decompose organic materials, transforming them into nutrient-rich humus. This process plays a crucial role in sustainable agriculture by recycling organic waste into valuable compost that enhances soil fertility and promotes plant growth.

Earthworms consume a wide range of organic materials, including food scraps, agricultural residues, animal manure, and industrial by-products, breaking them down into simpler, plant-available nutrients. The end product, known as vermicompost, serves as a highly beneficial organic fertilizer, while vermiculture refers to the rearing of earthworms specifically for this purpose.

Vermicomposting offers significant economic and environmental benefits, including reduced landfill waste, effective organic waste management, job creation, and low-cost implementation. These advantages make it particularly valuable in underdeveloped agricultural regions (Applehof, 1993). The three primary categories of organic waste suitable for vermicomposting originate from animal, plant, and urban sources (Thomas et al., 2012).

Vermicompost is rich in essential plant nutrients such as nitrates, calcium, phosphorus, potassium, ammonium nitrogen, and magnesium, which become more readily available to plants due to the action of earthworms (Edwards & Bohlen, 1996). Additionally, it contains beneficial

microbial populations and enzymes that enhance soil fertility, promote plant growth, and improve crop yield (Kale et al., 1992; Singh et al., 2013).

The recycling of organic waste into vermicompost not only provides an abundant source of organic manure but also facilitates eco-friendly waste management (Geetanjali, 2007). By improving soil structure, water retention, and aeration, vermicompost contributes to overall soil health and fertility, while its nutrient content surpasses that of raw organic materials (Ndegwa et al., 2000; Laxmiet al., 2014). It acts as an effective soil conditioner, enhancing microbial diversity and ensuring sustained nutrient availability for plants (Coyne &Knutzen, 2010).The digestive system of earthworms further enriches the compost by fostering beneficial microbial communities, creating a biologically active soil environment (Edwards, 2004). Furthermore, the mucus produced by worms helps prevent nutrient loss during irrigation, ensuring prolonged nutrient retention in the soil (Nancarrow& Taylor, 2012).

Vermicomposting can be implemented at both small and large scales. Small-scale systems utilize household waste, including fruit and vegetable scraps, coffee grounds, eggshells, and paper products. In contrast, large-scale operations rely on agricultural and industrial waste sources such as dairy manure, sewage sludge, brewery waste, and food processing residues (Sherman-Huntoon, 2000; Singh et al., 2013). The efficiency of vermicomposting depends on selecting suitable earthworm species. A commonly used species is Eiseniafetida (red wiggler), which thrives in decomposing organic matter and manure piles, making it ideal for vermicomposting. However, deep-burrowing species such as Lumbricusterrestris are not suitable for composting bins due to their natural habitat preferences (Dominguez & Gomez-Brandon, 2012). Analyze and compare the nutrient content of vermicompost prepared from different locally available organic waste sources using the red worm Eisenia fetida. Assess the growth-enhancing potential of the produced vermicompost on selected plants. By evaluating the efficiency of vermicompost derived from various organic materials, this research will contribute to optimizing waste management practices and promoting sustainable agricultural development.

MATERIALS AND METHODS

Vermicompost Production

Three types of vermicompost were produced using different organic waste substrates: neem leaves, cow dung, and vegetable waste. Initially, a small-scale experiment was conducted in pots on a trial basis, followed by large-scale production in tanks. The vermicompost samples obtained were analyzed for their composition and effects on plant growth. Vermicomposting was carried out during the monsoon season, from November to mid-January 2025, on the premises of Maruthupandiyar College, Vallam, Thanjavur, Tamil Nadu, India (Mean temperature: 35–40°C, humidity: 75–80%). The process involved the following steps:

Collection of Organic Waste

Locally available organic waste was collected from Maruthupandiyar College, Thanjavur. The selected waste materials included neem leaves, organic residues, and cow dung.

Preparation of Tanks for Rearing Eiseniafetida

A vermicomposting tank was constructed with the following dimensions:

Length: 400 cm
Width: 250 cm
Height: 200 cm

The tank was made using concrete, cement, and stones for durability. It was filled with a mixture of organic waste and cow dung to provide an optimal habitat for the red worm (Eiseniafetida).

Experimental Setup for Vermicompost Preparation

Pot Experiment

Cement pot used of 1-inch height × 1inchdiameter, each with a capacity of 1 kg, were used. Small holes were made at the bottom for aeration and drainage. The following steps were followed:

- Small pebbles were placed at the bottom of the pots.
- A2.55 cm layer of sand was added above the pebbles.
- 1/4 kg of cow dung was placed over the sand.
- 5 earthworms (Eiseniafetida) were introduced into each pot.
- 1/4kg of organic waste (either neem leaves, vegetable waste, or cow dung) was added separately to each pot.
- The mixture was covered with dried leaves and a wire mesh.
- The experiment was set up in duplicate for each substrate.
- Water was sprinkled every 2–3 days, and the composting mixture was turned after 15 days. Further incubation continued for another 15 days. A control setup (without earthworms) was maintained. After a successful trial run in pots, vermicomposting was scaled up to pits for larger production.

Pot Experiment Design

The experiment followed a randomized complete factorial block design with two factors: Fertilization treatment

Soil history

Each treatment was replicated five times, arranged randomly within each block.

Fertilization Treatments

Four fertilization treatments were applied:

- CK (Control): No fertilizer application.
- Urea (Chemical Fertilizer): Applied at a rate of 0.6 g N, 0.30 g P_2O_5, and 0.5 g K_2O per kg soil (equivalent to 1000 kg N, 580 kg P_2O_5, and 1000 kg K_2O per hectare).
- Vermicompost: Applied at 15.10 g per kg of soil (approximately 501 kg per hectare).
- Since phosphorus (P) was relatively higher in the compost and vermicompost treatments, the amounts were adjusted accordingly. Additional chemical nitrogen (N) and potassium (K) were added to balance nutrient levels using urea, superphosphate, and potassium sulfate.
- Planting and Irrigation
- On January 06, 2025chilli seedlings after 1 week 1-2 leaf stage were transplanted into each pot.
- Initially, the plants were irrigated to 100% field water-holding capacity using tap water.
- Throughout the experiment, soil moisture was maintained at 19–20% (w/w) (~80% of field capacity).
- Soil moisture levels were monitored gravimetrically, and additional water was applied when necessary.

Greenhouse Conditions

- The pots were randomly arranged inside a greenhouse and repositioned weekly to minimize environmental variation.
- Temperature:16–37°C
- Lighting:Natural light (no supplementary lighting)
- This experimental setup aimed to assess the impact of different fertilization treatments on soil fertility and plant growth under varying soil conditions.
- Harvesting of Vermicompost
- Vermicompost was ready for harvesting after 60 days. Once matured:
- Earthworms were separated from the compost.
- The harvested material was air-dried for 3–4 days in pots.
- The dried compost was sieved and packed in airtight plastic bags.
- Samples were stored at refrigeration temperatures for further chemical analysis.

Chemical Analysis of Vermicompost

- C, H, N, and S Analysis
- The carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content of the vermicompost samples was analyzed using a CHNS analyzer at the Soil Testing Laboratory, Aduthurai. The analysis process included:
- Sample Preparation:
- The vermicompost samples were dried and finely powdered.
- Samples were homogenized and weighed into tin containers.
- Combustion Process:
- The samples were introduced into a combustion reactor via an autosampler along with oxygen.
- The combustion process converted organic matter into gaseous compounds.
- This analysis provided valuable insights into the nutrient composition of vermicompost, essential for soil fertility and plant growth.

RESULT AND DISCUSSION

Vermicomposting has proven to be an efficient method for enhancing crop production and yield due to its high nutrient content and its ability to improve soil aeration and water retention. It also promotes plant growth by enhancing germination, improving root development, and enriching the soil with beneficial microbes. This study examines the effects of vermicomposting using Eiseniafetida (red worms) on different organic waste materials, including Cowdung, Organic waste and neem leaves. We analyzed the nutrient composition of the resulting vermicompost samples and assessed their impact on the growth of crops such as Chilli.

Table 1: C, H, N and S analysis of different vermicompost samples

Source of vermicompost	Organic carbon%	Nitrogen %	C/N ratio Control	C/N ratio Vermicompost	C/N ratio Cowdung	Hydrogen %	Sulfer %
Neem leaves	30.45	1.145	12.50	60	12.30	5.745	0.350
Cow dung (P=0.05)	20.45	1.120	0.30	1.50	1.25	0.140	0.040
Organic waste	18.40	1.110	31	40	15.17	2.700	0.175

The findings confirmed that E. fetida effectively converts organic waste into nutrient-rich vermicompost. The major nutrient and organic carbon content of the different vermicompost samples are presented in Tables 1 and 2.

Chilli plants growing A) On organic waste vermicompost B) Cowdung and C) Control

Carbon, Nitrogen, Hydrogen, and Sulfur Estimation

Our analysis revealed that vermicompost derived from neem leaves contained the highest organic carbon content (30.45%), followed by Cowdung-based vermicompost (20.25%). The highest total nitrogen content was also observed in Organic waste (2.150%), Similarly, neem leaves vermicompost exhibited the highest hydrogen and sulfur content (Table 1). The C/N ratio, a key indicator of nitrogen availability, remained below 20 for all samples, demonstrating enhanced nitrogen availability for plant uptake. These results align with previous findings, such as those of Thomas et al., (2012) for bagasse vermicompost. A lower C/N ratio (between 1 and 15) facilitates rapid nitrogen mineralization and release, making nutrients readily available to plants (Brust, 2019). Vermicomposting significantly reduced the C/N ratio of all organic wastes, with the most notable decrease observed in cowdung, from 12.50 to 12.30, followed by bagasse-

based vermicompost, which decreased from 12.50 to 60 This reduction highlights the improved fertilizer quality of the produced vermicompost.

Potassium, Phosphorus, and Humic Acid Estimation

Analysis of potassium, phosphorus, and humic acid content in the vermicompost samples revealed notable differences in composition (Table 2). Wheat straw-based vermicompost exhibited the highest potassium content (15.5 mg/g), followed by neem leaves-based vermicompost (20.4 mg/g). The phosphorus content across all samples ranged from 18 to 20 mg/g. Similar increases in nitrogen and phosphorus content in vermicompost have been reported by Liu et al.,(2012). The humic acid content further contributed to the improved nutrient availability.

Effect on Plant Growth

Vermicomposting enhances the bioavailability of nutrients by converting them into plant-accessible forms through the combined actions of earthworms and microbial activity (Edwards & Fletcher, 1988). In this study, plants grown with vermicompost showed significantly better growth in terms of fresh weight and dry weight compared to control plants without vermicompost. This improvement is attributed to the high nitrogen, phosphorus, potassium, humic acid, and plant growth hormones present in the vermicompost.

Table2: Potassium, Phosphorus and Humic acid content estimation of different vermicompost samples.

Source of vermicompost	Potassium content (mg/g of vermicompost)	Phosphorus content (mg/g) of vermicompost	Humic acid content (g/g of vermicompost)
Neem leaves	15.5	20.4	0.035
Cowdung	0.12	1.15	0.070
Organic waste	20.5	20	0.075

Beyond improving plant growth, this study demonstrates that agricultural waste can be efficiently transformed into valuable organic fertilizer rather than being discarded, burned, or sent to landfills, which would contribute to environmental pollution. Vermicomposting supports sustainable organic farming by providing a nutrient-rich alternative to chemical fertilizers. As a natural growth promoter, vermicompost enhances plant health and productivity while reducing environmental impact. Numerous studies have reported its positive effects on plant growth, physiology, and biochemistry, reinforcing its role as an eco-friendly and effective soil amendment.

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IDENTIFYING AND SEPARATING PIGMENTS FROM MARINE ACTINOMYCETES AND POSSIBLE APPLICATIONS

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ABSTRACT

In recent years, natural dyes made by marine microorganisms—especially actinomycetes—have become more important as a source of active compounds and medicines with potential medical uses. The experiment, which sought to ascertain the cytotoxic, antibacterial, and antioxidant qualities of a methanolic pigment extract derived from the marine actinomycetes VES 01 and VES 04, was based on this important discovery. Both extracts demonstrated significant toxicity activity according to the Brine Shrimp Lethality Test (BSLT). The findings showed that the pigment crude extracts of the actinomycetes VES 01 and VES 04 exhibited lethal concentration 50% (LC50) values of 92.64µg/mL and 134.21 mg/mL, respectively. The greatest inhibition rates were seen in Bacillus subtilis and Escherichia coli when those extracts were tested for antibacterial activity against a range of pathogens. Furthermore, we measured the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The results showed that the inhibitory concentration 50% (IC50) values for the actinomycetes VES 01 and VES 04 pigment crude extracts were, respectively, 228.08 µg/mL and 346.3 µg/mL. Using GC-MS analysis, we were able to determine the chemical components of actinomycete pigment crude extracts. According to the findings, the most common compounds were 3,7-dihydro-1,3,7-trimethyl (CAS) (44.65%) for VES 01 and VES 04, 5-Methoxypyrrolidin-2-one (30.23%) for VES 04, and 1H-Purine-2,6-dione. Two pigment crude extracts from actinimycetes actinomycetes VES 01 and VES 04.Actinomycete VES 04 pigment crude extract had the highest amount of antioxidant activity, whereas actnomycete VES 01 pigment crude extract demonstrated the strongest antibacterial activity against E. coli ATCC 8739.

INTRODUCTION

In recent years, a number of problems in the pharmaceutical industry have gained international notice. One of these is tolerance to different kinds of pathogens. bacteria and a rise in the number of people dying from illnesses that cause neurological disorders (Prestinaci et al., 2015; Kharirie and Andriani, 2020). Resistance results from long-term changes made by bacteria, viruses, fungi, and parasites that lessen the effectiveness of widely used antimicrobial drugs (Lounou et al., 2017). Furthermore, infections that are challenging to treat increase the likelihood of illness progression, mortality, and severity, all of which have long-term consequences (Roope et al. 2019). Prolonged bacterial, viral, or fungal infections can lead to degenerative disorders and autoimmune illnesses.Cell damage associated with aging or other factors affecting the body's free radical count can result in degenerative disorders (Sahardi and Makpol 2019). The accumulation of free radicals causes a variety of illnesses, including as Parkinson's, Alzheimer's, atherosclerosis, cardiovascular problems, hypertension, and type 2 diabetes (Stambler 2017). Naturally, the body can lessen free radicals to some degree since it produces antioxidant chemicals. The other bioactive material that may offer protection against degenerative diseases is the hazardous molecule. The brine shrimp lethality test (BSLT) has shown a good association between cytotoxic activity and the poisonous substance tested in several solid human tumors (Mclaughlin et al. 1998). Actinomycetes are gram-positive filamentous aerobic bacteria that contain a lot of G+C (Gong et al. 2018; Bhakyashree and Kannabiran 2018). Natural products have reportedly been rediscovered as a consequence of extensive study on actinomycetes isolated

from terrestrial habitats. However, marine actinomycetes are basically a new source of secondary metabolites, including terpenes, peptides, sterols, fatty acids, alkaloids, amino acids, and their derivatives, claim Liao et al. (2016) and Pudi et al. (2016). The marine ecosystem is capable of producing all of these substances due to its vast biodiversity and diverse environmental circumstances (Bibi et al. 2020).Hifnawy et al. (2020) state that Micromonospora sp. and other actinomycetes linked to marine sponges have been used recently to produce a range of bioactive chemicals with distinctive molecular scaffolds and notable pharmacological activity. Thus, Micromonospora is one of the genera that produces a large number of secondary metabolites, particularly bioactive chemicals. The Micromonosporaceae family, which includes 32 additional genera, includes the genus Micromonospora. This genus is found in a range of geographic locations, including soils, mangrove sediments, plants, marine habitats, and harsh conditions (Trujillo et al., 2015; Hifnawy et al., 2020). The pigment is one of the many bioactive substances produced by the more than 740 strains of Micromonospora that have been identified (Berdy 2005). Micromonospora colonies include carotenoid pigments, which can be yellow, orange, red, purple, brown, or black (Sineva et al. 2021). Soluble pigments are found in a number of species, including Micromonosporachalcea (yellow), M. halophytica (red-brown), M. olivasterospora (olive-green), M. purpureo chromogenes (dark-brown), and M. rosaria (wine-red) (Genilloud 2015). In order to ascertain the potency of pigment crude extract, this study sought to evaluate the antibacterial, antioxidant, and toxicological properties of the pigment methanol extracts made by the marine actinomycetes VES 01 and VES 04 as well.

MATERIALS AND METHOD

Isolation of actinomycetes

Five areas of the mangrove ecosystem in the Vellar Estuary in Tamil Nadu, India (Lat. 11°29'N; Long. 79°46'E) were the sites of soil sample collection. The soil samples were crushed using a mortar and pestle after being allowed to air dry for seven days at room temperature (27±1°C). A 0.5mm sieve was used to remove bigger particles, such as stone and plant debris, in order to isolate the ground soil samples using the soil dilution technique. Following that, the waste was stored independently in paper bags at 4°C. Two colored marine actinomycetes employed in this investigation, actinomycetes VES 01 (greenish-black) and VES 04 (orange), were isolated from mangrove soil. The five microorganisms employed in the antimicrobial test were Bacillus subtilis ATCC 19659, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 15442, Staphylococcus aureus ATCC 6538, and Candida albicans ATCC 10231.

Pigment extraction

Actinomycetes cultures were grown in 1 L of ISP-4 broth medium, which contained 10 g of soluble starch, 1 g of MgSO4.7H2O, 1 g of NaCl, 1 g of K2HPO4, 2 g of (NH4)2SO4, 2 g of CaCO3, 0.001 g of FeSO4.7H2O, 0.001 g of MnCl2.4H2O, and 0.001 g of ZnSO4.7H2O. The cultures were then centrifuged for 15 minutes at 6000 rpm. This procedure is based on the methodology slightly modified Dawoud et al. (2020).The cell biomass was heated in a water bath at 50°C for 15 minutes, or until the cell became colorless, then 1 liter of methanol was added as a solvent. Centrifugation at 6000 rpm for 15 minutes was then used to separate the cell biomass and methanol phase. The methanol solvent was evaporated at 50°C using a rotary evaporator. The crude extracts were collected, dissolved in 10% dimethyl sulfoxide (DMSO), and then stored in a cool environment until they could be utilized in further laboratory tests.

The toxicology investigation

According to Meyer et al. (1982), the toxicity test was conducted using the Brine Shrimp Lethality Test (BSLT). Four milliliters of seawater, twenty Artemia salina larvae, and crude

pigment extracts at concentrations of 0, 10, 100, 250, 500, 750, and 1000 µg/mL were all contained in each vial. The vials containing pigment extracts and larvae were cultured in light for a full day at room temperature. The percentage of mortality was calculated using the following formula after the dead larvae were counted:

$$\% \text{ Mortality} = \frac{(\sum \text{sample larvae mortality} - \sum \text{Control larvae mortality})}{(\sum \text{Total larvae})}$$

Probit analysis and a linear regression model were employed to calculate each extract's LC_{50} value.

Antimicrobial test

The disc diffusion method was employed to carry out the antimicrobial activity. A number of clinical isolates were tested against the pigment extracts. The following bacteria were added to a Mueller-Hinton Agar medium: B. subtilis ATCC 19659, E. coli ATCC 8739, P. aeruginosa ATCC 15442, and S. aureus ATCC 6538. In the meantime, C. albicans ATCC 10231 was added to a Potato Dextrose Agar medium. On the surface of the agar plate medium, a paper disc (6 mm) with different extract concentrations (250, 500, 750, and 1000 µg/mL) was placed. The positive control in this experiment was tetracycline at 100 µg/mL, while the negative control was 10% dimethyl sulfoxide (DMSO). Following a 24-hour incubation period at room temperature, the diameter of the inhibition zones that developed on the plates was measured.

Antioxidant test

The DPPH radical was used to measure the antioxidant activity of the pigment extracts according to with Batubara et al. (2009) with minor modifications. 500 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent (0.125µM in methanol) was combined with 500 microliters of each extract at different concentrations (0, 7.81, 15.63, 31.25%, 62.5, 125, 250, and 500 µg/mL). A spectrophotometer (Thermo spectronic-Genesis 20, Thermo Fisher Scientific, USA) was used to measure the sample's absorbance after the mixture was left to stand at room temperature for 30 minutes in the dark. A positive control was ascorbic acid. The following formula was used to determine the inhibition percentage

$$\% \text{ Inhibition} = \left[1 - \frac{\text{sample absorbance} - \text{control absorbance}}{\text{Blanko absorbance} - \text{control absorbance}} \right] X1$$

The IC50 value of every extract was found using a linear regression model.

Chemical composition examination

Gas chromatography was used in the Agilent 5977B GC/MSD (Agilent Technologies, USA) to analyse the pigment extract of the isolates VES 01 and VES 04. An extract solution diluted in methanol, weighing 0.6 µL, was introduced into an HP-5MS column (30 m x 250 µm x 0.25 µm). The oven was set to 80°C at first, and it was raised to 300°C over the course of 20 minutes at a rate of 15°C per minute. The carrier gas utilised was helium gas, flowing at a rate of 1 mL/min. Both the auxiliary temperature and the injection temperature were kept at 300°C. Using the GC-MS Pyrolysis programme (WILLEY9THN 08. L), the result was examined.

RESULT AND DISCUSSION

The pigment extraction

On ISP-4 agar medium, the Actinomycete VES 01 and VES 04 showed greenish-black and orange colonies after seven days of room temperature incubation. The VES 01 and VES 04 were cultivated in ISP-4 broth medium and were incubated on a rotary shaker for 14 days before the pigment was extracted. The crude extracts of the pigments showed greenish-black for VES 01 and brownish-orange for VES 04 (Figure 1). Actinomycete VES 01 and VES 04 pigment extract yields were 0.23% and 0.18%, respectively (Table 1).

Toxicity of pigment extracts

According to the BSLT test, both actinomycete pigment extracts VES 01 and VES 04 were toxic to *Artemia salina.* The toxicity was measured using the LC50 value. The LC50 value is a standard for the lowest concentration at which 50% of test organisms (A. salina) are executed. The lower the LC50 value, the lower the toxicity. The LC50 values for actinomycete pigment extracts VES 01 and VES 04 were 94.53 g/mL and 131.22 g/mL, respectively (Table 2).

Antimicrobial properties of pigment extracts

Actinomycetes pigment extracts were tested against Gram-positive and Gram-negative bacteria, as well as yeast. The pigment extracts of actinomycete VES 01 and VES 04 demonstrated antimicrobial activity against several tested microbes in an antimicrobial test. The VES 01 pigment extract was effective against B. subtilis, E. coli, and S. aureus. VES 04 pigment extract, on the other hand, could inhibit the activity of B. subtilis, E. coli, and P. aeruginosa (Table 3). Both extracts showed no inhibition zone against Candida albicans.

The antioxidant properties of pigment extracts

Actinomycete VES 01 and VES 04pigment extracts both exhibited scavenging activity against DPPH as a free radical. The pigment extracts of Actinomycete VES 01 and VES 04 showed IC50 values of 231.08 µg/mL and 369.30 µg/mL, in that order (Table 4). The lowest concentration that can prevent 50% of the activity of free radicals is known as the inhibitory concentration (IC50).

Identification of chemical compounds

The dominant extract compound was investigated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Actinomycete HV11.P3 pigment extract's main constituent was 1H-Purine-2,6-dione,3,7-dihydro1,3,7-trimethyl- (Figure 2; Table 5), whereas actinomycete SCA54's.5-Methoxypyrrolidin-2-one was the main compound in the P2 pigment extract (Figure 3; Table 6).

DISCUSSION

In our investigation, we discovered that VES 01 and VES 04 could produce orange and greenish-black intracellular pigments, respectively. The BSLT test revealed that the pigment extract of VES 04 and VES 01 had an LC50 value of less than 150 µg/mL, indicating a high in vivo toxicity effect. The BSLT test was employed as a screening tool in the hunt for anticancer drugs (Elsyana et al. 2016). Consequently, more research is required to assess the cytotoxic activity against human cells. Antimicrobial properties that show promise will result in high levels of toxicity (Pandit et al. 2018). It has been discovered that some compounds, like antimicrobial peptide (AMP), have both cytotoxic and antimicrobial properties (Felicio et al. 2017). The actinomycete VES 01 and VES 04 pigment crude extracts exhibited a moderate to low level of activity against the bacteria that were tested. The Gram-positive and Gram-negative bacteria that

were tested could both be inhibited by the extracts. As a representative of the eukaryotic cell, C. albicans, was not active against any of these extracts. Actinomycetes are the source of pigments that have attracted a lot of interest because of their potential therapeutic uses, including antimicrobial activity (Mumtaz et al. 2018). Both of our pigment extracts were evaluated for their in vitro antioxidant activities in order to look into their antioxidant activity. Actinomycete VES 01 and VES 04 pigment extracts had IC50 values of 23.08 g/mL and 369.30 g/mL against DPPH radicals, respectively. The antioxidant may reduce the level of oxidative stress in the cell. The low level of free radicals undoubtedly reduces the possibility of cell damage and the efficacy of antibiotics. Some antioxidants, on the other hand, have been shown to have potent antibacterial activity. The mechanisms involved in this process have previously been described (Naqvi et al. 2019; Dwyer et al. 2014). It's interesting to note that both of our crude extracts had antibacterial and antioxidant properties. Our findings were consistent with previous research using Streptomyces sp. VITSTK7, which had both antibacterial and antioxidant properties (Thenmozhi and Kannabiran 2012). GC-MS analysis was also used to determine the chemical composition of the pigment extracts in order to better understand the possible chemical compounds involved in their biological activities. Benzene, alcohols, esters, fatty acids, and amino acid groups are among those compounds. In our current study, the presence of n-Hexadecenoic acid (CAS), Octadecanoic acid (CAS), and 9,12,15-Octadecadienoic acid (Z, Z, Z) may be the main compounds that inhibit microbial growth. Our findings support previous research that found these compounds can inhibit the growth of bacteria like E. coli (Gram-negative bacteria), S. aureus (Gram-positive bacteria), and the fungus Aspergillus flavus (Krishnaveni et al. 2014). Meanwhile, n-Hexadecenoic acid (CAS) and 5- Methoxypyrrolidin-2-one, which may act as an antioxidant, may also play important roles (Dascalu et al. 2020). Antibacterial, antioxidant, and toxicity activities were demonstrated by pigments derived from two actinomycetes, Micromonosporachalcea VES01 and Micromonosporatulbaghiae VES04. M. tulbaghiae VES04 pigment extract demonstrated the best antibacterial activity against E. coli ATCC 8739, while M. chalcea VES01pigment extract demonstrated the best antioxidant activity.

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EVALUATION OF ACALYPHA INDICA EXTRACT FOR ANTIMICROBIAL PROPERTIES AGAINST PATHOGENS

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ABSTRACT:

Acalypha indica, commonly known as "Indian nettle" or "copper leaf," is a widely distributed weed in the Euphorbiaceae family, recognized for its significant medicinal properties in traditional medicine, especially in India. The plant has been traditionally used in Ayurveda for treating a variety of ailments, including skin infections, respiratory issues, menstrual irregularities, and snake bites. Its medicinal efficacy is largely attributed to its phytochemical constituents such as tannins, flavonoids, and glycosides, which contribute to its diverse pharmacological activities. These activities include anti-inflammatory, antioxidant, antimicrobial, anti-venom, wound healing, and potential anti-fertility effects. Despite its promising therapeutic potential, the safety and efficacy of *Acalypha indica* in clinical settings remain to be fully established. Therefore, further research is needed to explore its therapeutic applications and assess its potential toxicity. This study emphasizes the importance of continued investigation into the pharmacological properties and clinical viability of *Acalypha indica* as a natural remedy.

Keywords: *Acalypha indica*, traditional medicine, phytochemistry, pharmacological activities, antimicrobial, anti-inflammatory, antioxidant, anti-cancer.

INTRODUCTION

Acalypha indica, a medicinal plant belonging to the family Euphorbiaceae, is widely distributed in tropical and subtropical regions. Traditionally, it has been used in folk medicine for its diverse therapeutic properties, including antibacterial, antifungal, anti-inflammatory, and antioxidant activities. The phytochemical composition of Acalypha indica includes alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which contribute to its pharmacological properties (Annan & Houghton, 2008). The increasing resistance of pathogenic microorganisms to conventional antibiotics has driven the search for alternative natural antimicrobial agents, and plant-based bioactive compounds have emerged as potential candidates in this regard (Gopalakrishnan *et al*., 2016).

Pathogenic bacteria such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae are known to cause various infectious diseases, including skin infections, urinary tract infections, and respiratory illnesses. Similarly, fungal pathogens like Candida albicans and Aspergillus niger have been associated with opportunistic infections, particularly in immunocompromised individuals (Subramani *et al.*, 2017). Studies have demonstrated that Acalypha indica exhibits potent antimicrobial activity against these pathogens, which is attributed to the presence of secondary metabolites with bioactive properties. Methanolic and ethanolic extracts of the plant have shown significant inhibition of bacterial and fungal growth in vitro, suggesting their potential as natural antimicrobial agents (Mohan *et al*., 2011).

The antimicrobial activity of Acalypha indica has been evaluated using various methods, including the agar well diffusion assay, disc diffusion method, and minimum inhibitory concentration (MIC) determination. These methods help in assessing the efficacy of plant extracts against microbial strains and provide insights into their potential therapeutic applications (Jothy

et al., 2012). The agar well diffusion method, in particular, is widely used to screen antimicrobial activity due to its simplicity and reliability. Research indicates that the zone of inhibition observed in agar diffusion assays varies based on the solvent used for extraction, with polar solvents such as methanol and ethanol extracting higher concentrations of bioactive compounds compared to aqueous extracts (Sivakumar *et al*., 2014).

The phytochemical profile of Acalypha indica suggests that its antimicrobial activity is primarily due to the presence of flavonoids and tannins, which disrupt microbial cell walls and inhibit essential enzymes required for pathogen survival. Studies have also reported that the plant extract possesses synergistic effects when combined with conventional antibiotics, enhancing their efficacy against resistant bacterial strains (Duraipandiyan *et al*., 2009). This property is particularly relevant in the context of multidrug-resistant bacteria, which pose a significant challenge in clinical settings. The potential of Acalypha indica as an alternative antimicrobial agent highlights its importance in the development of plant-based pharmaceuticals and the need for further research to explore its therapeutic applications in medicine (Sundararajan *et al*., 2020).

MATERIALS AND METHODS

Collection of wound sample

Wound sample was collected from fungal infected person and immediately inoculated into normal saline in a plastic container and air tighted and then transferred laboratory and used for further investigation. The sample was collected from patient, Kavery Hospital, Trichy.

Isolation of bacteria from wound sample

Nutrient agar media was used for bacterial cultures revival. Nutrient agar medium was prepared and pH of the media was adjusted to 6.5. The media was then autoclaved for 121°C for 15 lbs pressure at 15 minutes. The medium was poured in to petridishes and allowed to solidify. The griseoflovin (20mg/lit) was added for the purpose of avoid fungal growth in the medium. The agar was allowed to solidification.

The collected wound sample was spread on petriplate containing sterilized nutrient agar medium. The inoculated plates were incubated in the room temperature was maintained at 37°C for 24 hours. Replicate plates were maintained for each sample. The colonies growing on nutrient agar plates with different morphology were counted separately.

Isolation of the fungi from wound sample

Potato Dextrose Agar (PDA) media was used for fungal cultures revival. Potato are weighing (200g) was peeled, sliced boiled and then sieved through a clean muslin cloth to get a filtrate, was made up to 1000µl of distilled water, to which agar (16g) and dextrose (20g) was added. pH of the media was adjusted to 6.5. The media was then autoclaved for 121°C for 15 lbs pressure at 15 minutes (Downes and Ito, 2001). The medium was poured in to petridishes and allowed to solidify. The streptomycin (20mg/lit) was added for the purpose of avoid bacterial growth in the medium. The agar was allowed to solidification.

Wound sample was spread on petriplate containing sterilized PDA medium. The inoculated plates were incubated in the room temperature was maintained at 28 ± 2°C for 72 hours. Replicate plates were maintained for each sample. The colonies growing on PDA plates with different morphology were counted separately.

Identification of bacteria

Gram's staining

A thin smear of bacterial isolates were separately made on a clean glass slide and heat fixed. After that the smear was stained by crystal violet for 1 minute and then washed with water,

followed by flooded with Gram's iodine. After 1 minute the slide was washed again in tap water and decolorized with alcohol. The slide was washed and air dried. Finally, the slide was observed under microscope to find out the staining.

Indole production

Peptone broth inoculated with 48 hours old bacterial cultures and was incubated at 28±0.1°C for 24 hours. To this test tube was added 0.5 µl Kovac's reagent and vials shaken gently. Indole production was indicated by deep red colour in the reagent layer.

Methyl Red test

MR-VP broth inoculated with 48 hours old bacterial cultures and was incubated at 28+0 1°C for 24 hours. To this test tube was added 3-4 drops of methyl-red reagent. Positive test was indicated by a change in the medium colour from yellow to red.

Voges Proskauer test

MR-VP broth inoculated with 48 hours old bacterial cultures and was incubated at 28±0.1°C for 24 hours. After incubation period to add 3-4 drops of Barritt's reagent A and Band shaken well. Positive test was indicated by the appearance of strong red colour and no colour change indicate negative results.

Citrate utilization test

Simmon's citrate medium inoculated with 48 hours old bacterial and was incubated at 28±0.1°C for 24-48 hrs. Citrate utilization was indicated by a change in green colour of the medium to blue.

Catalase test

A drop of 3% hydrogen peroxide was added to 48 hours old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase positive.

Identification of the fungal isolates

The cultures were identified on the basis of macroscopic (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic characteristics (separation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium). Identification of fungi was done by using standard manual of soil fungi by Gillman (1957).

Lactophenol cotton blue staining

Lactophenol cotton blue staining –mounting medium is commonly used for microscopic identification of fungi. Immerse the specimen in the drop of alcohol .Add 2 drops of lactophenol cotton blue mountant. Holding the coverslip and avoiding air bubbles.

Collection of Plant Material

Fresh healthy plant of *Acalypha Indica* was collected from Marthupandiyar collage,vallam,Thanjavur (Dt), Tamilnadu, India. Collected material was washed throughly in running tap water, rinsed in distilled water, stored in sterile polythene bags and used for further studies.

Sterilization of plant materials

The disease free and fresh plant stems were selected for this investigation. About 2 gm fresh plant materials were taken for washed with distilled water. Then, surface sterilized with 0.1% mercuric chloride and alcohol for few seconds. Again the plant materials were washed thoroughly with sterile distilled water (Three times).

Preparation of plant extracts (Alade and Irobi, 1993; Essawi and Srour, 2000).

Two grams of leaves was kept taken in the 10µl of different organic solvent acetone, ethanol and aqueous. Crushed with use of cleaned sterile mortar and pestle. Then the solvent extracts were filtered through Whatsman No.1 filter paper and series of sterile filter. These prepared stem extracts were stored in sterile glass bottles at 4°C for further use.

Determination of antimicrobial activity

Preparation of culture inoculums

The stock cultures of bacteria and fungi isolated from skin lesion sample of the patient was used in this study was maintained on nutrient agar slants and PDA slants respectively at 4°C Inoculums was prepared by suspending a loop full of bacterial cultures into 10 µl of nutrient broth and for fungal culture 10µl of PDA broth was used and incubated at 37°C ± 2°Cfor 24 to 48 hours.

Agar well-diffusion method (Mahalingam, *et al*., 2011)

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. Solvent alone was used as a control. About 100µl of different solvent leaves extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions The plates were incubated in an upright position at 37°C 2°C for 24 h for bacterial pathogens and 28°C 2° C for fungi. The organic solvents alone were acted as a negative control. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The meters of the zones were measured using diameter measurement scale. The effect of plant extract was compared with standard antibiotics. Triplicates were maintained and the average values were recorded for antimicrobial activity.

Table 3:Biochemical characterstics of the pathogenic bacteria

S.No	Test Name	E.coli	Staphylococcus aureus	Klebsiella
1.	Morphology	Rod	Coccus	Rod
2.	Arrangement	Single	Irregular	Single
3.	Gram staining	Gram-ve	Gram+ve	Gram-ve
4.	Motility	+	-	-
5.	Indole	+	-	-
6.	MR	+	-	-
7.	VP	-	+	+
8.	Citrate	-	+	+
9.	Urease	-	+	+
10.	Glucose	+	+	+
11.	Sucrose	+	+	+
12.	Lactose	+	+	+

Table:4 Identification of fungi from wound sample.

S.No	Name of the fungi
1	*Aspergillus niger*
2	*Aspergillus flavus*
3	*Candida albicans*

Table: 5 Antibacterial activity of *Acalypha indica*

Name of the bacteria	***Zone of inhibition(mm)***		
	Acetone (µl)	***Ethanol (µl)***	***Water (µl)***
E. coli	**18**	**27**	**11**
Staphylococcus	**28**	**32**	**10**
Klebishella	**17**	**24**	**12**

Table: 6 Antifungal activity of *Acalypha indica*

Name of the fungi	**Zone of inhibition(mm)**		
	Acetone (µl)	**Ethanol (µl)**	**Water (µl)**
Aspergillus niger	**11**	**17**	**11**
Klebsiella	**16**	**22**	**7**
Aspergillus flavus	**18**	**16**	**10**

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COMPARATIVE STUDIES ON THE ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF *ALOE VERA* AND *OPUNTIA FICUS-INDICA* AGAINST PLANT PATHOGENS

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ABSTRACT

The present study examines the antimicrobial activity of different plant extracts from Aloe vera and Opuntia ficus-indica. The antimicrobial study confirmed that a combination of ethanol and methanol fractions exhibited maximum inhibition against plant pathogens, with a zone of inhibition measuring 20 mm. However, aqueous and ethanolic extracts alone did not demonstrate significant activity compared to the standard drug. Phytoconstituents identified included sterols, tannins, alkaloids, flavonoids, and terpenoids through preliminary investigations. The present study may provide valuable information regarding the identification of active fractions as potent antimicrobial agents.

INTRODUCTION

Plants have been used as medicinal sources since time immemorial. Ethnobotany and ethnopharmacology involve the scientific evaluation of traditional medicinal practices, which have been effective in treating gastrointestinal, inflammatory, and dermatological ailments. The plant kingdom holds immense potential for drug discovery, with only 10-15% of the estimated 750,000 species of higher plants having been explored for biologically active compounds. Natural products derived from plants, fungi, bacteria, insects, and animals serve as biologically active pharmacophores. Approximately one-third of the world's top-selling drugs are either natural products or their derivatives. Natural products are widely recognized in the pharmaceutical industry for their structural diversity and extensive pharmacological properties. Traditional, empirical, and molecular approaches have led to the discovery of new medicines (Harvey, 1999).

MATERIALS AND METHODS

Phytochemical Screening

To screen the phytochemicals present in Aloe vera and Opuntia ficus-indica.

Collection and Processing of Plant Materials:

Dried plants of Aloe vera and Opuntia ficus-indica were collected from Thanjavur. The samples were air-dried, powdered, and stored in polythene containers for further use.

Phytochemical Screening:

Chemical tests were conducted on aqueous extracts using standard procedures to identify phytoconstituents as described by Sofowara (1993), Trease and Evans (1998), and Harborne (1973).

Qualitative Analysis of Phytochemicals:

- **Test for Tannins (Mace, 1963):** A brownish-green color upon adding ferric chloride indicates the presence of tannins.
- **Test for Phlobatannins (Iyenger, 1995):** A red precipitate upon boiling with hydrochloric acid confirms the presence of phlobatannins.

- **Test for Saponins (Ramakrishnan, 1994):** Formation of a stable froth and emulsion with olive oil indicates the presence of saponins.
- **Test for Flavonoids (Iyenger, 1995):** A yellow color upon the addition of ammonia and sulfuric acid confirms flavonoids.
- **Test for Steroids:** A color change from violet to green confirms the presence of steroids.
- **Test for Terpenoids (Salkowski test):** A reddish-brown coloration at the interface indicates terpenoids.
- **Test for Cardiac Glycosides (Keller-Killani test):** A brown ring at the interface suggests the presence of cardiac glycosides.
- **Test for Anthraquinones:** A color change upon adding diluted ammonia to the chloroform extract indicates anthraquinones.

Estimation of Antioxidant Activity – DPPH Method (Lin *et al*., 1999)

To estimate the total antioxidant content in *Aloe vera* and Opuntia ficus-indica using the DPPH method.

Principle:

The antioxidant activity of plant extracts was measured based on their ability to decolorize a purple methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Calculation:

Inhibition of the DPPH radical (%) was calculated as follows:

I% = [(A_blank – A_sample) / A_blank] × 100

Determination of Antimicrobial Activity

The antimicrobial activity of Aloe vera and Opuntia ficus-indica was tested against two bacterial and two fungal strains:

- **Bacteria:** *Pseudomonas solanacearum* and *Xanthomonas citri*
- **Fungi:** *Aspergillus niger* and *Aspergillus oryzae*

The results were compared using nutrient agar for bacteria, potato dextrose agar for fungi, and Mueller-Hinton agar for comparative studies.

RESULTS

Phytochemical screening indicated the presence of tannins, saponins, flavonoids, and anthraquinones in both plants. Terpenoids were present in *Opuntia ficus-indica* but absent in *Aloe vera*. Phlobatannins, steroids, and cardiac glycosides were absent in both plants. Antioxidant activity were determined by using 2, 2 Diphenyl - 1 - Picylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) assay methods. *Aloe vera* shows 0.908 % antioxidant capacity in 2, 2 Diphenyl - 1 - Picylhydrazyl (DPPH) assay and 0.228 mM/100gm in Ferric reducing antioxidant power (FRAP) assay. Opuntia ficeus indica shows 0.903 %antioxidant capacity in 2, 2 Diphenyl - 1 - Picylhydrazyl (DPPH) assay and 0.125 mM/100gm in Ferric reducing antioxidant power (FRAP) assay. This result were tabulated in Table-II.

Antimicrobial activity test were determined by using Minimum Inhibitory Concentration (MIC) disc diffusion assay method. *Aloe vera* and Opuntia ficeus indica extracts in different concentration on nutrient agar and potato dextrose agar and muller hinton agar plates. Here, muller hinton agar plates were used for comparative studies. The four bacterial species of *Xanthomonas citri* ,*pseudomonas solanacearum* from papaya and Tomato respectively and four fungal species of *Aspergillus niger*, *Aspergillus oryzae* plant pathogens were taken for antimicrobial activity studies(Table III).

Table I. Quantitative Analysis of Phytochemicals:

Sl. No	Test for	*Aloe vera* (g/100g)	*Opuntia ficus-indica* (g/100g)
1	Alkaloids	0.4	1.2
2	Flavonoids	1.41	1.22
3	Phenols	0.186	0.324
4	Saponin	5.695	2.132
5	Tannin	2.644	0.231

Table II. Determination of Antioxidant Activity:

Sl. No	Antioxidant Activity Test	*Aloe vera*	*Opuntia ficus-indica*
1	DPPH Method (Inhibition %)	0.908%	0.903%
2	FRAP Method (mM/100g)	0.228	0.125

Table III. Antimicrobial activity of *Aloe vera* and *Opuntia ficeus indica*

Organisms	Aqueous extract Concentration (%)		Ethanolic extract Concentration (%)	
	AV	OFI	AV	OFI
Bacteria *Xanthomonas citri Fruit*	6mm	5mm	7mm	9mm
Xanthomonas citri Stem	11mm	11mm	8mm	14mm
pseudomonas solanacearum papaya	43mm	20mm	14mm	15mm
pseudomonas solanacearum Tomato	17mm	6mm	16mm	12mm
Fungi *Aspergillus niger Mango*	12mm	10mm	12mm	7mm
Aspergillus niger Grape	14mm	12mm	20mm	18mm
Aspergillus niger Citrus	16mm	15mm	15mm	10mm
Aspergillus oryzae	8mm	Nil	16- mm	15mm

Antimicrobial Activity of *Aloe vera* and *Opuntia ficus-indica*:

Both extracts showed significant antimicrobial activity, with inhibition zones ranging from 6 mm to 43 mm.

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PHYTOCHEMICAL ANALYSIS AND I*N VITRO* ALPHA AMYLASE INHIBITORY ACTIVITY OF AQUEOUS EXTRACT OF E*UPHORBIA HIRTA*

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ABSTRACT

Euphorbia hirta have been used as traditional herbal drugs in Indian medicine for the treatment of many ailments diseases such as diabetes and cancer. The aim of the present study was to examine the phytochemical analysis, *in vitro* antidiabetic activity of aqueous extract of E*uphorbia hirta.* The dried leaves of *Euphorbia hirta* were successively extracted with water by a continuous hot extraction process using a Soxhlet apparatus. The results of the phytochemical analysis demonstrated the presence of secondary metabolites such as saponin,tannins,flavonoids, alkaloids and phenolic compounds. The alpha amylase inhibitory activity was investigated. The results revealed that the aqueous extract of *Euphorbia hirta* showed the highest inhibitory activity against alpha amylase (IC_{50} value of 50.56 ±0.02 at a concentration of 250μg/ml), compared to the standard acarbose. In *vitro* studies demonstrated *Euphorbia hirta* showed high potent antidiabetic activity and could be used as herbal drugs in the treatment of diabetes.

Keywords- Antidiabetic, Acarbose, alpha amylase, *Euphorbia hirta*

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion. Type II diabetes is characterized by insulin resistance, where peripheral cells do not respond to insulin, or β-cell dysfunction, which takes about 90-95% of all diabetes patients. The global burden of DM has increased significantly over the past few decades, with the majority of cases occurring in low-income and middle-income countries (Dar *et al*., (2024).

According to the International Diabetes Federation (IDF) Atlas 10th edition reports 537 million adults (20-79 years) are living with diabetes - 1 in 10. This number is predicted to rise to 643 million by 2030 and 783 million by 2045. The complications associated with DM can be broadly into categories: diabetic retinopathy (blindness), diabetic nephropathy (kidney failure), diabetic neuropathy (nerve damage) and cardiovascular disease (heart attacks, strokes).This disorder is associated with many complications that may lead to the morbidity and death of a patient. The burden of DM has increased globally, particularly in low-income and middle-income countries. Metformin, sulfonylureas, thiazolidinediones, SGLT2 inhibitors and DPP-4 inhibitors can currently be used as synthetic drugs for the treatment of diabetes. These drugs have undesirable pathological effects like weight gain, diabetic ketoacidosis, edema and sudden declined sugar level in blood. (Padhi *et al*., (2020).

Herbal medicines or natural materials have been safer, cheaper and more efficient than synthetic drugs. The effect of herbal medicines on hypoglycemia is usually the inhibition of the

absorption of glucose by inhibition of carbohydrate-hydrolyzing enzymes such as alpha-amylase and alpha-glucosidase (Yadav *et al*., (2024).

Euphorbia hirta (E. hirta) belongs to the family Euphorbiaceae, commonly known as Amman Pacharisi in the Tamil language. It is widely used for the treatment of several ailments such as gastrointestinal disorders, skin diseases, diabetes and kidney stones (Sharma, 2024). It is also known as Asthma plant has been traditionally used for centuries to treat respiratory issues, particularly asthma and bronchitis. Additionally, the latex of this plant has been used to treat skin lesions to promote healing and reduce inflammation (Tripathi, 2022).

Figure: 1 *Euphorbia hirta*

MATERIALS AND METHODS

Collection and preparation of plant materials

The fresh leaves of *Euphobia hirta* was collected from Lalgudi region of Tiruchirappalli, Tamil Nadu. The leaves were washed with distilled water, cut into small pieces and shade dried at room temperature. These dried leaves made into a coarse powder using a mechanical blender and stored in an airtight container for further use.

Preparation of plant extract

100g of leaves powder was extracted by hot extraction in 400 ml of water with frequent agitation. The mixture was filtered through muslin cloth. The filtrate was concentrated by rotary evaporator with vacuum at 50 °C.

Phytochemical studies

The aqueous leaves extract were subjected to various phytochemical analysis to detect the secondary metabolites (Harborne, J.B. (1998).

Inhibition of α -amylase enzyme

The α-amylase inhibitory activity was measured by the dinitrosalicylic acid method. (Yadav,2023).0.5 ml of extract samples were pre-incubated with α-amylase 2U/ml for 15 minutes. Then the mixture was added 0.5 ml of 1% starch solution. The mixture was further incubated at 37°C for 10 minutes. Then the reaction was stopped by adding 1 ml DNS reagent and heated in a boiling water bath for 10 minutes. The blank was prepared without plant extracts Acarbose was used as positive control. The absorbance was measured at 540 nm. The percentage inhibition was calculated.

RESULT AND DISCUSSION

Medicinal plants are enriched with essential phytochemicals, which are a variety of primary and secondary plant metabolites. These phytochemicals have been extensively studied for their potential therapeutic applications and their ability to manage various diseases, including diabetes, arthritis, and infectious diseases. The aqueous extract was subjected to the preliminary phytochemical screening to identify the presence of secondary metabolites in Table 1. The results revealed the presence of alkaloids, flavonoids, saponins, tannins, sugars, coumarins, and phenols.

Polyphenols have been extensively researched for their numerous health benefits, which include scavenging free radicals, exerting protective effects against cardiovascular diseases, cancers, and other age-related diseases, and preventing inflammation and allergies, ultimately contributing to the promotion of overall health and well-being (Manach *et* al., 2004). Saponin's diverse medicinal properties make it a valuable compound with potential applications in various health-related areas.

Table 1: phytochemical screening of E*uphorbia hirta*

S.No	Phytochemical	Aqueous
1.	Alkaloid	+ +
2.	Flavonoid	+++
3.	Glycoside	+ + +
4.	Tannin	+ + +
5.	Coumarin	+ +
6.	Saponin	+ + +
7.	Phenol	+ +
8.	Sugar	+ + +
+ + + highly abundant		**+ + abundant**

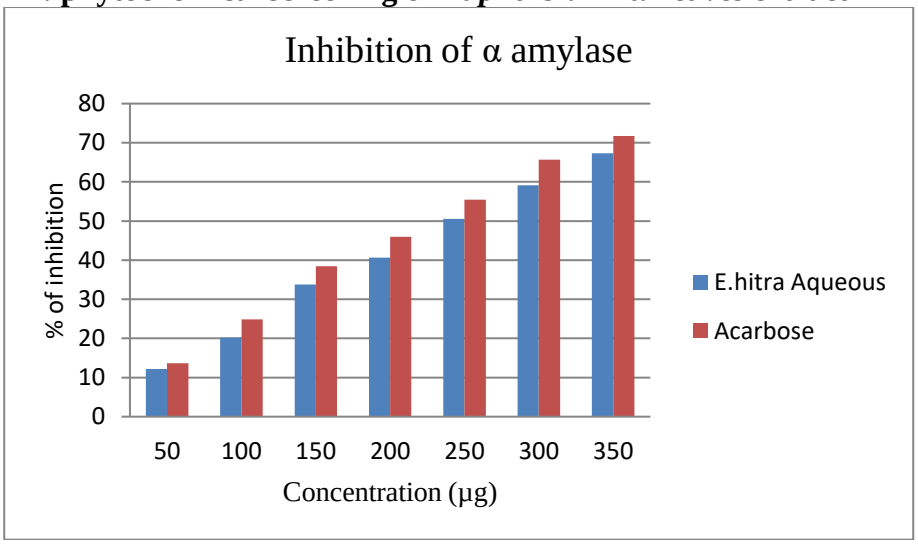
The antioxidant, anti-cancer, anti-inflammatory and antimicrobial activity of saponin is a promising natural remedy for preventing and treating a range of diseases (Biu *et al*., 2009). Yadav (2011) suggested that flavonoids have been extensively used for their diverse health benefits. They have been recognized for their antiallergic, anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic activities. Chang *et al.* (2002) further highlighted their protective effects of flavonoids against free radicals, platelet aggregation, and microbes, and they have potential as a valuable natural remedy for various health conditions. Tannins have been shown to have antioxidant and anti-inflammatory properties, which can help protect against chronic diseases such as heart disease, cancer, and neurodegenerative diseases (Kumar *et al.*, 2022).

Inhibition of α -amylase enzyme

The results demonstrated the inhibitory effect of *E.hitra* on the α-amylase enzymes compared to standard acarbose (Graph 1). The enzymes α-amylase is implicated in the

metabolism of polysaccharides, i.e., starch and glycogen, to disaccharides and glucose. The natural α-amylase inhibitors help to control hyperglycemia by lowering postprandial plasma glucose levels (Telagari 2015). *E.hitra* has shown highest α-amylase inhibitory potential with (IC_{50} value of 50.56 ±0.02 at a concentration of 250μg/ml), The dose dependent effect was observed on the increased concentrations of the extract solution. The similar finding was observed from antidiabetic activity of the leaves extract of *E. Japonica.* Aklima *et al.,* 2014 suggested phenolic compounds have been shown to exhibit anti-diabetic activity through the inhibition of two key enzymes α-amylase and α-glucosidase and potential natural remedy for managing type 2 diabetes and other metabolic disorders. The inhibitors of α-amylase from natural products may be safer than the conventional therapy (Kim *et al.*, 2005). The current study proved that the leaves extract of *E. hitra*, was the potential inhibitors of α-amylase.

Graph 1: phytochemical screening of E*uphorbia hirta* leaves extract



CONCLUSION

The present study demonstrated that *Euphorbia hirta* have high inhibitory activity of α-amylase due to the presence of high content of phenolic and flavonoid and has promising antidiabetic effect against α-amylase. This study proved that *Euphorbia hirta* could be used as herbal drug for the management of diabetes.

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ANTI MICROBIAL ACTIVITY OF *PERGULARIA DAEMIA* AGAINST BACTERIAL AND FUNGAL PATHOGENS

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ABSTRACT:

Antimicrobial potential of *Pergularia daemia*, a plant from the Apocynaceae family. The study highlights the plant's traditional use in medicine and examines its activity against various pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. By testing extracts from the plant in different solvents (methanol, ethanol, and aqueous), the researchers determined that *P. daemia* shows significant antibacterial and antifungal effects, with the methanol and ethanol extracts being particularly potent. The antimicrobial properties are attributed to bioactive compounds such as flavonoids, alkaloids, and saponins. The findings support the traditional medicinal use of P. daemia and suggest that it could be a promising source of natural antimicrobial agents, particularly in light of the growing issue of antibiotic resistance. The abstract also calls for further research to explore the plant's phytochemical composition and mechanisms of action.

Keywords: *Pergularia daemia*, Antimicrobial activity, Medicinal plants, Bioactive compounds, Bacterial pathogens, Fungal infections, Plant extracts, Secondary metabolites.

INTRODUCTION

Medicinal plants have long been a significant source of bioactive compounds with potential antimicrobial properties. Among them, Perugularia daemia (Forsk.) Chiov., a member of the Apocynaceae family, has gained attention for its diverse pharmacological activities, including antibacterial and antifungal effects. This climbing shrub, commonly found in tropical and subtropical regions, is traditionally used in folk medicine to treat various ailments, such as skin infections, inflammation, and respiratory disorders. The increasing incidence of antibiotic-resistant bacterial and fungal pathogens has led researchers to explore plant-derived compounds as alternative antimicrobial agents.

Perugularia daemia is rich in bioactive phytochemicals such as flavonoids, tannins, alkaloids, glycosides, saponins, and phenolic compounds, which contribute to its antimicrobial properties (Baskar et al., 2018). The antimicrobial potential of these phytochemicals has been demonstrated in several studies, highlighting their ability to inhibit bacterial and fungal growth. For instance, the ethanolic extract of Perugularia daemia has shown significant antibacterial activity against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae (Srinivasan et al., 2019). These bacterial pathogens are known to cause a wide range of infections, including respiratory tract infections, urinary tract infections, and wound infections.

Similarly, fungal infections caused by *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum* have emerged as major health concerns, particularly in immunocompromised individuals. Studies have reported that the methanolic and aqueous extracts of *Perugularia daemia* exhibit antifungal activity by disrupting fungal cell membrane integrity and inhibiting fungal growth (Rajasekaran et al., 2020). The presence of steroidal glycosides and flavonoids in the plant is believed to enhance its antifungal efficacy.

The antimicrobial action of *Perugularia daemia* is attributed to multiple mechanisms, including cell membrane disruption, enzyme inhibition, and interference with microbial metabolism. According to a study by Kumar et al., (2021), the plant extract significantly alters bacterial cell wall integrity, leading to increased permeability and cell lysis. In fungi, the extract affects ergosterol synthesis, a crucial component of the fungal cell membrane, resulting in fungal cell death (Madhavi et al., 2017).

Moreover, the antioxidant properties of Perugularia daemia contribute to its antimicrobial potential by reducing oxidative stress in infected tissues, thereby enhancing immune responses against microbial invasion (Arulmozhi *et a*l., 2016). The synergistic effect of various phytochemicals makes Perugularia daemia a promising candidate for developing novel antimicrobial agents.

With the rise of multidrug-resistant pathogens, the demand for alternative therapeutic agents has intensified. Research on Perugularia daemia has demonstrated its potential as a natural antimicrobial agent with minimal side effects (Banu et al., 2018). The integration of Perugularia daemia extracts into pharmaceutical formulations, such as ointments, creams, and herbal supplements, could provide effective treatment options for bacterial and fungal infections.

Additionally, further studies are needed to isolate and characterize the specific bioactive compounds responsible for antimicrobial activity. Advanced techniques, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), can aid in identifying potent antimicrobial compounds for drug development (Prakash et al., 2019).

MATERIALS AND METHODS

Collection of wound sample

Wound sample was collected from fungal infected person and immediately inoculated into normal saline in a plastic container and air tighted and then transferred laboratory and used for further investigation. The sample was collected from patient, Kavery Hospital, Trichy.

Isolation of bacteria from wound sample

Nutrient agar media was used for bacterial cultures revival. Nutrient agar medium was prepared and pH of the media was adjusted to 6.5. The media was then autoclaved for 121°C for 15 lbs pressure at 15 minutes. The medium was poured in to petridishes and allowed to solidify. The griseoflovin (20mg/lit) was added for the purpose of avoid fungal growth in the medium. The agar was allowed to solidification.

The collected wound sample was spread on petriplate containing sterilized nutrient agar medium. The inoculated plates were incubated in the room temperature was maintained at 37°C for 24 hours. Replicate plates were maintained for each sample. The colonies growing on nutrient agar plates with different morphology were counted separately.

Isolation of the fungi from wound sample

Potato Dextrose Agar (PDA) media was used for fungal cultures revival. Potato are weighing (200g) was peeled, sliced boiled and then sieved through a clean muslin cloth to get a filtrate, was made up to 1000µl of distilled water, to which agar (16g) and dextrose (20g) was added. pH of the media was adjusted to 6.5. The media was then autoclaved for 121°C for 15 lbs pressure at 15 minutes. (Downes and Ito, 2001). The medium was poured in to petridishes and

allowed to solidify. The streptomycin (20mg/lit) was added for the purpose of avoid bacterial growth in the medium. The agar was allowed to solidification.

Wound sample was spread on petriplate containing sterilized PDA medium. The inoculated plates were incubated in the room temperature was maintained at 28 ± 2°C for 72 hours. Replicate plates were maintained for each sample. The colonies growing on PDA plates with different morphology were counted separately.

Identification of bacteria

Gram's staining

A thin smear of bacterial isolates were separately made on a clean glass slide and heat fixed. After that the smear was stained by crystal violet for 1 minute and then washed with water, followed by flooded with Gram's iodine. After 1 minute the slide was washed again in tap water and decolorized with alcohol. The slide was washed and air dried. Finally, the slide was observed under microscope to find out the staining.

Indole production

Peptone broth inoculated with 48 hours old bacterial cultures and was incubated at 28±0.1°C for 24 hours. To this test tube was added 0.5 µl Kovac's reagent and vials shaken gently. Indole production was indicated by deep red colour in the reagent layer.

Methyl Red test

MR-VP broth inoculated with 48 hours old bacterial cultures and was incubated at 28+0 1°C for 24 hours. To this test tube was added 3-4 drops of methyl-red reagent. Positive test was indicated by a change in the medium colour from yellow to red.

Voges Proskauer test

MR-VP broth inoculated with 48 hours old bacterial cultures and was incubated at 28±0.1°C for 24 hours. After incubation period to add 3-4 drops of Barritt's reagent A and Band shaken well. Positive test was indicated by the appearance of strong red colour and no colour change indicate negative results.

Citrate utilization test

Simmon's citrate medium inoculated with 48 hours old bacterial and was incubated at 28±0.1°C for 24-48 hrs. Citrate utilization was indicated by a change in green colour of the medium to blue.

Catalase test

A drop of 3% hydrogen peroxide was added to 48 hours old bacterial colony on a clean glass slide and mixed using a sterile tooth-pick. The effervescence indicated catalase positive.

Identification of the fungal isolates

The cultures were identified on the basis of macroscopic (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic characteristics (separation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium). Identification of fungi was done by using standard manual of soil fungi by Gillman (1957).

Lactophenol cotton blue staining

Lactophenol cotton blue staining –mounting medium is commonly used for microscopic identification of fungi. Immerse the specimen in the drop of alcohol .Add 2 drops of lactophenol cotton blue mountant. Holding the coverslip and avoiding air bubbles.

Collection of Plant Material

Fresh healthy plant of *Pergularia daemia* was collected from Nattuchalai, pattukkottai (TK), Thanjavur (Dt), Tamilnadu, India. Collected material was washed throughly in running tap water, rinsed in distilled water, stored in sterile polythene bags and used for further studies.

Sterilization of plant materials

The disease free and fresh plant stems were selected for this investigation. About 2 gm fresh plant materials were taken for washed with distilled water. Then, surface sterilized with 0.1% mercuric chloride and alcohol for few seconds. Again the plant materials were washed thoroughly with sterile distilled water (Three times).

Preparation of plant extracts (Alade and Irobi, 1993; Essawi and Srour, 2000).

Two grams of leaves was kept taken in the 10µl of different organic solvent acetone, ethanol and aqueous. Crushed with use of cleaned sterile mortar and pestle. Then the solvent extracts were filtered through Whatsman No.1 filter paper and series of sterile filter. These prepared stem extracts were stored in sterile glass bottles at 4°C for further use.

Determination of antimicrobial activity

Preparation of culture inoculums

The stock cultures of bacteria and fungi isolated from skin lesion sample of the patient was used in this study was maintained on nutrient agar slants and PDA slants respectively at 4°C Inoculums was prepared by suspending a loop full of bacterial cultures into 10 µl of nutrient broth and for fungal culture 10µl of PDA broth was used and incubated at 37°C ± 2°Cfor 24 to 48 hours.

Agar well-diffusion method (Mahalingam, et al., 2011)

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. Solvent alone was used as a control. About 100µl of different solvent leaves extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions The plates were incubated in an upright position at 37°C 2°C for 24 h for bacterial pathogens and 28°C 2° C for fungi. The organic solvents alone were acted as a negative control. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The meters of the zones were measured using diameter measurement scale. The effect of plant extract was compared with standard antibiotics. Triplicates were maintained and the average values were recorded for antimicrobial activity.

RESULTS

The following results were obtained from the present investigation of antibacterial and antifungal activity of Pegularia daemia

Identification of bacteria

Selection of sample from human being to select and isolation of skin lesion from Human skin. Isolation and identification of bacteria from the head such as E. coli, Klebsiella pnemoniae. and Staphylococcus aureus from the dilution factors. The specific characters of the pathogenic bacteria by biochemical analysis were confirmed some specific test was determined such as morphology, arrangement, grams staining, motility, indole, MR, VP, Citrate, Urease and production of glucose sucrose and lactose confirmed (Table 2 and 3).

Isolates and Identification of fungi by using Lactophenol cotton blue method

Wound sample was evaluated for the isolation of fungi. Totally four fungi were isolated namely Aspergillus Niger, Candida albicans, and Aspergillus flavus. Identification was done by based characteristics (Table4). On the morphology and microscopic.

Antibacterial activity

Effect of medicinal plants with extracts of different concentration of 30 µl, 60 µl, 90 µl with E. coli, Kelabsiella pnemoniae. and Staphylococcus aureus was treated. The maximum concentration of ethanol extract was higher and bacterial activity was observed respectively. According to the *Pergularia daemia* ethanol was higher zone of inhibition was Staphylococcus 30mm zone of inhibition observed respectively (Table 5).

Antifungal activity

The effect of antifungal activity of some medicinal plants against Aspergillus flavus, Aspergillus Niger, and Candida albicans were treated with *Pergularia daemia* with different solvents was the Ethanol was highly suppressive activity when compared to other solvents of phytochemicals has effective activity was observed (Table 6).

Table 1: Isolation of bacteria and fungi from wound sample

S.NO	Sample
1.	Wound sample

Table 2: Identification of bacteria from wound sample

S.NO	Name of the bacteria
1.	*Escherichia coli*
2.	*Staphyococcus aureus*
3.	*Klebsiella pnemoniae*

Table 3 : Biochemical characteristics of the pathogenic bacteria

S.NO	Test Name	E.coli	Stephylococcus aures	Klebsiella pnemoniae
1.	Morphology	Rod	Coccus	Rod
2.	Arrangement	Single	Irregular	Single
3.	Gram staining	Gram-ve	Gram+ve	Gram -ve
4.	Motility	+	-	-
5.	Indole	+	-	-
6.	MR	+	-	-
7.	VP	-	+	+

8.	Citrate	-	+	+
9.	Urease	-	+	+
10.	Glucose	+	+	+
11.	Sucrose	+	+	+
12.	Lactose	+	+	+

Table 4 :Identification of fungi from wound sample

S.NO	Name of fungi
1.	Aspergillus niger
2.	Candida albicans
3.	Aspergillus flavus

Table 5 : Antibacterial activity of *Pergularia daemia* Zone of inhibition (mm)

Name of the bacteria	Acetone (ul)	Ethanol (ul)	Water (ul)
E.coli	17	26	10
Staphylococcus	28	30	10
Klebsiella pnemoniae	15	23	12

Table 6 : Antifungal activity of *Pergularia daemia*

Name of fungi	Acetone (uI)	Ethanol(uI)	Water(uI)
Aspergillus niger	10	16	10
Candida albicans	15	20	5
Aspergillus flavus	14	19	10

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STUDY ON ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA SP. FROM DIFFERENT URINARY TRACT INFECTED PATIENTS

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ABSTRACT

Candiduria has been considered as a challenging condition for physicians because of its colonization and infection Candida sp. are reportedly the most common human fungal pathogens, of about 200 different species of candida only few are human opportunistic pathogens. Several antifungals are available to treat such dreadful Candidal infections. During last decades resistance to antifungal has been increased. The present study aims to isolate the *Candida* sp. from different urinary tract infected patients and evaluate antifungal susceptibility to antifungal drugs of *Candida* sp. A total of 952 urine samples were collected from different hospitals and Candida sp. were isolated using HiCrome candidal differential agar. From this confirmed Candida sp. 48% were identified as *Candida albicans*, 21% *Candida dubliensis* , 17% *Candida tropicalis*, 8% *Candida glabrata*, 6% *Candida krusei* respectively. Isolated Candida sp. were screened for antifungal susceptibility using commercial broad and narrow spectrum antibiotics discs Amphotericin-B, Clotrimazole, Fluconazole, Itraconazole, Ketoconazole, Miconazole, Nystatin. The conformed five UTI fungal strains are maximum resistant to 6 antibiotics out of 7 (95%). All strains are sensitive maximum zone range 10 mm observed in Amphotericin-B, Fluconazole, Itraconazole and Ketoconazole compared than other antibiotics. The highly effective next antibiotics are Miconazole and Nystatin range 08-10 mm in diameter. Based on results five UTI strains were highly resistant criteria.

Key words : Candida sp., Antifungal susceptibility , UTI.

INTRODUCTION

A urinary tract infection (UTI) is an infection that affects both men and women, involving different components of the urinary system, including the kidneys, bladder, ureters, and urethra, typically with a focus on the lower urinary tract, such as the bladder and urethra . A UTI is more likely to occur when the causative pathogen's concentration in the urine culture reaches over 10,000 CFU/mL [1]. Developed regions like the United States and Europe see urinary tract infections (UTIs) diagnosed in around 3%–8% of girls and 1% of boys, with nearly seven million cases reported annually [2]. The normal flora of humans includes Candida species, these microorganisms reside in the gastrointestinal system, mouth, skin, mucosal regions and vagina. While Candida sp. are part of the normal microbiota in healthy individuals, they can lead to infections in immunocompromised situations. The Candida genus comprises over 100 species, but only a limited number are capable of infecting humans [3]. Over the past decade, Candida spp. have remained of significant medical importance, as they can act as opportunistic pathogens leading to life-threatening in immunocompromised patients, both systemic infections and long-lasting mucocutaneous infections can develop. The Candida species, which are fungal pathogens affecting humans, can result in genitourinary infections like vulvovaginal candidiasis in women, balanitis and balanoposthitis in men, and also oral candidiasis, gastrointestinal infections, and candiduria in both men and women. Several virulence factors contribute to its increased pathogenicity, such as the ability to adhere to tissues and surfaces, form biofilms, undergo phenotypic switching, exhibit dimorphism, and produce hydrolytic enzymes.

The incidence of Candida spp. infections is higher in females compared to males. There are several classes of antifungals, each targeting and either halting the growth or eliminating different fungal pathogens. While antifungal agents are essential in the treatment of UTIs, the selection remains limited, but more antibiotics are becoming available to combat fungal infections. Available antifungal treatments, including polyenes and azoles, target the fungal cell membrane, whereas echinocandins weaken the fungal cell wall structure. The growing resistance of 5 *Candida spp*. to antifungal treatments has gained worldwide attention in recent times. While *C. tropicalis* and *C. parapsilosis* are generally susceptible to azoles, *C. glabrata* and *C. krusei* inherently resist fluconazole. In present study antibiotic resistant fungi were identified from commercial broad and narrow spectrum antibiotics.

MATERIALS AND METHODS

UTI urine samples were collected from (different Hospital in Thanjavur, Tamil Nadu and India during the period from Nov 2021 to Oct 2022) by clean catch method. Urine samples were collected within seven days of symptom onset from each patient identified during a urinary tract infected sickness. A sterile container was used to collect a urine sample of 15-25 ml. Label the container(s) with the patient's name, patient's age, ID number and collection date. Store cooled box at 8 - 10ºC and transport on cooled gel packs [4].

The isolation of UTI Candida species was carried out using a streak method on Sabouraud Dextrose Agar and Hichrome Candida Differential Agar with loops for semi-quantitative analysis. The cultures were incubated aerobically at 22-38°C for 24-48 hours, and negative cultures at 24 hours were incubated for an additional 48 hours. For a specimen to be considered positive for UTI, a single organism must be cultured at a concentration of ≥105 cfu/ml [5]. The ATCC obtained strains such as *C. albicans* ATCC 90028, *C. dubliensis* ATCC 13883, *C. tropicalis* ATCC 750, *C. glabrata* ATCC 29212 and *C. krusei* ATCC 6258 were purchased and used as quality control.

The isolated all UTI Candida sp. were screened for antifungal susceptibility test on Muller-Hinton agar medium separately. The commonly available antifungal disc such as Amphotericin-B (50 mcg), Clotrimazole (10 mcg), Fluconazole (10 mcg), Itraconazole (30 mcg), Ketoconazole (50 mcg), Miconazole (50 mcg) and Nystatin (50 mcg) were used for isolated Candida sp. culture. The presence of a single organism at a concentration of ≥105 cfu/ml in a specimen was considered a positive result for UTI. The disc diffusion method used for antifungal sensitivity of isolated Candida sp. to the commercial antifungal agents. Antifungal activity test was carried out following the alteration of the method originally describes by Bauer et al., [6]. The isolated fungal cultures were swabbed on the Muller-Minton agar (2% Glucose) medium separately. Evaluate the turbidity of the inoculum in relation to the 0.5 McFarland reagent, with a turbid suspension having an OD range of 0.08–0.13 at 620 nm. After this swabbing different commercial antifungal discs were placed on each culture swabbed plate. The inoculated plates were incubated at 37˚C for 24 hours to allow for Candida species growth. After the incubation, measure the zones of complete inhibition and record their diameters to the nearest millimetre using a zone scale. The sensitivity conformed to zone size interpretation chart for list of antifungals, quality control limits and interpretative criteria [7].

RESULTS

The health of a country's population is its most valuable resource. Nature has supplied everything needed for our survival. Plants with medicinal properties are among nature's finest contributions to cure many illnesses in humans. These medicinal plants are crucial natural

resources, making it important to recognize them. Candida sp. diploid dimorphic fungus is the primary organism responsible for systemic candidiasis and fungal nosocomial UTI (Urinary Tract Infections) global issue. The growing prevalence of antibiotic-resistant fungi and the emergence of strains with diminished antibiotic effectiveness pose significant challenges to healthcare delivery and access, particularly due to untreatable urinary tract infections in humans.

Totally 952 UTI urine sample were collected from various hospital labs in Thanjavur, Tamil Nadu, India from November 2021 to October 2022. The collected samples were streaked on HiCrome Candida Differential Agar medium and Sabouraud Dextrose Agar (SDA) using calibrated loops for semi quantitative method. Urinary tract infected Candida sp. were isolated using HiCrome Candida Differential agar. The quality control results are presented in Table-1. The ATCC obtained strains such as *C. tropicalis* ATCC 750, *C. krusei* ATCC 6258, *C. dubliensis* ATCC 13883, *C. glabrata* ATCC 29212 and *C. albicans* ATCC 90028 were used as control.In present study comparing the sex female patients(88.57%) are affected more when compare with male patients and childrens 8.57%and 2.86 %respectively was illustrated in Figure 1.Candida albicans was the most common species (48%) compare with non albicans, Candida dubliensis(21%), Candida tropicalis (17%), Candida glabrata(8%), Candida krusei(6%) are given in Table 2 and Fig 2. In this investigation informed than C.albicans most common fungi in human urinary tract infection.

The isolated all UTI Candida sp. were screened for antibiotic susceptibility test on Muller-Hinton agar medium separately. The sensitivity results were comparing with interpretation chart for list of antibiotics, quality control limits and interpretative criteria (CLSI, 2017). The acceptable interpretation chart of antibiotics and quality control results were present in Table – 3. The ATCC obtained strains such as C. tropicalis ATCC 750, C. krusei ATCC 6258. C. dubliensis ATCC 13883, C. glabrata ATCC 29212 and C. albicans ATCC 90028 were used as quality control. The selected UTI Candida fungal antibiotic inhibition results were compared with CLSI acceptable Quality Control (QC) range interpretative criteria. The criteria range divided into three categories such as Sensitive, Intermediate and Resistant. The sensitive mean more than 13 mm in diameter zone of inhibition observe the susceptibility result, below 09 to 12 mm in diameter zone is intermediate and less than 09 mm in diameter zone indicate that's called resistant. The quality control all ATCC Candida fungal are given sensitivity results. In the all ATCC Candida fungal susceptibility results differed from one another. In this study seven commercial broad and narrow spectrum antibiotic discs were used for antibiotic sensitivity pattern. The investigated results were comparing with interpretative criteria chart than conformed UTI isolates criteria such as sensitive, intermediate and resistant. The all UTI isolates average range of sensitivity results was present in Table - 4. The isolated all fungal strains were analysed for antibiotic sensitivity, based on results all strains maximum intermediate zone of inhibition were observed in Itraconazole, Amphotericin-B, Ketoconazole and Fluconazole. The Clotrimazole Nystatin, Miconazole and Voriconazole antibiotics are having maximum observed resistant. Over all comparison five fungal UTI Candida strains have maximum resistant were observed. The resistant strains results were presented in Table4. Totally five different antibiotic resistant Candida fungal was screened from isolated UTI isolates which are named as UTIC1 – UTIC5. Preliminary level isolated five fungal were confirmed by antibiotic sensitivity assay method. In this experiment five highly antibiotic resistant Candida fungal strains were conformed (named as UTIC1, UTIC2, UTIC3, UTIC4 and UTIC5) and compared than quality control strains such as C. albicans ATCC 90028, C. dubliensis ATCC 13883, C. krusei ATCC 6258, C. glabrata ATCC 29212 and C. tropicalis ATCC 750. The conformed five UTI fungal strains are

maximum resistant to 6 antibiotics out of 7 (95%). All strains are sensitive maximum zone range 10 mm observed in Amphotericin- B, Fluconazole, Itraconazole and Ketoconazole compared than other antibiotics. The highly effective next antibiotic Miconazole and Nystatin range 08-10 mm in diameter. Based on results five UTI strains were highly resistant criteria (Table 4).

Table 1. Hi Crome UTI Candida Differential agar (Quality Control)

Quality Control				
Organism	**Inoculum (CFU)**	**Growth**	**Recovery(%)**	**Colour of Colony**
Candida albicans	50-100	Luxuriant	>=50	Light green
Candida glabrata	50-100	Luxuriant	>=50	Cream to white
Teunomyces krusei	50-100	Luxuriant	>=50	Purple, fuzzy
Candida tropicalis	50-100	Luxuriant	>=50	Blue to purple
Candida utilis	50-100	Luxuriant	>=50	Pale pink to pinkish purple
Candida parapsilosis	50-100	Luxuriant	>=50	White to cream

* The HiMedia Manual, HiMedia Laboratories™; CFU - Colony Forming Unit

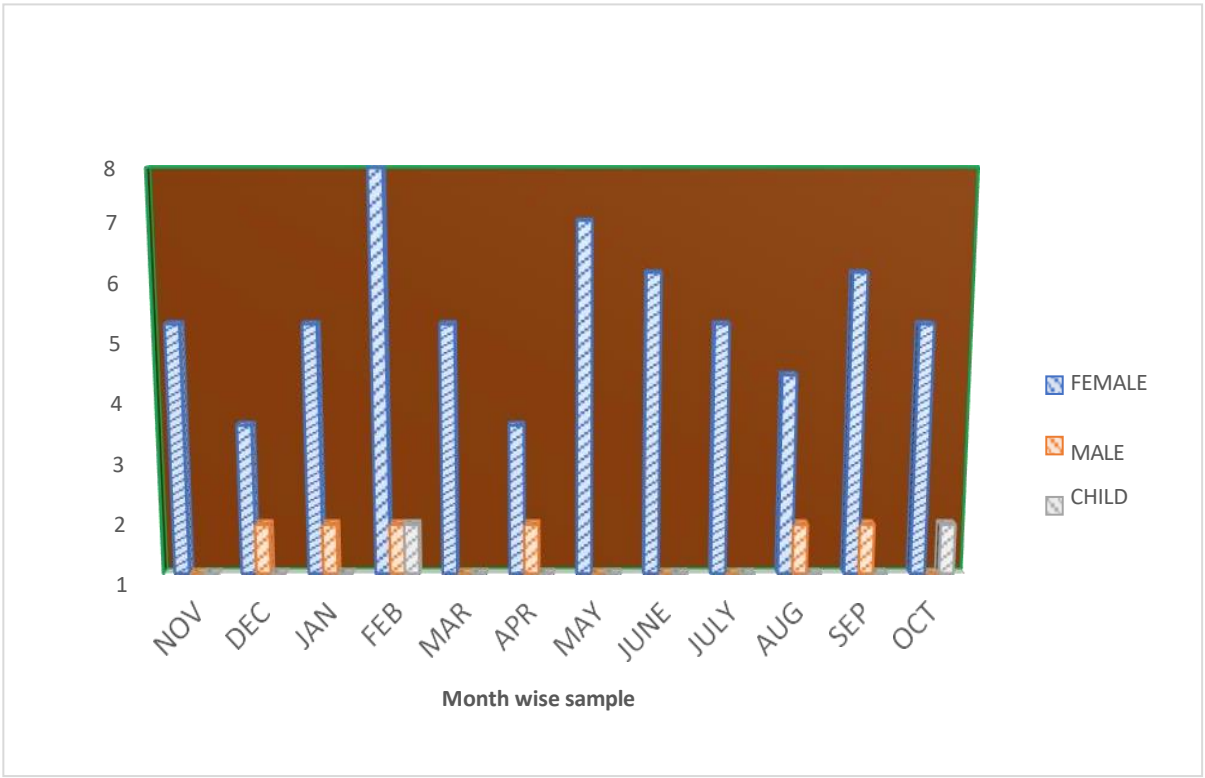

Figure 1. Percentage of sex factor affecting candida sp.

Table 2. Isolation of UTI Candida sp.

UTI candida sp.	Observation	
	No. of Positive UTI	**Percentage (%)**
Candida albicans	37	48
Candida dubliensis	17	21
Candida tropicalis	13	17
Candida glabrata	6	8
Candida krusei	5	6
No growth	874	-

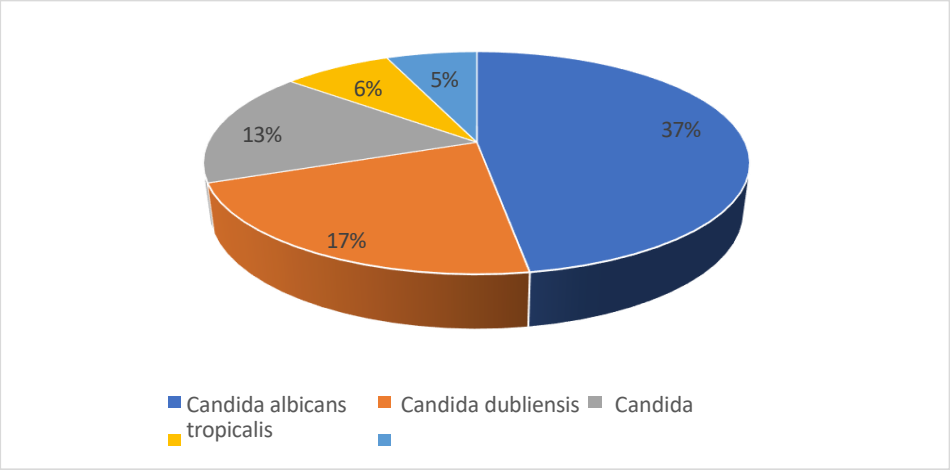

Fig 2. Percentages of isolated UTI Candida sp.

Table 3 Acceptable QC ranges of antifungal susceptibility test zone diameters for references strains

Interpretative Criteria				**Quality Control Zone of Inhibition (mm in diameter)**				
Antibiotics	S	I	R	C. albicans ATCC 90028	C.dubliensi s ATCC 13883	C.tropicalis ATCC 750	C. glabrata ATCC 29212	C. krusei ATCC 6258
Amphotericin-B	**14**	**11-13**	**10**	**10-17**	**11-20**	**8-12**	**11-18**	**9-14**
Clotrimazole	**13**	**11-12**	**10**	**18-32**	**10-17**	**10-20**	**13-17**	**14-24**
Fluconazole	**15**	**10-14**	**09**	**16-20**	**13-17**	**16-25**	**16-20**	**-**
Itracona zole	**16**	**11- 15**	**10**	**18-22**	**16-.30**	**8-13**	**17-24**	**8-15**
Ketoconazole	**13**	**09- 12**	**08**	**20-32**	**14-29**	**17-28**	**14-20**	**10-14**

Miconazole	**14**	**11- 13**	**10**	**22-26**	**14-17**	**14-20**	**15-21**	**19-26**
Nystatin	**15**	**11- 14**	**10**	**19-23**	**16-25**	**16-21**	**16-25**	**15-20**

S- Sensitive; I- Intermediate; R- Resistant

Table 4. Screening and conformation of *Candida sp*. antibiotic resistant fungal

Antifungal Agent	Zone of Inhibition (mm in diameter)				
	C. albicans ATCC 90028	C.dubliensis ATCC 13883	C. tropicalis ATCC 750	C. glabrata ATCC 29212	C. krusei ATCC 6258
Amphotericin-B	-	-	**10±1.30**	-	-
Clotrimazole	-	-	-	-	-
Fluconazole	**10±1.20**	**10±1.24**	-	-	-
Itraconazole	**10±1.42**	**09±1.30**	-	-	-
Ketoconazole	-	**08±1.74**	**09±1.31**	-	-
Miconazole	-	-	-	-	-
Nystatin	-	-	-	-	-

Results values are presented M±SD (Mean ± Standard deviation); n=6

DISCUSSION

Now a day's it's become the most vital trouble for clinical filed was antifungal resistant fungi. The developing country like India, Brazil, Russia, China, South Africa, Egypt and Ethiopia facing this problem from last ten decades. The reason of the problem was over use and misuse of antifungals. Antifungal resistant fungus was affecting everyone, every age, and in any country. In this situations we do two course of action, one is antifungal uses awareness for inhabitants and another one is discover new antifungals against resistance strains from natural sources because of the resistant power usually not present in ecological habit of fungi but that strains transfer or infecting to human become antifungal resistant power.

HiCrome Candida Differential agar was chromogenic medium that make easy rapid isolation in addition to presumptive identification of most UTI fungi including various species from urine. Enzymes produced by Candida species, including C. albicans and C. tropicalis, specifically cleave chromogenic substrates. The HiCrome Candida Differential agar contain chromogenic agent was cleaved to ß-glucosidase by formation of blue colonies resulting in Candida albicans were produce light green colonies due to the enzyme β-D-galactosidase that cleaves the other chromogenic agent. Candida glabrata were produced cream to white colored colonies due to cleavage of equally the chromogenic agents. The colonies of C. krusei, C. tropicalis, C. utilis and C. parapsilosis were formed in purple, blue to purple, pale pink to pinkish purple and white to cream for the reason that tryptophan deaminase activity reaction. The peptic digest of animal tissue or peptone special provides nitrogen, carbon source and other essential growth nutrients. In the present investigation 952 urine sample collected from Novembe 2021 to October 2022, UTI patients out of 952 (81%) were showed urine culture positive 224 (19%) patients urine culture negative. The results of our study on UTI and polymicrobial growths from urine cultures align with those from various studies in India [8].

HiCrome Candida Differential agar used for the growths of more than 75% isolates whereas Sabouraud dextrose agar (SDA) was given in 52.0% fungal growths. Both the blood agar and HiCrome Candida Differential agar are enriched media that provide all the necessary nutrients to support the growth of potential uropathogens, allowing all isolates to grow on these two media, a finding also reported by other researchers.

The present investigation maximum level of positive urine infected fungi is Candida albicans (48%). The isolation rate and distribution of major Candida species in urinary tract infections in this study were consistent with several studies conducted using both chromogenic and conventional media. Ciragil et al. [9] reported that 10 to 20% of urine samples from UTIs exhibit notable growth of Candida albicans, a major infective fungus, in both community and hospital acquired infections [10]. Infections of the urinary tract caused by Candida dubliensis seem to be increasing, posing a significant health concern, particularly in hospital environments [11]. The Candida dubliensis (17%) was positive urine infected bacterium, a similar laboratory-based study conducted in France found that Candida glabrata accounted for only 8% of isolates from UTI specimens [12]. In this study, Candida krusei (6%) was found to be the isolate in UTIs. The most of the investigation were done on Candida support the similar results which could be attributed to the dominance of Candida albicans in the body flora of the humen [13]. This finding was similar with the study of Tayebi et al. [14], Jombo et al., [15] in which Candida albicans was 8.7% and 12.4%, respectively. The most common organism isolated in these patients was Candida albicans (48%), Candida dubliensis (21%), Candida tropicalis (17%), Candida glabrata (8%) and Candida krusei (6%) (Table 2). The present study maximum patients were infected from Candida albicans in every month. The subsequently patients were infected from C. dubliensis, C. tropicalis, C. glabrata and C. krusei respectively.

The worldwide problem of antifungal resistance Candida sp. was rapid and attractive one of the most important methodical problem of current era. The improvement of new antifungals was slow and complicated to work but fungal resistance is lessening our arsenal of existing drugs posing a catastrophic threat as commonplace infections become untreatable. Our sensitivity results were comparing with interpretation chart for list of antifungals, quality control limits and interpretative criteria (Table 3). Five various candida sp. resistant to list of antifungals mentioned in the Table 4 antifungal resistant fungi were screened from isolated UTI fungi isolates which are named as UTIC1 – UTIC5. The UTI Candida albicans was entirely nontoxic and live on gladly in the human digestive system. Certain *C. albicans* strains causing urinary tract infections have been associated with serious conditions such as food poisoning, meningitis, and infections. With increasing antifungal resistance across several strains, these rare but concerning cases demonstrate the risks posed by uncontrolled antifungal usage. There are many ways for fungi to develop into antifungal resistant. The major one was from beginning to end discriminating force. Selective force occurs when not all fungi are susceptible to the antifungal, enabling the remaining resistant fungi to grow and proliferate. This leads to the development of a fungal population that is resistant to the drug. Selective pressure is a natural phenomenon that progresses gradually but cannot be fully prevented. Excessive use of antifungals contributes to the faster emergence of resistant fungi. Fungi can acquire resistance by transferring genetic material between individuals. One way this occurs is through plasmids. The pervious various literatures of Indian studies on antifungal agent Amphotericin-B has showed that approximately 5% resistance was prevalent. The maximum and minimum level Amphotericin-B resistance was detected in 5% and 1% Candida sp., correspondingly. The analysis of Amphotericin-B resistant Candida isolates using pulsed field gel electrophoresis showed two clones, with the majority of strains belonging to one clone, pointing to a clonal distribution [16]. The present investigation

sensitivity study Amphotericin-B was observed that zone of inhibition results. With the increasing emergence of strains resistant to commonly used antifungals, search for new antifungal products or naturally available plant antibiotics with lesser effects are required.

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ANTIBACTERIAL ACTIVITY OF *EPHEDRA SINICA* AND *ASTRAGALUS* AGAINST COMMON RESPIRATORY PATHOGENS

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ABSTRACT

Upper respiratory tract infections cases have been increasing days by days. The most common pathogens that responsible for infections is *Staphylococcus* and *Streptococcus*. Introduction of antibiotic for upper respiratory infection has been reduced tremendously. Emergence of new pathogens as well as resistance complicating the situation. This open area of study to seek new alternative. Herbs have been use since centuries in traditional medicine or as a supplement. *Ephedra Sinica* and *Astragalus* have been used to treat respiratory problem among Chinese population. In this project, *Ephedra Sinica* and *Astragalus* was macerated into two solvents which are ethanol and aqueous and was tested against *Staphylococcus pneumoniae, Streptococcus aureus* end *Klebsiella pneumoniae*. On the phytochemical revealed that *Ephedra Sinica* contain alkaloid, steroid, terpenoid, tannin, and polyphenol. Presence of terpenoid and tannins may be the reason for occurrence of antibacterial activity in the extract. The antibacterial activity of ethanolic and aqueous extracts of *Ephedra Sinica* and *Astragalus* was done using cup plate method. Only ethanolic extract of *Ephedra Sinica* was show significant result against *Staphylococcus aureus* and *Streptococcus pneumoniea*. MIC in ethanolic extract of *Ephedra Sinica* was recorded as 125 mg/mL for both *Staphylococcus pneumonaie Streptococcus aureus.* The MBC *in* ethanolic extract *Ephedra Sinica* against *Staphylococcus aureus* is 125mg/mL and ethanolic extract *Ephedra Sinica* against *Streptococcus aureus* is 250mg/mL. The outcomes from this current study confirmed that *Ephedra Sinica* have a potential that can be used in pharmaceutical industries to develop new formulation of antibiotic against common upper respiratory pathogens.

Keywords: *Ephedra Sinica, Astragalus*, common upper respiratory pathogens, antibacterial

INTRODUCTION

Upper respiratory tract infection (URTI) is a well-known sickness increase in the outpatient setting. Upper respiratory infections begin from the common-cold to life threatening illnesses. The upper respiratory tract involves the sinuses, nasal passages, pharynx and larynx. Upper respiratory infections involve direct invasion of the mucosa lining of upper airway. Inoculation of microbes or viruses happens when a person's hand comes in contact with pathogens and then touches the nose or mouth or when a person directly inhales respiratory droplets from an infected person who coughing or sneezing. After inoculation, viruses and bacteria encounter several boundaries, including physical, mechanical, humoral and cellular immune defences. Adenoids and tonsils contain immune cells that react to pathogens by getting swollen. Immunoglobulin A and cellular immunity act to decrease contamination all through the entire respiratory tract. Resident and recruited macrophages, neutrophils and eosinophils coordinate to engulf and destroy invaders. A host of inflammatory cytokines mediates the immune response to invading pathogens. Normal nasopharyngeal flora, defend against potential pathogens. Patients with suboptimal humoral and phagocytic immune function are at increased risk for contracting an upper respiratory infections and they are at increased risk for at any worsen infections.[Meneghetti, A. (2017)]

Any respiratory infections caused by bacteria may be treated using Ciprofloxacin. Ciprofloxacin belongs to fluroquinolones family, acts by preventing the pathogens from

duplicating. The examples of bacteria that sensitive to Ciprofloxacin are *Staphylococcus, Streptococcus, Salmonella* and *Anthrax*. Common side effects of Ciprofloxacin are headache, stomach ache, heartburn and loss of appetite. Any serious side effect, patients should consult the doctor immediately such as seizure, dizziness tremors or confusion Illiades, C. (2014).

Herbs is plants that prized for the scent, flavour, medicinal or other asset. Herbs usually classified depends on their scientific names. Herbs that practice among Chinese population is known as Chinese herbs. Chinese herbs were believed since ancient time can cure many diseases. Chinese herbal medicine dates back over thousands years ago approximately the rule of the Han dynasty. Two common herbs that use to treat respiratory infections are *Ephedra Sinica* and *Astragalus* [Yi-Wei Tanget al 2014].

Ephedra Sinica is a plant also known as Ma Huang or Chinese ephedra. Ephedra sinica is a green shrub growing up to 1 feet and 4 inch and it is in leaf from December to January and it is in flowering from May to June and the seed ripen starting August to September obtained from the woody stems, a shrub native to the quite dry and desert areas of Asia, Europe and Africa.[Songyuan Tang et al., 2023] Ephedra can produce side effect such as irritability, restlessness, anxiety, insomnia, headaches, nausea, vomiting and urinary problems. Worse side effects are high blood pressure, rapid heartbeat, stroke, seizures, addiction or even death.[Ehrlich, S. D. (2016)]

Astragalus has been used in Traditional Chinese Medicine for thousands years ago. Usually Astragalus was combine with other herbs to strengthen immune body against disease. Adaptogen, called for Astragalus means it helps defends the body against diseases include mental or physical illness. Astragalus contains antioxidants, which protects cells damage. Astragalus acts to defends and enhance the immune system, preventing colds and upper respiratory infections, lowering blood pressure, treating diabetes and protecting the liver. In United States, studies revealed that Astragalus may possible to treat person whose immune systems have been weakened by chemotherapy or radiation. Meanwhile, studies in China revealed that Astragalus may help people with severe forms of heart disease, relieving symptoms, lowering cholesterol levels, and improving heart function as it contains antioxidant. [Axe, J. (2017)] Root of Astragalus are believed that can act as adaptogen acts to protects body from stress and disease, also acts as anemia that may improve blood counts in people with aplastic anemia. Astragalus also can acts for cold and influenza, diabetes and to cover fatigue due to side effects of chemotherapy Rodriguez, C. (2015).

These Chinese herbs are using traditionally without any scientific proven yet. This study was done to determine how effective of these herbs against respiratory pathogens and this research was done to search for the corresponding phytochemicals from chosen Chinese herbs and its further anti-microbial properties.

MATERIAL AND METHODS

Plant Materials

Ephedra Sinica and Astragalus herbs were bought from amazon online shopping. These herbs were crushed into coarse powder form using blender.

Preparation of extracts

The coarse powder of Ephedra Sinica and Astragalus were equally separated to 200gm each and was transferred into aspirator bottle and was macerated with 900mL 80% ethanol and 900mL distilled water for 7 days at room temperature. After that, each extraction was filtered through Whatmann Paper No.1. The solvent was removed by using rotatory vacuum evaporator at 40˚C.[Zuo, G.-Yet al.,2012]

Collection of bacterial Strains causing respiratory infection

The bacterial strains, *Staphylococcus aureus*, *Streptococcus pneumoniae* and*Klebsiella pneumoniae* were obtained from Microbiology laboratory, Kavuery Hospital, Tiruchirappalli.

Maintenance of Inoculum

The stock culture of each strain was stored in blood agar at 4˚C. To maintain the stock culture, the bacteria were sub-cultured separately on prepared nutrient agarslant and maintained at 4˚C.

Standard drug

Ciprofloxacin 250mg/mL.have been used as standard antibiotic for bacterial infections.

Antibacterial activity using cup plate method

Antibacterial activity of extractions was assessed against respiratory pathogens by cup-plate method. Firstly, the Mc Farland solution was prepared. A loop full of organism was taken from the stock culture and was diluted into the prepared Mc Farland solution. The cloudiness of mixture was compared with the normal saline. This was done to ensure the accuracy of the concentration of the organism was prepared to be used for the streaking on the Muller Hinton agar plate in well plate method.Mueller Hinton Agar was inoculated with suitable inoculums of organism and then allowed to solidify. Each plate was divided into three equal areas with the diameter 6 to 8 mm. To each portion, a cylindrical cavity was made in medium with the help of sterile borer. Two cavities for extracts was added 0.1 mL for ethanolic and aqueous and one cavity was added with 0.1mL of standard. The petri dish then was placed in incubated for 8 hours at 37°C. Diameter of the zone of inhibition was measured and was noted. The diameter obtained by the extractions were compared with result of standard drug Ciprofloxacin 250mg/mL.

Minimum inhibitory concentration and minimum bactericidal concentration of the extracts.

The extracts with Inhibition Zone Diameter (IZD) ≥ 10 mm against respiratory pathogens was further subjected to the determine the value of minimal inhibitory concentrations and minimal bactericidal concentrations (MIC/MBCs) against isolated respiratory pathogens by serial dilution method. MICs/MBCs was done by the standardized broth (using MHB) microdilution techniques with starting inocolums of 5 × 105 CFU/mL for the bacteria according to CLS guidelines and incubated at 35˚C for 24 hours. For the MBC assaying, 0.1 mL aliquots from drug dilution wells with visual growth inhibition was platted onto MHA media. The lowest drug concentration that has no bacteria colonies was recorded as the MBC. (H- Zuo et al., 2012)

Statistical analysis

The data was expressed as mean ± S.E.M. the assessment for MIC study was performed in triplicate and the data was subjected to one-way analysis of variance (ANOVA) using Dunnet 'T' and p values < 0.05 was considered as significant.

RESULTS

The colour, consistency and percentage of yield of ethanolic and extract of Ephedra Sinica and Astragalus were shown in the Table 1 and Fig 1.

Table No 1 The nature and yield percentage

Extract of ***Ephedra Sinica and Astragalus***	Colour	Consistency	**Astragalus** Percentage Yield (%)	**Ephedra Sinica** Percentage Yield (%)
Ethanol extract	Brown	Sticky semi solid mass	55	45.0
Water extract	Dark brown	Sticky semi solid mass	40	39.5

Fig:1. Antibacterial activity of ethanolic and aqueous extracts ofEphedra Sinica and Astragalus

The antibacterial activity of ethanolic and aqueous extracts of *Ephedra Sinica* and *Astragalus* against common upper respiratory pathogens were performed using cup plate method. The diameter of zone inhibition (mm) was measured (Table no. 2) The results showed that the ethanolic and aqueous extract of *Ephedra Sinica* and *Astragalus* was active against *Staphylococcus* aureus, *Streptococcus pneumoniae*and *Klebsiella pnemoniae*Both ethanolic and aqueous extracts of *Ephedra Sinica* shows significant activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* with diameter inhibition zones are 26mm and 28mm respectively (Figure 2 and Figure 3)

Table no.2: Antibacterial activity of ethanolic and aqueous extracts of Ephedra Sinica and Astragalus

Isolated pathogens	Diameter of zone of inhibition (mm) (Mean ± SEM)				
	Ephedra Sinica		Astragalus		Standard
	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ciprofloxacin
Staphylococcus aureus	2.60±0.37 *	1.07±0.67*	1.00 ±0.12	1.53±0.15	4.93±0.67
Streptococcus pneumoniae	2.83±0.17*	1.20±0.12	1.13±0.13	0.93±0.07	5.33±0.6
Klebsiella pneumoniae	1.13±0.13	1.13±0.13	1.77±0.13	1.77±0.15	4.27±0.27

*p<0.05 indicates significant

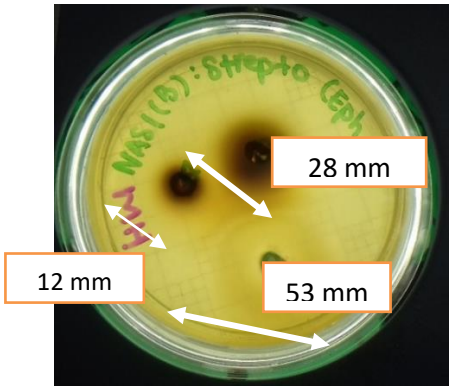

Figure 2. Zone of inhibition of ethanolic and aqueous extract of *Ephedra Sinica* against *Streptococcuspnemoniae*

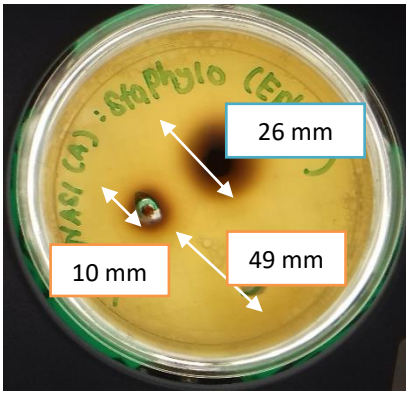

Figure 3: Zone of inhibition of ethanolic and aqueous extracts of *Ephedra Sinica* against *Staphylococcus aureus.*

As the highest diameter of zone of inhibition (mm) was observed for ethanolic and aqueous extracts of *Ephedra Sinica* against *Staphylococcus aureusand Streptococcus pnemoniae*, The Minimum Inhibition Concentration (MBC) and Minimum Bactericidal Concentration (MBC) were recorded as shown in Table no.3, Table no.4 and Table no.5.

Table no 3. : Result of growth of isolated pathogens

Isolated Pathogens	Concentration of ethanolic extracts of *Ephedra Sinica*					
	500 mg/mL	250 mg/mL	125 mg/mL	62.5 mg/mL	31.25 mg/mL	15.62 mg/mL
Staphylococcus pneumoniae	-	-	-	+	+	+
Streptococcus aureus	-	-	-	+	+	+

- = no bacteria growth; + = bacteria growth

-

Table no. 4 : Minimum Inhibitory Concentration of ethanolic extracts of *Ephedra Sinica* against *Staphylococcus aureus* and *Streptococcus pneumonia*.

Isolated pathogens	Minimum Inhibition Concentration
Staphylococcus pneumoniae	125 mg/Ml
Streptococcus aureus	125 mg/Ml

Table 5 : Minimum Bactericidal Concentration (MBC) of ethanolic extract of *Ephedra*

Isolated pathogens	Minimum Bactericidal Concentration
Streptococcus pneumonia	250 mg/Ml
Staphylococcus aureus	125 mg/Ml

Sinica* against *Stretococcus pneumonia* and *Staphylococcus aureus.

DISCUSSION

Upper respiratory tract infections have been reported increasing days by days. the infections commonly caused by bacteria such as *Streptococcus pneumonia*, *Staphylococcus aureus* and *Klebsiella pneumonia*. [Bosch, A et al 2013].

There are many antibiotics that intended to treat the infections but most of it slowly become resistant. Because of this, many researchers conducting study on new substances that derived from natural which are safe and economical to use. Besides that, people nowadays prefer to use natural based products than synthetics products to avoid preservatives and side effects of the chemicals.

The findings from this study explored that the ethanolic and aqueous extract of *Ephedra Sinica* and *Astragalus* was active against *Staphylococcus aureus, Streptococcus pneumoniae* and *Klebsiella pnemoniae* However, only ethanolic and aqueous extracts of *Ephedra Sinica* shows significant activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* with diameter inhibition zones are 26mm and 28mm respectively. In this study, presences of steroids, terpenoids and tannins may be the reason for antibacterial activity.[Singh, B et al., 2003].

Minimum inhibition activity (MIC) of ethanolic extract of *Ephedra Sinica* against *Streptococcus aureus* and *Staphylococcus pneumoniae* was done by serial dilution method. It was tested by the absence of turbidity in broth. The minimum inhibition concentration (MIC) of ethanolic extract of *Ephedra Sonica* against *Staphylococcus pneumoniae* and *Streptococcus aureus* was recorded as 125 mg/mL. The selected broths then were cultured on media plates to get the minimum bactericidal concentration (MBC). Result showed that minimum bactericidal activity (MBC) of ethanolic extract of *Ephedra Sinica* against *Staphylococcus aureus* is 125mg/mL and minimum bactericidal concentration (MBC) of ethanolic extract of *Ephedra Sinica* activity against *Streptococcus pneumoniae* is 250mg/mL.

CONCLUSION

In a conclusion, ethanolic extract of *Ephedra Sinica* is effective only against the gram-positive bacteria since there is no significant value occur in gram negative bacteria. This study can use as support study and need to go deeper about antimicrobial activity of ethanolic extract *Ephedra Sinica* against common upper respiratory pathogens.

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EVALUATION OF THE ANTIFUNGAL ACTIVITY OF LEMONGRASS (*CYMBOPOGON CITRATUS*) AND NEEM (*AZADIRACHTA INDICA*) EXTRACTS AGAINST DANDRUFF

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ABSTRACT

The antifungal potential of lemongrass (*Cymbopogon citratus*) and neem (*Azadirachta indica*) against *Malassezia* spp., the fungi associated with dandruff. As concerns about the side effects and resistance linked to chemical antifungal treatments grow, this research investigates the use of natural alternatives. Acetone, Ethyl acetate and aqueous extracts from both lemongrass and neem leaves were tested using the agar well diffusion method, with results showing that both plants exhibited significant antifungal activity. Lemongrass displayed a slightly larger zone of inhibition compared to neem. The ethyl acetate extracts, in particular, demonstrated stronger antifungal effects, suggesting that the bioactive compounds in these plants are more soluble in ethyl acetate.The study also determined the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) for both plant extracts, confirming their potent antifungal properties. The potential of lemongrass and neem as natural alternatives to chemical antifungals, offering a safer and sustainable approach to treating dandruff. The study recommends into the development of herbal formulations and clinical trials for their application in commercial anti-dandruff products.

Keywords: Antifungal, Dandruff, Lemongrass, Neem, *Malassezia* spp., Herbal treatment.

INTRODUCTION

Dandruff is a common scalp disorder that affects a significant portion of the global population, causing excessive scalp flaking, itching, and irritation. The primary causative agents of dandruff are lipophilic yeasts of the *Malassezia* genus, particularly *Malassezia furfur*, *Malasseziaglobosa,* and *Malasseziarestricta* (Gupta *et al.*, 2004). These fungi metabolize sebaceous lipids, breaking down triglycerides into free fatty acids, such as oleic acid, which disrupt scalp homeostasis and trigger inflammation (Ashbee& Evans, 2002). Conventional dandruff treatments primarily rely on synthetic antifungal agents such as ketoconazole, zinc pyrithione, and selenium sulfide. While effective, these treatments are associated with potential drawbacks, including scalp irritation, toxicity, and the emergence of antifungal resistance (Ranganathan&Mukhopadhyay, 2010). Concerns over these side effects have led to increased interest in natural, plant-based antifungal agents with fewer adverse effects. Medicinal plants like neem (*Azadirachtaindica*) and lemongrass (*Cymbopogoncitratus*) have been traditionally used for skin and scalp care due to their antimicrobial, antifungal, and anti-inflammatory properties (Biswas*et al.*, 2002; Saleem*et al.*, 2018).

Neem, commonly known as the "miracle tree," is widely recognized for its medicinal properties. It contains bioactive compounds such as azadirachtin, nimbidin, nimbin, and gedunin, which exhibit broad-spectrum antifungal activity (Biswas*et al.*, 2002). Studies have shown that neem extracts disrupt fungal cell membranes and inhibit ergosterol biosynthesis, an essential component of fungal growth and survival (Subapriya&Nagini, 2005). Research by Sultana *et al.*, (2015) demonstrated that neem leaf extracts significantly inhibited the growth of *Malassezia* species, highlighting its potential as a natural antifungal agent for dandruff treatment. Neem's

effectiveness is attributed to multiple mechanisms, including disrupting fungal cell integrity, inhibiting fungal proliferation, and reducing scalp inflammation (Saleem*et al.*, 2018).

Lemongrass is an aromatic medicinal plant known for its essential oils, which contain citral, geraniol, and myrcene—compounds with potent antifungal properties (Naik*et al.*, 2010). Several studies have confirmed the efficacy of lemongrass essential oil against *Malasseziaspp*., with its antifungal effects linked to its ability to alter fungal cell permeability and disrupt lipid metabolism (Park *et al.*, 2017). Research by Javed*et al*,. (2020) demonstrated that lemongrass oil exhibited stronger antifungal effects than some synthetic antifungal agents, making it a promising alternative for dandruff management. Lemongrass acts through various mechanisms, including disrupting fungal cell walls, interfering with lipid metabolism, and reducing oxidative stress, thereby preventing inflammation and scalp irritation associated with dandruff.

The combination of neem and lemongrass extracts may provide enhanced antifungal activity through their complementary mechanisms of action. Neem disrupts fungal cell membranes and inhibits ergosterol biosynthesis, while lemongrass essential oil interferes with fungal lipid metabolism, leading to synergistic antifungal effects (Kumar *et al.*, 2018). Several studies have shown that the combined use of neem and lemongrass extracts results in increased fungal cell membrane disruption, enhanced antifungal potency, and additional scalp health benefits, including anti-inflammatory and antioxidant effects (Singh *et al.*, 2014). Research by Singh *et al.*, (2014) confirmed that neem and lemongrass, when used together, exhibited stronger antifungal activity against *Malasseziaspp*. than when used individually, suggesting their potential for formulation into herbal shampoos or topical treatments for dandruff.

MATERIALS AND METHODS

Collection of Dandruff Sample

Dandruff samples were collected from the scalp of an myself, using a sterile comb in hair gently scrape visible flakes. The collected material was transferred into sterile Petri dishes and stored under appropriate conditions until further processing for fungal isolation.

Isolation of Fungi from Dandruff

Potato Dextrose Agar (PDA) medium was used for fungal culture revival. To prepare the PDA medium, 200 g of peeled and sliced potatoes were boiled, and the resulting filtrate was obtained by sieving through a clean muslin cloth. The filtrate was made up to 1000 mL with distilled water, and 16 g of agar and 20 g of dextrose were added. The pH of the medium was adjusted to 6.5 before autoclaving at 121°C for 15 minutes at 15 lbs pressure. The medium was poured into sterile Petri dishes and allowed to solidify. To prevent bacterial contamination, streptomycin (20 mg/L) was added. Dandruff samples were inoculated onto sterile PDA plates and incubated at 28 ± 2°C for 72 hours. Replicate plates were maintained for each sample. Fungal colonies with different morphologies were counted separately.

Identification of Fungal Isolates

Fungal isolates were identified based on:

Macroscopic characteristics: Colony morphology, color, texture, shape, diameter, and appearance.

Microscopic characteristics: Mycelial separation, presence of reproductive structures, shape and structure of conidia, and presence of sterile mycelium. Fungal identification was carried out using Gilman's Standard Manual of Soil Fungi (1957).

Lactophenol Cotton Blue Staining: Lactophenol cotton blue staining was performed for microscopic identification of fungi. The specimen was immersed in a drop of alcohol, followed by the addition of two drops of lactophenol cotton blue mountant. A coverslip was carefully placed to avoid air bubbles.

Collection and Preparation of Plant Material

Collection of Plant Material: Fresh, healthy neem (*Azadirachta indica*) and lemongrass (*Cymbopogon citratus*) plants were collected from Maruthupandiyar College, Vallam, Thanjavur, Tamil Nadu, India. The collected material was thoroughly washed under running tap water, rinsed with distilled water, and stored in sterile polythene bags for further studies.

Sterilization of Plant Materials: Disease-free and fresh plant leaves were selected. Two grams of fresh plant material was washed with distilled water, then surface-sterilized with 0.1% mercuric chloride and 70% alcohol for a few seconds. The plant material was then rinsed thoroughly three times with sterile distilled water.

Preparation of Plant Extracts: Two grams of leaves were crushed using a sterile mortar and pestle in 10 mL of different organic solvents, including acetone, ethyl acetate, and aqueous solutions. The extracts were filtered through Whatman No.1 filter paper and further sterilized using a series of sterile filters. The prepared extracts were stored in sterile glass bottles at 4°C for future use.

Determination of Antifungal Activity

Preparation of Culture Inoculums: Stock culture of fungi isolated from the dandruff sample were maintained on PDA slants at 4°C. Inoculums were prepared by suspending a loopful of10 mL of PDA broth was used for fungal culture. The cultures were incubated at 37 ± 2°C for 24 to 48 hours.

Agar Well Diffusion Method: The agar well-diffusion method was used to determine antifungal activity. PDA plates were swabbed with 48-hour fungal culture using sterile cotton swabs. Agar wells (6 mm in diameter) were created using a sterile cork borer. 25µL, 50µL, 100 µL of different solvent-based plant extracts were added into the wells using sterilized dropping pipettes. A negative control (solvent alone) was used. The plates were pre-incubated for 1 hour to allow diffusion before incubation at 28°C ± 2°C (for fungi). The presence of an inhibition zone around the well indicated antifungal activity (positive result), while the absence of an inhibition zone was recorded as a negative result. The diameters of inhibition zones were measured using a ruler, and antifungal effects of plant extracts were compared with standard antibiotics. Triplicates were maintained, and the average values were recorded.

Potato Dextrose Agar (PDA) Composition

Ingredients	Amount (g/L)
Potato Infusion	200
Dextrose	20
Agar	20
Distilled Water	1000 Ml
Final pH	6.5 ± 0.5

Potato Dextrose Broth (PDB) Composition

Ingredients	Amount (g/L)
Potato Infusion	200
Dextrose	20
Distilled Water	1000 Ml
Final pH	6.5 ± 0.5

RESULTS

To evaluate the antifungal activity of Neem and Lemongrass extracts against *Malasseziaspp*, the zone of inhibition was measured using the agar well diffusion method. The antifungal activity of neem extract prepared using different solvents at varying concentration. The zone of inhibition was measured to determine the effectiveness of neem extract.

Table 1: Antifungal Activity of Neem Extracts Against *Malassezia* species

S.No	Solvent	Concentration (µl)	Zone of Inhibition (mm)
1.	Acetone	25	8mm
		50	12mm
		100	16mm
2.	Ethyl acetate	25	9mm
		50	14mm
		100	18mm
3.	Aqueous	25	-
		50	6mm
		100	10mm

The results indicate that ethyl acetate and acetone extracts of neem exhibit higher antifungal activity compared to the aqueous extract. Highest inhibition was observed at 100µl ethyl acetate extract, suggesting a potent antifungal effect

Table 2: Antifungal Activity of Lemongrass Extracts against *Malassezia species*

S.No	Solvent	Concentration (µl)	Zone of Inhibition (mm)
1.	Acetone	25	9mm
		50	13mm
		100	18mm
2.	Ethyl acetate	25	11mm
		50	15mm
		100	19mm
3.	Aqueous	25	3mm
		50	8mm
		100	13mm

Lemongrass extract, particularly in ethyl acetate and acetone solvents, exhibited stronger antifungal activity compared to neem extract. Highest inhibition was observed at 100µl ethyl acetate extract, suggesting a potent antifungal effect.

Table 3: Positive and Negative control Results

Controls	Zone of Inhibition (mm)
Positive control (Ketoconazole)	22mm
Negative control (Solvent only)	No inhibition

As expected, the positive control (Fluconazole/Ketoconazole) exhibited the largest inhibition zone (22 mm), confirming its strong antifungal activity against *Malassezi*a species. Meanwhile, the negative control (solvent alone) showed no inhibition, confirming that the antimicrobial activity observed in neem and lemongrass extracts is due to their bioactive compounds rather than the solvents used.

DISCUSSION

Neem extract showed moderate to strong antifungal activity, with ethyl acetate and acetone extracts producing larger inhibition zones compared to aqueous extracts. This aligns with previous studies, where neem leaf extracts have been reported to exhibit antifungal activity due to bioactive compounds such as nimbidin, azadirachtin, and gedunin (Kumar et al., 2020). These phytochemicals disrupt fungal cell membranes, leading to inhibition of growth. Similarly, Singh et al. (2019) found that ethyl acetate extracts of neem leaves showed significant antifungal effects against dermatophytes and yeast-like fungi, supporting the present findings.

Lemongrass extract demonstrated higher antifungal activity than neem extract, particularly in ethyl acetate and acetone solvents. This is consistent with findings by Chouhan et al. (2017), who reported that lemongrass, rich in citral and geraniol, exhibited strong antifungal activity against *Malassezia*species. The presence of these volatile compounds enhances membrane permeability, leading to fungal cell lysis (Kamatou et al., 2018). Additionally, a study by Muniandy et al. (2021) confirmed that lemongrass extract inhibited *Malassezia* furfur effectively, with inhibition zones exceeding 18 mm at higher concentrations, supporting the present results.

The positive control, ketoconazole, exhibited the highest antifungal activity (22 mm inhibition zone), which aligns with previous reports that azole-based antifungal drugs are highly effective against *Malassezia* (Gupta et al., 2020). However, concerns about antifungal resistance highlight the need for natural alternatives. In contrast, the negative control (solvent only) showed no inhibition, confirming that the antifungal activity observed was due to bioactive compounds in the extracts rather than the solvents used.

The effectiveness of ethyl acetate and acetone extracts over aqueous extracts may be attributed to higher solubility of bioactive compounds in organic solvents. Similar findings were reported by Abdel-Mageed et al. (2022), where organic solvent extracts of medicinal plants exhibited stronger antimicrobial properties compared to aqueous extracts. This suggests that non-polar and semi-polar compounds in neem and lemongrass contribute significantly to antifungal activity.

Overall, these findings support the potential of neem and lemongrass extracts as natural antifungal agents for dandruff management. Future studies should focus on formulating topical treatments using these plant extracts and evaluating their clinical efficacy. Additionally, further phytochemical analysis and mechanism-based studies would help identify the key active compounds responsible for antifungal activity.

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STREPTOMYCES SP. MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AS AN ANTIBACTERIAL AGENT AGAINST ANTIBIOTIC-RESISTANT STRAINS

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ABSTRACT

The study investigated isolation and screening of antimicrobial producing *Streptomyces sp.* from soil sample and also in the biological synthesis of silver nanoparticles and their antimicrobial properties.Isolation of *Streptomyces sp.* from five different soil samples and about 10 different *Streptomyces sp.* isolates were separated and screened for the antimicrobial potentiality. From that the admirable isolates were selected based on the maximum diverse potential of antibacterial zone of inhibition. Furthermore, best isolate (ISB1)was used for the biological synthesis of silver nanoparticles and their synergistic antibacterial role is observed.Hence, this has been chosen for further optimization studies and biosynthesis of silver nanoparticles and confirmation of silver nanoparticles was carried by EDAX, size and morphology of the nanoparticles was determined by TEM and it was spherical; ranged between 20-50nm. Synthesized nanoparticles were subjected to minimum inhibitory studies and 40 µl gave significant results compared to other concentrations.The biosynthesized silver nanoparticles by *Streptomyces sp* showed better in killing pathogens that might be due to the synergistic effect. Thus, opens new platform for other application oriented studies.

Keywords: *Streptomyces sp*, silver nanoparticles, antimicrobial production, pathogens.

INTRODUCTION

The disturbance of traditional ecosystems due to human activities is an unavoidable reality, resulting in multiple types of pollution.Soil is a significant source of heavy metal pollution, which disrupts microbial communities and hampers essential processes such as nitrogen and potassium fixation, nutrient assimilation and the breakdown of organic matter (Raja and Prabakarana 2011).Although the population of Streptomyces species is relatively small compared to other soil microorganisms, their role is crucial as they participate in degradation, nitrogen fixation, and pathogen inhibition (Hanan *et al.,* 2016).Actinomycetes play a key role in the technology used for antibiotic production.Silver nanoparticles are gaining popularity over other metal nanoparticles because of their low toxicity, cost efficiency, and antimicrobial properties.As a result, nanoparticles can be synthesized through various methods, including chemical, physical, and biological approaches.The process of biologically synthesizing nanoparticles is economical, environmentally sustainable, and enhances the relationship between the biological source and silver nanoparticles (Sneha *et al.*, 2018; Mohamedin *et al.,* 2015).The objective of this research is to examine the biosynthesis of silver nanoparticles by Streptomyces sp. and their potential to kill antibiotic-resistant bacteria.

MATERIALS AND METHODS

Soil samples were gathered from the Muthupet Mangrove Forest (Latitude 10°46'N, Longitude 79.51'E) in Tamil Nadu, India, placed in sterile airlock polythene bags, and transported to the laboratory following a previously outlined procedure.The collected samples were kept at 4ºC for storage until they were needed for further use.The soil samples were pre-

treated by applying dry heat at 56°C for 10 minutes to increase the prevalence of mycelium-forming actinomycetes over other heterotrophic microbes.One gram of dried soil samples was then added to 10 ml of sterile water, followed by further dilution to a 10^6 ratio in sterile water.Each diluted sample (0.1 ml) was individually spread onto Streptomyces sp. isolation agar medium using a sterile glass rod.The media were treated with cycloheximide (40 µg/ml), nystatin (30 µg/ml), and nalidixic acid (10 µg/ml) after autoclaving to suppress the growth of fungi and non-filamentous bacteria.The plates, once inoculated, were incubated at 30ºC for 7 to 9 days, or until colonies exhibiting a tough leathery texture, dry or folded appearance, and branching filaments, with or without aerial mycelia, appeared.

Antimicrobial-producing Streptomyces sp. was selected for screening, and its antimicrobial activity was evaluated against pathogenic bacteria.The isolates selected were inoculated into Starch Casein broth and incubated at 28-30°C with continuous shaking at 250 rpm for 7 to 10 days.After incubation, the fermentation broth was centrifuged, and the supernatant was collected. Equal volumes of ethyl acetate (1:1 v/v) were added, and the mixture was shaken vigorously for 1 hour.The solvent phase was separated from the aqueous phase using a separating funnel, then the solvent was evaporated under reduced pressure in a rotary vacuum evaporator at 60°C and 100 rpm to obtain residue particles.The dried residue was measured and then dissolved in DMSO (Anupama Sapkota *et al.,* 2020). The single isolate was picked based on its ability to produce the largest inhibition zone and the strongest activity against the test isolates.The fresh single culture of Streptomyces sp. (ISB1) was inoculated into 100 ml of SCM broth.After the incubation, the culture was centrifuged at 1000 rpm for 15 minutes, and the supernatant was mixed with 2 mM of AgNO3 (1:1, v/v), followed by incubation at 26°C in a shaker for 3 days in the dark (Railean-PlugaruViorica*et al.,* 2021).A control sample containing only 2mM silver nitrate solution was maintained without inoculation to assess the involvement of actinomycetes in nanoparticle synthesis.The reduction of silver nitrate to silver nanoparticles was confirmed by the color change from light yellow to brown.Various characterization methods were employed to confirm the reduction of silver nanoparticles.

After centrifuging the silver nanoparticles solution at 1000 rpm for 15 minutes, the supernatant was separated for further processing.The solution was then analyzed using a UV-visible spectrophotometer for preliminary confirmation, followed by SEM (Zeiss EVO 18 at 20 kV) and TEM to assess size and morphology, and EDX to quantify the silver nanoparticles.Antibacterial properties of the silver nanoparticle solution supernatant were evaluated through the agar well diffusion method.

RESULTS AND DISCUSSION

The study involved collecting six soil samples, from which ten distinct strains of Streptomyces sp. were isolated based on their microscopic and macroscopic characteristics. The isolated strains were subjected to primary screening, where nearly all exhibited antibacterial activity against pathogens. However, Streptomyces sp. (ISB1) demonstrated the most promising results, showing a broad-spectrum effect against both gram-positive and gram-negative bacteria, as compared to the other Streptomyces isolates (Table - 1).Therefore, Streptomyces sp. (ISB1) was selected for further investigation. The ability of microorganisms to adapt to such harsh conditions is a complex process, demonstrating their capacity to form resistant structures like spores and survive alongside other harmful pathogens (Gurung et al, 2009). The antibacterial activity of Streptomyces sp. dry residues at concentrations of 10, 20, 30, and 40 µg (Fig. 1) was evaluated using the well diffusion method. The most significant zone of inhibition and broad-spectrum antibacterial effect was observed at the 40 µg concentration, surpassing other

concentrations and the control sample, DMOS.A comparable study using pigments extracted from Streptomyces sp. demonstrated inhibition of Escherichia coli growth, except for the yellow pigment, which had no effect on gram-negative bacteria (Parmar et al., 2016; Tandale et al., 2018). The morphology of microorganisms varies between gram-positive and gram-negative strains, leading to differences in their sensitivity to antimicrobial agents. Gram-positive bacteria have an outer peptidoglycan layer that restricts permeability, whereas gram-negative bacteria are protected by a polysaccharide membrane that renders the cell wall impermeable to lipophilic substances. The antimicrobial properties of Streptomyces sp. can be attributed to its selective permeability in targeting pathogens (Shirling and Gottlieb, 1996). Various environmental conditions affect the growth and antimicrobial effectiveness of Streptomyces sp. (Boroujeni et al., 2012). Thus, evaluating these factors provides valuable insights for enhancing the growth of Streptomyces sp. to fight against a broad spectrum of bacterial, fungal, and viral infections.

The primary aim of this study was to evaluate the ability of Streptomyces sp. (ISB1) to synthesize silver nanoparticles. The supernatant of Streptomyces sp. (ISB1) was mixed with silver nitrate solution, resulting in a colour change. Initially, the solution transitioned from colourless to pale yellow, and after incubation, it turned yellowish-brown, indicating the reduction of silver nitrate to silver nanoparticles. The progress was monitored using a UV-Visible spectrophotometer, with a peak observed at 427 nm. The size and morphology of the nanoparticles were examined using SEM (Fig. 2) and TEM (Fig. 3), revealing spherical nanoparticles with a size range of 21-50 nm.Elemental analysis and quantitative compositional data were obtained through EDX. As shown in Fig. 4, the results confirm the presence of silver, along with calcium and oxygen, which could be compounds derived from Streptomyces sp. (ISB1). The silver nanoparticles synthesized by microorganisms demonstrated remarkable antibacterial activity when compared to the control samples (silver nitrate and ampicillin), possibly due to the synergistic interaction between the silver nanoparticles and Streptomyces sp. (ISB1). Likewise, various concentrations of 10, 20, 30, and 40 µg were used, and the findings were compared. Interestingly, 30 µg yielded better results than 40 µg of Streptomyces sp. silver nanoparticles, demonstrating strong antibacterial activity and effective resistance against pathogens (Fig. 5).

The reduction of silver nanoparticles was indicated by a color change to yellowish-brown, which was further confirmed using UV-Vis spectrophotometry. This technique not only monitors the interaction between the Streptomyces sp. supernatant and silver ions but also validates the formation of silver nanoparticles through maximum absorbance values ranging from 400-450nm (Sastry et al., 2003; Sneha Paul et al., 2015). In our study, the peak absorbance was observed at 427nm, confirming the synthesis of silver nanoparticles. SEM and TEM analyses were employed to evaluate the size and morphology, and it was found that smaller nanoparticles facilitate better penetration and microbial invasion. Thus, the biological synthesis of silver nanoparticles shows promising potential for application-based studies.

CONCLUSION

The demand for a sustainable and efficient method for producing silver nanoparticles in nanotechnology has become significant, especially when compared to traditional methods. Streptomyces sp. (ISB1), which is primarily known for its role in antibiotic production, was isolated from soil samples to reduce silver nitrate into silver nanoparticles. The synthesized silver nanoparticles demonstrated a synergistic effect and exhibited a broader antibacterial spectrum than Streptomyces sp. (ISB1) alone.

Table 1: Screening of antimicrobial producing *Streptomyces sp.*

Isolated Streptomyces sp.	**Pathogenic Bacteria**				
	Escherichia coli	***Enterococcus faecalis***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Bacillus subtilis***
ISB1	+	+	+	+	+
ISB2	+	-	-	-	-
ISB3	+	+	-	-	+
ISB4	+	-	-	-	+
ISB5	-	-	-	-	+
ISB6	-	-	-	-	+

'+' Present antibacterial activity; '-' Absent antibacterial activity

Fig. 1. Antibacterial activity of *Streptomyces sp.* (ISB1) producing antibacterial substance

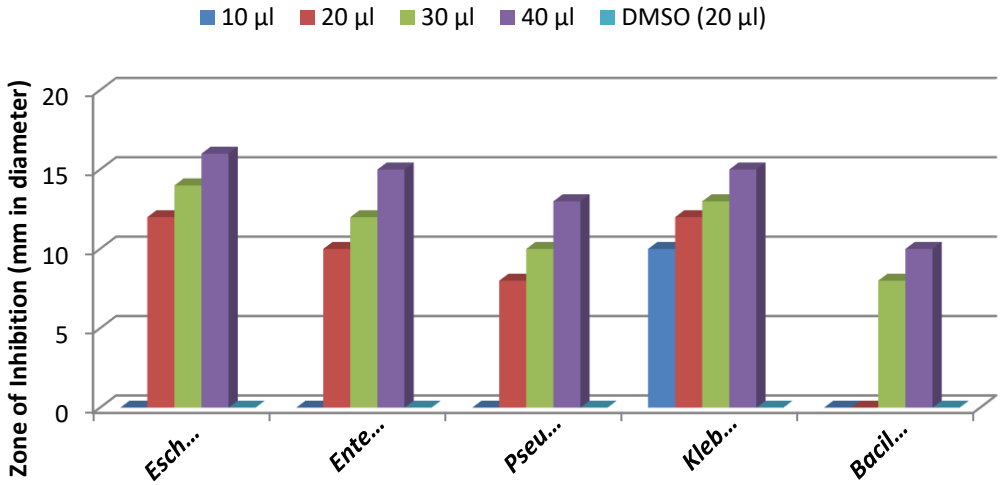


Fig. 2.SEM observation of AgNPs Synthesized using *Streptomyces sp.* (ISB1)

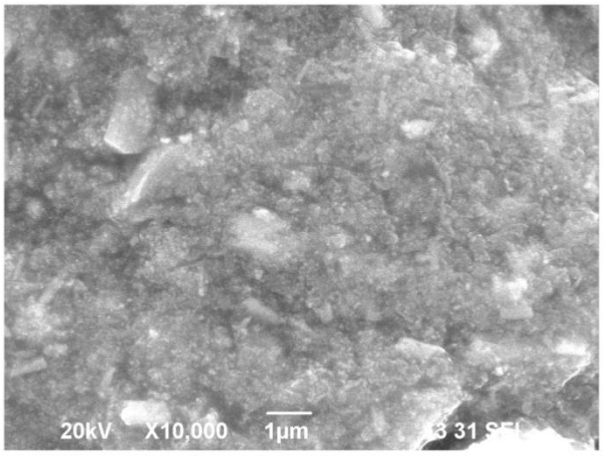


Fig. 3. TEM observation of AgNPs Synthesized using *Streptomyces sp.* (ISB1)

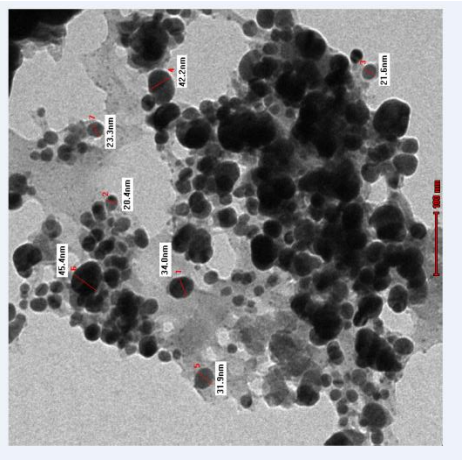


Fig. 4. EDAX analysis of AgNPs Synthesized by cell free supernatant of *Streptomyces sp.* (ISB1)

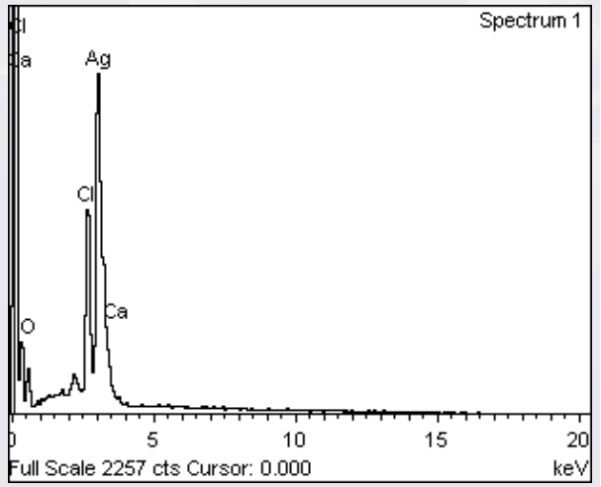
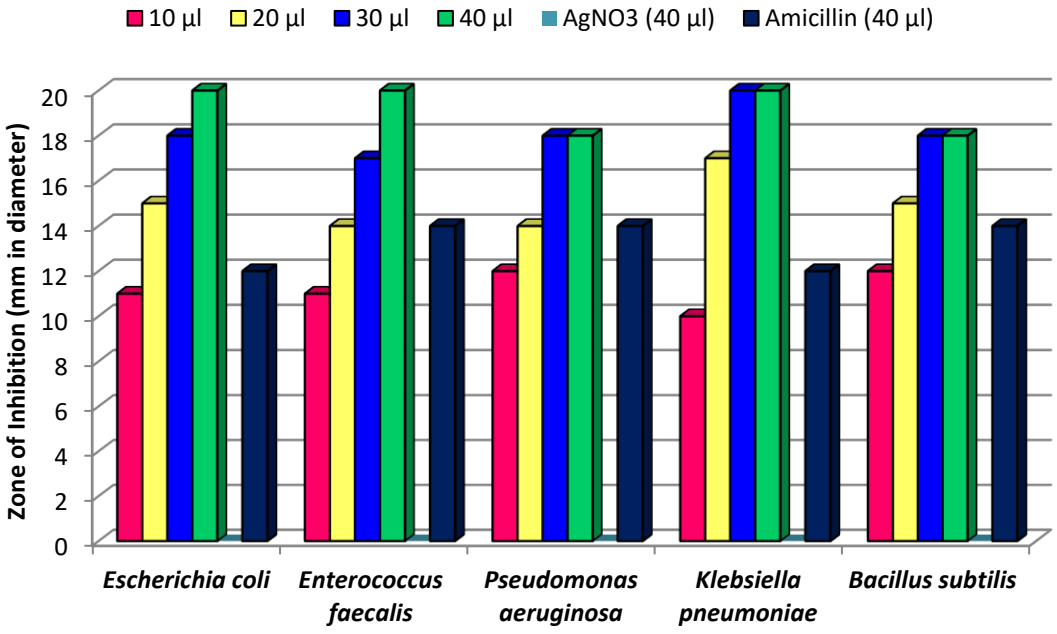


Fig. 5. Mean zone of inhibition (mm) of AgNPs synthesized using *Streptomyces sp.* (ISB1) against bacterial pathogens (well diameter was 6 mm)



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PRODUCTION OF ALKALINE PROTEASE BY *BACILLUS LICHENIFORMIS*

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ABSTRACTS

Screening of water and soil samples collected from Adirampattinam Lake, to know alkaline proteases producing bacteria, resulted 15 alkaline proteases producing alkaliphilic strains were isolated. AD-SK5 showed the highest enzyme production after 48 hours. This isolate was gram positive and was able to grow in the presence of NaCl up to 15%. Growth was observed at 25ºC, 37ºC, 45ºC and 55ºC but no growth was seen at 60ºC. It could grow at pH value from 8 to 11. No growth was detected at pH 7 after 48 hours incubation at 55ºC, which indicated this strain to be moderate halophilic thermophilic alkaliphiles. The crude alkaline protease showed reasonable activity at temperature range of 65 and 75°C with maximum activity at 70ºC and had a relatively wide pH range of activity between pH 8 to 11, with maximum enzyme activity at pH 10 in 50 mM Tris -HCl buffer maximum activity at 70°C and pH 10.0, indicating the enzyme to be thermo- alkaline proteases.

INTRODUCTION

Proteolytic enzymes are degradative enzymes, which catalyze the cleavage of peptide bonds in other proteins. Currently, proteases are classified on the basis of three major criteria, type of reaction catalyzed, chemical nature of the catalytic site and evolutionary relationship with reference structure. Alkaline proteases are referring to proteolytic enzymes that work optimally in alkaline pH (Barett, 1994 and Gupta *et al*., 2002). The vast diversity of proteases, in contrast to the specificity of their action, has attracted worldwide attention in attempts to exploit their physiological and biotechnological applications, (Fox *et al*., 1991 and Gupta *et al*., 2002) food and feed industry, peptide synthesis, leather industry, management of industrial household waste, photographic industry, medical usage, silk gumming and detergents industry.

Alkaliphiles are defined as organisms that grow optimally at alkaline pH, with pH optima for growth being in excess of pH 8 and some being capable of growing at pH > 11 (Grant and Jones, 2000; Horikoshi, 1999a). Although they were once considered to be curiosities, awareness of alkaliphiles has blossomed in recent years due to an interest in their physiological adaptation to high pH and their potential uses in biotechnological applications. Lakes and deserts represent the major types of naturally occurring highly alkaline environments, in which the indigenous microflora is subjected to number of extreme ecological pressures. They represent the most stable high pH environments on Earth, where large amounts of carbonate minerals can generate pH values >11.5. Lakes are widely distributed throughout the world; however as a result of their inaccessibility, few of such lakes have been explored from the microbiological point of view (Grant and Jones, 2000). One of those environmental niches, which have not been studied in details, is the Adirampattinam Lake in Tamil Nadu. The aims of this work were isolation of aerobic alkaliphilic bacteria from some lakes and screening for and alkaline protease producing alkaliphilic bacteria, characterization and identification of the interested strains and preliminary investigation of the alkaline protease.

MATERIAL AND METHODS

Soil and water samples were taken from Adirampattinam Lake in Tamil Nadu. The bottom of the area is 3 m below sea level and 8 m below the water levels. Soil and water samples

were collected from different locations of lakes. Samples were kept in sterile tubes in refrigerator at 4°C and were transferred to the laboratory within few hours

Isolation of Alkaline Protease Producing Bacteria

Isolation of alkaline protease producing alkaliphilic bacteria was carried out using rich alkaline agar medium containing skimmed milk (Ventosa *et al*., 1982). Aliquots (100 µl) of different dilutions of soil suspensions and water samples were plated and incubated at 37°C, 50°C and 60°C for three days. Formation of halo zone around the colonies, resulting from casein hydrolysis was taken as evidence of proteolytic activity. These colonies were isolated and streaked in fresh plates until single uniform colonies were obtained. The medium contained (g/l): Skimmed milk 100, yeast extracts 10, Na_2CO_3 15, agar 20. Skimmed milk, Na_2CO_3 and the other constituents were autoclaved separately (at 121 °C for 20 min) and mixed after cooling.

Production of Alkaline Proteases

A loopful of culture from agar plate was inoculated into 50ml glass tube containing 5 ml of alkaline protease production medium, and incubated overnight at 180rpm and 50°C. This culture was then inoculated into 500 ml capacity Erlenmeyer flask containing 95 ml of the same medium and incubated at 50°C for 48hours. Cells and insoluble materials were removed by centrifugation at 10,000 g for 10 min at 4°C and the cell free supernatant was filtered through a 0.45 µm pore-size membrane filter and was used as the source of crude alkaline protease enzyme. The production medium contained (g/l): Glucose 10, Peptone 5, yeast extract 5, K_2HPO_4 1, $MgSO_4$ 0.2, Na_2CO_3 15, pH 10.5. Na_2CO_3, glucose and the other constituents were autoclaved separately (at 121 °C for 20 min) and mixed after cooling.

Alkaline Protease Assay

Proteolytic activity was assayed by a modified method of Kunitz (1947). Samples containing 400 µl of 0.5 % (w/v) casein in 50 mM Tris –HCl buffer, pH 10, with 100 µl enzyme sample were incubated in a water bath at 50°C for 20 min. The enzyme reaction was terminated by addition of 500 µl of 10% (w/v) trichloroacetic acid and was kept at room temperature for 10min. The reaction mixture was centrifuged at 10,000 g for 10min at 4°C and the absorbance was measured against a blank (non-incubated sample) at 280nm.

Effect of Temperature on the Activity of the Crude Alkaline Protease

The crude alkaline protease was prepared as described above. The influence of temperature on the catalytic activity of the crude alkaline protease was determined by measuring the enzyme activity at temperatures range from 25°C to 90°C under the standard assay conditions.

Effect of pH on the Activity of the Crude Alkaline Protease

The influence of pH on the alkaline protease activity was determined by measuring the enzyme activity at varying pH values ranging from 5 to 12 at 70ºC using different suitable buffers, 50 mM sodium acetate (pH 5.0 and 6.0), 50 mM sodium phosphate buffer (pH 7.0 and 8.0), 50 mM Na_2HPO_4-NaOH buffer (pH 9.0, 10.0 and 11.0) and 50 mM KCl-NaOH (pH 12), respectively.

RESULTS AND DISCUSSIONS

Lakes are characterized by the presence of a high concentration of sodium carbonate formed by evaporative concentration, and are also associated with varying degree of salinity and low concentration of both Mg and Ca ions. (Grant and Tindall, 1986; Grant and Horikoshi, 1992;

Jones *et al*., 1998) reported and its alkaline inland saline lakes are an elongated depression about 90 km northwest of Cairo. Its average length is about 60 km and average width about 10 km. The bottom of the Wadi Natrun area is below sea-level and below the water-level of Rosetta branch of the Nile. (Imhoff *et al*., 1979 and Taher, 1999) studied the isolation of alkaline protease producing alkaliphilic bacteria was carried out using rich alkaline agar medium containing skimmed milk. Formation of clear zone around colonies was considered as indication of alkaline protease production. 15 isolates, showing large clear zone around their colonies, were isolated. It has been established that there is not necessarily good correlation between zones of clearing around colonies on milk-agar plates and levels of proteinase activity. Therefore, all the positive isolates were cultivated in the alkaline production medium and the proteolytic activity was measured. The results shown in Table 1 indicated that some isolates with considerable proteolytic activity could be isolated, AD-SK5, AD-SK6 and AD-SK7. The strain AD-SK5 showing the highest alkaline protease activity was selected for further characterization.

Cell-free supernatant was prepared as described in material and methods section and was filtered through a 0.45-µm pore-size membrane filter and used as the source of crude alkaline protease. For determination of the optimum temperature of the crude alkaline protease, the enzyme activity was measured at different temperatures at pH 10. The results illustrated graphically in Figure 1 indicated that the crude alkaline protease showed reasonable activity at temperature range of 65 to 75°C with maximum activity at 70ºC. A rapid decrease of enzyme activity was detected above 80ºC and the enzyme was completely inactivated at 90ºC.

The effect of different pH values of the reaction mixture on the activity of the crude alkaline protease was investigated in pH range from 5 to 11 at 70ºC. It was found that the crude alkaline protease had a relatively wide pH range of activity between pH 8 to 11, with maximum enzyme activity at pH 10 in 50 mM Tris -HCl buffer (Fig. 2). These preliminary properties of the enzyme are considered to be interested in comparison to other alkaline proteases (Gupta *et al*., 2002 and Raja *et al*., 2006). The results of this work indicated that the Adirampattinam lakes, in Tamil Nadu, are a rich source of many alkaliphilic bacteria which could be a good source of many interested enzymes from the industrial point of view and further studies are recommended on there lakes including study of microbial biodiversity and the biotechnological potent of the isolated strains.

Table 1: Production of alkaline protease by different isolated strains

S. No	Strains	Alkaline protease activity (U/ml)	
		24 hours	48 hours
1	AD-SK1	15.15	19.18
2	AD-SK2	5.51	10.12
3	AD-SK3	5.11	0.91
4	AD-SK4	22.15	29.11
5	AD-SK5	50.16	61.00
6	AD-SK6	41.12	31.51
7	AD-SK7	38.91	39.15
8	AD-SK8	0.90	3.50
9	AD-SK9	11.11	19.50
10	AD-SK10	8.88	16.11
11	AD-SK11	19.11	3.05

12	AD-SK12	6.55	19.12
13	AD-SK13	31.15	19.12
14	AD-SK14	6.16	11.15
15	AD-SK15	19.11	15.12

AD for Adirampattinam, SK for skimmed milk.

Fig. 1: Effect of temperature on alkaline protease activity from *B. halodurans* SK5

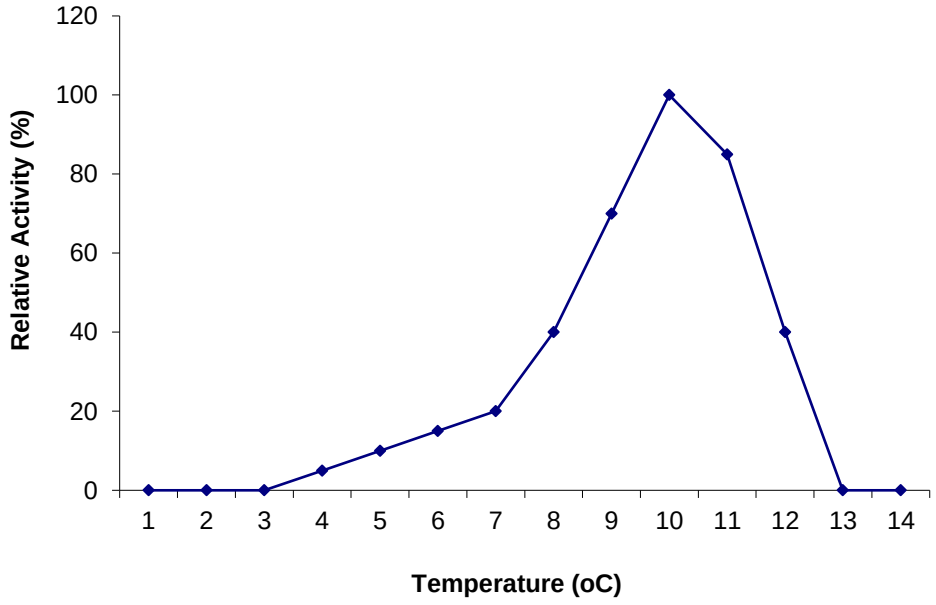
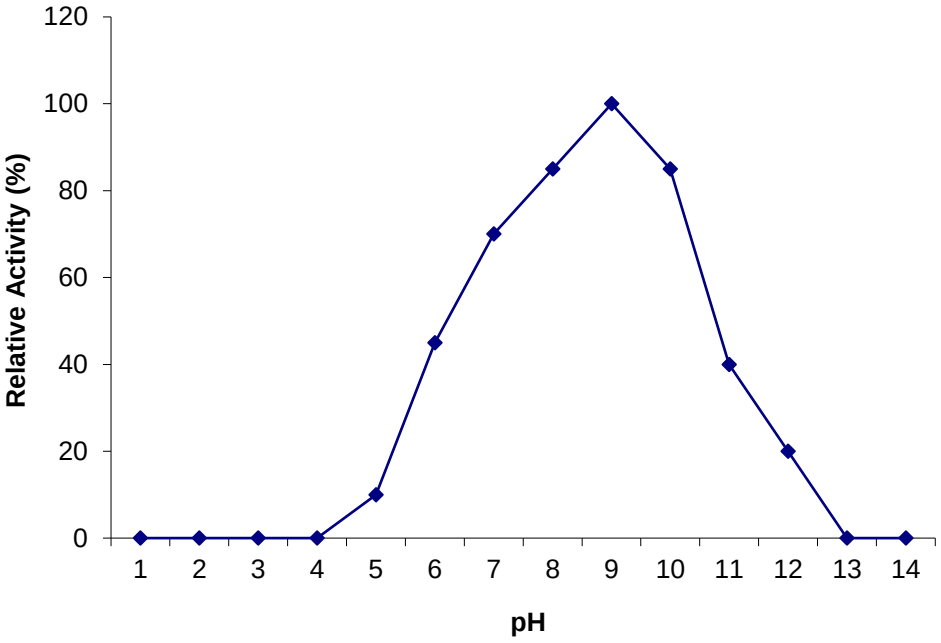


Fig. 2: Effect of pH on alkaline protease activity from *B. halodurans* SK5



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ICHTHYOFAUNAL DIVERSITY AND SPECIES RICHNESS OF LOWER ANICUT RESERVOIR, TAMIL NADU, INDIA: RECOMMENDATIONS AND CONSERVATION ACTION

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ABSTRACT

Faunal biodiversity is an essential for stabilization of an ecosystem, protection of environmental quality for understanding intrinsic worth of all species on earth. Ichthyodiversity focused to variety of species depending upon circumstance and scale it could refer alleles or genotypes within species of life. The branch of Cauvery, specifically Kollidam (Coleroon) river, Lower Anicut was selected as sampling point to the present work. Species identification was followed by colour patterns of body and fins, measurements and counts were clearly observed. The results of high-resolution image were used to extrapolate and assess clear morphological identification. Species richness was categorized as the availableof quantity of individual species. Following, the objectives and importance of this study to required and updated the information about diversity of fishes and its conservation through survey of sampling, fisherfolk, fish researchers, government personnel and experienced persons in relation to fisheries sectors. The assessment and documentation of the Ichthyodiversity and species richness in Lower Anicut reservoir, Tamil Nadu thereby to evaluate the clear conservation status of species, taking into reflection in riverine health and making the people more aware about local environment and its conservation for their existence.

Key words: Ichthyo diversity, Species richness, Taxonomy, Species identification, Classification and Conservation action.

INTRODUCTION

Freshwater ecosystems encompass distinctive properties both underpin and challenges for many key tenets of conservation biogeography. Freshwater fishes to exemplify this phenomenon, good health, high diversity and endemism stem were largely from the fact that the freshwaters are embedded within a terrestrial landscape that limits to dispersal within and among riverine basins. It broadly defines as the variety and variability among living organisms and ecological complexes in which they occur. These factors underlie an interesting observation such as regional to global scales, the most fishes were occupied only a fraction of localities where they might it and otherwise to thrive (Darlington, 1948). In addition, the constrained riverine habitat is at least partially responsible for the fantastic diversity of freshwater fishes around the world (Berra, 2001). Specifically one of author Balasundaram *et al*. (1999) were strongly quoted that the river has a varied population of important indigenous species of fish which form the basis for a fairly rich fishery. Likewise, the faunal diversity is an essential for stabilization of ecosystem, protection of overall environmental quality, for understanding intrinsic worth of all species on the earth. Ichthyo diversity mainly focused variety of species richness depending upon circumstance and scale it could refer alleles or genotypes within population of species or fish community of life across the aquatic system (Burton *et al.,* 1992). It is a vital tool to develop our knowledge and

understanding the biodiversity often the first footstep to embark on effectual conservation action. In addition, the information is also a fundamental assess to changes in species composition and distribution (Abraham *et al.*, 2011). The species diversity of an ecosystem is habitually related to the amount of living and non-living organic matter present in it. On the other hand, obvious species diversity depends upon the characteristics of a single ecosystem than on the interactions between ecosystems, *i.e.* transport of living animals across the different gradient region. Further, the effect of such transport is an important 'information' exchange to enhancing the diversity richness. In view of, the genetic imprint for various populations of lentic fish species is essential since the freshwater bionetwork constitute crucial parts of their life-support systems to providing nursery grounds and feeding areas (Hammer *et al.*, 1993). Additionally, species diversity is a property at population level while; the functional diversity concept is more strongly related to ecosystem stability, stress, physical and chemical factors to determining population dynamics in lentic ecosystem. Besides, the various organisms including the types of planktons play a momentous role in the dynamics of ecosystem (Kar and Barbhuiya, 2004).So far, around 750 species of freshwater fish taxa have been recorded in India whereas; out of 350 taxa have been estimated as an endemic species (Jayaram *et al.*, 1981; Sarkar and Kapoor, 1998; Basheer *et al.*, 1998). Similarly, the river of Cauvery is one of the major bionetwork systems and also is a foremost habitat of the richest freshwater fish fauna in Tamil Nadu, India. As well, one of the main tributary namely Kollidam (Coleroon) river, Lower Anicut is the prominent freshwater fishery resource and it play an important role in energy flow, cycling of nutrients and maintaining bio-community balance in an ecosystem. Further, the fast flowing river has been an excellent habitat and environment enabling to evolution of richest fish diversity. Hitherto,this river is believed to be an important feeding and spawning ground and conserve a variety of species which support to the commercial fisheries in throughout year. Thus, fishes occupy a remarkable position from socio-economical point of view for fisherfolk. As a result, the river was also supports livelihood and nutritional security for the above said people living alongside (Mathialagan *et al.*, 2014). Some, recent studies on biodiversity loss and its implications for ecosystem services have uncovered and unprecedented species extinctions at global and regional levels (Baille *et al.*, 2004). Though, the freshwater diversity has declined faster than either terrestrial or marine over past three decades (Jenkins, 2003). During the last century, riverine ecosystems have suffered from intense human intervention as a resulting in loss of habitat, degradation and as a consequence of many species have become highly endangered, particularly in rivers where is heavy demand is placed on freshwater bionetwork. Likewise, freshwater fish are one of the most threatened taxonomic groups (Darwall and Vie, 2005) because it their high sensitivity both quantitative and qualitative alteration of aquatic habits (Sarkar *et al.*, 2008; Kang *et al.*, 2009). At the present time, fish diversity and management is linked to habitats, in addition conservational view of aquatic diversity has gained great ecological magnitude over recent years (Dudgeon *et al.*, 2006). As well, habitat isolation and dispersal limitation that have been generated high in freshwater fish diversity and also can increase the risk of species extinction. Freshwaters are subjected to panoply of anthropogenic threats, including habitat loss and fall to pieces, hydrologic modification, climate flexibility, over-exploitation, pollution and dispersal of invasive species (Dudgeon *et al.*, 2006). According to Hossain *et al.* (2012) reported that most of the wild populations have seriously declined in rivers and streams due to exploitation of sources and it augmented by various biological changes and alarming conditions of natural habitats. The important causes behind the loss of biodiversity in freshwater were dreadful nature and breakup of habitats, water abstraction, industries and private use, introduction of exotic species, pollution and global climate change impacts. For harnessing the aquatic resources, a scientific understanding of fish species with respect to their morphological, biological and adaptive characters along with their natural distribution is imperative to back up their optimum

exploitation. In this context, it is aimed to assessment to fill in this gap the documentation of fish diversity and species richness in Lower Anicut reservoir, Tamil Nadu thereby to evaluating the conservation status of species, taking into reflection in riverine health and making the people more aware about local environment and its conservation for their existence. Moreover, to comparedthe Ichthyofaunal diversification with that of neighboring rivers for zoogeographical point of view. As a result the physical, chemical and biological characteristics of this reservoir were gradually changing and producing the harmful effect on aquatic biota and thereby also harming by human beings. Hence, there is an important necessity for documentary records of Ichthyofaunal diversity particularly in the riverine ecosystem.

MATERIALS AND METHODS

Sampling site

A branch of Cauvery, specifically Kollidam (Coleroon) river, Lower Anicut were selected the present study (Figure 1) which is located 11º 08' 03'' N latitude and 79º 27' 05" E longitude. Kollidam River flows from west to east forming with northern boundary of this block whereas Cauvery River at the central part of this blocks flanking at Kumbakonam (Tamil Nadu). The total catchment area of this reservoir is 29,693 square miles (sqm^2) and capacity to store 150.13 MCFT of water to use agricultural irrigation and fishing activities (Pazhanisamy and Ebanesar, 2008). This river concerning 500 peoples are actively engaged in fishing activities the whole time year. They are operating cast net (7x7 mm), gill net (8x8 mm and 10x10 mm), hand net, cage traps, rod and line and using some pots for catching fish through catamaran, thermacole raft teppam and four wheeler rubber tubes (floating device) are also used as a craft (Mathialagan *et al.,* 2014). The river is the main landing centre for fish fauna and varieties of fishes, where the Tamil Nadu State Fisheries Department has the sole authority for landing and marketing of fishes throughout the year.

Photographical image analysis

Ichthyological survey was carried out during from October 2013 to September 2014. After that, collected fishes were labeled along with vernacular names with assistance local skilled fishermen and brought to the laboratory. Colour patterns of the body and fins were clearly observed for morphological identification. Identification of large fishes was done in the field itself with the help of standard taxonomic field guide. Variety of fish samples in order to obtain more precise image of the each individual using digital camera (SAMSUNG-PL 20 with 6x level; Lens focal length: 4.9-24.5; mega pixel 16.2). Photographs were taken perpendicular to the subject were only used when the types of fins were fully expanded nature. Additionally, the results of high-resolution image were used to extrapolate by morphological identification (Mathialagan *et al.,* 2014). Subsequently, fish samples were fixed and preserved in 5% formalin based on their size in separate jars. Smaller ones are placed in directly while; the larger ones were preserved after giving a small incision on the abdomen before it was fixed in formalin.

Species Identification

Identification of specimens was done by standard reference materials (Jayaram *et al.,* 1982; Talwar and Jhingran 1991; Jayaram, 1999; Jayaram, 2010). Availability of fish species were determined onthe basis of their abundance during sampling through interviewing of fishermen with formerly prepared questionnaire.According toMosaddequr Rahman *et al.* (2012) reported that the species availability was categorized as TY (Throughout Year), TYLQ (Throughout Year in Little Quantity), TYBQ (Throughout Year in Bulk Quantity), SQM (Small Quantity during Monsoon), BQM (Bulk Quantity during Monsoon), R (rare), ER (Extremely Rare) and NE (Not Evaluated). As a result, the range of Standard Length (SL) attained by a

species (Table 1) and also number of species attaining the SL was estimated approximately during this study. The physico-chemical parameters were analysed using methods as described in APHA (1998). Furthermore, the required data and information about diversity of fishes were collected through survey of the fishers, fish farmers, fish researchers, government personnel and experienced persons in related to fisheries sectors and available literatures.

Species indicator test and Justification

Principles of identifying indicator species varied based on nature of the study and management objectives (Landres *et al.*, 1988). General requirements of this present work comprise the following rank in order to importance of (a) specialization to habitat (b) well-known and firmness taxonomy (c) simply observable (d) well-known biology and natural history (e) pattern of response reflected in other taxa (f) wide geographical range (g) and economical importance of species (Noss, 1990). The specialization of the habitat was awful important for identifying the species indicator system. Further, species that are specialized and it become more exceedingly rare with difficult to sampling exclude them as of serving as viable indicator to concerned species taxa (Landres *et al.*, 1988). In the same way, species that are exceedingly common and do not provide the statistical necessities to test indicator relationships since good quality of habitats where they are absent are rare. The limitation of indicator tests to species that encompass the total diversity and number of sampling sites through this survey. So far, the majority of species was feasible to provide the quality of habitat specialization exceedingly rare or common (Wesner and Belk, 2012). The potential indicator species for the reason that rare and threatened type of species can act as functional surrogates, provided they are not exceedingly specialized. Besides, the factors causing their turn down are also the major reason in focus during recovery and restoration, are likely to contribute to differential distributions of co-occurring species (Wiens *et al.*, 2008). The sampling area occurs in heavy livestock ranching, which can indirectly modify river fish distribution through alteration of the riverine habitat, increased siltation, channelization, loss of bank constancy, impaired water quality and increased the temperatures through removal of shading vegetation. Such habitat modification is commonly associated with reduced distribution of stream fishes (Allan and Flecker, 1993).

Statistical analysis

Statistical dataset analyses were consisted nearly four sampling sites together water and fishes. Descriptive statistical analyses were examined against seventy nine species between various families. Bi-variate correlation (Pearson Correlation) and multiple logistic or linear regression analysis were performed by SPSS, ver. 22.0 at $P<0.001\%$ level of the significant. Identification of habitat variable was associated with diversity of potential indicator species and common co-occurring native species. Following, the test value of using the species so as good indicators of fish diversity in the Kollidam River, Lower Anicut. The present work was mainly examined the residual species richness (D), Shannon–Wiener diversity (H′) and species evenness (J′) for each sampling site. Residual richness, diversity and evenness were defined as values derived for a given fish assemblage with potential species indicator (Matthews, 1998; Marsh-Matthews and Matthews, 2000). As a result, the *t*-test was measure up to residual richness, species diversity and evenness between sites with and without potential indicator species. For each variable (richness, diversity and evenness), were assessed by Levene's test and adjusted to degrees of freedom was used to test of significant (Quinn and Keough, 2002). Finally, the percentage composition analyses against the number of families, genera, species and number of individuals.

RESULTS

A total of 79 species under 11 orders and 21 families were recorded from Kollidam River, Lower Anicut during the study period (Table 1 and 2). Based on the species were analysed by percentage of number of individuals in each order and family. Following, family wise analysis of percentage composition the Cypriniformes was the most dominant order constituting about 23.8% of the total fish population followed by the Siluriformes 19.0%,Perciformes 14.3%, Clupeiformes 9.5%,Anguilliformes 4.8%, Beloniformes 4.8%, Osteoglossiformes4.8%, Gonorhynchiformes 4.8%, Mugiliformes 4.8%, Cyprinodontiformes 4.8% and Synbranchiformes 4.8% were the least numerous order and also it constituting only 13 individuals of the total order of fish population(Figure 2). On the other hand, genera wise percentage composition analysis were referred as the Cypriniformes was the most dominant order concerning about 55.5% number of genera were occurred. Subsequently, Siluriformes 11.1%, Perciformes 8.9%, Clupeiformes 6.7%, Osteoglossiformes 4.4%, Anguilliformes 2.2%, Beloniformes 2.2%, Synbranchiformes 2.2%, Gonorhynchiformes2.2%, Mugiliformes2.2% and Cyprinodontiformes 2.2% were recorded. Following, the results were mainly focused on firstly the descriptive statistical analysis to assess and compared to various sampling region of the fish species diversity in Table 3. Secondly, species richness were analysed and compared to sampling location was summarized in Table 4 and also were monitored fluctuate level of species richness was observed. Subsequently, the physico-chemical parameters of water samples were analysed and monitored in five sampling sites have been given in Table 5. Herein, all the parameters were compared within the types of sampling region.

As for, the status of biodiversity was concerning about the availability species were categorized as Throughout Year (TY) included 42 species were occurred in Least Concern (LC) category. However, the Throughout Year in Bulk Quantity (TYBQ) and Throughout Year in Little Quantity category (TYLQ) were included in 6 species were Vulnerable (VU) and 7 species was occur in Nearly Threatened category (NT). Whereas, the Bulk Quantity during Monsoon (BQM) and Small Quantity during Monsoon (SQM) were included in Data Deficient (DD) category were include in 3 species while the Lower Risk Near Threatened (LRNT) category were occurred in 9 species are also recorded. Likewise, the another group of Rare (R), Extremely Rare (ER) and Not Evaluated (NE) category were included in 7 species comprise not evaluated (NT) group and 3 species were include in Endangered (EN) category was recorded. On the other hand, genera wise percentage composition analysis were referred as the Cypriniformes was the most dominant order concerning about 55.5% number of genera were occurred. In order to, Siluriformes 11.1%, Perciformes 8.9%, Clupeiformes 6.7%, Osteoglossiformes 4.4%, Anguilliformes 2.2%, Beloniformes 2.2%, Synbranchiformes 2.2%,Gonorhynchiformes2.2%, Mugiliformes2.2% and Cyprinodontiformes 2.2% were also recorded. Subsequently, the species and number of individual wise analysis of percentage composition the Cypriniformes was the most leading order to comprise about 63.3% and 37.9% of the total fish population. Following, the Siluriformes 11.4% and 12.0%,Perciformes 6.3% and 11.6%, Clupeiformes 3.8% and 8.6%,Anguilliformes 2.5% and 5.1%, Beloniformes 2.5% and 4.2%, Osteoglossiformes2.5% and 4.5%,Gonorhynchiformes 1.3% and 4.7%, Mugiliformes 1.3% and 3.9%, Cyprinodontiformes 1.3% and 3.7% and Synbranchiformes 1.3% and 3.7% were the least numerous species and also it constituting only 13 individuals of the total order of fish population(Figure 3 and 4).

DISCUSSION

The River Cauvery has a diverse fish fauna and also needful to high conservation importance for upcoming days. The present study were highlighted the cyprinids species were widely distributed among the sampling region and it has widespread distributed in India (Talwar and Jhingran 1991; Jayaram, 1999) and it is a common and abundant species in Indian

freshwaters. However, the cyprinids were dominating fish assemblage as they occupy all possible habitats due to their high adaptive variability nature (Arunachalam *et al.,* 2003). Improvement of Ichthyodiversity management and species richness for assess the biological resources at local or national level and it is important to consider this information in a continental or global context. As well, the taxonomy and richness of species is a valid one and it is important surrogate in the context of Ichthyodiversity assessment. The assembled database and estimate the number of fish species were present in a large number of riverine ecosystem is a wide one. On the other hand, the readily available estimates of species richness have a good result from these studies at various times and reflect different approaches to fish taxonomy, so the species dataset is also even and in quality. Yet, both of the low intensity of field survey and slow rate at which the target species were experienced systematic can dealt with the available specimensdataset is greatly to be improved in near future. As a result, the data are refined through more accurate counts of species or families and to use of discharge rather than catchment area that to fit will improve somewhat and more interesting individual patterns of species will be emerge (Julian *et al.*, 2010). However, it is clear that the variable quality of the existing data at global level family diversity is a good surrogate for species and general patterns of species structure in one are well reflected to other. Presently, declinethe richness of species were observed to the present work due to proximity to human intervention, shortage of food availability, excessive fishing and scarcity of water in this reservoir.Thus, the biological diversity appears to play a substantial role in ecosystem plasticity (Folke*et al.,* 2004). Following,the physical and limnological properties of the present ecosystem combined with the interaction among species are amongst factors were responsible for promoting the novel structure and composition of the Ichthyodiversity at specific ecosystem (Agostinho *et al.*,1994 and 2008).

Potential species richness and availability in this riverine environment were predominantly influenced by water temperature and temperature stability. It is evident that the present result, the factors regional surface region of the riverine environment is a most influential determinant for species richness followed by habitat availability potential of freshwater network. Both of them, it has a positive impact on the species richness and alteration in those two factors would be drastically modified in the riverine background. Water temperature and stability have extremely little effect on species richness, though they are statistically significant nature for the present work carried out. Moreover, the water temperature has positive effect on richness of species, whereas temperature stability has the negative outcome on wealth of the fish species in riverine ecosystem (Manas *et al.,* 2012). As a results, to develop the scenarios of losses in riverine species wealth with respect to 5 and 10 percentage point reduction in the scores of the identified influential reason *viz.,* surface area of riverine region and species availability potential habitat for that region. Besides, loss of species richness in these rivers due to the percentage point loss in regional surface area of riverine region to provided there is no change in the fish habitat availability potential of the river, water temperature and water temperature stability. As the relative contribution, the temperature to change in species wealth is very low influential to hydrological factors and also change due to temperature will be nullify by the change due to mentioned factors. On the other hand, the hydrological parameters remain unchanged while temperature was increases during that time. In this situation, the option to increase species richness may occur due to invasion of exotic species and possible for shift of warm water species towards colder stretch (Vass *et al.,* 2009; Singh *et al.,* 2010). Based on this observation, the Kollidam River is being diversely rich in fish species, it can possibly be explained by the fact that prior to the coming up of the large number of hydraulic structures to maintain the overall physical and biological structure and function of this riverine system. The transport of water sediments and nutrients were downstream nature and also inadequate. Following, the floodplains were

connected to providing essential lateral link to the Cyprinids for breeding and recruitment. This information provided to a great variety of ecological habitats, for harbouring rich Ichthyofaunal diversity. Though, in recent years the breakup of the riverine ecosystem by series of dams have converted to riverine sections from lotic to lentic systems and it disconnected the main channel from their flood plain wetlands (Vass *et al.,* 2009 and Singh *et al.,* 2003). As a result, the fish habitat and availability potential has been reduced. Herein, this kind of fragmentation and continuity of the river remains unaccounted in the model system. Therefore, the model reflects the predicted richness of species to be much below the observed number of species.

According to Shrestha (2008) quoted that no one definite plan has been made to attempt restoration of damaged riverine area likewise dam, development of reservoir and improvement of irrigation facilities. Presently, the negative impact over-siltation, dredging and spoil disposal have been encountered in several rivers. Subsequently, the pollution has largely affected in fishery, aquatic living organism, enforceable conservation and pollution laws were fully necessitate and implemented. After that, the active restoration and habitat enhancement programs were desirable one particularly for riverine fish stocks. Thus, problem affecting the general riverine ecosystem includes dam construction, designing of reservoir, alteration of hydrobiological regime in tail water, paper mill effluents, domestic and industrial pollutants, agricultural runoff, channelization, dredging and ensuing spoil disposal, taking away of stream bank cover, eutrophication, riverine flood plain encroachment, degradation of wetlands, changing natural hydro-periods and development of watershed urbanization. Furthermore, the methods of detecting and forecasting the ecological changes in fish communities should be developed and utilized. Further, analysis of results must be readily understood through life histories and habitat requirement of rare and endangered species to living in the rivers should be investigated and appropriate measures taken to preserve them. Following, the efforts to restore decimated population, such as some species of carps, eels and catfishes should be continued. One the other hand the commercial fisheries should be monitored to evaluate overfishing potential and it possible need for regulatory controls. Finally the efforts should be made to educate the fisherfolk about the importance of native fishes land values, both aesthetic and economic of river ecosystem (Julian *et al.,* 2010). Yet, one of the scientific recommendations for fishermen to strictly avoid harmful fishing gear such as electrical shocker and dynamites also seriously affected the fish population as a result of overfishing. Presently, all ichthyologist of the world are aware the insecure condition and existence of unique fishes in the riverine waters and also give to devote more attention to the study and conserve them for future. For this reason, some potential fishing area in these regions can be developed into eco-friendly angling sites such as West and East potential spot of this riverine ecosystem. However, the Endemic species may be largely affected due to change in water temperature and other ecological factors to the probable effect of changes the typical climate. Therefore, it is necessary action taking that the habitat-specific plans for such species should be formulated with long-term ecological study to the highlighted region.

Spatial patterns of species richness and endemic species have often been used to a suitable guide the conservation of freshwater fish biodiversity (Chu *et al.,* 2003) however; such approaches do not provide the elasticity needed for an adaptive and multipart conservation planning process. Conservation planning that incorporates complimentarily (a gain in biodiversity when a site is added to an existing set of protected areas) yields more efficient and cost-effective conservation than ad-hoc scoring or ranking strategies (Margules *et al.,* 2002). A significant conservation goal was to develop the energetic biodiversity conservation management policy that can be adapt to changing environmental circumstances while maintaining the natural biogeographic patterns in biota (McClanahan *et al.,* 2008). Herein, fishes have commonly been

used as surrogates for biodiversity in freshwater conservation planning, possibly for the reason that the distribution and ecological requirements of fishes are comparatively well understood relative to most other freshwater groups in a biota. Hitherto, the extent to which group of fishes are effective surrogates for other aquatic biota and also right targets for conservation planning have not been comprehensively evaluated (Rodrigues and Brooks, 2007). Presently, alternative extinction scenarios can be expressed as potential losses in 'feature ichthyodiversity' in regard to the evolutionary history of the sampling region and may hence guide conservation priorities (Faith and Baker, 2006). The major challenge here is to assess the level of spatial resemblance between biodiversity measures and subsequent implication for prioritizing a network of freshwater areas and conservation management. The reflection of connectivity and its importance to maintaining natural ecological processes and biodiversity in freshwaters is also a key to effective conservation planning for these systems (Hermoso *et al.,* 2010). Designing well-organized conservation networks in freshwaters is challenging because the spatial hierarchies of fluvial ecosystems and networks and necessity to consider longitudinal, lateral, surface and groundwater connections (Fausch *et al.,* 2002). Evaluation of directional connectivity requirements based on knowledge of species life history traits, including migration patterns, offers one promising approach (Moilanen *et al.,* 2008). The scientifically defensible and conservation targets (*e.g.,* the number of populations or areas were mandatory to maintain species) is challenging, because minimum population sizes or habitat requirements for most of the freshwater species are not known (Nel *et al.,* 2009). Therefore, the multiple occurrences and diverse catchments allow for different genetic lineages to be conserved (Nel *et al.,* 2009) though, conservation plans must go beyond simple spatial configurations to deal with human induced stressors. So far, the improved integration of riverine ecological principles (*e.g.,* biogeographically, successional pathways and source-sink population structures is needed for conservation of freshwater fishes in an ever-changing world (Sarkar *et al.,* 2006). Therefore, measures of conservational were referred including stopping the illegal fishing, dynamiting, poisoning, identifying crucial breeding habitats and creating mass awareness are need to save the threatened fish fauna in this region. Without these efforts many freshwater fish species would become extinct. Collecting the juveniles and maintaining aquariums are pivotal to raising awareness and actively conserving species through conservational programmes as informed by the information made available through the species assessments published on the Red List.

Recommendation And Conservational Measures:

At present, species diversification wasgoing away to vulnerable, endangered and extinction. To conserve these valued endemic species, it is most important to ensure the following measures:

1. Firstly, to regulation of mesh size to prevent the catching of brooders and juveniles during breeding and larval rearing stages of fish. Declarations of sanctuaries in these areas where these species live are endemic nature.
2. Artificial recruitment may be made to revive the species in those areas where these fishes are less available and catch is declining. Prevention of entry of industrial pollutants in that area where these fish population is inhabits.
3. Conservation of gametes through gene banking is a good way for adopting future strategies of replenishment and stock enhancement of these valued species. Public awareness is required to save these fishes from extinction.

CONCLUSION

During this survey it has been found that religious inhibitions stand in the way of utilizing the rivers, canals and sluice all are stocked the juveniles to be neglected. Besides, the indiscriminate use of pesticides has risen in many eyebrows and also pesticides get washed into the streams and rivers. Herein, the residual effects are well known and thus can prove harmful to

the fish faunas. On the other hand, enormous growth in the industries which discharged effluents without proper treatment into the river streams resulting in destruction of fishes. One of the recent survey of Yamuna and its tributaries has shown that its dumping polluted materials have led to the disappearance of fishes. Essentially those periodic surveys should be undertaken the species are not lost to the scientific world. Public prejudice against fishing as a profession and against fish as an item in the menu stands in the way of proper exploitation of fisheries.

Table – 1. Status of Ichthyodiversity in Kollidam (Coleroon) River, Lower Anicut, Tamil Nadu

Order	Family	Species Name	VernacularName (Tamil)	SL(mm) Range	IUCN Status
Anguilliformes	Anguillidae	*Anguilla bengalensis* (Gray 1831)	Aaara	279-338	NT
		Anguilla bicolar (McClelland 1844)	Vilaangu	721-1230	NT
Beloniformes	Hemiramphidae	*Hyporhamphus xanthopterus* (Valaenciennes 1847)	Kokkurali, Kokku Meenu	118-150	VU
		Hyporhamphus limbatus (Valenciennes 1847)	Kokkurali	231-350	LC
Clupeiformes	Clupeidae	*Tenualosa ilisha* (Hamilton 1822)	Oolam, Karuva ulam, Sevva, llam	467-600	LC
	Pristigasteridae	*Ilisha novacula* (Valenciennes 1847)	Naattu matti	287-320	LC
	Engraulidae	*Thryssa dussumieri* (Valenciennes 1848)	Keela, Semporuva	83-110	NE
Synbranchiformes	Mastacembelidae	*Mastacembelus armatus* (Lacepede 1800)	Aara, Kal aara, Vilaangu meenu, Chettu aara	740-900	LC
		Macrognathus guntheri (Day 1865)	Vilaangu meenu, Aara	119-299	LC
Osteoglossiformes	Notopteridae	*Notopterus notopterus* (Pallas 1769)	Chenna valai, Ambattan valai, Ambatta kathi, Chotta valai, Chappatti kendai,	453-600	LC
		Chitala chitala (Hamilton 1822)	Ambattan walah	970-1220	NT
Perciformes	Ambassidae	*Ambassis dussumieri* (Cuvier 1828)	Kannadi thattai, Thattai podi	65-100	LC
		Chanda nama (Hamilton 1822)	Velicha podi, Kakkache	74-110	LC
	Cichlidae	*Etroplus maculatus* (Bloch 1795)	Setha kendai, Bommi, Puradi, Salli kasu, Chellai, Boorakasu	63-80	LC
		Etroplus suratensis (Bloch 1790)	Sella kasu, Setta kendai Puradi, Salladai podi	257-400	LC
		Oreochromis mossambicus (Peters 1852)	Jelebi kendai, Tilapi	234-380	NT
Gonorhynchiformes	Chanidae	*Chanos chanos* (Forsskal 1775)	Thullu kendai, Pal kendai, Paalai meenu	1123-1800	NE

Mugiliformes	Mugilidae	*Mugil cephalus* (Linnaeus 1758)	Madavai, Manalei, Kasi meenu	567-1000	LC
Cypriniformes	Balitoridae	*Balitora brucei* (Gray 1830)	Kallu kendai, Kallu Podi	86-105	NT
		Balitora mysorensis(Hora)	Kallu koravai	43-55	VU
		Ghatsa pillaii (Indra & Rema Devi 1981)	Kevi kendai	53-75	LC
	Cyprinidae	*Gibelion catla* (Hamilton 1822)	Kanavi, Kora kendai, Thoppa kendai, Yamaneri kendai,	1513-1882	LC
		Cyprinus carpio (Linnaeus 1758)	Sinai kendai, CC kendai	974-1200	VU
		Garra mullya (Sykes 1839)	Kallu koravai, Kallu kendai	123-170	LC
		Garra gotyla stenorhynchus (Jerdon 1849)	Kallotti meenu, Paarapadungi	130-155	LC
		Garra lissorhyncus (McClelland 1842)	Kallotti podi	62-94	LC
		Nemacheilus monilis(Hora 1921)	Sunnambu podi	47-48	LC
		Ctenopharyngodon idellus (Valaenciennes 1844)	Pullu kendai	965-1510	NE
		Salmophasia acinaces (Valaenciennes 1844)	Vellachikendai	94-153	LC
		Salmophasia bacaila (Hamilton 1822)	Salli podi	76-110	VU
		Salmophasia horai (Silas 1951)	Salli kendai	81-103	VU
		Barilius canarensis(Jerdon 1849)	Chinna kanan, Mattalai	133-157	EN
		Laubuka laubuca (Hamilton 1822)	Chinna parai, Mullu Parai	43-71	LC
		Devario devario(Hamilton 1822)	Pulli kendai	62-104	LC
		Devario aequipinnatus (McClelland 1839)	Selai paravai, Vannathipodi	67-143	LC
		Rasboracaverii(Jerdon 1849)	Kavuri kendai, Salli	82-100	LC
		Rasboradaniconius (Hamilton 1822)	Oovery kendai, Patta kanju, Purrovoo	87-150	LC
		Hypophthalmichthys nobilis (Richardson 1845)	Peruthalai kendai, Thoppai kendai	450-1460	DD
		Cirrhinus mrigala (Hamilton 1822)	Mirigal kendai	864-998	LC
		Cirrhinus reba (Hamilton 1822)	Uruttam podi, Aranjan kendai	69-239	LC
		Cirrhinus cirrhosus (Bloch 1795)	Venkendai, Pudukendai	640-1020	VU
		Cirrhinus fulungee(Sykes 1839)	Uruttu kendai	278-310	LRNT
		Puntius cauveriensis(Hora 1937)	Saani podi, Salli podi	54-74	EN

		Puntius amphibeus (Valaenciennes 1842)	Kulla kendai	89-200	DD
		Puntius sarana (Hamilton 1822)	Pungella, Uruttu podi, Saani kendai	322-420	LC
		Puntius conchonius (Hamilton 1822)	Mullu selli, Chenna kunni	71-140	LC
		Puntius ticto(Hamilton 1822)	Vennatii, Pulli kendai	59-100	LC
		Puntius sophore (Hamilton 1822)	Kulla kendai, Kurunchelli,	65-134	LRNT
		Puntius filamentosus (Valaenciennes 1844)	Pungella kendai, Poovali Kendai	120-180	LC
		Hypselobarbus dubius (Day 1867)	Kozhimeen, Kozharinjan kendai	540-610	EN
		Labeo rohita(Hamilton 1822)	Kannadi kendai	159-256	LRNT
		Labeo calbasu (Hamilton 1822)	Karupusel, Kakameenu, Selkendai,	342-900	LRNT
		Labeo bata (Hamilton 1822)	Kolarinja kendai	310-620	LC
		Labeo ariza (Hamilton 1807)	Kullarinjan	210-300	LC
		Labeo kontius (Jerdon 1849)	Curumuli kendai	590-620	NE
		Labeo fimbriatus (Bloch 1795)	Sel kendai, Gundumani sel kendai	340-910	NE
		Hypothalmichthys molitrix (Valaenciennes 1844)	Velli kendai	745-1055	NT
		Amblypharyngodon microlepis (Bleeker 1853)	Oori kendai	84-100	LRNT
		Amblypharyngodon mola (Hamilton 1822)	Oori kendai	171-203	LRNT
		Esomus thermoicos (Valaenciennes 1842)	Meesai paravai, Paravai meenu	97-127	LC
		Esomus barbatus(Jerdon 1849)	Meesai paravai	90-120	LC
	Anabantidae	*Anabas testudineus*(Bloch 1792)	Panaiyeri Kendai	83-121	DD
	Channidae	*Channa marulius* (Hamilton 1822)	Puveral, Iru viral, Coaree veralavuree	89-131	LRNT
		Channa punctata(Bloch 1793)	Paara koravai	230-310	LC
		Channa striata(Bloch 1793)	Karuppu viral, Viral	720-1000	LRNT
	Gobiidae	*Glossogobius giuris* (Hamilton 1822)	Ulluvai,Kal uluvai, Nullatan	420-500	LC
Cyprinodontiformes	Aplocheilidae	*Aplocheiluslineatus* (Valenciennes 1846)	Manankanni, Vaanampartha meenu	91-110	NE

		Aplocheiluspanchax (Hamilton 1822)	Killi meenu	82-97	NE
Siluriformes	Bagridae	*Mystus armatus*(Day 1865)	Katta keluthi	89-145	LC
		Mystus gulio(Hamilton 1822)	Uppang keluthi	78-112	LC
		Mystus cavasius (Hamilton 1822)	Nai keluthi, Vellakeluthi	84-131	LC
		Mystus vittatus(Bloch 1794)	Vella keluthi,	67-118	LC
		Mystus bleekeri (Day 1877)	Naattu Keluthi	90-141	LC
	Schilbeidae	*Neotropius atherinoides* (Bloch 1794)	Mandai keluthi, Chellei meenu	53-69	LC
	Siluridae	*Ompok bimaculatus*(Bloch 1794)	Savallai, Chota vaalai	74-89	NT
		Wallago attu (Bloch & Schneider 1801)	Vazhai meenu	700-750	LRNT
	Clariidae	*Clarias batrachus* (Linnaeus 1758)	Thalmeen, Thol keluthi	139-161	LC

Table – 2 Updated ichthyodiversity count some freshwater fishes in Kollidam River, Lower Anicut.

Sl. No.	Order	Families	Genera	Species	Number of individuals
1	Anguilliformes	1	1	2	15
2	Beloniformes	1	1	2	18
3	Clupeiformes	3	3	3	31
4	Synbranchiformes	1	1	2	13
5	Osteoglossiformes	1	2	2	14
6	Perciformes	2	4	5	43
7	Gonorhynchiformes	1	1	1	14
8	Mugiliformes	1	1	1	17
9	Cypriniformes	5	25	50	136
10	Cyprinodontiformes	1	1	2	16
11	Siluriformes	4	5	9	42
	Total	21	45	79	359

Table – 3 Descriptive statistics for species diversity in Kollidam River, Lower Anicut.

Sampling sites	'N' species	Mean SD	SE of Mean	'N' individuals	Mean	SD	SE of Mean
LC – 1	33	0.46±0.019	0.008	142	0.43	0.023	0.013
LC - 2	21	0.29±0.015	0.006	105	0.33	0.021	0.011
LC - 3	17	0.24±0.013	0.005	78	0.24	0.018	0.009
LC – 4	08	0.19±0.011	0.004	34	0.09	0.013	0.007
Total	79	0.99±0.047	0.019	359	1.00	0.010	0.021

Landing Centre 1-Vadavar River, Landing Centre 2 - Lower Anicut Town, Landing Centre 3 – UpperAnicut Town, Landing Centre – 4 catching centre near Thenkatchi, N – Number, SD –

Table – 4 Summary statistics for tests of species diversity indicators in Kollidam River, Lower Anicut

SS	NS	N	Richness (D)			Shannon-Wiener diversity index (H′)			Species evenness (J′)		
			SE	'r'	*P*	SE	'r'	*P*	SE	'r'	*P*
LC - 1	33	142	4.34	0.963**	0.087	2.74	0.912**	0.138	1.81	0.803**	0.089
LC - 2	21	105	3.63	0.942**	0.053	1.56	0.876**	0.093	0.78	0.787**	0.031
LC - 3	19	78	2.75	0.909**	0.034	0.87	0.811**	0.021	0.45	0.651**	<0.009
LC - 4	8	34	0.45	0.883**	0.006	0.58	0.781**	<0.006	0.03	0.614**	<0.003

SS – Sampling Sites; NS – Number of Species; N – Number of Individuals; SE – Standard Error; r – Correlation Co-efficient; r^2 – Regression Co-efficient; **Correlation is significant at the P<0.001; *P* – Probability.

Table - 5 Physico-chemical parameter analyses of water samples in Kollidam River, Lower Anicut.

Parameter	Range	Mean	SD	Parameter analysis (%)	
				5%	10%
Water temperature (°C)	21.7-32.0	26.8	1.79	0.18	0.36
Water transparency (cm)	39.5-80.5	59.7	3.59	0.08	0.16
P^H	6.2-8.7	7.45	0.76	0.67	1.34
EC(mS/cm)	0.16-0.29	0.23	0.018	21.73	43.4
Dissolved Oxygen (mg/L)	4.7-8.8	6.51	0.59	0.76	1.53
BOD(mg/L)	7.3-9.6	8.45	0.73	0.59	1.18
COD(mg/L)	4.2-7.2	5.71	0.52	0.87	1.75
Total Hardness (mg/L)	23.5-130.5	77.3	5.37	0.06	0.12
Total Alkalinity (mg/L)	32.0-85.3	58.6	4.13	0.08	0.16
Total Dissolved Solids (mg/L)	7.5-11.9	9.73	0.81	0.51	1.02
DOM(mg/L)	2.25-7.80	5.03	0.33	0.99	1.98
Free Carbon dioxide (mg/L)	2.3-8.7	5.52	0.38	0.90	1.80
Calcium (mg/L)	7.5-23.5	15.5	1.57	0.32	0.64
Magnesium (mg/L)	6.3-16.5	11.4	0.94	0.43	0.96
Phosphate (mg/L)	0.09-0.16	0.13	0.006	38.4	76.9
Chloride (mg/L)	1.9-2.6	2.25	0.14	2.22	4.43
Ammoniac Nitrogen (mg/L)	0.02-0.06	0.04	0.0006	12.3	23.6
Nitrate – NO_3(mg/L)	0.04-0.26	0.15	0.005	33.1	66.0

P^H – Power of Hydrogen concentration; SD – Standard Deviation;EC- Electrical Conductivity; BOD -Biochemical Oxygen Demand; COD - Chemical Oxygen Demand; DOM -Dissolved Organic Matter.

Figure 1- Map showing the geographical location of sampling area in Lower Anicut, Tamil Nadu.

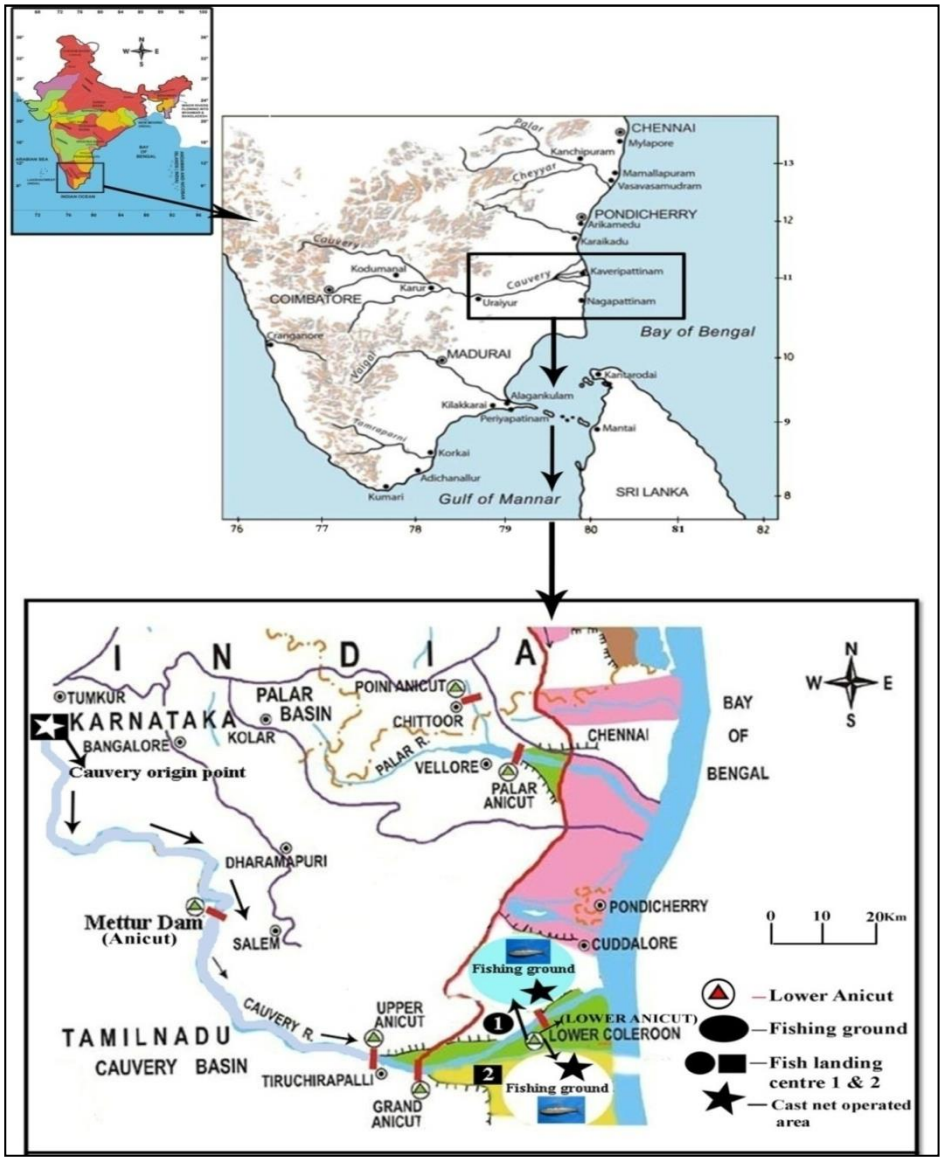


Figure 2 – Percentage value of family diversity in Kollidam River, Lower Anicut

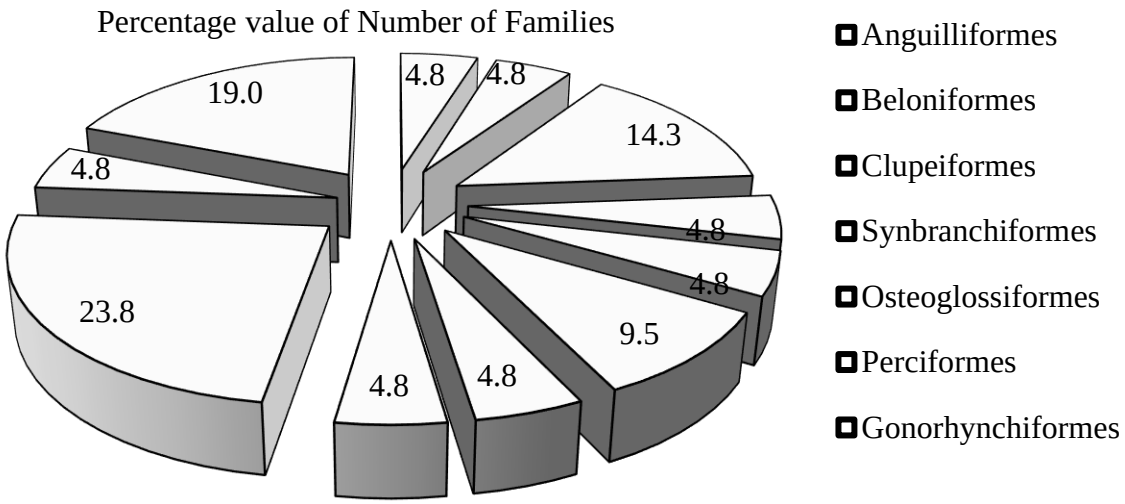


Figure 3 – Percentage value of genera diversity in Kollidam River, Lower Anicut

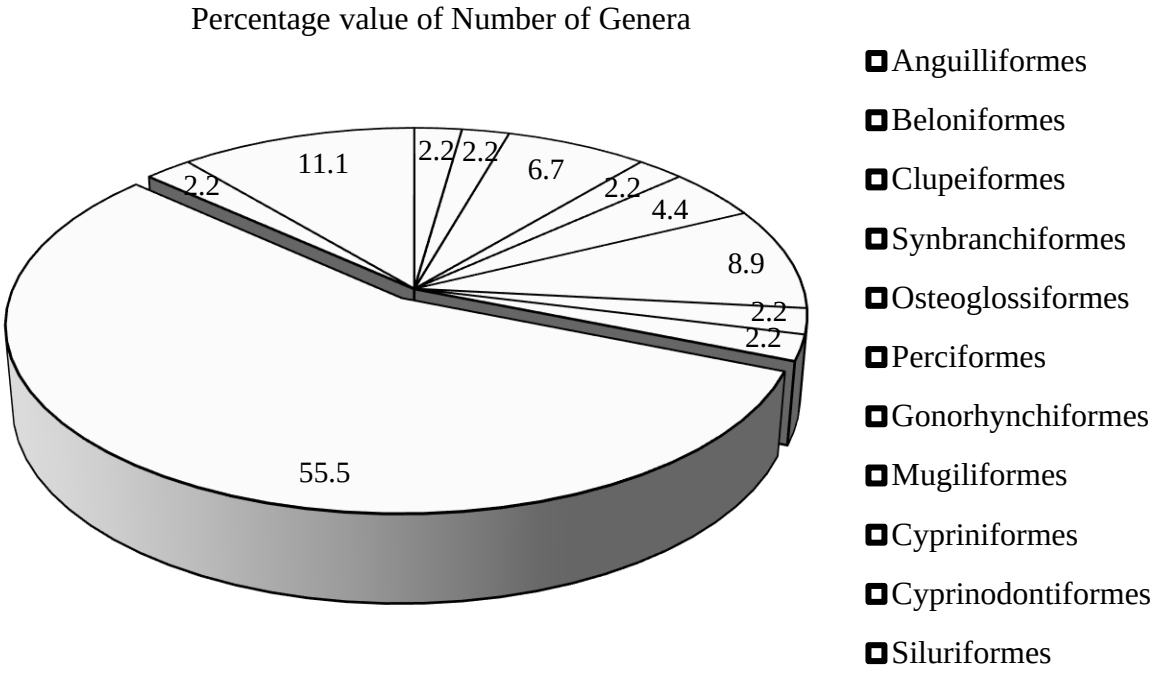


Figure 4 – Percentage value of species diversity in Kollidam River, Lower Anicut

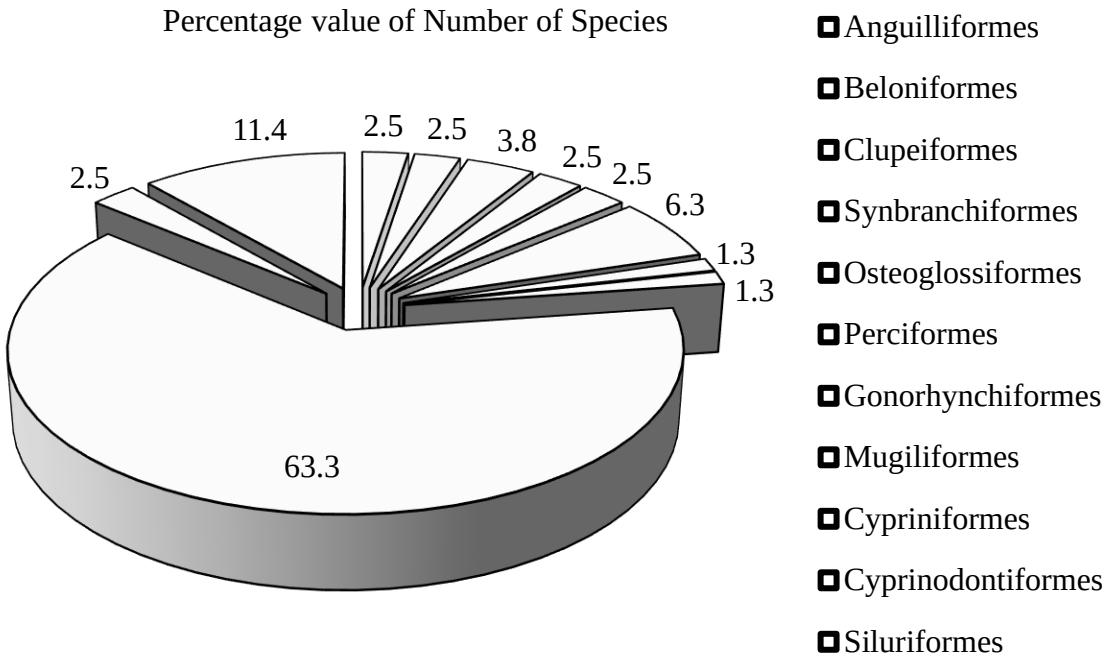


Figure 5 – Percentage value of individual diversity in Kollidam River, Lower Anicut

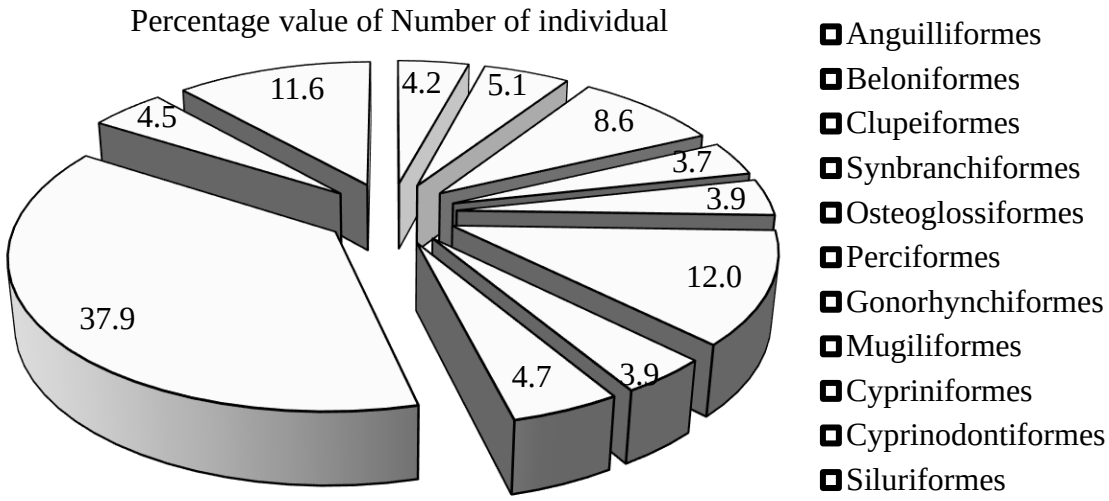
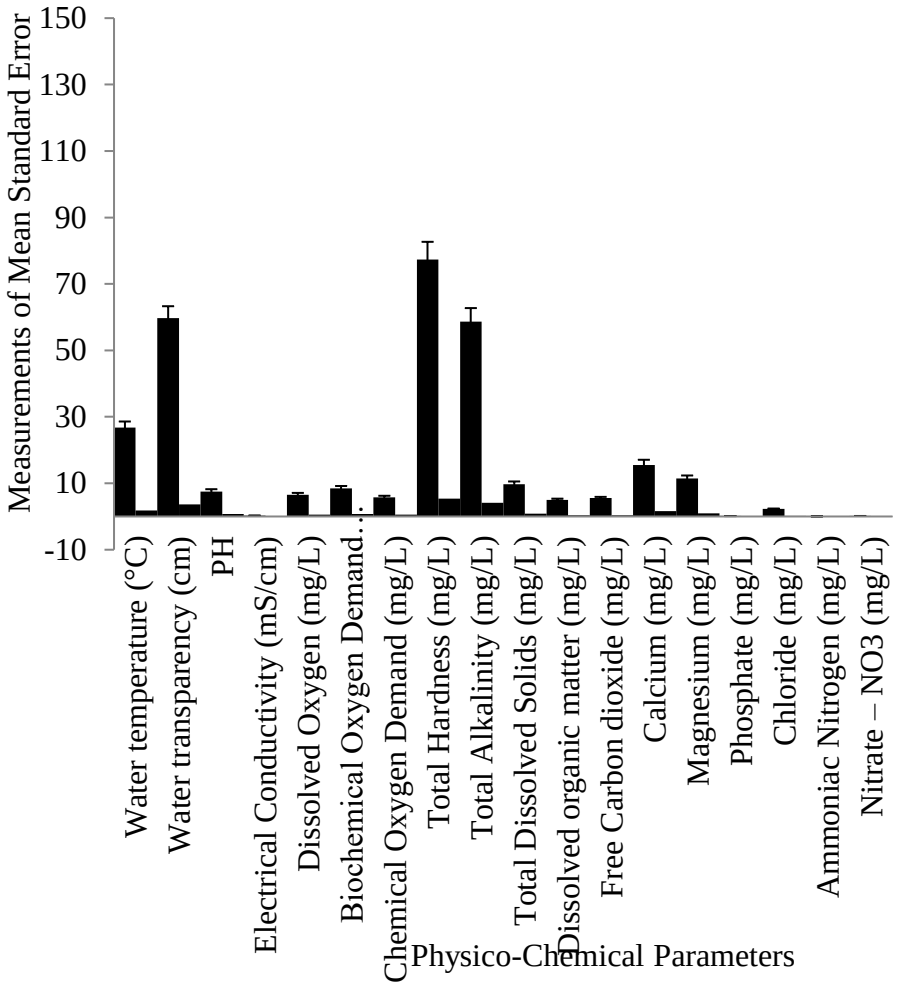


Figure 6 - Physico-chemical parameters analysis in Kollidam River, Lower Anicut



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IN-SILICO AND *IN-VITRO*PHOSPHOLIPASE A2 INHIBITION POTENTIAL OF PHYTOCONSTITUENTS PRESENT IN*ADIATUM LUNULATUM*

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ABSTRACT

PLA2 plays an important role in inflammation and is thought to be a promising target for anti-inflammatory drugs. Although the search for novel PLA2 inhibitors has slowed over the previous decade and substantial progress has been made in the sector in recent years. The purpose of this research is to determine the PLA_2 inhibition potential of *A.lunulatum* leaves using in-silico and in-vitro investigations. The leaves' ethanol extract was prepared and exposed to preliminary phytochemical examination. The extract was tested at 10, 50, 100, 200, 400, 600, 800 and 1000 µg/ml for PLA_2 inhibition and the percentage inhibition was calculated. The phytoconstituents found in the leaves were docked against the 1POE protein to determine their potential for binding to the PLA_2. The presence of flavonoids, phenols, carbohydrates, and alkaloids was discovered throughout the phytochemical screening process. The inhibition assay revealed a dose-dependent inhibition of 76.45 percent at 1000 µg/ml, with an IC_{50} of 248.7 µg/ml. Gallic acid was utilized as a control and shown an inhibitory potential of 83.17 percent at 1000 µg/ml with an IC_{50} of 174.5 µg/ml. Docking studies indicate that the phytoconstituentsrutin, isoquercetin, and 16-Hentriacontanone bind to the protein's calcium-based hydrophobic loop, comparable to the inhibitor present in the protein. These findings clearly imply that phytoconstituents are effective PLA_2 inhibitors and should be investigated further in preclinical and clinical research.

Keywords: anti-inflammatory; PhospholipaseA_2; docking; *A.lunulatum*; rutin; inhibition

INTRODUCTION

Inflammation is a complicated process that involves several cell types and serves as a body-protective reaction to injury, infections, cell damage, and irritants. When inflammation becomes uncontrolled and causes harm to healthy tissues, this is referred to as an inflammatory condition. PLA_2 is a robust tertiary structure enzyme with roughly eight disulfide links that protect it against heat denaturation and proteolysis [1]. There are around 25 human PLA_2 isoforms that have been classified into three primary classes: calcium-dependent and -independent intracellular enzymes, calcium-dependent secreted PLA_2, and calcium-independent secreted PLA_2 [2].

Because of its role in the inflammatory process, the $sPLA_2$ IIA enzyme has received the most attention among the PLA_2 isoforms [3]. $sPLA_2$ IIA, an inflammatory type, is found in a range of tissues and cells and is strongly up regulated in response to inflammatory stimuli in vitro and in vivo [4,5]. A high amount of $sPLA_2$ IIA isoform is discovered in several inflammatory illnesses and is believed to be responsible for inflammatory responses [6]. Injection of pure $sPLA_2$ IIA from synovial and pleural fluids into animal joints verified the formation of an initial inflammatory response with edoema, synovial cell enlargement, and hyperplacia [7].

Phospholipase A2 enzymes cleave fatty acids in the sn-2 position of glycerol phospholipids, releasing free fatty acid and lysophospholipid. When the fatty acid released from

the sn-2 position is arachidonic acid, the reaction is very important. Arachidonic acid is oxidatively converted by cyclooxygenase and lipooxygenase enzymes to prostaglandins, thromboxanes, prostacyclins, and leukotrienes, which are inflammatory mediators [8]. Both processes produce a variety of free radical intermediates as well as hydroxyl and superoxide radicals [9]. When the lysophospholipid product has a choline head group and an alkyl linkage in the sn-1 position, it functions as a precursor for proinflammatory platelet activating factor (PAF) [10]. Continuous synthesis of proinflammatory lipid mediators, in addition to free radicals, amplifies chronic inflammatory illness by many orders of magnitude. As a result, PLA_2catalyses the rate-limiting stages in the eicosanoid pathway and is recognised as a critical enzyme in a variety of inflammatory illnesses [11].

Recent research have revealed numerous natural substances that demonstrate inhibitory effect against PLA_2[12, 13]. However, therapy with a single anti-inflammatory medicine typically produces various side effects such as hepatoxicity, gastrointestinal bleeding, meningitis and asthma [14]. Therefore, a unique technique combining two or more possible chemicals that have inhibitory effects against PLA_2activity has been proposed to solve the problem [15].

Various anti-inflammatory drugs are used as PLA_2 inhibitors that also possess serious adverse effects. But natural phytoconstituents produce very less adverse events when compared to the synthetic drugs. Hence this undertaken to identify the PLA_2 inhibitory potential of *A.lunulatum* leaves through *in-silico* and *in-vitro* investigations.

Highlights

- *In-silico* molecular docking, standard precision methods were used to determine the binding affinity towards the PLA_2 protein which is a recent trending investigation of molecules.
- The ethanol extract showed a good inhibitory potential activity in the inhibition assay with gallic acid as a standard.
- The phytoconstituents were found to bind with the calcium based hydrophobic loop of the protein as cited in the literatures.
- The leaves of *A.lunulatum* have shown a good PLA_2 inhibition both *in-silico* and *in-vitro*.

METHODS AND MATERIALS

Collection and Preparation of extract

The Leaves of *A.lunulatum* was collected, washed under running water and then shade dried. The leaves were powdered and extracted using ethanol. The extract was then kept in incubator shaker for 48 hours, filtered using whattman filter paper (No.1) and evaporated to remove the excess solvent. The extract were store in an airtight container and kept for further use.

Preliminary Phytochemical screening

The following screening test were performed to identify the presence/ absence of phytochemical in the ethanol extract of A.lunualtum leaves [16-19].

Test for Flavonoids: A few drops of sodium hydroxide solution were added to the extract. The presence of flavonoids was detected by the formation of a bright yellow colour that became colourless with the addition of dilute acid.

Test for Phenols: 4 drops of ferric chloride solution were added to the extract. The presence of phenols was revealed by the formation of a bluish black colour.

Test for Carbohydrates: Extract was treated in a test tube with 2 drops of alcoholic –alpha naphthol solution, shaken, and conc. sulphuric acid was applied from the side of the test tube. The formation of a violet ring at the interface of two liquids proved the existence of carbs.

Test for sterols: Few ml of extract was dissolved in 10 ml of chloroform & equal volume of concentrated H 2SO 4 acid was added from the side of test tube, The upper layer turns red and H2SO 4 layer showed yellow with green fluorescence which indicates the presence of steroid.

Test for alkaloids: The filtrate was treated with a solution of potassium mercuric iodide (Mayer's reagent). The presence of alkaloids was suggested by the formation of a white yellow or cream-colored precipitate.

Test for tannins: Few ml of extract was treated with 4 ml FeCl3, formation of green colour indicates presence of tannin.

Test for proteins: A few drops of Millon's reagents was added to the extract. When warmed, a white precipitate forms that either becomes brick red or disappears which shows the presence of proteins

Test for saponins: The extract was diluted to 20 mL with distilled water and agitated in a graduated test tube for 15 minutes. The presence of saponins was shown by the formation of foam.

Test for terpenoids: 2 ml of chloroform was added to extract solution and conc. Sulphuric acid (3 ml) was added to form a layer. A reddish brown colouration of the interface indicated the presence of terpenoids.

Molecular Docking

Ligand Preparation: Rutin, isoquercetin, B-sitosterol,16-Hentriacontanone, Fern-9(11)-Ene-25-Oic Acid, FilicenolB, Fern-9(11)-en-6alpha-ol, Fernene, Filic-3-Ene, isoadiantone, Fern-9(11)-en-28-ol, Adiantone, 6-oxoFern-9(11)-ene, 22,29-Epoxy-30-Norhopane-13beta-Ol were utilised. The ligands were then prepared for docking using the Ligprep tool in the suite. The OPLS4 field force was chosen because it has been demonstrated to be more accurate than the previous field for protein-ligand docking [20]. The technique was developed so that each ligand returned with a pose of ten. Following ligand processing, the final ligands for docking were chosen based on state penalty ratings. The lower the state penalty score, the more likely it is that the ligands will be stable.

Protein Preparation: The free and inhibited Human Secretory Phospholipase (PLA_2) from inflammatory exudates (1POE) was obtained from the RSCB website and processed for protein preparation [21]. PRIME was first used to add all hydrogen, zero-order bonds, disuphide groups, missing atoms, and chains. The protein was then modified using the PROPKA option while the sample water orientation remained unchanged. Finally, the protein was minimized and integrated into the workspace of the suite [22].

Receptor Grid generation: The docking grid was created in the active site of the protein. The protein already contains a PLA2 inhibitor linked to its active site. This pocket was picked, and a docking grid was created.

Docking: The previously built receptor grid was selected, and the prepared ligands were selected from the workspace. The scaling factor and partial cut off charge were changed to soften the ligands in their non-polar regions. The standard precision technique was used, and the docking position was limited to a 0.10 tolerance. After that, the docking method was started, and the findings were saved with one posture per ligand-protein interaction.

Inhibition of PLA2 activity: Pla2 inhibitory activity was determined using the technique published by De Aranjo and Radvany [23]. The substrate was made up of lecithin (3.5 mM), a combination of NaTDC (3 mM), NaCl (100 mM), CaCl2 (10 mM), and red phenol (0.055 mM) as a colorimetric indicator, as well as 100 mL H2O. The pH of the reaction mixture was adjusted to 7.6, and 0.2 µg of pG-IB was solubilized in 10% acetonitrile at a concentration of 0.002 µg/µL. At room temperature, a volume of 2 µL PLA_2solution was incubated with a volume of 2 µL sample for 20 minutes. The solution was then mixed with 200 µL of PLA_2substrate. Kinetic hydrolysis was performed for 5 minutes, and the optical density was measured at 558 nm. PLA_2inhibitory activity was expressed in percentage of inhibition and was calculated as follows.

Enzyme activity (EA) = Optical Density (0 min) – Optical Density (15 min)/ 15 min

% inhibition = $EA_{\text{-ve control}} - EA_{\text{Sample}} / EA_{\text{-ve control}} \times 100$

RESULTS AND DISCUSSION

Table 1- Preliminary Phytochemical Screening of *A.lunulatum* leaves

S.No	Phytoconstituent chemicals	Ethanol
1	Flavonoids	+
2	Phenols	+
3	Carbohydrates	+
4	Sterols	-
5	Alkaloids	+
6	Tannins	-
7	Proteins	-
8	Saponins	-
9	Triterpenoids	-

The ethanolic extract of *A.lunulatum* leaves showed presence of Flavonodis, phenols, carbohydrates and alkaloids. No were no traces of steroids, tannins, proteins, saponins and triterpenoids. The presence of saponins has been reported in the water extract of the whole plant. Also, the presence of protein, sterols and triterpenes has been reported in the n-hexane extract of the whole plant [24]. The leaves of the A.lunuatum was reported to be used as anti-inflammatory that corresponds to the action of phytoconstituents presents in the ethanol extract of leaves. The *A.lunulatum* leaves is reported to contain flavonoids such as isoquercetin, rutin and other chlorophyll degradation products and higher carotenoids [25]. The plant also contains sugars, fat and phenols.

PLA2 inhibition activity:

The extract was subject to inhibitory PLA_2 activity and the results show a dose dependent inhibitory activity against PLA_2 (Figure 1)with 76.45% at 1000 µg/ml. Gallic acid was used as a positive control to support the method. The IC_{50} of the ethanol extract was found to be 248.7 µg/ml (Figure 2) and that of gallic acid was 174.5 µg/ml (Figure 3).

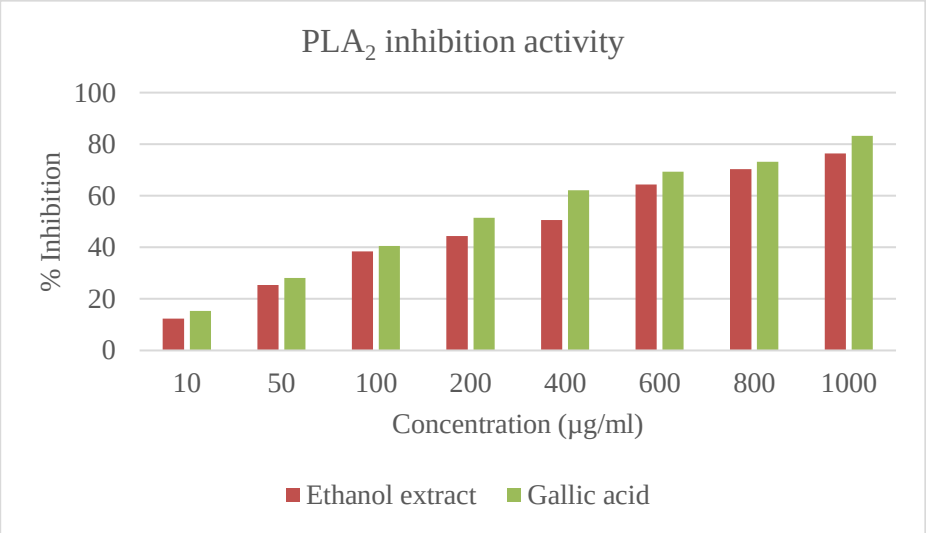

Figure 1- PLA_2 inhibition activity of leaves of *A.lunulatum*

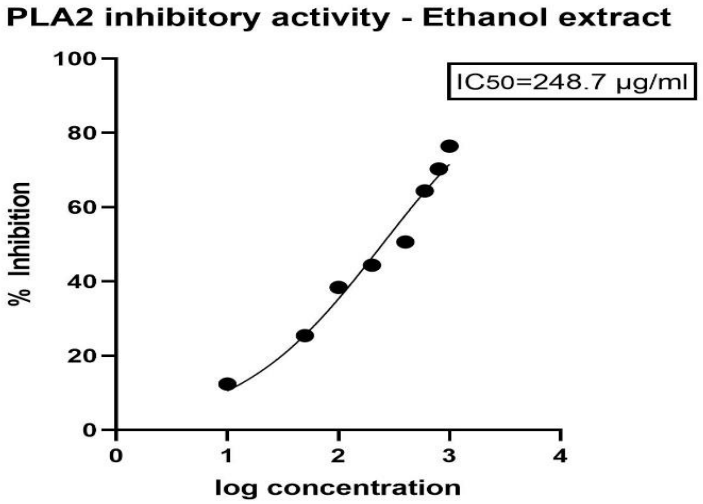

Figure 2- IC_{50} of Ethanol extract

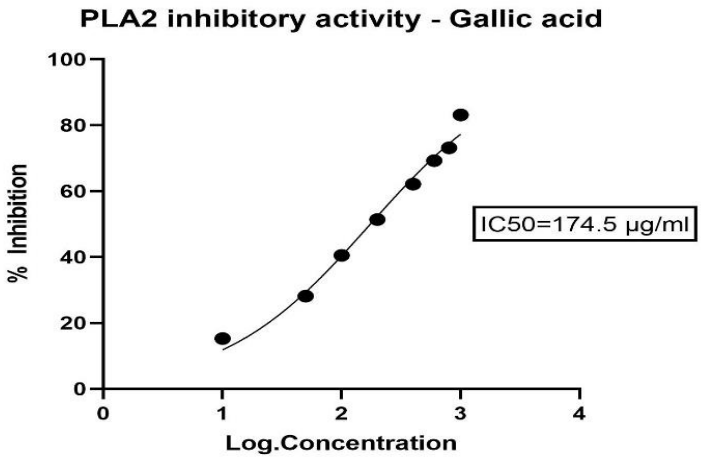

Figure 3- IC_{50} of Gallic acid

Docking

The docking investigation was done by finding the interaction of the ligand with the binding site of the protein. The docking score and data are tabulated. (Table 2). It was revealed that the protein already has a PLA_2 inhibitor at the active site which is calcium bound and showed inhibition at the hydrophobic channel by substrate binding. So, the molecule existing in the protein was treated as a reference for the ligand docking. The eight aminoterminal residues of uninhibited hnpsPLA_2 remain helical in the presence of calcium and at neutral pH, but are shifted toward the principal calcium ion. The amino-terminal helix shift, together with a minor displacement of the section of the calcium-binding loop that forms the opposing wall of the hydrophobic channel (residues 29–31), narrows the channel's entrance.

Table 2- Docking scores of ligands with 1POE binding site

S.No	Ligands	Docking score	Glide score	Glide e-model score
1	rutin	-6.634	-6.663	-61.043
2	isoquercetin	-4.646	-4.674	-58.463
3	B-sitosterol	-4.468	-4.468	-32.630
4	16-Hentriacontanone	-4.421	-4.421	-50.875

5	Ferna-9(11)-Ene-25-Oic Acid	-4.264	-4.268	-30.076
6	FilicenolB	-4.256	-4.256	-26.262
7	Fern-9(11)-en-6alpha-ol	-4.189	-4.189	-22.373
8	Fernene	-4.122	-4.122	-23.067
9	Filic-3-Ene	-3.924	-3.924	-26.167
10	isoadiantone	-3.793	-3.793	-34.613
11	Fern-9(11)-en-28-ol	-3.640	-3.640	-27.327
12	Adiantone	-3.628	-3.628	-23.394
13	6-oxoFern-9(11)-ene	-3.527	-3.527	-24.818
14	22,29-Epoxy-30-Norhopane-13beta-Ol	-3.480	-3.480	-29.583

PLA*2* activity needs calcium ions, and two calcium ions are present in each of the three crystallographically different representations of hnps-PLA_2. One calcium ion is connected to the archetypical "principal site" produced by the carboxylate oxygens of Asp and the carbonyl oxygens of residues 29, 30, and 32 [26, 27]. The secondary calcium ion in present at a distance from the calcium loop. Surprisingly, in the absence of an inhibitor, the hnps-PLA_2 binding site for the primary calcium ion lacks the two coordinated water molecules found in other crystal structures. In this docking investigation the molecule rutin was discovered to interact with the the Calcium ion, GLY29 and VAL30 identical to that of the inhibitor molecule in the protein. Similarly the isoquercetin was discovered to interact with HSP47 and ASP48 together with the Calcium ion. The molecule 16-Hentriacontanone was interacting with GLY29. Rest of the ligands did not show any interaction with the active site of the protein. The residues GLY29, VAL30 are found in the hydrophobic calcium loop therefore explaining the PLA_2 action. These interaction of ligands with the calcium loop of PLA_2 imply that the ligands blocks the PLA_2 enzyme from hydrolysis of the sn-2 position of membrane glycerophospholipids to liberate arachidonic acid.

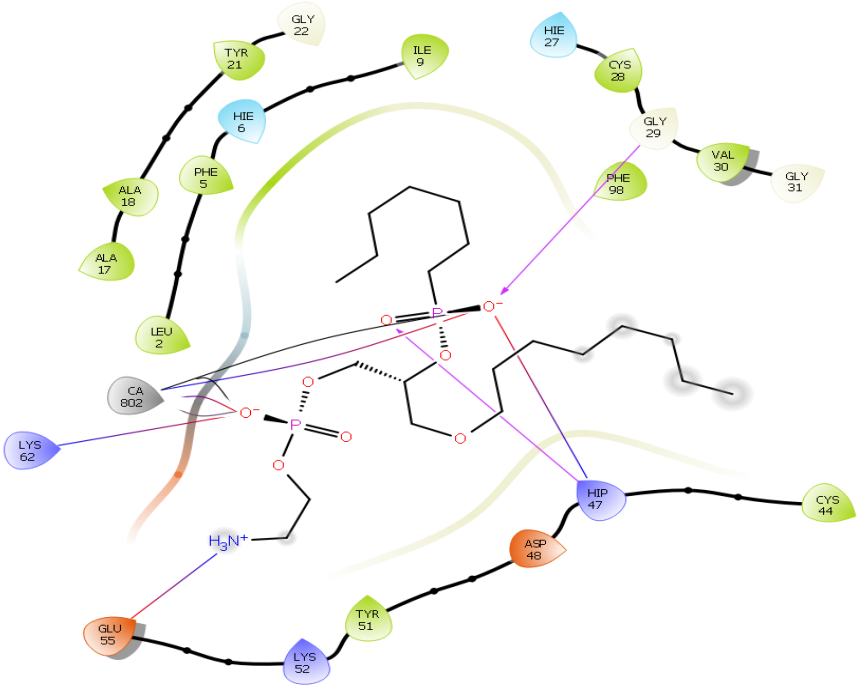

Figure 4 - 2D representation of inhibitor in 1POE

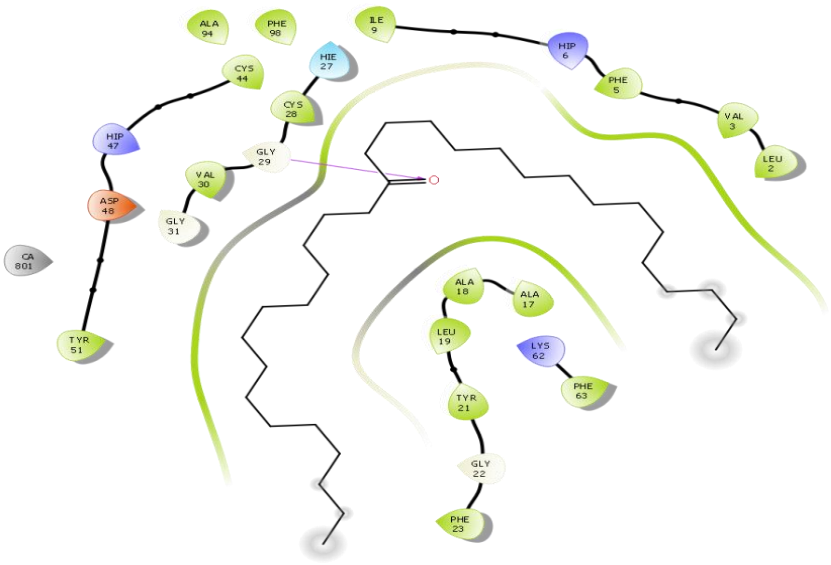

Figure 5- 2d representation of 16-Hentriacontanone with 1POE

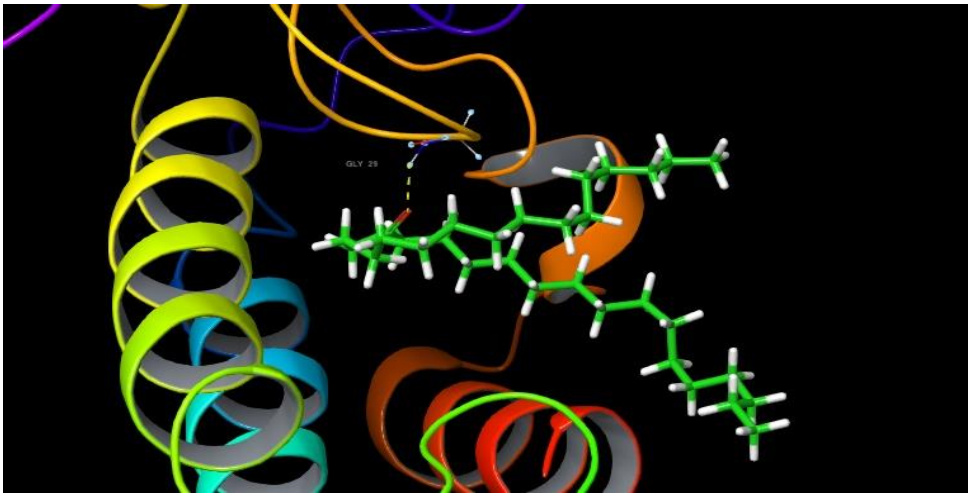

Figure 6- Interaction of 16-Hentriacontanone with active site of 1POE

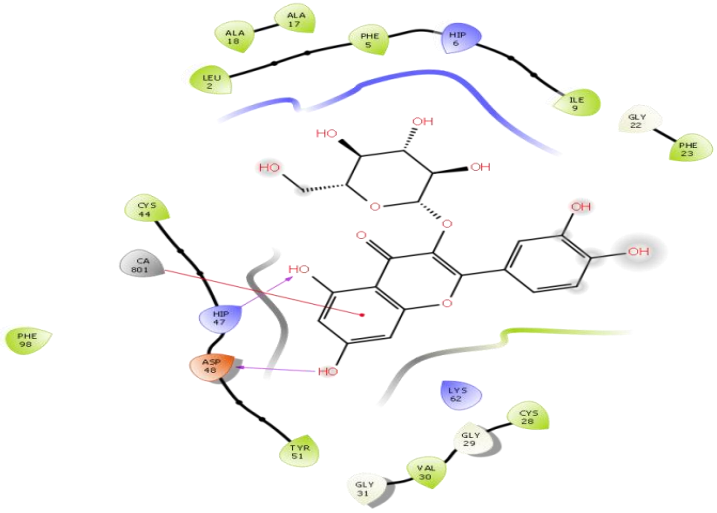

Figure 7- 2d representation of isoquercetin with 1POE

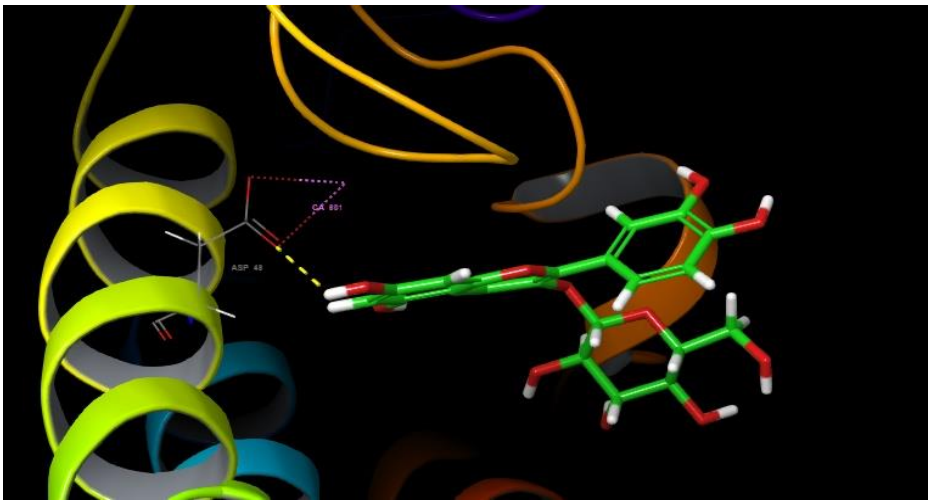

Figure 8- Interaction of isoquercetin with active site of 1POE

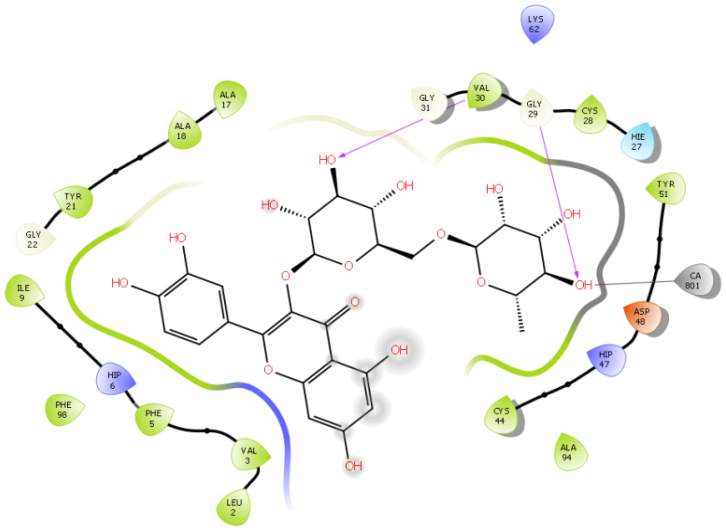

Figure 9-2d representation of rutin with 1POE

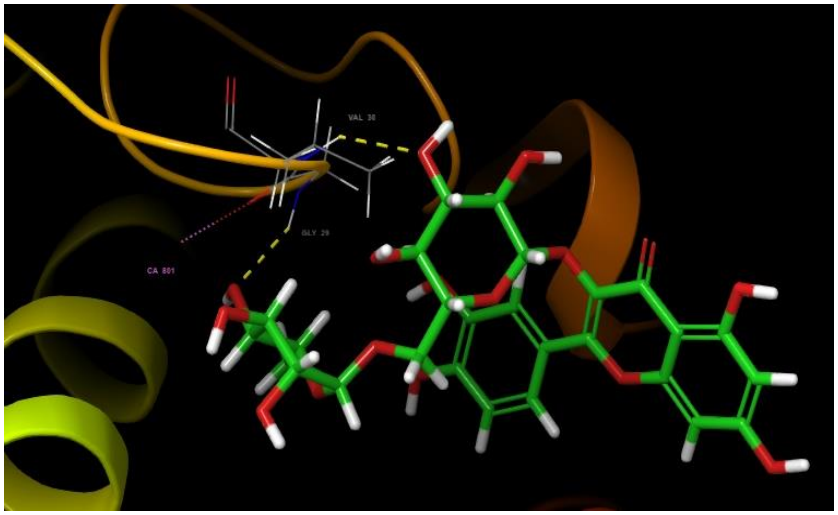

Figure 10- Interaction of rutin with active site of 1POE

CONCLUSION

PLA_2 is the largest target for inhibitor when compared to all isoforms, as several studies have established that this enzyme contributes in various inflammatory disorders, such as cancer, ulcer and arthritis. The current study reveals the PLA_2 inhibition potential of the leaves of *A.lunulatum* through *in-vitro* inhibition activity. Also the *in-silico* results explains the mechanism by which rutin and isoquercetin inhibit the PLA_2 by binding to the calcium loop of the receptor. The extract needs to be further subjected to preclinical and clinical studies to study in depth about the potential of the phytoconstituents.

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INVITO ANTIOXIDANT ANTI CERVICAL CANCER ACTIVITY OF *WITHANIA SOMNIFERA* USING HeLa cell LINE

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ABSTRACT

Worldwide, cervical cancer (CC) is the third most common malignancy in women, and it remains a leading cause of cancer-related death of women. Evergreen shrup (*Withania somnifera*) belongs to the family of Solanaceae contain several medicinal value in this study to evaluate anti-oxidant and anti-cancer activity of ethanol extracts of *Withania somnifera* using cancer cell line HeLa The antioxidant potential of ethanol extract of *Withania somnifera* extract is determined by DPPH radical scavenging activity and Nitric oxide scavenging activity. Cytotoxicity ethanol extract of *Withania somnifera* at different concentration (50µg/ml 300µg/ml) on HeLa cell line was carried out. The result suggested that the plant extract is more effective in inhibiting cell proliferation of HeLa cell line. Ethanol extract of *Withania somnifera* contain different content and composition of transition elements, which would imply on a potential for elevating and inducing ROS formation in HeLa cells after the treatment with the plant extracts. The loss of mitochondrial membrane potential was observed in extracts-treated cells and analysed using JCT dye. These staining results suggest that the extracts of *Withania somnifera* triggered apoptosis mediated cell death in HeLa ells. Assessed the apoptotic potential ethanol extract of the *Withania somnifera* in cervical cancer HeLa cells. Our results indicated that *Withania somnifera* may be used as an anti-cancer drug for cervical cancer in the future.

Key words: *Withania somnifera*, Cytotoxicity, DPPH radical scavenging activity and Nitric oxide scavenging activity.

INTRODUCTION

Cancer is categorized as a leading cause of mortality worldwide in the 21st century. According to the World Health Organization (WHO), cancer was the first or second leading cause of death before the age of 70 years in most countries in 2015 (Bray, *et al*., 2018), whereas new *cancer* cases and cancer-related deaths are expected to reach 21.4 and 13.2 million annually, respectively, by 2030 (Center*et al*.,2011). Overall, the incidence and mortality rates of cancer are rapidly increasing worldwide. The reasons for these increases are complex, but mainly reflect aging, population growth and changes to the major risk factors for cancer, several of which are related to socioeconomic development (Bray *et al*., 2018; Center*et al*., 2011).The classic model of cancer envisaged a normal cell transforming into an atypical or dysplastic cell with progression into an invasive or malignant cell (Idikio, 2011). Cancer can be defined as uncontrolled cell proliferation and division. Cancer cells can spread and invade other parts of the body through the blood and lymphatic circulation (Hanahan and Weinberg 2000.). At the cellular level, cancer classically develops through three defined stages: Initiation, promotion and progression. The second stage of cancer development is promotion. This is a prolonged stage, which begins with the proliferation of cells that become defective during the initiation process. Progression is the third and final stage, which is described as the metastasis of tumor cells that develop during the proliferation step (Doll and Peto 1981). However, a simple genetic mutation is not sufficient to drive cancer development; therefore, the multiple-hit hypothesis indicates that cancer is the result of accumulated genetic mutations in the DNA of a cell. This proposed hypothesis was first reported by Nordling and later by Knudson (Centelles 2013).Cancer cells

differ from normal cells in several aspects. Cancer cells are less stringently regulated compared with normal cells. The term 'tumor' is used to describe the abnormal proliferation of cells, and it may be either benign or malignant. The distinction between benign and malignant tumors is the most important issue in cancer pathology (Cooper 2000). The characteristics differentiating malignant from benign lesions are well established and comprise a rapid rate of growth, increased cell turnover, invasive growth, metastasis, and lymphatic and/or vascular channel invasion (Idikio 2011; Van Raamsdonkvan 2002 *et al.,,).*Benign tumors remain confined to their original location, without invasion of surrounding normal tissues or distant spread and dissemination .However, malignant tumors can both invade surrounding normal tissues and spread (metastasize) to other parts of the body via the circulatory or lymphatic systems.Only malignant tumors are referred to as cancers, and the ability to metastasize is what makes cancer dangerous. The spread of malignant tumors to distant body locations frequently makes them resistant to treatment. Over one million cases of cancer are diagnosed in the USA each year, and more than half a million Americans succumb to cancer annually (Cooper 2000).

Cervical cancers

Globally, in the year 2008, there were an estimated 12.7 million new cancer cases and 7.6 million cancer deaths. Cervical cancer is the third most common cancer among women worldwide, with an estimated 529 000 new cases and 275 000 deaths in 2008. More than 85% of the global burden of cervical cancer cases and 88% of cervical cancer deaths occur in developing countries. Cervical cancer is the most common cancer among Indian women and was estimated to have been responsible for 134 420 new cases and 72 825 deaths in the year 2008. India contributes to 25.4% and 26.5% of the global burden of cervical cancer cases and mortality, respectively. The age standardized incidence rate and age standardized mortality rate of cervical cancers are 27.0 and 15.2, respectively, among Indian women. Cervical cancer is responsible for 25.9% of all cancer cases and 23.3% of all cancer deaths among Indian women ([Ferlay*et al.*, 2010).

Diagnosis and pathology

Cervical cancer may be suspected on analysis of a Pap smear or visualisation of a lesion on the cervix. A biopsy sample must be taken from any suspicious lesion, because many Pap smears are non-diagnostic or falsely negative in the presence of invasive cancer. If a biopsy sample shows cells suggesting micro invasion, and if the patient does not have a grossly apparent invasive cancer, a cone biopsy should be done. For accurate staging of clinically occult lesions, sufficient underlying stroma must be obtained to allow for adequate assessment of the depth and width of invasion below the basement membrane. About 80% of primary cervical cancers arise from preexisting squamous dysplasia. Adenocarcinoma of the cervix accounts for about 20% of invasive cervical cancers; in more developed countries, the incidence of adenocarcinoma is rising in relation to that of squamous carcinoma. Although oncogenic HPV DNA has been identified in adenocarcinomas(Johnson *et al.*,1992; Noller*et al.*,1972) smoking does not seem to be a risk factor for this histological subtype. In most cases, adenocarcinoma-in-situ is probably the precursor lesion, but it is detected much less efficiently by Papsmear screening than are preinvasive squamous lesions. Clear-cell carcinoma is a rare adenocarcinoma subtype, which accounts for fewer than 5% of adenocarcinomas. Previously, many cases developing in young women were associated with in-utero exposure to diethylstilbestrol.Since use of diethylstilbestrol in pregnancy has been prohibited since 1971, the number of cases associated with this drug has diminished. In the absence of diethylstilbestrol exposure, clear-cell carcinomas most commonly occur in women who are postmenopausal. Other uncommon subtypes include adenosquamous cancers and small-cell (neuroendocrine) carcinomas.

Treatment options

In many more developed countries with established Papsmear screening systems, microinvasive or stage IA cervical cancers are commonly detected in women who are symptom free with cervices that seem normal on gross examination. The diagnosis is usually made after a cervical conisation, although many cases of superficially invasive cervical cancer are incidentally discovered after hysterectomy. If the focus of invasion extends no deeper than 3 mm below the basement membrane (stage IA1), the risk of pelvic nodal involvement is less than 1%. Cervical conisation is a reasonable treatment option for patients who want to preserve fertility(Tseng *et al.*, 1997) According to FIGO, the presence of invasion of the lymphatic or vascular space should not change the stage, but should be noted by the pathologist, because it may affect treatment recommendations owing to concerns about risk of nodal involvement (see later). If the patient has completed childbearing, the treatment of choice remains extrafascial hysterectomy, by the abdominal or vaginal approach For microinvasive squamous cancers invading 3–5 mm in depth and with less than 7 mm of horizontal extension (stage IA2), the risk of lymph-node metastasis is 2–8%. Most gynaecological oncologists would advise radical hysterectomy or radiotherapy as treatment. The extent of the surgery is open to debate(Creasman 1999 ; Creasman*et al.*, 1998) In view of the low risk of parametrial tumour extension with microinvasive carcinomas, a modified radical hysterectomy in which less parametrial tissue and vagina are removed is judged appropriate and may restrict associated complications such as bladder dysfunction(Jones *et al.*, 1993) Pelvic lymph adenectomy includes removal of lymph nodes from the common iliac, external iliac, internal iliac, and obturator regions. Paraaortic lymph-node dissection is not necessary unless suspicious pelvic lymph nodes are encountered. A current topic of interest is the use of more conservative surgery for patients with early cervical cancer who wish to cisplatin, this regimen has generally been accepted as the chemotherapy of choice. A systematic review and metaanalysis ofreports on the use of chemoradiotherapy generally supported the use of chemoradiotherapy over radiotherapy alone, or neoadjuvant chemotherapy followed by radiotherapy (Green *et al.*, 2001)

Chemotherapy

Chemotherapy for advanced or recurrent disease has been and continues to be considered palliative. Many agents have been investigated, as single or combined regimens(Park *et al.*, 1993). Response rates in multicentre phase-2 trials average 10–40%, with complete responses seen only rarely and for short duration. Cisplatin is at present deemed the most active single agent in recurrent disease. When it was combined with paclitaxel in a phase-2 study, an overall response rate of 46·3% was recorded (12·2% with complete responses and 34·1% with partial responses)(Rose *et al.*, 1999). As a single agent, cisplatin has been compared with the combination of cisplatin and paclitaxel in a randomised, phase-3 study. The combined regimen was superior to single-agent cisplatin in terms of response rate and survival, at a cost of reversible bone-marrow toxic effects (Moore *et al.*, 2001). The survival benefit is modest (a few weeks), and quality-of-life assessments for the two treatment groups have not yet been reported. A factor that seems to affect chemotherapy effectiveness adversely is whether a recurrence occurs within a previously irradiated field. About 25% of patients with recurrence outside the irradiated field respond to chemotherapy, compared with 5% if the recurrence is within the irradiated field(Brader*et al.*, 1998). Issues requiring further study (panel 2) include identification of the best chemotherapy regimen to be used in combination with primary radiotherapy and whether combination chemotherapy as a radiation sensitiser can produce long-term improvement in distant control. Advanced imaging modalities, including MRI, should be incorporated into multi-institutional clinical trials to investigate whether purported advantages in assessing extent of tumour can be translated to improvements in survival or quality of life over traditional staging

systems. Other areas of investigation are directed at improvement in both surgical and radiotherapy techniques to limit morbidity and improve quality of life.

Medicinal plants

Medicinal plants are considered as an upscale resources of ingredients which may be utilized in drug development either pharmacopoeial, nonpharmacopoeial or synthetic drugs. A neighborhood from that, these plants play a critical role within the development of human cultures round the whole world. Moreover, some plants are considered as important source of nutrition and as results of that they are recommended for his or her therapeutic values. The good interest within the use and importance of medicinal plants in many countries has led to intensified efforts on the documentation of ethnomedicinal data of medicinal plants. (Dhar*et al*.,1968) S. Aqueum, commonly referred to as water apple, one among the foremost valuable medicinal plant species under the Myetaceae. In Ayurveda, the plant extract has been evidenced to be pharmaceutically active as anti hyperglycaemic activity, anti inflammatory activity, Anti oxidant activity ect. The medicinal activities of this genus are such a lot vigorous that a broader range of study is required to be completing to assess the whole pharmacological role in various ailments.

Withaniasomnifera

Withania somnifera (family: *Solanaceae*) is a plant in which its fruits are favored by many. It is easy to grow and is usually planted in house yards not only for its fruits but also for protection purposes. Being native to Indonesia and Malaysia, the fruit of this plant is mainly known as the water jamboo, but other common names include water apple, bell fruit, water cherry, or watery rose apple.)ere are different variations of this plant with the main difference being the shape and color of the fruits. Various parts of *S. aqueum* have been utilized in traditional medicine due to the antibiotic activities, antioxidant, anticancer that are present [Cock and Cheesman 2019, Manaharan*et al*., 2012)

Figure -1 Morphology of *Withaniasomnifera*

Medicinal uses

Various part of this plant are used in traditional medicine; while the leaves has been shown to have antibiotic activity and relieving child birth pains. The dried leaves in powdered form have been used to treat mouth ulcers and a preparation of its root have been used to relieve

itching and reduce swelling. A decoction of the *Withaniasomnifera*astringent bark is used for thrush. The leaf extract has also been reported to have cosmeceutical properties, anti-tyrosinase, anticellulite and lypolitic. Health Benefits of Water Apple(Nakamura *et al.*,1986)

Pharmacological Actions

1. Antibacterial Activity: Studies suggest that extracts or compounds derived from rose apples exhibit antibacterial effects against various bacterial strains. These effects might be attributed to the presence of bioactive compounds like flavonoids, tannins, and phenolic compounds [Murugan*et al.*,2013].

2. Anticancer: Some investigations have highlighted certain aspects that suggest a potential role in cancer prevention or treatment. Certain review indicates that compounds derived from rose apples may exhibit inhibitory effects on tumor progression or metastasis [Chua *et al.*,2019].

3. Antidiabetic: Rose apples (*Withaniasomnifera)* have been studied for their potential antidiabetic properties due to their various bioactive compounds. [Zulcafliand , Lim 2018]

4. Antimalarial: While there's limited direct evidence on the antimalarial properties of rose apples *(Withaniasomnifera),* some studies have explored the potential of certain compounds present in rose apples against malaria parasites.

5. Antioxidant Effects: Antioxidants in rose apples may play a role in reducing oxidative stress associated with diabetes(Cabantchik 1995)

Anticancer activity: *W. Somnifera*leaves were extracted with methanol for the determination of cytotoxicity using sulforhodamine B (SRB) assay. The activity was tested on human breast cancer cell (MDA-MB-231) and compared with that of doxorubicin (standard cytotoxic drug). The extract was less toxic on cancer cell line (IC50 > 100 µg/mL).

Aim and Objective: Cervical cancer, the second most common gynecological malignant tumor seriously harmful to the health of women, remains a leading cause of cancer-related death for women in developing countries. Although a large amount of scientific research has been reported on plants as a natural source of treatment agents for cervical cancer, it is currently scattered across various publications. A systematic summary and knowledge of future prospects are necessary to facilitate further plant studies for anti-cervical cancer agents.

MATERIALS AND METHODS

Plant materials

Withania somnifera were collected in Mannargudi, Tiruvarur District, Tamil Nadu, India. The *Withania somnifera* were first washed well and dust was removed from the fruits. fruits was washed several times with distilled water to remove the traces of impurities from the fruits. The fruits were dried at room temperature and coarsely powdered. The powder was extracted with aqueous and ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Scavenging activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical

The effect of ethanol extract on DPPH radical was estimated using the method of Liyana-Pathiranan et al. (2005). About 0.1 ml of DPPH-methanol solution (0.135 mm) was mixed with 1.0 ml of different concentrations of ethanol extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Vitamin C were used as standard drugs. The percentage of free radical scavenging was calculated according to the following equation: %scavenging = 100 – (Abs sample – Abs blank)/Abs Control × 100.

Determination of nitric oxide radical scavenging activity

NO radical reducing capacity of ethanol extract was analyzed using the method described by Green et al. (1982). Briefly, 2 mL of 10 mM sodium nitroprusside and 0.5 mL of phosphate buffer were added along with 0.5 mL of extract and then maintained for 15 min at 25°C. After that, 0.5 mL of nitrite was taken and 1 mL of sulfanilic acid was mixed and incubated for an additional 5 min. Later, 1 mL of N-(1- naphtyl)-ethylenediaminedihydrochloride (NEDD) (1%) was mixed and incubated at 25°C for 30 min. Finally, absorbance was taken at 540 nm.The NO inhibitory percentage was examined using the formula depicted hereunder:Percentage (%) of NO radical scavenging = $[(A_0-A_1)/A_0] \times 100$.where A_0 was the control absorbance, and A_1 was the extract-treated absorbance.

Source of chemicals and reagents

Dulbecco's Modified Eagle's Medium, streptomycin, penicillin-G, L-glutamine, phosphate buffered saline, 3-(4,5 dimethylthiozol-2-yl)-2,5-diphenyltetrazoliumbromide, 2'7'diacetyl dichloro fluorescein, sodium dodecyl sulphate, trypan blue, trypsin-EDTA, ethylene diamine tetra acetic acid, acridine orange, ethidium bromide, rhodamine-123, triton X-100, ethanol, dimethyl sulfoxide (DMSO), and bovine serum albumin were purchased from Sigma Aldrich Chemicals Pvt. Ltd (India). All other chemicals used were of analytical grade, purchased from Hi media Laboratories Pvt. Ltd., India.

RESULT AND DISCUSSION

Cervical cancer will develop in about 500 000 women this year worldwide. In many less developed countries it is the most common cause of cancer death and years of life lost owing to cancer. The disease is most commonly diagnosed in the fifth decade of life—several years earlier than the median age at diagnosis of breast, lung, and ovarian cancers. Although this cancer poses a far greater health concern in less developed than in more developed countries, by necessity most of the information discussed here reflects work that has been done in more developed countries. Much of this work has been the result of studies or clinical trials under the direction of the European Organisation for Research and Treatment of Cancer (EORTC) and the Gynecologic Oncology Group (GOG). Members of these multidisciplinary organisations include gynaecological oncologists, radiation oncologists, medical oncologists, immunologists, and pathologists.

***In-vitro* antioxidant activity**

Oxidative stress has been implicated in the pathology of many diseases such as inflammation, cancer, diabetes, neurodegenerative disorders and aging. Reactive oxygen species and reactive nitrogen species such as superoxide anions, hydroxyl radical and nitric oxide inactivate enzymes and damage intracellular components causing injury through covalent binding and lipid peroxidation. Antioxidants are compounds that hinder the oxidative processes and thereby delay or prevent oxidative stress (Rajkapoor*et al.*, 2010).

DPPH Activity of *Withania somnifera*

Figure 1 shows the free radical scavenging activity of the *Withania somnifera* and standard antioxidant compound vitamin C. In this study, the free radical scavenging ability of various concentations (50-250µg) was evaluated through recording the change of absorbance produced by the reduction of DPPH· In the present study *Withania somnifera* extract showed a significant effect in inhibiting DPPH, reaching up to 87.53% at concentration of 250µg/mL. The IC_{50} value of *Withania somnifera* extract was 133.46µg/mL while the IC_{50} value of standard antioxidant ascorbic acid was 124.22µg/mL.

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. At a concentration of 50-250 µg/mL, the scavenging activity of *Withania somnifera* was 24.62, 34.53, 52.62, 70.43, and 87.53%, was respectively, whereas at the same concentration, the standard vitamin C was 90.74% (Fig. 1).

Table 1.DPPH Activity of *Withania somnifera*

Samples	Concentration					
	50 µg	100µg	150 µg	200 µg	250 µg	300 µg
Ethanol extract	27.81	33.27	43.17	50.17	61.26	75.93
Vitamin C	37.29	44.78	52.28	60.91	71.17	82.24

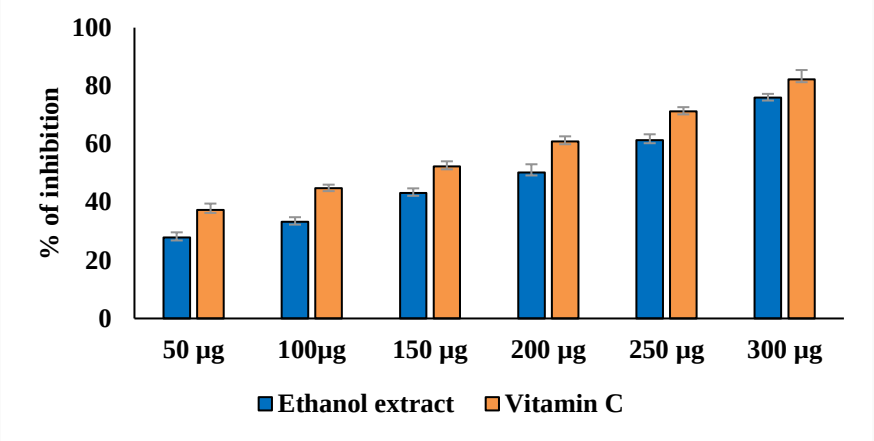

Fig.1.DPPH activity of *Withaniasomnifera*

Table 3.Cytotoxicity Assay of *Withania somnifera*

	Control	50 µg	100µg	150 µg	200 µg	250 µg	300 µg
Ethanol extract	100	87.12	71.42	50.79	44.09	30.51	23.28
	0	1.858	1.907	1.587	3.008	1.616	2.424

SUMMARY AND CONCLUSION

The following conclusion observed from the study.

The antioxidant potential of ethanol extract of *Withania somnifera* extract is determined by DPPH radical scavenging activity, Superoxide, and Nitric oxide Cytotoxicity of *Withania somnifera* extract at different concentration (50µg/ml - 400µg/ml) on HELA cell line was carried out. The result suggested that the plant extract is more effective in inhibiting cell proliferation of HELA cell line.

Withania somnifera extract induces intracellular ROS generation in HELA cells was assessed by DCFH-DA staining assay. extract contain different content and composition of transition elements, which would imply on a potential for elevating and inducing ROS formation in HELA cells after the treatment with the plant extracts. Determined the effects of *Withania somnifera* extract on the mitochondrial membrane potential of cervical cancer cells. In the present study, the loss of mitochondrial membrane potential was observed in extracts-treated cells and analysed using JC1 dye. These staining results suggest that the extracts of *xxx*

triggered apoptosis-mediated cell death in HELA cells. The results of the present study showed that the selected traditional medicinal plant possess the cytotoxic activity on cervical cancer cell lines and supported the folkloric usage of the studied plants and confirmed that the plant's extracts have the bioactive constituents with cytotoxic properties and their isolation can be useful for developing new anticancer drugs. The findings from this study indicated that ethanol extracts of *Withania somnifera* possessed vast potential as a medicinal drug especially in cervical cancer treatment.

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COMPARATIVE STUDY OF CULTIVATION OF PLEUROTUS SAJOR- CAJU AND PLEUROTUS CITRINOPILEATUS ON DIFFERENT AGRO WASTE MATERIALS.

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ABSTRACT

Growing oyster mushroom is becoming more popular throughout the world because of their abilitiesto grow in a wide range of temperatures, utilizing various lignocelluloses (Khan and Garcha, 1984). The present work was carried out to find out the effect of different agricultural wastes on yield of two strains Grey oyster mushroom (Pleurotussajor-caju) and yellow oyster mushroom (P. citrinopileatus)were investigated and recorded.The use of waste materials as the alternative for mushroom cultivationsubstrates, identified as a potential and viable way to generate new economic sources and minimising waste disposal. Cultivation ofedible mushroom with agricultural residues is a valued-added process to convert these materials,which are otherwise considered to be wasted, into human food (Zhang et al., 2002).

Key Words: *Pleurotussajor-caju, P. citrinopileatus, Agricultural waste residues. Substrates.*

INTRODUCTION

Oyster mushroom, *Pleurotus species* belongs to the family of Tricholomataceae and is the second widely cultivated mushroom worldwide. Obodai et al., (2003) reported that oyster mushroom is the third largest commercially produced mushroom in the world market. *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency. Moreover, the interest of oyster mushroom is increasing largely due to its taste, nutrient, and medicinal properties. *Pleurotus* species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures. In comparison to other edible mushrooms, *Pleurotus* species need a short growth time and their fruiting bodies are not often attacked by diseases and pests . *Pleurotus* species require carbon, nitrogen and inorganic compounds as their nutritional sources.

The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin which includes, Paddy straw, sugar cane bagassess, dried leaves of banana, black gram, corn leaves, rice bran, coconut coir pith, dried vegetable waste, and fruit peel waste had been used as mushroom substrates. Oyster mushroom can grow on a wide variety of substrate. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates.*Pleurotus citrinopileatus*, the golden oyster mushroom is an edible gilled fungus. The golden oyster mushroom, like other species of oyster mushroom, is a wood-decay fungus. In the wild, *P. citrinopileatus* most commonly decays hardwoods such as elm. The first recorded observation of naturalized golden oysters in the United States occurred in 2012 on Mushroom Observer, perhaps a decade after the cultivation of the species began in North America, and they have been found growing on oak, elm, beech, and other hardwoods. *Pleurotus sajor-caju* may refer to some warm weather varieties of *Pleurotus pulmonarius*, a commonly cultivated species of oyster mushroom, often incorrectly called *Pleurotus sajor-caju* , *Lentinus sajor-caju*, a species of mushroom formerly called *Pleurotus sajor-caju. Pleurotus species* are a rich source of protein, minerals (P, Ca, Fe, K, and Na) and vitamin (thiamine, riboflavin, folic acid, and niacin).Apart from food value, their medicinal value for diabetics and in cancer therapy has been emphasized Numerous mushroom

species contain a wide range of metabolites as antitumour, antigenotoxic, antioxidant, antihypertensive, antiplatelet- aggregating, antihyper-glycaemic, antimicrobial, and antiviral activities.

Large volumes of unused lignocellulosic by-products are available in the Kaveri Delta region as agro waste materials. These by-products are usually left to rot in the field or are disposed through burning. Using locally available lignocellulosic substrates to cultivate oyster mushroom is one solution to transform these inedible wastes into accepted edible biomass of high market and nutrient values. Use of agro-waste residues are the reasons why we need to identify alternatives for sustainable cultivation of oyster mushrooms. The study was conducted to compare the effects of different agro-wastes on the growth, yield, and nutritional composition of oyster mushrooms *P.citrinopilitus and P. sajor – caju.* The final aim is to find the best substrate formulas for effective cultivation of oyster mushrooms.

MATERIALS AND METHODS

Mother Culture Preparation

PureStrains of *Pleurotus* species*Pleurotus sajor-caju* and *Pleurotus citrinopileatus* strains were obtained from ICAR DMR Chambaghat, Solan.India. The cultures were preserved on 2% malt extract agarslants at 4ºC. Subculturing was done after every 15 days.

Spawn preparation

Sorghum grains were prepared in polythene bags. The grains were subjected to boiling in a water bath for a duration of 10 to 15 min, maintaining a ratio of 1:1 (Sorghum grains to water). Subsequently, the grains were combined with 4% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$. Following this, 250 g of the sorghum mixtures were placed into polythene bags measuring 200x300 mm and sterilized in an autoclave at 121ºC for 30 min. After the sterilization process, the bags were inoculated with actively growing mycelia of *Pleurotus sajor-caju* and *P. citrinopileatus* grown on malt extract slants and incubated at a temperature of 27±2ºC for 10 to 15 d in the absence of light, allowing the mycelia to completely colonize the grains (Garcha, 1994).

Substrate preparation and inoculation

Substrates for the present study were include Paddy straw, sugar cane bagassess, dried leaves of banana, black gram, corn leaves, rice bran, coconut coir pith, dried vegetable waste, and fruit peel waste had been used.These substrates were dried and cut into 3-4cm longpieces. The substrates were soaked in water for8-10 hours in cemented pond to obtained 70-75per cent moisture level. All the substrates weresterilized by boiling method where the substrateswere boiled for one hour at 70-75°C. Then theywere stalked on the steep cementedfloor so as toremove the excessive moisture from the substrates to get 65-75 per cent moisture level. The substrates were cooled up to room temperature (25oC). A local method was developed for determination of moisture. In this method moisture was determined by pressing a handful mixture. If there was no water runoff and the material stayed in form indicates that the moisture content was around 65 per cent. Five kilogram of each substrate was filled in transparent polythene bag (30x45cm and seeded with 150g of *P. sajor-caju* and *P. citrinopilitus.* The pinholes at 10- 12cm distance were also done in the bags with help of led pencil after sterilization in 2 per cent formaldehyde solution. The bags were incubated in dark cropping room where ambient temperature ranged between 22- 28+10C. The humidity 80 – 90 per cent of the room was maintained by spraying of water twice a day on the loor covered with jute bags. After complete colonization of substrate polythene was removed and bags were put on the bamboo made structure for fruiting. The humidity of the bags was accomplished by spraying of water on them twice a day. The experiment was laid out in complete randomized design (CRD) with three replications and live treatments. Time was recorded in days

for the completion of growth of mycelium on substrates, appearance of pinheads and maturity of fruit bodies in different treatments. The data on average values of observations were also recorded for the yield, number of fruit bodies. Biological eficiency of mushroom on fresh weight basis was calculated by using formula given by Chang and Miles (1989).

RESULTS AND DISCUSSION

Spawn running all the substrates were spawned at the same day. It was evident from the Table 1 that spawn running took 2-3 wk after spawning. Pinhead formation The pinhead formation is the second stage of mycelia during cultivation of mushroom. Small pinhead like structures were observed, these pinhead were formed 4-5 days after spawn running (Table1). Our results were corroborated with Ahmed (1986) who stated that Pleurotusostreatus completed spawn running in 17-20 d on different substrates and the time for pinhead formation was noted as 23-27 d. Fruit bodies formation This is the third and inal stage during the cultivation of mushroom. The fruit bodies appeared 4-5 wk after pinhead formation and took 25-34 d later after inoculation of spawn (Table1). Sharma and Jandaik (1981) reported that P. sajor-caju cultivation on wheat straw took 32 d for the first harvest.

$$\text{Biological efficiency (\%)} = \frac{\text{Yield of fruiting body (g)}}{\text{Total weight of substrate used (g)}} \times 100$$

Substrate	Days for completion of spawn running	Days for pinhead formation	Days of fruiting bodies formation	Average number of fruiting bodies
Paddy straw	12.67	12.66	7.33	27.66
Coir pith	17.65	16.77	12	12
Dried banana leaf	14.24	12.12	14	14
Dried black gram leaf	13.23	13.23	15	12
Rice bran	17.02	17.23	29	11
Corn leaf	15.03	14.98	21	12
Dried fruit waste	13.40	15.98	26	11
Dried vegetable waste	18.09	16.98	27	10

Proximate analysis

The assessment of moisture, protein, fat, crude fiber, total carbohydrates and ash content in the samples was conducted using established methodologies as outlined by AOAC (1995).

Proximate content	**Quantity**	
	Pleurotuscitrinopileatus	***Pleurotussajorcaju***
Ash (%)	7.23	8.61
Moisture (%)	9.94	9.54
Carbohydrate (µg/g)	10.04	19.70
Protein (µg/g)	9.71	8.11
Crude fat (µg/g)	0.86	1.10
Crude fibre (µg/g)	4.16	5.41

Vitamin estimation

Folic acid, thiamine (B1), riboflavin (B2), and niacin were estimated according to Kamman*et al.*, (1980). Vitamin C was estimated by the 2,6- dichlorophenolindophenol titration method (AOAC,1995). Vitamin A and E were estimated using the method described by Majesty *et al.*, (2019).

Determination of mineral elements

The prepared samples of *Pleurotus sajor-caju* and *Pleurotus citrinopileatus* were analyzed for Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Potassium (K) and Zinc (Zn) using spectrophotometric methods according to Afolabi*et al.*, (2023) and AOAC (2019).

Analysis of Bioactive compounds

(a) Qualitative bioactive analysis: The bioactive compounds such as alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and quinones were analysed with the solvents of aqueous, ethanol, methanol and diethyl ether extracts using standard method (Harborne, 1973) were followed.

(b) Quantitative bioactive analysis: The bioactive compounds such as alkaloids, amino acids, coumarins, phenols, steroids, terpenoids and quinones were analysed using standard method (Harborne, 1973). Flavonoids (Bohm and Mohammed, 1994), saponins (Obadone and Ochuko, 2001) and tannins (Van Buren and Robinson, 1981) were estimated.

Statistical Analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviations.

Species	Substrate	Spawn Run (d)	Pinhead formation (d)	Yield (g) per harvest (g/kg)			Total Yield (g/kg)	Bio-conversion Efficiency (%)
				I	II	III		
Pleurotuscitrinopileatus	Paddy straw	25	28	115	107	65	287	28.7
	Coconut coir pith	23	25	109	102	54	265	26.5
	Sugarcane trash	28	30	96	100	46	242	24.2
P. sajorcaju	Paddy straw	17	22	155	128	98	381	38.1
	Coconut coir pith	24	28	100	104	58	262	26.2
	Sugarcane trash	27	31	98	100	53	251	25.1

CONCLUSION

This study evaluated the proximate, mineral, vitamins contents and bioactive compounds of *Pleurotus citrinopileatus* and *P. sajor-caju*. The results of the proximate analysis indicated that *Pleurotus* sp. exhibited elevated levels of carbohydrates and moisture, moderate amounts of protein and fiber, and low levels of fat and ash. Vitamins A, E and B2 were found to

be present in high amounts in the *Pleurotus* spp.whereas other vitamins such as B1, B3 and C were found to be present much lower concentrations. The analysis of mineral content indicated a significant presence of calcium, iron and magnesium in comparison to other minerals. The maximum bioactive compounds were presented in the aqueous and methanol extracts of *Pleurotus* spp. The findings from other studies indicated that incorporating mushrooms into food products enhances both their nutritional content and physical attributes. Consequently, it is not unexpected that the food and pharmaceutical industries utilize mushrooms or their bioactive compounds to develop functional foods with nutraceutical benefits. By establishing a rapid, nutrient-dense food source, mushroom cultivation can provide individuals with a reliable income stream, thereby reducing their susceptibility to poverty and improving their overall quality of life. *Pleurotuscitrinopileatus* and *P.sajorcaju* have been identified as abundant sources of essential nutrients, vitamins, minerals and aminoacids which positions them as functional foods and significant contributors to dietary practices aimed at reducing the increasing prevalence of nutrition-related diseases.

Pleurotus species cultivated on paddy straw, coconut coir and sugarcane trash substrates exhibited a significant capacity for growth and yield. Among the 2 examined species of *Pleurotus*, *Pleurotus citrinopileatus* demonstrated a lower yield compared to *Pleurotus sajor-caju*. Recent research has extensively explored various aspects of oyster mushroom cultivation Notably,*Pleurotus sajor-caju* shows an increase in mycelial growth of up to 100%, irrespective of the substrate composition, when compared to *P. ostreatus* and *P. florida* (Olasupo*et al.,* 2019).

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PROTECTIVE EFFECT OF ETHANOLIC EXTRACTS OF *INDIGOFERA ASPALATHOIDES (LEAVE)* ANTIOXIDANT ACTIVITY OF FREE RADICAL SCAVENGING ACTIVITY

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ABSTRACT

Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge number of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. This study was devoted to the determination of *in vitro* antioxidant activity of *Indigofera aspalathoides* leaves. The antioxidant capacity of the plant extracts was measured by their ability to scavenge free radicals such as DPPH, superoxide anion scavenging, hydroxyl radical scavenging, nitric oxide scavenging and hydrogen peroxide scavenging activity. The effects were comparable with the standard antioxidant ascorbic acid and these antioxidant properties were concentration dependent. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine for the treatment of various diseases.

Keywords: Antioxidant, *Indigofera aspalathoides*. DPPH

INTRODUCTION

Medicinal plants, microorganisms, and animals are the leading sources of traditional medicines against different illnesses over many centuries. The plant-derived compounds share a major portion of medicines that have been used by humans against several diseases. Especially, people residing in developing countries rely on natural products as a major source of medicines due to their low cost, fewer side effects, and availability (Calixto, 2019; Abdel-Razek *et al.*, 2020; Otitolaiye *et al.*, 2023) The use of herbal medicines continuously plays a vital role in primary health care, and about 80% of the world's population is estimated to depend on traditional medicine for their primary healthcare (Newman *et al.*, 2000). The development of knowledge of the exercise and consciousness of medicinal plants by different human civilizations in the past has augmented the notions and capacity of pharmacists and physicians to tackle the challenges of spreading professional services to assist human health (Petrovska, 2012; Bani et al., 2023).

Free radicals are highly reactive molecules that contain one or more unpaired electrons. They either donate or take electrons from other molecules in an attempt to pair with their electrons and generate more stable species. Reactive oxygen species (ROS) are derivatives of oxygen (Fresquet *et al.*, 2006) and are constantly produced in the body during various metabolic activities such as aerobic respiration and by various exogenous factors (Hiroe and Nobuji, 1993). Therefore, a special interest to screen medicinal plants for the presence of natural antioxidants has greatly increased. Plant derived natural copounds such as flavonoids, terpenes, alkaloids etc. have received considerable attention in recent years due to their pharmacological properties including Antioxidant, Antimicrobial and Anti-inflammatory activities (Sahu *et al.*, 2010; Pereira *et al.*, 2009; McChesney *et al.*, 2007). Plant phenols, a diverse group of phenolic compounds

(flavonols, anthocyanins, phenolic acids, etc.) possess strong antioxidant activity and may help to provide the protection to the cells against the oxidative damage caused by free radicals. They are well known as "radical scavengers", "metal chelators", "reducing agents" hydrogen donors and singlet oxygen quenchers. They have an ideal structural chemistry for the removal of free radicals in the body. The present study has been conducted to evaluate the antioxidant properties of *Indigofera aspalathoides* leaves.

MATERIALS AND METHOD

Collection of plant

The leaves of *Indigofera aspalathoides* (Tamil name: Sivanar vembu) were collected in December 2022 from Thanjavur, Tamil Nadu, India from an herb. The collected *Indigofera aspalathoides* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The leaves were dried at room temperature and coarsely powdered using mixer grinder.

Preparation of extract

10grams of *Indigofera aspalathoides* leaves powder were used for extraction. Extraction was performed with cold extraction using the maceration method into ethanol solvent for 24 hours using the "intermittent shaking" method to obtain an extracts. The extracts were filtered using Whatman filter No 1 paper and filtrate was used for *in vitro* antioxidant assay.

In vitro antioxidant activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.,* (1992). The superoxide anion radicals scavenging activity was measured by the method of Liu *et al.* (1997). The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al*. (2004). Hydrogen peroxide scavenging activity of the extract was estimated by method of Zhang (2000). Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964).

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals' concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software. Results were expressed as graphically/ mean ± standard deviation.

RESULTS AND DISCUSSION

Biological combustion involved in various processes produces harmful products or intermediates called reactive oxygen species or free radicals. Excess of free radicals in living beings has been known to cause various problems like asthma, cancer, cardiovascular diseases, liver diseases, muscular degeneration, and other inflammatory processes (Sen *et al.,* 2010), resulting in the so-called oxidative stress. Oxidative stress is defined as imbalance between oxidants and antioxidants and causes damage in all types of biomolecules like protein, nucleic acid, DNA, and RNA (Dröge, 2002).

DPPH radical scavenging activity

DPPH has been used extensively as a free radical to evaluate reducing substances (Duan *et al.,* 2006; Kumar Nirbhay et al., 2023) and is a useful reagent for investigating the free radical scavenging activities of compounds (Meir *et al.,* 1995). Figure 1 represents DPPH radical scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid. The *I. aspalathoides* and ascorbic acid showed maximum DPPH inhibitory activity were 85.37% and 92.09% at 100µg/ml concentration range while minimum inhibitory activity were 20.55% and 22.92% at 20µg/ml concentration range respectively. The half inhibition concentration (IC_{50}) of *Indigofera aspalathoides* leaves extract and ascorbic acid was 60.04µg/ml and 51.59µg/ml

respectively (Table. 1). Their antioxidant activity is affected by phenolic compound on DPPH which able to donate hydrogen atoms to form stable (Sathishkumar *et al.*, 2009).

Table 1: DPPH radical scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid

	Indigofera aspalathoides	**Std. Ascorbic acid**
20	20.55±0.03	22.92±0.18
40	30.83±0.18	39.52±0.21
60	48.22±0.30	56.91±0.42
80	64.82±0.53	75.09±0.68
100	85.37±0.71	92.09±0.85
IC_{50} (µg/ml)	60.04	51.59

Values expressed as Mean ± SD for triplicates

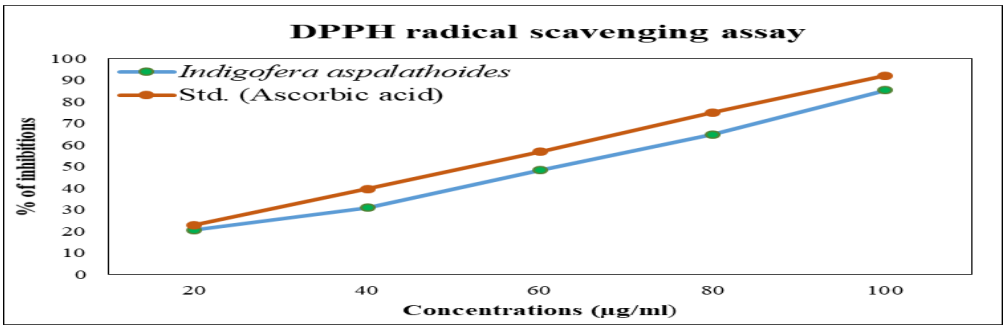

Figure 1: DPPH radical scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid

Superoxide Scavenging Activity

Superoxide anion is a weak oxidant produced during various biological reactions is highly toxic (Stief, 2003). In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT (Toda *et al.*, 1988; Lanzoni et al., 2023). Superoxide Scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid. At the minimum concentration of *Indigofera aspalathoides* and ascorbic acid (20µg/ml), were 17.84% and 21.16%, respectively while at the maximum concentration (100µg/ml), the corresponding activities were 82.15% and 91.28% respectively. The half inhibition concentration (IC_{50}) of *Indigofera aspalathoides* leaves extract and ascorbic acid were 61.88µg/ml and 51.54µg/ml respectively (Table 2 and Figure 2).

Table 2: Superoxide Scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid

	Indigofera aspalathoides	**Std. Ascorbic acid**
20	17.84±0.11	21.16±0.20
40	29.87±0.20	39.83±0.31
60	48.13±0.33	28.09±0.66
80	64.31±0.51	77.17±0.83
100	82.15±0.70	91.28±0.98
IC_{50} (µg/ml)	61.88	51.54

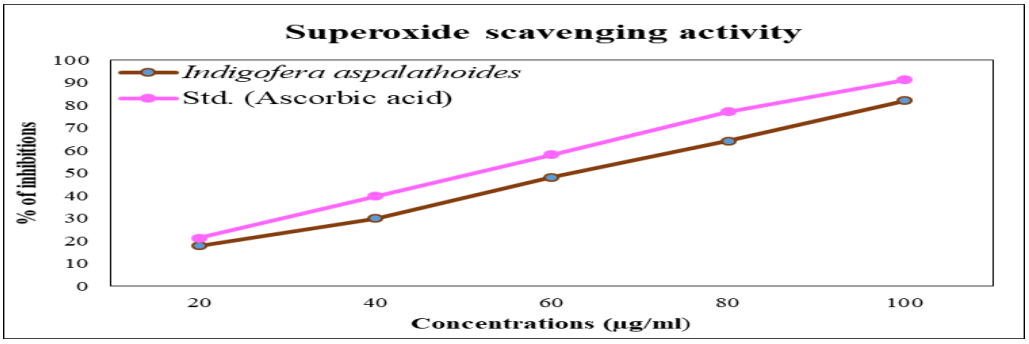

Figure 2: Superoxide Scavenging activity of *I. aspalathoides* leaves extract and compared with ascorbic acid

Hydroxyl radical scavenging activity

Hydroxyl radicals are highly reactive and are short-lived (Hayyan *et al.*, 2016). They are capable of inducing detrimental effects on the important macromolecules including proteins and nucleic acids. In the Haber-Weiss/Fenton reaction, hydroxyl radicals are generated from hydrogen peroxide in the presence of iron ions (Michiels, 2004). Figure 3 noticed the hydroxyl radical scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid. The leaves of *Indigofera aspalathoides* and ascorbic acid showed hydroxyl radical scavenging activity from 15.60 to 84.04% and 17.73 to 91.13% at concentration range from 20 to 100µg/ml respectively. The half inhibition concentration (IC_{50}) of *Indigofera aspalathoides* leaves extract and ascorbic acid were 60.81µg/ml and 54.40µg/ml respectively (Table 3). Similarly, many plant extracts and flavonoids including mangiferin, and naringin have been found to scavenge hydroxyl free radicals in a concentration-dependent manner (Shantabia, 2004; Lalrinzuali *et al.*, 2023). Several flavonoids synthesized by different plants as secondary metabolites have been reported to scavenge OH radicals earlier (Treml and Šmejkal,, 2016; Marković *et al.*, 2017).

Table 3: Hydroxyl radical scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid

Concerations (µg/ml)	**% of inhibitions**	
	Indigofera aspalathoides	**Std. Ascorbic acid**
20	15.60±0.08	17.73±0.21
40	28.72±0.23	36.17±0.33
60	51.06±0.48	56.38±0.55
80	67.02±0.63	74.46±0.73
100	84.04±0.85	91.13±1.02
IC_{50} (µg/ml)	60.81	54.40

Values expressed as Mean ± SD for triplicates

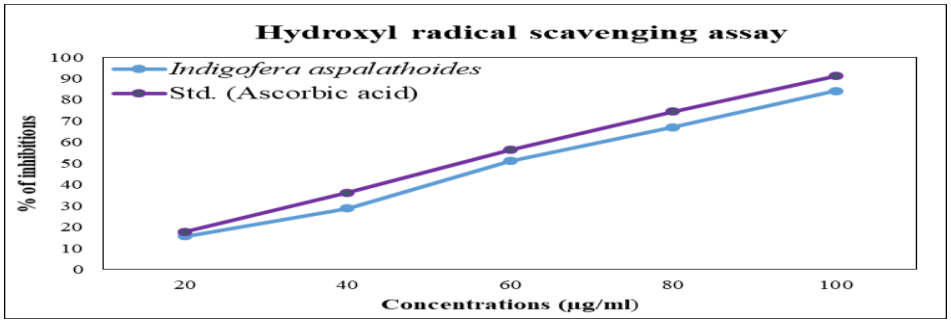

Fig. 3: Hydroxyl radical scavenging activity of *I. aspalathoides* extract and compared with ascorbic acid

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agent and tends to inactivate a free enzymes directly, usually by oxidation of essential thiol (-SH) groups. H_2O_2 can cross cell membrane rapidly once inside the cell it can react with Fe_{2+} and Cu_{2+} ions to form hydroxyl radical and this might be due to the origin of many of its toxic effects (Gutteridge, 1985). Figure 4 noticed the hydrogen peroxide scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid. The *Indigofera aspalathoides* and ascorbic acid showed maximum inhibitory activity were 83.20% and 90.94% at 100µg/ml concentration range while minimum inhibitory activity were 18.49% and 20.94% at 20µg/ml concentration range respectively. The half inhibition concentration (IC_{50}) of *Indigofera aspalathoides* leaves extract and ascorbic acid were 60.50µg/ml and 52.83µg/ml respectively (Table 4).

Table 4: Hydrogen peroxide scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Indigofera aspalathoides	**Std. Ascorbic acid**
20	18.49±0.15	20.94±0.22
40	29.24±0.23	38.67±0.38
60	50.37±0.32	56.41±0.42
80	66.60±0.50	74.52±0.71
100	83.20±0.69	90.94±0.95
IC_{50} (µg/ml)	60.50	52.83

Values expressed as Mean ± SD for triplicate

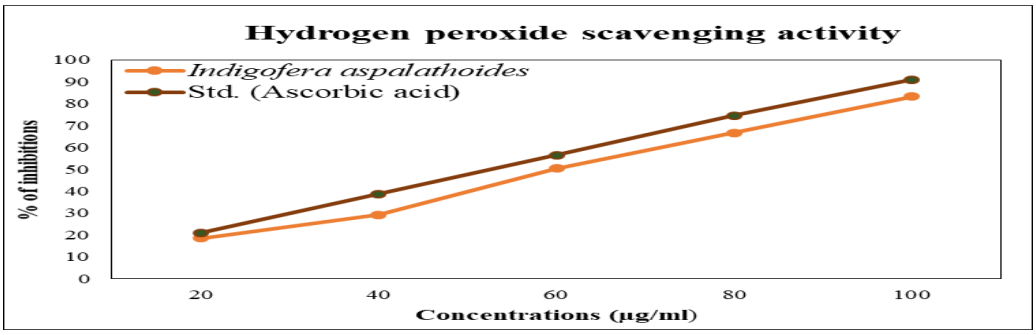

Figure 4: Hydrogen peroxide scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid

Nitric oxide scavenging activity

Nitric oxide is a potent pleiotropic mediator of various physiological processes. It is a diffusible free radical, which plays many roles as an effectors molecule in diverse biological systems (Miller *et al.*, 1993). Figure 5 noticed the nitric oxide scavenging activity of *Indigofera aspalathoides* leaves extract and compared with ascorbic acid. At the minimum concentration of *Indigofera aspalathoides* and ascorbic acid (20µg/ml), were 19.14% and 22.87%, respectively while at the maximum concentration (100µg/ml), the corresponding activities were 85.10% and 93.61% respectively. The half inhibition concentration (IC_{50}) of *Indigofera aspalathoides* leaves extract and ascorbic acid were 57.42µg/ml and 50.48µg/ml respectively (Table 5).

Table 5: Nitric oxide scavenging activity of *Indigofera aspalathoides* extract and compared with ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Indigofera aspalathoides	**Std. Ascorbic acid**
20	19.14±0.10	22.87±0.19
40	36.17±0.21	40.95±0.32
60	50.53±0.35	58.51±0.44
80	69.68±0.72	76.06±0.85
100	85.10±0.81	93.61±1.02
IC_{50} (µg/ml)	57.42	50.48

Values expressed as Mean ± SD for triplicate

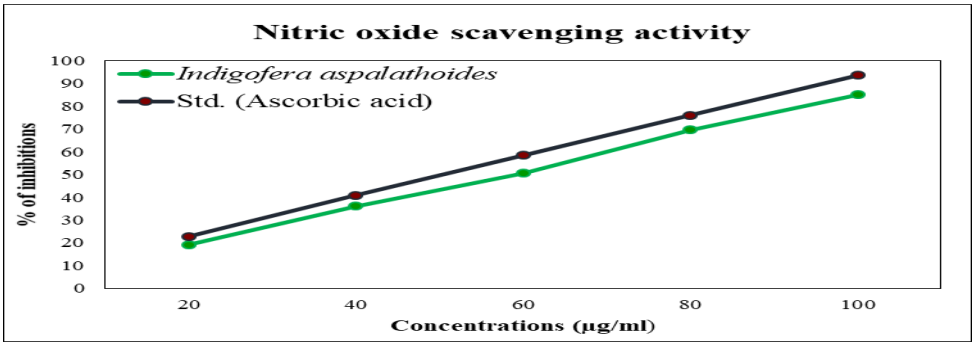

Figure 5: Nitric oxide scavenging activity of *Indigofera aspalathoides* extract and compared with ascorbic acid

Antioxidants present in plant products help in the stimulation of cellular defence system and biological system against oxidative damage. Medicinal plants are an important source of antioxidants (Rice-Evans, 2004). Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke (Prior and Cao, 2000). The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann, 1979). Radical scavenging activities are very important to prevent the deleterious role of free radicals in different diseases, including cancer. DPPH free radical scavenging is an accepted mechanism for screening the antioxidant activity of plant extracts resulting in DNA breakdown and therefore playing an important role in cancer formation (Scully, 1993).

CONCLUSION:

On the basis of the results obtained in the present study, it is concluded that an ethanolic extracts of leaves of *Indigofera aspalathoides* exhibits high antioxidant and free radical scavenging potential. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compound(s) present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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THE PROTECTIVE EFFECT OF ORAL ADMINISTRATION OF HONEY AND INDUCED DIABETIC MELLITUS AND GLUCOPHAGE ON THE SERUM BIOCHEMICAL PARAMETERS IN ALBINO RATS

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ABSTRACT

The effects of feeding honey on normal and alloxan induced diabetes rats treated and untreated were studied. In the experimental design, 25 rats were divided into five groups of five rats each, with Groups I and II serving as the normal and diabetic control, while Groups III, IV and V were the diabetic test groups administered with glucophage 500 mg/kg, glucophage in combination with honey (500 and 10 mg) and only honey (10 mg wet wt) per kilogram body weight respectively. All groups, (I - V) were fed with growers mash and water ad libitum for six weeks. The following parameters were assayed using standard methods; serum blood glucose, lipid profile, urea and creatinine. The differences observed in the serum level of HDL, triglycerides and total cholesterol in the test groups and diabetic control were statistically significantly ($p \geq 0.05$) compared to the normal control. The same was the case for low- density lipoprotein(LDL) serum level in the test groups which was statistically insignificant to the normal control while LDL serum level in diabetic control was significantly ($p \geq 0.05$) higher than the normal control. The difference in the LDL and total cholesterol level in the test groups were statistically significant to the diabetic control except cholesterol level of the Test III that was statistically in significant to the diabetic control. High-density lipoprotein (HDL) and triglycerides in the test groups were statistically in significant to the diabetic control. The blood glucose level in the combined therapy group (Test II) gave an acceptable range in both the fasting and 2 h postprandial compared to the diabetic and honey control group respectively. In conclusion, honey should be administered along with hypoglycemic agent in diabetic condition for use as alternative sweetener

Keywords: Diabetes, honey, glucophage.

INTRODUCTION

The diabetes mellitus is sweeteners are ingredients that add sweetness to foods. There are two categories of sweeteners: nutritive and non-nutritive sweeteners. Example of nutritive sweetener is honey (Lynn, 2001). Honey is sweet and viscous fluid produce by honey bees (genus Apis) and other insects from the nectar of flowers. Honey is also a popular sweetener and groups as a common house hold product used throughout the world. Popularity comes not only of its being a natural sweetener but also many benefits proven or unproven associated with it. It has many medicinal uses described in traditional medicine. Modern system of medicine is also finding the honey efficacious in various medicinal and surgical conditions (Frankel et al., 1998; Lubsy etal., 2003). Antimicrobial, antioxidant and wound healing properties of honey are being evaluated with successful outcome. Prevention and treatment of various infections due to wide variety of organisms and promoting surgical wound healing are some of the areas where honey is making its mark (Bansal et al., 2005). Obi et al. (1994) reported 5% v/v concentration of honey decreases the duration of diarrhoe in cases of bacterial gastroenteritis. Honey lowers glycaemic index in patients with diabetes (Chen et al., 2000; Ahmed et al., 2008). In one of the clinical trials of Type I and II diabetes, the use of honey was associated with significantly lower glycaemic

index than with glucose or sucrose in normal as well as Type I diabetes (Al-Walli, 2004). Type II diabetes had values similar to honey. Honey compared with dextrose caused a significantly lower rise in plasma glucose levels in diabetes subjects. It also causes reduction of blood lipids, homocystein levels and protein levels in normal and hyperlipidaemic subjects (Al-Walli, 2004). The active ingredient is honey is fructose. Fructose generates a small hyperglycaemic effect as it is absorbed slowly by our body as opposed to either sucrose or glucose (Brand, 2003).

Diabetes is a metabolic disorder which is due to insulin resistance or deficiency (Shulaman, 2000). It is a complex disease characterized by grossly abnormal fuel usage where by glucose is over produce by the liver and underutilized by organs. It is the most common serious metabolic disease in the world.

Type I is caused by auto immune destruction of the insulin-secreting beta cells in the pancreas, Type II diabetes, by contrast, has a different cause and it is the most prevalent while gestational diabetes occurs during pregnancy. Diabetes and its associated complication have affected about 200 million people worldwide representing 6% of the population. In diabetes condition, the blood sugar level is high, a condition referred to as hyperglycaemia. In this condition, the renal tubular glucose re absorption threshold is exceeded and glucose is excreted in urine, a process called glucosuria. The metabolic derangement is frequently associated with permanent and irreversible function and structural changes in the cell of the body, those of the vascular system being particularly susceptible. The changes lead to the development ofwell-defined clinical entities, when glucose concentration in the blood exceeds the capacity of the renal tubules to reabsorb, its forms a glomerular filtrate, glucose uria occurs. Glucose increases the osmolality of the glomerular filtrate and thus prevents the reabsorption of the water as its passes down the renal tubular system. This way, the volumes of urine is markedly increase and polyuria occurs.This in turns lead to loss of water and electrolyte which result in thirst and polydipsia (Stanley and Passmore, 1973; Allan et al., 2004). A stricking feature of diabetes is the shift in fuel usage from carbohydrate to fats. Triacylglycerols are mobilized and ketone bodies are formed to an abnormal extent. Since ketones are acids, this high concentration put a strain on the buffering capacity of the blood and on the kidney which controls the PH by excreting excess H into the urine. H excretion is accompanied by Na, K, PO4 andH2O excretion causing severe dehydration leading to a decreased blood volume. Diabetes complication may lead kidney failure thereby causing changes in urea and creatinine levels. Urea level become elevated in the blood principally due to increase in the breakdown of amino acids for energy since insulin uptake of glucose by cell is impaired (http://www.cufpallief.com/test.htm).Acceleratedketone body formation can lead to acidosis, coma and death in untreated insulin-dependent diabetes. Hyper lipidemias are common with patients with diabetes and further increase the risk of ischemic heart disease, especially in Type II diabetes. Detection and control of hyperlipidemia can reduce myocardial infarction, coronary deaths and overall mortality. Indeed, even when low density lipoprotein (LDL) cholesterol concentration is normal or slightly raised in Type II diabetes (the major abnormalities being low HDL cholesterol and high triglycerides concentrations) the LDL particles may be qualitatively different and more atherogenic than those in non diabetic patients (Watkins, 2003).

Objectives

The research is aimed at evaluating the effect of administration of honey alone, glucophage alone or their combination for use as an alternative sweetener in the management of diabetes mellitus.

MATERIALS AND METHODS

Experimental design Male albino rats, weighing 150–200 g, were obtained from the Department of Animal Science, Bharathidasan University, Trichy. They were housed in clean polypropylene cages under standard conditions of humidity (45±4%), temperature (25±20℃), and light (12 h light/12 h dark cycle).and fed a standard diet and water ad libitum. This study was approved by the Institutional Animal Ethics Committee (IAEC) (1416/PO/a/11/CPCSEA). They were kept in a well-ventilated animal house and weighed weekly for four weeks. After which they were grouped into five groups of five (Making a total of 25 rats) as follows:

Group I: Normal control: Rats were not induced and untreated. They were given normal feed and parameters from this group serve as a base live data (control).
Group II: Rats were induced with Alloxan monohydrated (0.2 ml/200 g body weight) and untreated, this serves as the diabetic control group and in addition were given normal feed/water adlibitum.
Group III: Rats were induced with Alloxan monohydrate(0.2ml/200 g body weight) there after they were treated with Glucophage hydrochloride tablet B.P500mg/100gbodyweight twice a day with urine sugar monitored, this group served as the test Group (I).
Group IV: Rats were induced with Alloxan monohydrate(0.2ml/200 g body weight and then treated with glucophage (as described in group B) for one week then treated with 1 ml of honey which was administered for 1 week this represents test Group II.
Group V: Rats were induced with Alloxan monohydrate as described above and then treated with honey (1 ml/200 g body weight) for 1 week. (Honey control).

Table1.Shows the effect of feeding honey on lipid profile in normal rats

Groups	Total cholesterol	HDL	LDL	Triglycerides
Normal control	2.06±0.15^{a}	1.86±0.21^{a}	0.36±0.1^{a}	0.96±0.5^{a}
Diabetic control	2.86±0.38^{a}	1.26±0.64^{a}	0.96±0.11^{b}	1.9±0.63^{a}
Test I	2.06±0.17^{a}	1.62±0.19^{a}	0.52±0.13^{a}	1.06±0.21^{a}
Test II	2.22±0.3^{a}	1.82±0.4^{a}	0.48±0.13^{a}	1.18±0.61^{a}
Test III	2.3±0.4^{a}	1.4±0.12^{a}	0.54±0.11^{a}	1.44±0.79^{a}

Values are mean ± SD, n = 5, Values with different superscript along a column vertically are statistically significant ($p \leq 0.05$).

Figure 1.Shows the effect of feeding honey on lipid profile in normal rats

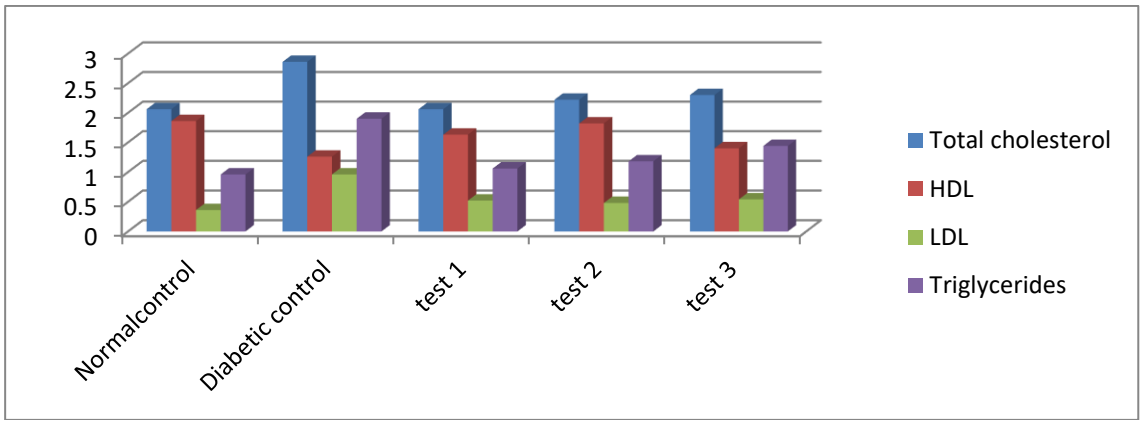
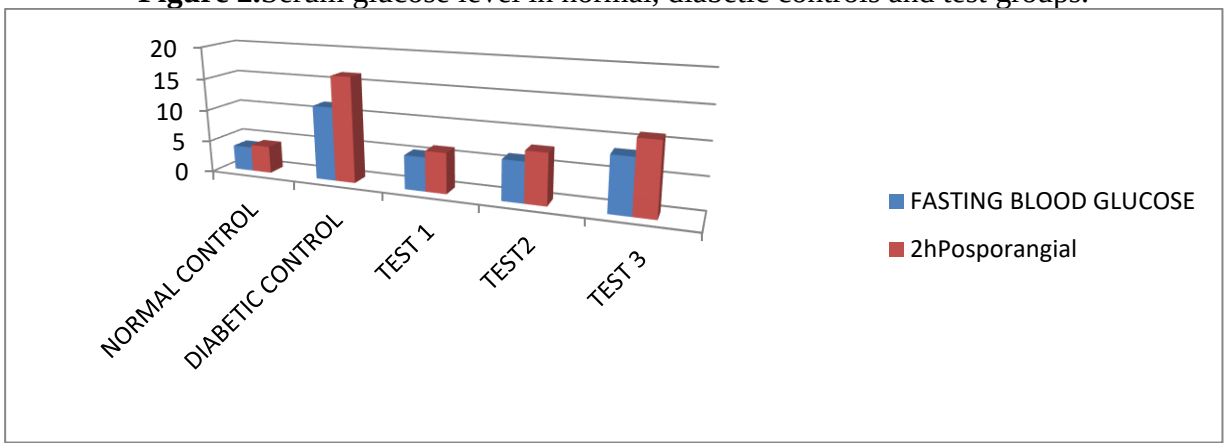


Table2.Serum glucose level in normal, diabetic controls and test groups

Parameters/Groups	Fasting blood glucose (Mmol/l)	2hPosporangial
Normal control	3.86±0.38[a]	4.26±0.64[a]
Diabetic control	11.57±2.22[b]	16.45±3.11[b]
Test I	5.28±1.33[b]	6.26±1.00[b]
Test II	6.22±1.03[b]	7.82±1.04[b]
Test III	8.44±1.66[b]	11.05±2.11[b]

Values are mean ±SD, n = 5, Values with different superscript along a column vertically are statistically significant ($p \leq 0.05$).

Figure 2. Serum glucose level in normal, diabetic controls and test groups.



Method of intubation

The intubations were done using stomach tube. The rats were maintained on a daily administration of glucophage 500 mg/100 g body weight b.d. (twice a day) 1 ml of honey /100 g body weight was administered to the test group treated with honey.

Method of blood collection (Serum)

At the end of the experiment, the rats were sacrificed and the blood was collected in a plain container. The blood was allowed to clot and centrifuged in an ultra centrifuge at 3500 r pm to obtain the serum. The serum was used for analyzing cholesterol, HDL, LDL, Triglycerides and Blood glucose level.

Determination of serum cholesterol

Free and esterified cholesterol in thes ample originates by means of the coupled reactions with a colored complex formation that was measured spectrophotometrically as described by the (National Cholesterol Program Expert Panel, 2001; Fossati and Prencipe, 1982)

Determination of high density of lipoprotein (HDL)

VLDL and LDL in the sample precipitate with phosphor tungstate and magnesium ions. The supernatant contains HDL. The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described by Bustein et al. (1980), Bucole et al. (1973) and National Cholesterol Program Expert Panel (2001).

Determination of blood glucose

The enzymatic method of glucose oxidase was used as described by Trinder (1965) and urine sugar was estimated using the clinistex test strips (Burgett, 1974).

RESULTS AND DISCUSSION

Table 1 and figure 1 shows the result of total cholesterol HDL, LDL, and total triglycerides level before and after the administration of honey. A test of significant was carried out between the normal control against the diabetic control and within test groups. The serum level of total cholesterol was significantly higher ($p \geq 0.05$) than the normal control; the same pattern was also observed in the test groups. HDL serum level in diabetic control was insignificantly lower than normal control and the same was observed down the groups. Triglycerides and LDL in the diabetic control were significantly higher than the normal control, except the serum level of LDL in the diabetic control that was significantly ($p \leq 0.05$) higher than normal control. The level of total cholesterol in the Test I was significantly lower than the diabetic control, the same was observed in Test II expect Test III that was statistically insignificant to the diabetic control.

HDL serum level in the test groups was significantly ($p \geq 0.05$) higher than the diabetic control. LDL seruml evel in the test groups was statistically significantly ($p \leq 0.05$) to the diabetic control while triglycerides serum level in the test groups was statistically insignificant to the diabetic control. In Table 2 and figure 2 the diabetic control which is the groups that have not received any treatment after diabetic induction, they have a high cholesterol level than those that received treatment. In the test groups, the group that have received treatment only glucophage have lower cholesterol level compared to those that have received both glucophage and honey or those that received only honey.

Table3. The effect of administration of honey on ASAT, ALAT and total protein in treated and untreated diabetic rats

Group	PARAMETER		
	ASAT	ALAT	TOTAL PROTEIN
Normal control	437.7±5.8	132.7±25.0	97.2±1.0
Diabetic control	486.3±108.5	167.3±44.5	95.2±18.0
Test I	426.8±29.8	143.0±41.9	90.5±2.7
Test II	377.6±41.3	117.2±30.5	82.6±64
Test III	422.7±143.1	94.7±41.9	80.3±7.6

Values are mean ± SD, n = 5, Values with different superscript along a column vertically are statistically significant ($p \leq 0.05$).

Figure 3.The effect of administration of honey on ASAT, ALAT and total protein in treated and untreated diabetic rats.

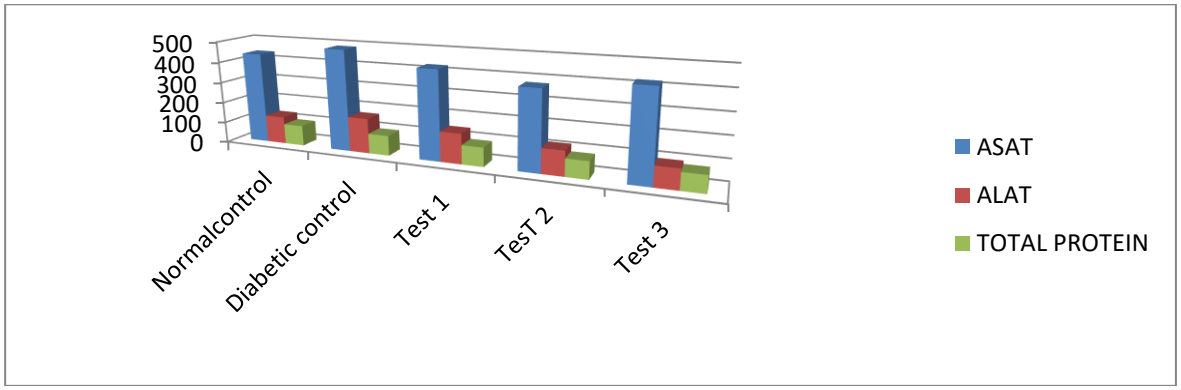
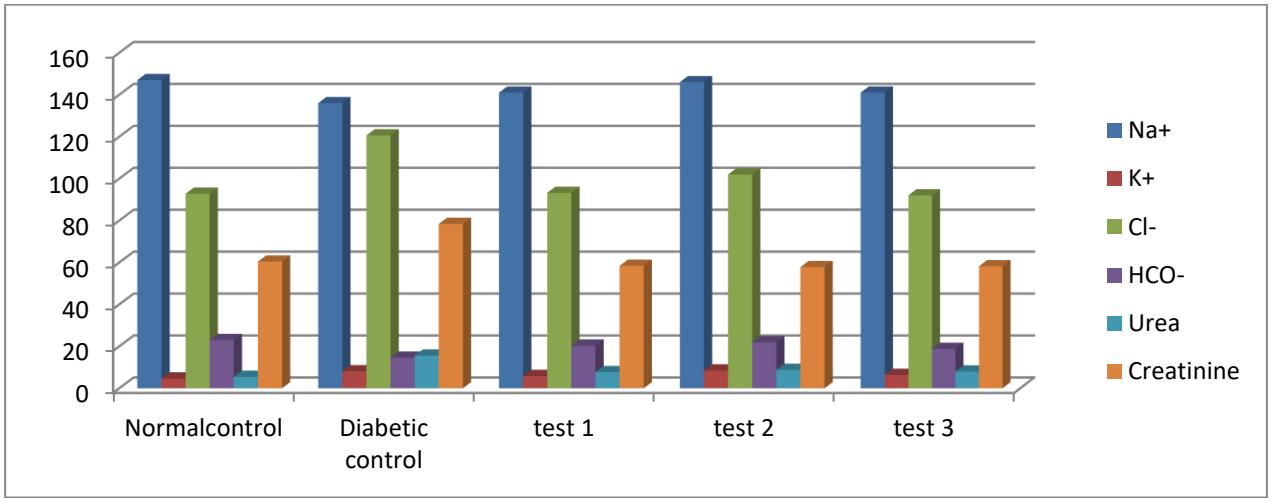


Table4.Electrolytes,urea and creatinine in normal, treated and untreated alloxan induced diabetic rats

Group	Parameter					
	Na^+	**K^+**	**Cl^-**	**HCO^-**	**Urea**	**Creatinine**
Normal control	147.2±1.3	4.7±0.4	92.8±1.3	23.0±2.1	5.5±0.7	60.4±4.7
Diabetic control	136.0±3.8	8.1±0.7	120.6±4.2	14.6±0.6	15.6±3.1	78.4±4.2
Test I	141.2±1.9	5.9±0.6	93.2±2.6	20.4±1.1	7.7±0.9	58.4±3.5
Test II	146.6±7.1	8.5±1.3	102.0±7.5	22.0±4.3	8.8±3.4	57.8±2.4
Test III	141.2±0,8	6.4±0.8	92.0±2.8	18.8±1.8	8.0±3.3	58.2±4.9

Values are mean ± SD, n = 5, Values with different superscript along a column vertically are statistically significant ($p \leq 0.05$).

Figure 4.Electrolytes, urea and creatinine in normal, treated and untreated alloxan induced diabetic rats



The HDL also shows that it is higher in the group that received honey alone or honey with glucophage.LDL is higher in the diabetic control than in the group that received treatment. While those that received only honey have the lowest LDL level. Triglycerides are higher in the diabetic group when compared to the test groups. While in the test groups, the group that received combination therapy with honey and glucophage has a higher value of triglycrides compared to those that received only honey or glucophage.

Table 2 shows the serum blood glucose level of in the test groups in compares' with the normal and diabetic control. The blood glucose of the diabetic control is significantly higher when compared to both the normal control and the all the test groups, both at fasting and2 h postprandial. This is consistent with earlier reports by Modu et al. (2008). The group that was given administered only glucophage, had a near normal blood glucose level both at fasting and 2 h postprandial compared to diabetic and honey control (Test III). This effect might be attributed to the increased peripheral absorption of glucose by Glucophage (Sfikakis,1988).But the group that was administered glucocphage in combination with honey recorded a slightly higher glucose level both at the fasting and 2 h postprandial. Even though the increase is within the normal range.This shows that honey when used in combination with a hypoglyceamic drugs, can serve as an alter native sweetener (DaisyandEzira,2007).While the group that served as the honey control, recorded much higher blood glucose concentration both at fasting as well as 2 h postprandial

compared to the normal control and Test I and II respectively. This result revealed that, the use honey singly in diabetic condition will result into hyperglyceamia and its continuous use under such conditions might result into complications associated with diabetes mellitus.

Table 3 and figure 3 ALAT level is found to be higher in the normal control than in the test groups, while the diabetic controls have the highest level of ASAT. The ALAT is higher in the normal than the test group. The diabetic controls also have higher value of ALAT than the test group. The diabetic controls have higher ALAT valuet han the other test groups but the group that received only honey have the lowest ALAT concentration, followed by the group that received both glucophage and honey. The normal controls have higher concentration of total serum protein than the other groups. The diabetic control also has higher on concentration of total protein compared to the other test groups. The group that received only honey has the lowest total protein level. From Table 4 and figure 4 the normal control has higher Na and HCO3 concentration than all the other groups, while creatinine level is higher in the normal control than the test group, but the creatinine on the other hand is higher in the diabetic control than in the test groups as well as the normal control. The K level is higher in the diabetic control and those taking only honey, while it is lower in the normal control group. The normal control has lowest urea level, while diabetic control has the highest urea level which is followed by the group treated with only honey.

CONCLUSION

Our data suggest that, the honey could inhibit the diabetes-induced damages in diabetic mellitus and Glucophage Moreover, honey administration showed better results versus other forms of application. Thus it could be suggested that simultaneous administration of honey could be considered as appropriate form of application, as the testes of honey-received groups were manifested with improved histological features. Moreover, honey could improve diabetic mellitus and Glucophage activities partly by regulating diabetic mellitus and Glucophage levels.

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RAW AND PASTEURIZED COW'S MILK COLLECTEDAT THE DAIRY FARMS IN THANJAVUR (SOUTH INDIAN)

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ABSTRACT

Milk is a widely consumed food that supports biological functions and growth, especially in children. This study aimed to evaluate the microbiological, chemical, and sanitary quality of raw and processed milk in Thanjavur, South India. Twelve milk samples were randomly selected, comprising three raw and three processed (two pasteurized and one UHT-treated) samples. These samples were analysed for microbial contaminants, including coliforms, Escherichia coli (E. coli), Staphylococcus aureus, and other pathogens, using standard microbiological techniques. Chemical and physicochemical tests such as alcohol, clot-on-boiling, and acidity tests were also performed, along with the detection of adulterants like formalin and sucrose. The specific gravity and fat content of raw and pasteurized milk samples were determined, and water content, total soluble solids (TSS), fat, lactose, protein, and ash were also analysed. The results showed that both raw and pasteurized milks exhibited substandard chemical and sanitary quality, with high bacterial contamination levels, including pathogenic strains. However, UHT-treated milk demonstrated excellent chemical quality and was free from bacterial contamination. Additionally, some raw and pasteurized milk samples were found to be adulterated with water and sucrose. These findings underscore the significance of pasteurization and UHT treatment in reducing microbial risks and ensuring milk safety, while also highlighting the ongoing issue of milk adulteration in Thanjavur.

Keywords: Adulteration, bacterial distribution, chemical composition, pasteurized milk, sanitary quality, titratable acidity, UHT-milk

INTRODUCTION:

Milk is often regarded as one of nature's most complete foods (O'Mahony, 1988), given its rich composition of proteins, fats, carbohydrates, vitamins, and minerals, all of which play a vital role in promoting growth, development, and overall health. Its significance as a dietary staple cannot be overstated, as it provides essential nutrients that support various bodily functions.

However, milk's nutrient-rich environment also makes it highly susceptible to bacterial contamination, rendering it a highly perishable (Kim*et al.*, 1983; Steele*et al.*, 1997) product. This inherent perish ability, coupled with the widespread issue of milk adulteration, particularly in developing regions, necessitates rigorous evaluation of its quality to ensure both safety and nutritional integrity.

Consumers prefer wholesome and nutritious food produced and processed in a sound and sanitary manner, such that it is free from pathogens. To meet consumer demand, the production of quality milk is essential. Quality milk is defined as milk that has a normal chemical composition, is completely free from harmful bacteria and toxic substances, and is devoid of sediment and extraneous matter. It should have a low degree of titratable acidity, a good flavor, adequate preservation quality, and a low bacterial count. Raw milk, when freshly secreted from a healthy animal, naturally contains a relatively low bacterial load, typically ranging from 500 to 1,000 bacterial cells per milliliter. However, this count can increase exponentially if the milk is exposed to unsanitary conditions during milking, handling, and storage. Bacterial populations can

rapidly escalate, sometimes reaching millions of cells per milliliter, especially in cases where hygiene practices (Khan *et al.,* 2008) are inadequate or the animals themselves are diseased. Among the various bacterial groups that may proliferate in contaminated milk, coliform bacteria, such as *Escherichia coli* (E. coli), are of particular concern, as their presence is often indicative of fecal contamination and poor sanitary practices.

The ingestion of raw or improperly pasteurized milk poses a significant risk to public health (Ryser, 1998), as it may harbor a range of harmful pathogens, including *Salmonella*, *Listeria*, *Campylobacter*, and various strains of *E. coli*. These microorganisms are known to cause severe food borne illnesses, with symptoms that can range from gastrointestinal distress to more serious conditions such as systemic infections or neurological damage. Moreover, the consumption of milk tainted with such pathogens is linked to outbreaks of diseases such as bovine tuberculosis, brucellosis, anthrax, and listeriosis, further highlighting the gravity of the situation.

Milk is an excellent growth medium for bacteria and can easily be contaminated from various sources, including the udder and body of cows, dust from the air, litter, floor, flies, insects, and rodents, water supply, hands and clothes of the milker, utensils, bottles, and the atmosphere (Ensminger*et al.,* 1994; Heinemann, 1919; Cousin, 1982). Therefore, milk's ability to support bacterial growth makes it a particularly challenging product to handle. While pasteurization—heating milk to a specific temperature for a set period of time—can effectively destroy most harmful microorganisms, the risk of recontamination remains. If the milk is exposed to unsanitary conditions post-pasteurization, bacteria can proliferate once again, undermining the benefits of heat treatment.

Consequently, the efficacy of pasteurization in ensuring the safety of milk is heavily reliant on maintaining proper hygiene during all stages of processing and storage. Thus, milk and dairy products can be important sources of food borne pathogens (Oliver *et al.,* 2005; De Buyser*et al.,* 2001; Harrington et al., 2002).

In addition to microbial contamination, milk quality can be compromised through adulteration. In some cases, water or other substances are added to milk to increase its volume. This not only dilutes the milk's nutritional value but can also introduce harmful chemicals or contaminants, depending on the substances used. Adulteration is particularly prevalent in regions with informal or unregulated milk markets, where oversight and quality control systems are often lacking or inadequate. In such contexts, consumers may unknowingly ingest substandard or unsafe milk, putting their health at risk (sivapalasingams*et al.,* 2004).

Given the multiple challenges associated with milk quality—ranging from bacterial contamination to adulteration—routine monitoring is essential to safeguard public health. According to World Health Organization (WHO) standard and other scientific words, quality ilk contains 2.6%fat, 3.5% protein,0.17 Titratable Acidity(TA) ,7.71% solid-not-fat(SNF) and SG 1.030, total bacterial count $1.3x10^6$cfu/l. The PH 6.6 ensures the ilk freshness at boiling point 100^{o}c to 117^{o}c of milk with different substances especially with water. This is especially critical in regions where milk production is predominantly carried out by smallholder farmers, and formal regulatory structures may be minimal or nonexistent. Effective testing protocols, coupled with stricter enforcement of quality control measures, are needed to ensure that milk reaching consumers is both safe and nutritionally adequate.

Efforts to address these concerns should focus on improving milk quality at every stage of the supply chain, from farm to table. This may involve enhanced education and training for milk producers regarding hygiene practices, the establishment of robust regulatory frameworks, and the implementation of regular testing for both microbial contamination and adulteration. Only through these concerted efforts can the dairy industry effectively meet the growing demand for

safe, high-quality milk and dairy products, ultimately protecting public health and promoting consumer confidence.

METHODS

Collection of sample:

Milk was typically sold in two forms: raw milk, which is brought by farmers in open pots and sold directly in markets without processing, and processed milk, which is collected by companies, pasteurized or UHT-treated, and packaged for retail sale. In this study,12 milk samples were randomly selected for analysis—6 raw milk samples (R-1 to R-6) from local vendors and farms, and 6 processed milk samples, including 4 pasteurized (P-7to P-9) and 3 UHT-treated (U-10,U-11,U-12) from different brands. These samples were transported to the laboratory under cold conditions and tested for coliforms, Escherichia coli (E. coli), and Staphylococcus aureus using culture methods, biochemical tests, and bacterial identification techniques. The study aims to compare the microbial contamination in raw and processed milk, providing insights into the safety and hygiene of different milk types.Before sample collection, the sampling glass bottles were sterilized in an autoclave for 15–20 min at 120 °C. Then 250 mL of raw milk samples in sterile glass bottles and 250 mL of plastic bottles of pasteurized milk were collected aseptically. Then, samples of raw and pasteurized milk were placed in the icebox (4 °C) for transportation. Milk samples were mixed thoroughly before analysis to ensure the homogeneity of the samples. In addition, analysis was performed within 24 h of sampling.

Experimental Procedure:

Chemical Analysis:

Preliminary quality analysis of milk:

It looks like you're describing the process for testing the quality of milk using three different methods: alcohol test, clot on boiling test, and total acidity test. These methods are part of the AOAC (Association of Official Agricultural Chemists) standard procedures for determining milk quality. Here's a breakdown of each method:

Alcohol Test:

This test involves mixing equal volumes of milk and 68% ethanol in a test tube. If the milk coagulates (clots) and fine particles appear after inverting the tube several times, the result is considered positive. This indicates that the milk may have a higher level of acidity or has been contaminated by microorganisms, causing it to react with alcohol.

Clot on Boiling (COT) Test:

In this test, the milk sample is boiled in a test tube. If the milk coagulates or forms curds after boiling, it indicates a positive result. This suggests that the milk has a higher acidity, which could be a sign of spoilage or fermentation.

Acidity Test:

For the acidity test, lactic acid in the milk is neutralized with a 0.1 N sodium hydroxide (NaOH) solution. The amount of NaOH required to neutralize the lactic acid is measured, and from this, the percentage of lactic acid in the milk is calculated using the provided equation:

This equation gives the acidity percentage in the milk based on the volume of NaOH needed for neutralization. Higher acidity generally indicates older or spoiled milk.

$$\text{Acidity (\%)} = \frac{\text{0.1N NaOH (ml)} \times 0.009}{\text{ml of milk sample}} \times 100$$

Physiochemical quality analysis

The physicochemical quality of raw and pasteurized milk (specific gravity, freezing point, TSS, lactose, protein, lacto reading, fat, snf, and ash content of milk) was determined using a

calibrated milk analyzer (Lacto scope). The total solid content was calculated using the Formula: % total solid = %fat+% snf. The other parameters, pH and temp, were determined using a digital multiparameter probe.

Milk Fat

Gerber method was used to determine the milk fat content. Milk samples were kept at 84°F for pasteurized milk in a water bath (Raw milk room temp).Ten ml of concentrated sulphuric acid was pipetted into a butyrometer. Then, 10.7 ml of milk was added using milk pipette into a butyrometer having the sulphuric acid, and then one milliliters of amyl alcohol was added. The butyrometer stopper was put on and the sample was shaken and inverted several times until all the milk was digested by the acid. The sample was placed in a Gerber centrifuge for five minutes at 1100 rpm. Finally, take the butyrometer from the Gerber centrifuge, and measure the fat percentage was read from the butyrometer.

Solids-not-fat (SNF):

The solids not fat (SNF %) was determined with the equation below by subtracting the percent fat from total solids.

Total solids

To determine the total solids, five grams of milk sample was place in a pre-weighed and dried triplicate of crucibles. The samples were kept at 102°C in a hot air oven overnight. Then, the dried samples were taken out of the oven and placed in a desiccators

Total protein:

Formaldehyde titration method was used to determine the total protein content. Ten ml of milk was added into a beaker. Then, 0.5 ml of 0.5 percent phenolphthalein indicator and 0.4 ml of 0.4 percent Potassium Oxalate was added into the milk. Then, the sample was titrated with 0.1N Sodium Hydroxide solution. The titration was continued until pink color becomes intense. Finally, the burette reading was recorded. The reading was multiplied by a factor 1.74 and total protein was calculated using the formula.

Total protein-Burette reading×1.74

Lactose

Percent lactose was determined by subtracting the fat, protein and total ash percentages from the percentage of the total solids [1].

Percent lactose Percent total solids-(%fat+%protein+% total ash)

Analysis of Milk Adulteration:

Adulterants such as carbonate, starch, formalin, hydrogen peroxide, sodium chloride, sugar, urea and addition of water were determined using standard procedure.

1. **Neutralizers**: 20 mL of milk was evaporated in a silica crucible and burned in a muffle furnace. The ash was dissolved in 10 mL distilled water and titrated with N/10 hydrochloric acid using phenolphthalein. A titre value exceeding 1.2 mL indicated adulteration with neutralizers.
2. **Formalin**: 10 mL of milk was mixed with 5 mL concentrated sulfuric acid. A violet or blue ring at the interface indicated the presence of formalin.
3. **Sucrose**: 10 mL of milk was combined with 5 mL hydrochloric acid and 0.1 g resorcinol, heated for 5 minutes in a boiling water bath. A red color indicated the presence of added sugar.
4. **Starch**: 3 mL of milk was boiled, cooled, and treated with 2-3 drops of 1% iodine solution. A blue color confirmed the presence of starch.

5. **Glucose**: 3 mL of milk was mixed with 3 mL Barford's reagent, heated for 3 minutes, cooled, and treated with 1 mL phosphomolybdic acid. A blue color confirmed the presence of glucose.
6. **Salt**: 5 mL of 0.8% silver nitrate was mixed with 2-3 drops of 1% potassium dichromate and 1 mL of milk. A yellow color indicated the presence of salt, while a chocolate color indicated its absence.

Microbial Analysis: Standard Plate Count (SPC) method recommended for dairy products (APHA 1960) was followed for quantitative analysis of bacteria: Enumeration of total viable bacteria: Nutrient agar medium (Difco) was used for enumeration of total viable bacteria. PH of the medium was adjusted at 6.8 prior to sterilization. Inoculated plates were incubated at 37°C for 24 to 72 hours to facilitate viable bacterial growth. After incubation, the inoculated plates having 30 to 300 colonies were considered for counting using colony counter (Gallenkamp, England) and following back calculation total count was expressed as colony forming units per milliliter (cfu/ml).
Microbial Counts: The pasteurized milk samples were assessed for Total Plate Counts (TPC) and Coliform Counts (CC). Dilutions were selected so that total number of colonies on a plate was between 30 and 300 for TPC, while for CFC; dilutions were selected for plate counts between 15 and 150.
Total Plate Count (TPC): Homogenized pasteurized milk sample was serially diluted by adding 1mL into 9 mL of Maximum Recovery Diluent, until a solution is obtained that is expected to give a plate count between 30-300. One millilitre of the sample from a chosen dilution was placed on the petri dish with pour plated in nutrient agar (10-15 ml) allowed to solidify for 15 min and incubated for 48 hours at 37°C . Finally, the counts were made using digital colony counter. The plate counts were calculated by multiplying the count on the dish by 10n, in which n stands for the number of consecutive dilutions of the original sample.
Enumeration of Total Coliform Bacteria: Total coliform was determined by the same method used in the enumeration of total viable bacteria. The medium used for coliform was MacConkey agar. Inoculated plates were incubated at 37°C for 24 hours. After incubation, typical pinkish and centrally red colonies were counted by using colony counter and total coliform was calculated.
Enumeration of Total Fecal Coliform Bacteria: Fecal coliform (much) agar medium was used for the enumeration of fecal coliform. The media were inoculated and after incubation at 44°C for 24 hours, typical bluish colonies were counted using colony counter and using back calculation, total fecal coliform count determined.
Enumeration of Total Staphylococcus Bacteria: Staphylococcus medium was used for the enumeration of Staphylococcus bacteria. Media were inoculated and after incubation at 37°C for 24 hours, colonies were counted using colony counter and following back calculation, total Staphylococcus count was obtained in cfu/ml.
Enumeration of Total Psychrophilic Bacteria: Nutrient agar medium was also used to enumerate total psychrophilic bacteria. Inoculated plates were incubated at 4°C for 15 days to facilitate the growth of psychrophilic bacteria. After incubation, colonies were counted using colony counter and following back calculation, total psychrophilic bacterial count was determined.

Morphologically dissimilar well-spaced colonies were picked up with the help of a sterile loop from the plates, which had from 30 to 300 colonies. Each colony was streaked on to freshly prepared plates of the same media and incubated at 37°C for 24 hours or more. After incubation, typical pure colonies were taken as isolates. The selected isolates were then purified through repeated streak plating. When plating produced only one type of colony in a particular plate, it

was considered to be pure. The purified isolates were then transferred to nutrient agar slant in one drum screw capped culture vial and preserved as stock culture.

Identification was done up to genus by following the 'Bergey's manual of determinative bacteriology,' (Buchanan and Gibbons 1974). For identification, different morphological characteristics including shape, size, form, texture, opacity, edge, elevation of the isolated colonies were studied carefully and after Gram staining, microscopic examination was carried out.

The biochemical tests performed were catalase test, oxidase test, methyl-red test (MR Test), Voges-Proskauer test (VP Test), production of hydrogen sulphide (KIA Test), and hydrolysis of starch and fermentation tests.

RESULT AND DISCUSSION

Chemical Composition

The percentage of water, total soluble solids (TSS), fat, solids-non-fat (SNF), lactose, protein, and ash has been presented in Table 1. Five (R-1 and R-6) of the six raw milks contained more than 80% water which is above the usual range i.e. 84.0 to 90.0% (Eckles*et al* 1951), suggesting that they were adulterated with water. Among the heat-treated milk, only P-8 contained high percentage of water i.e. 90% (Table 1). Addition of water dilutes milk reducing its TSS content. Reduced TSS was observed in five raw (R-2,R-4) and one pasteurized (P-7) milk; none of these samples had TSS over 9.5% though milk TSS usually ranges from 10.5 to 14.5% (O'Mahony 1988). The UHT-milks were comparatively rich in TSS content each having at least 11.0% TSS (Table 1).

Commercially, the fat of milk is unquestionably the most valuable commercially; the fat of milk is unquestionably the most valuable constituent of milk. Milk having a fair amount of fat is more valuable as a food than milk which is poor in fat. The Food and Drug Administration (FDA) requires not less than 3.25% milk fat for fluid whole milk. The U.S. public health service (USPHS) Milk Ordinance and Code also recommended a minimum of 3.25% butterfat in farm milk (Graf 1976). However, in this study, one of the raw milks (R-2,R-6) contained less than 3.25% fat. The other raw milk , however, satisfied the criterion each having at least 3.3% fat. The fat content of pasteurized milk is a minimum of 3.5% which is fulfilled by only one (P-8) of the three pasteurized milks. The other two had fat contents of 3.34% and 3.4% respectively. The fat content of U-1, one of the UHT-processed milks, was even not less (3.09%). Data have been presented in Table 1.

FDA standard for SNF content of whole milk is a minimum of 8.25% (Graf 1976). None of the raw milks maintained this standard. Five of these even contained SNF of less than 6.5% indicating that these might have been adulterated with water. The pasteurized milks also failed to maintain the minimum SNF requirement set by fssai which is 8.0%. Two (P-6) of these had SNF values of more than 7%, whereas SNF of the other (P-7) was exceptionally low, 5.83%. In case of the UHT processed milk, two (U-6) had SNF contents of more than 8.0.Table 1).

The percentage of lactose of most of the raw milks was around 4.0%, similar to that reported by Lingathurai et al (2009). The lactose content of milk though can range from 3.6 to 5.5% (O'Mahony 1988). The specifications for pasteurized milk, established by fssai require at least 4.4% lactose in milk. All the three pasteurized milks fulfilled the requirement. The lactose content of the UHT-milks was even higher, around 4.8%, the highest being 4.5% obtained in U-6 (Table 1).

Table 1: Chemical composition of the samples.							
Sample	**% of constituents**						
	water	**TSS**	**Fat**	**SNF**	**Lactose**	**Protein**	**Ash**
R-1	86.0	10.0	3.75	7.69	5.30	3.09	0.82
R-2	90.0	8.0	3.0	7.83	4.38	3.10	0.71
R-3	89.0	10.98	4.1	7.97	4.10	3.29	0.81
R-4	90.1	9.08	3.37	7.79	4.89	3.57	0.70
R-5	90.39	11	4.2	8.0	4.38	3.3	0.72
R-6	83.9	10.6	3.08	7.8	4.08	3.19	0.65
P-7	89	8.09	3.34	7.99	4.4	3.24	0.73
P-8	90	12.7	3.2	5.9	4.78	3.0	0.69
P-9	88.08	11.6	3.45	8.4	4.56	3.36	0.68
U-10	88.9	12.1	3.56	8.5	4.39	3.7	0.71
U-11	89.0	10.8	3.41	8.43	4.84	3.59	0.70
U-12	90.09	11.9	3.4	7.99	4.55	3.44	0.72
R=Raw milk; P= Pasteurized milk; U=UHT-Treated milk; TSS=Total Soluble Solids; SNF=Solids-not-Fat.							

The protein content of the raw milks varied from 3.0% to 3.6 %. Lingathurai*et al* (2009) reported slightly higher (3.77%) protein content. The three pasteurized milks were of acceptable quality with respect to protein content. All the pasteurized milks satisfied this requirement each containing a minimum of 3.35% protein. The UHT-milks were also of good quality regarding protein content each having at least 3.4% of protein (Table 1).

It was interesting to find that the raw milks, though inferior in fat, sugar and protein contents in most cases, had minerals greater than the pasteurized milks. The ash content of the raw milks varied from 0.69% to 0.8% which falls within the usual range of 0.6 to 0.9% (O'Mahony 1988). But It is higher than that (0.33- 0.69%) found by Elmagli and El Zubeir (2006). Ash content of the pasteurized milks ranged from 0.64% to 0.71%,. On the other hand, two of the UHT-milks (U-6) were quite rich in the mineral content, each containing 0.75% of ash.

Table 2 depicts the titratable acidity of the sample.

Table 2: Titratable acidity of the samples.	
samples	**Titratable Acidity (% lactic acid)**
R-1	0.218
R-2	0.120
R-3	0.168

R-4	0.148
R-5	0.171
R-6	0.148
P-7	0.131
P-8	0.144
P-9	0.128
U-10	0.184
U-11	0.170
U-12	0.184

Titratable acidity is a measure of freshness and bacterial activity in milk. Popescu and Angel (2009) reported that high quality milk has to have less than 0.14 percent acidity. The acidity of the raw milk samples varied largely from one sample to another. The highest value was 0.136 % (R-1) indicating high bacterial activity and the lowest was 0.116 % (R-2) indicating it's relatively better quality with regards to freshness. The acidity of the pasteurized milks ranged from 0.128 % to 0.160%. Elmagli and El Zubeir (2006) observed a greater range of acidity (0.14 to 0.86%) in pasteurized milks. No bacteria were found in the UHT-milks U-10 and U-12, but both these showed high degree of titratable acidity (0.189% and 0.175% respectively) suggesting that the high acidity might have developed prior to the heat treatment (Table 2).

Titratable acidity of milk has long been recognized and employed as an indicator of quality (Jaynes et al 1980). It is expressed in terms of percentage lactic acid since lactic acid is the principal acid produced by fermentation after milk is drawn from the udder. Fresh milk, however, does not contain any appreciable amount of lactic acid and therefore an increase in acidity is a rough measure of its age and bacterial activity (O'Mahony 1988; Lampart 1947). Within a short time after milking, the acidity increases perceptibly due to bacterial activity. The degree of bacterial contamination and the temperature at which the milk is kept are the chief factors influencing acid formation. Therefore, the amount of acid depends on the cleanliness of production and the temperature at which milk is kept. For this reason, determination of acid in milk is an important factor in judging milk quality. Acidity affects taste as well. When it reaches about 0.3%, the sour taste of milk becomes sensible. At 0.4% acidity, milk is clearly sour, and at 0.6% it precipitates at normal temperature. At acidity over 0.9%, it moulds (Heineman 1919; Tzouwara-Karayanni 2000).

Adulteration

Result for the presence of adulterants is given in table 3. No neutralizer, preservative, added sugar, glucose, starch, or salt was found in raw milks. Five (R-2, R-3 and R-5) of the raw milks. however, had been adulterated with water which is very common in village in thanjavur particularly in case of raw milk. Addition of water dilutes the amount of total solids in milk and it also involves the danger of introducing germs into milk including the pathogens. Adulteration of milk with water therefore may introduce chemical or microbial hazards to health. It reduces nutritional and processing quality, palatability as well as marketing value of milk (Swai and Schoonman 2011).Water had also been added in one (P-8) of the pasteurized milks. The other adulterant detected was added sugar (sucrose) which was found in five (R-3,R-6,R-2) of pasturised milk.

Table 3: presence of adulterants.							
Samples	Added water	neutralizer	formalin	sucrose	starch	Glucose	salt
R-1	-	-	-	-	-	-	-
R-2	+	-	-	+	-	-	-
R-3	+	-	-	+	-	-	-
R-4	-	-	-	-	-	-	-
R-5	+	-	-	-	-	-	-
R-6	-	-	-	+	-	-	-
P-7	-	-	-	-	-	-	-
P-8	-	-	-	+	-	-	-
P-9	+	-	-	-	-	-	-
U-10	-	-	-	-	-	-	-
U-11	-	-	-	+	-	-	-
U-12	-	-	-	-	-	-	-
"-"=Absent		"+"= Present					

Microbial analysis of milk samples:

All the raw milks had high bacterial load which ranged from 1.60×10^6 to 1.20×10^8 cfu/ml. The most frequent cause of high bacterial load is poor cleaning of the milking system. Bacterial count was high due to milking dirty udders, maintaining an unclean milking and housing environment, and failing to rapidly cool milk to less than 50°F. The TVBC (total viable bacterial count) of the pasteurized milk samples ranged from 8.3×10^7 to 1.26×10^8 cfu/ml. (Jay 2003). The reason for high bacterial count in the pasteurized milks may include defective pasteurization machinery, surviving pasteurization, and post-pasteurized contamination due to poor processing and handling conditions and/or poor hygienic practices by workers. However, TVBC of each of the UHT processed milks was nil, indicating their excellent sanitary quality (Table 4).

Results revealed that Total Plate Counts (TPC) were not significant between the samples. However, one of the samples (S) is significant at $p<0.07$ in terms of Coliform Counts (Table 1). Total plate count is the most accurate method for counting live microorganisms in raw milk and heat-treated milk [17]. Milk produced under hygienic conditions from healthy cows should not contain more than 6×10^4 bacteria per millilitre. Therefore, the total bacterial counts of the collected samples were higher than acceptable standard, which could be associated with poor efficiency of pasteurization. Coliform bacteria (CC) could contaminate milk from dung, bedding materials, polluted water used for cleaning, soil and inadequately cleaned milking utensils. This could possibly expose the milk to high risk of contamination, which in turn increases the microbial count. Coliform count is especially associated with the level of hygiene during production and subsequent handling since they are mainly of fecal origin.

Coliforms do not survive pasteurization and their presence in the pasteurized milks indicates recontamination after pasteurization. If coliform count of any milk is higher than a certain level, say over ten coliform organisms per millilitre of pasteurized milk, it means the milk was produced under improper procedures. The existence of coliform bacteria in high proportion is suggestive of unsanitary condition or practices during processing.

Table 4: Distribution of bacteria (cfu/ml)

sample	TVBC (cfu/ml)	TCC (cfu/ml)	TFCC (cfu/ml)	TSC (cfu/ml)	TPBC (cfu/ml)
R-1	1.20×10^8	$1.27X10^6$	$3.0X10^5$	$2.66X10^5$	$1.99X10^5$
R-2	$3.0X10^6$	$5X10^8$	$10.5X10^5$	$1.90X10^5$	$9X10^4$
R-3	3.0×10^7	$4.5X10^7$	$2.8X10^6$	$1.3X10^6$	$1.6X10^4$
R-4	$2.5X10^6$	$5.4X10^8$	$1.9X10^5$	$1.58X10^6$	$1.9X10^5$
R-5	3.3×10^7	$3.6X10^8$	NIL	$1.53X10^6$	$2.0X10^4$
R-6	$1.23X10^8$	$2.09X10^8$	$4.8X10^3$	$5.4X10^6$	$1.12X10^3$
P-7	$8.3X10^7$	NIL	NIL	$1.0X10^9$	$1.96X10^2$
P-8	1.26×10^8	$3.0X10^7$	$1.9X10^3$	$8.1X10^6$	$4.0X10^4$
P-9	$7.5X10^8$	$1.27X10^7$	$5.0X10^3$	$1.6X10^6$	$1.87X10^5$
U-10	NIL	NIL	NIL	NIL	NIL
U-11	NIL	NIL	NIL	NIL	NIL
U-12	NIL	NIL	NIL	NIL	NIL

cfu/ml = colony forming units per milliliter, TVBC = Total viable bacterial count, TCC = Total coliform count, TFCC = Total fecal coliform count, TSC =Total staphylococcal count, = Total psychrophilic bacterial count

Coliforms are considered as 'indicator organisms' because their presence in food indicates some form of contamination. Coliform count in the raw milks ranged from 5.0×10^3 to 1.27×10^7cfu/ml. These results are higher than that obtained by Saitanu et al (1996), who found TCC (total coliform count) of <1000 cfu/ml. (Table 4)

Among the raw and pasteurized milks, two samples, R-1 and P-5, didn't have any fecal coliform but others showed quite average count, higher than that found by Sraïri et al (2006) in the raw milk of some dairy farms in tanjore . The fecal coliforms are more closely related to fecal contamination than are the total coliforms. The organisms can originate from improperly sanitized working surfaces in a processing plant. In these cases, theirpresence would reflect the quality of sanitation and not the direct pollution of the product (Banwart 2004). The UHT-milks didn't contain any fecal coliform (Table 4). A large percentage of all cases reported as food poisoning or food infection is actually Staphylococcus poisoning and many people encounter this illness during their lifetime. The staphylococcal food intoxication accounted for over 15% of all the outbreaks and almost 30% of the cases of reported foodborne illnesses in the United States in 1981 (Frazier and Westhoff 2005; Banwart 2004). In this study, Staphylococcus was found in all of the raw and pasteurized milks but not found in the UHT-milk. TSC (total staphylococcal count) in the raw milks ranged from 5.4×10^6 to 1.0×10^9 cfu/ml. These counts are less than the findings of Khan and Abdul (2002) where the mean staphylococcal counts were 4.5×10^8 cfu/ml in raw milk, but higher than that of Sraïri et al (2006).(Table 4).

TPBC (total psychrophilic bacterial count) of raw milks varied from 1.5×10^4 to 3×10^5 cfu/ml and that of the pasteurized milks ranged from 3.78×10^2 to 1.0×10^4 cfu/ml. The UHT-milks didn't possess any psychrophiles as usual (Table 4). Presence of psychrotrophs in the pasteurized milks indicates post-pasteurization contamination. Psychrotrophs are becoming increasingly dangerous to the dairy industry because they produce extracellular heat- resistant lipases and

proteases. Milk altered by the activity of these enzymatic systems is depreciated and must be eliminated from processing. TPBC is used as a supplementary indicator of milk quality. Data on TPBC are required by some dairies because of specific technological requirements and quality-dependent payment for raw milk supplies. The current EU standards for top quality milk require that TPBC shall not exceed 6,000 CFU/ml. (Cempírková 2002).A majorreason of the poor bacterial quality of the raw milks is adulteration with addition of water. Water is added to milk to increase its volume. Addition of water reduces the percentages of the soluble solids including fat and the other vital components in milk and at the same time it involves the danger of introducing germs that may even be pathogenic. Diluting milk with pure water, however, may lead only to malnutrition; but adding impure water may cause intestinal problems (KDB Training guide 2004, Kurwijila 2006).

CONCLUSIONS

The UHT-treated milks were much better than the raw and pasteurized milks particularly from sanitary point of view and two of these, U-7, were the bests of all samples considering most parameters. The hygienic standard of the raw and pasteurized milks was very poor. All the raw and pasteurized milks had high bacterial loads and some contained pathogenic bacteria. The UHT milks didn't contain any. Few of the raw and pasteurized milks were also inferior in fat content.Two adulterants, added water and sucrose, were identified in a number of raw and pasteurized milks. The presence of the pathogenic organisms, the high counts of coliforms and the high levels of adulteration are indicative of a potentially hazardous product which is likely to be posing a serious health risk to the consumers. The government therefore should conduct frequent inspection of the marketed milks to check whether they meet the minimum legal standards and should monitor the overall hygienic condition surrounding the production and handling of milk. Realistic standards for the raw milks need to be devised and appropriate training should be given to the raw milk producers in hygienic handling of milk.

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ANTI-INFLAMMATORY, ANTI –ARTHRITIC PROPERTIES OF *GLYCYRRHIZA GLABRA* L.

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ABSTRACT

High mobility group box 1 (HMGB1) is increased in osteoarthritis (OA) tissue and chondrocytes stimulated with interleukin-1β (IL-1β). Suppression of HMGB1 expression is correlated with reduced inflammatory responses induced by IL-1β. Therefore, the discovery of novel drugs for the treatment of osteoarthritis diseases is urgently needed. *Glycyrrhiza glabra* has been largely used for thousands of years in traditional Chinese medicine. *Glycyrrhiza glabra* and its derived compounds possess antiallergic, antibacterial, antiviral, anti-inflammatory, and antitumor effects. These pharmacological properties aid in the treatment of inflammatory diseases. In this review, we discuss the pharmacological potential of bioactive compounds derived from Licorice and address their anti-inflammatory and antioxidant properties. To investigate how inhibition of HMGB1 by glycyrrhizin might affect inflammatory responses and viability of OA patient–derived chondrocytes treated with IL-1β.

Key Words: *Glycyrrhiza glabra* L., Anti-inflamatory, Anti-ortheratic, Interlukines

INTRODUCTION

Glycyrrhiza glabra L. (Fabaceae) (common name liquorice) is a valuable medicinal plant. Its name derives from the Greek words 'glykos' that means sweet and 'rhiza' that means root (Biondi et al., 2003). The genus Glycyrrhiza comprises more than 30 species that are extensively dispersed worldwide mainly at Mediterranean regions of Asia (Sharifi-Rad et al., 2021). This species is a native of Mediterranean areas, but it is now also present in India, Russia, and China. The extracts are currently used in pharmaceutical and food industries Hayashi & Sudo, 2009). *G. glabra* and its bioactive phytochemicals holds the prevention of painful swellings, cough, colds, and influenza, they have also multiple pharmacological activities like, antidemulcent, expectorant, antiulcer, anti-inflammatory, anticancer and antidiabetic (Aly et al., 2005; Babushetty et al 2012; Biondi et al., 2003; Hosseini et al., 2020; Nasiri et al., 2020).

The flavonoids identified belong to different classes, including flavanones, flavones, flavanonols, chalcones, isoflavans, and isoflavanones. The major flavonoids are glycosides of liquiritigenin (4′,7-dihydroxyflavanone) and isoliquiritigenin (2′,4,4′-trihydroxychalcone), such as liquiritin, isoliquiritin, liquiritin apioside, and licuraside (Babushetty et al., 2012). Glabridin is the principal isoflavone identified, ranging between 0.08% and 0.35% of roots' dry weight (Nazari et al., 2017). The minor phenolic compounds are isoprenoid-substituted flavonoids, chromenes, coumarins, dihydrostilbenes, coumestans, benzofurans, and dihydrophenanthrenes. Furthermore, many volatile components are present in roots, such as geraniol, pentanol, hexanol, terpinen-4-ol, and α-terpineol, conferring the characteristic odour. The essential oil obtained from *G. glabra* is also rich in propionic acid, benzoic acid, furfuraldehyde, 2,3-butanediol, furfuryl formate, maltol, 1-methyl-2-formylpyrrole, and trimethylpyrazine (Chouitah et al., 2011). These kinds of flavones to induced antioxidant activity of *G. glabra* are one of the major

reasons for its uses. The phenolic content is probably responsible for the powerful antioxidant activity observed (Rackova et al., 2007). Varsha and Sonam (2013) attributed this activity to flavonoids, lawhereas Rackova et al. (2007) reported that mostly isoflavones, such as glabridin, hispaglabridin A, and 30-hydroxy-4-*O*- methylglabridin, are the responsible compounds. Biondi et al., (2003) reported a huge antioxidant activity of the dihydrostilbene derivates present in *G. glabra* leaves. These phenolic compounds are effective in the protection of biological systems against oxidative stress, being able to inhibit the onset of skin damages (Haraguchi et al., 1998). According to Castangia et al. (2015), the topical application of liquorice extract formulations may be of value in innovative dermal and cosmetic products as it counteracts oxidative stress damage, maintaining the skin homeostasis due to its high antioxidant content. Table 1 summarizes the most important studies of antioxidant activity.

In fact, the anti-inflammatory effects of glycyrrhizin were described as similar to those of glucocorticoids and mineralocorticoids. Furthermore, *G. glabra* is used in renal and liver complications on the basis of its strong anti-inflammatory effects (Xiao et al., 2010). Xiao et al. (2010) reported the inhibition of liver granuloma formation and the inflammatory cytokine production by glycyrrhizin, wherea Wang et al. (2020) described the anti-inflammatory effects on endometriosis. Moreover, Li et al. (2016) proved the anti-inflammatory activity of glabridin on RAW cells.

Rheumatoid arthritis (RA) is a chronic, inflammatory, and systemic autoimmune disease [Chunxia et al, 2011]. The primary symptoms of RA include pain, swelling, and destruction of cartilage and bone as a result of which permanent disability occur. The exact pathophysiology is still unknown but release of certain free radicals such as nitrous oxide and superoxide radicals generated as by-products of cellular metabolism. The release of such free radicals may induce the production of interleukins (IL) and tumor necrosis factor (TNF-α) from T-cells which ultimately influence the production of growth factors, cytokines and adhesive molecules on immune cells as such factors may cause tissue destruction and inflammation [Kasper et al., 2005].

In certain cases, TNF-α neutralizing agents like infliximab, etanercept etc; IL-1 neutralizing agents like anakinra; and the drugs which interfere with T-cell activation such as abatacept can also be included in treatment of chronic cases. Finally, immunosuppressive and cytotoxic drugs such as cyclosporine, azathioprine, and cyclophosphamide are used for the treatment of chronic patients [Ngoc et al, 2005; Mazumder et al, 2012; Rajkapoor et al, 2007].

To the best of our knowledge, a limited number of research works have been published on this plant, particularly in what concerns to pharmacological aspects (Fiore et al., 2008). The objective of this work has to examine the bioactive compounds of *G. glabra* and the biological activities associated with these compounds have been investigated. Further, the utility through novel drug delivery specially nanomedicine approaches, cosmeceutical application and as an animal feed alternative are well discussed and reviewed this work (Rani et al., 2021).

MATERIAL AND METHODS

Collection of plant material

The rhizome of *G. glabra* linn (Fam: Polypodiaceae) were collected from thanjavur District. The plant material was dried, powdered and stored in air tight containers until further studies.

Determination of total ash value

Accurately weighed 5gms of powdered rhizome of *G. glabra* was taken in a dried silica crucible. It was incinerated at temperature 450°C, until free from carbon and then cooled. The

weight of total ash was taken and the percentage of it was calculated with reference to the air dried sample.

Determination of acid insoluble ash value

The total ash obtained was boiled for 5 mins with 25 ml of 2N HCI, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in tarred crucible cooled and the residue obtained was weighed. Finally the percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of water soluble ash value

The total ash obtained was boiled with 25 ml of water for few mins. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 mins at temperature not exceeding 450°C. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Determination of moisture content

Accurately weighed 5gms of powdered rhizome of *G. glabra* was taken in a china dish. It was kept for 30 mins in a hot air oven at 105 - 110°C. The percentage of moisture content was then calculated.

Qualitative chemical examination of extracts

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloid reagents.

a. **Mayer's Test:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.

b. **Wagner's Test:** Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of brown or reddish brown precipitate indicated the presence of alkaloids.

Detection of flavonoids

Lead acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids

a. **Millons Test** The e tracts were treated with 2 ml of Millons reagent The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.

b. **Biuret Test**: The extract: were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation, of purplish violet color indicated the presence of proteins.

Preparation of rhizome of G. glabra extract

The Rhizome of *G. glabra* was shade dried and powdered well using a mixer and stored in an airtight container. The rhizome powdered (100g) were taken and subjected to successive solvent extraction (500ml) with Hexane, Chloroform, Ethyl acetate, Methanol, and aqueous extracts using Soxhlet apparatus. The plant extracts were concentrated by rotary evaporator and stored in an airtight vial for further studies.

Determination of inflammatory markers

To investigate the cytokines levels for osteoarthritis patients. Blood was drawn from osteo arthritis and rumatoid arthritis donors into heparinized tubes. These tubes were further centrifuged at 400 X g for 30 min at RT. To the supernatant of plasma obtained, followed by centrifugation at 3220g for 10 min at 4 C. Plasma was frozen in liquid nitrogen prior to storage at 80 C. Inflammatory cytokines interleukins (IL) are IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13 and TNF-α were measured by using Luminex bead-based multiplex ELISA assays obtained from EMD-Millipore (Milliplex MAP Assay catalogue number HSTCMAG-28SK)

according to manufacturers' instructions. Assays were read on a Bio-Plex 200 bead analyzer and cytokine levels determined using Bio-plex Manager Software v6.2 (Bio-rad).

***In Vitro* antiarthritic activity**

To evaluate the *in vitro* antiarthritic activity was evaluated using albumin denaturation test. 1ml of different concentrations of aqueous extract and ethanolic extracts of *G. glabra* and Diclofenac sodium ranging from 100-500 µg/ml were prepared was homogenised with 1 ml of aqueous solution of 1% bovine serum albumin and incubated at at 27 ±1˚C for 15 minutes. The mixture of distilled water and BSA constituted the control tube. Protein denaturation was caused by placing the mixture in a water bath for 10 minutes at 70°C. The mixture was cooling inside the ambient room temperature, and the activity each mixture was measured at 660 nm. Percentage inhibition of denaturation was calculated using control in which no drug was added. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated.

RESULTS & DISCUSSION

Proximate Analysis The rhizome of *G. glabra* linn was subjected to evaluate its total ash value, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value and moisture content.

Determination of total ash, acid and insoluble ash value

Phytochemical evaluation helps in the determination of quality and purity of *G. glabra*. In this determination of foreign matter and ash value gives us an idea about earthy matter or inorganic composition and other impurities present along with the drug, whereas acid insoluble ash value specifically indicates siliceous impurities in the sample. In the present study no foreign matter were found on analysis of the drug. The percentage of total ash, acid insoluble and water insoluble ash were 8.25 %, 3.09% and 7.90 % respectively.

Table 1: Ash value of *G. glabra* rhizome linn

Total	Acid insoluble	Water soluble
8.25%	3.09%	7.90%

Determination of moisture content

The moisture content of a drug is the factor responsible for decomposition of the drugs, either by producing chemical change or by microbial growth. So the moisture content of the drug was determined and the value was 15.98 %. The result of total ash, acid insoluble ash and moisture content were comparable with results has been reported (Hasan et al., 2021).

Table 2: Extractive value of *G. glabra* rhizome

Alcohol soluble	Water soluble
7.87%	21.94%

Determination of water soluble ash value

The moisture content of a drug is the factor responsible for decomposition of the drugs, either by producing chemical change or by microbial growth. So the moisture content of the drug was determined and the value was 15.82 %.

Table 3: Moisture content of *G. glabra* L rhizome

Time (mins)	Moisture content (%)
30	**15.82**
45	**13.94**
60	**9.84**
75	**7.92**
90	**5.03**

All the extracts at a concentration of 500ppm have shown very good antioxidant activity. Among the rhizome extracts of *G. glabra* L only methanolic extract at 500ppm has shown activity above 90%. Higher activity has been shown by the methanolic extract than standard α-tocopherol.

Detection of flavonoids

Qualitative analysis of ash obtained from the powdered drug performed in this study showed the presence of carbonates, phosphates, sulphates and absence of chloride and potassium. Quantitative estimation of phytochemicals such as flavonoids, saponins, phenols, tannin and alkaloids were done by Hosseini et al. (2020) using the methanolic extract of the rhizome. In our study quantitative estimation of powdered drug for phenol and tannin were done and the result was 63.13 % and 28.5% respectively. These results found were less as compared to the previous research works. This may be due to the changes in secondary metabolites of plants according to seasonal and geographical variations. In the present study the results of quantitative estimation of volatile oil, fibre content, total sugar and reducing sugar were 0, 37.3%, 12.92% and 4.18% respectively.

Table 4 . Occurrence of bioactive compounds in different solvent extracts of *G. glabra* L

Phytochemical groups	Hexane	Chloroform	Ethyl Acetate	Methanol	Water
Flavonoids (F)	+	+	++	++	++
Alkaloids (A)	-	-	-	-	-
Saponins (S)	+	-	-	++	++
Tannin (TA)	-	-	-	-	-
Phenolics (P)	-	-	-	-	-
Terpenoids (TE)	-	++	++	-	-
Glycosides (G)	-	-	-	-	-
Steroids (ST)	+	-	+++	++	+

Highly present (+++), Moderately present (++), Lightly present (+), Absent (-)

In the present study these two were analyzed and the results were 4.26% and 5.36 % respectively. In addition to this hot alcohol and hot water soluble extractives were determined in

this study and the result were 6.20 % and 12.20 % respectively. In the present study petroleum ether, cyclohexane, acetone and alcohol were the solvent used and the result obtained were 3.82 %, 5.98%, 3.72% and 15.38 % respectively. Out of the three researches on successive solvent extraction the results in the present study was comparable with results of Pandey et al., 2017. A comparison of extraction yield in different extraction solvents showed that solvent type had a significant effect on the extraction yield, where acetone maintained the lowest percentage of extract (3.72%) and alcohol maintained the highest (15.38%). Phenol was present in all the four types of extract of the powdered drug (Honda et al., 2014).

. Determination of inflammatory markers by ELSIA

Osteoarthritis and rumatoid arthritis patients were individually blood extracted and subjected to total protein determination was observed through ELISA method. When the Patients samples were exposed to different kinds of interleukins (IL), a significant increase in level was observed. The value of cytokine markers of IL were IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13 and TNF-α has been shown in Table 5. The Immune cytokine markers like IL-2, IL-5, IL-6 and IL-10 were significantly increased than the control patients. When treated with IL-2 the values of the above markers were significantly 4 fold increases in the chronic patients than the acute patients. Exposures of IL-6 to the acute and chronic patients' blood sample, the values are 356.6 ± 27 and 510.52 ± 25 respectively. When compare to the control patients almost 4 and 6 fold increases in IL-6 treated samples were observed. Similar results were obtained when treated with IL-5 and IL-10. From this investigation the study suggested that the IL-2, IL-5, Il-6 and Il-10 should be considered as markers for osteoarthritis in acute and chronic stages (Shakhsi et al.,2021).

Table.5. Anti-inflammatory assay performed using in different solvent extracts of *G. glabra* L by ELSIA method

Sl. No	Cytokines	Control Patients (N=30)	Acute Oste Oarthritis (N=30)	Chronic Osteoarthritis (N=30)	*p- Value*
1	IL-1β	17.32±0.81	19.27±0.92	25.35±0.72	0.210
2	IL-2	105.82±5.02	118.72±5.25	145.52±5.81	0.147
3	IL-4	2.03±0.53	3.12± 0.6	6.5±0.56	0.001**
4	IL-5	121.50±3.24	128± 2.10	156.±0.48	0.076
5	IL-6	143.54±6.25	354.6±27	510.52±25	<0.001**
6	IL-10	126.78±5.25	139.15±1.14	165.37±3.4	0.050
7	IL-12	2.42±1.5	4.52±0.49	8.141±2.35	0.080
8	IL-13	21.32±3.21	24.20±0.52	31.32±5.06	0.283
9	TNF-α	7.63±0.58	8.5±1.24	10.26 ± 4.25	0.157

Osteoarthritic activity:

In vitro anti-osteo arthritic activity was assed by protein denaturation assay. The inhibition protein denaturation was observed when treated with different concentration (100-500 µg/ml) of *G. glabra* L plant extracts. When treated with 500 µg/ml of ethylacetate and methanolic extrate to inhibite 74.32% and 68.22 % of protein denaturation respectively. Whereas the 500

µg/ml aqueous extract of *G. glabra* L inhibits 56.24 % of protein denaturation significantly when compared to the standard drug Diclofenac sodium (85 ± 0.14**) shown in table. 6.

Table 6. Anti-osteo arthritic activity performed using in different solvent extracts of *G. glabra* L by ELSIA method

Conc. (µg/ml)	% inhibition of protein denaturation			
	Diclofenac sodium-Positive Control	Ethyl acetate extract	Methanol extract	Aqueous extract
100	42.02 ± 0.62**	32.04 ± 0.41*	29.6 ± 0.21*	23.41 ± 0.41*
200	58.14 ± 0.12**	41.24 ± 0.24*	38.42 ± 0.14**	39.41 ± 0.29**
300	64.42 ± 0.21***	53.42 ± 0.42*	47.42 ± 0.64**	42.38 ± 0.60*
400	72.25 ± 0.24**	60.20 ± 0.24**	52.63 ± 0.34**	50.24± 0.32*
500	85 ± 0.14**	74.32 ± 0.32**	68.22 ± 0.41**	56.24 ± 0.40**

CONCLUSION

In conclusion the present study experimentally validated that the *G. glabra* L extracts Ethyl acetate and methanol constitutes bioactive medicinal compounds which have the efficacy to treat Osteoarthritis. Through our study it can be different extracts of *G. glabra* L leaves possesses good phenolic profiles have potent to anti-inflammatoryand anti-arthratic activity. Certainly *G. glabra* have been widely researched and found to be effective remedies as anti-inflammatory, antivirals and immunomodulation. Therefore, the current study designed to deliver a content to be useful for development of future medicine and development of multiple value added product. Specifically, to establish liquorice and its bioactive compounds through research and review article for delivering anti-inflammatory, antivirals and immunomodulatory action is established by including several molecular mechanisms.

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SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF SOME NEW MANNICH BASES

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ABSTRACT

The newly synthesized Mannich bases were characterized in this study on the basis of UV-Vis, FT-IR, FT-Raman, 1H NMR, 13C NMR, 2D-NMR, Mass spectral techniques and elemental analysis. Antibacterial and antifungal study has been carried out for the Mannich bases against a panel of five Pathogenic bacterial strains namely, (Staphylococcus aureus, Klebsielloa Pneumonia, Bacillus anthracis, Escherichia coli, and, Bacillus cereus) and antifungal activity against a panel of five pathogenic fungal strains namely, (Aspergillus fumigates, Aspergillus nidulans, Aspergillus terreus , Aspergillus flavus , Aspergillus niger). Using gentamycin and tetracycline as standards. The synthesized compound contains significant antibacterial and antifungal activities.

Keywords: Mannich bases, formaldehyde, cinnmaldehyde derivatives, antibacterial activity, Antifungal activity,

INTRODUCTION

The Mannich reaction is an organic reaction which consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or either primary or secondary amine. The final product is a β-amino-carbonyl compound is known as a Mannich base.[1] Microbial infections are a growing problem in contemporary medicine, and the use of antibiotics is common across the world. Antimicrobials are among the most commonly purchased drugs worldwide. They are essential treatments especially in the developing world where infectious diseases are a common cause of death. The health problem demands to explore and synthesize a novel class of antimicrobial compounds effective against pathogenic microorganisms that developed resistance to the antibiotics used in the current regimen.[2] The organic heterocyclic compounds containing hetero atoms are well known to possess significant biological activities[2]. Such as bactericidal, fungicidal, herbicidal and insecticidal activates.[3] Shortly developments in biomedical point to the involvement of free radicals in many diseases.[3-4] For these reasons, antioxidants and biological activities are of interest for the treatments of many kinds of cellular degeneration.[5] So that it received great attention to synthesis some new organic molecules contains heteroatom such as O, N, and S, with enhanced biological activity[4].

The present study was undertaken in an attempt to synthesis some new Mannich bases of 1-((o-tolylamino) methyl) pyrrolidine-2, 5-dione (TOFS) and (*E*)-N- (3 -phenyl -1-((o-tolylamino)allyl)acrylamide (TOCA) by condensation reaction using formaldehyde, cinnamaldehyde, o-toluidine, succinamide, and acryalamide, carry out their antibacterial and antifungal studies. [6-8]

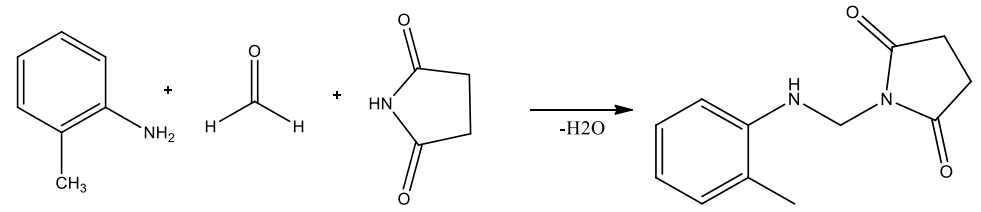
MATERIALS AND METHODS

All reagents were commercially available and used without further purification. Solvents were distilled from appropriate drying agents subsequently prior to use. The human pathogenic bacterial and fungal species were purchased from Department of Botany and Microbiology

AVVM Sri Pushpam College (Autonomous) Poondi Thanjavur-613503. (*Staphylococcus aureus*, *Klebsielloa Pneumonia* , *Bacillus anthracis* ,*Escherichia coli*, and *Bacillus cereus*) and fungal species (*Aspergillus fumigates*, *Aspergillus nidulans* , *Aspergillus terreus* , *Aspergillus flavus* , *Aspergillus niger*) and were used for the antimicrobial studies.

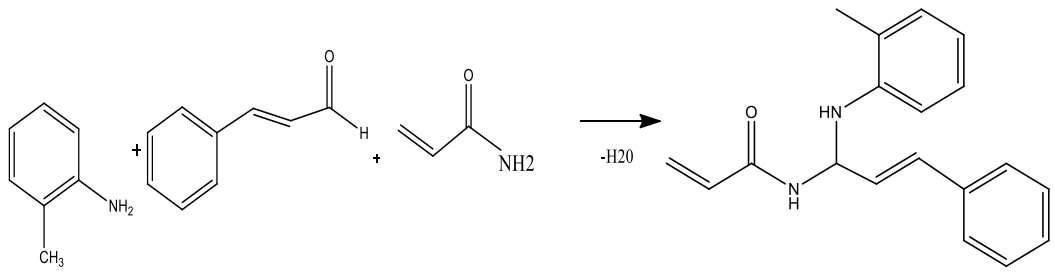
Synthesis of Mannich bases

1-((o-tolylamino) methyl) pyrrolidine-2, 5-dione (TOFS) o-toluidine (1.06mL, 0.01M), succinimide (0.99g, 0.01M),and formaldehyde (0.32mL,0.01M) were taken in equimolar ratio. A concentrated ethanolic solution of succinimide and o-toluidine was prepared. Formaldehyde was added in drops with stirring of the solution. The mixture first becomes oily and then slowly turned into a white solid mass which was separated by suction filtration and washed with water several times. The product (Fig,1) was dried at 45^{o}C in an air oven and recrystallised using methanol by slow evaporation method. [9-11] (*E*)-N- (3 -phenyl -1-((o-tolylamino)allyl)acrylamide (TOCA) o-toluidine (1,06mL, 0.01M), acrylamide (0.71g, 0.01M), were taken in equimolar ratio. A concentrated ethanolic solution of acrylamide and o-toluidine was prepared. To this solution ethanolic solution of cinnamaldehyde (1.32mL, 0.01M) was added slowly with constant stirring in an ice bath. A paste like semisolid was observed. After 2 h a pale yellow oily turned into brown solid mass which was separated by suction filtration and washed with distilled water. The product (Fig.2) was dried at 50^{o}C in an air oven and recrystallized from ethanol by slow evaporation method [11]



o- toluidine formaldehyde succinimide 1-((o-tolylamino) methyl) Pyrrolidine-2, 5-dione

Fig,1 The Reaction Scheme for 1-((o-tolylamino) methyl) Pyrrolidine-2, 5-dione



o- toluidine Cinnamaldehyde Acrylamide (*E*)-N- (3 -phenyl -1-((o- tolylamino) allyl) acrylamide

Fig,2 The Reaction scheme for (*E*)-N-(3-Phenyl-1-(o-tolylamino)allyl)acrylamide

Spectral data

1-((o-tolylamino) methyl) pyrrolidine-2, 5-dione (TOFS)

Molecular formula: C_{12} H_{14} N_2 O_2, Yield: 92%, MP: 67 ± 2^{oC}, Mol.Wt:218.25. FT IR KBr in cm^{-1} :3230 (-NH), 3050,2968 (CH aromatic and aliphatic), 1653 (C=O stretching), 1135 (C-N-C), 1401(C=C,C=N stretching), 751 (benzene CH out of plane bending), FT Raman Polycrystalline

powder ν in cm^{-1} : 3065 (-NH), 3017 (CH aromatic),2978 (CH aliphatic),1570 (δ NH) 1359-1296 (C-H stretching), (δ CH) 1167, (C-N-C),1591 (Skeletal vibration of benzene ring),450 (C=O bending) 115,87 (skeletal bending vibration), 845 (CH opb of pyridine and benzene ring), ^{1}H NMR(500 MHz, DMSO- d_6) δ 2.04 (S,4H), δ 4.84 (d, 2H), δ 5.42 (t, NH), δ 6.56 -7.91 (m,4H o- toluidine ring) ^{13}C NMR (500 MHz, DMSO- d_6) δ 17.35 (S,C methyl carbon), δ 27.89 (S,2C $(CH_2)_2$) δ (S,1C,CH_2), δ 109.90 (S,C), δ 117.36 – 143.54 (m, 4C,o-toludine ring). 2D NMR Homo Cosy (500 MHZ, DMSO d6), The ^{1}H-^{1}H and ^{1}H-^{13}C correlation of TOFS. This shows the better results corresponding to those of ^{1}H NMR and^{13}C NMR spectral data. The 2D homo cosy NMR spectral in the (TOFS) substantiated the ^{1}H NMR and^{13}C NMR spectral Assignment. Mass (EI) m/z: 218.95 ($C_{12}H_{14}N_2O_2^{\cdot +}$), m/z: 98.02 ($C_4H_4N\,O_2^{\cdot +}$), m/z: 120 ($C_8H_{10}N^{\cdot +}$), m/z: 106 ($C_7H_8N^{\cdot +}$), Elemental analysis : C 66.04 %, H 6.47 %, and N 12.84 %, O 14.66 %. Found: C 66.02%, H 6.45%, and N 12.80%, O 14.64 %.

(*E*)-N-(3-Phenyl-1-(o-tolylamino) allyl) acrylamide

Molecular formula: $C_{19}\,H_{20}\,N_2\,O$, Yield: 98%, MP: 65 ± 2^{oC}, Mol.Wt: 292.37. FT IR KBr in cm^{-1}:3429 (-NH), 3035,2933 (CH aromatic and aliphatic), 1674 (C=O stretching), 1590 (δ NH) ,1148,1040 (C-N-C), 985 (pyridine ring),687,748 (CH of mono substituted benzene ring),451 (C=C), 980(CH opb of vinyl group). FT Raman Polycrystalline powder ν in cm^{-1} :3059 (-NH), 3206, (CH aromatic),2970 (CH aliphatic),1589 (δ NH), 1359- 1296 (C-H stretching), (δ CH) 1150 ,1111 (C-N-C),1483,1359 (CH symmetrical and asymmetrical stretching) 982 (CH opb of Vinyl group)1630 (C=O bending) 186,88 (skeletal bending vibration), 862 (CH opb of pyridine and benzene ring), ^{1}H NMR(500 MHz, DMSO- d_6) δ 2.26 (S,4H), δ 2.50 (3H CH_3) δ 8.25 -8.27 ppm (S,NH), δ 6.95,6.96 ppm (d 2H), δ 7.09-7.23ppm (m 4H o-toluidine aromatic ring) δ7.34-7.70 (m 5H benzene ring). ^{13}C NMR (500 MHz, DMSO- d_6) δ 18, 126 (1C,CH_3) δ 118.05 (s,C) 126.01 -131.67 (m,4C,o-toluidine aromatic ring) 129.40 – 135.92 (m 5C, benzene ring) δ 144.46(s,2C) δ 135.92 (s,1C,CH), δ 151.25 (s,2C), δ 162.04 (s 2C,C=O). 2D homo cosy NMR(500 MHZ, DMSO d6), (^{1}H-^{1}H and ^{1}H –^{13}C correlation of (TOCA). Which is the better results corresponding to those of ^{1}H NMR and ^{13}C NMR spectral data of the 2D homo cosy NMR study of the (TOCA) substantiates the 1H NMR and 13CNMR spectral assignments. Mass (EI) m/z: 292 ($C_{19}H_{20}N_2O^{\cdot +}$) m/z: 221($C_{16}H_{16}N^{\cdot +}$), m/z: 116($C_9H_8^{\cdot +}$), m/z: 106 ($C_7H_8N^{\cdot +}$), m/z: 77 ($C_6H_5^{\cdot +}$), m/z: 70 ($C_3H_4NO^{\cdot +}$), Elemental analysis : C 78.05 %, H 6.89 %, and N 9.58 %, O 5.47 %. Found: C 78.01%, H 6.81%, and N 9.55%, O 5.42 %.

RESULT AND DISCUSSION

The synthesized compounds in the study are depicted in (TOFS) and (TOCA). Based on the analytical and spectral data, the structure of the ligand was confirmed. The important FT- IR spectrum of the compound was observed. which shows the absorption bands at 1653 and 1135 cm^{-1} for aromatic and aliphatic C-H,N-H,C-N,C-N-C,C=N,C=O, NH and CH3 groups. ^{1}H NMR spectra given under the number of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds, the molecular mass of the synthesized compounds were nearer to the molecular mass of the expected compounds.

Antibacterial Activities

The Antibacterial Activities of Mannich bases TOFS and TOCA were carried out against the panel of *Staphylococcus aureus*, *Klebsielloa Pneumonia* , *Bacillus anthracis* ,*Escherichia coli*, and *Bacillus cereus* by Agar diffusion plate method[11-13], The maximum zone of inhibition was observed against *Klebsielloa Pneumonia* (18 mm) for TOFS. The maximum zone of inhibition was observed against Escherichia *coli* (17mm) *and Klebsielloa Pneumonia (*17mm) for TOCA. The Mannich base ligands TOFS and TOCA shows significant inhibition activity with respective bacterium was recorded when compared with standard drug Gentamycin.

Table: 1 Antibacterial Activities of Mannich Bases

S.NO	Compound(50µg)	Zone of inhibition(in mm) against				
		E. coli	***S. auerus***	***K. pneumo***	***B. cereus***	***B.anthracis***
1	TOFS	17	11	18	15	16
2	TOCA	17	11	17	10	7
3	Gentamycin (std)	16	18	17	17	15
4	DMSO (Control)	0	0	1	0	0

Antifungal activities

The antifungal activities of Mannich bases TOFS and TOCA were carried out against the panel of *Aspergillus fumigates*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus flavus* , *Aspergillus niger* by Agar diffusion plate method[13-17]. The maximum zone of inhibition was observed against *Aspergillus terreus* (25mm) for TOCA,The maximum zone of inhibition was observed against *Aspergillus fumigates* (15mm) and *Aspergillus flavus* (13mm) for TOFS. The Mannich base ligands TOFS and TOCA shows significant inhibition activity with respective fungi was recorded when compared with standard drug tetracycline.

Table: 2 Antifungal Activities of Mannich Bases

S.NO	Compound(50µg)	Zone of inhibition(in mm) against				
		A. fumigats	***A. nidulans***	***A. terreus***	***A. flavus***	***A. niger***
1	TOFS	15	5	Resistant	13	3
2	TOCA	16	19	25	20	7
3	Tetracycline(std)	Resistant	18	13	16	19
4	DMSO(Control)	0	0	0	0	1

CONCLUSION

The newly synthesized organic compounds have been characterized on the basis of above spectroscopic method and these compounds have been assigned for antibacterial and antifungal studies. The synthesized compounds TOFS and TOCA have significant activities against the microbes.

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COMPARATIVE STUDY OF CULTIVATION OF PLEUROTUS SAJOR- CAJU AND PLEUROTUS CITRINOPILEATUS ON DIFFERENT AGRO WASTE MATERIALS.

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ABSTRACT

Growing oyster mushroom is becoming more popular throughout the world because of their abilitiesto grow in a wide range of temperatures, utilizing various lignocelluloses (Khan and Garcha, 1984). The present work was carried out to find out the effect of different agricultural wastes on yield of two strains Grey oyster mushroom (Pleurotussajor-caju) and yellow oyster mushroom (P. citrinopileatus)were investigated and recorded.The use of waste materials as the alternative for mushroom cultivationsubstrates, identified as a potential and viable way to generate new economic sources and minimising waste disposal. Cultivation ofedible mushroom with agricultural residues is a valued-added process to convert these materials,which are otherwise considered to be wasted, into human food (Zhang et al., 2002).

Key Words: *Pleurotussajor-caju, P. citrinopileatus, Agricultural waste residues. Substrates.*

INTRODUCTION

Oyster mushroom, *Pleurotus species* belongs to the family of Tricholomataceae and is the second widely cultivated mushroom worldwide. Obodai et al., (2003) reported that oyster mushroom is the third largest commercially produced mushroom in the world market. *Pleurotus* species are popular and widely cultivated throughout the world mostly in Asia, America and Europe because of their simple, low cost production technology and high biological efficiency. Moreover, the interest of oyster mushroom is increasing largely due to its taste, nutrient, and medicinal properties. *Pleurotus* species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures. In comparison to other edible mushrooms, *Pleurotus* species need a short growth time and their fruiting bodies are not often attacked by diseases and pests . *Pleurotus* species require carbon, nitrogen and inorganic compounds as their nutritional sources.

The main nutrients are less nitrogen and more carbon so materials containing cellulose, hemicellulose and lignin which includes, Paddy straw, sugar cane bagassess, dried leaves of banana, black gram, corn leaves, rice bran, coconut coir pith, dried vegetable waste, and fruit peel waste had been used as mushroom substrates. Oyster mushroom can grow on a wide variety of substrate. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates.*Pleurotus citrinopileatus*, the golden oyster mushroom is an edible gilled fungus. The golden oyster mushroom, like other species of oyster mushroom, is a wood-decay fungus. In the wild, *P. citrinopileatus* most commonly decays hardwoods such as elm. The first recorded observation of naturalized golden oysters in the United States occurred in 2012 on Mushroom Observer, perhaps a decade after the cultivation of the species began in North America, and they have been found growing on oak, elm, beech, and other hardwoods. *Pleurotus sajor-caju* may refer to some warm weather varieties of *Pleurotus pulmonarius*, a commonly cultivated species of oyster mushroom, often incorrectly called *Pleurotus sajor-caju* , *Lentinus sajor-caju*, a species of mushroom formerly called *Pleurotus sajor-caju. Pleurotus species* are a rich source of protein, minerals (P, Ca, Fe, K, and Na) and vitamin (thiamine, riboflavin, folic acid, and niacin).Apart from food value, their medicinal value for diabetics and in cancer therapy has been emphasized Numerous mushroom

species contain a wide range of metabolites as antitumour, antigenotoxic, antioxidant, antihypertensive, antiplatelet- aggregating, antihyper-glycaemic, antimicrobial, and antiviral activities.

Large volumes of unused lignocellulosic by-products are available in the Kaveri Delta region as agro waste materials. These by-products are usually left to rot in the field or are disposed through burning. Using locally available lignocellulosic substrates to cultivate oyster mushroom is one solution to transform these inedible wastes into accepted edible biomass of high market and nutrient values. Use of agro-waste residues are the reasons why we need to identify alternatives for sustainable cultivation of oyster mushrooms. The study was conducted to compare the effects of different agro-wastes on the growth, yield, and nutritional composition of oyster mushrooms *P.citrinopilitus and P. sajor – caju.* The final aim is to find the best substrate formulas for effective cultivation of oyster mushrooms.

MATERIALS AND METHODS

Mother Culture Preparation

PureStrains of *Pleurotus* species*Pleurotus sajor-caju* and *Pleurotus citrinopileatus* strains were obtained from ICAR DMR Chambaghat, Solan.India. The cultures were preserved on 2% malt extract agarslants at 4ºC. Subculturing was done after every 15 days. **Spawn preparation**

Sorghum grains were prepared in polythene bags. The grains were subjected to boiling in a water bath for a duration of 10 to 15 min, maintaining a ratio of 1:1 (Sorghum grains to water). Subsequently, the grains were combined with 4% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$. Following this, 250 g of the sorghum mixtures were placed into polythene bags measuring 200x300 mm and sterilized in an autoclave at 121ºC for 30 min. After the sterilization process, the bags were inoculated with actively growing mycelia of *Pleurotus sajor-caju* and *P. citrinopileatus* grown on malt extract slants and incubated at a temperature of 27±2ºC for 10 to 15 d in the absence of light, allowing the mycelia to completely colonize the grains (Garcha, 1994).

Substrate preparation and inoculation

Substrates for the present study were include Paddy straw, sugar cane bagassess, dried leaves of banana, black gram, corn leaves, rice bran, coconut coir pith, dried vegetable waste, and fruit peel waste had been used.These substrates were dried and cut into 3-4cm longpieces. The substrates were soaked in water for8-10 hours in cemented pond to obtained 70-75per cent moisture level. All the substrates weresterilized by boiling method where the substrateswere boiled for one hour at 70-75°C. Then theywere stalked on the steep cementedfloor so as toremove the excessive moisture from the substrates to get 65-75 per cent moisture level. The substrates were cooled up to room temperature (25oC). A local method was developed for determination of moisture. In this method moisture was determined by pressing a handful mixture. If there was no water runoff and the material stayed in form indicates that the moisture content was around 65 per cent. Five kilogram of each substrate was filled in transparent polythene bag (30x45cm and seeded with 150g of *P. sajor-caju* and *P. citrinopilitus.* The pinholes at 10- 12cm distance were also done in the bags with help of led pencil after sterilization in 2 per cent formaldehyde solution. The bags were incubated in dark cropping room where ambient temperature ranged between 22- 28+10C. The humidity 80 – 90 per cent of the room was maintained by spraying of water twice a day on the loor covered with jute bags. After complete colonization of substrate polythene was removed and bags were put on the bamboo made structure for fruiting. The humidity of the bags was accomplished by spraying of water on them twice a day. The experiment was laid out in complete randomized design (CRD) with three replications and live treatments. Time was recorded in days for the completion of growth of mycelium on substrates, appearance of pinheads and maturity of

fruit bodies in different treatments. The data on average values of observations were also recorded for the yield, number of fruit bodies. Biological eficiency of mushroom on fresh weight basis was calculated by using formula given by Chang and Miles (1989).

RESULTS AND DISCUSSION

Spawn running all the substrates were spawned at the same day. It was evident from the Table 1 that spawn running took 2-3 wk after spawning. Pinhead formation The pinhead formation is the second stage of mycelia during cultivation of mushroom. Small pinhead like structures were observed, these pinhead were formed 4-5 days after spawn running (Table1). Our results were corroborated with Ahmed (1986) who stated that Pleurotusostreatus completed spawn running in 17-20 d on different substrates and the time for pinhead formation was noted as 23-27 d. Fruit bodies formation This is the third and inal stage during the cultivation of mushroom. The fruit bodies appeared 4-5 wk after pinhead formation and took 25-34 d later after inoculation of spawn (Table1). Sharma and Jandaik (1981) reported that P. sajor-caju cultivation on wheat straw took 32 d for the first harvest.

$$\text{Biological efficiency (\%)} = \frac{\text{Yield of fruiting body (g)}}{\text{Total weight of substrate used (g)}} \times 100$$

Substrate	Days for completion of spawn running	Days for pinhead formation	Days of fruiting bodies formation	Average number of fruiting bodies
Paddy straw	12.67	12.66	7.33	27.66
Coir pith	17.65	16.77	12	12
Dried banana leaf	14.24	12.12	14	14
Dried black gram leaf	13.23	13.23	15	12
Rice bran	17.02	17.23	29	11
Corn leaf	15.03	14.98	21	12
Dried fruit waste	13.40	15.98	26	11
Dried vegetable waste	18.09	16.98	27	10

Proximate analysis

The assessment of moisture, protein, fat, crude fiber, total carbohydrates and ash content in the samples was conducted using established methodologies as outlined by AOAC (1995).

Proximate content	**Quantity**	
	Pleurotuscitrinopileatus	***Pleurotussajorcaju***
Ash (%)	7.23	8.61
Moisture (%)	9.94	9.54
Carbohydrate (µg/g)	10.04	19.70
Protein (µg/g)	9.71	8.11
Crude fat (µg/g)	0.86	1.10
Crude fibre (µg/g)	4.16	5.41

Vitamin estimation

Folic acid, thiamine (B1), riboflavin (B2), and niacin were estimated according to Kamman*et al.*, (1980). Vitamin C was estimated by the 2,6- dichlorophenolindophenol titration method (AOAC,1995). Vitamin A and E were estimated using the method described by Majesty *et al.*, (2019).

Determination of mineral elements

The prepared samples of *Pleurotus sajor-caju* and *Pleurotus citrinopileatus* were analyzed for Calcium (Ca), Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Potassium (K) and Zinc (Zn) using spectrophotometric methods according to Afolabi*et al.*, (2023) and AOAC (2019).

Analysis of Bioactive compounds

(a) Qualitative bioactive analysis: The bioactive compounds such as alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins, terpenoids and quinones were analysed with the solvents of aqueous, ethanol, methanol and diethyl ether extracts using standard method (Harborne, 1973) were followed.

(b) Quantitative bioactive analysis: The bioactive compounds such as alkaloids, amino acids, coumarins, phenols, steroids, terpenoids and quinones were analysed using standard method (Harborne, 1973). Flavonoids (Bohm and Mohammed, 1994), saponins (Obadone and Ochuko, 2001) and tannins (Van Buren and Robinson, 1981) were estimated.

Statistical Analysis

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviations.

Species	Substrate	Spawn Run (d)	Pinhead formation (d)	Yield (g) per harvest (g/kg)			Total Yield (g/kg)	Bio-conversion Efficiency (%)
				I	II	III		
Pleurotuscitrinopileatus	Paddy straw	25	28	115	107	65	287	28.7
	Coconut coir pith	23	25	109	102	54	265	26.5
	Sugarcane trash	28	30	96	100	46	242	24.2
P. sajorcaju	Paddy straw	17	22	155	128	98	381	38.1
	Coconut coir pith	24	28	100	104	58	262	26.2
	Sugarcane trash	27	31	98	100	53	251	25.1

CONCLUSION

This study evaluated the proximate, mineral, vitamins contents and bioactive compounds of *Pleurotus citrinopileatus* and *P. sajor-caju*. The results of the proximate analysis indicated that *Pleurotus* sp. exhibited elevated levels of carbohydrates and moisture, moderate amounts of protein and fiber, and low levels of fat and ash. Vitamins A, E and B2 were found to

be present in high amounts in the *Pleurotus* spp.whereas other vitamins such as B1, B3 and C were found to be present much lower concentrations. The analysis of mineral content indicated a significant presence of calcium, iron and magnesium in comparison to other minerals. The maximum bioactive compounds were presented in the aqueous and methanol extracts of *Pleurotus* spp. The findings from other studies indicated that incorporating mushrooms into food products enhances both their nutritional content and physical attributes. Consequently, it is not unexpected that the food and pharmaceutical industries utilize mushrooms or their bioactive compounds to develop functional foods with nutraceutical benefits. By establishing a rapid, nutrient-dense food source, mushroom cultivation can provide individuals with a reliable income stream, thereby reducing their susceptibility to poverty and improving their overall quality of life. *Pleurotuscitrinopileatus* and *P.sajorcaju* have been identified as abundant sources of essential nutrients, vitamins, minerals and aminoacids which positions them as functional foods and significant contributors to dietary practices aimed at reducing the increasing prevalence of nutrition-related diseases.

Pleurotus species cultivated on paddy straw, coconut coir and sugarcane trash substrates exhibited a significant capacity for growth and yield. Among the 2 examined species of *Pleurotus*, *Pleurotus citrinopileatus* demonstrated a lower yield compared to *Pleurotus sajor-caju*. Recent research has extensively explored various aspects of oyster mushroom cultivation Notably,*Pleurotus sajor-caju* shows an increase in mycelial growth of up to 100%, irrespective of the substrate composition, when compared to *P. ostreatus* and *P. florida* (Olasupo*et al.,* 2019).

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ISOLATION AND IDENTIFICATION OF FUNGI FROM MANGROVE SOIL

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ABSTRACT

The mangrove ecosystem is a crucial coastal habitat that supports a diverse array of flora and fauna, including microorganisms like fungi, which are essential for nutrient cycling, decomposition, and maintaining ecological balance. This study was conducted to isolate and identify fungal species from soil and water samples collected from two mangrove areas in Kallimedu and Naluvedhapathi in Vedaranyam, Nagapattinam Dt. The primary goal was to explore the diversity of fungi in this ecosystem and assess their ecological functions. Soil and water samples were collected from two distinct locations within the mangrove area. The first sampling site was characterized by a more exposed, tidal zone with frequent saltwater immersion, while the second site was situated in a sheltered, less disturbed area, with more stable environmental conditions. These contrasting environments provided an opportunity to examine how different mangrove conditions influence fungal distribution. Soil samples were processed using the serial dilution method to isolate fungi, while water samples were filtered to capture fungal spores. The fungal isolates were then cultured on selective media, such as Potato Dextrose Agar (PDA) under controlled laboratory conditions. After the colonies developed their macroscopic and microscopic characteristics.

Keywords: Mangrove soil, Potato Dextrose Agar, Fungi, Mangrove forest.

INTRODUCTION

The occurrence of Aspergillusis worldwide but mainly in tropical and subtropical regions (Domsch et al. 1980; Christensen & Tuthill 1985). Mangroves are the unique forests, representing intermediate vegetation between land and Sea that grow in oxygen deficient water logged soils. All mangrove species have mechanism to provide air to their root system from the atmosphere. Mangrove ecosystems, characterized by their unique coastal environment, host a diverse array of microorganisms, including fungi, which play vital roles in nutrient cycling and ecosystem stability. These ecosystems, located in the inter tidal zones, are subjected to a variety of environmental stressors such as salinity fluctuations, tidal movements, and low oxygen conditions. The mangrove soils support a rich diversity of fungi that contribute to the decomposition of organic matter and the formation of symbiotic relationships with plants and other organisms. The isolation of fungi from mangrove soils is crucial for understanding their ecological functions and potential biotechnological applications. Fungi in mangrove soils exhibit unique adaptations to survive in extreme conditions, By isolating and characterizing these fungi. This study aims to isolate fungi from mangrove soils, identify their fungal species from mangrove soil, and explore their functional physicochemicalproperties in mangrove soil and water samples.

METHODS AND MATERIALS

Collection of samples

The mangrove soil and water samples were collected from Kallimedu and Naluvedapathi mangroves of Vedaranyam, Nagapattinam District, Tamilnadu, India. The soil sample were collectedat0-15cmdepthandstoredinsterileplasticbags.

Image1.mangrovesoil & water samples collecting locations

Isolation of fungi

The two soil samples were isolated from each sample by serial dilution and spread plate method. The collected soil samples were serially diluted using sterile water and plated on Potato Dextrose Agar Medium. The plates were incubated at 48 hours.

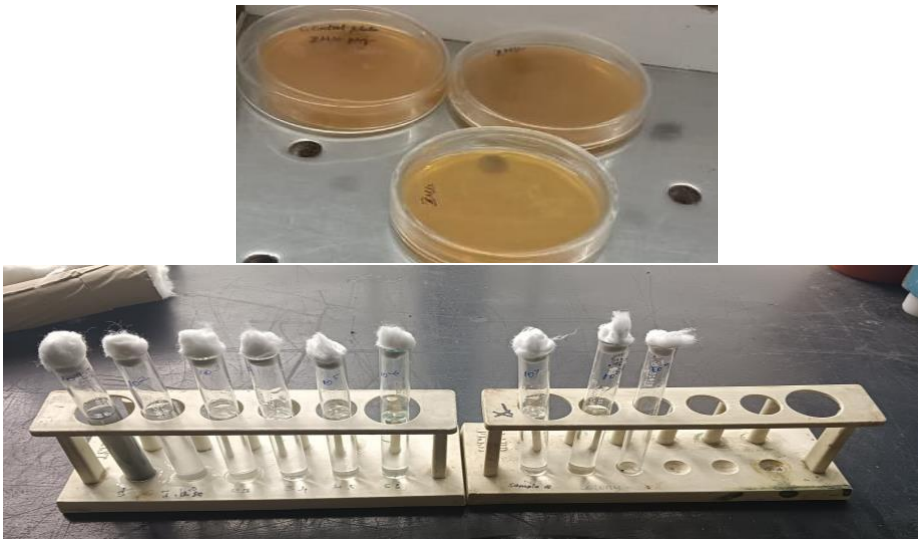


Image 2.Serial dilution method for isolation of fungi in mangrove soil

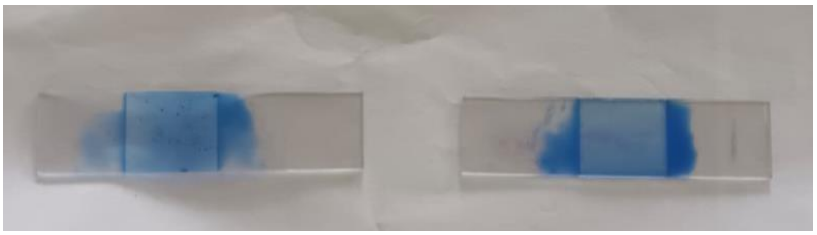
RESULTS AND DISCUSSION

The mangrove ecosystems are known for high productivity, studies on mangrove mycobiota have been initiated onlyrecently (Hyde and Lee 1995). Isolation and characterization of phosphate solubilizing fungi from the soil sample of muthupet mangroves studies (Arulselvi, et al. 2018), Aspergillusspecies isolated from mangrove forests (Jaya SeelanSathiyaSeelan, et al. 2009), FusariumSpecies in Mangrove Soil in Northern Peninsular Malaysia and the Soil Physico-Chemical Properties Wafa S. Mohamed Zubi, et al. 2021).

Microscopic observation

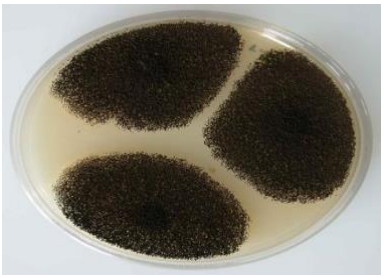
Lacto phenol cotton blue is a strain commonly used for making semi-permanent microscopic preparations of fungi. Place a drop ofLacto phenol cotton blue on a clean slide. Transfer a small tuft of the fungus with the sterilized inoculation needle with spores and spore bearing structure in to the drop. Gentle tease thesampleusing thetwo mounted needles. Mix gently thestrain with the mold structure place a cover glass over the preparation and taking care to avoid trapping air bubbles in the strain. The slides were observed under bright field microscope with oil immersion objective. All the fungi were identified with help of the standard manual ofGillman, 1957.

Lacto phenol cotton blue strain for fungal identification



Identification of fungi

The identify the fungi in this study were isolated from mangrove soil and isolating two fungal species only they are, Aspergillus and penicillium sp. Identification of fungal isolates was done by observing colony characteristics on PDA plates. This was confirmed by microscopic analysis of colony using lacto phenol cotton blue strain.



Aspergillus spp ***Penicillium spp***

Image3.Morphological character of isolating fungi in mangrove soil

Table1.Physicochemicalproperitiesofthemangrovewatersample

S. No	Sampling Places	Hardness (Mg/L)	Ph	Salinity	Alkaliniy (Mg/L)
1	Kallimedu	300ppm	8.0	3.4	196.03
2	Naluvedapathi	600ppm	8.1	20	372.22

Table2.Physicochemical properties of the mangrove soil sample

S. No	Sampling Places	Texture	Ph	Salinity (Ppt)	N
1	Kallimedu	Claysoil	7.01	5.44	1.0
2	Naluvedapathi	Claysoil	7.04	10.99	2.99

The physicochemical properities of mangrove soil and water parameters such as pH, salinity, alkalinity and etc. The analysis from mangrove soil and water were reported to fungal populations to (Table 1&2) referred (T. Arulselvi, et al 2018).

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GREEN SYNTHESIS OF SILVER NANOPARTICLES, CHARACTERIZATION AND ANTI-DANDRUFF ACTIVITY STUDIES BY USING *PIPER NIGRUM* L. EXTRACT

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ABSTRACT

In recent years the synthesis of metal nanoparticles using plants has been extensively studied and recognized as a non-toxic and efficient method applicable in the biomedical field. The use of biological substances in the production of nanoparticles is currently a highly talked-about subject in contemporary nanoscience and nanotechnology. This study aims to investigate the role of different parts of the medical plant *Piper nigrumin* synthesizing silver nanoparticles and characterize the produced nanoparticle. Our study showed that silver nanoparticles (AgNP) synthesized via whole leaf extract exhibited a blue shift in absorption spectra with increased optical density, which correlates to a high yield and small size. Characterization of nanoparticles was done using different methods, which include; ultraviolet-visible spectroscopy (UV–Vis), Fourier transform infrared (FTIR), powder X-ray diffraction (XRD), scanning electron microscope (-SEM), energy dispersive X-ray analysis (EDAX), Brunauer-Emmett-Teller (BET). Further, in vitro anti-dandruff green synthesized AgNPs were assessed against Malassezia furfur., The minimal inhibitory concentration (20 and 40µL) of AgNPs for dandruff causative agent M. furfur was found at 10.0 and 10.5µg/mL-1, respectively. The half maximal inhibitory concentration (60µm) value of the AgNPs for Malassezia cells was calculated as 12.0 µgmL-1 and complete inhibition was observed at a concentration of 16.0µgmL-1 (80 µm). Finally, our results proved that green synthesized AgNPs using Piper nigrum L. have great potential in biomedical applications such as anti-dandruff and treatment.The anti-dandruff activity of AgNPs against generally found fungi was assessed to find their potential use in silver-containing anti-dandruff products.

Keywords: anti-dandruff, silver nanoparticles, Piper nigrum, SEM, nanotechnology

INTRODUCTION

One of the most active research fields in contemporary materials science is nanotechnology. Based on particular attributes including size, distribution, and shape, nanoparticles display entirely new or enhanced features. Nanomaterials and nanoparticles are finding new uses rapidly (1). Silver nanoparticles' unique physiochemical and biological characteristics have led to their widespread use in a variety of industries in recent years, including antimicrobial agents, filters, microelectronics, biolabeling, sensors, and catalysis(2,3,4) The development of bacteria, viruses, and other eukaryotic microbes is inhibited by these nanoparticles, but they do not harm humans. Together with their unique qualities, they also have comparatively low production costs.

The widespread skin ailment known as dandruff mostly affects the scalp (5). It will impact nearly half of the prepubescent population, regardless of gender or race. Epidermal development and the discharge of dead skin cells from the scalp are common causes of dandruff (6,7,8). The condition was linked to seborrhoeic dermatitis. The expression and production of

immune responses during the development of dandruff are influenced by keratinocytes. It affects around 50% of people on the planet. Males are likely more affected than females.

A fungal infection was the cause of the infectious dandruff. Flaking and occasionally mild itching are symptoms. According to earlier research, dandruff is caused by the fungus Malassezia furfur, formerly known as *Pityrosporum ovale*. *Malassezia globosa*, a fungus unique to the scalp, was shown to be the causative agent. It produces oleic acid as a lipid byproduct by using lipase to break down the triglycerides found in sebum. Malassezia levels rise by 1.5 to 2 times their typical level during dandruff. In susceptible individuals, oleic acid causes an inflammatory reaction that disrupts homeostasis and causes irregular cleavage of stratum corneum cells. Oleic acid also penetrates the stratum corneum, the top layer of the epidermis. The primary signs of dandruff are flakiness and an itchy scalp. Other symptoms include a tingling sensation on the skin and red, oily areas.

Many traditionally utilized medicinal plants possess therapeutic properties and have been employed to enhance the immune system and treat various health conditions. Numerous natural and synthetic methods have been established for dandruff management. Individuals predominantly rely on commercial shampoos, ointments, and creams for dandruff control. The ingredients found in these products often include Zinc Pyrithione, Ketoconazole, and antifungal agents such as Clotrimazole, Amphotericin B, Miconazole, and Nystatin. In contrast, plants contain naturally occurring bioactive compounds, including alkaloids, flavonoids, tannins, and terpenoids. White pepper (*Piper nigrum*) was selected for this study due to its antifungal and antidandruff properties, which demonstrate superior effectiveness in treating dandruff(9). While black pepper also possesses antifungal and antibacterial characteristics that may aid in diminishing dandruff, the focus here is on the antidandruff activity of green synthesized silver nanoparticles (AgNPs) derived from the extraction of Piper nigrum L. This research aims to evaluate the efficacy of these nanoparticles against Malassezia furfur utilizing the disk diffusion method.

MATERIAL METHODS

2.1 Collection and extraction of Piper nigrumL.

The white pepper (*P. nigrum*) wasprocured from the local market in Kumbakonam, Tamilnadu, India, based on cost-effectiveness, ease of availability, and medicinal properties. Healthy pepper seeds were rinsed thoroughly first with tap water followed by distilled water to remove all the dust and unwanted visible particles, and crushed into small pieces. About 20 g of these fine pepper powder was weighed separately and transferred into 250 mL beakers containing 100 mL distilled water and boiled for about 20 min. The extracts were then filtered thrice through Whatman No. 1 filter paper to remove particulate matter and to get clear solutions which were then refrigerated (4°C) in 250 mL Erlenmeyer flasks for further experiments. In everystep of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

2.2 Synthesis of AgNPs by using P. nigrumL.

Aqueous solution (1 mM) of silver nitrate (AgNO3) was prepared in 250 mL Erlenmeyer flasks and leaf extract was added for reduction into Ag+ ions for pepper aqueous extract. The composite mixture was then kept on turntable of the microwave oven for complete bio-reduction at a power of 300 W for 4 min discontinuously to prevent an increase of pressure. In the meantime, the colour change of the mixture from faint light to yellowish brown to reddish brown to colloidal brown was monitored periodically (time and colour change were recorded along with

periodic sampling and scanning by UV-visible spectrophotometry) for maximum 30 min. This was separately performed with each type of pepper extract. The reactions were carried out in darkness (to avoid photoactivation of AgNO3) at room temperature. Suitable controls were maintained all through the conduction of experiments. Complete reduction of AgNO3 to Ag+ ions was confirmed by the change in colour from colourless to colloidal brown. After irradiation, the dilute colloidal solution was cooled to room temperature and kept aside for 24 h for complete bioreduction and saturation denoted by UV-visible spectrophotometric scanning. Then, the colloidal mixture was sealed and stored properly for future use. The formation of Ag NPs was furthermore confirmed by spectrophotometric analysis.

2.3 Characterization studies of AgNPs P. nirgrum NPs

2.3.1 UV-Vis Spectra analysis

Samples (1 mL) of the suspension were collected periodically to monitor the completion of bioreduction of Ag+ in aqueous solution, followed by dilution of the samples with 2 ml of deionized water and subsequent scan in UV-visible (vis) spectra, between wave lengths of 200 to 700 nm in a having a resolution of 1 nm.

2.3.2 FTIR analysis

FTIR analysis of the dried Ag NPs was carried out through the potassium bromide (KBr) pellet (FTIR grade) method in 1:100 ratio and spectrum was recorded using Jasco FT/IR-6300 Fourier transform infrared spectrometer equipped with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a resolution of 4 cm−1 (JASCO, Tokyo, Japan).

2.3.3 SEM analysis

P. nigrum extract solution containing Ag NPs were centrifuged at 4,000 rpm for 15 min, and the pellets was discarded and the supernatants were again centrifuged at 25,900 rpm for 30 min. This time, the supernatants were discarded and the final pellets were dissolved in 0.1 mL of deionized water. The pellet was mixed properly and carefully placed on a glass cover slip followed by air-drying. The cover slip itself was used during scanning electron microscopy (SEM) analysis. The samples were then gold coated using a coater (Hitachi S-4500 SEM). The images of NPs were obtained in a scanning electron microscope (ZEISS EVO-MA 10, Oberkochen, Germany). The details regarding applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

2.3.4 XRD (X-ray Diffraction)

The x-ray diffraction (XRD) analysis of the samples AgNPs was carried out on an XRD instrument with the scanning range between 10 0 and 90 0 . The surface area of nanoparticles were assessed by the method of Brunauer-Emmett-Teller (BET) using metrometrics ASAP 2010 surface area analyzer (USA) with N 2 adsorption-desorption isotherms at degassing Temperature of 110℃.

2.4 Antifungal activity

2.4.1 Isolation and identification of dandruff causing fungus

Samples were collected from scalp of 6 persons suffering from dandruff (sample collection person details will be maintained to be confidential). Collected samples was inoculated into sterile Sabouraud Dextrose Agar (SDA, Himedia) media and incubated at 320C for 3-5 days.Microscopic and macroscopic identification were done to specifically identify fungus. Microscopic identification was done by Lactophenol Cotton Blue (LPCB) test. Macroscopic identification was done by morphological, cultural characteristics on SDA.

2.4.2 Antifungalassay

The commercially available shampoos (ketaconazole and loreal paris), antibiotic, and Piper nigrum were diluted using dimethyl sulfoxide (10).To check the Minimum Inhibitory

Concentration (MIC) of siver nanopaerticles of Piper nigrum were made in the range of 20µl, 40µl, 60µl and 80µl samples and their antifungal activity were checked using disc diffusion method. Agar disc diffusion method was done to determine the zone of inhibition of extracts against dandruff causing fungus and recorded against the corresponding concentration(11).

2.5 Statistical Analysis

The mean ± standard error was used to express the values for each parameter. To evaluate normality of data a Kolmogorov-Smirnov analysis was run and Leven's test was used to evaluate homogeneity of variance. The analysis was used SPSS software (version 21.0; SPSS for Windows).

RESULTS AND DISCUSSIONS

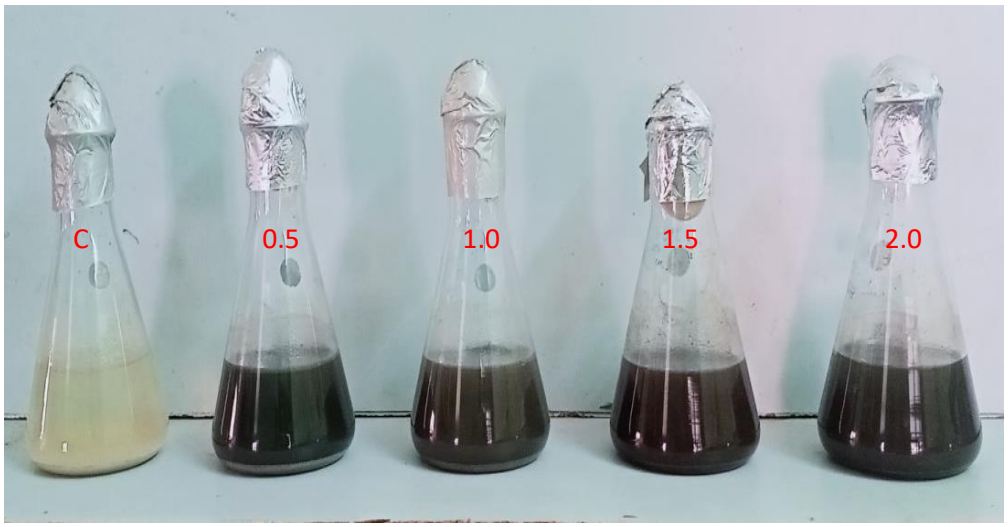

Fig 1: Green synthesis of silver nanoparticles from Piper nigrum L.

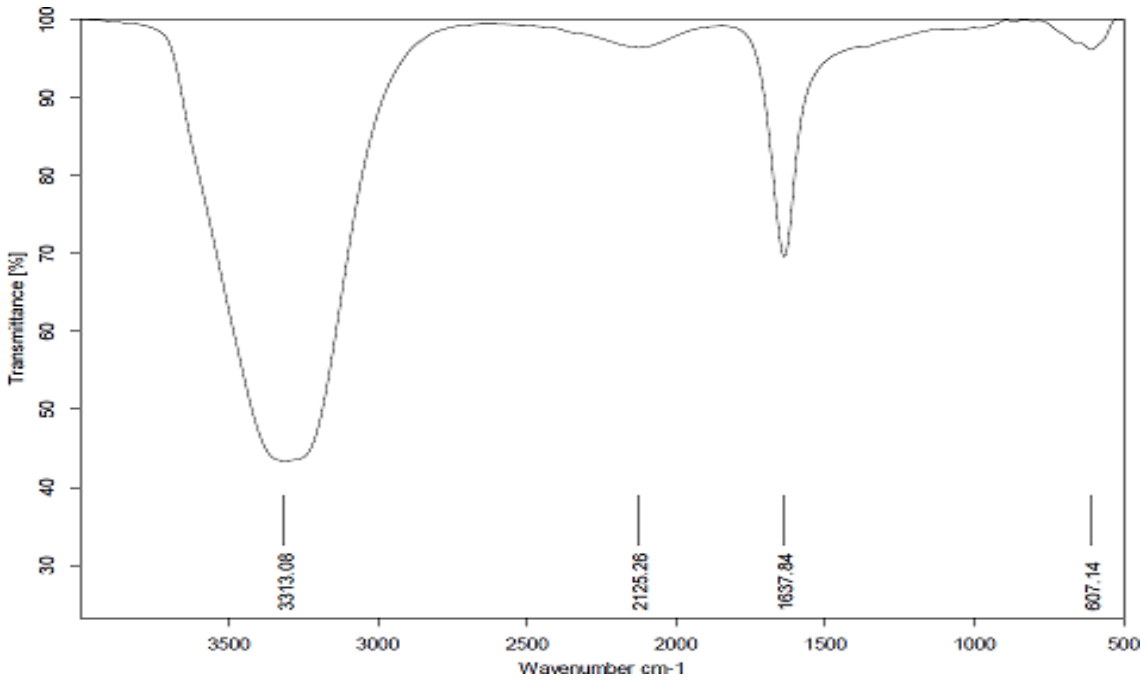

Fig.2: FT-IR analysis of highest value in concentration (2.0mM) fromP. Nigrum L.

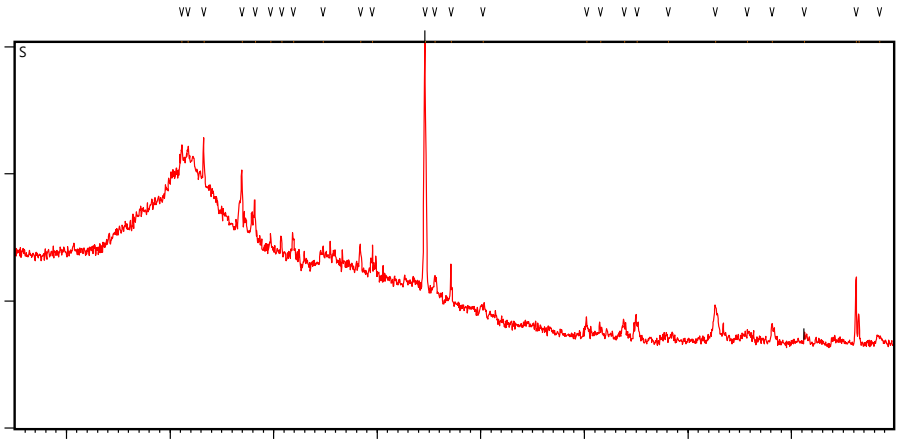

Fig.3: XRD analysis of highest value in concentration (2.0mM) from P. nigrumL.

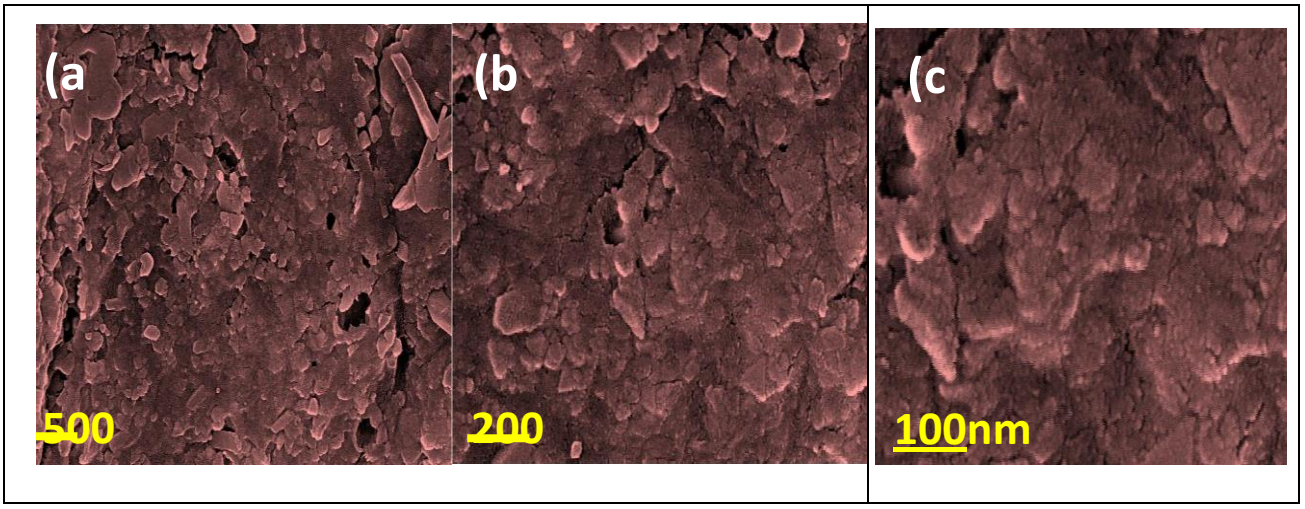

Fig 4: SEM analysis of highest value in concentration (2.0mM) from P. nigrum L

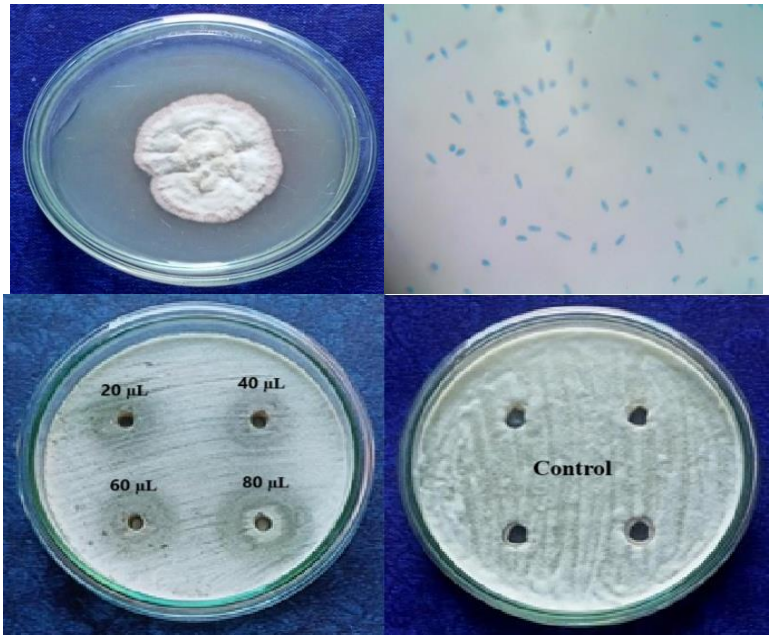

Fig 5: Isolation of dandruff samples and antidandruff activity by using P. Nigrum L. coated with silver nanoparticles

Table 1: Green synthesis of silver nanoparticles from P. nigrumL

Different concentration of silver nitrate (mM)	Percentage of absorbance (%)					
	Different wavelength (nm)					
	400	420	440	460	480	500
Control	0.518	0.660	0.735	0.802	0.681	0.501
0.5	0.609	0.693	0.776	0.973	0.713	0.608
1.0	0.715	0.882	0.903	1.326	0.867	0.745
1.5	0.807	0.953	1.126	1.404	1.108	0.887
2.0	0.872	0.978	1.241	1.572	1.227	0.964

Table2: FT-IR analysis of highest value in concentration (2.0mM) from P. Nigrum L

S.No.	Range(cm-1)	Functionalgroupname
1.	3313.08	Amines, N-H Stretching vibrations, Imines (=N-H); oneband,Amine salts.
2.	2125.26	UnsaturatedNitrogenCompounds,C≡NStretchingvibrations,Isocyanides
3.	1637.84	UnsaturatedNitrogenCompounds,O-NO2,Nitrates
4.	607.14	HalogenCompounds,C-XStretchingVibrations,C-Br

Table 3: Isolation of dandruff sample

Sample	No of colonies	No of species
Dandruff	298	1(Malassezia sp.)

Table4:Antidandruff activity by using P. nigrum L. coated with silver nanoparticles

Name of the fungi	**Synthesized silver nanoparticles from P. nigrum L.**	**Zone of inhibition(mm)**			
		Different concentration(µl)			
		20	**40**	**60**	**80**
Malassezia sp.	Control	-	-	06.0±0.18	07.0±0.02
	Concentration	10.0±0.11	10.5±0.04	12.0±0.13	16.0±0.19

The values are expressed in terms of (Mean ±Standard deviation

UV-analysis of AgNO3 P. nirgrum NPs

The solubility and stability of these reductants were the most important factors that drive the formation of corresponding AgNPs. AgNO3 was added separately to white pepper solutions and formation of the AgNPs was monitored spectroscopically (Table 1.). Formation of pep-AgNPs occurred at RT only when the concentration of AgNo3 was increased as observed by the color change from straw yellow to dark brownish orange color and was monitored using a UV-Visible spectrophotometer. When AgNO3 was added to a solution of White papper small Surface Plasmon Resonance (SPR) peak at 460 nm was observed indicating the formation of pap-AgNPs. Intense and narrow SPR peak at 460 nm with no shoulder at NIR region was observed when 2mM of AgNO3 was raised.

FTIR analysis of AgNO3 P. nirgrum NPs

The FTIR spectrum of biosynthesized silver nanoparticles (AgNPs) derived from Gomphrena serreta was analyzed. As illustrated in Fig. 2, the FTIR spectra reveal distinct peaks corresponding to OH, alkene, and aldehyde groups, which likely play a role in both the reduction and stabilization of the silver nanoparticles. The compounds present in the extract, including OH, aldehyde, and alkene, function as capping and stabilizing agents in the synthesis of silver nanoparticles, as noted in previous research (Netai et al., 2017). Our findings align with these earlier studies, indicating that various functional groups present in the pepper extract contribute to the capping and stabilization of the synthesized silver nanoparticles. The spectrum of pure pep-AgNPs showed a deep band at 3313 cm-1 representing the –OH stretch of the phenolic groups at the N-H region (Fig.2 and Table 2). Broadening of the band in fa- AuNPs indicated the involvement of this hydroxyl group in the formation of pep-AgNPs. Three strong bands observed in the pep-AgNPs spectrum at 607 cm-1, 16355 cm-1 and 21256 cm-1 were assigned to the -NO2 and O–H out-of-plane bending vibrations of the phenolic groups. Presence of these bands in the PeP-AgNPs spectra was evidence for the involvement of the -OH groups in the reduction of Ag1+. A strong band at 1034 cm-1 assigned to the O-CH3 stretching vibrations appeared in spectra indicating that the anisole ring remained unaltered at the end of the reaction. FTIR results of Pep-AgNPs also suggested a shift in band at 3437 cm-1 representing the vibration attributed to –OH stretch of the phenolic groups at the C4–OH region. A strong band at 1034 cm-1 assigned to the O-CH3 and CCC ring stretching vibrations appeared in AgNPs spectra indicating the presence of perper on the NPs. The band at around 600 cm-1, indicated the presence of -OH groups on peper and this –OH group was completely absent in pep-AgNPs.

XRD analysis of AgNO3 P. nirgrum NPs

Research on X-ray powder diffraction The Piper nigrum L extract's biosynthesised AgNPs' crystal nature was investigated using the XRD technique. The synthetic AgNPs' XRD profiles are shown in Fig 3. The diffraction peaks at 2θ values of 38.13°, 46.72°, 64.44°, and 76.93° can be attributed to the face-centered cubic structure of AgNPs' (111), (002), (022), and (113) planes (12). This confirmed the presence of silver and the highly crystalline nature of the particles (13). The results are in agreement with several studies that reported the cubic nature of biologically synthesized AgNPs. The pattern of AgNPs synthesized from Piper nigrum L extract showed some additional peaks, which might be due to the presence of organic molecules in the extract and these peaks are shown in Fig 3. For pep-AgNPs the diffraction peaks appeared at 38.14 º, 44.25 º, 64.49 º and 77.25 º . The crystallinity of the synthesized AgNPs was further confirmed by XRD analysis (Fig.3). A bulk amount of the samples was analyzed by XRD which assessed the number of crystallites. Our results showed the presence of almost all the peaks in the spectrum corresponding to the lattices planes of silver.Thus, the results of X-ray diffraction pattern validated the presence of organic molecules that facilitate the synthesis of nanoparticles.

SEM analysis of AgNO3 P. nirgrum NPs

At very high concentration of synthesized silver nanoparticles from Piper nigrum L. Spherical shaped agglomerated patches were observed. It is seen that AgNPs of different shapes were obtained in case of different leaf extracts being used as reducing and capping agents. Banana, neem and tulsi extracts formed approximately spherical, triangular and cuboidal AgNPs, respectively. This may be due to availability of different quantity and nature of capping agents present in the pepper extracts. This is also supported by the shifts and difference in areas of the peaks obtained in the FTIR analysis.

Anti-dandroff activity

Karnasphotha is experiencing Tikta Rasa and Katu. It can be suggested that Katu Vipaka has antidandruff properties because it serves as Krimi's Prakruti Vighatana Chikitsa. It eliminates Nidana for dandruff and stops new dandruff formation with its Prakruti Vighata action. Its Vishahara characteristic aids in dandruff healing. (15). The minimal inhibitory concentration (20 and 40) of AgNPs for dandruff causative agent M. furfur was found to be at 10.0 and 10.5µgmL(-1), respectively. The half maximal inhibitory concentration (60) value of the AgNPs for Malassezia cells was calculated as 12.0µgmL(-1) and complete inhibition was observed at a concentration of 16.0µgmL (80).

CONCLUSION

In this present investigation, AgNPs were green synthesised using Piper nigrum L. extract. The physicochemical properties of AgNPs were characterised using UV-visible spectrophotometer, Scanning electron microscopy Fourier transformed infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and Brunauer-Emmett-Teller (BET) analysis. Further, in vitro anti-dandruffof green synthesised AgNPs were assessed against Malassezia furfur. The minimal inhibitory concentration (20 and 40) of AgNPs for dandruff causative agent M. furfur was found to be at 10.0 and 10.5µgmL(-1), respectively. The half maximal inhibitory concentration (60) value of the AgNPs for Malassezia cells was calculated as 12.0µgmL(-1) and complete inhibition was observed at a concentration of 16.0µgmL (80). Finally, our results proved that green synthesised AgNPs using Piper nigrum L have great potential in biomedical applications such as anti – dandruff and treatment.

In this study, AgNPs were green synthesised using Piper nigrum L. extract. The phytochemical screening result proved flavonoids are mainly responsible in the reduction process of Ag+ to Ag0. The green synthesised AgNPs showed excellent anti-dandruff activity M.furfur .The method used in this study is very simple, eco – friendly and economically viable, making it amenable to large – scale industrial production of AgNPs. However, further investigation of this green synthesised AgNPs for its biocompatibility will bring it into effective nano- drug for in vivo medical application.

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EFFECT OF METALS ON HAEMATOLOGICAL CHANGES IN THE FRESH WATER FISH

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ABSTRACT

The physiological, biochemical and haematological changes in *Channa punctatus* after exposure to sublethal concentrations of the copper sulphate of the heavy metal have been investigated. The toxicity of the two-ppm copper sulfate with respect to behaviour, and haematological paramaters of the fish *Channa punctatus* has been studied. Red blood cell (RBC) count and haemoglubin (Hb) content were decreased 0.9% with the increasing concentrations of the copper sulfate. However, the white blood cell (WBC) count was increased 1% with increasing concentrations of the copper sulfate. A dissimilar relationship was established with respect to RBC and WBC. The constant increase in the differential count clearly indicates that the heavy metal stress certainly stimulate the white blood cells to produce more at all times of exposure. Biochemical changes of protein, lipid and carbohydrate were recorded. Also, such fish when consumed as food leads to the deposition of the heavy metal in the soft tissues of the human body leading to exposure to a health effects.

Key words: Copper sulfate, *Channa punctatus*, Biochemical, Haematology

INTRODUCTION

Water harbours all types of biotic and abiotic components including metals essential for life processes. The effects of heavy metals and trace elements in water have been well known since the episodes of the Minimata and Nigata bay incidents and with the existence of itaital disease caused due to mercury pollution in Japan. Because of these disasters the study of toxicity of heavy metal to the aquatic life is very much required. Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. Heavy metals are dangerous because they tend to bioaccumulation. As trace elements, some heavy metals like copper, zinc are essential to maintain the metabolism of the human body. Copper has been used to control fungal diseases in plants. High concentrations of this heavy metal were detected in some aquatic ecosystems, collecting runoff water and it is also highly concentrated in ground water (Gerbe, 1996). There are also, anthropogenic sources of environmental contamination by copper including mining, smelting, foundries, municipal waste incinerators, burning of coal for power generation and a variety of copper based products in building and construction. Cadmium (Cd) is a well-known heavy metal toxicant with a specific gravity 8.65 times greater than water. Heavy metals become toxic when they are not metabolized by the body and accumulate in the soft tissues. The target organs for Cd toxicity have been identified as liver, placenta, kidneys, lungs, brain and bones (Ramamurthy *et al.*, 2008).

Copper is an essential trace metal in small concentrations for several fish metabolic functions. Essentiality of copper arises from its specific incorporation into a variety of enzymes, which play important roles in physiological processes (e.g. enzymes involved in cellular respiration, free radical defense, neurotransmitter function, connective tissue biosyntheses & other functions), as well as, into some structural proteins. Although the crucial role of copper in several enzymatic processes (Baker, 1969), this heavy metal can exert adverse toxicological effects, when present in high concentrations in water (Pelgrom *et al.*, 1995). In fact, it is potentially toxic when the internal available concentration exceeds the capacity of physiological detoxification processes.

Copper sulfate is one of the most widely used algicides for the control of phytoplankton in lakes, reservoirs and ponds; it is also used for aquatic weed control (Karan *et al.,* 1998). Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The present study aims to document the toxicity of copper sulfate is on biochemical and haematological parameter of *Channa punctatus.*

MATERIALS AND METHODS

For the experimental purpose, the healthy live *Channa punctatus* were collected from the local fish farm at Adirampattinam. The collected fish were brought to the laboratory without injury in order to avoid dermal infection and they were made to acclimate themselves to the conditions prevailing in the lab for about a week. *C. punctatus* were fed with groundnut oil cake, coconut oil cake, rice bran; they were given feeds for 7 to 15 h. The food found unused by them was cleared periodically from that tank, during the acclimation period. The chlorine free water was changed daily. The feeding was stopped one day prior to the experiment.

In a preliminary study the toxic and sublethal levels of 0.5 to 5 ppm copper sulfate were found out for 96 h exposure. The fish were observed for behavioural changes and mortality at 96 hours and 21 days of exposure were recorded and tabulated employing each of the two test media. The 4 ppm concentration at which 100 percent mortality was observed within 96 hours was considered as the lethal concentration (96 h LC_{100}) and the 0.5 ppm concentration at which 100 percent survival was observed by the end of 96 hours was considered as the sublethal concentration (96 h LC_0). The LC_{50}, the lethal concentration, which kills 50% of individuals at 96 h exposure, was found out and the data obtained were tabulated. For these present study heavy metals copper was taken and this was prepared at different concentrations.

The experiments were carried out in such a manner that the *Channa punctatus* were subjected to 0.5 to 5 ppm different sublethal concentrations in order to assess the effect of copper on selected biochemical and haematological parameters. Before the actual starting of the experiments, the test fishes were divided into 3 groups of the same weight one control and two experimental. They were selected from the stock tank and transferred into the test chamber with test solution of various concentrations of copper viz., 1 and 2ppm. Each group consisted of 10 fish as per aquarium. These groups were actually subjected to both short (96 h) and long term (21 days) exposure periods. Both long term and short tem exposure were given to all the groups involving the 1 & 2 ppm concentrations of copper sulfate. A control third group (copper free water 0 ppm) was also maintained in the above said manner. After the exposure period was over, fish was taken out and sacrificed for the analysis of selected haematological parameters viz., RBC, WBC, Hb, MCV, MCHC, and MCH with respect to short term and long term exposure periods of fish to the different concentrations of copper. After 21st day analysis of biochemical and enzymes was estimated in muscle of fish. The estimation of glucose (Dubois *et al.,* 1956), protein (Lowry *et al.,* 1951), Alanine transaminase and Aspartate transaminase (Mohun and Cook, 1957), Lipase (Oser, 1965) were estimated.

RESULTS

Survival Capacity: The study recorded that 96 h LC_{50} value of copper was 3.0-ppm. Percentage of mortality of *Channa punctatus* is varied with effect of copper concentrations and exposure time (Table 1). The mortality of fish was increased with increasing concentrations of copper sulfate. No mortality was observed up to 2-ppm.

Haematological parameters: With respect to haematology, the study illustrative of the fact that there was a gradual decrease in the haematological parameters such as RBC number, Hb content and gradual increase in WBC number, such was actually caused by the use of the heavy metal. The copper sulfate shows significant concentration dependent. The reduction of red blood cells was noticed at lower concentration and also at short term (Acute) exposures i.e. in 1 and 2-ppm for 96 h. The same individual were treated at a higher a concentration of 1 and 2-ppm even though there was a short term exposure results a considerable number of decreased RBC. At the same times for a long period, the maximum reduction was observed.

It is evident that the normal blood parameter values of RBC count was 2.80 X 10^6/cmm, WBC 9640 X 10^3/cmm, Hb 10.40 g/100 ml; PCV 30.40%; MCV 108.57 ulX10^9; MCH 37.14 P.gm and MCHC 34.21%. The RBC counts found in the 1-ppm copper treated fish after an exposure of 96 h was 2.70 X 10^6/cmm. Later when the fish was introduced at high concentration at 2 ppm, its count was 2.60X10^6/cmm with a decrease of 7.14%. When the fish was introduced of choronic exposure period at higher concentration of 2 ppm the RBC count was 2.55 x 10^6 cmm with a decrease 8.92%. Like wise, the same results are also true for haemoglobin contents. For a prolonged exposure period of different concentrations of copper medium, the Hb content was decreased in *Channa punctatus* by 6.7% at low concentration and by 9.6% at higher concentration. During the experiments, white blood cell count (WBC) increased more and more at prolonged exposure period. The consistent increase in the WBC indicates that the heavy metal stress certainly stimulates the production of more WBC's at all time of longer exposures.

There are variations in the values of MCV, MCH and MCHC when the fish are exposed to 1 and 2ppm of copper for 96 h and 21 days (Tables 2 & 3). It has been suggested that enumeration of different cell ratio count provides a useful diagnostic procedure to assess physiological stress in the fish as well as the water quality. There are variations in the values of enzymes and biochemical content when the fish are exposed to 1 and 2ppm of copper for 96 h and 21 days. The biochemical and enzymes results presented here have clearly demonstrated that the elevated metal ion concentrations for copper substances (Table 4).

DISCUSSION

Heavy metals are present in aquatic environment from mining activities and industries that use these metals in various processes (Lloyd, 1992). Aquatic ecosystems polluted with heavy metals, may therefore threaten human nutrition and health directly. Fish are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution. The survival of aquatic animals depends on not only the biological state of the animals and physico-chemical characteristics of water but also on kind, toxicity, type and time of exposure to the toxicant. In the present study, the mortality increased with an increase in concentration of copper and also the duration of the exposure. Table 1 depicts the percentage mortality for different exposure periods at different concentrations of copper. LC_{50} value of copper for the fish *C. punctatus* was determined.

A capacity of copper sulfate destruction fish and aquatic animals is largely a function of its toxicity, exposure time, does rate and persistence in the environment. Immediately after transfer to the test solution, *Channa punctatus* became hypersensitive and showed a rapid of opercular movements accompanied by occasional gulping of air. The higher the concentration, the more pronounced was this behaviour. After several minutes of exposure the individual lost their equilibrium. They were floated with complete cessation of movements and finally dead.

The haematology of fishes has gained recognition, as an applied science. Haematology tests have become important diagnostic tools in medicine. Recent studies have shown that the haematological parameters may be equally valuable, in indicating the disease or the stress in the fish. The composition of blood of fishes varies with the changing conditions of the environment and responds immediately to any change in water quality because of intimate contact. Out of varied haematological parameters differential red blood cells counts are of immense physiopathological importance. In the present investigation, an attempt has been made to elucidate the effects of copper with different sublethal concentration on certain physiological properties of the blood of *C. punctatus. C. punctatus* exposed to sublethal concentrations of copper resulted in a significant decrease in RBC's count leading to anaemia as a result of inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in haemopoietic organs. Natarajan, (1981) reported a reduction in Hb content. RBC count and PCV values resulting in hypochronic anaemia due to deficiency of iron and decreased utilization for Hb synthesis.

The anaemic condition recorded in the present study could be due to the destruction of mature RBC or inhibition of erythrocyte production. Such decreases in RBC and anaemic suspense have been observed by Koundinya and Ramamurthy, (1979) in *Sarotherodon mossambius* after exposure to lethal concentration of submithion. Chouhan *et al.,* (1983) in *Puntius ticto* treated with herbicide. Lal *et al.,* (1986) in *H. fossilis* exposed to Malathion. It is evident that in the present study the reduction in number of RBC and Hb content and decrease in MCV values might have caused microcytic anemia as suggested by Venkataramana *et al.,* (2004) in *Glossogobicus giuris* after exposure to sublethal concentration of Malathion. Varadharaj *et al.,* (1993) have opined that a reduction in the number of RBC and Hb content with an increase in MCV and MCHC values might cause macrocytic anaemia in *Oreochromis mossambicus.* Since the Hb and RBCs are oxygen-carrying devices, the quantitative decrease in their levels might have led to the rearrangement of the oxidative metabolism with a concomitant decrease in the tissues of respiratory potential.

The fish facing asphyxia of undergoing physical exercise and facing hypoxic stress required increased amount of Hb to cope with the decreased oxygen availability. This consequently is partially achieved by immediately releasing mire and more cells, which also bring more erythroblasts in circulation. In the present study also, the RBC and Hb content are more or less the same due to the tolerance to short term exposure, at longer exposure period, depletion or reduction of RBC numbers and Hb content was obtained.

The long- term exposure to copper treated fish reduced the red blood cell count and haemoglobin value. This indicates that he high doses of copper produce anaerobic condition and limits the oxygen carrying capacity and there by decrease the mobility. Most of the blood corpuscles were very thin after long-term exposure and the hypochromic cells naturally contained the decreased concentration of haemoglobin. Total leucocytes count showed an increase in their number in the higher concentrations, but at lower concentration, there is a gradual increase in their number. In the present investigation at higher concentration and longer exposure period produces an increase in total W.B.C and MCHC count as in *G. hiuris* suggested by Radhakrishnan and Prasad, (1994) in *Oreochromis mossambicus* after exposure to malathin and Ekalak Ec-25 respectively. Achuttan Nair *et al.,* (2000) suggested that this leucocytosis was the result of direct stimulation of the immunological defenses due to the presence of toxic substance or may be associated by induced tissue damage. A linear relationship was established with respect to heavy metal copper and total leucocytes. The constant increase in the differential count

clearly indicates that the copper stress certainly stimulate the white blood cells to produce more at all times of exposure. It has been suggested that the enumeration of differential cell ratio counts provide of useful diagnostic procedure to assess the physiological stress in the fish.

A significant hyperglycaemia was also recorded after exposure to this wastewater e.g. control fish had a mean plasma glucose of 56.80 mg/100 cm3 while the-treated fish exhibited an increase in the levels of plasma glucose to 65.30 and 81.00 mg/100 cm3, respectively. This means that the fish were subjected to some sort of hypertoxic stress (Ramamurthy *et al.*, 2008). In this study, tissue biochemical were generally influenced by this heavy metal that may be attributed to the relative changes in the mobilization of protein and carbohydrates. Changes in the biochemical concentrations may be a result of increased production of metals, which is a sequestering agent. On the other hand, the elevation of enzymes that runs parallel to a decrease in muscle biochemical content may be on indication of a gluconeogenetic response. This additional source of biochemical may support the fish with the required energy highly demanded to manage with the presence of a potentially harmful substances such as copper sulphate.

Table 1. Effect of different concentration of copper sulfate on mortality (%) of *Channa punctatus* as a function of different exposure time

S.NO	Concentration of copper (ppm)	Exposure time (h)			
		24	48	72	96
1	0.5	-	-	-	-
2	1.0	-	-	-	-
3	1.5	-	-	-	-
4	2.0	-	-	-	-
5	2.5	-	10	20	30
6	2.0	-	-	-	-
7	3.5	30	40	50	80
8	4.0	40	60	80	100
9	4.5	60	80	90	100
10	5.0	70	90	100	100

Table 2. Effect of sublethal concentration at 1ppm of copper sulfate on selected haematological parameters in *Channa punctatus*

S. No	Blood parameters	Control	Acute 96 h exposure	Chronic 21 days exposure
1	RBC X 10^6/cmm	2.80 ± 0.52	2.70 ± 0.75	2.60 ± 0.59
2	WBC X10^3/cmm	9640 ± 0.12	9790 ± 0.85	9795 ± 0.73
3	Hb g / 100ml	10.40 ± 0.11	10.35 ± 0.76	9.70 ± 0.55
4	PCV %	30.40 ± 0.72	29.50 ± 0.76	28.60 ± 0.85
5	MCV µl X 10^9	108.57 ± 0.12	109.25 ± 0.85	110.00 ± 0.85
6	MCH (Pg)	37.14 ± 0.72	38.33 ± 0.29	37.30 ± 0.11
7	MCHC (%)	34.21 ± 0.75	35.08 ± 0.23	33.91 ± 0.35

Table 3. Effect of sublethal concentration at 2-ppm of copper sulfate on selected haematological parameters in *Channa punctatus*

S. No	Blood parameters	Control	Acute 96 h exposure	Chronic 21 days exposure
1	RBC X 10^6/cmm	2.80 ± 0.52	2.65 ± 0.75	2.55 ± 0.59
2	WBC X 10^3/cmm	9640 ± 0.12	9990 ± 0.85	10110 ± 0.73
3	Hb g / 100ml	10.40 ± 0.11	10.30 ± 0.76	9.40 ± 0.55
4	PCV %	30.40 ± 0.72	29.10 ± 0.76	23.10 ± 0.85
5	MCV µ l X 10^9	108.57 ± 0.12	109.81 ± 0.85	110.19 ± 0.85
6	MCH (P g)	37.14 ± 0.72	38.86 ± 0.29	36.96 ± 0.11
7	MCHC (%)	34.21 ± 0.75	35.39 ± 0.23	33.45 ± 0.35

Table 4: Effect of sublethal concentration at copper sulfate on selected biochemical and enzyme parameters in *Channa punctatus*

S. No	Parameters	Control	1ppm copper sulfate		2ppm copper sulfate	
			Acute 96 h exposure	Chronic 21 days exposure	Acute 96 h exposure	Chronic 21 days exposure
1	Total protein	24.7	22.3	15.9	16.2	12.8
2	Carbohydrate	10.5	10.2	9.0	9.1	8.2
3	Phosphatase	18.7	16.5	15.8	16.0	14.5
4	Protease	6.8	5.9	5.5	5.4	4.5
5	Lipase	1.08	1.5	1.0	1.2	0.95
6	AST	4.52	3.8	3.2	3.5	2.36
7	ALT	0.44	0.55	0.74	0.68	0.96

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IMPACT OF MONOCROTOPHOS ON RESPIRATORY AND CHANGE OF BIOCHEMICAL CONSTITUENTS OF *CHANNA PUNCTATUS* (BLOCH)

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ABSTRACT

India is rich in inland fishery resources, but the indiscriminate use of the pesticides causes serious threat to such resources like water and land. Pesticides are stable compounds and they enter into the aquatic ecosystem through the agricultural run off. The pesticides, which enter the body tissues of the fish, affect the physiological activities and the nature of main food stored like glycogen and protein. Based on the fish were treated with various concentrations of the monocrotophos the amount of protein and glycogen contents in the muscle of the fish decreases slowly sometimes rapidly on prolonged exposure even in the same concentrations. There was clear and steady increased in the rate of opercular movements and follows decreased rate of oxygen consumption was observed with increasing concentrations of the pesticide monocrotophos.

Key words: Pesticide, Monocrotophos, *Channa punctatus*, biochemical composition of fish muscle.

INTRODUCTION

Monocrotophos is the single largest selling agrochemical in India. monocrotophos in aquatic organisms, especially fish, are lacking. The fish used in this study are *Channa punctatus*, which are edible, commercially valuable and distributed all over India. Pesticides are useful tools in agriculture and forestry, but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored (Konar, 1975; Basak and Konar, 1976 & 1977). The aquatic ecosystem as a greater part of the natural environment is also faced with the threat of a shrinking genetic base and biodiversity. *Anabas testudineus, Channa punctatus* and other indigenous small fishes use paddy fields as breeding and nursery grounds (Shaun *et al.*, 2007). *Barbodes gonionotus* is an important species for integrated rice-fish farming. Pesticides at high concentrations are known to reduce the survival, growth and reproduction of fish (Mckim *et al.*, 1975) and produce many visible effects on fish (Johnson, 1968).

Pesticides are stable compound. Indiscriminate liberal and injudicious use of pesticides by man to control the crop pests and diseases for higher agricultural productivity has led to a slow but steady deterioration of the aquatic ecosystem, since water as the ultimate sink. These pollutants also destroy the quality of the aquatic media and render it unfit for various aquatic organisms particularly fishes. A variety of organophosphate, organchlorine and carbamide pesticides are extensively used in agriculture for the control of pests. It can be seen that the toxic action is specific for a particular animal in a particular toxicant. The toxicity levels were influenced by the sex and the nutrient supply (Arunachalam, 1980) by the pH (Mathivanan, 2004). The effects of several insecticides and pesticides on various physiological responses of fishes were reported by Anderson, (1971); Monoharan and Subbiah, (1982); Devi Sewtharanyam, (2000); Prashanth *et al.*, (2003).

The fish species showed several abnormal behaviors which included restlessness, arena movements, loss of equilibrium, increased opercular activities, strong spasm, paralysis and sudden quick movements during the exposure. For histopathological studies, *A. testudineus*, *C. punctatus* and *B. gonionotus* were exposed for 7 days to sublethal concentrations of Diazinon 60 EC, respectively (Gupta, 2002). The fish exposed to sublethal concentration of LC(10) (7.74 mg/l) were under stress and altered their locomotor behavior, such as distance traveled per unit time (m/min) and swimming speed (cm/sec) with respect to the length of exposure. Inhibition in the activity of brain AChE and deformities in the primary and secondary lamellae of gill may have resulted in failure of exchange of gases. The maximum inhibition of 95% of AChE activity was observed on days 20 and 24.Morphological aberrations in the gills were also studied during exposure to the sublethal concentration of monocrotophos for a period ranging from 8 to 24 days. The extent of damage in gill was dependent on the duration of exposure. The findings revealed that inhibition in brain AChE activity and structural alteration in gill were responsible for altering the locomotor behavior of exposed fish (Venkateswara *et al.*, 2005). Sensitive biomarkers to study the genotoxic effects caused by monocrotophos in aquatic organisms, especially fish, are lacking. The study reveals that the comet assay is a sensitive and rapid method to detect genotoxicity of monocrotophos and other environmental pollutants in sentinel species.

Omitoyin *et al.*, (2006) indicated that lindane exacts toxic effects on fish. The 96 h LC50 value (0.38 mg/L) for *C. gariepinus* juveniles suggest that the fish show a quick response to the toxicant. This is in agreement with the findings of (Joshi *et al.*, 2002), who reported that lindane creates haematological disturbances and causes metabolic disorders to fishes which may ultimately lead to the deterioration of general health of fish. The present investigation has been takes up to elucidate the effects of monocrotophos at sublethal concentration on certain biochemical constituents of the fish muscle and also to examine the sublethal effect of the behaviour and respiratory activity of the fish *Channa punctatus.*

MATERIALS AND METHODS

The fish for the experimental purpose healthy live *Channa punctatus* weighing 15.52 ± 2.52 g with a mean body length of 9.11 ± 2.02 cm were collected from culture ponds. The experiments were carried out with help of small circular troughs of 25 liters capacity in which an iron wire gauge covered the trough at the surface level of water to avoid for aerial respiration. The Lethal concentration 0. Lc 50, and Lc 100 values were calculated and tabulated. Before the actual starting of the experiments, the test fishes were divided into 6 groups of the same weight were selected from the stock tank and transferred into the test chamber with test solution of various concentrations of monocrotophos ie. 0.0 ppm, 1.0 ppm, 2.0 ppm, 3.0 ppm, 4.0 ppm and 5.0 ppm. Each group is consisted of 10 fish per trough. These were actually subjected to both short and long term exposure periods, the former lasting for about 24 to 48 hrs and the latter lasting for 72 to 96 hrs. Both the long term and the short-term exposures were given to all the five groups involving the different concentrations on monocrotophos. A control (pesticide free water) was also maintained in the above said manner.

After the exposure period was over one of the fish was taken out and scarified for the analysis of selected biochemical constituents viz., protein Lowry method (Lowry *et al.*, 1951) and glycogen Antheron method. Behavioural effects were also studied in normal and treated fishes, which were maintained at room temperature.

RESULTS AND DISCUSSION

Pesticides are one of the major xenobiotic substances that have been used in India for a longer period for the management of pests in agriculture fields and control of vectors in public

health operations. Most of the insecticides are hydrophobic that they can easily be absorbed by soil particles and can migrate to natural water systems such as rivers, lakes and ponds through the run off causing severe aquatic pollutions. The indiscriminate use of the pesticides, however, resulted in the pollution of our eco-system causing hazards to non-target organisms including fishes.

The fish *Channa punctatus* were treated with 0.0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0 ppm of monocrotophos and the mortality percentage was performed on survival percentage after 24, 48, 72 and 96 h. (Table 1) and also to examined the sublethal effect (Gupta 2002)

Glycogen and proteins are the chief nutrients of the animals. They have a variety of functions. The glycogen supplies energy in the form of ATP molecules, which are formed during TCA cycle. Glycogen is autoketogenic and aids in the utilization of body fats. Similarly proteins are made up of amino acids which form the building blocks of the body like essential constituents of protoplasm of all the cells. The proteins in different tissues differ in composition and properties.

In the present study the results obtained clearly indicate that there was a decreased amount of protein and glycogen content to resist the effects of pesticides. That is to provide immediate energy to the fighting elements of the body and protect all systems of the body from the harmful effect of the pesticides. As the fish was constantly kept in the medium of different concentration of pesticides dissolved in water there was no way for the animal to move away from the toxic medium. The effect of pesticides dominates. The organ system and the glycogen content are slowly depleted due to the utilization of the already stored glucose contents of the body. At particular level poison overtakes the organisms so that the level of the carbohydrates content is very low in the muscles because of the utilization.

With regard to the exposure of fishes to different concentrations of Monocrotophos pesticide there was no much change within 24 hours of treatment with mild concentrations of 1.0 ppm and 2.0 ppm but the same on prolonged exposure upto 96 hours the glycogen content was observed in decreased amount (Table 2 & 3) when the concentration was increased to 3.0 ppm, 4.0 ppm and 5.0 ppm. The glycogen content was observed in the decreasing order with increasing concentrations and with more exposure periods the glycogen content was found more and more in decreased. Because of the stress the fish makes suitable adjustments for which the stored energy is utilized. This may be the reason for the decreased amount of glycogen content (Anonymous, 2005). Such reduction in stored glycogen content has been reported in *Tilapia mossambica exposed* to methyl parathion (Rao and Rao, 1929) effect of endosulfan (Vasanthi and Ramasami, 1987) in *Saratherodon mossambicas* and in *Channa punctatus following* malathion intoxication reported (Shah and Double, 1983) and in *O. mossambicus* to metacid (Baskaran, 1991).

The protein content in the muscle of *C. punctatus* is decreased with increasing concentrations of pesticide monocrotophos. Even with the same concentrations longer exposure resulted in decreased amount of protein content (Table 2), which indicates that the tissue protein undergoes proteolysis. This results in the production of free amino acids, which are used in the TCA cycle for energy production under stresses (Kabeer Ahamed, 1979). There are similar reports of the effects of toxicants on total protein in other fishes (Rath and Mishara, 1980; Shah and Double, 1983; Palanichamy *et al.*, 1989).

In the present study, the opercular beats increased with increasing concentrations of pesticides (Table 3). The initial increase in opercular movement in the less concentrated medium as a primary response to sudden stress was reported by Anbu and Ramasamy, (1991) as in *Channa striatus* exposed to carbomate pesticide sevien. Baskaran and Palanichamy, (1990) reported that the opercular beats increased with increasing concentrations of fertilizer in *A. scandens.*

Oxygen consumption of pesticide treated fish showed an initial increase with lower concentration of pecticides and decreased with increasing concentrations (Table 3). The reduced oxygen consumption could be attributed to gill damage or to hypochronic mirocytic anemia as suggested by Baskaran and Palanichamy, (1990). Similar decrease in oxygen consumption was observed by Lars Collivin, (1984) in *Perca fluviatilis.*

The decrease in oxygen consumption appears to be a protective measure to ensure that there is a low intake of the toxic substance. Reduced oxygen consumption at higher concentrations of pecticides could also arise as a result of respiratory inhibitory factors that come into play as suggested by Rafia Sultana and Uma Devi, (1995) in *Mystus gulio* under heavy metal pollution.

Oxygen consumption and opercular activity of the fish were concentration dependent. The initials increase oxygen uptake probably to meet energy demand during early periods of exposure. Which was followed by gradual decrease in oxygen uptake in later period of exposure, which may be due to onset of toxicity as suggested by White, (2006). In order to meet the increased demand for oxygen by the tissues opercular activity is enhanced but this too has a limit beyond which the activity stops resulting in the death of the animal (Palanichamy and Baskaran, 1995).

The pesticide monocrotophos dissolved in water brings about extensive changes in the physical parameters of water such as pH, salinity, alkalinity and severe depletion in the dissolved oxygen content. So the aquatic systems are drastically altered due to this new stress in the environmental medium that may alter the biochemical contents and respiratory parameters to cope with those situations.

Table.1. Effect of different concentration of pesticide monocrotophos on mortality percentage of *C. punctatus* as a function of 24, 48, 72, 96, hrs exposure

S. No	Concentrations (ppm)	% of survival & hours of exposure				Remarks
		24	48	72	96	
1	1.0	0	0	0	0	Lc 0/96 hr
2	2.0	0	0	0	0	”
3	3.0	0	0	0	0	”
4	4.0	0	0	0	0	”
5	5.0	0	0	0	0	”
6	6.0	0	0	0	10	Lc 10/96 hr
7	7.0	10	20	40	50	Lc 50/96 hr
8	8.0	10	20	40	70	Lc 70/96 hr
9	9.0	10	20	50	80	Lc 80/96 hr
10	10.0	40	80	90	100	Lc 100/96 hr

Table. 2. The total protein content and total Glycogen content of muscles of *C. punctatus* exposed to pesticide monocrotophos

S. No	Concentration of monocrotophos (ppm)	Exposure Period (hrs)	Amount of Protein Content (mg /g)	Amount of Glycogen Content (mg /g)
1	Control 0 ppm	24	23.50±0.43	3.25±0.15
		48	22.10±0.25	3.10±0.17
		72	21.10±0.17	2.90±0.18
		96	20.75±0.22	2.50±0.23
2	Treated 1.0 ppm	24	22.25±0.51	3.10±0.22
		48	21.75±0.62	2.95±0.13
		72	20.25±0.25	2.35±0.18
		96	19.25±0.15	1.90±0.14
3	Treated 2.0 ppm	24	22.00±0.25	2.85±0.11
		48	21.25±0.15	2.50±0.12
		72	18.50±0.16	2.25±0.13
		96	18.25±0.17	1.90±0.14
4	Treated 3.0 ppm	24	21.50±0.13	2.65±0.22
		48	20.40±0.12	2.35±0.55
		72	1810±0.14	1.70±0.51
		96	15.25±0.11	1.50±0.62
5	Treated 4.0 ppm	24	20.20±0.11	2.50±0.55
		48	19.50±0.12	1.95±0.62
		72	16.50±0.11	1.15±0.63
		96	15.25±0.12	0.50±0.25
6	Treated 5.0 ppm	24	19.76±0.10	2.25±0.92
		48	17.50±0.20	1.70±0.72
		72	16.00±0.23	1.10±0.62
		96	15.25±0.22	0.40±0.50

(Each value represents the mean ± SD of five values).

Table. 3. Sub lethal effects of monocrotophos on oxygen consumption and opercular beats of *Channa punctatus*

S. No	Concentration (ppm)	Opercular beats (No./min)	Oxygen consumption (ml/g/h)
1	0	125.32 ± 3.85	0.98 ± 0.05
2	1.0	130.00 ± 3.25	0.98 ± 0.03
3	2.0	160 .21± 4.65	0.75 ± 0.08
4	3.0	180.34 ± 6.25	0.60 ± 0.18
5	4.0	195.46 ± 3.50	0.40 ± 0.25
6	5.0	225.12 ± 4.50	0.25 ± 0.45

(Each value represents the mean ± SD of five values)

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PREPARATION OF M-TEA (MENSTRUAL TEA) AND PRELIMINARY ANALYSIS OF ITS NUTRITIONAL COMPONENTS

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ABSTRACT

Menstrual pain is a burden on women. Menstrual pain, also known as dysmenorrhea is characterized by cramping abdominal discomfort and other symptoms. It can significantly impact a womens daily activities and overall well - being. The prevalence of menstrual pain is a common issue among women, leading to significant discomfort and reduced quality of life. M teas consist of herbal materials that are often folk recipes with known health benefits. M teas containing theofAshwagandha, Shatavari, Ashoka, Ginger, Licorice, Tea leave, Turmeric, Rose petals, Mint, Pepper, Clove, Hibiscus were developed and evaluated for its physicochemical properties. The M tea generally showed good physicochemical properties. It showed stability properties that are characteristic of a good finished product. M -Tea analyzsed using GC-MS. The tea also showed effective free radical scavenging properties and consistent with its antioxidant benefits. Some of its physicochemical and antioxidant properties were diminished when stored in a stress environment.

Keywords: M-tea, tea bags, Physicochemical properties, antioxidant, GC-MS analysis

INTRODUCTION

Menstruation is the natural process that occurs in women reproductive age. However, many women experiences menstrucral cramps, bloating and other symptoms that can distrupt daily life. Menstrual Tea is also called as M-Tea. M-Tea using a combination of herbal ingredients and analyse its nutritional components of M-Tea, including vitamins, minerals and antioxidants, were analysed using various analytical techniques.M-tea have been used for centuries to alleviate menstruals symptoms. M-tea is a blend of herbal ingredients that are rich in nutrients and antioxidants[1].

Clinically PCOD women diagnosed with associate inflated sterility risk like dysfunctionalhaemorrhage, obesity, carcinoma, sort a pair of polygenic disorder, dyslipidemia, cardiovasculardisease, and presumably disorder. Symptoms of Polycystic Ovarian Syndrome includeAmenorrhea or infrequent menstruation, irregular bleeding, infrequent or no ovulation, multipleimmature follicles, increased levels of male hormones, male pattern baldness or thinning hair,excess facial and body hair growth, acne, oily skin or dandruff, dark coloured patches of skinspecially on neck, groin, underarms, chronic pelvic pain, increased weight or obesity, diabetes,lipid abnormalities and high blood pressure[2].

MATERIALS AND METHOD

Preparation of M-Tea:

Preparation of M-Tea powder: All the ingredients are in dry form and grinded to make fine powder. Required tea ingredients were accurately weighed individually by using digital balance.All these fine ingredients were mixed thoroughly with the help of Mortar and Pestle to get uniform mixing. Fine tea powder was passed through sieve no.63, to get the sufficient

quantity of fine powder. Tea Powder mixture was collected and stored in suitable air tight container. [3]

M-TEA Extract: Tested samples are weighed by given value from Ingredients table and weighed samples are dipped into 100ml hot water for 15miutes. Take the liquid sample and store into the centrifuge tube.

Preliminary Phytochemical Analysis:

Test for Alkaloids [Mayer's Test]

Boiled 2ml of each extract with dilute hydrochloric acid then filtered and add to the filtrate a few drops of Mayer's reagent.A cream or while colour precipitate produced immediately indicate the presence of alkaloids.

Carbohydrate [Benedict's test]

Added 2 ml of each extract to 1ml of benedict's reagent. The mixture is heated on a boiling water bath for 2 minutes solution appeared green showing the presence of reducing sugar.

Phenol [Lead acetate]

1ml of each extract was added to 3 ml 10% lead acetate solution. A bulky white precipitate indicates the presence of phenolic compounds.

Glycosides [kellerkilanin test]

5ml of each extract taken added with 2ml of glacial acetic acid which was followed by addition of few drops of ferric chloride solution and 1ml of concentrate sulphuric acid. Formation of brown ring at interface confirms the presence of glycoside.

Terponoids [salkowskitest]

5ml of each extract was taken in the test tube and 2ml of chloroform was added to it followed by the addition of 3ml of concentrated sulphuric acid formation of reddish brown at the junction of 2 solutions confirm presence of terpenoids.

Flavonoids [alkaline reagent test]

2ml of extract was treated with few drops of 20% sodium hydroxide solution formation ofintence yellow colour which becomes colourless on addition of dilute hydrochloric acid indicate the presence of flavonoids.

Sapnonins[Foam test]

2ml of extract was taken in thetest tube and 6ml of distilled water was added to it. The mixture was shaken vigorously and observed the formation persistent foam that confirms the presence of sapnonins.

Proteins [Biuret test]

5ml of each extract was mixed with 10% NaOH solution and added few drops of copper sulphate to it. The formation of reddish violet color indicate the presence of proteins.

Steroids [Salkowskitest]

1ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicate the presence of steroids.

Tannins [Lead acetate]

2 ml of extract was added to few drops of 1% lead acetate. A yellow precipitate indicates the presence of tannins. [4]

Estimation of Protein by Lowry's Method:

Pipette out various concentration of working standard solution into a series of test tubes andmade up the volume to 0.2ml with distilled water (10µl to 100µl).To Each test tube add 1ml of the mixed reagent and mix thoroughly and allow to stand at room temperature for 10min or longer Add 0.3ml of diluted FolinCiocalteu reagent rapidly and mix properly.Incubate all tubes for 60 minutes. Measure OD of the standard and test solution at 660nm and plot the standard

graph. Run the blank.The test protein sample is performed as like the standard solution and calculate the amount of protein present in the given sample.Blue colour is noted and read using spectrophotometer.

Estimation of Carbohydrate by Anthrone Method:

Prepare various concentration of the working standard solution in a series of test tube from 0.1ml to 1ml. [10µl to 100µl].Make up the volume to 1ml with distilled water. Keep the tubes in an ice bath and slowly add 5ml of the cold anthrone reagent and mix properly.Close the tubes with aluminum foil and place it in a boiling water bath for 10min.Cool the tubes and measure OD at 620nm. Black should be prepared as per previous steps without adding test or standard solution.Blank should be prepared as per previous steps without adding test or standard solution.Plot the graph and calculate the carbohydrate content of the sample given Green colour standard solution.Blank should be prepared as per previous steps without adding test or standard solution.Plot the graph and calculate the carbohydrate content of the sample given Green colour formation is noted and measured OD at 620nm.[5]

Antioxidant Activity of Reducing Power:

Take a 10mg/ml of sample and added to 0.25ml of H2O & 0.5Ml of PO4. Then added to the 0.5ml of Pottasium Ferric Cinode and the solution was incubate for 50°c for 20mins. Then added 10% TCA OF O.5ml.centrifuge to 2500 RRPM in 10mins. Supernatant was collected. Then added 0.5ml of H_2O &add 0.1%-0.1ml of ferric chloride and measure OD value at 700nm.[6]

Gas Chromatography Mass Spectroscopy (GC-MS):

The M-tea extracts was subjected to GC-MS analysis to identify the presence of unknown compounds. The extract was mixed with GC grade methanol at 1mg/mL concentration. The extract was purified through syringe filter to ensure that the sample was devoid of impurities that may block the GC-MS column. 1µL of purified extract injected into the GC-MS (agilent GC 7890A/MS5975C). The column used was aglient DB 5ms with a dimension of 30mx 250µm × 0.25µm. The carrier gas used was helium at a column temperature of 325°C. The mode was set as split less. The oven was programmed at 50°C for 1min and then increased at a rate of 10°c/ min to 300°c/min with total runtime of 28°c. The pressure was maintained at 3psi. The MS acquisition was performed in scan mode and the m/z ratio ranged between 50 to 550amu. The compounds were identified using NIST library.[7]

RESULT AND DISCUSSION:

M-tea ingredients were collected and prepared. We have prepared the tea pouch. Many Incredients has been put into the tea pouch. That the natural incredients are help us to healthy beneficial. The ingredients have the ability to cure the Polycysticovarien disease.

pHAnalysis:

We have analyse the pH level in the M-tea extract of about 6.7

Qualitative Phytochemical Analysis for M-TEA Extract:

The qualitative phytochemical analysis was carried out the extract of M-TEA. Theresult shows that the Tannins are present high amount of compound present in the extract of M-TEA. Tannins is the major compound to this activity presence in the m-tea. The other compound like Phenol, Carbohydrate, Alkaloids, Terponoids, Glycosides are present in the moderate and trace amount in the M-tea extract.

Estimation of Protein & Carbohydrate:

The total protein content and total carbohydrate content was also conducted.In that the protein content 200µl of M-TEA contains 79µg of protein was present. In 200µl ofM-tea contains 63µg carbohydrate was present in the M-tea sample.

Antioxidant Activity of Reducing Power:

Antioxidant activity of theM-tea extract was analysed by reducing power. The IC50 values of all parameters weredetermined while ascorbic acid was used as standard. In this studyof M-tea extract was present isa potential source of natural antioxidant.

FTIR Analysis:

The M-tea extract was injected into the FTIR to analyse the chemical compounds present in thePlants. In there are many compounds present in the Ingredients like carboxylic acids, esters, Amides, Aromatics, Alkanes,Alkyl Halides, Esters, Ethers, Alcohols, Alkenes,were present.[8]

GC-MS Analysis:

The M-tea extract was injected into the GC-MS to analyse the chemical compounds present inthe Plants. In there are many compounds (2(5H)- Furanone, 2-Butenal, 2-4-Diamino,5,6- Epoxyheonal-1, 2H-Pyran, d-Glucitol, 3-Methyl-2-Butene-1-thiol, tetrahydro-2, Phenol,3,5-bis, Distannoxane, Pentadecanoic acid, Dibutyl Phthalate, Ethane,1, Tetrasiloxanedeamethyl, 5-methyl-2-Phenylindolizine) present in the Plants By conducting the preparation ofM-tea(menstrual tea) were the ability to cure the pcod/pcos.

CONCLUSION

The above study concludes that the M-tea has many medicinal and the ability to cure thepcod/pcos. The plant has many phytochemical analyses and the presence aresapnonins, tannins, terpenoids, and other chemical compounds are present in this M-tea were analysed. The M-tea has the nutritional values like high amount of protein, high amount of carbohydrate and also rich source of fatty acids like omega 3 fatty acid and some flavonoids are present the plant. Flavonoids play a major role in this study. In this Biochemical analysis of Total Protein content higher than compared to the total carbohydrate content.

Polycystic ovarian syndrome were selected for this activity ability to cure them by using the M-tea extract. For this study the M-tea has selected for the solvent. While conducting FTIR analysis to M-tea extract that shows that the presence of carboxylic acid in the plant, in that also another compound called amides, alkanes, ester was also present in this M-tea extract.

Antioxidant activity of reducing power also present the potential source of natural antioxidant. While GCMS analysis to M-tea extract the chemical compounds are present in the M-tea extract. The M-tea extract was injected into the GC-MS to analyse the chemical compounds present in the plant. In there are many compounds present in the plant.By conducting the preparation of M-tea(Menstrual tea) we observing were the ability to cure the pcod/pcos.

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HEPATOPROTECTIVE STUDIES ON *SARCOSTEMMA BREVISTIGMA* ON ALBINO RATS

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ABSTRACT

The hepatoprotective activity of an alcohol extract of *Sarcostemma brevistigma* (Asclepiadaceae) was studied against CCl_4 induced acute hepatotoxicity in rats. Estimation of serum enzyme activities of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, protein and bilirubin was carried out to assess liver damage. Pre and post-treatment with alcohol extract showed a dose-dependent reduction of CCl_4 induced rats were elevated levels of enzyme activity with parallel increase in total protein and bilirubin, indicating the extract could preserve the normal functional status of the liver.

Key Words: Hepatoprotective activity, *Sarcostemma brevistigma*, Rats, Biochemical studies.

INTRODUCTION

Liver diseases are a serious health problem. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However, we do not have satisfactory remedy for serious liver disease; most of the herbal drugs speed up the natural healing process of liver. So the search for effective hepatoprotective drug continues.

The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrate, protein, and fat metabolism, detoxification, and secretion of bile. Therefore, the maintenance of a healthy liver is vital to overall health and well being. Unfortunately, the liver is often abused by environmental toxins, poor eating habits, alcohol, prescription and over-the-counter drug use, which can damage and weaken the liver and eventually lead to hepatitis, cirrhosis, and alcoholic liver disease. Conventional medicine is now pursuing the use of natural products such as herbs to provide the support that the liver needs on a daily basis. Many Ayurvedic herbs, such as *Sarcostemma brevistigma*, have a long history of traditional use in revitalizing the liver and treating liver dysfunction and disease. Many of these herbs have been evaluated in pharmacological studies and are currently being investigated phytochemicals to understand their actions better.

Sarcostemma brevistigma Wight (family Asclepiadaceae) grows throughout India and other tropical regions of the world. It is found to be active as anti-rheumatic, anti-allergy, anti-emetic and branchodilator (Kirtikar and Basu, 1993). This plant is used for the treatment of several ailments, including respiratory infections and pain. Our research group has previously investigated the phytochemical and pharmacological properties of this plant. In this context, its analgesic, anti-microbial and anti-diabetic effects, were described and related with the presence of terpenes and flavonoids, including kaurenoic acid and luteolin. Hernandez-Munoz *et al* (1994) and Muriel (1998) showed that kaurane diterpenes from *S. brevistigma* display trypanosomicidal activity and smooth muscle relaxant effect. Due to the widespread use of this plant by the rural communities to treat several diseases, the objective of the present study was to obtain data on the safety of the crude extract. The acute and sub-acute oral toxicity of the hydroalcoholic extract from aerial parts of this plant in mice was assessed. The changes in selected biochemical and

haematological parameters were also determined. The aim of this study was to determine whether circulating liver enzyme levels, serum bilirubin and protein are raised at liver injury, during the earliest phases of implantation. The liver damaged rat blood was collected and analyzed with several parameters of biochemical impacts.

MATERIALS AND METHODS

The medicinal plant of *Sarcostemma brevistigma* belongs to family of Asclepiadaceae was collected from in and around area of Pattukkottai, Tamil Nadu, South India. The plant was identified (Gamble 1967; Krtikar & Basu 1935). A voucher specimen has been preserved in our laboratory. The plant leaf was dried and powdered and 50 g powdered sample was extracted with ethanol using soxhlet apparatus and concentrated *in-vacuo*. Approximately, 0.50 g of extract was obtained from 10 g of dried leaf material. The extract was suspended in 5% gum acacia and used for studying hepatoprotective activity.

Albino rats weighing between 100 and 200 g were selected. The rats were divided into five groups, each group consisting of six individuals. Hepatoprotective activity of *S. brevistigma* was evaluated using CCl_4 induced model. Group I was kept on normal diet and served as control; the group II received CCl_4 (0.2 ml/100 g) by oral route, III and IV groups received by extracts of *S. brevistigma* (25 mg 100 g & 50 mg/ 100 g) and group V received by silymarin (2.5 mg/ 100 g) respectively once daily, for ten days. On the tenth day, CCl_4 was given by oral route 30 min after the administration of silymarin and test drug. After 36 hours of CCl_4 administration, blood was collected and the serum separated was analyzed for various biochemical parameters.

Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) by the methods of Reitman & Frankel (1975), alkaline phosphatase (Kind & King 1971), total bilirubin (Mallay & Evelyn 1937) and protein (Lowery *et al*. 1951) were analyzed. The liver was examined grossly, weighed and stored in formalin 10% and processed for paraffin embedding using the standard microtechnique (Galigher & Kozloff 1971). A section of the liver (5 mm) stained with alum haemotoxylin and eosin and the stained sections were observed microscopically for histopathological studies (Galigher & Kozloff 1971).

RESULTS

The treatment with the extract did not decrease water and food consumption. The body weight of the rats treated with hydroalcoholic extract once a day during 10 days (sub-acute treatment) did not show any significant change when compared with the control group, although had a tendency to decrease body weight (200 & 400 mg/kg). This decrease can be associated with the decrease of liver weight at the dose of 400 mg/kg in comparison with the control group without any concomitant alteration in the activity of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase. Estimation of the serum activity of total bilirubin, protein, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase is one of the most widely used means of measuring hepatocellular injury (Table 1). The macroscopic analysis of the target organs of the treated rats (liver, lung, heart, spleen & left kidney) did not show significant changes in colour and texture when compared with the control group (Table 2).

Histologically, individuals in control showed normal hepatic architecture, the group II rats exhibited intense centrilobular necrosis, vacuolization and macrovesicular fatty changes. CCl_4 treated individuals showed a normal hepatic architecture. Moderate accumulation of fatty lobules and cellular necrosis were observed in the rats treated with aqueous extract. However, ethanol extract treated animals exhibited significant liver protection against CCl_4 induced liver damage, as evidenced by the presence of normal hepatic cords, absence of necrosis and fatty infiltration.

DISCUSSION

The results of biochemical parameters revealed the elevation of enzyme level in CCl_4 treated group, indicating that CCl_4 induces damage to the liver (Table 1). Liver tissue rich in both transaminases increased in patients with acute hepatic diseases, SGPT, which is slightly elevated by cardiac necrosis, is a more specific indicator of liver disease (Rodwell *et al* 1983 and Ramamurthy *et al* 2008). A significant reduction ($P < 0.001$) was observed in SGPT, SGOT, ALP, total bilirubin and protein levels in the groups treated with silymarin and alcoholic extract of *S. brevistigma*. The enzyme levels were almost restored to the normal.

It was observed that the size of the liver was enlarged in CCl_4 intoxicated rats but it was normal in drug treated groups. A significant reduction ($P < 0.001$) in liver weight supports this finding. It was found that the extract decreased the CCl_4 induced elevated levels of the enzymes in-groups III & IV, indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with silymarin and extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evidenced from formation of normal hepatic cards and absence of necrosis and vacuoles.

Decrease in serum bilirubin after treatment with the extract in liver damage indicated the effectiveness of the extract in normal functional status of the liver. The preliminary phytochemical studies revealed the presence of flavonoids in ethyl acetate extract of *S. brevistigma*; various flavonoids have been reported for their hepatoprotective activity (Scevola *et al* 1984). So the hepatoprotective effect of *S. brevistigma* may be due to its flavonoid content.

It is observed that *S. brevistigma* has a significant hepatoprotective effect in CCl_4-administrated rats that hepatocellular degenerative and necrotic changes are slight without advanced fibrosis and cirrhotic process in *S. brevistigma* treated group. However, Turkdogan *et al* (2001) found that *Nigella sativa* can prevent liver fibrosis and cirrhosis, suggesting that *N. sativa* protects liver against fibrosis possibly through immunomodulator and antioxidant activities. Ramamurthy *et al* (2008) found that *Wedelia chinese* can prevent liver fibrosis and cirrhosis that protects liver against fibrosis possibly through immunomodulator and antihepatotoxicity activities.

CCl_4 has been widely used to induce experimental hepatic damage (Yao *et al* 2005). It induces liver cell necrosis and apoptosis, and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration (Constandinou *et al* 2005; Chang *et al* 2005; Shi & Li, 2005). Liu *et al* (2001) investigated the effect of manual acupuncture at ST36 and LR3 on CCl_4-induced acute liver damage. In this effect of manual acupuncture at GB34 on chronic liver damage induced by long-term CCl_4 administration.

ALT and AST are the specific markers to assess hepatocellular damage leading to liver cell necrosis (Amacher 1998). Slight to moderate increases in ALP (1-2 times normal) occurred in liver disorders (Isselbacher *et al*. 1994). Serum cholesterol is one of the general indications of the synthetic and general metabolic capacity of the liver (Fregia & Jensen 1994). In the present study, CCl_4 injection significantly increased serum ALT, AST, ALP and cholesterol levels, indicating induction of hepatic damage.

Table 1. Effect of ethanol extracts of *Sarcostemma brevistigma* on some serum biochemical parameters of CCl_4 intoxicated rats.

Parameters	Normal control	CCl_4 (0.2ml/100g)	EESB (200 mg/kg)	EESB (400 mg/kg)	Silymarin (25 mg/kg)
Bilirubin (mg/dl)	0.95± 0.4	2.65 ± 0.36	1.91 ± 0.3	1.10 ± 0.45	1.09±0.2
SGOT (U/l)	61.13±0.23	198.33± 4.8	155± 12.0	108.01±12.9	95.28± 0.69
SGPT (U/l)	46.36 ± 1.8	100.1± 0.9	84.0± 7.1	69.67± 7.2	56.08± 0.86
ALP (U/l)	129.1± 0.6	382.9±12.2	235.1±4.0	186.5±12.6	163.2±10.8
Protein (g/dl)	8.02 ± 0.6	6.41 ± 0.7	7.93 ± 0.8	7.98 ± 0.9	8.0 ± 0.8

Values are mean ± S.E.M. number of rats = 6. CCl_4 control group compared with normal control group $p<0.001$. **Note. EEAB: Ethanol Extracts of *Sarcostemma brevistigma***

Table 2. Effect of oral administration of *S. brevistigma* extract on body and organs weight.

Dose (mg/kg)	Control	CCl_4 (0.2ml/100g)	Silymarin (25 mg/kg)	EESB (200 mg/kg)	EESB (400 mg/kg)
Body (g)	54.1 ± 3.17	54.7 ± 1.90	56.0 ± 3.77	52.0 ± 3.8	53.0 ± 2.77
Liver (g)	1.59 ± 0.20	1.45 ± 0.14	1.46 ± 0.22	1.32 ± 0.17*	1.12 ± 0.16
Heart (g)	0.14 ± 0.018	1.45 ± 0.140	1.48 ± 0.225	1.35± 0.177*	1.16 ± 0.176
Left lung (g)	0.21 ± 0.03	0.19 ± 0.01	0.23 ± 0.04	0.22 ± 0.06	0.18 ± 0.04
Spleen (g)	0.18 ± 0.092	0.15 ± 0.031	0.16 ± 0.051	0.12 ± 0.013	0.14 ± 0.096
Kidney (g)	0.17 ± 0.021	0.16 ± 0.017	0.16 ± 0.025	0.18 ± 0.027	0.15 ± 0.029

Mean values of 6 individuals ± S.D. *$p<0.05$; **$p< 0.01$ vs.
Note. EESB: Ethanol Extracts of *Sarcostemma brevistigma*

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AN *IN VITRO* AND TOXICITY STUDIES OF *AMORPHOPALLUS PAEONIIFOLIUS* AQUEOUS EXTRACT BY USING ZEBRAFISH

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ABSTRACT

Amorphophallus paeonifolius is a traditional medicinal plant used to treat various ailments. However, its toxicity profile remains unclear. This study aimed to evaluate the invitro and toxicity effects of *Amorphophallus paeonifolius* aqueous extract using zebra fish as an animal model. First, Analyse the physiochemical, phytochemicals, and in vitro antioxidant properties of aqueous extracts. DPPH radical scavenging assay aqueous extract expressed relatively low radical scavenging property. The present study suggests that the corm of *Amorphophallussylvaticus* have great potential as a natural source of antioxidant. GC-MS analysis revealed the presence of 15 bioactive compounds, including essential oils, fatty acids, and terpenoids. The study provides valuable information on the phytochemical constituents and spectral characteristics of *Amorphophalluspaeonifolius* , which can be useful for its pharmacological applications.

Keywords: *Amorphophalluspaeoniifolius,* Toxicity, DPPH, Phytochemical, Aqueous extract

INTRODUCTION

Tamil name –senaikizhangu, Hindi –senakand,Malayalam – pattalakilann, Telugu – armigaddadinusu*Amorphophalluspaeoniifolius* the elephant foot yam or white spot giant arum, is a tropical tuber crop grown primarily in Africa, south asia, southeast Asia and the tropical pacific islands. Because of its production potential and popularity as a vegetable in various cuisines, it can be raised as a cashcrop.

The yam is used as a food in island southeast Asia, mainland southeast Asia, south asia, new guinea, oceania, and Madagascar. Its origin and center of domestication was formerly considered to be india.

The flower bud emerges from the corm as purple shoot, and later blooms as a purple inflorescence this allows the pollen to be deposited on the emerging insects to pollinate other flowers, while preventing the pollen from the same inflorescence fertilising itself, preventing inbreeding.

In 24-36 hours, after the first bloom of the inflorescence, the inflorescence's female flower start developing into berries bright red fruiting bodies, and other parts of the inflorescence start wilting away. In southern India, especially kerala, it is known as chena, the tuber has been a part of people's diet for centuries.

***AMORPHOPHALLUS PAEONIIFOLIUS* (YAM)**

CLASSIFICATIONOF *AMORPHOPHALLUS PAEONIIFOLIUS*	
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Order	Alismatales
Family	Araceae
Genus	Amorphophallus
Species	A. paeoniifolius
Binomial name: *Amorphophalluspaeoniifolius* (Dennst. Nicolson,1977)	

As medicine

The yam is widely used in Indian medicine and is recommended as a remedy in all three of the major Indian medicinal systems:Ayurveda, siddha and unani. The corm is prescribed in those systems for a variety of ailments. The tuber is reported to be useful in treatment of piles.Women with PCOS have an increased risk for metabolic abnormalities and T2 DM, infertility, obstetricl complications, endometrial cancer and mood disorders. These women also probably have an increased risk for thromboemobolism and ovarian cancer.

Treatment of polycystic ovary syndrome is individualized based on the patient's presentation and desire for pregnancy. For patients who overweight, weight loss is recommended. Clomiphene and letrozole are first-line medications for infertility(Daniel., 2011). The sexuality levels of PCOS fish, however, could be improved after short periods of rearing in conditions that lack of males. After only 3 days of rearing alone, the PCOS fish showed an increase in sexuality levels and displayed characteristic swimming patterns for mating after 30 days of separation from males, not only the sexual activity, but also the mating rate was improved in the PCOS fish together, the data suggests the zebra fish can serve as a new type of research model to further develop strategies for the treatment of reproductive disorders, such as those related to PCOS. High levels of testosterone cause clinical symptoms and possibly, alterations in sexuality (Moghetti P *et al.*, 2010).

MATERIALS AND METHODS

Sample Collection:

The *Amorphophalluspaeoniifolius*tubewere collected form Thanjavur Market, Tamil Nadu, India. The collected sample were Shade dried, Powdered, and used for further process.

Preparation of Extract:

Yam extract:25g of *Amorphophalluspaeoniifolius* tuber were dried the power and 500 ml of distilled water was add to it shaking for 3 days and filtered using gauze cloth and placed it in the water bath at 60℃ for 5-6 days to evaporation the water and the remaining extract was used for further activities

Qualitative Phytochemicals Tests:

Carbohydrate (Benedict's test): 1ml of *Amorphophalluspaeoniifolius*extract was added to 1ml of Benedict's reagent. the mixture was heated at a boiling water bath for 2 minutes, solution appeared green colour showed the presence of reducing sugar.

Phenol Llead acetate test): 1ml of *amorphophalluspaeoniifolius*extract was added to 3ml of 10 % lead acetate solution. A bulky white precipitate indicates the presence of phenolic compounds.

Glycosides (kellerkailianin test): 5ml of *Amorphophalluspaeoniifolius*extract was added with 2mlof glacial acetic acid which was followed by the addition of few drops of ferric chloride solution and 1 ml of concentrated sulpuric acid the brown ring was formed at the interface of two junctions confirmed the presence of glycosides.

Terpenoids(Salkowski test): 5ml of each *Amorphophalluspaeoniifolius* extract was taken in a test tube and 2ml of chloroform wasadded to it followed by the 3ml of concentrated sulphuric acid was also added. The formation of reddish –brown at two solutions confirms the presence of terpenoids.

Flavonoids (Alkaline reagent test): 2ml of *Amorphophalluspaeoniifolius* extract was treated with few drops of 20% sodiumhydroxide solution. The intenseyellow colour was formed then it becomes colourless on addition of dilute hydrochloric acid, it indicates the presence of flavonoids.

Saponins (Foam test): 2ml of *Amorphophalluspaeoniifolius* extract was added in a test tube and 6 ml of distilled water was added to it. The mixture was shaken vigorously and observed. The persistentfoam was formed that confirms the presence of saponins.

Proteins (Biuret test): 5ml of *Amorphophalluspaeoniifolius* extract was mixed with 10% NaoH solution and added few drops of copper sulphate. The reddish violet colour was formed and that indicates the presence of proteins.

Tannins (Lead acetate): 2ml of *Amorphophalluspaeoniifolius* extract was added to few drops of 1% lead acetate.A yellowish precipitate was formed and indicated the presence of tannins.

Total Protein Content

0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml,1.0 ml of the standard protein(BSA) was added in the fresh tube and make into1 ml. 0.2 ml of *Amorphophalluspaeoniifolius* extract was added to new tube. The blank was prepared byadding1 ml of distilled water in a new tube and follow the procedure. Then 4.5 ml of Lowry's reagent was added to all the tubes and incubate the tubes for 10 minutes. Then 0.5 ml of folin's reagent was added to all the test tubes and incubated at 20 minutes at dark room. The colour change was observed and the optical Density was noted at 660 nm under UV spectrophotometer.

Total Carbohydrate Content

0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml of the working standard glucose was in the fresh tube and make into 1ml. 0.2 ml of *Amorphophalluspaeoniifolius* extract was added to new tube. Keep the tube in an ice bath and slowly add 5 ml of the cold anthrone reagent and mix properly.Close the tubes with aluminium foil and place it in a boiling water bath for 10 minutes. Cool the tubes and measure OD at 620nm. Blank should be prepared as previous steps without adding test or standard solution. Plot the graph and calculate the carbohydrate content of the sample given.

FTIR Analysis:

The spectral data of whole pulse flours as well as its protein extracts were collected and corrected with the air background using a PerkinElmer ATR-FTIR (Spectrum 1; Perkin Elmer, Norwalk, CT, USA) equipped with the Spectrum software (ver. 5.3.0; Perkin Elmer). The spectra were generated in absorption mode with mid-IR (ca. 4000-800 cm1) with a resolution of 4 cm1.The regions of specific interest in this study included the heights of the protein amide I, II and III groups.From the deconvoluted spectra (in anodization mode with an enhance factor of 1.5), the spectral intensity of the a-helix (1650 cm^1) and sheet (1635 cm1) secondary structures as well as the intermolecular (Inter) (1616 cm1) and intramolecular (Intra) (1630 cm1) interactions were acquired as described by Cameron & Moffatt (1984) and Cai& Singh 2004).

DPPH Procedure:

Stock solutions of DPPH were preparedin methanol,andMethanol buffered with acetic acid buffer (0.1M,PH 5.5), respectively. Buffered methanol was prepared by mixing 40 ml of 0.1 M AcetateBuffer (PH 5.5) with 60 ml methanol. The solvents and other chemicals were of analytical grade.The reaction tubes, in triplicates, were wrapped in aluminium foil and kept at 30°C for 30 min in dark.All measurements were done under dim light. Spectrophotometric measurements were done at517 nm using spectrophometer**.**

Reducing Power:

Sample-10mg/ml&0.25ml H2O(125ul)+PO40.5ml(250ul). PFC PottasiumFerriccinode 0.5ml (250ul). 50°c (incubate 20 minitues). 10% TCA0.5ml (250ul). Centrifuge 2,500 RPM (10minitues). Supernatant collective. 0.5 of H2O (250ul). 0.1%-0.1 ml of ferricchloride (50ul). OD value700 nm.

GCMS Analysis:

The aqueous and Ethanolic yam extract of *Amorphophalluspaepniifolius* was subjected to GC - MS analysis to identify the presence of unkown compounds. The extract was mixed with GC grade methanol at 1 mg/ mL concentration. The extract was purified through syringe filter to ensure that the sample was devoid of impurities that may block the GC-MS column.1 µL of purified extract was injected into the GC-MS (Agilent GC 7890A / MS5975C). The column used was Agilent DB 5ms with a dimension of 30m × 250 µm × 0. 25µm .The carrier gas used was helium at a column temperature of 325° C. The mode was set as split less. The oven was programmed at 50° C for 1 min and then increased at a rate of 10°C/ min to 300°C/min for 2min with a total runtime of 28°C.The pressure was maintained at 3psi. The MS acquisition was performed in scan mode and the m/z ratio ranged between 50 to 550µm. The compounds were identified using NIST library

Toxicity Studies:

The three different concentrations of the yam extract were separated. The various concentration of the yam extract sample (10µl, 50µl, 100µl) was mixed with the fish feed composition. The zebrafish were differentiated in to four groups for each group 10 fishes were separated. One group was marked as control. The three different concentrations of the yam extract were mixed with fish feed is added to the fish tank. The fishes were observed for the seven days. The fishes were dissected after seven days and the dissected organs were analyzed through the histological observation (McGrath, & Li, 2008)

RESULTS AND DISCUSSION

The results for Qualitative phytochemical analysis were carried out for the yam and ethanol extract of *Amorphopalluspaeoniiflius* shows that tannins are present in the high amount in ethanol extract comparing to the yam extract. Tannin is the major compound to this activity present in yam.

Like tannin one more compound called Saponin was present in the high amount in the ethanol extract and moderate the yam extract, other compound like phenol, carbohydrate, alkaloids, terpenoids, glycosides, are present in the moderate and trace amount in the *Amorphophalluspaeoniifolius*

The total protein content of yam extract of *Amorphophalluspaeoniifolius* was analysed by Lowry's method. Protein is a macronutrient that is essential for the building block of the muscles. Many amino acids are combined to form a protein. In this *Amorphophalluspaeoniifolius* there is 0.2% of protein in yam.

The yam was collected cut and dried the extract was filtered with what man filter paper no.1 and placed in water bath for 60° C for evaporating the solvent to take the yam extract. The qualitative phytochemical analysis was analysed. In this phytochemical analysis there are many compounds like tannins, phenol, saponins, terpenoids, were present in this plant . The total protein content and total carbohydrate content was also conducted. In that the ethanol extract 220µg of protein was present in the 1ml of ethanol extract contains 112µg of carbohydrate and in 1 ml of ethanol extract contains 142µg of carbohydrate present in the yam.

FTIR: Analysed the FTIR function group of yam Carboxylic acids, Amides, Aromatics, Alkanes, Aromatics, Alkyl halides, Esters, Ethers, Alcohols, Aromatics, Alkenes.

DPPH: The DPPH content was analysed then graphed and showed in the 100µg.concentration of aqueous extract inhibition of 75µl. The denotes ascorbic acids 100 µg concentration of inhibition 50µg. and then 500µg of aqueous extract of in inhibition of 90µl .the denotes the ascorbic acid 500 µg concentration of inhibition in 65µg

REDUCING POWER : The result that the percentage of inhibition in the yam sample is higher the standard. The Ascorbic acid is used as standard. 73.45 percentage of inhibition was noted in 100 µg/ml of sample and 69.65 percentage of inhibition was noted in 100 µg/ml of ascorbic acid.89.90 percentage of inhibition was noted in 500 µg/ml of sample and 79.96 percentage of inhibition was noted in 500 µg/ml of ascorbic acid.

GCMS : The GCMS analysis yam Provide fifteen components identified and ethanol extract will shows the presence of the linolenic acid and docosahexaenoic acid as omega 3 fatty acids.

Toxicity studies

The various concentration of yam extract (10µl, 50µl and 100µl) were mixed with the prepared fish feed. The sample added fish feed was fed to the zebrafish as single dosage. After first day the normal fish feed was given to the zebrafish. The acute toxicity studies were conduct to seven days. The organs like liver and gills were dissected form the treated fishes and analyzed for histoarchitecture studies.

Histo-architecture

Histoarchitecture studies were performed removing gills and liver tissues were dissected form the treated and non-treated zebrafishes. The sample yam extract results shows that organs are not damaged. The tissues were healthy and there is no alteration were observed in yam extract

sample. The samples were grouped into three different concentration and there were no changes in the organs.

CONCLUSION

The present study concludes that the results show the positive interaction with the regulation. The phenol, flavonoid content also deals with the increase of hormonal regulation. Antioxidant also has the ability to increase and regulate the hormone level, in this study reducing power and antioxidant assays were carried out and the result shows higher amount of antioxidant present in the YAM. In various studies the antioxidant has the ability to increase. So, we can conclude that the YAM has the ability to regulate the sex hormones. The non-toxic effect of YAM was also proved by using zebrafish as an animal model.

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EARLY DIAGNOSIS OF ALZHEIMERS DISEASE BY DEEPNET FRAMEWORK

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ABSTRACT

In this project, we present a novel approach for the early diagnosis of Alzheimer's disease by employing deep learning algorithms. Alzheimer's disease is a devastating neurodegenerative disorder that affects millions of individuals worldwide, and its early detection is crucial for timely intervention and effective management. Leveraging the power of deep learning, our algorithm harnesses extensive datasets of medical images, including MRI and PET scans, in combination with clinical data to discern intricate patterns and abnormalities associated with the disease. Through a systematic analysis of these multimodal datasets, our approach can effectively identify potential indicators of Alzheimer's disease at an early stage, facilitating more timely and accurate diagnoses. The integration of deep learning techniques in Alzheimer's disease diagnosis holds great promise for enhancing our understanding of this condition and improving patient outcomes by enabling early intervention and personalized treatment strategies. Overall, the steps and considerations in creating an automated Alzheimer's detection system using a deep neural network (Deep Net) framework for the Alzheimer's disease detection system.

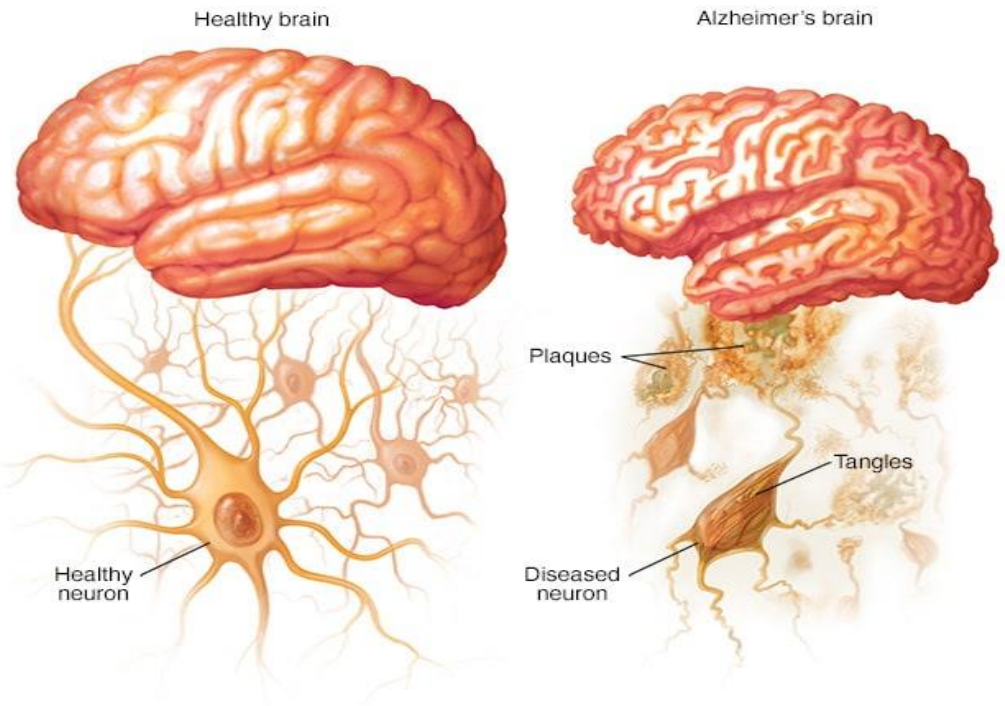
We present a new automated system for the detection of brain magnetic resonance images (MRI) affected by Alzheimer’s disease (AD). The MRI is analyzed by means of Multi Scale analysis (MSA) to obtain its fractals at six different scales. The extracted fractals are used as features to differentiate healthy brain MRI from those of Alzheimer’s disease by a deep learning for inceptionv3 classifier. The result of classifying the brain MRIs consisting of images of healthy brains and brains affected by AD, using leave-one-out cross-validation method, yielded high classification accuracy, sensitivity and specificity. The results indicate that the proposed approach may be an efficient diagnostic aid for radiologists in the screening for Alzheimer’s disease.

Keywords: Alzheimer’s disease, deep learning, Positron emission tomography, Magnetic Resonance Imaging.

INTRODUCTION

Alzheimer's disease is a progressive and devastating neurodegenerative disorder that poses a significant public health challenge globally. [1]As the aging population continues to grow, the prevalence of Alzheimer's disease is expected to increase, making early diagnosis and intervention more critical than ever. Early detection of the disease allows for timely medical intervention, which can potentially slow down the progression of symptoms and improve the quality of life for affected individuals. Deep learning, a subset of artificial intelligence, has emerged as a powerful tool in the field of medical imaging and healthcare[2].

This project aims to explore the potential of deep net framework for inceptionv3 algorithms to enhance the early recognition of Alzheimer's disease, providing a foundation for more accurate and timely diagnosis and ultimately contributing to more effective treatment and care for those affected by this debilitating condition.



Alzheimer's disease can range from mild to severe. The scale ranges from a state of mild impairment, through to moderate impairment, before eventually reaching severe cognitive decline. The sections below will discuss the stages of Alzheimer's Trusted Source and some of the symptoms that characterize them. Advanced neuroimaging strategies, such as Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET), have been employed to uncover the structural and molecular biomarkers pertaining toAlzheimer's disease [3].

Existing System:

Alzheimer's is a neurological illness and affects the nerve cells especially those that are involved in memory and thought processes [4]. The early identification of Alzheimer's disease raises ethical concerns because there is currently no known treatment for Alzheimer's disease and medications from therapeutic trials show to moderately reduce the illness's progression while having occasionally significant side effects. Therefore, medical image analysis has proven to be very useful due to its application in this setting for both treatment follow-up and diagnosis. These pictures can best be handled by use of Computer Assisted Diagnostic Systems (CAD). We suggested an application to identify Alzheimer's disorders in our work [5]. We employed three sections to identify the disease at an early stage: frontal to extract the hippocampal region (H), sagittal to analyze the corpus callosum (CC), and axial to deal with the cortical variation features (C). Support Vector Machine is the foundation of our classification technique (SVM). The suggested method produces an early AD diagnosis accuracy of 90.66%. We suggest using this programme to identify the illness early on [6]. Hippocampus, Corpus Callosum, and Cortex are the three areas that were extracted using segmentation in our programme, which made use of the Region of Intert ROI. Subsequently, SVM-based classification steps are followed (Support Vector Machine) [7]

Proposed System:

The core of the suggested system is the creation and application of the InceptionV3 algorithm, which is based on deep learning and is especially designed for the early detection of Alzheimer's disease. This novel method makes use of the powerful powers of deep learning—

specifically, InceptionV3—to carefully examine and categorize medical pictures, including MRI and PET scans, together with relevant clinical data. Fundamentally, the system is designed to identify complex patterns and anomalies linked to Alzheimer's disease, hence promoting early identification and assistance [8]. To facilitate early detection and intervention, the main goal is to discover complex patterns and abnormalities linked to Alzheimer's disease. The system has a powerful image processing built in to efficiently preprocess the input images. What makes this method distinct from others is the algorithm's ability to differentiate between a healthy brain and one infected by Alzheimer's disease by itself. This computer-based classification augments accuracy, contributes towards swift decision-making for immediate actions so as to lower the diagnostic burden on healthcare practitioners. On this note, the proposed technique employs large and intricate datasets in these records and images. For better comprehension of the condition for detection of subtle clues that might precede actual clinical features. It is an anticipatory intervention policy which has a potential to enhance their quality of life as well as overall healthcare experience by giving them earlier access to support and treatment while diagnosed with Alzheimer's disease in its early stages. In other words, therefore, this suggested system signifies furthering Alzheimer's diagnosis while concurrently reaching at personalized medicine. Its purpose is to get clinicians such that it will become a useful tool in helping them quickly identify who may be potentially affected by Alzheimer disease in future [9]. Therefore, this will lead to improved patient outcomes and greater awareness about this intricate neurological ailment (Refer Figure 1)

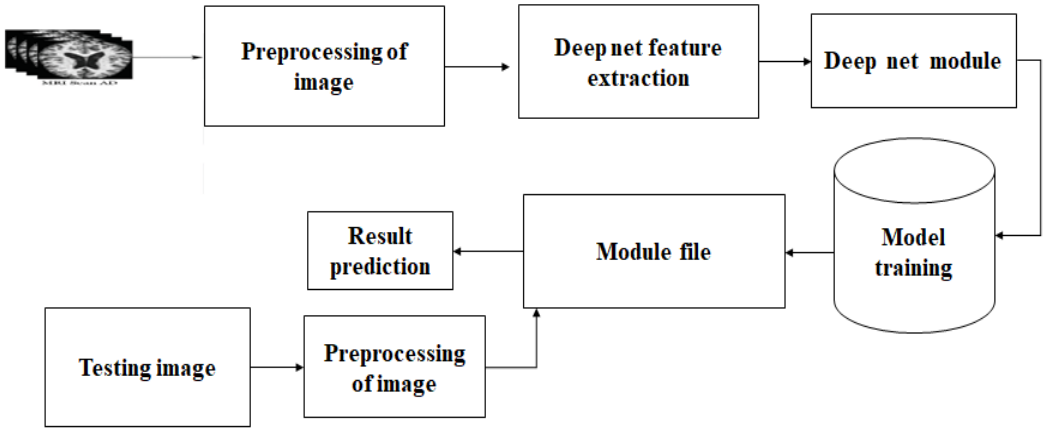


Figure: 1 ALGORITHM

INCEPTION V3: InceptionV3 is the most sophisticated deep learning system used in image recognition. CNN by Google's researchers is a major milestone in computer vision. It represented a complete change of focus from looking for more effective and precise models of image classification. Its distinctive design philosophy gives it an exceptional architecture that contains multiple inception modules making it possible to correctly capture and process information on different scales, all within one layer. This technique maximizes computational resources spent while improving accuracy of the model providing practicality. Since its introduction, InceptionV3 has been at the forefront in various fields including auto-driven cars, medicine and agriculture [10]. In short, practitioners as well as scientists find this an important tool due to its adaptability plus efficiency which contributes to evolution of AI thereby leading to visual perception or interpretation and other intricate applications (Refer figure 2)

WORKING PROCESS:

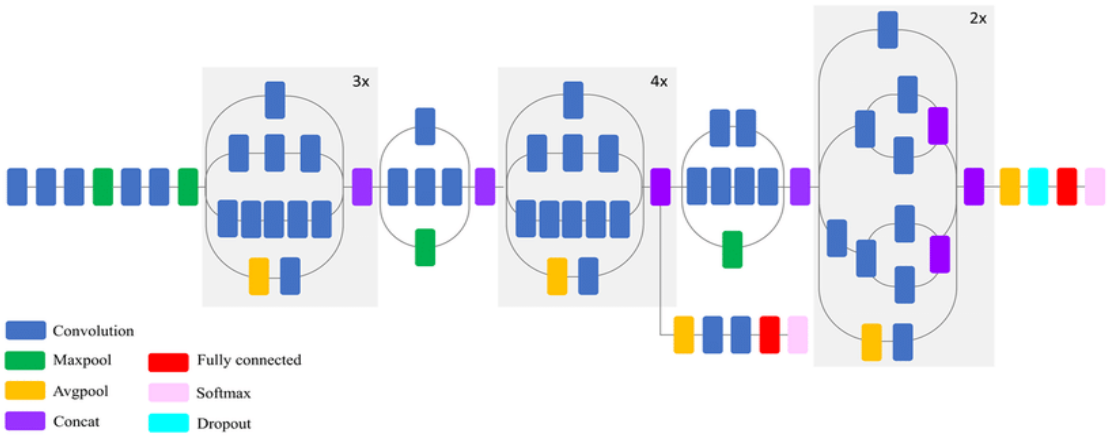


Figure 2: InceptionV3 Architecture

Input Layer: The size is always fixed, with three RGB color channels for all input photos, usually 299 by 299 pixels.

Preprocessing Layer: Performs standardization and normalization of input images to ensure consistency and optimal processing conditions.

Convolutional Layers:

InceptionV3 comprises numerous convolutional layers, responsible for extracting features from the input images.These layers consist of learnable filters that convolve across the input images, detecting patterns at different spatial scales.

Inception Modules:

The hallmark of InceptionV3 architecture is the inception module, which is a combination of parallel convolutional operations of different sizes.Each inception module typically consists of 1x1, 3x3, and 5x5 convolutions, as well as max-pooling operations.By incorporating multiple convolutions at different scales within a single layer, the network can capture rich hierarchical features efficiently.

Pooling Layers:

After each set of convolutional operations, pooling layers are often employed to downsample the feature maps, reducing spatial dimensions while retaining important information.

Fully Connected Layers:

Towards the end of the network, fully connected layers are utilized to aggregate the extracted features and perform high-level reasoning.These layers typically include dense connections between neurons, allowing the model to learn complex relationships between features.

Output Layer:

The final layer of the network is usually a softmax layer, which produces probability distributions over the possible classes.Each node in this layer represents a class, and the output values represent the likelihood of the input image belonging to each class.

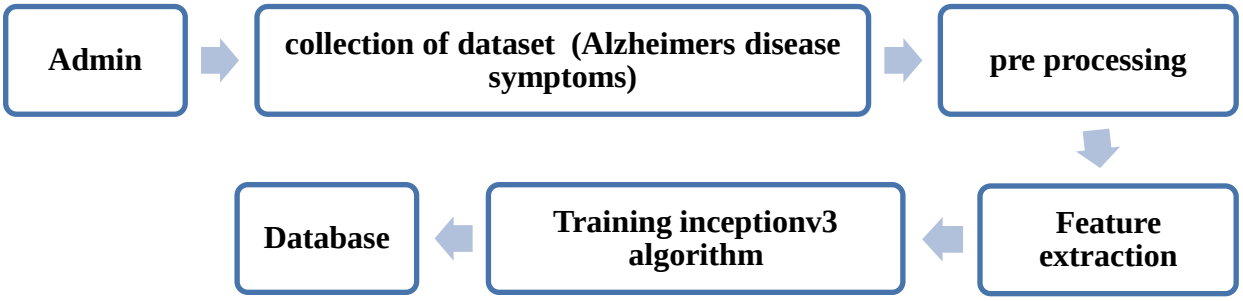
RESULT

The proposed technique mainly contributes enhancing the detection accuracy of Alzheimer's disease from brain images like MRI, PET. Thus, it becomes helpful for diagnosis of Alzheimer's disease using brain images, and it can be a useful CAD for diagnosis of the

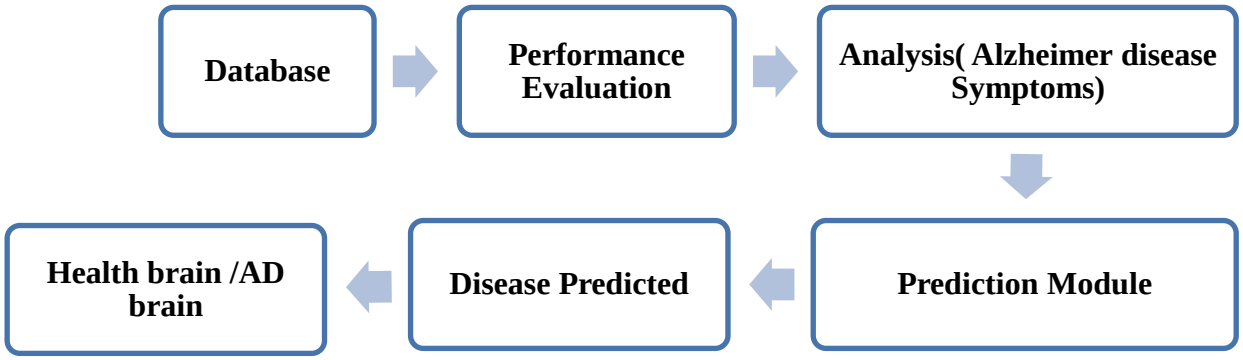
disease.Software engineers design, develop, test and maintain software applications that they create for their clients according to the requirements.Web developers create web applications to serve their users using the client-server model.

A Data Flow Diagram (DFD) is a graphical representation that illustrates how data moves within a system. It consists of processes, data stores, data flows, and external entities. Processes represent activities or transformations, data stores signify where data is stored, data flows depict the movement of data between elements, and external entities are sources or destinations of data. DFDs provide a clear visualization of the data flow within a system, aiding in the understanding of information processes and interactions. They are particularly useful in system analysis and design, helping to identify data sources, processing steps, and data destinations in a concise and intuitive manner.

Level 0



Level 1



CONCLUSION

In conclusion, our project is a significant step forward in the quest for early diagnosis of Alzheimer's disease using deep learning. The large datasets of medical images and clinical data, we have developed an algorithm that holds the potential to identify patterns and abnormalities associated with the disease. Our innovative image processing approach distinguishes between a healthy brain and an Alzheimer's disease-affected brain, providing a valuable tool for healthcare professionals and researchers. Early diagnosis is critical in Alzheimer's disease management, and our system offers the promise of timely intervention and personalized treatment. As we continue

to build on our knowledge about Alzheimer's disease, and perfect our deep learning approaches, we come closer to making the lives of those impacted by this challenging condition better.

FUTURE ENHANCEMENT

Privacy measures in medical data should be developed when private medical data are used. There has to be more emphasis on strong data encryption while taking into consideration the changes that take place in data security laws.

A real-time monitoring system that continuously reviews patient information for early detection of change in brain health or cognitive performance should be installed. Those who are at risk or in early stages may find it particularly helpful.

Examine how wearable technology and mobile apps might be combined to collect and monitor data. Patients may be able to actively engage in their own care and provide insightful data for analysis as a result.

Provide a prognostic model that can both diagnose Alzheimer's and forecast how quickly the illness will advance. This can assist in customizing care regimens and patient assistance for individuals at varying phases of the illness.

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AN EVALUATIONOF THE PHYTOCONSTITUENTS AND PROXIMATE COMPOSITION OF *SALICORNIA BRACHIATA* MARINE HALOPHYTES

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ABSTRACT

The phytochemical and proximate analysis of maritime halophytes, *Salicorniabrachiata*, were examined in this work.*S.brachiata*, a quantitative analysis of the phytoconstituents was also conducted. Quantitative measurements were made of the phytochemicals, including flavonoids, phenols, and tannins, in various solvent extracts of *S.brachiata*. After petroleum ether (27.20±0.2mg CE/g) and hexane extracts (12.32±1.22 mg CE/g), the ethanolic extract had the highest total flavonoid concentration (32.32±2.01mg CE/g). Carbohydrate was the largest proximate composition in the *S.brachiata* followed by ash, and moisture was the largest single content among the proximate compositions in this study. The biochemical composition of *S. brachiata* revealed significant levels of total chlorophyll, ascorbic acid, and β-carotene. The maritime halophytes *S.brachiata*shown encouraging phytochemical and biochemical contents in the current investigation.

Key words: Proximate, phytochemical, chlorophyll, *Salicorniabrachiata*, solvent extracts.

INTRODUCTION

A vital source oftherapeutic assistance for numerous human problems, medicinalplants have long been a part of man's modern existence. Even now, the majority of therapeutic plants are gathered fromthe wild. The number ofmanyspecies intheir native habitat has decreased as a result of the ongoing commercial exploitation of these plants. Therefore, it is imperative that these plants be cultivated in order to guarantee their availability to the industry and to those involved in traditional medicine. The pharmaceutical industry's constantly rising demands cannot be satisfied by in situ conservation of these sources alone.Halophytes, often known as salt-tolerant plants, can withstand salinity through a variety of eco-physiological processesand thrive in severely saline environments."Halos" is a term that denotes salty and"Phyte" refers to a plant. Saline semi-deserts, mangrove swamps, marshes, sloughs, degraded soils,and seashores are among the salt-affected areas where these plants are naturally found or grown (Ksouri et al., 2008). A sizable section of the populace in various nations has been using traditional medicine for manygenerations. The number of medicinalplants that contain pharmacologicallyactive chemicals has grownglobally. The primarysourceofmedicine used to treat infectious disorders in India is plants. The ongoing search for novel bioactive active compounds is reflected in medicinal plant extracts.

Through synthesis or secretion, marine halophytes offer a good supply of natural and therapeutic health products or chemicals. In terms of lowering salt stress, these plant families offer a significant number of antioxidants, phenol compounds, enzymes, biomolecules like carbohydrates, and other biochemical compounds including free amino acids and phytochemicals. The phenyl propanoid pathway is used to synthesise phenolic chemicals, which serve as a defensive mechanism against both biotic and abiotic stress. The majority of plants cannot be grown on salt-affected land because they cannot withstand high soil salt concentrations. These plants are referred to as glycophytes, nonhalophytes, or non-salt-

tolerating plants (Xiong and Zhu, 2002). Due to various mechanisms for salt tolerance, some plants maythrive insalinity;these plants are referredto as halophytes, salt-resisting plants, or salt-tolerating plants (Flowers et al., 1986). Salicornia has gained greater attention lately due to its ability to treat conditions like organic disorders or inappropriate nutrition that cause constipation, diabetes, heart disease, cardiovascular disease, and hypertension. Particularly noteworthy are its substantial salt-accumulating qualities and potential for exploitation. Infact, the vacuole can withstand 500–600 mM salts (Maathuiset al., 1992), which permits its cultivation in extremely salinised areas with the goal of removing salts from the soil through repeated cropping (Flowers et al., 1977 and Ravinndran et al., 2007). Only the investigations on the mineral composition, the properties of various salt kinds, and the production structure of rock salt and purified salt by Jeong (1988) have been noteworthy (Jo et al., 1988). Many Salicornia species have antibacterial and antihypertensive qualities, and they are used in traditional medicine to treat cancer, obesity, diabetes, constipation, chronic rheumatism, and toothaches. *Salicorniabrachiata* extracts phytochemical characterization and proximate analysis are the focal points of the current investigation.

MATERIALSANDMETHODS

Collection of Plant

*Salicorniabrachiata*was gathered from the coastal regions of Tamil Nadu, India, and Nagapattinam. The plant material was allowed to air dry before being ground into a coarse powder with a mixer grinder.

Preparation of plant extract

50 g of coarse plant powder was macerated for 48 hours with an adequate amount of methanol, petroleumether, and water, andthe resulting extracts werethen filtered. Additional experiments were conducted using this (UdhayaPrakash et al., 2012).

Proximateanalyses

Estimation of moisture content

Using a Bunsen burner, an empty porcelain crucible with a lid was thoroughly cleaned and heated to red hot. After cooling in desiccators, the crucible was weighed. One gramme of the material was cooked for three hours at 105°C in an oven. After cooling in desiccators, the crucible was weighed. The sample's moisture content was indicated by the weight difference (AOAC, 1990).

Estimation of Protein content

The protein content of foods has been determined on the basis of total nitrogen content, while the Kjeldahl (or similar) method has been almost universally applied to determine nitrogen content (AOAC, 1990). Accurately 1 gm of sample was weighed and put inadigestion flask.10gmpotassiumsulphate,0.7gmmercuricoxide and 20ml sulphuric acid were added. The flask was heated gentlyat an inclined angle until frothing subsides and then boiled until the solution clears. Boiling went on for an additional half hour. On cooling, about 90 ml. Distilled water was added, re cooling was done, 25ml. Sulphide solution was added and mixed. Small pieces of boiling chip added at prevent bumping and 80ml. of sodium hydroxide solution while tilting the flask so that two layers were formed. The condenser unit wasconnectedrapidly, heated, andcollecteddistilledammonia in50ml.Boric acid/indicator solution. 50ml of distillate was collected. On completion of distillation; the receiver was removed and titrated against standard acid solution.

Estimation of total carbohydrate (AOAC,1990)

Nitrogen free extract (NFE) of a sample was determined by difference after the analysishasbeencompleted forash,crude fiber,crudefat andcrudeprotein.CalculationNFE on dry

basis = 100 %-(% ash on dry basis+% crude fiber on dry basis+% crude fat on dry basis+% protein on dry basis)

PreliminaryPhytochemicalanalysis

The preliminary qualitative phytochemical investigation of *Salicorniabrachiata*extract indifferent solventswasperformed to detectthe phytoconstituentssuchasFlavonoids, Tannins, Steroids, Saponins, Cardiac glycoside, Phlobatannins, Alkaloids, Terpenoids and Phenols was performedbythestandardprocedureas describedbyTrease and Evans 1989, Harborne,1973.

Analysisofphytochemicalsquantitatively

Estimation of total phenolic content

Total phenolic compounds were determined in sample extracts using the Folin–Ciocalteu reagent (Dewanto*et al.,* 2002). An aliquot of 0.125ml of diluted extracts were mixedwith0.5mlofdistilledwaterand01.25ml oftheFolin–Ciocalteureagent.After6min,1.25ml of Na2CO3 (7 %) and 1ml of distilled water were added and the obtainedpreparation was mixed thoroughly then incubated. After 90min, the absorbance was monitored at 760nm and the results are expressed as mg of gallic acid equivalents per gram of dry residue (mg GAE/g). The assay was done in triplicate.

Estimationoftotaltannin

Condensed tannins (proanthocyanidins) were determined following Sun and Ricardoda-Silva (1998) protocol. 50µl of each solvent extract was mixed with 3ml of vanillin solution (4 %) and 1.5 ml of sulfuric acid then incubated at room temperature for 15minutes. The absorbance was read at 500nm and, as for total flavonoids, the results were expressed as mg CE/g. All samples were analyzed in triplicates.

Estimationoftotalflavonoids

The total flavonoid was determined by colorometric method described by Dewanto*et al.*, (2002). To 0.25ml of each sample, 75µl of sodium nitrite and 0.15ml of aluminium chloride were added. After 5min, 0.5ml of sodium hydroxide (1M) and 1.525ml distilled water was added to the mixture. Absorbance was measured at 520nm and results were presented as mg of catechin equivalent per gram of dry residue (mg CE/g), using acatechin calibration curve (concentration range: 100-400 µg/ml). The assay for each sample was analyzed in three replications.

Determination of Biochemical composition

Estimationofβ-Carotene

To determine the total amount of carotenoids, β-carotene and its Z and E isomer content, approximately 15g of the samples, plus 3g of celite 454 (Tedia, Ohio, USA) were weighed in a mortar on a digital balance (Bel Engineering, model MA0434/05). For the carotenoid extraction, successive additions of 25ml of acetone were made to obtain a paste, which was transferred into a sintered funnel (5µm) coupled to a 250ml Buchner flask and filtered under vacuum. This procedure was repeated three times or until the sample became colorless. The extract obtained was transferred to a 500mlseparatory funnel containing 40ml of petroleum ether. The acetone was removed through the slow addition of ultrapure water (Milli-Q- Millipore)to prevent emulsion formation. The aqueous phasewas discarded. This procedure was repeated four times until no residual solvent remained. Then, the extract was transferred through a funnel to a 50ml volumetric flask containing 15g of anhydrous sodium sulfate. The volume was made up by

petroleum ether, and the samples were read at 450 nm. The total carotenoid content was calculated

Estimationof chlorophylls

The method of Arnon (1949) was used to estimate the chlorophyll pigments in the leaves.All of the locations' fully grown leaves were gathered in polythene bags and brought to the lab. Distilled water was used to properly wash the leaves. For every plant, three duplicates were used.Fresh leaf material that had been weighted was homogenised and extracted three times using cooled 80% acetone (v/v). Aspectrophotometerwasusedto measure the opticaldensity at 645nm and 663nm wavelengths after the volume of the acetone extract was adjusted to a known value.

Estimation of Ascorbic Acid (Vitamin-C)

The AOAC (1990) technique was used to quantify ascorbic acid (Vitamin-C). Five gram of sample powder were thoroughly crushed in a mortar and pesticide with oxalicacid. Test tubeswere filled with a different aliquot (0.2to 2ml) of the working standard' sde-hydroform, and the volume was adjusted to 3ml using water. One millilitre of DNPH and two drops of thiourea were added to each tube.The tubes were incubated at 37°C for 3 hours. After incubation the orange-red oxazonecrystals formed was dissolved by adding 7ml of 80% H_2SO_4. The absorbance was measuredat 540nmandstandardgraphwas plotted. De-hydroform of sample was taken in a liquots and preceded it for plotting on the standard curve. The absorbance was compared with the standard graph and the percentage of ascorbic acid was calculated.

Proximatecomposition

The greatest single component among the proximate constituents of S.brachiata (fresh weight) was moisture, at 85.43±1.6%. Among dry biomass, it was clear that the largest proximate components in S.brachiata were carbohydrates (5.45±0.5g/100g fresh weight) and ash (4.58±0.2g/100g fresh weight). There was a moderate amount of crude protein (1.64±0.1 g/100g fresh weight) and fibre (0.85±0.12 g/100g fresh weight).Of these, the S.brachiata. has a very low total lipid content (0.33±0.01 g/100g fresh weight) (Table 1).

According to Ahmed Ali (2017), Suaedavermiculata had the highest percentage of crude fat content (5.54%), while Atriplexleucoclada had the lowest (0.5%). According to the earlier study, the bulk tissue weight of the succulent and fleshy stem contributed to the fresh entire plant P. pellucida's 93.14% moisture content.The entire P. pellucida plant maintained a mean moisture content of 8.33% after oven drying (DerJiunOoi et al., 2012).

RESULTSANDDISCUSSION

Phytochemical screening

Qualitative analysis Standard procedures were used to qualitatively assess the preliminary phytochemical assays of S.brachiata. in various solvent extracts, including ethanol, petroleum ether, and hexane. All of the extracts of S.brachiata. (Table 2.2) demonstratedthe presence of significant active chemical components, including flavonoids, alkaloids, tannin, saponin, terpenoids and phenols.Onlythe ethanolextract contains the steroids, cardiac glycoside, and anthraquinones. The presence of steroids and anthocyaninsin Boerrhhaveaorellanaseeds and alkaloids andsteroids inCardiospermumofficinalis (Adeniyietal., 2005);terpenoids, tannins, andguaabins from Psidiumguajava and polygalactorunasesin Mangiferaindica (Akinpelu and Onakoya, 2006); alkaloids, tannins, steroids, and flovonoids from the ethanolic and aqueous extracts of Picralimanitida's stem and bark (Nkere and Iroeghbu,2005); comparable studies by earlierresearchers.

Quantitativeanalysis

The total flavonoids content was showed the highest value in ethanolic extract (32.32±2.01mgCE/g) followed by petroleumether(27.20±0.2mgCE/g)and hexane extracts (12.32±1.22 mg CE/g). These differences may be attributed to the solvent types. The highest content of total tannin content was found (27.85±2.3 mg CE/g) in ethanolic extracts and moderate level was present in the petroleum ether (25.54±2.5 mg CE/g) and hexane (11.56±7.4 mg CE/g) extracts respectively. The standard curve of gallic acid was preparedand the total phenolic contents of examined extracts werecalculated based upon thisstandard and presented as gallic equivalents (GAE) per gram of dry sample. The highest total polyphenol content was present the ethanolic extract (54.23±5.2 mg GAE/g) when compared with petroleum ether (48.75±2.5 mg GAE/g) and hexane (38.56±3.2 mg GAE/g) extracts respectively. This variability may be depending on the influence of the solvent polarity (Table.3). Phenolic compounds are secondary metabolites and play an imperfect in profiting the plants from biotic and abiotic stresses (Ksouri*et al.,* 2008The current study found that all halophyte species analysed from these regions had a substantial diversity in their total polyphenol content.Therefore, using this standard, the total phenolic contents of the extracted materials were computed and given as Gallice Equivalents (GAE) per gram ofdry sample. According to Hamdoon et al. (2013), the quantitative assessment of the phenolics and flavonoids in the extracts of our current plants provides unambiguous proof of the large percentages of these byproducts.Rajakumar and Allwinpremsingh (2017) reported that the identified phytochemical compounds maybe the bioactive constituent of the marine algae is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Biochemicalcontents

Biochemical compositions of the *S.brachiata* showed high values of β-carotene, ascorbicacidandtotalchlorophyll.Theβ-carotenecontent(155.0±6.4mg/kgfreshweight)was rich in *S.brachaita* which made the plant a good source of Vitamin A. The Ascorbic acid was presented in 53.2±1.2 mg/kg fresh weight (Fig.1). In plants, the green colour is due to chlorophyll located in chloroplasts. Loss of green colour is normally considered as the major consequence of chlorophyll degradation. Total chlorophyll content was 573.2±7.3 mg/kg fresh weight presented in *S.brachiata*. Similarly Ayala-Astorga and Alcaraz- Melendez (2010) reported significant decrease in β-carotene content in *Paulownia imperialis*and *P. fortune* plants grown under NaCl salinity. Fathi and Asem(2013) while working with Chlorella, also reported that high salinity (50 gL-1) leads to decrease in β-carotene content. According to Adams *et al.,* (1989) the decrease in chlorophyll a and b content is due to the increase intheir degradationunder saline condition. The changes occur inthe chlorophyll a/b ratio are used a sanindicatorforrelativephoto system stoichiometry(Pfannscmidt *et al.,*1999).

Table.1Proximatecompositionsof*Salicorniabrachiata*

S.No	Proximate content	Composition(g-100g-1FW)
1	Moisture	85.43±1.6%
2	Crude protein	1.64±0.1
3	Totallipids	0.33±0.01
4	Crude fiber	0.85±0.12
5	Totalcarbohydrate	5.45±0.5
6	Ash	4.58±0.2

Table.2Qualitativephytochemicalanalysisofdifferentextracts of *S.brachiata*

S.No	Phytochemicals	Extracts		
		Ethanol	Petroleum ether	Hexane
1	Flavonoids	Present	Present	Present
2	Alkaloids	Present	Present	Present
3	Tannin	Present	Present	Present
4	Steroid	Present	Absent	Absent
5	Saponins	Present	Present	Present
6	Terpenoids	Present	Present	Present
7	Anthraquinones	Present	Absent	Absent
8	Cardiacglycoside	Present	Absent	Absent
9	Phenols	Present	Present	Present

Table.3Quantitative phytochemical analysis for the extracts of *S.brachiata*

Solvent	Phenol(mgGAE/g)	Flavonoid(mgCE/g)	Tannin(mgCE/g)
Ethanol	54.23±5.2	32.32±2.01	27.85±2.3
Petroleumether	48.75±2.5	27.20±0.2	25.54±2.5
Hexane	38.56±3.2	12.32±1.22	11.56±7.4

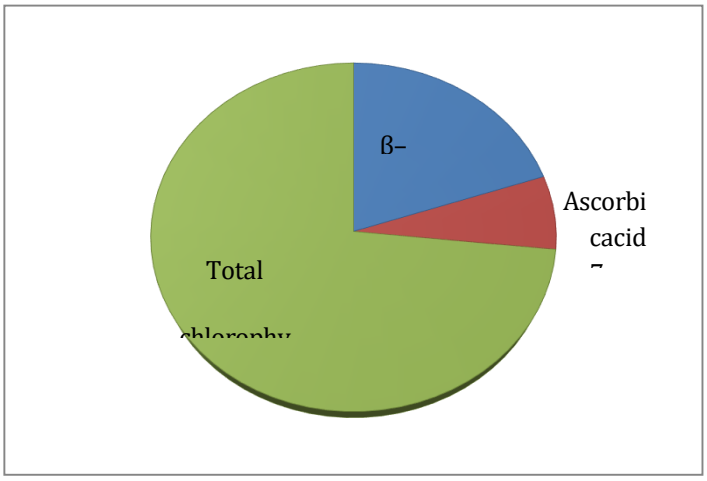

Fig.1Biochemicalcontentsof*Salicorniabrachiata*

CONCLUSION

Methanolic extracts of S.brachiata contained the majority of the phyoconstituents that were discovered. The current investigation came to the conclusion that the marine halophytes S. brachiata contain very promising minerals and phytoconstituents. Asaresult, it plays acrucial part in the synthesis of active ingredients that give medicines their therapeutic effects. They create medicinally significant phytocomponents in the salt-stressed environment, which couldlikewise be helpful in upcoming initiatives. S.brachiate is used to quantitatively quantify the phytocompounds. Ethanolic extracts of S.brachiate and associated biological activities were found in the current study.

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ASSOCIATION BETWEEN DIABETES AND PESTICIDES

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ABSTRACT

Diabetes is a chronic condition resulting from either insufficient insulin production by the pancreas or the body's inability to use insulin effectively. As per Indian Council of Medical Research – India Diabetes (ICMR INDIAB) study published in 2023, the prevalence of diabetes is 10.1 crores. Pesticides could affect the pancreases and interfere with insulin secretion by damaging the mitochondria cells. Eco-friendly alternatives to chemical pesticides are biological control, botanical pesticides, organic home remedies, natural predators and companion planting, physical barriers, cultural practices and bio pesticides. That are increasing the soil fertility and decreasing the mortality rate due to diabetes.

Key words: Diabetes mellitus, pesticides, pollutants and Odd Ratio

INTRODUCTION

Diabetes is a chronic condition resulting from either insufficient insulin production by the pancreas or the body's inability to use insulin effectively. Insulin is essential for regulating blood glucose levels, and without proper insulin function, blood sugar can rise uncontrollably, a condition known as hyperglycemia. Uncontrolled diabetes, particularly over time, can damage various body systems, especially the nerves and blood vessels. As per Indian Council of Medical Research – India Diabetes (ICMR INDIAB) study published in 2023, the prevalence of diabetes is 10.1 crores. Experience using any types of pesticides was positively associated with diabetes but the association were statistically significant only for rodenticide (adjusted OR = 1.35; 95%CI 1.04–1.76). For insecticides, year of exposure to insecticides and cumulative days failed to predict risk of diabetes. For fungicides, diabetic risk seems to correlate with both year of exposure and exposure days, but none with statistical significance. For herbicides and molluscicides, both year of exposure and exposure days were inversely associated with the disease. For rodenticides, only exposure days was positively correlated with diabetes (Juntarawijit and Juntarawijit, 2018).

Association Between Diabetes and Pesticides

In this study, the OR(Odd Ratio) of diabetes was statistically associated with three types of insecticides investigated, including one organochlorine (endosulfan), one organophosphate (mevinphos), and one carbamate (carbaryl/Sevin), as well as one fungicide (benlate). The results were well supported by previous findings either from epidemiological studies or an animal study. A recent study also suggested that pesticides could affect the pancreases and interfere with insulin secretion by damaging the mitochondria cells (Ozmen et al.,2010)

Persistent organic pollutants (organochlorine insecticides)

Studies using NHANES data have found associations of POPs with both diabetes and insulin resistance and have noted in particular the association of diabetes with organochlorine insecticides. A metabolite and an impurity of chlordane were most strongly associated with insulin resistance in non-diabetics. Animal studies of exposure to chlordane have demonstrated increased lipids and triglycerides in liver and altered glucose metabolism. Our finding that

chlordane exposure followed a dose-response association with diabetes incidence strengthens the chlordane-diabetes hypothesis (Montgomery et al., 2008).

CONCLUSION

Eco-friendly alternatives to chemical pesticides are biological control, botanical pesticides, organic home remedies, natural predators and companion planting, physical barriers, cultural practices and bio pesticides. That are increasing the soil fertility and decreasing the mortality rate due to diabetes.

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A STUDIES ON BACTERIA FROM HOME MADE VEGAN CURD

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ABSTRACT

Curd is a traditional food which is high in nutrition and helps in boosting immunity.Curd has been consumed worldwide and has health gut bacteria that fights against harmful pathogenic bacteria.Curd is coagulated using milk.Since some people experience lactose intolerance,plant based curd is advised for their intake.In this study we will explore the good gut bacteria and other microorganisms present in the homemade vegan curd.This plant based curd is made of peanut milk and rice milk and green chilli pediceles are added for fermentation process that helps in curdling of milk.Peanuts are rich in antioxidants and they have heath gut bacteria like Bradyrhyzhobium, proanthocyanins, polyphenols and fibres. Rice contains bacteria like Bacilli.Green chillies consist of Lactobacilli and is also a starter for lacto fermentation.The homemade vegan curd are rich in protein and carbohydrates. The bacteria which are present in homemade vegan curd are analysed through plating methods.

Keywords: Lactofermentation,Lactose intolerance, Peanut, Antioxidant, Curdling, Bacilli.

INTRODUCTION

Curd is obtained by coagulating milk that is normally called curdling. It is often caused by adding edible acidic substance like lemon juice or vinegar, then permitting it to coagulate. The accrued acidity causes the milk proteins to breakdown at its iso-electric point and the solids separate from the whey liquid.The regular consumption of curd produces a higher level of immunity boosting interferon as these bacteria cultures stimulate infection fighting white cells in the blood stream with anti-tumor effects(Maltock,2007).It is widely consumed as a functional food due to it is good taste and is also rich in potassium,calcium,protein and in vitamins and also acts as an excellent vehicle to deliver probiotics to the customer(Reid et al.,2003). Regular consumption of curd is thought to be beneficial in strenghthening of the immune system, improves in lactose digestion,blood glucose management (Yadav, Jain,&Sinha, 2007). Fermentation is the metabolic process that converts sugar into acids, gases, or alcohols by microorganisms in available or environmentally controlled conditions. Yeasts for example, produce alcohol under anaerobic conditions, while most bacteria produce toxins and acids under aerobic conditions. Yeast and plenty of alternative microbes normally use fermentation to produce different compounds to hold on for their survival, in their surrounding environment.Alcoholic fermentation one mole of glucose into two moles of ethanol and two moles of carbon dioxide, producing two moles of ATP in the process.(Aren van Waarde;Maria Verhagen,1993).

Probiotics causes an intestinal inner lining which prohibits from causing any harmful infections to the human body.The most common probiotics in curd are Lactic Acid Bacteria which is also known as LAB and Bifidobacteria. Some of the other probiotics are *Bifdobacterium longum, Bifdobacterium breve, Brifdobacterium infantis, Brifdobacterium bifdum, Brifdobacterium adolescentis, Streptococcus cremoris, Steptococcus diaceylactis ,lactococcus cremoris,Lactococcus lactis, Lactobacillus acidophilus, Lactobacillus fermeum and Latobacillus casei.* These kind of probiotic strains can be seen in curd (Corthier G and Marteau ,2011).

MATERIALS AND METHODS

Preparation of vegan curd

125 grams of raw peanuts and raw rice are soaked in 250 ml of water overnight.The soaked peanuts are washed thoroughly and blended along with 250 ml of water.This process is repeated for two times. After blending the mixture is filtered using a nut strain bag.Later the peanut milk is separated and heated at a medium flame.Meanwhile 125grams of raw rice is washed thoroughly and blended and later the extract is filtered.The rice milk is added to the peanut milk and stirred continuously in a medium flame.After pasteurization the milk is allowed to come back to it is luke warm temperature. The milk should be in lukewarm temperature so that fermentation process takes place easily.When the milk is extremely hot fermentation of milk does not take place.When the milk is in luke warm temperature green chilli pediceles are added on the top and the vessel is closed for 24 hours and is kept in an ambient temperature.

Preparation of Milk Curd

200ml of fresh raw milk is heated at a medium flame.Four tablespoons of milk is taken in a separate vessel and one full green chilli and a tea spoon of lemon juice is added and is kept aside for 24 hours. The heated milk is later poured in a container and kept in an ambient temperature overnight.Later,the milk which contains green chilli and lemon juice is added to the fresh heated milk and left undisturbed for 12 hours. If the temperature is cold the curd can be kept near a microwave oven or stored in a heat place.If the temperature is normal the curd takes 24 hours to set.The setting of the curd depends upon the surrounding temperature.The maximum time for setting of the curd is around 12 to 24 hours or sometimes 48 hours.

Nutrition Analysis Vegan Curdand Milk Curd

Soxhlet Method (Fat test)

The apparatus is rinsed with petroleum ether and is kept in hot air oven for 120^0C.5gram of curd sample was weighed and placed it under thimble. The thimble was attached to the soxhelet extractor. In a apparatus curd was added along with ammonia solution and petroleum benzene and was mixed thoroughly until the pressure was released.Later,150ml round bottom flask was taken and washed with petroleum ether and dried thoroughly.

Later the round bottom flask was filled with 90ml of petroleum ether and is placed on the mantle and was heated for six hours and the same process was repeated again for several hours and the extraction unit was removed from the condensation unit allowing the sample to cool down.The conical flask was later kept in hot air oven to evaporate the solvents and weight of the fat is noted.

Kjeldahl method (Protein test)

This method is helped in oxidising the organic compounds the basic principle was discovered by Johan Kjeldahl hundred years ago. This method has three process digestion, distillation and titration.Digestion is a process where in a kjeldahl flask 5grams of curd sample and 0.5grams of copper sulphate and 25ml of sulphuric acid is added and kept for protein digestion.After 24 hours the flask appears to be colourless.

The liquid is cooled down by adding distilled water and 40ml of sodium hydroxide and 5gel crystals are added and transferred to a kjeldahl round bottom flask. After protein extraction. Receiver solution is prepared by adding 40ml of sulphuric acid and 12 drops of methyl red indictor. After the kjeldahl method is complete. The receiver solution is titrated against 50%NaOH after it reaches to 150ml.

pH value for vegan curd

In the digestive system stomach helps to digest the food without causing any harm to the human body.Hydrochloric acid produced in the body helps in the digestion of the food. But when the amount of acid goes beyond a certain limit due to indigestion,pain and irritation causes in the stomach.When there is normal pH in the body there is no harm.Different types of body has

different values.The food that is consumed regularly has a certain pH that helps the body to undergo proper digestion.

Titratable acidity

Natural acidity of milk is due to its constituents such as casein, albumin, citrates, phosphates and carbn dioxide. The acidity can be measured by titrating milk against standard alkali using an indicator like phenolphthalein and is expressed as terms of lactic acid.10ml of curd is taken in a beaker and 2 to 3 drops of phenolphthalein indicator was added and shaken well and later titrated with standard NaOH solution. The titration was completed in 20 seconds. The titratable acidity of milk curd and vegan curd was performed.

Isolation of Bacteria from Vegan Curd

Preparation of Nutrient agar

Suspend 1.6grams of nutrient agar powder in a 250ml conical flask which contains 60ml of distilled water. Mix and dissolve them completely. Cover the conical flask using cotton balls and wrap a parafilm on the neck of the flask. Sterilize by autoclaving at 121^0C for 15 minutes at 15lbs. After sterilizing pour the liquid into the petridish and wait for the medium to solidify.

Serial dilution method

Dilution process is usually done to make a solution weaker and to reduce it is concentration.In microbiology serial dilution technique is performed to decrease the bacterial concentration so that it is easier to count the colonies on the agar plate.Ten test tubes, 200ml of distilled water and six petridishes were autoclaved for sterlization at 121^0Cfor 15 minutes at 15lbs.Each test tube is filled with 9ml of distilled water and named as 10^{-1} to 10^{-9} respectively.1ml of small amount of well mixed curd is added to the 10^{-1}test tube and using a 1000ml micropipette the curd is diluted upto nine test tubes.1ml of the solution is diluted in all the nine test tubes and the last 1ml dilution is discarded using a micropipette.

Serial dilution and solidified agar plates

Spread plate technique

This technique is used to plate a liquid sample for isolating and counting the bacteria present in that sample Spread plate technique provides visible and isolated colonies that are evenly distributed in the plate. To avoid contamination,the L rod which is also known as spreader should be sterilized or wiped with ethanol to kill the unwanted bacteria.From serial dilution three plates are of duplicate to compare the colony growth from the original.

1ml of liquid sample is poured on the agar plate and by using L rod the bacteria is evenly spread through the surface of the plates.After spreading the bacteria the plates are incubated at 37^0Cfor 24 hours and later the colony growth is noted.

Spread plate technique

Colony growth

A visible mass of microorganisms is called as colony growth.Bacteria needs a special nutrient medium to grow and be visible and they grow on solid media as colonies.The colonies that are grown on the agar plats may be different. They differ in size,shape and colour and growth.After incubating the plate for 24 hours visible colonies are identified and are characterized.

The temperature should be ranged around 40 when the plates are kept in the incubator.If the temperature increase the bacteria will not survive and it stops its growth.The plates should be wrapped tightly with paraflm to avoid melting of agar and contamination.

Streak plate technique

After performing spread plate technique several colonies were grown in the agar plates.Streak plate method is one of the easiest method to isolate a pure bacteria strain from several species of microorganisms.

The dilution and streaking method was first identified by Leffeler and Gaffky in Koch laboratory.This is also a simple and a rapid process.These are done by aseptic techniques and sterile tools are used such as cotton swabs ethanol Bunsen burner and an inoculation loop.

By using this method the bacteria or any microorganisms can be identified.When numerous colonies are grown in a plate it is difficult to identify them as a group.Streaking helps the bacterial colonies to be identified.After the colony growth three plates were selected and different colour colonies were identified and selected.Further streak plate technique were performed on the agar plates and incubated it for 37^0Cfor 24 hours and later the

Total plate count

This method is done by pour plate method to count the number of colonies present in a specimen.In this method a fixed amount of innoculumn which is 1 ml is poured on a sterile petridish and 15ml of plate count agar is autoclaved for 15 minutes at 15lbs and poured on the half closed petridish. The mixture is now gently rotated and the medium should be covered evenly.

The agar should not spilt in the end of the petridish. Parafilm is wrapped around the petridish and is incubated for 37^0C and observed for the growth.

The agar should be lukewarm so that that bacterium inoculum survives and grows during incubation.When the medium is at high temperature bacteria growth or any colony growth cannot be seen.While pouring the agar the neck of the conical flask should be slightly heated while pouring it on the petridish. Later colonies were counted.

Characterization of the Bacteria

Gram staining method

This method is performed to differentiate between gram positive and gram negative bacteria.This method was first discovered by the Danish bacteriologist Hans Christian Gram.The cell walls has different physical and chemical properties,by using gram staining technique the thick layer and the thin layer of peptidoglycan in the cell reacts to the stains that added to the bacteria.For example: thick layer absorbs the violet stain and the thinner peptidoglycan absorbs the red stain which is called as saffranin.

Take a clean glass slide and pick a colony from the agar plate by using a sterilized inoculation loop.The inoculation loop is heated thoroughly before scraping a colony. The bacteria is heat fixed slightly.The slide should not be overheated.Temperature is checked by placing the back of the slide on the wrist.

Sterilized distilled water droplet is added on the slide and after it is dried crystal violet stain is added and kept for one minute on the slide and washed away.

After placing the crystal violet stain for a minute the slide should be washed in a running tap water.Later,Gram's iodine is added and is left for one minute and washed away.

Decolorizer is added after washing off the gram's iodine solution. Adding too much decolorizer can give a wrong result. Ethanol should be on the slide only for 15 seconds. Placing decolorizer for a long time can kill the purple stain.

After washing off the decolorizer saffranin solution is added and is kept for one minute on the slide and later washed away with water. After adding all the reagents allow the slide to dry and view it under microscope.

Biochemical Tests for Bacteria

Catalase test

Catalase is an enzyme which converts Hydrogen peroxide into oxygen and water.This is a primary test used for identifying gram negative and gram positive organisms and also differentiates aerobic and anaerobic bacteria. Louis Jaccques Thunard was the first scientist to discover the catalase and hydrogen peroxide.Take a clean glass slide the suspected colonies are

placed on each slide and a loopful of culture was taken with a wire loop and 3 to 5% of Hydrogen Peroxide was added and the reaction was observed.

Urease test

Urease was discovered by Jack bean in 1975.Urease test is performed to identify those organisms that produce ammonia and carbon dioxide.Slant was prepared with Christenson Urea agar and the culture was inoculated in the slant and incubated for 24 hours at 37^{o}C and later observed for reaction.

Voges Prokauer test

VP test is used to detect acetoin in bacterial culture. Voges and Proskauer in 1898 first observed the red colour after adding potassium hydroxide to cultures that were grown on a specific media.Later it was discovered that the production of red colour was due to acetyl-methyl carbinol production. Tryptone broth was prepared for 5ml in a test tube and the culture was added.The test tube was incubated for 24 hours and 1ml of alpha naphthol solution and potassium hydroxide solution was added and shaken well.By adding few drops of solutions the test tube was observed for any colour changes.

Methyl Red Reaction test

5ml tryptone broth was taken in a test tube and the culture was added.It was incubated for 24 hours and after incubation two three drops of Methyl red solution was added to observe the reaction.

Citrate utilization test

This test helps to identify the ability of a microorganism to use citrate as a carbon source.The citrate depends upon enzyme citrase that breaks down citrate to oxalo acetate and carbonat. Sterlized citrate agar was made into slant and bacterial cultures were streaked in the slant and kept for incubation for 24 hours.After incubation tubes were checked for colour change.

Indole Production test

5ml of tryptone broth was taken in a test tube and culture was added.It was inoculated for 24 hours and add two drops of Kovac's reagent and the test tube is observed for any reaction

Starch hydrolysis test

In a clean sterilized petridish nutrient agar was prepared and poured.The culture was streaked on the petridish and was kept in an incubator for 24 hours.After incubation the streaked plate has some growth.two to three drops of iodine solution was flooded and excess of iodine solution was removed to observe any changes in the Petridish.

Bile tolerance test

The culture were grown in a nutrient broth at 37^{o}Cwhich contains 2%,4%,6% of bile salt mixture. The test tubes were checked at an interval of 1 hour 3 hour and 4 hour.The growth was checked using pour plate technique where 1ml of culture was placed in a nutrient agar.The plates were incubated and checked for plate counts as colony forming units

Indole reagent test

A filter paper is taken and culture was placed with a plastic loop and two three drops of indole reagent was added to observe any changes

Triple Sugar Ion test

This test is to identify the microorganisms that can ferment sugars.This test was originally discovered by Sulkin and Willet in 1976. A slant was prepared using TSI agar and inoculated with culture by stabbing and streaking the slope.The test tube was inoculated for 24 to 48 hours and later was observed for any black appearance due to H_2S production and gas fermentation.

Total Number of E.Coli Present in Vegan Curd

In a 250ml of conical flask 1.44grams of EMB Agar was prepared and diluted in 40ml of distilled water. Two petridishes were taken and marked as control and the other plate for observing growth of E.Coli. The dishes were autoclaved for fifteen minutes for 15lbs and the agar

was poured on the petridish to solidify. Meanwhile the curd sample was diluted with distilled water and spread plate technique was performed and incubated at 37°C for 24 hours.

RESULT AND DISCUSION

Nutritional Analysis

Fat content value for milk curd 2.58%, Fat content value for vegan curd is 4.70%. Protein content value for milk curd 0.4 %, Protein content value for vegan curd 0.7%

Total Plate Count

Colony growth on 10^{-4},10^{-6},10^{-8} plates

Colony forming unit

DILUTIONS	VEGAN CURD	CFU
4	50	5.0*10-5
6	40	4.0*10-6
8	37	3.7*10-8

Identification of bacteria

ORGANISMS	GRAM STAINING
A	Positive, rod shaped
B	Positive, rod shaped
C	Positive,rod shaped
D	Positive, cocci shaped
E	Negative, rod shaped

Biochemical tests

Biochemical tests are usually performed to characterize the bacteria colonies that were grown on the Agar plates. These tests differentiates several organisms.

Characteristics	A	B	C	D	E
Colony Morphology	White,opaque buldged	Transparent Bright chained	Single colony, White, opaque	White, Large Opaque smooth	Yellow colony
Gram Staining	+	+	+	+	-
Spore Formation	-	-	-	-	-
Indole Test	+	-	-	-	-
Methyl Red	-	-	-	-	+
Vp	-	-	-	-	-
Citrate Utilization	+	+	+	+	-
Urease	+	+	+	-	-

Total E.Coli Count in Vegan Curd

E.Coli count Total plates	Growth observation	Colony count
Control	No growth	None of the colonies
EMB agar plate	colonies were observed after incubation	Ten E.Coli colonies

Characterization of bacteria

ISOLATED BACTERIA	CULTURE CHARACTERISTICS	COLONY MORPHOLOGY
Lactobacillus acidophilus	Small to medium grey colonies	Convex,small, rough edges and white
Lactobacillus lactis	Bright orange	They occur in pairs and short chains
Lactobacillus cremoris	Transparent white chains	non spore forming.
Streptococcus thermophilus	White colony and circular	They occur in chains and pairs and are non motile.
Bacillus anthracis	Flat, slightly convex	Colonies have a sticky consistency with irregular edges and ground glass
Micrococcus	Yellow colony	They grow in clusters

CONCLUSION

Milk curd has many rich sources and helps in digestion and has good gut bacteria which fights the pathogens in our body.They produce lactic acid bacteria through fermentation.They have the highest nutritive value and contains more probiotics.The bacteria present in the curd is Lactobacillus bulgaricus.But due to lactose intolerance in few people milk curd cannot be consumed.As a substitute vegan curd can be taken for consumption and they are good for digestion.The bacteria present in vegan curd are Lactobacillus acidophilus,Streptococcus thermophilus,Bacillusanthracis which are probiotics and are equally healthy and nutritive than the milk curd.This curd can also be prepared by adding toppings and other flavours for good appearance and taste.Vegan curd does'nt cause lactose intolerance.This determines that the organisms present in the Vegan curd fights against harmful infections and has high protein content which is good for the gut and also boosts immunity.

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BIOSENSOR-BASED SMART ENVIRONMENTAL MONITORING: AN AI-DRIVEN APPROACH FOR REAL-TIME POLLUTION DETECTION

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ABSTRACT

Environmental pollution has become a major global concern, demanding efficient and real-time monitoring solutions. Traditional environmental monitoring systems rely on chemical and physical sensors, which often face challenges such as low sensitivity, high maintenance, and limited scalability. This paper proposes a biosensor-based smart environmental monitoring system that integrates biosensing technology, IoT, and AI-driven analytics for real-time pollutant detection. The study explores different biosensor types, including electrochemical, optical, and microbial biosensors, to assess their accuracy, response time, and adaptability. The proposed system leverages cloud computing and AI-based predictive modeling to analyse pollution patterns, enabling proactive environmental management. Experimental results demonstrate that biosensors provide higher specificity, rapid detection, and cost-effective monitoring compared to conventional sensors. Furthermore, integrating blockchain technology ensures secure environmental data storage, enhancing reliability and transparency. This research highlights the potential of biosensorbased smart monitoring as a scalable, sustainable, and intelligent solution for pollution detection and environmental protection. Future directions include hybrid biosensor models, AI-enhanced analytics, and real-time pollution forecasting for improved decision-making.

Keywords : Biosensors, IoT, Electrochemical Sensors, Optical Sensors, Microbial Biosensors, Blockchain, Cloud Computing

INTRODUCTION

Biosensor-based smart environmental monitoring systems represent a transformative approach to pollution detection and management. By integrating biological recognition elements with IoT, AI, and blockchain, these systems enable real-time tracking of pollutants with high accuracy and efficiency. Unlike traditional sensors, biosensors offer enhanced specificity, rapid response, and cost-effective scalability, making them ideal for large-scale environmental applications. The use of AI-driven analytics further improves predictive capabilities, allowing for early detection of pollution trends and proactive intervention. With advancements in technology, biosensor-based monitoring has the potential to revolutionize environmental protection, ensuring a cleaner and healthier ecosystem for future generations.

Problem Statement

The ambient contribution is a growing gray challenge, have an impact in public health, my password and the station of ecosystem. Traditional systems of the traditional feature, which are mainly chemicals, face of significant restrictions, including operational tens, the sensitivity and challenges. Also, these conventional systems often miss real-processing capabilities, that makes it difficult to detect pollution the proactive peaks .

To discount these challenges, needs an advanced, efficient environmental and frightened environmental system and frightening a minimum and minimum maintenance. This search is to develop a bio cact environmental surveillance system that includes biokaters, IoT, him and blockchain analysis for safe data management. The proposed system seeks to enhance pollutant

detection, enable predictive analysis, and facilitate proactive environmental protection, overcoming the limitations of existing monitoring solutions.

METHODOLOGY

The methodology for biosensor-based environmental monitoring involves several key stages, including sensor selection, data collection, signal processing, and real-time analysis. The process begins with biosensor integration, where biological recognition elements such as enzymes, antibodies, or DNA probes are incorporated into the sensor to detect specific pollutants like heavy metals, microbial contaminants, or airborne allergens. These biosensors generate measurable electrochemical, optical, or colorimetric signals in response to pollutant interaction. Next, the data acquisition phase involves real-time pollutant detection, where the biosensor captures environmental samples from air, water, or soil. The biochemical reactions produce signals that are recorded using microcontrollers or portable devices. Ticked Signals are subjected to sign-in processing, where the algorithms is driven by he interpreted the data interpreted to improve the accuracy noise and identify polluters. The signature system, the date transmission and storage information is stored on the platforms transmitted to the cloud or wireless in the distance monitoring systems. This allows real-time access score of polluting, allowing environmental agencies and environmental researchers to take immediate action. Finally, the decision system and alarm the system provides users that pollutants that pollutants are the limits of security, Support the preventive measures and policy application. Metogia are frisclation, devising and distant effectiveness, sustainability and public healthy improvement thanks to advanced biodic technology.

Existing System

Mediotic Surveillance Systems are based on chemical sensors and laboratory analysis to detect the pollutants in the air, water and ground. These conventional methods include spectrimometry, shortmarket and spectrometer mass, that provides the very prized results, but I need the dear equipment and safe equipment. Fixed monitoring stations are commonly used to measure pollution level, but lack real flexibility and real realizations. In the past few years, electrochemical age and optical based on sensors were introduced for ambient supervision in the country. These systems use the furniture analysis to detect instagrient metals, unstable organic compounds as the coal dioxide (CO) and nitrogen (No2). Some existing solutions, such as Aeroqual's air quality monitors, integrate wireless communication for remote access to pollution data. However, these systems often suffer from limited selectivity, high maintenance requirements, and sensitivity to environmental factors such as temperature and humidity.

Proposed System

The proposed system to improve environment integrating the biospector technology in the focused analysis on him and the IOT link to detect the right contaminations. Unlike traditional chemical sensors, this system uses enzyme -bated, anticative, anticative or detects such aholding, aircraft and microwos. These biocipitors generate measurable electrochemical signals during interactions with pollutants, ensure high and specific sensitivity. The system includes a wireless ot frame, where sensor data is conveyed to a cloud deck for the remote monitoring and storage. The algorithms of automisa Advanced Automans analyzed the data collected, improve detection precision, leaking trends and pollution pollution. A mobile app and dashboard online provides real updates, visual analysis and alarm notifications when the levels of superior contaminants Dreams. The best of of this proposed system includes upper-time account access, preventive analysis and the stability of the long run in a integration with biocatory. This hybrid approach improve sensor and assicure a continuous monitoring even to varial environmental contoses. The

proposed system is a lucrative, escalating and effective methods of monitoring: an ambidity methods and environmental swimmers and Industries in -bedic decisions on the contamination control and sustainable management.

Future Scope

Future advancements in biosensor-based monitoring can focus on hybrid sensor technologies, combining biological and nanomaterial-based sensing elements to improve durability and stability in harsh environmental conditions. The integration of blockchain technology could enhance data security and transparency, ensuring reliable pollution monitoring records. Additionally, self-powered biosensors using energy-harvesting techniques may eliminate the need for external power sources, making the system more sustainable. Expanding the system to detect a wider range of pollutants, including microplastics and pharmaceutical residues in water, can further enhance its environmental impact. With continuous improvements. This biosensor-based system has the potential to revolutionize smart environmental monitoring, supporting global efforts for pollution control and sustainable resource management

CONCLUSION

The environment-based soverencing system has prosensitive prosers tried a red solution, very sensitive and lucrative to detect the polluters in the air, water and terrian. Plinging the anisage in the analysis IoT and he improved the accuracy, automated data data and provide distance access to the effective decision. Comparise to traditional survivity methods, this approach enable the fastest detection of pollutants, reduce operational costs and a improvement, Make it is suitable for great-yield environmental applications.

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AN *IN VITRO* INVESTIGATION INTO THE ANTIOXIDANT PROPERTIES OF *LANATA CAMERA* LEAVES

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ABSTRACT

To demonstrate the plant's potential for treating free radical-mediated illnesses like diabetes, heart disease, and cancer, tests were conducted on the antioxidant activity of a methanolic extract of the entire *Lanata camera* plant. In vitro antioxidant activity was assessed using nitric oxide radical scavenging, oxygen radical scavenging (including DPPH scavenging), superoxide anion radical scavenging, total antioxidant, metal chelation, and hydroxyl and nitric oxide scavenging activity at varying concentrations in the methanolic extract. The extract exhibited significant antioxidant activity throughout the investigation. The phytochemicals in the extract might be the cause of its antioxidant properties. The concentration-dependent antioxidant activity was discovered and might be explained by the *Lanata camera's* bioflavonoid content. All things considered, the plant extract contains natural antioxidants that may help slow the progression of a number of diseases caused by oxidative stress, including ageing.

Key words: Radical Nitric Oxide DPPH Radical Hydroxyl Superoxide Radical Scavenging of Radical Ascorbic Acid Antioxidant Activity

INTRODUCTION

The detrimental impact of oxidative stress on human health has grown to be a significant problem. Reactive oxygen species (ROS) such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide are produced in greater quantities by our bodies under stress than by enzymatic antioxidants like catalase, glutathione peroxidase (GPx), and superoxide dismutase (SOD) and non-enzymatic antioxidants like ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids. Health issues (Steer et al., 2002) and cell damage (Bhatia et al., 2003; Peuchant et al., 2004) are caused by this imbalance. Degenerative disorders, such as cardiovascular disease, cancer, neurological diseases, Alzheimer's disease, and inflammatory diseases, are facilitated by a shortage of antioxidants, which can squelch reactive free radicals (Velavan, 2011; Alma et al, 2003). Both synthetic and natural antioxidants help treat diseases caused by free radicals. Widely employed as antioxidants in the food business, synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) may cause liver damage and cancer (Grice, 1988). This has led to a rise in interest in using natural antioxidants.

According to estimates from the World Health Organisation (WHO), 80% of people on the planet receive their primary medical treatment from traditional medicine, which mostly uses plant extracts and their active ingredients (Winston, 1999). Rich in phytochemicals, plants and their byproducts have been shown to have a range of biological properties, including the capacity to act as antioxidants (Velavan et al, 2007; Velavan, 2015). Flavonoids, isoflavones, flavones, lignans, coumarins, anthrocyanins, catechins, and isocatechins make up the majority of the active antioxidant components. Vitamins C and E, beta-carotene, and tocopherol are recognised to have antioxidant potential in addition to the aforementioned substances present in natural meals (Prior, 2003). Given this context, plants are a rich source of special active ingredients.The medicinal

plant of choice is *Lanata camera*, also known as Unni chedi in Tamil. Lanata cameras are regarded as antiseptics, aperients, and stomachic. Tender branches and flow are used as a diaphoretic for fevers. As a result, *Lanata camera's* capacity to scavenge free radicals was not assessed. Consequently, the goal of the current study was to examine the Lanata camera's capacity to scavenge free radicals using DPPH scavenging, superoxide anion radical scavenging, total antioxidant, metal chelation, and iron reduction power activities.

MATERIALSANDMETHODS

Chemicals

Sisco Research Laboratories Pvt. Ltd., India, was the supplier of nitro blue tetrazolium (NBT), potassium hexa cyano ferrate [K3Fe(CN)6], sodium nitroprusside (SNP), ethylene diamine tetra acetic acid (EDTA), trichloro acetic acid (TCA), thio barbituric acid (TBA), and L-ascorbic acid. Every other chemical and solvent that was utilised was of analytical quality and could be purchased commercially.

Plant materials

In January 2015, the completely grown leaves of *Lanata camera* were harvested from a single plant at the Tamil University campus in Thanjavur District, Tamil Nadu, India. The director of the Rabinat Herbarium and Centre for Molecular Systematics at St. Joseph's College in Trichy, Tamilnadu, India, Dr. S. John Britto, identified and verified the leaves. At the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India, a voucher specimen (RCS001) has been placed.

Preparation of alcoholic extract

The gathered *Lanata camera* leaves were repeatedly cleaned with purified water to get rid of any remaining contaminants. After drying at room temperature, the leaves were ground into a coarse powder. The powder was extracted for 48 hours using a variety of extracts, including methanol, petroleum ether, ethanol, and water. Alcohol was completely eliminated at low pressure, yielding a semi-solid extract. The extract from *Lanata camera* leaves (LCLE) was kept in a refrigerator until it was needed. For the in vitro antioxidant activity, doses of 20, 40, 60, and 80 µg/ml were used.

In vitro antioxidant activity

The phosphomolybdenum technique, as described by Prieto et al. (1999), was used to assess the extracts' antioxidant activity. The Lanata camera's ability to scavenge superoxide anion radicals was assessed using the Liu et al. (1997) approach. The Dinis et al. (1994) method was used to assess the extracts' chelating activity for the ferrous ions Fe2+. The Oyaizu (1986) approach was used to calculate the extract's Fe3+ reduction power. Yu et al. (2004) used the Fenton process to test the scavenging activity for hydroxyl radicals. The nitric oxide radical scavenging activity was measured using the Garrat (1964) method.

Statistical analysis

Three to five distinct experiments were tested in triplicate. IC50, or the quantity of extract required to reduce the concentration of free radicals by 50%, was visually calculated using a nonlinear regression technique according to Garrat (1964).

RESULTSANDDISCUSSION

Plants contain naturally occurring chemical substances called phytochemicals that are physiologically active and beneficial to human health. Although plants make these compounds to

defend themselves, new studies show that many phytochemicals can also shield people from illness. Fruits and plants contain a variety of phytochemicals, and each one functions in a unique way. Flavonoids, terpenoids, steroids, tannin, saponins, glycosides, phlopatannins, carbohydrates, triterpenoids, protein, alkaloids, and anthroquinones were all found in *Lanata camera* phytochemical screening (Sanjeeb Kalita, 2012).

DPPH Assay

Figure 1 displays the *Lanata camera* extract's and ascorbic acid's DPPH radical scavenging activities. A common method for assessing antioxidants' ability to scavenge free radicals is the DPPH radical (Nuutila et al., 2003). The DPPH• reaction has been frequently utilised recently by food technologists and academics to assess the scavenging activity of free radicals on plant extracts, food materials, or individual chemicals.The antioxidant successfully converted the stable radical DPPH into the yellow 1, 1-diphenyl-1, 2-picryl hydrazine in the DPPH assay. The delocalisation of the spare electron over the 2, 2-diphenyl-1-picryl hydrazine molecule makes it a stable free radical.

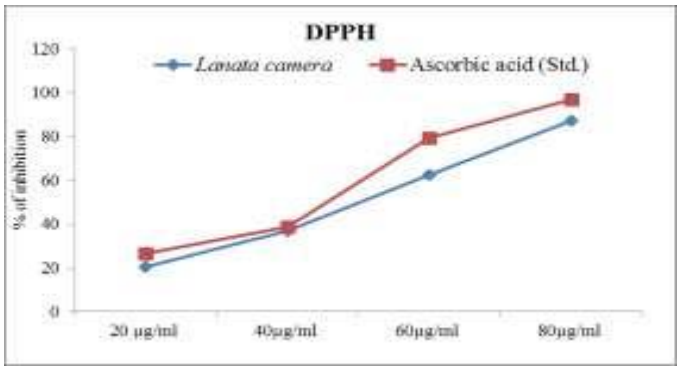

Fig. 1. DPPH radical scavenging activity of *Lanatacamera*

The entire molecule. A standard spectrophotometer placed in the visible spectrum can detect the drop in absorbance at 517 nm caused by the proton transfer reaction of the DPPH• free radical by a scavenger (A-H). It is believed that antioxidants' capacity to donate hydrogen is what causes their impact on DPPH• (Sindhu and Abraham, 2006). Ascorbic acid and plant extract had corresponding half inhibition concentrations (IC50s) of 41.81 µg ml-1 and 48.51 µg ml-1. The plant extracts significantly reduced DPPH activity in a dose-dependent manner. L-ascorbic acid's capacity to scavenge DPPH radicals is directly correlated with its concentration. Ascorbic acid and the DPPH assay activities are nearly identical.

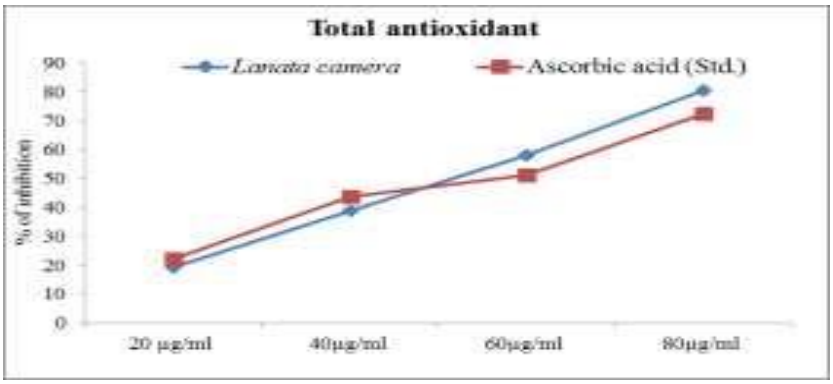

Fig.2.Total antioxidant assay of *Lanata camera*

Superoxide anion radical scavenging activity

Superoxide is crucial to biology because it can break down into more potent oxidative species like hydroxyl radicals and singlet oxygen, which can be extremely damaging to a biological system's cellular constituents (Korycka-Dahl & Richardson, 1978). Figure 3 displayed the extract from Lanata camera's superoxide anion radical scavenging activity as determined by the PMS-NADH method. The Lanata camera's capacity to scavenge superoxide was enhanced.

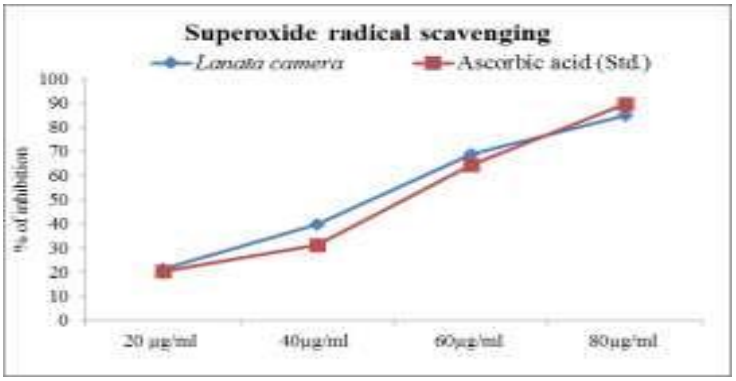

Fig. 3.Super oxide scavenging activity of *Lanatacamera*

Total antioxidant activity

Fig. 2 shows the overall antioxidant capacity and the yield of the plant extract's methanol extract. The number of ascorbic acid equivalents represents the *Lanata camera* extract's total antioxidant capability. Based on the antioxidant compound's reduction of Mo (VI) to Mo (V) and the creation of a green phosphate/Mo (V) complex with a peak absorption at 695 nm, the phosphomolybdenum technique was developed. Since the test is straightforward and unaffected by other widely used antioxidant measurements, it was chosen to expand its usage to plant extract after it was successfully used to measure vitamin E in seeds (Prieto et al., 1999). Furthermore, because the antioxidant activity is reported in terms of ascorbic acid equivalents, it is a quantitative one. According to the study, the extract's antioxidant activity is trending upward as the plant extract's concentration rises. Ascorbic acid and plant extract had corresponding half inhibition concentrations (IC50s) of 45.48 µg ml-1 and 50.80 µg ml-1.significantly as concentrations rise. Lanata camera's half inhibitory concentration (IC50) was 48.89 µg ml-1, while ascorbic acid's was 46.60 µg ml-1. These findings showed that the Lanata camera's superoxide radical scavenging capabilities were noticeably better.

The ferrous ion chelating activity

Ferrous ions and ferrozine can form complexes. The complex's red colour decreases when chelating chemicals are present because they prevent the complex's red-colored production. Therefore, the rate of colour loss can be used to measure the chelating effect of the coexisting chelator. The addition of *Lanata camera* aqueous extract disrupts the formation of the ferrozine–Fe2+ complex, suggesting chelating action with an IC50 of 51.36µg ml-1 and ascorbic acid of 45.91µg ml-1, respectively (Fig. 4). By breaking down lipid hydroperoxides into peroxyl and alkoxyl radicals, ferrous iron can speed up peroxidation and start it through the Fenton reaction (Halliwell, 1991; Fridovich, 1995).In lipid peroxidation, metal chelating activity may help lower the concentration of the transition metal that catalyses the reaction. Additionally, because they lower the redox potential and stabilise the oxidised form of the metal ion, chelating compounds that create s bonds with a metal work well as secondary antioxidants (Gordon, 1990).

Consequently, Lanata cameras exhibit a notable capability for iron binding, indicating their potential as a peroxidation protector that is related to this property.

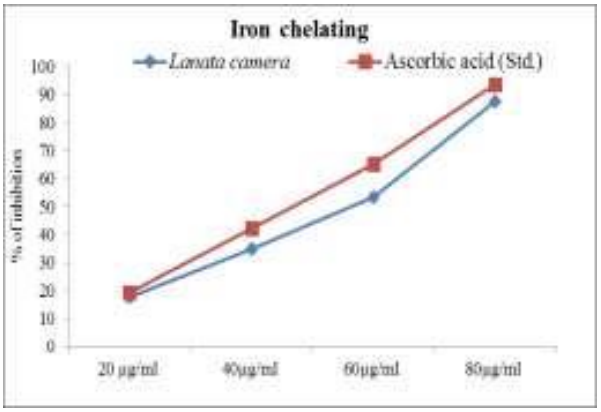

Fig. 4. Ferrous iron chelating activity of *Lanatacamera*

Hydroxyl radical scavenging activity of *Lanata camera*

The 1, 10 phenanthroline-Fe2+ complex oxidation technique was used to test the ethanolic extract's capacity to scavenge hydroxyl radicals. When hydrogen peroxide was mixed with ferrous sulphate, Fe2+ was produced. In an oxidation-reduction reaction, this generated ferrous ion combines with 1, 10 phenanthroline to form the 1, 10 phenanthroline Fe2+ complex, which serves as an indicator. Phenanthroline-Fe2+ is oxidised into the Phenanthroline-Fe3+ complex at the same time by the hydroxyl radical generated from the H2O2-Fe2+ reaction mixture. The extract's presence of a free radical scavenger lowers the oxidation reaction and, therefore, the absorbance, which can be quantitatively evaluated at 560 nm (Olabinri et al., 2010). As the dosage was increased, *Lanata camera's* hydroxyl radical scavenging activity rose as well (Fig 5). Lanata camera's half inhibitory concentration (IC50) was 46.10 µg/ml-1, while ascorbic acid's was 35.26 µg/ml-1.

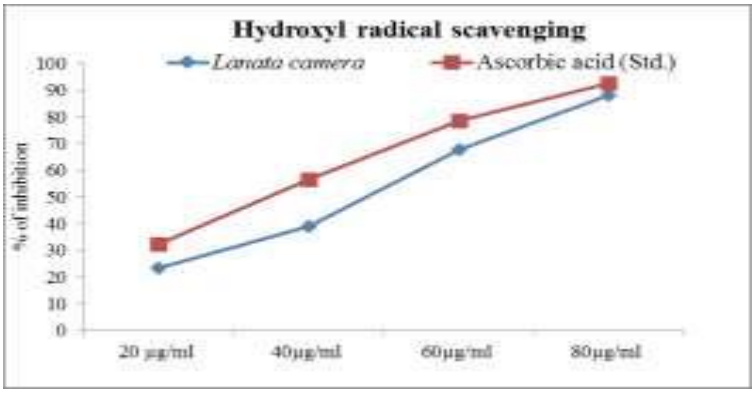

Fig.5. Hydroxyl radical scavenging activity of *Lanata camera*

Nitric oxide scavenging activity of *Lanata camera*

Many cellular components' structure and function can be changed by the strong NO+ character of nitric oxide (NO•), which is generated from sodium nitroprusside (SNP). The nitrite concentration in the assay medium decreased as a result of the *Lanata camera* extract's strong NO• scavenging action. With 80µg/ml scavenging the most effectively, the NO• scavenging capability was concentration dependant. When compared to normal ascorbic acid, the *Lanata*

camera in SNP solution greatly reduced the formation of nitrite, a stable oxidation product of NO• released from SNP in the reaction medium over time. When NO• combines with superoxide to form the peroxynitrite anion (•ONOO-), a potentially potent oxidant that can break down to produce •OH and NO2, its toxicity rises (Pacher et al., 2007). The results of this investigation demonstrate the strong nitric oxide scavenging ability of *Lanata camera* leaf extract. As concentrations rose, Lanata camera's nitric oxide scavenging capacity rose as well (Fig 6). Lanata camera's half inhibitory concentration (IC50) was 46.10 µg/ml-1, while ascorbic acid's was 35.26 µg/ml-1.

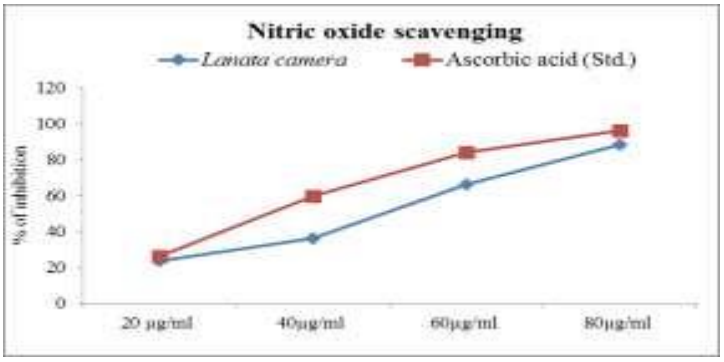

Fig 6 Nitric oxide scavenging activity of *Lanatacamera*

CONCLUSION

The current study's findings demonstrated that the *Lanata camera* extract includes flavonoids and polyohenols. These phytochemicals have the highest antioxidant activity, including the ability to scavenge DPPH, superoxide anion radicals, total antioxidants, metal chelation, hydroxyl radicals, and nitric oxides. These compounds are involved in the pathophysiology of a number of diseases, such as cancer, diabetes, ageing, and more. Because of its ability to scavenge reactive oxygen and nitrogen species and shield cells and organisms from oxidative damage, the *Lanata camera* extract has been experimentally proven to be a natural antioxidant in this work. As a result, it may be useful in combating oxidative stress.Furthermore, a significant amount of total phenols, which are crucial in regulating antioxidants, was discovered in the *Lanata camera* extract. Therefore, it can be said that *Lanata camera* extract is a readily available natural antioxidant source that has health benefits.

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PHYSICO-CHEMICAL CHARACTERISATION OF TEXTILE INDUSTRY EFFLUENT

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ABSTRACT

Pollution has arisen as a serious environmental concern to the present world after industrialization of human societies. It has severely affected our air, soil and water sources. Looking to its global, national, regional and local dimensions, it is now imperative to check it at each and every level. In the present study, effluent on the microbial diversity viz. bacteria, fungi and cyanobacteria. Results of one year ecological study revealed that altogether 10 species of bacteria, 11 species of fungi and 20 species of cyanobacteria were isolated from the effluent stream. The species isolated, *Pseudomonas* with two species and others with single each were recorded. Among the fungi recorded the *Aspergillus* was found to be dominant with four species viz., *A. flavus, A. fumigatus, A. luchensis* and *A. niger*. Higher amounts of phosphates and nitrates with sufficient amount of oxidizable organic matter, limited dissolved oxygen content and slightly alkaline pH were probably the factors favoring the growth of microbes especially cyanobacteria. The utilization of dominant species of cyanobacteria to monitor pollution in detergent effluent has been discussed.

Keywords: textile industry effluent, bacteria, fungi,

INTRODUCTION

Water is essential to all forms of life and makes up to 50-97% of the weight of all plants and animals and about 70% of human body. Water is also a vital resource for agriculture, manufacturing, transportation and many other human activities. Despite its importance, water is the most poorly managed resource in the world. The availability and quality of water always have played an important role in determining the quality of life. Water quality is closely linked to water use and to the state of economic development (Ramamurthy *et al*., 2014). Ground and surface waters can be contaminated by several sources. In urban areas, the careless disposal of industrial effluents and other wastes may contribute greatly to the poor quality of water (Ramamurthy *et al*., 2015). Most of the water bodies in the areas of the developing world are the end points of effluents discharged from industries.

Non-hazardous industrial wastes are those that do not meet the EPA's definition of hazardous waste - and are not municipal waste. The EPA estimated in 1980 that more than 70,000 different chemicals were being manufactured in the U.S., with some 1,000 new chemicals being added each year. Industrial waste has been a problem since the industrial revolution. Industrial waste may be toxic, ignitable, corrosive or reactive. If improperly managed, this waste can pose dangerous health and environmental consequences. In United States, the amount of hazardous waste generated by manufacturing industries in the country has increased from an estimated 4.5 million tons annually after World War II to some 57 million tons by 1975. In 1990, this total had shot up to approximately 265 million tons. This waste is generated at every stage in the production process, use and disposal of manufactured products. Thus, the introduction of many new products for the home and office - computers, drugs, textiles, paints and dyes, plastics also introduced hazardous waste, including toxic chemicals, into the environment. These, too, must be managed with extreme care to avoid adverse environmental or human health impacts.

Textile industries represent a very diverse sector in terms of raw materials, processes, products and equipments and have very complicated industrial chain (Savin and Butnaru, 2008). A number of dyes, chemicals and other materials are used to impart desired grade and quality to fabrics. These industries generate substantial quantity of effluents, which contaminates the natural water bodies altering their physical, chemical and biological nature. Textile effluents can seep into aquifer, thus, polluting the underground water. The impact of textile industry on environment, both in terms of the discharge of pollutants and of the consumption of water and energy has long been recognized (Lacasse and Baumann, 2006).

Textile processing employs a variety of chemicals, depending on the nature of the raw material and product (Aslam et al., 2004). Major pollutants in textile wastewaters are high suspended solids, chemical oxygen demand, heat, colour, acidity, and other soluble substances. Colour is imparted to textile effluents because of various dyes and pigments used. In addition to dyes, various salts and chemicals are the major sources of heavy metals in wastewater.

Sediments, suspended and dissolved solids are important repositories for toxic heavy metals and dyes causing rapid depletion of dissolved oxygen leading to oxygen sag in the receiving water (Alihameed and Ahmed, 2008). The metals and contaminants like dyes tend to persist indefinitely, circulating and eventually accumulating throughout the food chain. The dyes and metals have direct and indirect toxic effects in the form of cancers and allergies besides, inhibiting growth at different trophic levels (Kant Rita, 2012). Taking the above facts into consideration, a survey was undertaken in detergent effluent to explore the nature of microbial flora such as bacteria, fungi and cyanobacteria are screen in order to biodiversity index.

MATERIALS AND METHODS

Effluent was collected from textile industry waste, Tirupur, Tamil Nadu, India. Samples were collected in large sterilized container and brought to the laboratory. Physico-chemical characteristics were done on the same day when the samples were brought to the laboratory. The effluent samples were filtered through cotton to remove suspended coarse particles before use. Population of microbes was isolated from the effluent samples by serial dilution technique. Bacteria were identified based on colony characteristics, Gram staining methods and by various biochemical studies as given by Bergey's (1984) Manual of Determinative Bacteriology. Fungi were identified by using standard manuals, such as Mannual of Soil Fungi (Gillman, 1957), Dematiaceous Hyphomycetes (Ellis, 1971). Physico-chemical characteristics of effluent were done according to the Standard Methods (APHA, 1995). Temperature and pH of the effluent were measured at the station itself.

RESULTS AND DISCUSSION

The results of physico-chemical characterization of textile effluent are analyzed. The effluent was slightly alkaline and contained high amounts of nitrate, nitrite and ammonia, total phosphate, inorganic and organic phosphate and calcium in all the seasons examined. Very low level of dissolved oxygen and high levels of BOD and COD were recorded during the study period. High amount of total suspended solids were recorded in summer and pre-monsoon seasons (Table 1). Total dissolved solids were high in summer followed by post monsoon and pre monsoon seasons. Bicarbonate was observed only during monsoon seasons. Nutrients such as nitrate, nitrite and inorganic phosphates were high in monsoon, whereas total and organic phosphates were maximum during monsoon season. BOD and COD were very high during summer on the other hand high level of DO was recorded during monsoon. Dissolved oxygen

level was very low during summer and high in monsoon. Most of the parameters tested were slightly higher in summer than monsoon, post monsoon and pre monsoon seasons.

The physicochemical analysis of the effluent revealed that it is slightly alkaline in nature and also the presence of high quantity of both organic as well as inorganic nutrients in all the seasons examined (Table 1). Values of DO were very low indicating highly obnoxious conditions. Though BOD and COD levels in the present study were high as per WHO standards, their levels were not so much high as compared to other types of effluents such as paper (Somashekar and Ramasamy, 1983; Manoharan and Subramanian, 1992b), distillery (Jain *et al.*, 2001, Veerasamy *et al.*, 2011) and dye effluent (Sulaiman *et al.*, 2002). Most of the parameters tested were slightly higher in summer than in other seasons. Somashekar and Ramaswamy (1983) reported similar results in paper mill effluent in different seasons. They recorded objectionable amounts of BOD and COD, oil and grease, total dissolved solids, and algal nutrients such as ammonia nitrogen, nitrate nitrogen, silicates, phosphates and calcium. Such a trend was observed in the detergent effluent also. Sahai *et al.* (1985) analyzed pollution load of four different effluents such as fertilizer, sugar, distillery and domestic sewage. Among these, highly objectionable amounts of various pollutants including BOD and COD were recorded in distillery followed by sugar, fertilizer and domestic sewage.

Bacteria isolated from the textile effluent were identified based on colony morphology, Gram staining and various biochemical characteristics. The characteristics of isolated bacteria are given in the table 2. Totally ten different bacteria were isolated from the effluent sample. The species isolated were *Escherichia coli, Enterobacter aerogens, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas* sp, *Pseudomonas aerogenosa, Shigella sonnei, Streptococcus faecalis, Bacillus subtilis* and *Staphylococcus faecium*. All the species were recorded in all the seasons. All the species were recorded in all the seasons. There was not been much work regarding the isolation and identification of bacteria from textile industry and other related effluent samples. Jain *et al.* (2001) isolated three different bacterial strains from the distillery sludge to treat predigested distillery wastewater. In the present study totally eleven different species of fungi belonging to eight genera were isolated from the effluent (Table 3). Among the fungi recorded the *Aspergillus* was found to be dominant with four species viz., *A. flavus, A. fumigatus, A. luchensis* and *A. niger*. The rest of the genera such as *Candida* sp, *Penicillium javanicum, Saprolespgia* sp, *Trichoderma viride, Alternaria* sp, *Fusarium oxysporum* and *Rhodosporium* were recorded with single species each. Similarly *Neurospora crussa* was not observed during rainy season. Kousar *et al.* (2000) isolated 23 species of fungi from dye effluent polluted habitat with *Aspergillus* as the dominant genus.

Table 1. Characteristics of effluent observed in four seasons

S.No.	Parameters	Summer	Premonsoon	Monsoon	Post monsoon
1.	Temperature^{0}C	22.75 ± 1.05	20.17 ± 0.65	18.85 ± 0.17	20.45 ± 0.16
2.	pH	8.95 ± 0.26	8.76 ± 0.28	8.52 ± 0.37	8.80 ± 0.22
3.	Total suspended solids	1990 ± 15.5	1976 ± 28.7	1951 ± 58.2	1968 ± 22.5
4.	Total dissolved solids	1430 ± 15.2	1385 ± 14.5	1374 ± 18.3	1393 ± 11.7
5.	Free carbon-di-oxide	25.3 ± 1.06	24.9 ± 1.07	17.8 ± 1.12	21.7 ± 1.16
6.	Carbonate	2.38 ± 0.15	2.16 ± 0.18	3.68 ± 0.14	2.58 ± 0.16
7.	Bicarbonate	56.5 ± 1.81	57.3 ± 2.13	59.8 ± 1.19	58.9 ± 1.26
8.	BOD	291 ± 5.27	248 ± 6.51	236 ± 9.15	259 ± 7.26
9.	COD	512 ± 8.12	438 ± 4.84	385 ± 6.71	440 ± 5.18

10.	Dissolved oxygen	3.21 ± 0.12	4.18 ± 0.17	4.93 ± 0.24	4.09 ± 0.17
11.	Nitrate	92.7 ± 2.44	93.9 ± 2.81	96.9 ± 2.29	94.1 ± 2.52
12.	Nitrite	66.1 ± 2.27	69.9 ± 2.17	78.2 ± 2.12	71.5 ± 2.16
13.	Ammonia	41.3 ± 3.24	44.6 ± 3.08	46.2 ± 3.17	45.4 ± 3.42
14.	Total phosphate	70.02 ± 4.18	72.05 ± 4.13	80.25 ± 4.19	71.45 ± 4.71
15.	Inorganic phosphate	36.52 ± 2.19	38.93 ± 2.17	39.25 ± 2.18	38.25 ± 2.32
16.	Organic phosphate	35.50 ± 3.15	38.06 ± 3.18	40.50 ± 3.42	39.24 ± 3.72
17.	Calcium	191 ± 2.10	178 ± 2.42	170 ± 2.17	181 ± 2.18
18.	Magnesium	150 ± 2.17	143 ± 2.54	132 ± 2.52	141 ± 2.17
19.	Chloride	69.2 ± 4.62	63.1 ± 4.57	62.3 ± 4.43	65.4 ± 4.35

(Each value represents mean ± SD of three observation)

Table 2. Bacterial flora observed from textile effluent of four different seasons

S.No.	Name of bacteria	Summer	Premonsoon	Monsoon	Post monsoon
1.	*Escherichia coli*	+	+	+	+
2.	*Enterobacter aerogens*	+	+	+	+
3.	*Klebsiella pneumoniae*	+	+	+	+
4.	*Proteus vulgaris*	+	+	+	+
5.	*Pseudomonas* sp.	+	+	+	+
6.	*P. aerogenosa*	+	+	+	+
7.	*Shigella sonnei*	+	+	+	+
8.	*Streptococcus faecalis*	+	+	+	+
9.	*Bacillus subtilis*	+	+	+	+
10	*Staphylococcus faecium*	+	+	+	+

+ : Observed in all the months.

Table 3. Fungal flora observed from textile effluent of four different seasons

S.No.	Name of fungi	Summer	Premonsoon	Monsoon	Post monsoon
1.	*Aspergillus flavus*	++++	+++	+++	+++
2.	*A. fumigatus*	++	+	+	+
3.	*A. luchensis*	++	++	++	+
4.	*A. niger*	+++	++++	+++	++++
5.	*Candida* sp.	+	+	-	+
6.	*Penicillium javanicum*	+++	++	++	++
7.	*Saprolespgia* sp.	++	+	+	+
8.	*Trichoderma viride*	+++	+++	++	+++
9.	*Alternaria* sp.	+	-	-	-
10.	*Fusarium oxysporum*	++++	+++	+++	+++
11.	*Rhodosporium* sp.	+++	+++	++	+++

++++ : Observed in all the months

+++ : Observed in above five months

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STUDIES ON THE LARVICIDAL EFFICACY OF OCIMUM SANCTUM AND ANNONA SQUAMOSA AN AGAINST *DYSDERCUS SINGULATUS*

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ABSTRACTS

The red cotton bug, *Dysdercuscingulatus* (Fab).is an important pest of cotton and okra. Although synthetic chemical insecticides can control it, the side effects are enormous. *Dysdercuscingulatus* (Fab.) is a serious pest of cotton and distributed all the cotton growing region of India. The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors as an alternative source for the synthetic pesticides.The study indicated that essential compounds were the only chemical used for the control of mosquito larvae while extract was used as the control of adult mosquitoes. The essential compounds were extracted by steam distillation and their chemical composition determined by Gas-chromatography coupled to mass spectroscopy. A study was made to monitor the effect of plant extracts on different instars of larvae and pupae of mosquito vector *D.cingulatus*. Bio-assay was made using the solvent acetone to find out the median lethal concentration. Plants, like *Ocimum sanctum* and *Annonasquamosa* which possess insecticidal properties are seemed to be better vector control agents than the synthetic xenobiotics. These results suggest a potential utilization of the extracts of these two plant species for the control of *Dysdercuscingulatus*.

KEY WORDS: *Dysdercuscingulatus*, insecticide,larvicidal activity, mosquito control, phyto-compounds, GC-MS analysis, *Ocimum sanctum* and *Annonasquamosa*

INTRODUCTION

The red cotton bug (*Dysdercuscingulatus*) has wide distribution; it is a major pest in cotton growing regions of northern India particularly Punjab and Uttar Pradesh. This pest also occurs throughout the Maharashtra state but is of minor importance. It is commonly known as ‘cotton stainer’. Host plants of cotton stain rare cotton, bhendi, ambadi, hollyhock and several other malvaceous plants. The adult bug measures about 12-15 mm in length. The females are longer (15 mm) than the males (12mm). It is blood red in color except eyes, scutellum, anal style and antennae which are black colored. Besides, there is black spot on each of the membranous forewings. Cotton stainer feeds both on immature and mature seeds. Their penetrations into the developing cotton boils transmit fungi on the immature lint and seed, which latter on stain the lint with typical yellow color, hence the name “cotton strainers”. Heavy infestations on the seeds affect the crop mass, oil content and the marketability of the crop. The cotton stainer*Dysdercuscingulatus* (Fab.), commonly known as red cotton bug causes serious damage by feeding on developing bolls and ripe cotton seeds (Natarajan and Rajendren, 2005). It is distributed all over the cotton producing regions of India (Sahayaraj and Illayaraja, 2008). In India cotton production is about 295 million bales (H” 480 1b bales) during 2009-2010 against 113.9 million bales in the rest of the world. Also, India has the largest area under cotton cultivation (10.31million ha) and yield was 486 kg ha-1 during 2009-2010 (Cotcorp, 2010). Due to hazards associated with the increased use of synthetic pesticides the use of biopesticides

especially from marine algae has gained considerable attention on the eco-friendly approaches for the management of insect pest.

*Dysdercuscingulatus*is a serious pest of cotton and distributed all the cotton growing region of India (Chari, 1998; Venugopal, 1994; David andAnanthakrishnan, 2004). It is difficult to control by insecticidebecause itis highly mobile, Polyphagous (Iwata, 1975) and Polymorphic (Sahayaraj and llayaraja, 2008) pest of many Malvaceae crops. Terrestrial plants like *Catharanthusroseus*G.Don,*Paratheniumhysterophorus* Don andNephrolepsextracts were have insecticidal activity against red cotton bug (Rajendran andGopalan, 1980; Gahukan, 1995; Gawande andBurkhade, 1998).Moreover, neem based pesticide like neem gold (Abraham andAmbika, 1979) also shows nymphicidal activity against this pest. Ovicidal activity of *Pedalium murex* (Linn.)(Sahayaraj*et al*., 2006) on *D. cingulatus*was reported earlier.

In India, herbs have long been used for promotion of health, prevention and treatment of diseases. *Ocimum sanctum* L., commonly known as 'Tulasi' in Tamil and holy basil in English, has been claimed to be valuable against a wide variety of diseases. Indian MateriaMedica describes the use of the plant in the treatment of a number of ailments like bronchitis, rheumatism and pyrexia (Nadkarni, 1976). Studies on the immunomodulatory effect of *O. sanctum* have been reported for various animal species (Singh *et al*., 1996; Singh andMajumdar, 1997).

Tulasi (Holy Basil) is a traditional plant considered sacred by the Hindus. This religion links the plant with the Goddess figure as described in the Puranas. Hindus regard it as an earthly manifestation of goddess Vrindavani, who is dear to Lord Vishnu. The name "Tulasi" in Sanskrit means "the incomparable one." The ShyamaTulasi or Krishna Tulsi (*Ocimum sanctum* L. syn.*Ocimumtenuiflorum*) possesses great medicinal value as mentioned in CharakSamhita, an ancient Indian literature.

It is a most common household plant in India and grows wild in tropics. Native to India, it is a short lived perennial herb or small shrub of Mint family Labiatae (Lamiaceae). It has small leaves with a strong smell and purple flowers. The foliage is green or purple, strongly scented. Oil extracted from leaves of this plant possesses significant insecticidal properties (Nanasombat and Lohasupthawee, 2005). *Ocimum sanctum* has been extensively studied for therapeutic potentials in various areas like immuno-stimulation, anticancer antioxidant, as adjuvant to radiotherapy, antiulcer, analgesic and antidiabetic (Hammer *et al*., 1999).

Annonasquamosa Linn, belonging to family Annonaceae is commonly found in India & cultivated in Thailand & originates from the West Indies & South America. It is mainly grown in gardens for its fruits & ornamental value. *Annonasquamosa* L. (Annonaceae), commonly known as the custard apple tree is a native of West Indies. But the cultivation is present throughout India, because of its edible nature. It is a fruit tree considered as a native of Central America also and hence has a wider cultivation throughout the regions of tropics. The taste of the pulp of the fruit is really sweet because of its higher sugar content of about 58% of dry mass, and hence it is found clear that the fruit pulp possess a high calorie value. This plant was reputed to contain several medicinal properties (Gajalakshmi*et al*., 2011).

One of the major obstacles hindering cotton cultivation is insect pest manifestations. In particular, the sucking bugs such as *Dysdercus* Spp., (Hemiptera) cause severe injury or losses by feeding on developing cotton balls and ripe cotton seeds. These pests are difficult to control by synthetic insecticide application because the nymphs and adults of *Dysdercus* Spp., are highly

mobile and have many alternate host plants such as castor, lady's finger, turnip, cabbage (Sahayaraj and MajeshTomson, 2010). In the present work alcoholic extract of *Ocimum sanctum* and *Annonasquamosa*were investigated for potential larvicidal activity. To identify and characterized the compounds of therapeutic value extracted from *O.sanctum* and *A. squamosa.* The analytical methods chosen are Gas Chromatography/Mass Spectrometry (GC/MS). The methods were applied to characterize the infusion prepared from this plant and to make a comparison between the alcoholic extracts of the leaves.

MATERIALS AND METHODS

Plant material and oil distillation

The medicinal plant of *Ocimum sanctum* and *Annonasquamosa*were collected from in and around area of Pattukkottai, Thanjavur District, Tamil Nadu and South India. The plant was identified with the help of flora presidency, Tamil Nadu and Karnatic flora (Gamble1967; Matthew1983) and standard references (KrtikarandBasu1935). A voucher specimen has been preserved in our laboratory. The plants leaf were dried and powdered of 50 g powdered sample were extracted with ethanol using soxhlet apparatus and concentrated *in-vacuo*. Approximately, 5 g of extract was obtained from 100 g of dried powder material. The extracts were dried in an air conditioned room at 25°C, milled and submitted to hydrodistillation in a Clevenger-type apparatus for 4 hours. The extractwere dried in anhydrous sodium sulphate, filtered, stored in amber glass bottles in a refrigerator (4°C) for investigation of chemical constituents and larvicidal activity.

GC-MS ANALYSIS

The extracts of *Ocimum sanctum* and *Annonasquamosa*washed with sterile distilled water, and they were shade dried and powdered by using Pestle and Mortar and for the alcoholic extracts (96% alcoholic solution) roots and leaves. The tincture was prepared by mixing all parts of the plant with a 50% alcoholic solution for 30 days. The infusion was also prepared by mixing parts of the plant with hot water for 20 min and the alcoholic extracts by mixing the fresh parts of the plant with a 96% alcoholic solution for 12 days.

The dry fractions (20g) were dissolved in 75ml of alcohol and than soaking for 24 hrs. After soaking, collect a filtrate and evaporate under liquid nitrogen. Then concentrate the filtrate for GC-MS analysis.

For the GC-MS analysis a 30m x 0.25mm I.D x 1.0 µmdf fused Elite-1 (100% Dimethyl Poly Siloxane) column; GC Clarus 500 Perkin Elmer gas chromatograph with Mass detector-Turbo mass gold- Perkin Elmer, Software- Turbo mass 5.1. The samples (1µl) were introduced *via* an all – glass injector working in the split mode (10:1), with Helium as the carrier gas.

Oven temperature programme: 110 deg-2min hold, upto 280 deg at the rate of 5 deg/9min hold. Injector temperature: 250 deg C. GC time – 45 mins.

MS Programme: Inlet line temperature: 200^{o} C, Source temperature: 200^{o} C, Electron energy: 70eV, Mass scan: (m/z) 45-450. MS time – 46 mins.

The identification of the constituents was performed by computer library search, retention indices and visual interpretation of the mass spectra. Compounds were identified by comparing their mass spectrum to those of the database of the GC-MS (NIST 62.lib), literature (McLafferty and Stauffer 1989) and retention indices (Adams 2007).

Collection and storage of experimental animals

Larvae of *Dysdercuscingulatus*were obtained from a permanent colony. The larvae were cultured and maintained in the laboratory at 27 ± 2^{0}C and 50 - 75% of relative humidity. Larval forms were maintained in tray by providing dog biscuit and yeast powder in the 3:1 ratio.

Test for Larvicidal activity (WHO, 1996)

The laboratory colonies of *Dysdercuscingulatus*were used for the larvicidal activity. The instarII and instarIV larvae and pupae of the selected mosquito species were kept in 1 litreglass beaker and different concentration of selected plant extract was added to find out LC_{50}.

Larvicidal bioassay

Different concentrations of extract (300, 250, 100, 50 and 25 µg/ml) were prepared using distilled water. The mosquito larvae were treated with extract by using the method of WHO (1981). Ten larvae of *Dysdercuscingulatus*were introduced in different test concentration of both plant extracts along with a set of control containing distilled water without any test solution. After adding the larvae, the glass dishes were kept in laboratory at room temperature. By counting the number of dead larvae at 24hrs of exposure, the mortality rate and the median lethal concentration were obtained. Three replications were maintained for each concentration. Dead larvae were removed as soon as possible in order to prevent decomposition which may cause rapid death of the remaining larvae. The water used for the study was analyzed by usingthe method of APHA (1996).Mortality was recorded after 24 h of exposure during which no nutritional supplement was added.

The experiments were carried out 27 ± 2^0C. Each test comprised of three replicates with four concentrations (300, 250, 100, 50 and 25 µg/ml). Data were evaluated through regression analysis. From the regression line, the LC_{50} values were read representing the lethal concentration for 50% larval mortality of *Dysdercuscingulatus*.

RESULTS AND DISCUSSION

Physical and chemical characteristics of water used for the study, like temperature 27 ±0.5^{0}C, pH 7.3 ± 0.5, dissolved oxygen 3.6 ±0.5 mg/l, dissolved carbondioxide 1.2 ±0.5 mg/l,salinity 1.5 ±0.5 ppt and alkalinity 125 ±0.5 mg/l were within the permissible limits throughout the study periods.

The 24h bioassay is a major tool for evaluating the toxicity of phytotoxins, and a number of researchers have been applying this method to assess the toxiceffect of different plant extractionagainst mosquitoes (Sakthivadivel and Daniel 1999). The mosquito larvae exposed under plant extracts showed significant behavioral changes. The changes were observed within 30 minutes of exposure. The most obvious sign of behavioral changes observed in*Dysdercuscingulatus*was inability tocome onthe surface. The larvae also showedrestlessness, loss of equlibrium andfinally led to death.Remia and Logaswamy(2010) reported that these behavioral effects weremore pronounced in case of *Catharanthusroseus* than*Lantanacamara* extracts after exposures. These effects may be due the presence of neurotoxic compounds in both the plants. In the present study the behavioral effects were more pronounced in case of *Ocimum sanctum* and *Annonasquamosa*extracts after exposures. These effects may be due the presence of neurotoxic compounds in both the plants. No such behavioral changes were obtained in control groups.

Results of the experiment conducted for evaluating the larvicidal efficacy of both plants showed that they are toxic to the*D.cingulatus*larvae. Three replicates of each extract and control were performed in order to ascertain the consistency of the results (Tables 1- 3). The corrected percent mortalities were analyzed using Abbott's formula (Abbott 1925). The mortality data were analyzed using Prism Version 3 from which lethal concentration (LC_{50}) values (24 h) and 95% confidence intervals (CI) were determined. The LC_{50} value of the test extract was compared with that of *Ocimum sanctum*reflecting the potencies of the two; the one with a lower LC_{50} value being more potent of the two.

The crude extract of *Annonasquamosa*was found to be active on the IV th instar larvae of *D.cingulatus*. The larvicidal activity varied with the concentration and exposure. The larvicidal activity of *Annonasquamosa*was comparable to that of *Ocimum sanctum*. The exact active principles in *Ocimum sanctum*responsible for the larvicidal effect have been reported to contain sufficient amount of tetranortriterpenoids (Pegeland Rogers 1990). The observed mosquito larvicidal effects could possibly be due to these compounds (Siddiqui*et al*. 2000).

The results from the *D.cingulatus*larvicidal assay using two different plants are shown in Table 4. The most active essential compounds against third instar larvae of *D. cingulatus* were those of *Ocimum sanctum* and *Annonasquamosa*. Sukumar et al. (1991) reported that *C. citratus* causes significant growth inhibition and mortality in later developmental stages of *A. aegypti*. The analysis of the essential oil of this plant from the state of Ceará, showed that its major components are geranial (60.3%) and neral (39.7%).

Lippiasidoides essential oil and its main constituent thymol were shown to be very active against *A. aegypti* larvae. Sukumar et al. (1991) studied of *Ocimum americanum* showed that solvent extracts from the whole plant have ovipositional deterrence against *A. aegypti*. Matos (2000) reported that *Ocimumgratissimum* essential oil displays antifungal (*Aspergillus* and *Trichoderma*) and antibacterial (S*taphylococcus*) activities. *O. gratissimum* oil presented antihelmintic activity against *Haemonchus contortus*, the main nematode of ovines and caprines in Northeastern Brazil (Pessoa et al. 2002). The citrus oils, although they have insecticidal activities (Ezeonu et al. 2001) and *Hyptissuaveolens* that is used as mosquito repellent (PalssonandJaenson 1999) were not effective in the larvicidal test. Supavarnet al. (1974) tested 36 vegetable extracts on *A. aegypti* and found that 11.1% were capable of producing mortality at a concentration of 500 ppm but only 2.8% produced the same effect at a concentration of 100 ppm.

The use of vegetable oil presents a better option in comparison to chemical pesticides forthelarvalmosquitocontrol,aschemicalsmaycauseenvironmental hazardsand proved troublesome in the long run (Ranapukar*et al.* 2001). Extensive research has been carried out on the effect of botanical derivatives of the neem tree and its derivatives (Mullaand Su 1999).

Methanolic extract of the leaves of *Ocimum sanctum* and *Annonasquamosa*were evaluatedfor mosquitocidal activity against the immature stages of mosquitoes, *Culex quinquefasciatus, Anophelesstephensis* and *D.cingulatus*in the laboratory (Sivagnaname and Kalyanasundaram, 2004). A survey of literature on control of different species of mosquito revealed that assessment of the efficacy of different phytochemicals obtained from various plants has been carried out by a number of researches on the field of vector control *Ageratina adenophora* (Spreng.) showed toxic effects on the mosquito species of *D.cingulatus* and *Culexquinquefasciatus* (Rajmohan and Ramaswamy 2007). *Albiziaamara*and *Ocimum sanctum* showed larvicidal andrepellant properties against *Aedesaegypti* and neem seed kernel extracts showed higher larvicidal activityof *Aedesaegypti*(Palsson and Janeson 1999; Sakthivadivel and Daniel 1999). A detailed laboratory study on extracts of fruit of *Piper nigrum* against larvae of *Culexpipines, Aedesaegypti* and*Aedestogoi* was carried out (Park *et al*. 2002). The authors determined the LC_{50} and observed the behavioural changes and mortality in the larvae. Similar observations were noticed in the present study and support the potential applications of these herbs in mosquito control measures.

Molluscicidal and mosquito larvicidal efficacy of *Ocimum sanctum*and mosquitolarvicidal property of *Momordicacharantia* have already been reported (ManishaSrivastava*et al.* 2007 and

Singhet al. 2006) and observed them safe for humanhealth. In conclusion the leaf extract of *Ocimum sanctum* and *Annonasquamosa*are highly toxic even at low doses these plants may eventually prove to be useful larvicide. Further analysis is required to isolate the active principles and optimum dosages, responsible for larvicidal and adult emergence inhibition activity in*D.cingulatus*. The product of these plants canbe well utilized for preparing phytochemicals from which all the non-target organisms can be rescued from harmful vectors. These plants would be eco-friendly and mayserve as suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and are readily available in many areas of the world.

Table 1. Larvicidal effects of ethanolic extracts of *Ocimum sanctum* and *Annona squamosa* on larvae of *Dysdercus cingulatus* after a 24 h treatment at room temperature

S. No	Concentration of the extract (mg/ml)	No. of larvae Dead/No. exposed (*O. sanctum*)	No. of larvae Dead/No. exposed (*A. squamosa*)	Mortality
1	Control	0/30	0/30	0
2	0.025	3/30	3/30	10
3	0.050	6/30	6/30	20
4	0.075	9/30	9/30	30
5	0.100	12/30	12/30	40
6	0.150	15/30	15/30	50
7	0.200	18/30	18/30	60
8	0.200	24/30	24/30	70
9	0.250	30/30	30/30	80
10	0.300	30/30	30/30	100

Table 2. Phyto-components of extract of *O. sanctum* identified by GC-MS study

S.No	Components	Formula
1	Benzene acetaldehyde	C_8H_8O
2	5H-1-Pyrindine	C_8H_7N
3	2-Furan carboxaldehyde, 5-(Hydroxymethyl)-	$C_6H_6O_3$
4	Benzene acetic acid	$C_8H_8O_2$
5	Dodecanoic acid	$C_{12}H_{24}O_2$
6	Phenol, 3-Isopropoxy-5-Methyl-	$C_{10}H_{14}O_2$
7	3`-Acetyllycopsamine	$C_{17}H_{27}NO_6$
8	Squalene	$C_{30}H_{50}$
9	Octanoic acid, Ethyl ester	$C_{10}H_{20}O_2$
10	Benzaldehyde, 3-Hydroxy-4-Methoxy-	$C_8H_8O_3$
11	Benzaldehyde, 4-Hydroxy-3, 5-Dimethoxy-	$C_9H_{10}O_4$
12	4-((1E)-3-Hydroxy-1-propenyl)-2-	$C_{10}H_{12}O_3$
13	Benzaldehyde, 4-Hydroxy-	$C_7H_6O_2$
14	Butanoic acid, 2-Methyl-	$C_5H_{10}O_2$
15	Nonanoic acid	$C_9H_{18}O_2$
16	Benzene acetic acid, 2,5-Dihydroxy-	$C_8H_8O_4$
17	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	$C_{20}H_{40}O$
18	Phytol	$C_{20}H_{40}O$
19	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$
20	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$
21	Phytol	$C_{20}H_{40}O$
22	9,12-Octadecadienoic acid (Z, Z)-	$C_{18}H_{32}O_2$

23	9,12-Octadecadienoic acid, Ethyl Ester	$C_{20}H_{36}O_2$
24	Squalene	$C_{30}H_{50}$
25	Methyl Salicylate	$C_8H_8O_3$
26	1-(+)-Ascorbic acid 2,6-Dihexadeconate	$C_{38}H_{68}O_8$

+: Present; -: Absence

Table 3. Phyto-components of extract of *A. squamosa* identified by GC-MS study

S. No	RT	Name of the Compound	Molecular Formula	Name of the compound
1	3.95	Benzene, 1,2,3-trimethyl-	C_9H_{12}	Aromatic compound
2	11.32	Undecanoic acid	$C_{11}H_{22}O_2$	Fatty acid
3	13.03	E-7-Tetradecenol	$C_{14}H_{28}O$	Alkene compound
4	13.79	Tetradecanoic acid	$C_{14}H_{28}O_2$	Myristic acid
5	16.61	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid
6	16.89	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Fatty acid ester
7	18.89	Phytol	$C_{20}H_{40}O$	Diterpene
8	19.31	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	Linoleic acid
9	19.61	Oleic Acid	$C_{18}H_{34}O_2$	Oleic acid
10	23.12	Eicosane, 2-methyl-	$C_{21}H_{44}$	Alkane compound
11	23.48	Oxirane, tetradecyl-	$C_{16}H_{32}O$	Oxirane compound
12	25.18	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	Plasticizer compound
13	27.41	Heptacosane	$C_{27}H_{56}$	Alkane
14	29.51	Squalene	$C_{30}H_{50}$	Diterpene

**Source: Dr.Duke's Phytochemical and Ethnobotanical Databases

Table 4. Percentage larval and pupal mortality of *Dysdercus cingulatus* for different concentrations of extract of *O. sanctum* and *A. squamosa* for 24 h exposure

Plants used	Stages of exposure	Parameters	Effective concentration in µg/ml					
Annona squamosa	II instar	Larval mortality (%)	Control	50	100	150	200	250
			0	5	24	37	50	64
	IV instar	Larval mortality (%)	Control	160	180	200	220	240
			0	16	24	32	48	55
	Pupae	Pupal mortality (%)	Control	200	225	250	275	300
			0	10	19	27	53	66
Ocimum sanctum	II instar	Larval mortality (%)	Control	25	50	75	100	125
			0	15	22	31	42	55
	IV instar	Larval mortality (%)	Control	50	100	150	200	250
			0	14	35	52	63	75
	Pupae	Pupal mortality (%)	Control	200	225	250	275	300
			0	29	42	53	66	81

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ULTIVATION OF SPIRULINA PLATENSIS IN DIFFERENT MEDIUM AND OIL CAKES

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ABSTRACT

Experiment were carried out to assess the optimum culture conditions for the growth of *S. platensis* in different natural medium and synthetic medium*viz* ,zarrouk medium, and different oil cakes of groundnut oil cake, castor oil cake,coconut oil cake .Growth analyses and total biomass were monitored for 30 days of regular basis.pH was found to be ranged from 9.1to10 in zarrouk medium,8.9to9.5 in oil cake was different concentration 200mg,400mg,600mg, 800mg, 1000mg .Room temperature was maintain at 30◦c under 12/12 hours light-dark cycle,light illuminate (4500lux) and spectrophotometer used at 550nm,680nm for ten days interval .total biomass (DW) was gradually increased along with the age of culture and 1.92dw/L in oil cakes.The*S.platensis* inoculated in zarrouk medium were survived but well the growth was not flourished , achived the maximum dry weight of 1.86 dw/L on 30 day of cultivation .However, result of the present investigation could be considered for commercial cultivation of *Spirulina* using natural substrate.
(**Key words**:*Spirulina*,zarroukmedium,oil cakes and total biomass)

INTRODUCTION

Spirulina is a primitive organism originating some 3.5 billion years ago that has established the ability to utilize carbon dioxide dissolved in oil cakes as a nutrient source for their reproduction. vigorously in strong sunshine under high temperature and high alkaline condition.The cyanobacterium, *spirulina* has been commercialized in several countries for its use in health *spirulina* is a photosynthesizing cyanophyte(blue-green algae) that grows foods and for therapeutic purpose due to its valuable constituents particularly proteins and vitamins.,Benneman (1988) andvenkataraman*etal*.,(1985).The growing awareness of importance of natural colour especially food and cosmetic colourants has placed great demand on biological sources of natural colours.cyanobacteria and algae posses a wide range of colored components including carotenoids ,chlorophyll and phycobiliproteinHenriksonetal.,(1989). Principal phycobiliproteins are phycocyanin, allo-phycocyanin and phycoerythrin which are made up of dissimilar ‘a’ and ‘b’ polypeptide subunit Henriksonetal.,(1989).*Spirulina* contain a wide spectrum of nutrients that as include proteins, carbohydrates,andvitamin,minerals,carotene and super antioxidants apart from trace elements . protein content is as high as 60-70% of its dry weight Cifferi (1983) .*Spirulina* has a high concentration of vitamins and lipid in the amount of 4-7%is also present in it . The essential fatty acid , linolenic acid and also 13.6 % of carbohydrates are present Mahajan *etal*.,(1995) and Cohen (1997) .*Spirulina* is a planktonic photosynthetic filamentous cyanobacterium that forms massive population in trobical and subtrobical bodies of water which have high level of carbonate and bicarbonate and alkaline pH value of up to 11.*Spirulina* from chad lake in Africa and Texco lake in Mexico have been harvested as a source of food Vonshak (2002) .several cultivation methods like open ponds,Lee (1997).tubular photobioreactors Torzillo *et al*.,(1986).inclined glass panels have been tried Hu *etal*.,(1996). The cost and composition of cultivation media along with growth rate of the algae are the challenging factor for commercially viable production . Media have been tried for cultivation of spirulina such as zarrouk’s media , Zarrouk (1966),Rao’s medium ,Singh (2006),CFTIR media ,Venkataraman ,(1995),OFERR media ,Singh (2006), Revised media , Raoof*etal* (2006),Bangladesh medium ,Khatum *et al*.

,(1994). cultivation of *Spirulina* platensis in zarrouk media .Effect on growth behavior of *S.plantensis* in oil cakes enriched with adaptation of *S.platensis* in natural medium .

MATERIALS AND METHODS

Collection of sample

The culture of *Spirulina* platensis were obtained from national repository for Microalgae and cyanobacteria –Freshwater, Department of Microbiology, Maruthupandiyar college ,Thanjavur .

Preculturing

The starter inoculums and culture were maintained in the traditional Zarrouk medium .The present study emphasis was on the use of the oil cake filtrate medium for the cultivation of *Spirulinaplatensis* . The groundnut cake ,castor cake, coconut cake , in different concentrations. The preliminary work was done to analyze 10 g of oil cake soaked into 100ml of water then fermented water diluted in 1000 ml of distilled water . 0.5ml and 1ml of oil cake fermented water used in20 ml of medium .

Maintenance of culture

The experiment was performed in the closed chamber and the temperature was maintained at 30◦C .The *Spirulina platensis* maintained in zarrouks medium at ambient temperature and the culture was maintained using light energy sources under 12 hours dark and light photoperiod .

Aeration system

The nutrients and *Spirulina* cells are to be mixed properly by aeration system . The concentration of biomass was monitored daily and the proper aeration of *Spirulina* is required to fulfill its CO2 requirement and also aeration does not allow algae to settle down and form a layer at bottom .Aeration can be achieved by either mechanical stirring or air pump system. Aeration is needed to gain the sufficient nutrients as an energy sources for the growth of *Spirulinaplatensis* .the light temperature are the important factors in the growth *Spirulina platensis* .

Microscopic identification

The procured cultures were identified using the morphological characters (spiral shape) of blue –green algae (*Spirulina*) identified through the light microscope (Hernando Cortez 1519). The culture grows on different oil cakes fermented water medium for the protection of biomass such as groundnut cake, castorcake, coconut oil cake with and without zarrouk medium. The pH medium was measured by using a pH meter .*Spirulina* platensis grows at a pH range of 8.9 to9.5±0.2 as it maintained at the alkaline point . Then culture was allowed to grow for 30 days of biomass was harvested by filtration method. Initially, the paper weight was measured by electronics balance then 1ml of culture filtered by filter paper .The suspensions in the filter paper were dried at 750◦C for 4 to 6hours and dried filter paper containing *spirulina* biomass was measured. The concentration of biomass was calculated by dry weight and the difference between the premliminary and final weight were calculated. The weight of the filter paper and weight of the sample was keenly observed by the dry weight was calculated by the following formula process .

Dry weight =Weight of suspended particles in the filter paper –Weight of fresh filter paper and it was represented in g/l

Composition of zarrouk's medium

Nacl : 1.0gm/l
CaCl2.2H2O : 0.01gm/l
NaNO3 : 2.5gm/l
FeSO4.7H2O : 0.01gm/l
EDTA (Na) : 0.01gm/l
K2SO4 : 1.0gm/l
MgSO4.7H2O : 0.2gm/l
NaHCO3 : 16.8gm/l
K2HPO4 : 0.1gm/l
A5 micronutrient : 1ml (H3BO3,MnC12.4H2O,ZnSO4.4H2O Na2MoO4, CuSO4.5H2O)

Alternative to zarrouk 's medium
The medium was modified with different concentration of oilcake in fermented water

Composition of groundnut cake

Protein :20-30%
Carbohydrate :30-40%
Fiber :10-20%
Fat :5-10%
Moisture : 5-10%
Ash : 5-10%
Phosphorus : 1-2%
Potassium : 1-2%
Calcium :0.5-1%
Magnesium : 0.5-1%

Composition of castor cake

Protein : 21-48%
Carbohydrate:30-40%
Fiber:10-20%
Fat: 5-10%
Moisture:5-10%
Ash: 5-10%
Nitrogen:4-5%
Phosphorus :1.8-2.55
Calcium:0.5-1%
Magnesium: 0.5-1%
Potassium: 1-1.5%

Composition of coconut cake

Protein :15-25%
Carbohydrate : 40-50%
Fiber :10-20%
fat : 10-20%
moisture : 5-10%
ash : 5-10%
nitrogen :1.5-3 %

phosphorous : 1-2%
potassium : 1-2%
calcium :0.5-1 %
magnesium : 0.5-1%

Table 1. pH,Temperature and total biomass of *Spirulina* cultivation in medium and oil cake 0 day

Natural substrate	pH	temperature	Dw/l
Groundnut	8.1	30◦c	0.10
Castor	8. 1	30◦c	0.10
Coconut	8.1	30◦c	0.10
Zarrouk	8.7	28◦c	0.10

Table 2. 15th Day of pH, Temperature and total biomass of *Spirulina* cultivation in medium and oil cake

Natural substrates	pH	Temperature	Dw/l
Groundnut	8.5	30◦c	0.86
Castor	8.5	30◦c	0.82
Coconut	8.5	30◦c	0.81
Zarrouk	8.7	29◦c	0.83

Table 3. 30th day of pH,Temperature and total biomass of *Spirulina* cultivation in medium and oil cakes

Naturel substrate	pH	Temperature	Dw/l
Groundnut	**9.5**	**30◦c**	**1.92**
Castor	**8.9**	**30◦c**	**1.85**
Coconut	**9.2**	**30◦c**	**1.83**
Zarrouk	**9.6**	**30◦c**	**1.86**

Fig .1-Range of pH during cultivation of *Spirulina platensis* of in different oil cakes

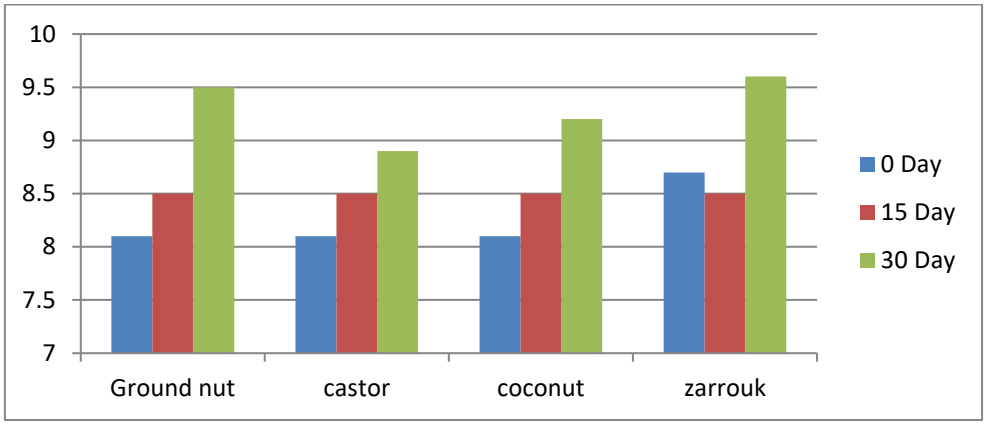


Fig .2 Range of Temperature during the different cultivation of *S.platensis*

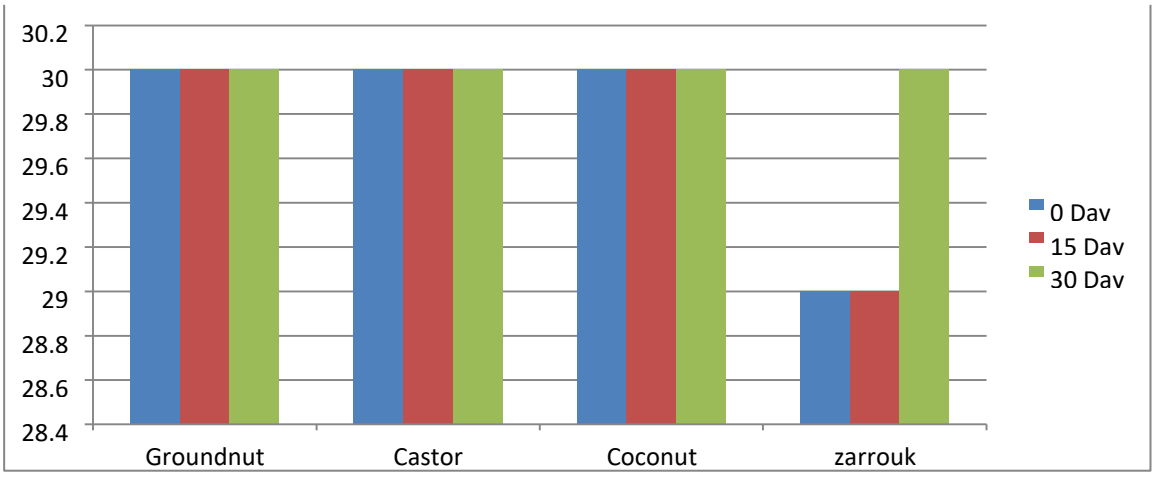
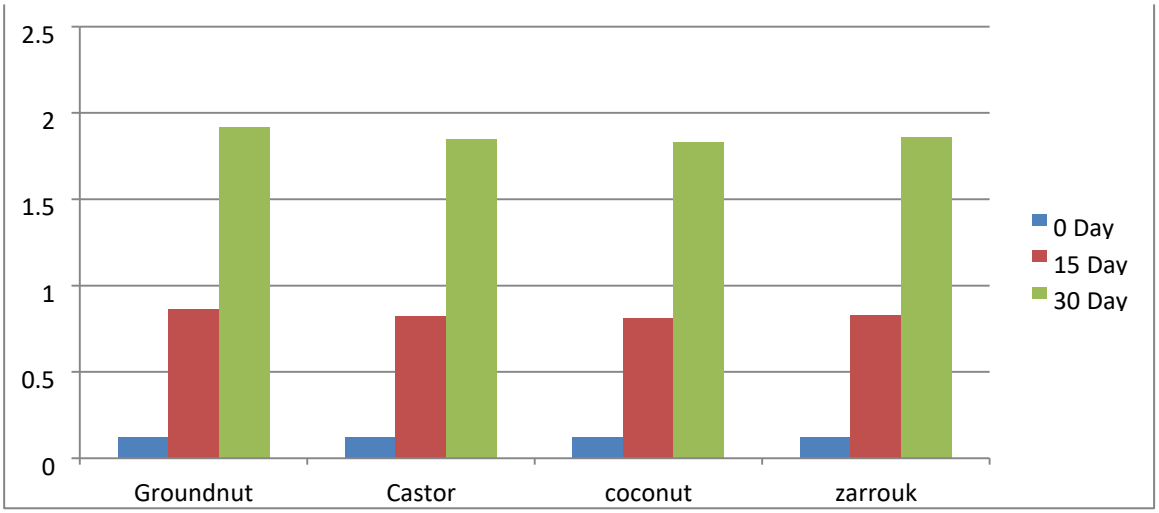


Fig .3-Total biomass concentration amount in(g/l) experiment in cultivation (value are average 30 days).



RESULT AND DISCUSSION

Spirulinaplatensis was successfully cultured in selective liquid medium *viz*.zarrouk medium ,groundnut oil cake ,castoroil cake ,coconut oil cake for 30 days.total biomass of the *S.platensis* (Table 3) were determined on daily basis on the above respective medium.Inzarrouk medium ,groundnut oil cake ,castor oil cake ,coconut oil cake,the pH becomes more basic as culture has become older .Appearance of culture has also shifted from light green to dark green in proportion to the increasing cell mass .While the cultivation of *Spirulina* in oil cake , both pH and appearance dose not changed as compared to cultivation in zarrouk medium . Microscopic &visual observation revealed that the culture was grown healthy and morphology of *S.platensis* filament also maintain its colour and shape as reported earlier.

The *S.platensis* in conical flask has its limitation to provide complete information related to growth ,development and production of value added chemicals . However it would give premliminary information for further demo or commercial level of cultivation . Environmental factors particularity irradiance lux and temperature are important evaluators of biomass production and their general characterization .In general the growth of S.*platensis* is maximum at 30-35◦c while high alkalinity is mandatory for growth of *Spirulina* .In the present investigation ,temperature and irradiance lux was found to be good for the growth earlier as mentioned .The growth behavior and total biomass yield of *S.platensis* culture in oil cake medium clearly indicate that environmental factor as mentioned in Fig.2&3 were supporting the growth .Result of *Spirulina* cultivation in oil cake indicated that oil cake composition is supportive to growth but survived in oil cake medium which indicated that gradul exposure to oil cake and further enrichment of oil cakes would favour the growth of Spirulina.

In the present study oil cake enriched with explored alone as well as in combination at different concentration for *Spirulina* cultivation. The *Spirulinaplatensis*cultured in fortified oil cakes medium was observed for dry weight and microscopic investigation for 30 days (Table 3) Total of three result *viz* 0th day,15th day , and 30th day for each form it is depicted that has some influence in *Spirulina* cultivation in oil cake .In the maximum of 1.92g/l dry weight on 30th was achieved in groundnut oil cake fortified which was comparatively high when compared only in zarrouk medium (1.86g/l). The dry weight of *S.platensis* in oil cake medium was good enough to scale it for commercialization but it would consider as simulation study .During cultivation ,of the pH of behavior of coiled filament of *Spirulina* is good indicator to study effect of light ,temperature ,cultivation vessels ,medium components .Daily microscopic observations were taken from each combination are representing filament behavior in natural and zarrouk medium at 15th day of cultivation and 30th day of cultivation respectively with low growth and dry biomass concentration from zarrouk medium .It was clearly predicated from both figures that initially coiled filament of *S.platensis* became straight as culture it was old .During the period of this study, having many cloudy and rainy days, sunlight also seems to play a significant role apart from medium combination .

CONCLUSION

The present study indicates that groundnut oil cake medium has a potential one among the other medium to grow *Spirulinaplatensis* .

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ANTICARCINOGENIC EFFECT OF LACTOBACILLUS HELVETICUS

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ABSTRACT

Cancer is an emerging public health problem that urges more serious attempts to combat the disease. This study aimed to investigate the protein from Lactobacillus helviticus that shows the anticarcinogenic activity. At 24 hours of growth, *L.helveticus* had the highest free radical scavenging activity, but this activity significantly decreased at 48 hours when compared to the other strains and the control. Throughout the growth period, *L. helveticus* strains contained compounds with anti-colon cancer activity. After 24 hours of growth, the extract with the highest activity (19.03–50.98% inhibition of growth) significantly decreased (5.4–9.94%) at the end of the growth period. When compared to the other strains, *Lactobacillus helveticus* produced compounds that inhibited the growth of the colon cancer HT-29 cell line by 50.98 percent in the culture supernatant after 48 hours. More importantly, T4056 normal primary colon cells were not significantly inhibited by these compounds. These findings, on the other hand, suggested that *L. helveticus* strains might release bioactive compounds with important properties that can serve multiple purposes.

Keywords —Lactobacillus helveticus, T4056 normal primary colon cells.

INTRODUCTION.

Lactobacillus helveticus is a probiotic with high proteolytic activity, producing bioactive peptides and exopolysaccharides. Bioactive compounds released by proteolytic cleavage of milk proteins during milk fermentation have a role beyond their nutritional importance. Due to their biodegradability and biocompatibility in human tissue, as well as their capacity to protect interactions in blood and tissues, the metabolites of lactic acid bacteria (LAB) and bifidobacteria (Bb) have recently received a lot of attention. Bacterial exopolysaccharides (EPS) have long been used with outside effects in medical and other industrial applications. In this regard, antioxidant, antitumor, and periodontal regeneration properties ofEPSs from LAB and Bb culture supernatants were identified and characterized. The ability of bacteria to make a wide range of polysaccharides is well-known. These polysaccharides can either be released as exopolysaccharides (EPSs)orremaintightlyboundto the cell surface as capsular polysaccharides (CPSs). During fermentation, many bacterial taxa, especially lactic acid bacteria (LAB) and bifidobacteria (Bb), produce a variety of carbohydrate polymers (Sanalibabaand Cakmak,2016).Accordingto Surayot et al., LAB is generally regarded as safe microorganisms (GRAS, which stands for generally recognized as safe). They are also capable of producing EPSs with a wide variety of structures withoutposinganyhealthrisks.2014).Gram-positive bacteria, which are frequently isolated from fermented natural products and are frequently utilized in industrial processes, arer eferred to as LAB. LA Band their metabolic items have been displayed to upgrade insusceptibility, gastro-digestive capability, protection from stoutness, cell reinforcement action, and blood glucose and cholesterol levels (Mathur et al., 2020; Wang and others, 2020). They may also have health benefits,suchastheabilitytofightcancer(Tukenmez et al., 2019), the ability to stimulate the immune system (Adebayo-Tayo et al., 2018).

Over90%oftheocean'sbiomassismadeupofmarine flora, which includes bacteria, actinobacteria, cyanobacteria, fungi, microalgae, seaweeds, mangroves, and other halophytes. They are systematically different, generally useful, organically dynamic, and syntheticallyone ofa kind contribution anincredible breadthfor revelationofnewanticancer medications. Due to its probiotic property, Lactobacillus is a well- studiedmarinemicrobe.Ananaerobicmicrobe with gram-positiveaerotolarity,lactobacillusDuringlactic fermentations, lactobacilli produce a variety of antibacterial compounds like organic acids, diacetyl, hydrogen peroxide, reuterin, and bacteriocin, also knownasbactericidalproteins(Holzapfeletal.,2001; Hirano et al., 2003). The majority of bacteriocins delivered by grampositive microorganisms are from lactic corrosive microbes (Ennahar et al., 2000; Garneau and other, 2002). Bacteriocins are biologically active proteins or complexes proteins with antimicrobial and antitumor properties. Due to the distinct receptors that are present in various bacterial species or types, bacteriocins have a highly specific membrane interaction. The antineoplasmic movementofbacteriocinisbasicallycreditedthrough the acceptance of modified cell passing or apoptosis. Therefore, the research's goal is to suggest a natural anticancer drug without any side effects from marine *Lactobacillus helveticus*.

MATERIALSANDMETHODS

Lactobacillusisolation:

Tests were sequentially weakened up to 10-5 with sanitized half seawater and plated with deMan- Rogosa-Sharpe (MRS) mode for lactobacilli. One milliliter ofthe seriallydiluted sediment samples was pipette into a sterile Petridish for plating. Afterthat, thoroughly mixed sterile media were poured into dishes in an aseptic manner and swirled. All measurements were carried out in duplicate after the plates had solidified in an inverted position at 28±2 degrees Celsius. The number of microbial colonies was counted after the incubation period. The counts are represented as a colony-forming unit (CFU) per gram of sediment. For further research, the isolated colonies were cultured in MRS agar slant and purified using the pureculture method. Thefollowingstandard morphology, physiological, and biochemical test was used to differentiate and characterize pure cultures (Bergey's manual, John Holt, 1994).

Identificationofisolates(*Lactobacillushelveticus*):

The isolated lactobacilli were identified using Gram staning, Motility, biochemical test and molecular characterization.

Isolatebacterialsequencingusing16SrRNA:

Applied Bio-Systems, Bangalore, India, provided the facility for the sequential generation of consensus sequence for the 16S rRNA gene from forward and reverse sequence data.

Lactobacillus culture extract in its purest form:

MRSbrothwasusedtocultureLactobacillus*helveticus*for24hoursat30°C.Thecell-free supernatantwas adjustedtopH 5.0with 1MNaOH afterthecells were harvested (8000xg,10 minat4°C), heat-treated(80°Cfor10min),andthebacteriocinwas precipitated with an 80 percent saturated ammonium sulfatesolution (Sambrooketal., 1989). The bacteriocin fraction was extracted,dissolvedin distilled water,anddialyzedovernightat 4°Cagainst distilled water.

DeterminationofProteolyticActivity:

Thereleaseoffreeaminogroupswasmeasuredusing the o-phthaldialdehyde (OPA) method to determine theextentofproteolyticactivityingrowthtimeat0,4, 8, 12, and 24 hours of fermentation (Church et al., 1983), and by following the previously described method (Elfahri et al., 2014). To quickly precipitate largeproteins,10mLofeachsamplewasdiluted with 10 mL of 1% (wt/vol) TCA. The mixture was centrifuged for 30 minutes at 4°C at 4,000 g, and the supernatant was vacuum-filtered with a 0.45- millimeter filter. After that, 150 mL of the collected suspensionwasmixedwith3mLofOPAreagent,and it was leftfor two minutes at room

temperature (20°C). The Spectrophotometer was used to measure each mixture's absorbance at 340 nm, and the proteolytic activity was calculated using the absorbance of OPA derivatives at 340 nm. At the beginning of growth time, the relative proteolytic activity of each sample was compared to that of control.

Cytotoxicityassayinvitro:

Twovarietiesofimmortalized cellswereinhibitedby theproducedantitumorextract;HT-29acelllinefrom the colon, and T4056 a cell line. This cytotoxicity assay was carried out with the help of 3-[4,5- dimethylthiazole-2-yl]-2,5 diphenyltetrazolium bromide (MTT) [Arullappan et al., 2015].

Determination of Radical Scavenging Activity:

Theabilityofextractedsamplestoscavengefree radicalswasassessedusingtheradical1,1-diphenyl-2-picrylhydrazyl(DPPH)(Elfahrietal.,2014).Ina nutshell,0.2mLofeachsolubleextractwasmixed with 800 L of 0.1 mM DPPH dissolved in 95 percent methanolinglasstesttubes.Afterbeingvigorously shaken,thesolutionsweresealedwith parafilm and left to incubate for thirty minutes in the dark at room temperature. Methanol wasusedforthebaseline correction,andthemethanolicDPPHservedasthe blank. UsingaUV-Visspectrophotometer(Biochrom Ltd.,Cambridge,UK),the reductionin absorbance was measured at517nmfollowingtheincubation period. Each experiment was conducted in triplicate. Using the following formula,the radical scavenging activity was calculated as the inhibition percentage: Radicalscavengingactivity(%)=[1−(absorbanceof sample/absorbance of blank)] × 100.

Maintenanceandthawingofcells:

The human primary colon cell line, T4056, and the human colonic epithelial carcinoma cell line, HT29, colorectal adenocarcinoma, was obtained from Applied Biological lab (Bangalore). Toproliferateand enacteachculture,1mLofHT-29orT4056(106/mL fixation) was immediately defrosted and quickly weakened in 20-mL aliquots of RPMI-1640 development medium containing 10% fetal cow-like serum (Invitrogen, Waltham, Mama), and the cell societieswerepelletedbycentrifugationat200×gfor 5 min at 20°C. After being pelleted, the cells were resuspended in 20 milliliters of complete growth mediumwithonepercentpenicillin-streptomycinand incubated for one week in 75 cm2 cell culture flasks. After this, the aggregated colon cancer and primary colon cells were dissociated by washing them with PBS and replacing the medium with 5 mL of trypsin/EDTA and incubating them for 5 minutes at 37°C. The complete growth medium was added to 15 mL to inactivate the trypsin solution. In a 50-mL falcon tube, the trypsinized cell suspension was removed by centrifugation (200 g for 5 minutes at 20°C), and the cells were resuspended in complete medium.Beforebeingcounted usinga hemocytometer (SigmaAldrich,St.Louis,MO),thecellswerestained with trypan blueina1 :1ratioforthreeminutes.Using a 400-magnification light microscope (Olympus CH- 2, model CHT, Olympus Optical Co. Ltd., Tokyo, Japan), cells were counted inthe four outer quadrants ofthe hemocytometer. Thediluted cells were divided into two parts: on ewasused for passingagingintoa new flask for continued propagation (1.0 $x10^6$/mL), and the other was used for an antiproliferative assay (1.0 $x10^3$ /100 L).

AssayforMTTProliferation:

TheMTT(CellTiter96AQueousOneSolutionCel ProliferationAssay)[3-(4,5-dimethylthiazol-2-yl)-5- (3 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H- tetrazolium] Assay was carried out in accordance with thedirectionsprovidedbythemanufacturer.Ina nutshell,cells(HT-29orT4056) were firstpassed throughandcountedusingahemocytometerto determine the appropriate seeding. The MTT assay was usedtomeasuretheinhibitionofcellproliferation after cells were plated at a concentration of 1.0x 10^3 cells per well. In a nutshell, the cells received 20 mL of the soluble extract sample (1.0 $x10^3$ in 80 mL of complete medium). The MT Sreagent was

added after the sample had been exposed to the cells for72hours, and it was incubated for an additional 4 hours in the same conditions. Formazan's absorbance was measured at495nm,and cellsthathadnotbeen stimulated served as a control. The results of each assay were presented as mean values standard error. Each assay was carried out intriplicate. The percentage ofnormalcoloncellsinhibitedby colon cancer cells was calculated (Kimetal.,2000)fromthe following ratio of treatment values to controls: Proliferativeinhibition (%) = (Treatment A495/Control A495) × 100,
whereA495=absorbanceat495nm.

StatisticalAnalysis:

Eachbacterialculturewasthesubjectofthreeseparate experiments. The obtained results were examined using a blocked split plot in a time design and two primary factors: time as a subplot and strains and replications as the main plot. As a block, the replications were used. The general linear model of SPSS (version 13.5) was utilized for the statistical analysis of the data. ANOVA was used to look for significant differences between treatments, and Fisher's least significant difference method was used tocomparetreatmentswithalevelofsignificanceofP 0.05.

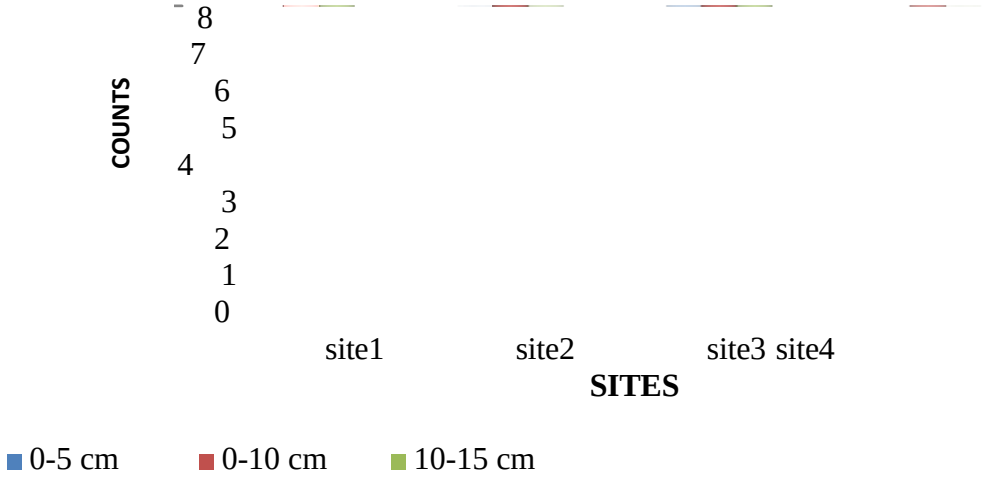
RESULTANDDISCUSSION

The study areas had a variety of marine lactobacilli counts, ranging from $5.0x10^2$ to $285x10^2$ CFU. g^{-1} at various depths and sediments. The counts were generally higherat sediment depthsof10-15cmthan at any other depth (0-5, 6-10).Lactobacilli counts were higher in subsurface sediments than in surface sediments in this study table1 and fig-1.

Table-.Lactobacillicountsindifferentdepthandsiteofmarinebiotopesof PattukkottaiCoast

Pattukkottai coastal area	Lactobacilli(cfu×102.g-1ofsediment)			
	0-5cm	0-10cm	10-15cm	Average
Site1	3	8	6	5.6^d
Site2	4	7	5	5.3^d
Site3	5	7	6	6.0^d
Site4	3	6	4	4.3^d

Fig-1.LactobacillicountsindifferentdepthandsiteofmarinebiotopesofPattukkottaiCoast.



Since,lactoseisavailablejustinendlesslynutrients,it is conceivable that these strains have developed from conditions related with warm blooded creatures, as was recommended for other lactose positive lactobacilli(Garvie,1984).Lactosemightbeavailable in the climateasa waste; resulting fromtheproduction of livestock and dairy factories' wastewater. Their DNA guanine plus cytosine (G+C) (fig-2) content ranges from 32 to 54%, indicating a wide range of genetic diversity. According to Kendall and Weiss (1986), this is about twice as big as what is typically considered to be a well-defined genus (Schleifer and Stackbrandt,1983). The resolve dprecipitate with80% saturation of ammonium sulfate contained the most antibacterial activity when the precipitation of bacteriocin was attempted at various ammonium saturation levels (20percent, 30percent,40percent, 50percent, 60percent, 70percent, 80percent,and90 percent). However, even when 90% saturation of ammonium sulfate was added, it continued to exhibit high activity. Bacteriocin was sanitized 1.57 crease and explicit action of the to some degree cleaned planningwas280 U/mgprotein,addressinganall-out recuperation of 28.07 (Table 2). Bacteriocins are proteinsorproteincomplexes withbiologicalactivity that have a bactericidal mode of action against typically closely related species. In the last two decades, numerous strains of organisms that produce bateriocin from various ecological niches have been isolated from various sources.

Table2.PartialpurificationofbacteriocinproducedbyLactobacillushelveticus

Purification Stages	Volume (ml)	Activity (Au100µl/)	[a]Total activity	Protein (µg/100 µl)	[b]Total Protein (mg)	Specific activity[c]	Purification factor[d]	Recovery (%)[e]
Culture supernat a nt (crude bacterioc in)	50	22	10100	63	6 3	179	1.08	100
(NH4)2S O4 precipitat ion 40% -	20	25	5000	24	2 5	240	1.38	41.3
(NH4)2S O4 precipitat ion 80%-	10	28	5600	13	1 3	280	1.57	28.07

It has been demonstrated that cell wall proteases are the primary cause of the high proteolytic activityof the *L. helveticus* strain chosen for our research (Virtanen etal.,2007 ;Nielsenandother,2009;Elfahriandother, 2014) and metabolic processes that result in therelease of a variety of peptides. The primary metabolite productionofor ganicacids, asmeasured by adecrease inpH, was used to evaluate the culture's performance (Table3). Viable cell counts wereusedtomeasurethe growth of the selected strains (Figure 3), which significantly (P 0.05) increased after 24 hours of growth culture compare than 48 hours. Indicating strain dependence and acid resistance (Elfahri et al., 2014). A starter culture needs highly developed proteolytic and glycolytic systems that are able to provide essential compounds for the growth of the culture (AA, glucose; Kunji and co., 1996). While glucose is primarily needed tomeetenergyneeds,AA isneededtosupportasufficientgrowthrate.Acell wall-bound protease isthestartingpointforacomplex

proteolytic apparatus that yields these AA (Elfahri et al., 2014). Oligopeptides that have been liberated by this enzyme are transported across the cell wall and into the cytoplasm, where they are further degraded into simpler peptides and, as a result, AA. Even oligopeptides appear to have a very potent physiologicalactivity, bu titisunclear which enzymes are responsible for the release of bioactive peptides (Asharand Chand, 2004). According to Donkoretal., apparent bioactivity in water extracts was probably time-dependent,indicatingthatsomeofthesepeptides may be further degraded, losing or gaining potency. 2007). The ability of OPA to react with primary amines to form fluorescent moieties was used to first assess the proteolytic activity of a few strains of L. helveticus in different duration. All strains outperformed the control in terms of proteolytic activity, as shown in Table 3.

Table 3. During the growth of *Lactobacillus helveticus* strains in sterile reconstituted modified MRS medium for up to48hoursat37°C,theo-hthaldialdehyde(OPA) method measured the declinein Ph and the extent of proteolysis.

Incubationtime(h)	Straincode	pH	OPAat340nm
0	Control	6.61 ± 0.01^{A}	0.31 ± 0.01^{B}
	Lh	6.48 ± 0.06^{B}	0.33 ± 0.02^{B}
8	Control	6.61 ± 0.01^{A}	0.33 ± 0.01^{E}
	Lh	6.29 ± 0.03^{B}	0.37 ± 0.01^{D}
12	Control	6.58 ± 0.01^{A}	0.34 ± 0.01^{D}
	Lh	4.91 ± 0.26^{B}	0.83 ± 0.02^{B}
24	Control	6.54 ± 0.01^{A}	0.35 ± 0.03^{C}
	Lh	6.85 ± 0.03^{C}	0.90 ± 0.01^{B}
48	Control	6.53 ± 0.07^{A}	0.35 ± 0.02^{C}
	Lh	6.46 ± 0.1^{B}	1.52 ± 0.16^{BA}

A–C Meansinthesamecolumnatparticularincubation time with different uppercase letters aresignificantlydifferent($P<0.05$).Valuesaremean of 3 replicates (mean ± SE).

By measuring the decrease in the concentration of DPPH radicals when crude peptide extracts encountered radical scavengers, the antioxidant activity of the extracts was evaluated. From 0 to 48 hours, the samples' free radical scavenging activity significantly changed (P 0.05) in comparison to the control. This change varied between specific strains of L.helveticus. This may be because antioxidant capacityislikelyrelatedtostrainselectionandproteolytic enzyme specificity rather than continuing proteinhydrolysisorbacterialgrowth(Virtanenetal., 2007).In the present investigation, antioxidantactivity peakedat12hours,butsignificantlydecreased(P 0.05) after 48 hours of fermentation (table-4). Antioxidant activity was highest in L.helveticus (0.526%at0h to7.67%at24h)(P0.05).At48 h,*L. helveticus* showed similar trends (7.67, 5.97 and 4.59 percent, respectively) table-4. The DPPH radical scavenging activity might also be attributed to fermentation progresses (37°C, final pH of 3.7; Nishino et al., 2000).

Table-4Duringthegrowthof*Lactobacillushelveticus* strainsinsterilereconstituted modifiedMRSmedium for up to 48 hours at 37°C the DPPH assay.

Lactobacillus helveticus	Averageoptical density230nm	Concentration (µg/µl)
Blank	0.143	0
12 hrs	0.526	4.59± 0.137
24 hrs	0.371	7.67± 0.114
48 hrs	0.354	5.97± 0.111

DNA damage and mutation, which can increase the incidence of cancer, can result from an imbalance betweenthepresenceofantioxidantsandthe formation of free radicals. As a result, cancer prevention and treatment may benefit from antioxidants; By minimizing reactions that influence cell proliferation induction not only in vitro, they may reduce the incidence of cancer (Kim et al., 2000), in vivo in rats, and in clinical trials on humans (Tsudaet al.,2002;Kozuetal.,2009).The cell viability (MTT) assay was used to evaluate in vitro the inhibition of both celllines(HT-29andT4056) after treatment with crude peptide extract. The current study evaluated the inhibition effect of crude peptide extracts during fermentation on the proliferation of colon cancer cell (HT-29) and healthy colon cell line (T4056).The effect of incubation period the strains of *L. helveticus* on the proliferation of the HT-29 colon cancercelllineisdepictedintable-5.Whencompared to the control, all samples displayed antiproliferative effects at varying levels. The sample taken after 12 hours of incubation showed the greatest (P 0.05) decrease in HT-29 proliferation. When the 24 h after incubation sample was used, the effect was significantly reduced. After 12 hours of incubation with *L. helveticus*, the percentage of proliferative inhibition increased significantly (P 0.05) and was higher than that of other strains. The normal T4056 cellsexhibitednosignificantinhibitionofanysoluble extract. The increased concentration of a potential bioactive compound in the medium in which certain cell death mechanisms, such as apoptosis (programmed cell death), were activated could have contributed to the HT-29 cell line's decrease in proliferation. Colon cancer cell lines (T 4056 and HT29) were found to be dose-dependently cytotoxic when helveticin, a peptide derived from *L. helveticus* protein, was used at various concentrations (50 and 100, g/mL) (Mader et al., 2006).

Table-5AnticanceractivityofmarineL.helveticus Wecan concludethat*Lactobacillus helveticus*strains haveastatistically significant effect on thereleaseof great potential antioxidant and anti-colon cancer compounds during different culture time.

Lactobacilushelveticus	Conc.(µg/ml)	HT-29	T4056
	Growthinhibition(%)		
0	50	-	-
	100	-	-
12	50	34	64
	100	82	93
24	50	83	68
	100	96	79
48	50	66	14
	100	89	41
Mito-C	-	-	-
Adriamycin	-	-	-

CONCLUSION

In this study, four proteolytic strains of L. helveticus were used aspotential function aldairystarter cultures that could extract numerous bioactive compounds from modified MRS medium. Under acidic conditions, strains of Lactobacillus helveticus thrived in modified MRS medium. Medium appears to have released compounds that are anti-oxidative and anti- colon cancer as a result of their growth. However, fermentation time, temperature, pH, and the concentration of released bioactive compounds all appeared to have an impact on the level of bioactivities, which appeared to be strain-specific.

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ANTI-BACTERIAL ACTIVITY AGAINST THULASI EXTRACT (*OCIMUM SANCTUM*)

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ABSTRACT

Leaves of Ocimum tenuiflorum leaves contains alkaloids like morphine, boldine, tannins, saponin, terpenoid, glycosides, Phlobatannins and steroid. Methanolic extract of ocimum teniflorum posses antimicrobial potential against both gram positive and gram negative bacteria. It is therefore confirmed as a useful antimicrobial agent. The present study provides evidence that solvent extract of ocimum tenuiflorum contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. Ocimum tenuiflorum extracts, the methanol extract was found to be most active against all of the bacterial species tested when compared to aqueous extract. Furthermore, extracts prepared from root were shown to have better efficacy than leaf parts demonstrate that the secondary metabolites and antimicrobial agents are present in root in better amount than leaves. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic protypes. The results confirm the validity of the use of Ocimum sanctum plant as medicine in ancient medicinal traditions and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens.

Key Words: Ocimum tenuiflorum; Anti-Microbial activity; Gram +Ve Bacteria, Gram-Ve Bacteria, Pathogens

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). The use of plants and plant products as medicines could be traced as far back as the beginningof human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in “Rigveda”, which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi and Mehrotra, 2002).

Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Akerele, 1993). Our approach involved to explore the antibacterial activity of four medicinal plants and study their antimicrobial constituents. The drugs which are already in use to treat infectious diseases is of concern because, drug safety remains an enormous global issue. It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106,000 died in a single year in the USA.

Ocimum sanctum is a grassy annual plant originated from Iran, Afghanistan and India. Some of the phytochemicals of medicinal importance present in Ocimum sanctum have already been identified. Some of these phytochemicals have been shown to possess useful biological

activities belonging mainly to phenolic, flavonoid, and carotenoid compounds. The ability of this plant to be used in traditional medicine in the treatment of headches, cough, diarrhea, constipation, warts, kidney malfunctions, nasal polyps and ulcers has also been reported . Further, its action as insecticide, nematicide, fungicide and antimicrobial compound also has been reported . As such, extract of these chemicals from Ocimum sanctum plant possess useful pharmacological applications. However, reports on the antibacterial activity of Ocimum sanctum extracts against food borne pathogens are limited.

The aim of present study was to investigate the antibacterial activity of different extracts of Ocimum sanctum leaves against above four food borne microbial pathogens. It is suggestible that like any other field of medicine, dentistry should also consider restraining the use of antibiotics as inappropriate and inadvertent administration can result in destruction of physiological bacteria, allergic manifestations, and resistant bacterial strains.The principal advantages of using plant derived products over allopathic drugs are- low risk of side-effects and costeffectiveness, making them much more safer and convenient than the artificially synthesized substitutes.

Tulsi has been India's pillar of the Ayurvedic holistic health care system. Since time immemorial, various plant parts have been used extensively in treating several systemic diseases like upper respiratory infections, bronchitis, skin diseases, malaria, etc. Several investigators have tested the antimicrobial properties of Tulsi extracts and oil against various bacterial and fungal pathogens.

The extract and essential oil of Ocimum sanctum possess insecticidal and bactericidal activity. In recent years, Tulsi has Arti Jain et al / Int. J. Res. Ayurveda Pharm. 13 (4), 2022 173 been employed in numerous forms in Indian medicines like the aqueous extract of leaves (fresh and dried powder) or oil extract. The use of medicinal plants in traditional medicine has been described in literature dating back several 1000 years (Chang et al., 2016). Books on Ayurvedic medicine, written in the Vedic period (3500–1600 B.C.) describe practices, including the use of medicinal plants, that formed the basis of all other medical sciences developed on the Indian subcontinent(Pattanayak et al., 2010).

In modern complementary and alternative medical practice, plants are the primary source of therapeutics and each part of the plant, including the seeds, root, stem, leaves, and fruit, potentially contains bioactive components (Jiang et al., 2014, 2015; Mandave et al., 2014; Sun et al., 2014). The main bioactive components in medicinal plants are considered to be combinations of secondary metabolites (Singh et al., 2010; Wu et al., 2016). There are many advantages and benefits associated with the use of medicinal plants, the main ones being their cost-effectiveness and global availability. The plant extracts and phytochemicals, with known antimicrobial properties, could be a potential alternative to antibiotics in managing common infections.

The secondary metabolites of plants were found to be a source of various phytochemicals that could be directly used as intermediates for producing new drugs. One of the possible strategies is the rational localization of bioactive phytochemicals with antibacterial activity. Tulsi extract is often used therapeutically to treat many diseases like fever, headache, malaria, heart diseases, and inflammation.

The antimicrobial properties of Aqueous extract of Tulsi leaves and essential oil of Tulsi seed have been utilized in ‘Food Preservation’ to inhibit the bacterial and fungal infestation. In the past few years, it has become difficult to manage skin and soft tissue infections (SSTIs) due to the increasing prevalence of multidrug-resistant pathogens. To avoid the spread of multidrugresistant pathogens in clinical settings, it is essential to identify SSTIs that need antibiotic treatment. A recent European survey has reported that a large proportion of physicians use systemic antibiotics only when it is imperative to treat conditions like MRSA colonized ulcers or broken skin surfaces.

Plant extracts has been used to treat for microbial disease from ancient time in traditional medical systems. Ability of using most of the medicinal plants for the treatments for various diseases may lie in the antioxidant and antimicrobial effect of phytochemicals. Antimicrobial activities of some phytochemicals have been investigated and the possibility of using them in the development of new antimicrobial drugs also been documented. Due to the development of resistance in pathogenic microorganisms to antibiotics used in modern medical science, there is a growing attention towards plant extracts as a source of new antimicrobial drug discoveries. As such investigations on the composition, activity, as well as validation of the use of extracts obtained from medicinal plant is important.

Medicinal properties Heart disease can be treated with tulsi. 1)Tulsi reduces Blood Pressure. 2) Diabetics benefit from tulsi. Total cholesterol levels aredecreased by tulsi. 3)Tulsi lowers blood glucose levels and contains antioxidanteffects. 4)It had occasionally been used as an antispasmodic for cough. 5)It helps with cramps in the stomach. 6)Basil has digestive, carminative, galactagogue, antispasmodic, and appetiser properties. Vernacular names of Tulsi. Trittavu Malayalam: Marathi Tulsi Tamil, Telgu Tulasi, , and Holy Basil There are 4 varieties. Rama tulsi, (Ocimum sanctum). Krishna tulsi (Ocimum tenuiflorum). Amiti Tulsi (Ocimum tenuiflorum). Vana (Ocimum iratissum). Vietnamese basil. Lemon basil . American Basil. Blue African basil. Genovese basil from Italy. Basil lettuce. Basil with green frills . Cardinal Basil, age 15 Summertime basil. Greek basil. Spicy globe basil. Mediterranean basil (B) Asia, Europe, America, and Africa all have sweet basil. .

Tulsi used as traditional Indian ayurvedic medicine One of the characteristics that make the tulsi plant sucheffectivemedical herb is its capacity to decrease stress, according to organic india, an organisation devoted to organic agriculture and sustainable development. Tulsi is rich in antioxidants and essential oils that are incredibly good at lessening the damaging effects of stress on the body. Though traditionally utilised by Hindus or Indians, tulsi has a variety of restorative benefits that are increasingly being recognised by various cultures. A tulsi plant's ability to function as an adaptogen. It helps the body's various functions run in harmony and is very effective in reducing stress. Tulsi extracts have been employed in the traditional Indian ayurvedic medical system. Additionally, the unani system of medicine makes use of it. The tulsi is used in Ayurvedic treatments for cataracts, malaria, common colds, headaches, stomach problems, inflammation, infections, and heart disease. The tulsi enhances the neurological system by acting on it. It makes the heart stronger. It aids with digestion and serves as an appetiser. It helps to secrete digestive enzymes more easily and reduces flatulence. possessing detoxifying qualities Blood is cleansed of any potential poisons by the tulsi. Tulsi might protect against radiation poisoning. Tulsi may possibly have cancer-causing qualities, according to certain reports. The notion that daily use of tulsi leaves will provide cancer protection has gained traction. In addition to its religious significance, this herb has great medicinal value and is a staple in ayurvedic medicine.

MATERIALS AND METHODS.

Sample collection Flowering branches of plants were collected and brought to the laboratory. Different parts (young and mature leaves, inflorescence and stem) were separated and cleaned properly and washed under running water to remove dust and other debris. The materials were air dried at room temperature. The stems were sliced before allowed to dry. After removal of surface water, the materials were wrapped with brown paper and allowed sun drying for complete dryness (less than 1-2 % moisture content). The materials were grounded to fine powder using mortar and pestle and then in electric grinder. The fine powder was kept in air tight bottles for further analysis.

Collection Of Soil Sample

Soil samples collected from a flowering plant area Kumbakonam, Thanjavur Dt., Tamil Nadu.

Isolation And Identification Of Soil From Bacteria Soil bacteria form the soil food web that supports soil health and other living organisms within the soil. This procedure will facilitate the guidelines for isolation and estimation of soil fungi and bacteria populations which after calculation, can aid in knowing the population of these organisms in a landscape. Using the pour plate method, a volume of 1ml of the diluted soil sample is transferred into a sterile Petri dish and then molten agar is poured into the plate and mixed. The inoculated Petri dishes are then incubated for 7 days at 35.5°C and 23°C for bacteria and fungi, respectively. This method yields isolates that form colonies throughout the agar. The colonies are then counted and the colony forming units (CFUs) analysed to give the bacteria: fungi ratios.

Preparation of Inoculum:-

Stock cultures were of gram positive and gram negative bacteria maintained at 4°C on slants of nutrient agar. Active cultures for experiments were prepared by transferring a loopful bacterial cells from the stock cultures to Erlenmeyer flask of nutrient broth followed by incubation with agitation at 37°C for 24 hrs. The bacterial cultures of gram positive and gram negative bacteria were maintained on nutrient agar medium. These microorganisms were allowed to grow at 35°C - 37°C temperature. A fresh inoculum of test microorganism in saline solution was cultured on freshly grown agar slant before every antibacterial assay by adjusting the concentration of microorganism in the medium using spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm. Each organism was recovered for testing by sub culturing on fresh media. A loopful inoculum of each bacterium was suspended in 5ml of nutrient broth and incubated overnight at 37 °C. These overnight cultures were used for the experiment.

GRAM STAINING REAGENTS:

Crystal Violet, the primary stain

- Iodine, the mordant
- A decolorizer made of acetone and alcohol (95%)
- Safranin, the counterstain PROCEDURE OF GRAM STAINING
- Take a clean, grease free slide.
- Prepare the smear of suspension on the clean slide with a loopful of sample.
- Air dry and heat fix • Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
- Flood the gram's iodine for 1 minute and wash with water.
- Then ,wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
- Add safranin for about 1 minute and wash with water.
- Air dry, Blot dry and Observe under Microscope.

Antibacterial Activity Against Soil Microbes

Antibacterial tests is performed using the Kirby-Bauer disc diffusion method. The leave lates are tested for their inhibition zones on colonies of isolated soil microbes bacteria.

Kirby Bauer Disc Diffusion Method

Materials Required

Mueller- Hinton agar, Antibiotic discs, Cotton swabs, Petri dishes, 0.5 McFarland Turbidity standard, Inoculum, Forceps, Metric ruler or caliper.

Procedure

- Sterilize the area with disinfectant and open burner before performing the test.

• A sterile cotton swab is dipped into the inoculum and remove excess medium by pressing the swab onto the wall of the tube.
• Swab the surface area of the plate completely by rotating the plate. This technique is called lawn culture or carpet culture.
• Allow the plates to dry for 5 minutes so that the medium absorbs the inoculum properly.
• Firstly sterilize the forceps with alcohol before picking up antibiotic discs.
• Discs should be placed at a distance of 24mm.
• Lightly touch each disc with forceps to ensure that it is in good contact to avoid misplacement.
• Incubate the plate upside down for 24 hours at 37ºC.
• After 24 hours of incubation, use a metric ruler to measure the zone of inhibition and include the diameter of the disc in the measurement.
• Compare the result with CLSI guidelines to report the result.
• The results are reported as Susceptible (S), Intermediate (I), or Resistant ®

MOTILITY TEST MATERIALS REQUIRED Cavity Glass slides Paraffin wax, Inoculating loop, Coverslip, Microscope, Bunsen burner, Culture Sample HANGING DROP METHOD PREPARATION

• Take a clean glass slide and apply a paraffin ring, adhesive-tape ring to make circular concavity. (This step is not needed if a glass slide with depression is available).
• Hold a clean coverslip by its edges and carefully dab vaseline on its corners using a toothpick.
• Place a loopful of the fresh broth culture to be examined in the center of the prepared coverslip. Use a light inoculum (not visibly turbid).
• Turn the prepared glass slide or concavity slide upside down (concavity down) over the drop on the coverslip so that the vaseline seals the coverslip to the slide around the concavity.
• Turn the slide over so the coverslip is on top and allow organisms to “settle” for a minute. The drop can be observed hanging from the coverslip over the concavity.
• Observe the slide under the microscope.

INDOLE TEST MATERIALS REQUIRED

• 15 ml test tubes, bacterial culture, peptone water, Kovac’s reagent

PROCEDURE

• The peptone water tubes were inoculated with bacterial broth culture using sterile needle technique. • An uninoculated tube was kept as control.
• Both tubes were incubated at 37°C for 24-48 hours.
• After proper incubation, 1 ml of Kovac’s reagent was added to both tubes including the control.
• The tubes were shaken gently after an interval for 10 – 15 minutes. METHYL RED TEST MATERIALS REQUIRED MR broth, 24 hours broth cultures, Methyl red indicator, inoculating loop PROCEDURE

• Using sterile technique experimental organisms were inoculated into appropriately labeled tubes containing MR broth by means of loop inoculation.
• Uninoculated tube was kept as control • Both tubes were incubated at 37°C for 24-48 hours.
• After proper incubation 5 drops of MR indicator was added to both tubes including control.
• It was mixed well and colour was observed.

VOGES PROSKAUER TEST MATERIALS REQUIRED

MR broth, 24 hours broth cultures, Barrett’s reagent A & B, inoculating loop

PROCEDURE

• Using sterile technique, the experimental organism was inoculated into VP broth by means of loop inoculation.
• One tube is kept uninoculated as control.
• The tube will be incubated at 37°C for 24-48 hours.
• After proper incubation, about 3 ml of Barrett's reagent A & 1 ml of Barrett's reagent B was added into both tubes including control.
• The tubes were shaken gently for 30 seconds with the caps off to expose the media to oxygen.
• The reaction was allowed to complete in 15 – 30 minutes and tubes were observed.

CITRATE UTILIZATION TEST MATERIALS REQUIRED

Bacterial broth, Simmons Citrate Agar Slants, Inoculation Loop PROCEDURE

• Using sterile technique Simmons citrate agar slant was inoculated with the test organism by means of a stab and streak inoculation.
• An uninoculated tube was kept as control.
• Both tubes were incubated at 37°C for 24 – 48 hours & was observed

CARBOHYDRATES FERMENTATION TEST MATERIALS REQUIRED

8 ml Test Tube, Durham's Tube, Phenol Red Indicator, Sugar (Glucose, Lactose, Sucrose)
PROCEDURE

• Using sterile technique, culture was inoculated into its appropriately labeled medium by means of loop inoculation.
• Care was taken during this step not to shake the fermentation tube.
• 1 tube of each fermentation broth was kept uninoculated as a comparative control.
• All the tubes were incubated at 37°C for 24 hours and the reaction was observed. Observation All carbohydrate broth cultures were observed for colour and presence or absence of gas bubble by comparing with the uninoculated tube (control).

OXIDASE TEST MATERIALS REQUIRED

Small piece of filter paper in 1% Kovács oxidase reagent and bacterial culture
PROCEDURE FILTER PAPER TEST METHOD

• Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry.
• Use a loop and pick a well-isolated colony from a fresh (18- to 24-hour culture) bacterial plate and rub onto treated filter paper.
• Observe for color changes.
• Microorganisms are oxidase positive when the color changes to dark purple within 5 to 10 seconds. Microorganisms are delayed oxidase positive when the color changes to purple within 60 to 90 seconds. Microorganisms are oxidase negative if the color does not change or it takes longer than 2minutes.

CATALASE TEST MATERIALS REQUIRED

24 hours old bacterial culture, glass slide, petridish, 3% H_2O_2, applicator sticks
PROCEDURE

• Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
• Place a drop of 3% H_2O_2 on to the slide and mix.
• A positive result is the rapid evolution of oxygen (within 5-10 s) as evidenced by bubbling.
• A negative result is no bubbles or only a few scattered bubbles.
• Dispose of your slide in the biohazard glass disposal container.

RESULT AND DISSCUSION.

Ethanolic extract of Tulsi was prepared by cold extraction method. Extract was diluted with an inert solvent, dimethyl formamide, to obtain five different concentrations (0.5%, 1%, 2%, 5%, and 10%). Doxycycline was used as a positive control and dimethyl formamide, as a

negative control. The extract and controls were subjected to the microbiological investigation against Aggregatibacter actinomycetemcomitans, Prevotella intermedia, and Porphyromonas gingivalis. Agar well diffusion method was employed to determine the concentration at which Tulsi gave an inhibition zone, similar to doxycycline. Data were analyzed using oneway analysis of variance and Tukey post-hoc test was used for inter- and intra-group comparisons.At 5% and 10% concentrations, Tulsi extracts demonstrated antimicrobial activity against A. actinomycetemcomitans, similar to doxycycline with similar inhibition zones ($P > 0.05$). P. gingivalis and P. intermedia, however, exhibited resistance to Tulsi extract that showed significantly smaller inhibition zones ($P < 0.05$)

Table 1: Isolation of bacteria from soil sample

Dilution factors	Number of colonies (CFU/ml)
10^{-4}	TNTC
10^{-5}	TNTC
10^{-6}	TNTC

TNTC – Too Numerous To Count

Plate 1: Isolation of bacteria from soil sample

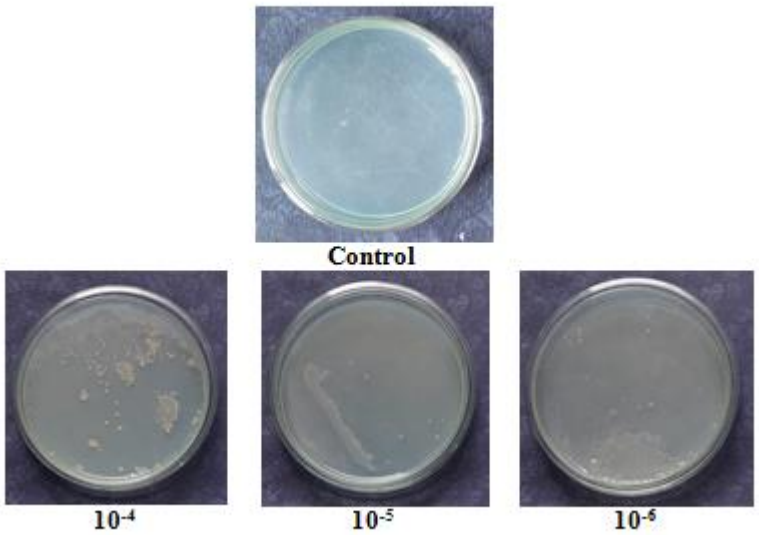

Table 2: Morphological characteristics of isolated bacteria

Name of the strains	Growth	Shape	Surface	Margin	Color	Elevation	Consistency	Opacity
N 1	Slow	Punctiform	Smooth	Wavy	White	Flat	Buttery	Opaque
N 2	Slow	Rod	Smooth	Entire	Pink/buff	Convex	Facultative anaerobic	Translucent
N 3	Rapid	Punctiform	Smooth	Even	White	Convex	Viscous	Transparent
N 4	Slow	Irregular	Smooth	Even	White	Convex	Adhesive	Translucent
N 5	Slow	Circular	Smooth shiny	Entire	Pale yellow	Pulvinate	Buttery	Opaque

Table 3: Biochemical characteristics of isolated bacterial strains

Name of the biochemical test	Code of the bacteria				
	N 1	N 2	N 3	N 4	N 5
Gram Staining	-	-	-	+	+
Indole broth	+	-	-	-	-
Methyl Red reaction	-	-	-	+	-
Voges-Proskaure reaction	+	+	+	-	-
Citrate Utilization	-	+	-	+	+
Motility	+	+	+	-	
Catalase	-	+	+	-	+
Lactose	-	+	-	-	
Oxidase	+	-	+	-	+
Triple Sugar Iron Agar	-	Acid/Acid Gas	-	+	A/AH_2S
Name of the bacteria	***Escherichia coli***	***Enterobacter aerogenes***	***Staphylococcus aureus***	***Klebsiella pneumoniae***	*Bacillus subtilis*

A/A – Acid butt Acid slant, H_2S – Hydrogen sulphide production

Plate 3: Antibacterial activity of *Ocimum sanctum* extract against isolated bacteria

Name of the bacteria	Zone of inhibition (mm)			
	Different concentration of *Ocimum sanctum* (µg/ml)			
	20	40	60	80
Escherichia coli	-	05.05±0.01	07.05±0.25	09.50±0.03
Staphylococcus aureus	12.05±0.06	14.50±0.32	14.00±0.12	17.05±0.00
Enterobacter aerogenes	09.25±0.18	11.05±0.17	15.00±0.07	20.05±0.11
Klebsiella pneumoniae	-	-	06.50±0.34	10.50±0.14
Bacillus subtilis	-	-	-	05.00±0.07

The values are expressed in terms of (Mean ± Standard deviation)

SUMMARY & CONCLUSION

Leaves of Ocimum tenuiflorum were collected and extracted in methanol, acetone and water solvent and evaluated for its phyto constitutes present in them. These leaves contains alkaloids like morphine, boldine, tannins, saponin, terpenoid, glycosides, Phlobatannins and steroid. Methanolic extract of ocimum teniflorum posses antimicrobial potential against both gram positive and gram negative bacteria. It is therefore confirmed as a useful antimicrobial agent. The present study provides evidence that solvent extract of ocimum tenuiflorum contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. Among all the extracts, the methanol extract was found to be most active against all of the bacterial species tested when compared to

aqueous extract. Furthermore, extracts prepared from root were shown to have better efficacy than leaf parts demonstrate that the secondary metabolites and antimicrobial agents are present in root in better amount than leaves. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic protypes. These plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological Investigations are necessary [31; 32]. Concluding Remark: The results confirm the validity of the use of Ocimum sanctum plant as medicine in ancient medicinal traditions and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It is quit safer to use as an herbal medicine as compare to chemically synthesized drug. Results of present study provided information about percentage yield, antimicrobial and antifungal activity measurement and minimum inhibitory concentration in both aqueous and ethanolic extracts. Based on elucidation of this study on antimicrobial/ antifungal activities of ginger, neem and green tea it can be inferred that these spices can be used as effective antimicrobial and antifungal force against Xanthomonas campestris and Alternaria alternata. Amalgamation of their extracts can impart additional or collaborative inhibitory outcomes making them supplementary valuable as antimicrobial/antifungal agents. Although conventionally, spices herbs and plants are used for food preservation and as disinfectant, it is essential to set up their antimicrobial/antifungal qualities by methodizing their concentrations in amalgam extracts so that most advantageous inhibitory effect may be acquired. Advance researches are obligatory to explore interaction and microbial growth inhibition mechanisms f divergent phytochemicals from different plants. The awareness on effectiveness of pooled extracts can be expanded from food implementations to pharmacological and chemical field.

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FORMULATION AND SENSORY EVALUATION OF BARLEY BITES INCORPORATED WITH CARROT

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ABSTRACT

This study investigates the formulation and sensory evaluation of functional snack bites using *Hordeum vulgare* L (barley) and carrot (Daucus carota L). The objective was to develop a nutrient-rich snack and assess its sensory acceptability. (*Hordeum vulgare* L) Barley is known for its high fiber and beta-glucan content, promoting cardiovascular health and its nutritional qualities have anti-oxidant, anti-carcinogenic, anti-inflammatory, and cardio and neuro protective benefits, helping to avoid a variety of metabolic illnesses, while(Daucus carota L) carrots contribute beta-carotene and antioxidants. barley bites incorporated with carrot was prepared in three different variations such as variation 1 contained 5%, variation 2 contained 10% and variation 3 contained 15% of the carrot respectively. A standard bites was also prepared. Among the variations, the variation 2 (V2) of the bites was highly accepted when subjected for sensory evaluation. Sensory analysis indicated high consumer acceptability. The study highlights the potential of barley and carrot as valuable ingredients in functional food development.

Keywords: *Hordeum vulgare* L , Carrot, Functional Snack, Nutrient Composition, Sensory Acceptability.

INTRODUCTION

One of the earliest cereals to be domesticated is barley (*Hordeum vulgare* L), which dates back to about 10,000BC. It is one of the first cereals to be domesticated and is widely grown for human use, as feed for cattle, and for brewing, malting, and pearling (Ana Badea *et al* 2021).Barley (*Hordeum vulgare* L.) is widely recognized for its health benefits due to its high beta-glucan and fiber content Since there are essential biochemical components in barley, it is regarded as a significant food item (beta-glucan, starch, amylase and protein). Additionally, it is regarded as a food with numerous health benefits for humans due to its nutritional makeup and impacts on a variety of systems (reduces and prevents cardiovascular diseases and has a positive effect on reducing appetite and obesity, etc. (Jasmine *et al* 2022). Vegetables and fruits are an essential part of our nutrition. They supply a variety of micronutrients, including minerals, vitamins, and antioxidant compounds like carotenoids and polyphenols, in addition to them a in dietary fibre component of food. (Judita bystricka *et al* 2015)Daucus carota, often known as carrot, is a member of the Apiaceae family. (Krishnan Sharma *et al* 2012). Carotenoids, phenolics, vitamin C, and tocopherol are all abundant in carrots, making them a special type of vegetable crop. Its antioxidant power guards against free radicals produced both internally by normal food and metabolic activity and externally from external sources (Khyati Varshney *et al* 2022). This study explores the formulation and sensory evaluation of barley-carrot snack bites.
To develop a nutrient dense barley bites incorporated with carrot.
To assess the sensory acceptability of the barley bites prepared with various proportions of carrot.

MATERIALS AND METHODS

A) Procurement and processing of Barley bites incorporated with carrot

Barley, carrot, jaggery, milk, butter are available at stores throughout the year in Salem. The sample were cleaned to remove dust and foreign particles. These raw ingredients were incorporate in the particular ratio.

B) Standardisation and formulation of Barley bites incorporated with carrot

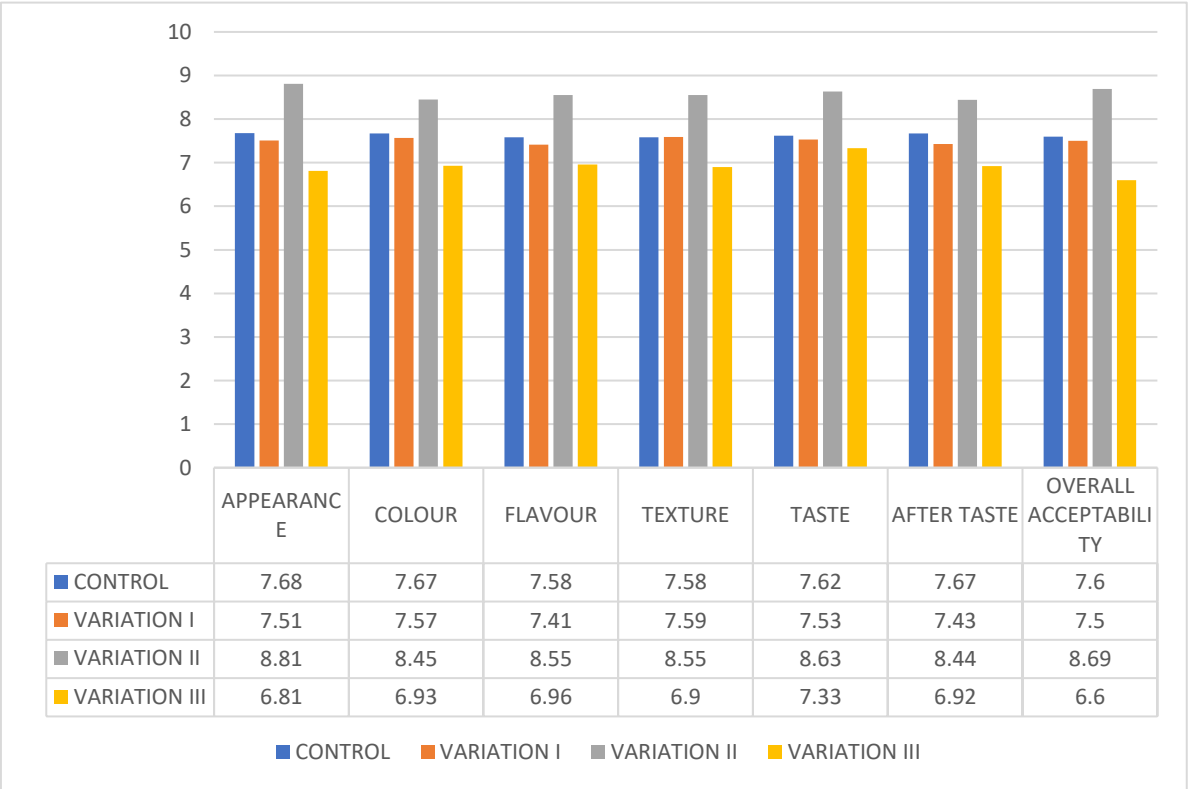
A Standard bites was prepared using the ingredients with different quantity like barley flour (50g), jaggery(20g),butter(5g) and milk (50ml) without carrot powder. Barley bites incorporated with carrot was prepared in three different variations. Variation 1 carrot powder(5 g), barley flour (50g), jaggery(20g),butter(5g) and milk (50ml). Variation 2 contained carrot powder (10g), barley flour (50g), jaggery(20g),butter(5g) and milk (50ml).Variation 3 contained carrot powder(15g), barley flour (50g), jaggery(20g),butter(5g) and milk (50ml).

C) Sensory evaluation and acceptability of Barley bites incorporated with carrot .

The discipline of hedonic response flourishes swiftly in 20th century along with the growth of food processing industries. It encompasses a set of techniques required for the precise measurements of human reactions to foodstuff ultimately persuading the consumer perceptions (Mian ***et al***., 2017). The hedonic rating test is used to measure the consumer acceptability of food products. The consumer is asked to rate the acceptability of the food product on a scale usually of a point, ranging from 'like extremely' to 'dislike extremely' (Srilakshmi,2018). This nine point hedonic scale was used to evaluate the sensory attributes like colour, appearance, flavour, taste, texture and overall acceptability for Barley bites incorporated with carrot by a trained panel member group consisting of ten members.

RESULT AND DISCUSSION

Sensory evaluation and mean score of the acceptability of Barley bites incorporated with carrot. The overall acceptability of Barley bites incorporated with carrot revealed that the variation 2 got the highest mean score of 8.9±0.2. The least accepted sample was variation 3 with a mean score of 6.4±0.5. The mean score of the variation 2 was higher than all the variations.



	APPEARANCE	COLOUR	FLAVOUR	TEXTURE	TASTE	AFTER TASTE	OVERALL ACCEPTABILITY
CONTROL	7.68	7.67	7.58	7.58	7.62	7.67	7.6
VARIATION I	7.51	7.57	7.41	7.59	7.53	7.43	7.5
VARIATION II	8.81	8.45	8.55	8.55	8.63	8.44	8.69
VARIATION III	6.81	6.93	6.96	6.9	7.33	6.92	6.6

FIGURE- 1: MEAN SCORE OF THE ACCEPTABILITY OF BARLEY BITES INCORPORATED WITH CARROT

The mean score of the sensory evaluation showed that the barley bites incorporated with carrot were within acceptable range, while the variation 2 had a better appearance(8.9), colour(8.6), flavour(8.5), texture(8.7), taste(8.5) and after taste (8.5) among all the variations. Of all the bites, variation 2 (v2) with the incorporation of 10% carrot with barley bites had the highest level of the overall acceptability.

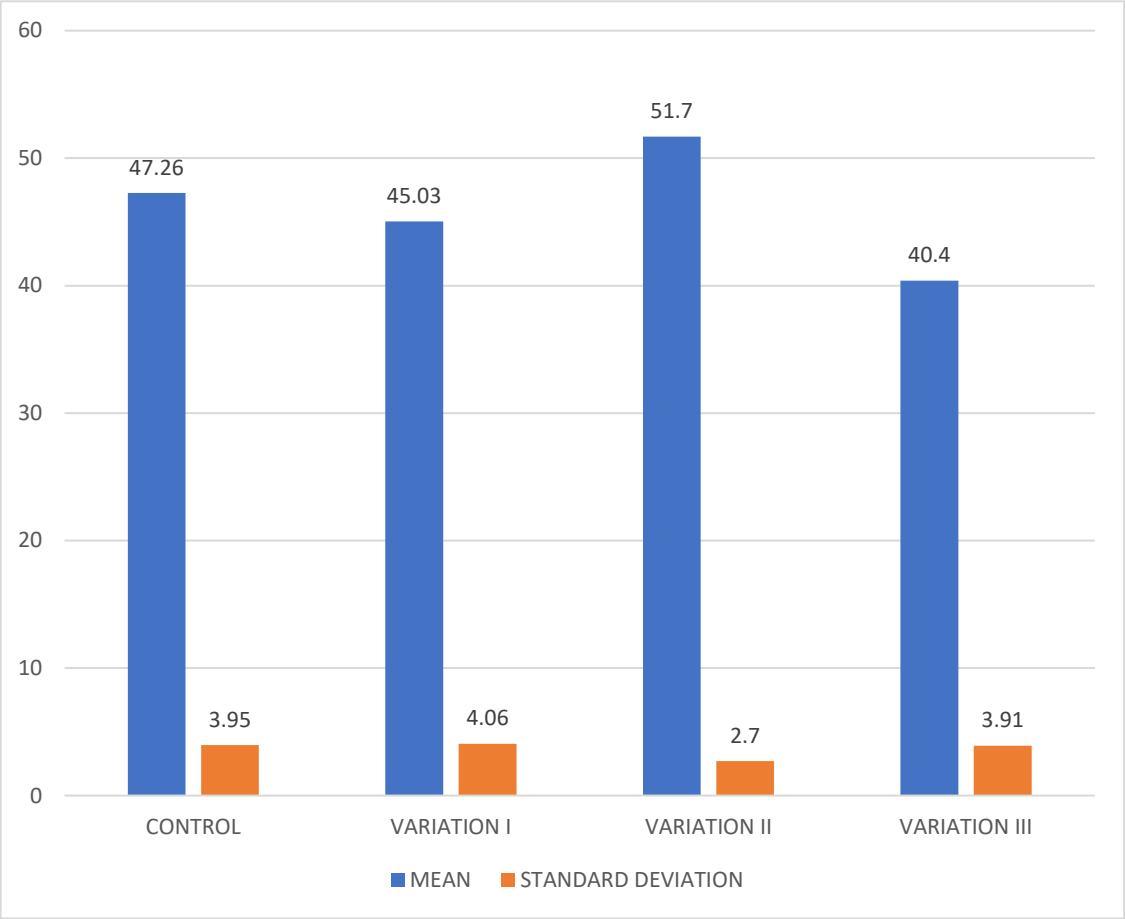


FIGURE- 2: OVERALL MEAN AND STANDARD DEVIATION VALUE OF BARLEY BITES INCORPORATED WITH CARROT

The overall mean and standard deviation value of the sensory evaluation showed that the barley bites incorporated with carrot were within acceptable range, while the variation-2 had a highest mean (51.7),standard deviation(2.7) among all the variation.

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CONCLUSION:

Cereals, particularly barley, have gained recognition as functional foods that improve consumer health and lower their chance of developing numerous diseases. This cereal is a great source of soluble dietary fiber, particularly beta glucans, and it also includes vital vitamins and minerals. Barley's nutritional qualities have anti-oxidant, anti-carcinogenic, anti-inflammatory, and cardio and neuro protective benefits, helping to avoid a variety of metabolic illnesses. This study successfully developed barley-carrot snack bites with improved sensory and nutritional properties. The findings support the incorporation of barley and carrot in functional snacks, with future research recommended on storage stability and commercialization potential.

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MOLECULAR CHARACTERIZATION OF PIGMENT-PRODUCING HALOPHILIC BACTERIA FROM THE SALT PANS OF TUTICORIN

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Hypersaline environments serve as reservoirs for extremophilic microbial communities capable of withstanding high salt concentrations while producing novel bioactive metabolites. This study aimed to isolate and characterize red pigment-producing halophilic bacteria from the salt pans of Tuticorin, Tamil Nadu, India. Saltwater and soil samples were collected and serially diluted before being plated on nutrient agar with salt concentrations ranging from 5% to 25%. Red-pigmented bacterial colonies were successfully isolated at 20% and 25% salt concentrations. The isolates were subcultured, preserved as stock cultures, and further propagated in liquid media for molecular characterization.

Genomic DNA was extracted from the bacterial pellets, and 16S rRNA gene sequencing was performed for species identification. BLAST analysis of the sequenced data revealed two distinct halophilic bacterial species. One isolate showed 100% identity with Salinicoccus roseus strain PIGB1, while another exhibited 99.76% identity with Sutcliffiellacohnii strain DSM 6307, with 100% query coverage and an E-value of 0.0. These findings confirm the presence of multiple pigment-producing halophilic bacterial species in the hypersaline conditions of Tuticorin salt pans. Given the potential of microbial pigments in various biotechnological applications, the identified strains warrant further investigation into their pigment production capabilities, stability, and industrial applicability, particularly in fields such as textiles, cosmetics, and pharmaceuticals. This study highlights the significance of halophilic bacteria as a promising source of bioactive pigments, emphasizing the need for further exploration into their metabolic pathways and commercial potential.

TRADITIONAL MEDICINAL PLANTS USED FOR THE TREATMENT OF DANDRUFF

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Dandruff is a common scalp disorder characterized by white, flaky scales and itchiness, affecting approximately 20–60% of the population. The condition is primarily caused by Malassezia species, a type of fungus. This study focuses on the isolation and microscopic examination of Malassezia sp. using selective media. Despite the availability of numerous synthetic shampoos and serums, a complete cure for dandruff remains elusive. Therefore, this study explores the anti-dandruff potential of natural plant extracts derived from Azadirachta indica (neem), Cassia auriculata (tanner's cassia), and Cymbopogon nardus (lemon grass). The extracts were obtained using three different solvent extraction methods—ethanol, acetone, and aqueous. The antimicrobial activity of these plant extracts was evaluated using the agar well diffusion method, and their inhibitory concentrations were measured. Results were analyzed in comparison with positive controls (fluconazole, ketoconazole, and clotrimazole) and a negative control.

Keywords: Flaking, itchiness, Malassezia sp., inhibitory concentration, microscopy

EVALUATION OF ANTIBACTERIAL PROPERTIES OF SELECTED MEDICINAL PLANTS AGAINST *SALMONELLA SPP.*

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Medicinal plants have been widely used for centuries in traditional medicine to treat various diseases. Azadirachta indica (Neem) is one such plant known for its diverse therapeutic properties, including antibacterial, antifungal, antidiabetic, anti-inflammatory, and antifertility effects. This study investigates the antibacterial activity of selected medicinal plants—Azadirachta indica, Coccinia grandis, Euphorbia hirta, Hybanthus enneaspermus, Psidium guajava, and Ricinus communis—against clinical pathogens, particularly Salmonella typhi. Blood samples from typhoid patients were collected and cultured for bacterial isolation and identification using biochemical and morphological methods. Extracts from medicinal plants were prepared using aqueous and methanol extraction techniques. The antibacterial efficacy was assessed through agar well diffusion methods. Preliminary phytochemical screening was also conducted to identify bioactive compounds. The findings emphasize the potential of medicinal plants as natural alternatives in antimicrobial treatments.

Keywords: Azadirachta indica, Antibacterial activity, Salmonella typhi, Phytochemical analysis, Traditional medicine, Plant extracts, Antimicrobial properties, Neem, Herbal therapy.

BIO PROCESSING OF SUGAR CANE INDUSTRYWASTE TO PRODUCTION OF ITACONIC ACID

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ABSTRACT

The potential for the profitable use of sugarcane bagasse and other agro-industrial leftovers was the focus of this study, which focused on developments in industrial biotechnology. A complex substance, sugarcane bagasse is the main by-product of the sugarcane industry. Investigating the potential of employing agricultural waste as a source for itaconic acid production from different fungus is the goal of the current project. Penicillium sp., Aspergillus oryzae, Aspergillus niger, and Aspergillus flavus were chosen and cultivated to produce itaconic acid in solid state fermentation utilizing sugarcane baggasse powder, a less expensive raw material. At 35°C, solid state fermentation yielded the greatest itaconic acid content (8.241 ±1.5 mg/kg) from A. niger. The ideal pH and fermentation medium for the synthesis of itaconic acid were determined to be 3.5. The fact that shows the recent developments onprocesses and products developed for the value addition of sugarcane bagasse through the biotechnological means.

Keywords: Sugar cane bagasse, itaconic acid, fermentation medium.

BIOCHEMICAL CHANGES OF CIGARETTE SMOKERS AND NON-CIGARETTE SMOKERS

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There is growing evidence that several biochemical constituents of cigarette smoking play a significant role in the development and progression of the heart and blood vessel damage, especially atherosclerotic lesions Nicotine is one of many substances that may be acquired through active and passive smoking of tobacco. In man, nicotine is commonly consumed via smoking cigarettes, cigars or pipes. The addictive liability and pharmacological effects of smoking are primarily mediated by the major tobacco alkaloid nicotine. There are elevated serum protein levels in smokers resulting in dysfunction with an increased incidence of microalbuminuria progressing to proteinuria, followed by type-1 diabetes mellitus induced renal failure. Nicotine, or concurrent intake significantly increases lipid peroxidation in liver, and decreased Hb, HDL, Cholesterol activity and increased Enzyme activity in the Blood. haematologic changes are a consequence of cigarette smoking exposure. This Conclusion describes the effects of smoking, smoke extracts and other tobacco constituents on renal and cardiovascular functions, and associated effects on the nervous system. Both active and passive smoking is toxic to renal function

Key words: Cigarettes smoking, lipid peroxidation, cholesterol, protein, enzyme activity,

BIOCHEMICAL CHANGES OF LIPID PROFILE IN LEPROSY PATIENTS

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Biochemical changes of serum cholestrol and lipoprotein in leprosy patients were investigated. The serum triglyceride level was lower than normal in tuberculoid leprosy (TT), showed no alteration in Borderline tuberculoid (BT) or Borderline borderline (BB) and was insignificantly increased in Borderline lepromatous (BL) and Lepromatous leprosy (LL) patients. The total cholesterol was lower than normal in TT, showed no alteration in BT or BB and was insignificantly increased in Bland LL patients. The total cholesterol was lower than normal in TT, whereas in BT, BB, BL and LL groups the levels were statistically decreased. The HDL cholesterol was within normal range in TT, significantly decreased in BT and LL patients, showed no significant alteration in BB and was insignificantly decreased in BL group. The LDL cholesterol in TT was low but was not so low statistically when compared with the controls, whereas in BT, BB, BL and LL groups the levels were statistically decreased. The VLDL cholesterol was within normal range in TT and BT, was raised insignificantly in 3 of 12 cases of BB, was within normal range in BL and in LL leprosy it was raised in one out of 9 cases. In the absence of any derangement of liver function tests, it can be concluded that leprosy per se leads to alterations in lipid metabolism. However, no correlation could be established between the group/type of leprosy, bacterial indices and levels of different lipid fractions.

Keywords: Leprosy, Lipid profile, Biochemical changes

SYNTHESIS AND CHARACTERIZATION OF SNO_2/ZNONANOCOMPOSITE AND ITS BIOMEDICAL APPLICATION

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ABSTRACT

NanocompositesZnO/SnO_2were produced by the sol-gel method. In this study, two semiconductors (ZnO-SnO_2) are examined. The Miller indices of the ZnO-SnO_2nanocomposites were used to determine their particle sizes through powder XRD analysis.UV–vis and X-ray photoelectron spectroscopies (XPS) were carried out for the optical characterizations. FTIR is a spectroscopy method that detects changes in biomolecule composition by analyzing functional groups.Using photoluminescence, optical properties were investigated.It was used to calculate the absorbance band and band gap energy of nanocomposites. Model pollutants such as methylene blue were used to assess the photocatalytic activity of ZnO-SnO_2 and SnO_2/Al_2O_3photocatalysts under UV light. There are many uses for nanocomposites in the biomedical industry. Both NCs' antimicrobial activity was investigated.

Keywords: SnO_2/ZnO, Nanocomposites, Sol-Gel Method, Characterization, Biomedical Applications.

SYNTHESIS OF NANOPARTICLES SYNTHESIS FROM NATIVE STRAIN OF RHIZOBIUM

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ABSTRACT

The most successfully studied nanoparticles today are those made from noble metals, in particular Cu (copper), Ag (Silver), Ti (Titanium), Pt (Platinum), Au (Gold) and Pd (Palladium). Among all metal oxides, silver nanoparticles (AgNPs) has gained more attention due to its distinctive properties and applications. Green routes of synthesis are simple, safe, nontoxic and eco-friendly methods to synthesize nanoparticles of various metals and their oxides by the application of bioactive compounds of plants, algae, fungi, yeast, etc. In the present study is to synthesis of Ag nano particles using *Rhizobium* and evaluating its effect on plant growth. The selected sample sites of this study covered were in the major *Vigna mungo* soil field of the Orathanadu region. Silver nanoparticles were synthesized using *Rhizobium*. The formation of silver nanoparticles was noted by detecting the change in color of the solution. The presence of nanoparticles was detected by performing UV visible spectroscopy and monitoring the spectrum from 400 nm to 800 nm. A small peak at 420nm suggested the presence of silver nanoparticles. In a later part of the study, the growth effect of green synthesized silver nanoparticles was monitored. The the application of the synthesized nanoparticles to plant showed the better growth was confirmed.

Keywords: *Rhizobium*, Silver nanopartilces, Soil, UV visible spectroscopy

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