# SMC SCIENCE UPDATES

## PROCEEDING OF UG PROJECT UNDER DBT - STAR COLLEGE SCHEME

## (2020-2021)

सत्यमेव जयते

**Department of Biotechnology**
**Ministry of Science & Technology**
**Government of India**

**ST. MARY'S COLLEGE (AUTONOMOUS)**

**Re-accredited by NAAC with `A+' Grade**

**THOOTHUKUDI, TAMIL NADU**

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# PREFACE

This proceeding reflects the impact of the DBT Star College Scheme for the undergraduate science research students during the academic year 2019-20. The collaboration with DBT-STAR has special meaning and relevance to St. Mary's College (Autonomous), Thoothukudi, as it caters to the academic needs especially in the domain of science education to the rural women in the backward coastal belt of south India. By giving impetus to science education at the undergraduate level and meticulously guiding students in research pathway, the college has enabled to understand better the cognitive and analytical skills more deeply to produce results. Our undergraduate students in the departments of Botany, Zoology, Physics and Chemistry were empowered enough to bridge connections with other levels of science education and technology and turn out quality research work at their level with genuine concern for society. The productive combination of our infrastructure, management support, faculty expertise and generous funding of DBT will surely pay enormous dividends in their lives and the public's awareness and appreciation of science.

It is with divine grace that we could accomplish various projects and these proceedings in a meticulous way. The reviewers did a brilliant job in refining the papers. We would also like to express our gratitude to our Secretary Sr. Flora Mary, Principal Dr. Sr. A. S. J. Lucia Rose, Heads of the Department of Botany, Chemistry, Physics and Zoology, faculty members, students and the CBA publishers.

With immense pleasure we proudly present this proceeding, the compilation of the research work undertaken by students and faculty using the resources procured with DBT STAR College fund.

***Editorial Team***
***DBT-STAR College Proceeding***
***(2019-20)***

# MESSAGE

**Rev. Sr. Flora Mary**
**Secretary**
**St. Mary's College (Autonomous)**
**Thoothukudi.**

Gaining knowledge has been an extensive process, from understanding the water cycle to creating genetically modified species and human curiosity is the reason why natural sciences have been explored in depth. Today technology is evolving at a dizzying rate and our classrooms may not be designed to keep pace with it. Practical training and research exposure to undergraduate students has almost become mandatory and STAR COLLEGE SCHEME funded by DBT, New Delhi is a boon to UG students from the Departments of Physics, Chemistry, Botany and Zoology. The financial assistance granted by DBT helped to procure sophisticated instruments in the lab and carry out project work with ease. It has played a significant role in kindling the spirit of research in the young minds of UG students and inculcate project-based learning experience to students. I am indeed very happy that the star Departments have taken up this initiative in publishing the proceedings to create research awareness among the UG students. This proceeding is a compilation of research papers carried out by the UG students in their final year. I congratulate the Star College Scheme coordinators, Heads of the Department of Physics, Chemistry, Botany and Zoology, all their faculty members for taking the initiative in compiling this. This proceeding provide a written record of the project work carried out by the students and represents a solid framework from which new research will evolve in the future.

**Rev. Sr. FLORA MARY**

# MESSAGE

**Dr. Sr. A. S. J. Lucia Rose**
**Principal**
**St. Mary's College (Autonomous)**
**Thoothukudi.**

Greetings from St. Mary's College (Autonomous), Thoothukudi. Our institution aims to hone the potential of our students, impart knowledge and promote research aptitude. The Star College Scheme sponsored by the Department of Biotechnology, New Delhi has provided an opportunity to learn more with confidence and enthusiasm every student. DBT Star College Scheme has broadened their horizon of knowledge and provided suitable platform for research activity. DBT has enhanced the quality of practical training among students. It has been instrumental in making them to take up new challenges in research and avail the opportunities to explore their analytical skills. It is with great pleasure I congratulate all the undergraduate final year students and faculty for their meticulous hardwork in contributing to the proceedings.

The research ambience has been enhanced with a productive outcome from students. This proceedings is a promising enterprise in pooling upcoming scientific research from young minds. It would enhance the quality research in the institution. My special appreciation and due acknowledgement to the organisers of the Star College Scheme and the overall coordinator and member secretary Dr. Sr. Arockia Jenecius Alphonse.

I wish you the best in all your endeavours.

Lucia Rose

**Dr. Sr. A. S. J. LUCIA ROSE**

# MESSAGE

**Prof. G. Paruthimal Kalaignan M.Sc., Ph.D.**
**Senior Professor and Head**
**School of Chemical Sciences**
**Department of Industrial Chemistry**
**Alagappa University, Karaikudi**

I am happy to note that students from Departments of Botany. Zoology. Physics and Chemistry of St. Mary's College (Autonomous), Thoothukudi have done group projects in the academic year 2019-2020 under the STAR COLLEGE SCHEME sponsored by Department of Biotechnology, New Delhi, India.

All the project works have been written in the form of research papers and published in the Proceedings. All the science departments have richly contributed to the Academic ambience and quality enhancement of St. Mary's College, Thoothukudi.

I am sure that, the Research Proceedings will encourage researchers to learn about recent developments in Science and Technology. My compliments are due to the Faculty and students of all the science Departments of St. Mary's College, Thoothukudi for their enthusiasm in preparing the Research Proceedings with an objective to undertake studies and research under the STAR COLLEGE SCHEME.

I hope that, the Research Proceedings will provide unique opportunity for the young researchers to publish their research articles.

I wish the release of Research Proceedings under the STAR COLLEGE SCHEME a grand success.

**Dr. G. PARUTHIMAL KALAIGNAN**

# MESSAGE

**Dr. L. Ranjith, M.F.Sc., Ph.D., ARS**
**Scientist**
**Marine Biodiversity Division**
**ICAR-Tuticorin Regional Station of CMFRI**
**Thoothukudi**

The Star College Scheme has been initiated by the Department of Biotechnology (DBT) under the Ministry of Science & Technology, Government of India since 2008 to support colleges and universities offering undergraduate education to improve science teaching across the country.

In this view, UG students from various Departments (viz., Botany, Zoology, Physics and Chemistry) of St. Mary College, Thoothukudi has carried out various research activities including group projects for the academic year 2019-2020 under the above-mentioned scheme.

I was delighted to see that many of the group activities/projects have been brought into a shape as a research article and few of them are in the form of proceedings. I congratulate all the faculty member of the St. Mary College, Thoothukudi for their sincere efforts in the successful organisation various activities under the Star College Scheme.

I once again wish all the faculty members and the students for the future endeavours and bring laurels to St. Mary College, Thoothukudi.

**Dr. L. RANJITH**

# MESSAGE

**Dr. Sr. Arockia Jenecius Alphonse**
**(Assistant Professor of Botany)**
**The STAR College Scheme**
**Overall Coordinator & Member Secretary**
**St. Mary's College (Autonomous)**
**Thoothukudi.**

Dear All,

Every kid starts out as a natural-born scientist. But in a rural system with inadequate infrastructure, access to science education, lack of scientific inquisitiveness the innate craving gradually dies out and the country would lose the best talents undeveloped or underdeveloped. It is in this context, the initiatives of DBT become relevant with its efforts to attract and support science education at the undergraduate level in our country. The academic guidelines and financial support provided by DBT helped the higher education centers to shift the colleges to next generation science standards by encouraging student inquiry, feeding their curiosity and deepening their understanding of scientific concepts.

St. Mary's College (Autonomous), being a women's college, situated in the most vulnerable and the backward coastal belt of South India, with thousands of women students who were the direct beneficiaries of the financial and academic assistance extended by the Department of Biotechnology "Star College Scheme", well acclaim the support with immense gratitude and gratefulness. The science departments of the college with a well backed-up infrastructure, postgraduate programmes and research facilities leading to Ph.D. endeavoured to shift its human resource and institutional approach to share the benefits of scientific fervent among the undergraduate students. This in turn attracted, involved and empowered the undergraduate students to use their knowledge and imagination to develop scientific ideas about the concepts being discussed. With the DBT Star College Scheme, our students are on the way of "learning out" to "figuring it out" level.

Surely, we have a long wayto go. The college lacks the most advanced and sophisticated lab equipments and other facilities. The current project and the financial support were not adequate to cater to our dreams. We are looking for major collaboration and assistance from DBT to enhance the lab facilities of our college

at par with international standards. However, we are happy that with the available resources, we were able to do things meticulously and scrupulously. Every science department produced multiple results.

I place on record my immense thanks to the DBT authorities, my college authorities, faculty members, students, other institutions, agencies and a great array of experts who visited the college in connection with various programmes under the scheme. I acknowledge the benevolence of God almighty and all those who have helped me in successfully accomplishing the project.

Thank you

**Dr. Sr. AROCKIA JENECIUS ALPHONSE**

# ABOUT THE COLLEGE

St. Mary's College (Autonomous) established in the year 1948 by the Congregation of Mother of Sorrows Servants of Mary as the first women's college in Thoothukudi has contributed a lot to the development and well-being of women in the coastal region. As the first and the only autonomous college in Thoothukudi, it has progressed ahead in the arena of education attaining notable accolades in every discipline. Disadvantaged, underprivileged and first generation learners are given priority in the admission so that they are given an opportunity to come up in life raising their economic and social status resulting in generational transformation in this remote part of the country.

St. Mary's College (Autonomous) achieved A+ Grade in the 4$^{th}$ cycle of reaccreditation by NAAC on 1st May 2019 with 3.29 CGPA, one among the 65 higher educational institutions to be awarded the higher status of academic excellence across the country. With the implementation of autonomy and CBCS, the institution has been designing its own innovative curricula and evaluation system.

The academic activities of all disciplines are grouped into five Schools of Excellence to augment research, design pioneering courses, enable efficient teaching and facilitate effective outcome based learning and to empower the student community to face the global demands of the 21st century. The IQAC endeavours to optimise the integration of modern methods of teaching, learning and evaluation.

The institution offers 18 undergraduate programmes (Aided: 10; Self-Supporting: 8), 13 postgraduate programmes (Aided:5; Self-Supporting:8), 6 M.Phil. programmes which are globally relevant and competent. Six departments have been upgraded as Research Centres. At present, 1805 students are in the regular stream and 1314 students (Total 3119) are enrolled in the Self Supporting Courses with the faculty strength of 95 permanent and 6 management in Regular Stream and 70 in Self Supporting Courses. It also offers Career-oriented Courses to all the III UG students and certificate courses for the I & II year students.

The curriculum is enriched with ICT enabled teaching, domain specific CLIL based on the Common European Framework of Languages for Part II English. The certificate courses and the MOOCs ensure employability to the students.

The activities of various clubs and forums are effectively grouped under Eight Centres of Excellence to promote the skills of the students. The Co-curricular activities viz., NCC wing and NSS units instill discipline, responsibility, patriotism and social awareness among the students to uplift the society. In sports and games

the college has made a landmark achievement by retaining the overall Championship trophy in team games of M.S. University 28 times consecutively.

For the past 72 years, St. Mary's College has been imparting value conscious integrated education to the young women and has established itself as the knowledge hub in the Southern India.

# ABOUT THE STAR COLLEGE SCHEME

"Star College Scheme" has been initiated by Department of Biotechnology (DBT) to support colleges offering undergraduate programmes to promote and facilitate science teaching. The scheme aims to hone the skills of teachers by helping the institution to organise faculty training programmes to offer improved curriculum keeping up with the current trends. It emphasises applied learning and practical training for students by providing access to specialised infrastructure and consumables.

Star College Scheme is formulated with an aim to enhance conceptual clarity, develop scientific thinking and creativity among students. It enables interactive participation among different scientific disciplines and departments with a holistic approach towards science rather than compartmentalised thinking.

St. Mary's College (Autonomous) sent the proposal for Star College Scheme on 10$^{th}$ May 2018. The funds under STAR COLLEGE SCHEME was sanctioned on 16$^{th}$ March 2019. Four departments of the college namely Physics, Chemistry, Botany and Zoology have been recommended by the Department of Biotechnology (DBT), Ministry of Science & Technology, Government of India, New Delhi, to receive financial assistance under the Star College Scheme. For implementation of the Star College Scheme, the DBT, New Delhi has sanctioned Rs 82, 00,000 and an initial release of a sum of Rs.40 lakhs(Rs.10 lakhs per dept) under non-recurring head and Rs.14 lakhs (Rs.3.5 lakhs per dept) under recurring head for the financial year 2019 - 2020.

The objective of the Star College Scheme is to strengthen Life Science and Biotechnology education and training at the undergraduate level to encourage and attract students to pursue a career in Life Sciences. The scheme envisages:

- To strengthen the academic and physical infrastructure to enhance the teaching and learning process of the students and to stimulate critical thinking by providing 'hands on' exposure in experimental work.
- To increase the core instrumentation resources by procuring new equipment and upgrading existing facilities.
- To provide students access and exposure to Research Laboratories and Industries in the country.
- To provide better library facility to students and teachers in the department.
- To organise workshops/Seminars/Guest lectures for the benefit of the students.
- To conduct field trips/excursions for students to optimise learning.

# ACKNOWLEDGEMENT

I am extremely contented to express our truthful and sincere thanks to God Almighty whose blessings had given us the right chance of initiating and completing this proceeding successfully.

I owe an immense depth of gratitude and heartfelt thanks to our Secretary Sr. Flora Mary for her constant support and encouragement. I express my deep sense of gratitude and profound thanks to our Principal Dr. Sr. A. S. J. Lucia Rose for equipping the institution with all the necessary facilities to carry out this research work.

I wish to extend my earnest thanks to the Overall Coordinator and Member Secretary, DBT Star College Scheme Dr. Sr. Arockia Jenecius Alphonse for her prompt guidance and vital help consistently rendered to us wholeheartedly in every situation to have this work completed in time and made it triumphant. I extend my sincere thanks to Heads of the Departments of Botany, Chemistry, Physics and Zoology for proving us support to develop an interdisciplinary team work.

With profound sense of gratitude, I would like to thank the Department Coordinators, DBT STAR College Scheme for their encouragement, support and cooperation in completion of this work. I would like to express a great deal of appreciation and cooperation extended by all the faculty members who have contributed their research works for this proceeding.

I would like to express my sincere gratitude to the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi for the financial aid granted under Star College Scheme to publish this proceeding. I would like to acknowledge Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi who gave access to the laboratory and research facilities. Without their precious support it would not be possible to conduct this research. This scheme had imbibed undergraduate students to complete their course with research-oriented outcome. It instilled scientific aptitude among undergraduate students by the execution of projects.

I am indeed grateful to the Laboratory Assistants, for their timely help and support. I sincerely thank CBA Publishers, Chennai for publishing this proceeding.

I also thank Mr. P. Kandasamy for his technical assistance to bring this proceeding.

Arockia Jenecius Alphonse

**Dr. Sr. AROCKIA JENECIUS ALPHONSE**

# FROM THE EDITORIAL DESK

**Dr. Sr. A. S. J. Lucia Rose**
**Principal**

**Dr. Sr. A. Arockia Jenecius Alphonse**
**The STAR College Scheme**
**Overall Coordinator & Member**
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**Assistant Professor of Botany**

**Dr. P. Subavathy**
**Assistant Professor of Zoology**

Research leads to quality learning. With this objective and the support of faculty members, we publish this proceeding with great pleasure. This proceedings work has enriched our undergraduate students of Botany, Chemistry, Physics and Zoology. We express our thanks to God Almighty for showering blessings and guidance in this academic venture.

We also express our sincere gratitude to our Secretary Sr. Flora Mary, Principal Dr. Sr. A. S. J. Lucia Rose, Heads of the Department of Botany, Chemistry, Physics and Zoology, faculty members and students for their continuous support and enormous effort to publish this proceeding. We express our sincere thanks to the reviewers for refining the work and for their timely assistance. We also thank CBA Publisher for their support towards this proceedings.

**Editorial Team**

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**Dr. M. Glory**
**Head and Associate Professor**
**Department of Botany**
**St. Mary's College (Autonomous)**
**Thoothukudi.**

The world today is driven by a knowledge economy where technology plays a prominent role. In the context of the sustained growth and diversification of higher education systems, the academic world is more and more concerned about the quality of programmes offered to students. The socio-economic and scientific development of the country is conditioned to the quality of human resources produced in our higher education system. The higher education system in India has grown into one of the largest systems of its kind in the world, particularly in the post-independent period. However, the system has many issues of concern at present such as access, equity and relevance. Moreover, it tends to be contextually disconnected. The values, ethics and quality of higher education are of great significance as we strive to engage higher education as a powerful tool to build a knowledge-based information society of the 21st century.

In this regard, we value and admire the enthralling support of the DBT in enhancing the quality of science education in the country especially those of the leading higher education centres in rural settings. We are thankful to the Department of Biotechnology, Government of India for the financial assistance to the college by which the Department of Botany benefited much. This gave an opportunity for the department to strengthen the basic infrastructure and enabled the faculty and students to carry out interdepartmental and intercollegiate activities. The DBT Star College fund allocation has definitely enhanced the potential and prospects of the department of Botany. We the faculty members of the department of Botany would like to place on record the deep sense of appreciation and gratitude to DBT, College authorities especially the principal and the coordinator of the DBT Star College Programme.

**DR. M. GLORY**

# MESSAGE FROM THE REVIEWER

**Dr. Uthayakumari Kalavathy,**
**Head and Associate Professor (Retd.),**
**Department of Botany**
**St. Mary's College (Autonomous)**
**Thoothukudi.**

Incorporating a research component along with a sound academic foundation enables students to develop independent critical thinking skills along with scientific writing skills. Faculty members can enhance learning experiences for students while benefiting from a productive research agenda. It is evident from the various research papers of the final year The students of the Department of Botany, St. Mary's College (Autonomous), Thoothukudi, presented for critical review. The students might have gained deeper understanding of the selected topic, strengthened their comprehension of research and research methods. It is learnt that the students carried out the research projects under the DBT Star college scheme. They have chosen diverse topics like marine algae, mushrooms, phytochemical analysis, taxonomic analysis, and environmental issues as the focus of their group project. All the papers are of excellent quality at the undergraduate level, eligible to be published.

Authors of the research work "Characterization of Seaweeds and Effect of Seaweed Liquid Fertilizer on Growth Parameters and Nutrition Content of *Brassica Juncia*" reconfirm the utilization of seaweed fertilizer as a more effective and sustainable mean in crop improvement. The standardised formula can be made use of while producing algae based fertilizers. The research paper entitled "Comparative Study of Effect of Different Substrates on Yield of Oyster Mushroom and its Nutritional Value" investigates the effect of various substrates like paddy straw, sugarcane bagasse, banana leaves, and their combinations on the growth yield, biological efficiency, and nutritive values of *Pleurotus ostreatus*. The authors have used standard methods to explore the yield and nutritional values for the comparative study purpose, with a remarkable interpretation based on the available literature.

The work "Macroscopic and Microscopic Studies on the Aerial Parts of Selected Species of Lamiaceae" extracted the morphological and anatomical characters of the *Anisomelesmalabarica* and Lucas *aspera*. Though the anatomical studies are previously reported by other researchers, the comparative analysis attributes to its

novelty that might have helped in rekindling the scientific research skills in the students. Five important medicinal herbs are inspected for their pharmacognostic characteristics in the work titled "Preliminary Phytochemical Screening and Pharmacognostic Studies on Leaves of Selected Weeds". The powder microscopic data presented can be refined, and used in the identification of the crude drugs sourced from the plants studied.

The authors of the paper "Isolation and Screening of Microalgae from Natural Habitats in the Thoothukudi Coast for Antibacterial Sources" have standardised the method of growing marine *Chlorella sps*. The work can be further exploited in growing the specific Chlorella sps in huge bioreactors for its industrial production. The research work entitled "Phytochemical Screening and Antioxidant Activity of *Tecomastans* (L.) Juss. Ex Kunth" explores the radical oxygen scavenging activity of *Tecomastans*. The work may be further extended in purifying the active compound from *T. stans* ethanolic fraction and test for their biological significance.

The authors of "Extraction of Natural Dye and its Application on Selected Fabrics" have standardized a method for the extraction of dye from *Zinnia elegans*, and can be transformed for the industrial extraction of dye from the *Zinnia* plant. The research work presented in "Assessment of Water Quality Parameters along the Selected Coastal Areas of Thoothukudi" exposes that still much effort needs to be taken to protect the local marine environment. It also might have helped in evoking the social responsibility of the student participants engaged in the research work.

All the papers invariably contributed some knowledge addition and tried to address socio-economic aspects through scientific inquisitiveness that would enhance the life of the coastal community. I do appreciate the faculty mentors, students and the editorial team for their efforts to publish the findings of the best student projects in the proceedings. I wish the very best for the publication of the proceedings.

Uthayakumari

**DR. UTHAYAKUMARI KALAVATHY**

# Characterization of Sea Weeds and Effect of Sea Weed Liquid Fertilizer on Growth Parameters and Nutrition Content of *Brassica juncea*

**S. Maria Jenifer, S. Rabiya, K. Muthu Esakkiammal, P. Muthu Santhiya, C. Muthu Valliammal and M. Glory***

PG and Research Department of Botany, St. Mary's College (Autonomous), Thoothukudi, Tamilnadu, India.

(Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India)

Received on 5 November 2020

## Abstract

The present study was carried out to find out the effect of seaweed liquid fertilizer on morphological parameters and biochemical constituents of *Brassica juncea*. Seaweeds were collected along the coast of Thoothukudi and it was identified and preserved in 4% buffered formalin. From the collected seaweeds *U. lactuca, C. scalpelliformis, P. tetrastromatica* and *S. linearifolium* were selected to evaluate the impact of SLF as organic fertilizer. The result of the investigation revealed the positive effect of SLF on various parameters and it was found to be concentration-dependent. The significant effects of SLF were well pronounced only after 14 days of analysis. Among the various SLF used *P. tetrastromatica* at 10% concentration showed better results in the morphological parameters. The amount of protein was found to be higher in 10% SLF of *P. tetrastromatica* and *S. linearifolium*. Similarly, the amino acid content was also higher in *B. juncea* treated with 10% SLF of *P. tetrastromatica* and *S. linearifolium*. The result of sugar content indicated that the SLF of *C. scalpelliformis* was more effective. SLF of *U. lactuca* found to be less effective than other selected SLF. The overall investigation suggested the efficacy of SLF as a good source of manure for crop growth.

***Keywords:*** Seaweed Liquid Fertilizer, Morphology, Nutrients, Legumes, Plant growth

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## Introduction

Seaweeds are the macroscopic algae, grow in intertidal and subtidal regions of the sea. It serves as an excellent source of food, fodder, fertilizer, and industrial raw material for the production of phycocolloids like agar, algin, carrageenan[1], and antimicrobial agent[2].

Several thousand species of macroalge live in the oceans throughout the world, some are free-floating and most are found attached to the substratum. The best quality organic fertilizer is known to provide all the nutrients required by the plants and helps to increase the quality of the soil by enhancing the natural microbial environment. Biofertilizer contains a wide range of plant nutrients, trace elements, carbohydrate, amino acids, and other growth-promoting substances. The seaweed biofertilizer checks the leaching of minerals from the field to a considerable extent. Seaweeds are used in different parts of the world as fertilizer for land crops. In India, freshly collected and coastal ashore seaweed are used as manure for coconut plantation either directly or in the form of compost in coastal areas of Tamilnadu and Kerala. Seaweed fertilizers enhance the potash and mineral contents of the soil. The presence of gelatin substance in the seaweeds improve the physical and chemical properties of the soils. The occurrence of calcium carbonates in the seaweeds increases the pH of the soil.

Recent research proved that seaweed liquid fertilizers are better substitutes for chemical fertilizer[3] they not only have N, P, K but also possess some trace elements and growth regulators[4]. Today, SLF (Seaweed Liquid Fertilizer) has been widely used with excellent results in all kinds of plants in all countries. Therefore constant effort is taken by scientists to use SLF in agricultural practices as they are eco-friendly.

Thoothukudi coast situated along the Gulf of Mannar is an excellent source of seaweeds, which are being not studied on their application. So, the present study pertains to study the effect of SLF on the growth and biochemical constituent of *Brassica juncea (L)* with the following objectives. Collection of seaweeds from Gulf of Mannar, characterization of seaweeds, study the effect of SLF on the morphological character of *Brassica juncea* and to understand the role of SLF on the biochemical constituent of *Brassica juncea*.

## Materials and Methods

#### Seaweed Collection:

Collection of seaweed was carried out from Thoothukudi cost (Lat 9.15'N long 79'E) during the low tide period. The algal species were handpicked and washed thoroughly with running tap water to remove all the unwanted impurities, adhering particles, and epiphytes. The collected seaweeds were identified and characterized with the help of manual Phycologia Indica (Srinivsan KS, 1969)[5]. From the collection, a part of

healthy seaweeds was preserved wet in 4% buffered formalin. Seaweeds include *Ulva lactuca, Caulerpa scapelliforms, Padina tetratromatica* and *Sargassum linearfollium* were used for the preparation of SLF.

#### Seaweed liquid fertilizer Preparation (Rama Rao 1990)[6]:

Well cleaned seaweeds were dried in sunlight for 5 days then oven dried for 24 hr at 60-64°C. The dried material was hand crushed and made into a coarse powder using a mixer grinder. The coarse powder was soaked in water for 5 minutes before extraction. Seaweeds were cooked within pressure cooker for 2 hr. 1:10(w/v). The extract was removed from the pressure cooker and filtered through a double-layered cheesecloth. The filtrate was centrifuged at 1000 rpm to remove most of the suspended impurities. The filtrate was dried in a hot air oven at 65°C for 48 hr and the powder was stored in airtight bottles. The dried seaweed samples were considered as 100% seaweed extract. It was used to prepare various concentrations of SLF for the experiments.

#### Experimental design:

Mustard seeds were initially surface sterilized with 0.1% mercuric chloride for one minute and repeatedly washed with distilled water to remove the trace of mercuric chloride. The seeds were soaked separately in 100ml of two different concentrations such as 5% and 10% of the SLF prepared with *U. lactuca, C. scalpellifromis, P. tetrastromatica* and *S. linearifolium*. The soaked seeds (15 seeds/pot) were sown in pots, filled with garden soil. Morphological parameters such as length of root, length of shoot and leaf area were studied. Biochemical constituent like soluble sugar[7], soluble protein[8], and amino acid[9] were also estimated based on standard methods.

## Results and Discussion

#### Wet method of preservation of algae:

Nine seaweeds namely *Ulva lactuca, Cauler pataxifolida, Gracillaria verrucosa, Gracillaria corticata, Padina tetrastromatia, Stoeihospermum, Graiilaria iortiata, Graiilaria verruiosa and Sargassum linearifolium* were collected from the study area. The collected macroalgae's were displayed and tied on glass plate to reveal the plant parts and it was kept in glass bottles with 4% formalin. It was labeled properly and kept for future reference purpose (Figure 1).


**Figure 1: Wet preservation of macroalgae**

## Characterization of Seaweeds:

***Ulva lactuca* Linn**

Division - Chlorophyceae

Order - Ulvales

Family - Ulvaceae

Genus - Ulva

Species - lactuca

Plants were initially attached, later on at times becoming detached and drifting in broadly expanded and torn sheet. Holdfast very small and inconspicuous. Thallus was foliaceous, membranaceous, expanded plane, reaching up to 18cm tall, obovate in young condition, and Broadly ovate or as rounded expansion in older plants. Margin of the thallus was ruffled, wavy and folded. Suface glossy. Colour bright green to light green, fading to yellowish and sometimes darker when young. Chloroplast was cup shaped.

***Caulerpa scalpelliformis* - C. Agardh**

Divison - Chlorophyceae

Order - Caulerpales

Family - Caulerpaceae

Genus - Caulerpa

Species - scalpelliformis

Plants were seen in large association, with prostrate rhizome like stolons, rooting from the lower surface at intervals and erect branches above; simple stolon or slightly branched, glossy, glabrous, 15-20 cm or longer with the presence of erect assimilators on the upper faces at 1-2 cm intervals of assimilators with distinct stipe below, 1-2 cm length, rarely forked, simple. Branches on the main broad thickened flat rachis were closely pinnate, alternately linear, subacute, plano-compressed, 1-2 cm long, 0.3 mm broad. Sections of thallus with a spongy network of anastomosing filaments were filled with semifluid matter. Colour bright yellowish-green to olive green.

***Padina terastromatica* hauck**

Division - Phaeophyceae

Order - Dictyotales

Family - Dictyotaceae

Genus - Padina

Species - tetrastromatica

Plant body was erect, several blades arising from the same stupose basal attachement, 12-15 cm or more in height. Rhizome was prostrate, branched and attached to substratum by tufts of rhizoids. Frond stalked, varying in size, numerous, fan shaped to reniform, thin, flat, much lobed, somewhat plicate, the larger blades loosely rolled on their longitudinal axis like a cornet, conspicuously zonate. Blades were frequently split into numerous narrow segments. Colour was straw coloured to brown or dark, olive green and reddish hues in older parts. They appeared whitish, also due to thin encrustation of carbonate of lime.

***Sargassum linearifolium***

Divison - Phaeophyceae

Order - Fucales

Family - Sargassaceae

Genus - Sargassum

Speices - linearfolium

They were yellowish brown in colour.Thallus was 35cm tall, attached with discoid holdfast. Axis was cylindrical alternatively arranged branches, bearing leaves and the vesicles; in young the stalk is 2.5-11.5 mm wide. Leaves were generallyoblong slightly tapered retuse (slightly rounded) or emarginated leave, smaller7-15 mm long including stalk and 17.4 mm wide oblanceolate, oblong. The apices were rounded,

obtuse to acute outer margin in coarse; prominent midrib. Pendulate vesicles, ovule with adiameter of 1.5-33 mm, vesicles may besolitary or may form clusters attached to the primary and secondary branches.

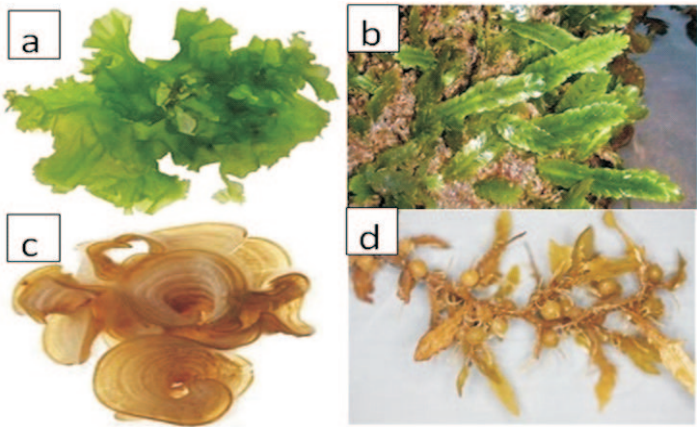


**Figure 2: Morphology of the seaweeds**

**a - *U. lactuca*, b - *C. scalpelliformis*, c - *P. tetrastromatica*, d - *S. linearifolium***

**Effect of SLF of various algae on the growth parameters of *B. juncea*:**

The effects of SLF on morphological character of *B. juncea* (L) are presented in Table 1. The study revealed that selected seaweed liquid fertilizers might have significant effect on growth parameters like root length, shoot length and leaf area. The increase in growth was found to be concentration dependent. The SLF of *P. tertrastomatica* at 10% concentration showed maximum shoot length (12.1 cm) over the control (8 cm) on 21st day. However the seedlings showed the pronounced growth effect only in the later period on 35th day the shoot is significantly more in *B. juncea* treated with 10% SLF of *P. tetrastromatica*. Increasing root length shoot length and leaf area over the control for all treatments indicate that nutrients in the SLF were absorbed and translocated efficiently by *Brasscia juncia*. Seaweeds contain all the trace elements which improves the growth and yield in cereal crops, vegetables and fruits[10]. Our results were corroborated with the findings of ThirumalThagam *et al.*, (2003)[11] who observes highest values of shoot and root length and leaf area after the applications of seaweed liquid fertilizers of *Caulerpa scalpelliformis* and *Gracilaria corticota*. The low concentration of aqueous extract prepared from *Caulerpa racemosa* and *Gracillaria edulls* promoted the seedling growth biochemical content in *Vignacatajung* and *Dolichos biflorus* (Anantharaj and Venkatesalu, 2001 and 2002)[12]. SLF will be useful to reach the great level of agriculture production due to the presence of growth promoting hormones (IAA and IBA), Cytokinins, Gibberellins, trace elements, vitamins, aminoacids, antibiotics and micronutrients[13].

**Effect of SLF of various algae on the biochemical constituent of *B. juncea*:**

Different concentration of SLF from selected seaweeds showed positive results in the biochemical constituents of the *B. juncea* (Table 2).

**Protein:**

The protein content estimated from *Brassica juncea* on the 7th day of analysis did not show much difference between treated and the control plant. Significant variations are noted in the treated plants on 21st and 35th day of analysis. Among the various SLF used, SLF of *P. tetratromatica* at 10% concentration showed a significant increase in protein content over the control at about 45% on 35th day of analysis.

**Table 1: Effect of SLF of the shoot length, root length and leaf surface area of *B. juncea***

| SI. No | Algal sample | Concent-ration of SLF | Shoot length (cm) | | | Root length (cm) | | | Leaf surface area | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Day | | | Day | | | Day | | |
| | | | 7 | 21 | 35 | 7 | 21 | 35 | 7 | 21 | 35 |
| 1 | Control | 0% | 3.5 | 8 | 13.8 | 0.8 | 3.3 | 3.6 | 1.5 | 1.9 | 2.5 |
| 2 | *U. lactuca* | 5% | 4 | 9.7 | 15.1 | 0.8 | 2.7 | 3.5 | 1.5 | 1.9 | 2.6 |
| | | 10% | 3.9 | 9.5 | 15.1 | 0.9 | 2.7 | 3.4 | 1.6 | 2.6 | 2.8 |
| 3 | *C. scalpelli-formis* | 5% | 4.1 | 9.7 | 15.0 | 0.8 | 2.9 | 3.8 | 1.5 | 2.1 | 2.5 |
| | | 10% | 3.8 | 9.8 | 14.8 | 0.8 | 2.7 | 3.5 | 1.5 | 1.9 | 2.7 |
| 4 | *P. tetrastro matica* | 5% | 4.2 | 9.6 | 15.4 | 0.8 | 2.6 | 3.8 | 1.6 | 1.9 | 2.7 |
| | | 10% | 4.1 | 12.1 | 20.8 | 0.9 | 2.7 | 3.7 | 1.7 | 2.3 | 2.9 |
| 5 | *S. linearfo lium* | 5% | 3.7 | 10.3 | 15.9 | 0.8 | 2.8 | 3.3 | 1.6 | 2.0 | 2.8 |
| | | 10% | 3.8 | 10.1 | 16.2 | 0.9 | 3.0 | 3.9 | 1.5 | 2.2 | 2.8 |

Similar trend was also noted for *S. linearifolium*. The increase in protein content of *B. jnucea* due to SLF of *P. tetrastromatica* and *S. linearifolium* can be attributed to high content of protein in these seaweeds[14].

**Table 1: Effect of SLF of the shoot length, root length and leaf surface area of *B. juncea***

| Algal sample | Concentration of SLF | Protein mg/g | | | Aminoacid mg/g | | | Sugar mg/g | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day | | | Day | | | Day | | |
| | | 7 | 21 | 35 | 7 | 21 | 35 | 7 | 21 | 35 |
| **Control** | 0% | 50 | 63 | 66 | 2.9 | 4.4 | 5.6 | 3.2 | 4.4 | 6.3 |
| ***U. lactuca*** | 5% | 51 | 70 | 68 | 3.3 | 4.5 | 7.2 | 4 | 4.6 | 6.4 |
| | 10% | 5 | 72 | 70 | 3.7 | 4.8 | 5.7 | 4.3 | 4.4 | 8.4 |
| ***C. scalpelli-formis*** | 5% | 52 | 60 | 69 | 3.5 | 5.3 | 7.5 | 3.8 | 4.8 | 6.6 |
| | 10% | 51 | 65 | 71 | 3 | 5 | 6.3 | 3.6 | 4.9 | 8.1 |

| Algal sample | Concentration of SLF | Protein mg/g | | | Aminoacid mg/g | | | Sugar mg/g | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Day | | | Day | | | Day | | |
| | | 7 | 21 | 35 | 7 | 21 | 35 | 7 | 21 | 35 |
| ***P. tetrastro-matica*** | 5% | 50 | 91 | 90 | 3.3 | 5.9 | 6.5 | 3.4 | 5.1 | 6.5 |
| | 10% | 52 | 100 | 96 | 4 | 6.8 | 9.8 | 4 | 5.8 | 7.9 |
| ***S. linear folium*** | 5% | 53 | 72 | 70 | 3.6 | 4.5 | 8.7 | 4.5 | 5.2 | 8.8 |
| | 10% | 5 | 79 | 82 | 3.5 | 5.5 | 9.6 | 4.2 | 5 | 7.1 |

**Amino acids:**

The 7th day analysis on the amino acid content of *B. juncea* treated with the 10% SLF of *P. tetrastromatica* was 37% more over the control. Same treatment showed (54%) more over the control on 21st day. Among the various SLF used SLF of 10% *P. tetrastromatica* and *S. linearifolium* were resulted as the best nutrient source of the growth of *B. juncia*. An array of literature indicates that *Ulva* is the rich source of amino acid whereas in the present study *B. juncea* did not show much response to the SLF of *U. lactuca*. The reason yet to be explored.

**Sugars:**

The SLF of *P. tetrastromatica* at 10% concentration showed 21% increase over control on 21st day of analysis. When compared to the control, the pronounced effects were noted on the plant treated with SLF of *C. scalpelliformis* at 10% concentration this may be possible due to the increasing photosynthetic pigments and enhancing photosynthetic activity[15]. Similar results were obtained by Sreelatha et al., (2017)[16] who observed higher concentration of sugars in the plants treated with seaweed extracts.

Our results of biochemical analysis of the present study were in accordance with the findings of Sathya et al., 2010[17] who reported highest biochemical composition of *Cajanus cajan* with the addition of seaweed liquid fertilizer. Erulan et al. (2009)[18] also obtained enhanced morphological characters and biochemical constituents with the application of SLF.

## Conclusion

The present study has found that utilization of seaweed fertilizer is more effective in crop improvement. It is a sustainable and eco-friendly way. The present investigation on the effect of SLF on *B. Juncea* showed substantial increase in growth parameters and biochemical constituents. The variation in the growth over control was pronounced after 14 days of analysis. Species wise variation in growth induction was distinct. SLF from *P. tetrastromatica* showed enhance growth than the other species. The investigation has reported the concentration depended growth. Effect of 10% SLF of *P. tetrastromatica* was found to be enhancing the growth of *B. juncea*.

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# Comparative Study of Effect of Different Substrates on Yield of Oyster Mushroom and its Nutritional Value

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## Abstract

Mushrooms have been consumed and cherished for their flavour, medicinal properties, economical and ecological significance. They can be grown oncheap and readily available waste materials under crude conditions. Oyster mushroom (*Pleurotus ostreatus*) is one of the most popular edible mushroom varieties under cultivation. Advantage of growing oyster mushroom is that a high percentage of the substrate is converted to fruiting bodies, increasing profitability as compared to other mushrooms making *P. ostreatus* an excellent choice for cultivation. The present study brings out the efficiency of various substrates used in mushroom production. By using these materials in mushroom cultivation an eco-friendly mean of waste disposal is achieved. The present study represents an economic strategy to convert waste products into a nutritious food source.

***Keywords:*** Mushroom, *Pleurotus*, agrowaste, nutrients

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## Introduction

Mushrooms have been identified as an important food source and play an important role in human health, nutrition, and medicinal application, and their significance is increasing ever since its discovery[1]. Oyster mushroom (*Pleurotus species*) is the world's third-largest commercially produced edible mushroom with a fleshy fruiting body belongs to the class Basidiomycetes grows on dead branches of trees. *Pleurotus*

*species* are also rich in medicinal values and they are effective against certain life-threatening diseases. Important medicinal properties endorsed to oyster mushrooms comprise anti-biotic, anti-viral activities, anti-cancer, anti-inflammatory immune - modulator effect and anti-cholesterol effects[2]. Oyster mushrooms attract commercial attention because of its ability in transforming a wide variety of agricultural waste converting into nutritious nicely flavoured quality food by acting on their lignocelluloses.

In India, a huge amount of agricultural wastes is produced annually and is of no use. These agricultural wastes could be used as a source of nutritious food by utilising them as a substrate for mushroom cultivation. *Pleurotus species* can be cultivated on different substrates containing cellulose, hemicellulose, and lignin such as soybean straw, paddy straw, coffee pulp, cotton wastes, corn cobs waste[3], bean straw, crushed bagasse and molasses wastes[4]. In India, mushroom cultivation has never been the priority of farmers despite its delicious taste, highly nutritious, and gives high production with little input. The majority of the mushrooms exhibit substrate type depended on growth that leads to remarkable utilization of nutrients in the agro-industrial bio-wastes. Therefore, the present study is planned to investigate the effect of various substrates like paddy straw, sugarcane bagasse, banana leaves, and their combinations on the growth yield, biological efficiency, and nutritive values of *Pleurotus ostreatus*.

## Materials and Methods

#### Substrate and spawn procurement:

Agro-waste materials such as paddy straw, sugarcane bagasse, and banana leaves were used as the substrates for the present study. Paddy straw and banana leaves were collected from farmers, and sugarcane bagasse was purchased from sugarcane vendors. These substrates were dried and stored. Sorghum grain-based spawn of *Pleurotus ostreatus* was procured for the present study from certified cultivation centre, MSM Mushroom Corner, Mushroom Cultivation Training and Seed Sale, Rediyarpatti, Tirunelveli.

#### Mushroom Cultivation method:

In the present study, the edible oyster mushroom *Pleurotus ostreatus* was cultivated using the standard procedure given by Tamil Nadu Agricultural University. *Pleurotus ostreatus* was cultivated by bag method using three different agro-waste materials as substrates such as paddy straw (Substrates A), sugarcane bagasse (Substrates B), and banana leaf (Substrates C). A mixture of paddy straw, sugarcane bagasse and banana leaf was prepared in the ratio 1:1:1 and used as substrates D.

#### Morphological Parameters:

Length and width of stipe and pileus were measured immediately after harvesting with the help of thread and measuring scale.

#### Biological Efficiency:

Biological efficiency was calculated with the help of the following formula[5]

$$\text{Biological efficiency} = \frac{(\text{Fresh weight of the mushroom})}{(\text{Dry weight of the substrate})} \times 100$$

#### Moisture Content:

A known amount of sample was dried in shade for 12 hours and then moisture content was calculated by using the following formula[6]

$$\text{Moisture content} = \frac{(\text{Initial weight} - \text{Final weight})}{(\text{Initial weight})} \times 100$$

#### Biochemical Analysis:

Phytochemical constituents were analyzed using different extracts of *Pleurotus ostreatus*. Standard procedures were followed for the same[7,8,9]. Total Soluble Protein[10], Carbohydrate[11], Amino Acid[12] and Lipid[13] were estimated using standard procedures.

## Results

#### Duration of Spawn Running

The spawn running duration was calculated by counting the days taken to produce the first pin head from the day of spawning. The time required for the completion of spawn running varied on different substrates ranged from 22 to 37 days (Figure 1a). As per the findings of this study, the growth of *P. ostreatus* mycelia was relatively faster on substrate A (rice straw) followed by substrates C, and D used for the study. The longest spawn running was observed in the case of substrate B (37 days).

#### Number of Pinheads and rate of fruit body development

The average number of pin heads produced in each bag of replicate was taken. Maximum numbers of pinheads (51) were recorded on substrate A followed by substrate C (33) and substrate D (29). Minimum numbers of pinheads were observed on substrate B (12) (Figure 1b). The data presented in Figure 1c and Figure 2A and 2B showed that there a difference between the substrates for the percentage of fruit bodies developed from pinheads. The highest percentage of fruit bodies (84%) were produced by substrate C followed by substrate D (75%), substrate A (66%) and substrate B (64%).

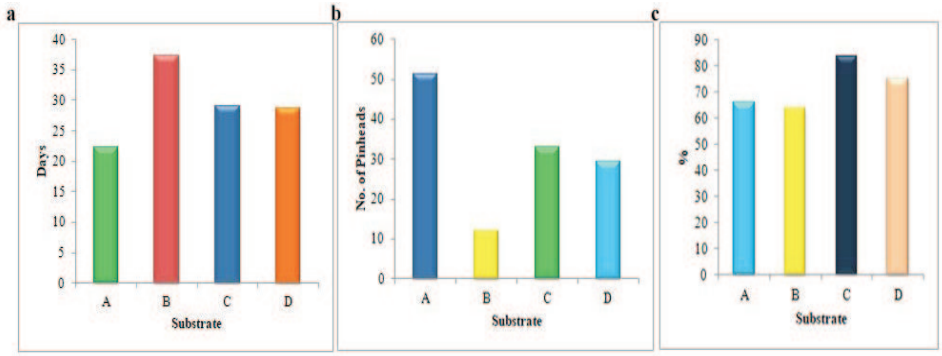


**Figure 1:** Effect of different substrates. (a) on spawn running duration, (b) on number of pin heads, (c) percentage of fruiting body developed from pin heads. Substrate A: paddy straw, Substrate B: sugarcane bagasse, Substrate C: banana leaf

### Yield and Biological Efficiency

Yield was calculated by taking average of the total weight of the fruiting body produced by the triplicates in each set of experiment. Among all the four substrates, substrate A showed the highest percentage of biological efficiency and the yield followed by substrate C respectively. The lowest biological efficiency and yield of *P. ostreatus* was obtained on substrate B (Table 1).

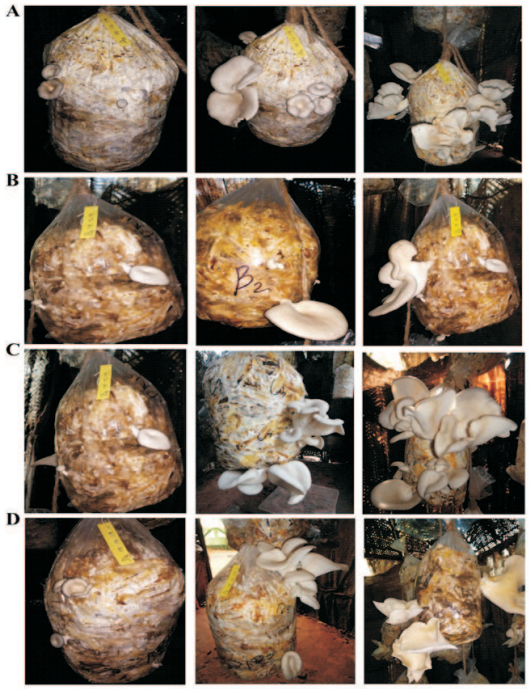


**Figure 2:** Fruiting bodies developed on various substrates. A.Paddy straw, B. Sugar cane bogasse, C. Banana leaves D. Mix of all these

#### Percentage of Biomass Loss

The biomass loss was calculated by comparing the initial and final weight of the substrate and the fruiting bodies produced. Paddy straw biomass loss was 26.3% which shows that degradation and solubilization was more intensive in the substrate A (Table 1).

#### Morphological analysis of the Pileus and Stipe

The length and width of the pileus and stipe were calculated by using thread and scale ruler. The maximum length and width of pileus was obtained on substrate C followed by substrate D. The minimum length and width of pileus was noted on sugarcane bagasse (Table 2). The maximum length of the stipe was obtained on substrate D and substrate A. Similarly, the Maximum width of stipe was obtained on the substrate A and substrate D. Minimum length of stipe was observed on substrate C while the minimum width was noticed on substrate B.

**Table 1: Effect of Different Substrates on the Performance of Pleurotus ostreatus**

| Substrate | Yield (g) | Biological Efficiency (%) | Organic Mass Loss (%) | Pileus | | Stipe | |
|---|---|---|---|---|---|---|---|
| | | | | Length (cm) | Width (cm) | Length (cm) | Width (cm) |
| A | 587.7 | 78.4 | 26.3 | $9.8 \pm 2.2$ | $18.5 \pm 7.8$ | $2.3 \pm 1.5$ | $2.6 \pm 1.1$ |
| B | 101.7 | 13.6 | 12.5 | $5.8 \pm 0.8$ | $8.2 \pm 2.9$ | $2.6 \pm 1$ | $1.2 \pm 0.3$ |
| C | 571 | 76.1 | 24.1 | $11.3 \pm 2.3$ | $21.5 \pm 6.7$ | $1.3 \pm 0.3$ | $2.0 \pm 0.4$ |
| D | 525.7 | 70.1 | 21.6 | $11.3 \pm 3.4$ | $20.3 \pm 6.1$ | $3.1 \pm 1.5$ | $2.3 \pm 0.7$ |

#### Phytochemical content

**Table 2: Effect of different substrates on the nutrient content of *Pleurotus ostreatus* A.E. - Aqueous extract, E.E. - Ethanolic Extract**

| Substrate | Moisture content % | Carbohydrate (mg/g DW) | | Protein (mg/g DW) | | Aminoacid (mg/g DW) | | Lipid (mg/g DW) | |
|---|---|---|---|---|---|---|---|---|---|
| | | A.E. | E.E. | A.E. | E.E. | A.E. | E.E. | A.E. | E.E. |
| **A** | 89.8 | 3.02 | 5.6 | 80.5 | 134.2 | 136.6 | 206.5 | 1.8 | 5.3 |
| B | 91 | 3.7 | 11.6 | 144.1 | 194.1 | 155.9 | 220.3 | 4.7 | 8.6 |
| C | 90.3 | 3.6 | 8.6 | 91.4 | 169.3 | 147.4 | 229.7 | 1.7 | 5.7 |
| D | 90.1 | 3.3 | 6.8 | 99.1 | 184.2 | 144.1 | 243.8 | 3.5 | 7.8 |

The presence of various phytochemical contents was explored using standard procedures. The various extracts of *P. ostreatus* revealed the presence of medicinally important bioactive ingredients. The alcoholic extracts of *P. ostreatus* showed the presence of alkaloid, protein, carbohydrate, phenol, tannin, flavonoid, and saponin, whereas the aqueous extract was found containing protein, carbohydrate, tannin and coumarin (Table 3).

**Table 3: Qualitative Analysis of *Pleurotus ostreatus***

| Test | Water | | | | Hexane | | | | Chloroform | | | | Ethyl acetate | | | | Ethanol | | | | Methanol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D | A | B | C | D |
| **Alkaloid** | - | - | - | - | - | - | - | - | + | + | + | + | - | - | - | + | + | + | + | + | + | + | + | + |
| **Protein** | + | + | + | + | - | - | - | + | + | + | + | + | - | - | - | + | + | + | + | + | + | + | + | + |
| **Carbohydrate** | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - | + | + | + | + | + | + | + | + |
| **Glycoside** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Saponin** | - | - | - | - | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | - | + | + | + | + |
| **Phenol** | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | + | + | + | + | + | + | + | + |
| **Tannin** | + | + | + | + | - | + | + | + | + | + | + | + | - | - | - | - | + | + | + | + | + | + | + | + |
| **Flavonoid** | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | + | + | + | + | + | + | + | + |
| **Steroid** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Phytosterol** | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Quinone** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **Terpenoid** | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | - | - |
| **Cardioglycoside** | - | - | - | - | - | + | + | + | + | + | + | + | - | - | - | - | + | + | + | + | - | + | + | + |
| **Coumarin** | + | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | + | - | - | - | - |
| **Phlobatannin** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

**'+' Indicates Presence '-' Indicates Absence**

### Nutrient content

The moisture content in the substrate was calculated by taking initial and final weight of the substrate. The present study revealed that the highest moisture content was observed in substrate B followed by substrate D and A. The carbohydrate content calculated using standard procedure of Dubiose *et. al.* 1956 revealed that carbohydrate in mushrooms represents the bulk of fruiting bodies ranged from 5.6 to 11.6 mg/g on a dry weight basis in ethanolic extract. Carbohydrate content was found to be maximum in substrate B followed by substrate C. Protein content of *P. ostreatus* was found maximum in substrate B followed by substrate D while the fruit bodies developed from substrate A. The free amino acid content of *P. ostreatus* ranged from 137 mg /g to 156 mg / g in the alcoholic extract of dry thallus and 207 mg/g to 244 mg/g in the alcoholic extract of the dry thallus. The highest amount of amino acids was recorded in the ethanolic extract of substrate B while the lowest amount of amino acids was registered in ethanolic extract of substrate A. The highest lipid content was found in substrate B followed by substrate D while the fruit bodies developed from paddy straw had the lowest lipid content (5.3 mg/g) in ethanolic extract (Table 2).

## Discussion

Our finding in the present experiment is almost similar to the findings of Lalithadevi and Many (2014) who reported that the spawn running period is between 16 - 25 days on paddy straw [14]. It was followed by banana leaves (29 days) and a mix of all the three substrates (29 days) used in the present study. The longest spawn running was observed in the case of sugarcane bagasse (37 days). The results of the spawn run on sugarcane bagasse was not in agreement with the report of Hossain (2017) who stated that *P. ostreatus* completes the spawn run in 17 days on sugarcane bagasse[15]. An increase in the duration for spawn running on lignocellulosic waste materials might be due to the slow hyphal growth of mushroom on substrates[14].

The difference in duration of full mycelial running might be due to the variation in the chemical composition and C:N ratio of the substrate[17]. The spawn running takes 16 - 25 days after inoculation[29]. The variation in the number of days taken for a spawn to complete colonization of a given substrate depends on the function of the fungal strain, growth condition, and substrate type. Our finding was further supported by Hague (2004) and Al Amin (2004) who reported that the highest number of pinheads of oyster mushroom was found on paddy straw[19,20]. Minimum numbers of pinheads were observed on sugarcane bagasse. Almost similar results were reported by Hasan *et.al* 2015 who observed the minimum number of pinheads of oyster mushroom on sugarcane bagasse[21].

The results of the present work agree with the result of Sardar *et. al.* (2016) who reported that the lowest biological efficiency was obtained on sugarcane bagasse[22]. Higher the biological efficiency of different substrates represents its higher suitability for the cultivation of mushroom. According to a report, the increase in the yield of *P. ostreatus* on paddy straw substratum is due to the easy extraction of sugars from cellulosic substances[23]. The superiority of paddy straw over other substrates in the cultivation of *P. ostreatus* with respect to yield and biological efficiency has been reported earlier[24]. The results of the present study also agrees with the result of Ragunathan *et. al.* (1996) who reported that maximum yield is obtained by the cultivation *P. sajor - caju* on paddy straw[25]. Present results were in agreement with the findings of Sardar *et. al.* (2016) who observed the minimum diameter of pileus ($4.10 \pm 0.07$ cm) on sugarcane bagasse[22]. Oyster mushroom quality depends on the length of the stipe. The higher the stipe length, the poorer the quality of the mushroom[26]. Hence growers should use substrates that do not promote excessive growth of stipe length at the expense of marketable yield.

An increased capacity of mushrooms was observed to degrade lignocellulosic materials during the idiophase stage following severe nitrogen and carbon depletion[27]. Protein is an important constituent of the dry matter of mushrooms. The protein content of mushrooms depends on the composition of substratum, size of the pileus, harvest time, and species of mushrooms[28]. The fat and protein content in the mushroom mycelium vary with variations in carbon-nitrogen ratio[29]. The variation in fat protein content depends not only on the species of mushroom but also substratum used, age, part of the fruitification and atmospheric conditions[30]. The phytocompounds present in this edible mushroom are known to play an important role in promoting health. In the present study the changes occurred in the nutrient content of mushrooms cultivated upon different substrates may due to these factors.

## Conclusion

The selection of substrate for the cultivation of oyster mushroom plays a central role in colonization, pinhead formation, fruiting body development, and nutrient content. Among the selected substrates viz. paddy straw, sugarcane bagasse and banana leaves, the highest yield and biological efficiency were recorded in paddy straw. Therefore, paddy straw can be recommended to the cultivators of *Pleurotus ostreatus* for optimum yield and nutrient content.

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# Macroscopic and Microscopic Studies on the Aerial Parts of Selected Species of Lamiaceae

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## Abstract

Morphological and microscopical characters of *Anisomeles malabarica* and *Leucas aspera* of family Lamiaceae are compared. Both taxa are used in traditional medicine. The quantitative morphological characters of stem, leaves and inflorescence are examined and measured. The cross-section of leaf and stem are taken and photographed using TC capture software. Many characters of the plants like leaf arrangement, the color of the flower, and types of trichomes help to identify species.

***Keywords:*** *Leucas, Anisomeles*, Lamiaceae,Morphology, Anatomy

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## Introduction

Lamiaceae is the sixth largest and one of the most important families of angiosperms. It consists of about 236 genera 7173 species. The members of Lamiaceae are mostly aromatic and widely used in folk medicine and essential oil production[1]. *Anisomeles malabarica*R. Br. is commonly known as 'Malabarcatmint' distributed throughout South India. The infusion of the leaf is used for fever, epilepsy and fever. The decoction of the leaf and oil is used externally in rheumatic arthritis[2]. The plant *Leucas aspera* (Willd.) Link. is commonly called *Thumbai* is distributed throughout India. It is used as a laxative, anthelmintic, used to cure bronchitis, jaundice, inflammation, asthma, indigestions, fever, coughs and colds[3].

The morphological characters are essential in the identification and delimitation in many species of Lamiaceae[4]. The anatomical features of the vegetative parts provide important taxonomic information[5]. The present study is carried out to find

the morphological and anatomical characters of the A. *malabarica* and *L. aspera* which can be used to identify the taxa studied.

## Materials and Methods

The fresh plant materials of *Anisomeles malabarica* and *Leucas aspera* were collected from the Harbour beach road and St. Mary's College campus, Thoothukudi in January 2020. The plants were identified with the help of local floras. The collected plants were preserved as per the standard procedure[6]. Voucher specimens of the selected taxa were deposited in the St. Mary's College Herbarium, Research Centre for Plant Sciences, St. Mary's College, Thoothukudi, Tamil Nadu, India.

The macroscopical characters of stem, leaf, inflorescence and flowers of the selected taxa were examined by physical observation. Ten quantitative macromorphological characters viz. plant height, leaf length, leaf width, petiole length, spike length, bract length, calyx length, corolla length, stamen length and pistil length were measured.

The cross-section of the stem and lamina of the selected taxa were taken and examined under a binocular microscope and photographed. The stomatal characteristics were studied by using a stripped epidermal peel.

## Result and Discussion

### Macroscopical characters

The morphological characters of the studied taxa are presented in (Table 1 and Figure 1 and 2). *A.malabarica*, was a shrubby herb and *L. aspera* is a small herb. Both taxa were aromatic. The arrangement of the leaf was opposite decussate in *A. malabarica* and whorled in *L. aspera*. Variations in leaf shape, margin, base and apex of the two taxa are noted. In *Anisomeles* leaf was oblanceolate with acute apex, asymmetric base, leaf yellowish-green, hairy, astringent with a characteristic odour. In *Leucas aspera* leaves were linearly lanceolate, obtuse with crenate margin, pubescent and aromatic. In the selected taxa, the inflorescence position, corolla color, and calyx shape had shown some variation. The corolla color is purple in *A. malabarica* and white in *L. aspera*. The quantitative macromorphological characters were measured and summarized in Table 2. Morphological characters were taxonomically significant for taxa identification and delimitation in Lamiaceae. This result agreed with that of Anhar *et. al.*[7].

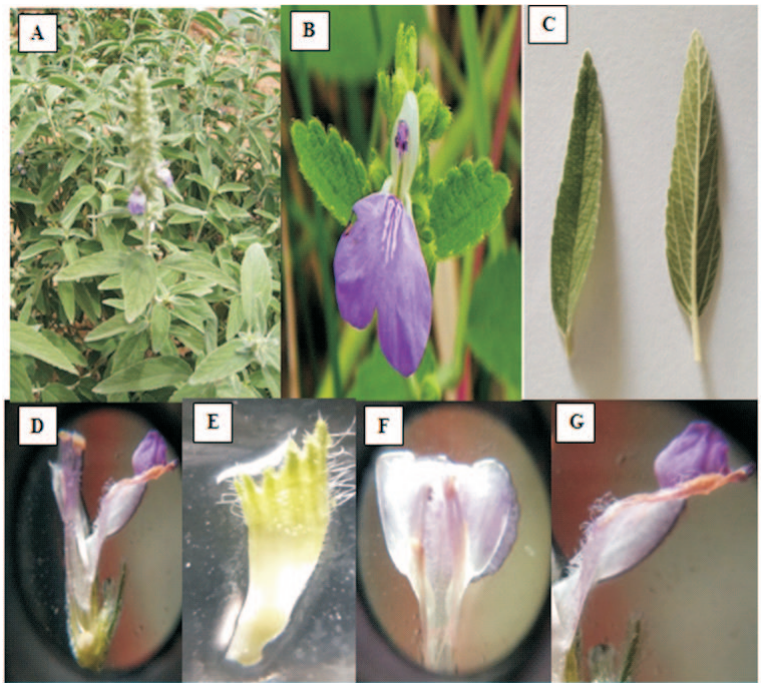


**Figure 1: Morphology of Anisomeles malabarica A.Habit, B. A twig with flower, C. Leaf, D. L.S. of flower, E.Calyx, F. Corolla, G.Epipetalous stamen**

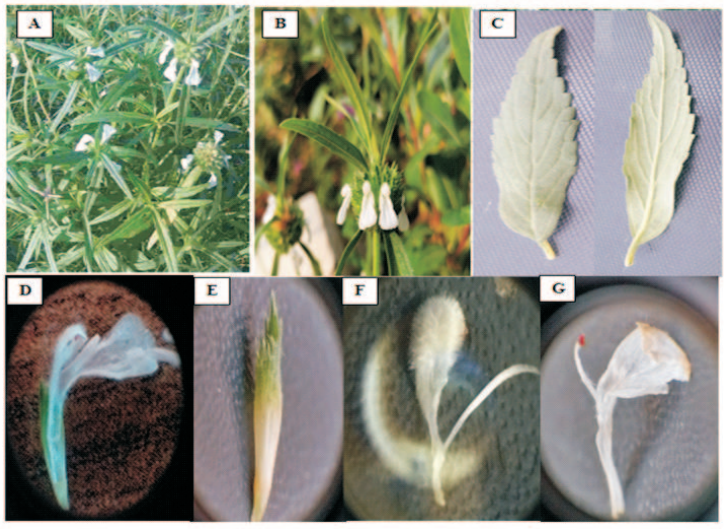


**Figure 2: Morphology of *Leucas aspera*A.Habit, B. A twig with inflorescence, C. Leaf, D. L.S. of flower, E.Calyx, F. Corolla, G. Epipetalous stamen**

**Table 1: Morphological characters of selected species of Lamiaceae**

| Characters | | *Anisomeles malabarica* | *Leucas aspera* |
|---|---|---|---|
| **Duration** | | Perennial | Annual |
| **Stem** | Strength | Erect | Erect |
| | External appearance | Subquadrangular | Quadrangular |
| | Internal | Solid | Solid |
| | Texture | Wolly | Tomentose |
| **Leaf** | Arrangement | Opposite-decussate | Whorled |
| | Composition | Simple | Simple |
| | Shape | Oblanceolate | Linearly lanceolate |
| | Margin | Serrate | Crenate |
| | Apex | Acute | Obtuse |
| | Base | Round | Cuneate |
| **Inflorescence** | Position | Axillary | Terminal |
| | Bract shape | Linear | Linear |
| | Calyx shape | Ovoid or tubular | Tubular |
| | Corolla colour | Purple | White |
| | Corolla shape | Bilipped | Bilipped |
| | Stamen number | 4 | 4 |

**Table 2: Quantitative macromorphological measurements of studied taxa**

| Parts | Size | |
|---|---|---|
| | ***A. malabarica*** | ***L. aspera*** |
| Plant height | 1 -1.6 meter | 0.4 - 0.6 meter |
| Leaf length | $5.1 \pm 0.9$ cm | $7.2 \pm 0.9$ cm |
| Leaf width | $1.5 \pm 0.4$ cm | $1.8 \pm 0.3$ cm |
| Petiole length | $0.8 \pm 0.4$ cm | $1 \pm 0.2$ cm |
| Spike length | $25 \pm 4.2$ cm | $1.8 \pm 0.7$ cm |
| Bract | $1.3 \pm 0.4$ cm | $0.7 \pm 0.2$ cm |
| Calyx length | $0.9 \pm 0.2$ cm | $1 \pm 0.2$ cm |
| Corolla length | $1.3 \pm 0.3$ cm | $1.8 \pm 0.4$ cm |
| Stamen length | $1.1 \pm 0.1$ cm | $0.8 \pm 0.1$ cm |
| Pistil length | $1.2 \pm 0.1$ cm | $0.8 \pm 0.1$ cm |

## Microscopic Observation:

#### *Anisomeles malabarica:*

T.S. of the stem (Figure 3) was a semi quadrangular with four ridges. The epidermis was a single layer covered witha thin cuticle, traversed by glandular trichomes. 2-4 layer of collenchymatous cortex present. The endodermis was distinct. Pith was four angled and wide parenchymatous. Pith has starch grains and calcium oxalate crystals. The leaf was dorsiventral and amphistomatic. The midrib was concavo-convex in

outline. 4-5 layers of collenchyma were present in both sides. Collateral vascular bundle present in the centre of midrib surrounded by parenchyma. Both the epidermis consisted of single-layered polygonal cells. The lower epidermis was covered by thick cuticle than the upper epidermis. Paracytic stomata are seen in both the upper and lower epidermis. The laminar portion of the leaf had a single layer of elongated palisade cells and 3-5 layers of spongy parenchyma. Trichomes were seen on both epidermises[8]. The lamina had many vascular bundles.

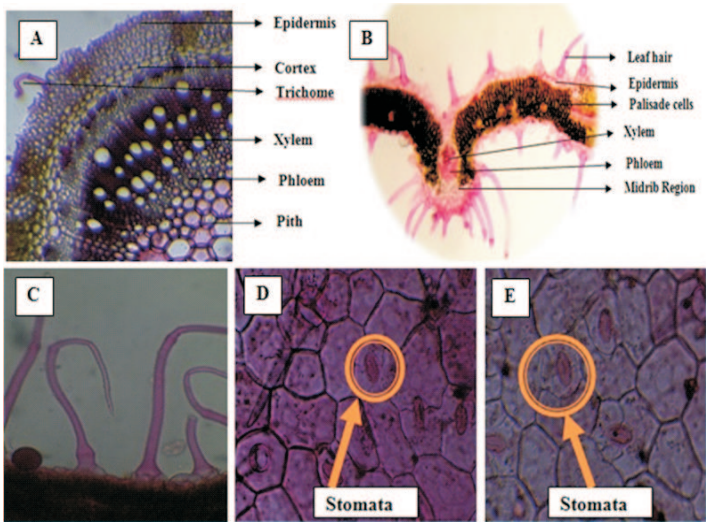


**Figure 3: Microscopic observation of *A. malabarica*.A.Stem (a portion enlarged), B. Leaf, C. Trichome, D. Upper epidermal layer, E. Lower epidermal layer**

***Leucas aspera*:**

In cross-section, the stem (Figure 4) was quadrangular in outline. Collenchymous tissues were present at the corner. The outermost layer epidermis is covered with a thick cuticle. Cortex consisted of parenchymatous cells. The endodermis was distinct. Four collateral vascular bundles were present beneath the ridges. The pith consisted of thin-walled parenchyma cells. The cells have crystals. The presence of glands and exudates on the stem surface indicate the presence of aromatic substances of medicinal values[9].

The leaf was dorsiventral and ampistomatic. T.S. of the leaf showed distinct midrib covered by a thin layer of epidermis, collenchymatous tissues present under the epidermis. The lamina consisted of a thin epidermis covered by a thick cuticle. Mesophyll was with 1 or 2 layers of palisade and spongy parenchyma. Uniseriate

multicellular trichomes were found on both sides of the leaves. Paracytic stomata were observed in the upper and lower epidermis.

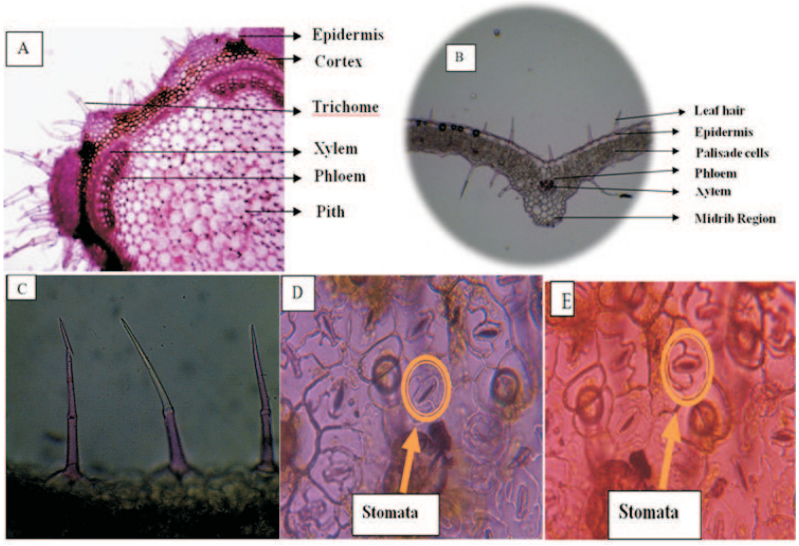


**Figure 4: Microscopic observation of *L. aspera* A- stem (a portion enlarged), B -Leaf, C- Trichome D - Upper epidermal Layer, E - Lower epidermal layer**

## Conclusion

From the present study, it was concluded that the morphological and anatomical features of plants are important in diagnosing the species. The results provide some pharmacognostic standards for the quality control of preparations from the plant in future. In both the taxa studied leaves are aromatic, with trichomes and glandular hairs. The epidermis in both species is with paracytic stomata on both surfaces. The taxa are characterized by the quadrangular stem with crystals and trichomes.

## Acknowledgement

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# Preliminary Phytochemical Screening and Pharmacognostic Studies on Leaves of Selected Weeds

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## Abstract

The present work summarizes the phytochemical and pharmacognostic screening of five selected weeds. The preliminary phytochemical screening was carried out in the different extract of leaves of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Aerva lanata* L. *Boerhaavia diffusa* L. *Bryophyllum calycinum Salisb*. and *Centella asiatica (L)* Urban. After performing an analysis of the following phytochemicals such as alkaloids, terpenoids, steroids, coumarin, tannins, saponin, flavones, phenols, protein, carbohydrate and quinones have screened. The phytochemicals present in these plant extracts can be used for the treatment of different type of acute and chronic disease. The pharmacognostic characters studied for the selected medicinal weeds in the present study could serve as a valuable source of information and provide suitable standards for the identification of these plant materials in future investigation and application.

***Keywords:*** Weeds, Phytochemicals, Pharmacognosy

***Abbreviation:*** T.S - Transverse Section

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## Introduction

Weed "An herbaceous plant" considered unwanted, not valued for use or beauty, growing wild, and was considered to cumber the soil or impede the growth of superior vegetation. The good news is, some of the most popular weeds produce incredible

healing skills, and they're all legal. Many of today's most popular medicines are made from medicinal plant components. *Abutilon indicum* G. Don.*Achyranthus aspera* L. *Aerva lanataL.Boerhaavia diffusa* L. *Bryophyllum calycinum Salisb*. and *Centella asiatica* (L) Urban.interesting plants that have been used for many years in traditional medicine and have been described as one of the best-known remedies for bladder and kidney stone treatment. Everywhere in the plains of India, these plants are common weeds that grow wild. Ethonomically, they have been used as therapeutic agents for a number of diseases[1−4]. With relation to the modern medicine method, it is important to perform thorough investigations of many historically used medicinal plants[5]. The frequent and widespread use of herbs worldwide has raised serious questions about their consistency, protection and productivity[6]. Different criteria, such as photochemical, macroscopic, and microscopic analysis for drug detection and purity, have been evaluated in the present work.

## Materials and Methods

#### Collection and processing:

The whole plant *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Aerva lanataL. Boerhaavia diffusa* L. *Bryophyllum calycinum Salisb*. and *Centella asiatica* (L) Urban was collected from the Korampallam village Thoothukudi District, Tamilnadu. The samples obtained were cut into small pieces and dried in the shade until the fracture was uniform and smooth. Using a blender, the dried plant material was granulated or powdered and sieved to obtain uniform particles using the NO.60 sieve. The final uniform powder was used to extract the active constituents.

### Preparation of extracts for phytochemical screening

#### Cold and Hot maceration method using Soxhelet apparatus:

The 100 gm of the coarse powder of leaf samples were extracted successively with 250 ml of acetone, chloroform, methanol petroleum ether and water in a Soxhlet apparatus by hot maceration method. All the extracts were filtered through Whatman No. 41 filter paper[7,8]. All the extracts (acetone, chloroform, methanol petroleum ether, and water) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedure[9,10]. The alcoholic and aqueous extracts of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Aerva lanata L. Boerhaavia diffusa* L. *Bryophyllum calycinum Salisb*. and *Centella asiatica* (L) Urban. were subjected to various qualitative tests to reveal the presence or absence of common chemical constituents.

## Pharmacognostic study

#### Morphology of Leaf:

Morphology of leaf was studied by naked eye and microscope

#### Microscopic Study:

Transverse sections of leaf were taken, stained with safranin, and mounted in glycerin. Semi-permanent slides were prepared and observed under a compound microscope. Photographs of the sections were taken by photomicroscope under 10X and 45X magnifications.

#### Pattern and Distribution of Stomata:

Pattern and distribution of stomata were studied by the stomata peel method[11]. The peels of the epidermis were stained with safranin and mounted in glycerine. Semi-permanent slides were prepared and observed under the compound microscope.

#### Powder microscopy:

Shade-dried leaves were finely powdered and microscopically studied. Small amounts of different plant powder were put on slides separately and 2-3 drops of chloral hydrate were mounted on each slide and each slide was covered with a coverslip and then examined under the microscope[12]. Different types of cells, such as cork cells, fibres of sieve tubes, lignified fibres, cortex cells, crystals of calcium oxalate, and stomatal cells were noted and photographed.

## Results

The preliminary photochemical screening was carried out on alcoholic and aqueous extract of five selected leaf samples and the results are shown in table 1-11.

#### Leaf Morphology

The leaves are simple, alternate mostly ovate to broadly ovate in *Abutilon indicum, Aerva lanata, Boerhavia diffusa*, and *Centella asiaticaopposite* in *Achyranthus aspera* and *Bryophyllum calycinum. Petiolated leaves* in all the leaf samples studied. Margin is crenate in Abutilon *indicum Bryophyllum calycinum* and *Centella asiatica*. A wavy margin is observed in *Achyranthus aspera*, *Aerva lanata* and *Boerhavia diffusa*. Except for *Bryophyllum calycinum* the leaves of other plants selected are hairy in nature. Length and width of the leaves are 4-12×3.5-8.5 cm in *Abutilon indicum* G. Don. 4-9×2-4 cm in *Achyranthus aspera* L. 2-2.5×1-1.6 cm in *Aerva lanata* L. 3-4.5×2-3 cm *Boerhaavia diffusa* L. 5-13×2-5 cm in *Bryophyllum calycinum Salisb*. and 2.5-3.5×1.5-2.5 cm in *Centella asiatica* (L) Urban (Figure1).

**Leaf Anatomy**

***Abutilon indicum:***

The leaf was dorsiventral and amphistomatic. T. S of *Abutilon* leaf consisted of midrib and lamina. The midrib consisted of single-layered short epidermal cells with cuticle and three different types of trichomes. Below the epidermis, several cells rows of parenchymatous cells on the abaxial side of the midrib but on the adaxial side, there were 3-4 rows of angular collenchyma and 2-3 rows of parenchymatous cells. The vascular bundle was collateral consists of phloem and xylem[13]. Phloem cells were several rows with phloem fibres. Xylem parenchyma cells containing, simple ovoid starch grains. (Figure 1)

***Achyranthes aspera:***

The leaf was dorsiventral. The midrib showed a single-layered epidermis, on both the surfaces; epidermis followed by 4-5 layered collenchymas on the upper side and 2-3 layered on the lower side; ground tissue consisting of thin-walled, parenchymatous cells having a number of vascular bundles; each vascular bundle showed below the xylem vessels, thin layers of cambium, followed by phloem and a pericycle represented by 2-3 layers of thick-walled, non-lignified cells; rosette crystals of calcium oxalate found scattered in ground tissues. Leaf lamina showed single-layered, tangentially elongated epidermis cells covered with thick cuticle having a covering trichomes which were similar to those of stem found on both surfaces; mesophyll differentiated into palisade and spongy parenchyma; palisade 2-4 layered of thick parenchyma larger, slightly elongated in upper, while smaller and rectangular in the lower surface; spongy parenchyma 3-5 layers thick, more or less isodiametric parenchymatous cells; idioblast containing large rosette crystals of calcium oxalate distributed in palisade and spongy parenchymacells; stomata anisocytic and anomocytic in both surfaces (Figure 1).

***Aerva lanata:***

The leaf was dorsiventral and amphistomatic with a prominent midrib and thick lamina[14]. The midrib vascular bundle was single and collateral. It consisted of a dense cluster of thick-walled, narrow angular Xylem elements and a narrow arc of phloem with three sclerenchyma layers beneath the vascular bundle. The adaxial epidermis had wide, rectangular thin-walled cells with a prominent cuticle. The mesophyll consisted of an adaxial zone of palisade tissue of a single row of vertically oblong cylindrical cells[15]. The spongy mesophyll was with 6 or 7 lobed cells that form a filamentous structure (Figure 1).

***Boerhaavia diffusa:***

The leaf was dorsiventral and amphistomatic transverse section of the leaf passing through the midrib showed a single convex shape on the abaxial side. It had crescent-shaped three large central vascular bundles, collateral along with thick-walled parenchyma cells adjoining the phloem were embedded in the ground parenchyma tissue. The lamina of the leaf showed a uniseriate epidermis covered with a thin cuticle. It consisted of stomata and trichomes on both, upper and lower surfaces. The epidermis was accompanied by tissue mesophyll one-two layers of palisade stratum and 3-4 spongy tissue layers (Figure 2).

***Bryophyllum calycinum:***

T.S of the leaf showed upper and lower epidermis with cuticle. The midrib region is broad. The ground tissue is homogenous. The cells were circular and compact with 1-2 vascular bundles. Each vascular strand was conjoint, collateral with xylem facing toward the upperside. The lamina was with flat and uniform epidermal cells. The mesophyll region of the lamina was homogenous and chlorenchymatous and not differentiated into palisade and spongy tissues (Figure 2).

***Centella asiatica:***

The leaf was dorsiventral and amphistomatic. The leaf was dorsiventral differentiated and made up of epidermis, mesophyll, and vascular tissue. Both epidermises were uniseriate, composed of compactly arranged rectangular cells with moderately striated outer walls. Some of the upper and lower epidermis located at the midrib portion were provided with uniseriate, trichomes. The abaxial epidermis contained a patch or band of sclerenchymatous tissue consisted up of 4-5 layer. There was an epidermis, collenchyma, mesophyll, and vascular bundle in the midrib. Lamina was dorsiventrally differentiated with adaxial mesophyll having compact palisade parenchyma with one layer of elongate and barrel-shaped cells. Spongy parenchyma consisted of 3-4 layers of oval-shaped cells. A parenchymatous bundle sheath was encircled the vascular strand[16]. Crystals were present in the mesophyll. Lamina also possessed vascular traces, but not differentiated into distinct metaxylem and protoxylem (Figure 2).

**Pattern and Distribution of Stomata**

In *Abutilon indicum* stomata occurred mostly on the lower surface of the leaf. They were anomocytic type, lacking distinct subsidiary cells and epidermal cells were angular. *Achyranthus aspera* were amphistomatic and possessed anomocytic stomata. Epidermal cells with angular walls and the stomata were well developed with two guard cells. Stomata of *Aerva lanata* were anomocytic with no distinct subsidiary cells. Stomata were circular with slit like stomata pores. Epidermal cells were wide with thin, wavy anticlinal walls. In the case of *Boerhaavia* and *Bryophyllum* the

stomata were anomocytic and epidermals with waxy margin. In *Centella asiatica* the stomata were described to be mostly rubiaceous and anomocytic and epidermal cells were angular in outline (Figure 2).

#### Powder microscopy

Crude drug assessment was an important part of determining the proper identity of the drug. Pharmacognostical parameters were necessary for the confirmation of the identity of the crude drug[17]. The powder microscopic evaluation of leaf of *Abutilon indicum ,Boerhaavia diffusa, Centella asiatica, Achyranthus aspera, Bryophyllum calycinum* sample was useful for setting standards for the identification and the authentication of drugs. Not only in crude form, but also in finished goods. Under microscopical examination, the leaf of *Abutilon indicum, Boerhaavia diffusa, Centella asiatica, Achyranthus aspera, Bryophyllum calycinum* leaf powder shows the fibers, cortical cells, xylem vessels, tracheid cells, trichomes, and stellate hairs. The photographs were presented in Figure 2.

**Table 1: Carbohydrate in the cold and hot leaf extracts of different plant samples. + present, - absent**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| **1** | **Aqueous Solution (hot)** | + | + | + | + | + | + |
| **2.** | **Acetone (cold)** | - | - | - | - | - | - |
| **3.** | **Acetone (hot)** | - | - | - | - | - | - |
| **4.** | **Chloroform (cold)** | - | - | + | - | - | - |
| **5.** | **Chloroform (hot)** | - | - | + | - | - | - |
| **6.** | **Methanol (cold)** | - | - | - | - | + | - |
| **7.** | **Methanol (hot)** | + | + | + | + | + | + |
| **8.** | **Petroleum Ether (cold)** | - | - | + | - | - | - |
| **9.** | **Petroleum Ether (hot)** | - | - | + | - | - | - |

**Table 2: Protein in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | + | + | + | + | + | + |
| 2. | Acetone (cold) | - | - | - | - | - | - |
| 3. | Acetone (hot) | - | - | - | - | - | - |
| 4. | Chloroform (cold) | - | - | - | - | - | - |
| 5. | Chloroform (hot) | - | - | - | - | - | - |
| 6. | Methanol (cold) | - | - | - | - | - | - |
| 7. | Methanol (hot) | + | + | + | + | + | + |
| 8. | Petroleum Ether (cold) | - | - | - | - | - | - |
| 9. | Petroleum Ether (hot) | - | - | - | - | - | - |

**Table 3: Alkaloids in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | + | + | + | + | + | + |
| 2. | Acetone (cold) | - | - | - | - | - | - |
| 3. | Acetone (hot) | - | + | - | + | - | - |
| 4. | Chloroform (cold) | + | + | + | + | - | - |
| 5. | Chloroform (hot) | - | + | + | + | - | - |
| 6. | Methanol (cold) | + | + | + | + | + | + |
| 7. | Methanol (hot) | - | + | - | + | - | - |
| 8. | Petroleum Ether (cold) | - | + | - | - | - | - |
| 9. | Petroleum Ether (hot) | - | + | - | + | - | - |

**Table 4: Terpenoids in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | + | + | + | + | + | + |
| 2. | Acetone (cold) | - | - | - | - | - | - |
| 3. | Acetone (hot) | - | + | - | + | - | - |
| 4. | Chloroform (cold) | - | - | - | - | - | - |
| 5. | Chloroform (hot) | - | + | - | + | - | - |
| 6. | Methanol (cold) | + | - | - | - | - | - |
| 7. | Methanol (hot) | + | + | + | + | + | + |
| 8. | Petroleum Ether (cold) | - | - | - | - | - | - |
| 9. | Petroleum Ether (hot) | - | + | - | + | - | - |

**Table 5: Steroid in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | - | - | + | - | - | - |
| 2. | Acetone (cold) | - | - | - | - | - | - |
| 3. | Acetone (hot) | - | + | - | + | - | - |
| 4. | Chloroform (cold) | - | - | - | - | - | - |
| 5. | Chloroform (hot) | + | + | - | + | - | - |
| 6. | Methanol (cold) | - | - | - | - | + | + |
| 7. | Methanol (hot) | - | - | - | - | - | + |
| 8. | Petroleum Ether (cold) | - | - | - | - | - | - |
| 9. | Petroleum Ether (hot) | - | + | - | + | - | - |

**Table 6:Coumarin in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | + | + | + | - | + | - |
| 2. | Acetone (cold) | - | - | - | - | - | + |
| 3. | Acetone (hot) | + | + | - | + | + | + |
| 4. | Chloroform (cold) | - | - | - | - | - | - |
| 5. | Chloroform (hot) | + | + | - | + | + | - |
| 6. | Methanol (cold) | - | - | - | - | + | + |
| 7. | Methanol (hot) | - | + | - | + | + | - |
| 8. | Petroleum Ether (cold) | + | + | - | + | - | - |
| 9. | Petroleum Ether (hot) | + | - | - | + | - | - |

**Table 7: Tannins in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | + | + | - | - | + | + |
| 2. | Acetone (cold) | - | - | - | - | - | + |
| 3. | Acetone (hot) | + | + | - | + | + | - |
| 4. | Chloroform (cold) | + | + | + | + | + | + |
| 5. | Chloroform (hot) | + | + | - | + | + | - |
| 6. | Methanol (cold) | + | + | + | + | - | + |
| 7. | Methanol (hot) | - | - | - | - | - | + |
| 8. | Petroleum Ether (cold) | - | + | + | + | - | + |
| 9. | Petroleum Ether (hot) | + | - | - | - | + | - |

**Table 8: Saponin in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | - | - | + | - | - | - |
| 2. | Acetone (cold) | - | + | - | + | - | - |
| 3. | Acetone (hot) | - | - | - | - | - | - |
| 4. | Chloroform (cold) | + | + | - | + | + | - |
| 5. | Chloroform (hot) | - | - | - | - | - | - |
| 6. | Methanol (cold) | - | - | + | - | - | + |
| 7. | Methanol (hot) | - | - | - | + | - | + |
| 8. | Petroleum Ether (cold) | + | + | - | + | + | + |
| 9. | Petroleum Ether (hot) | - | - | - | - | - | - |

**Table 9: Flavonoids in the cold and hot leaf extracts of different plant samples**

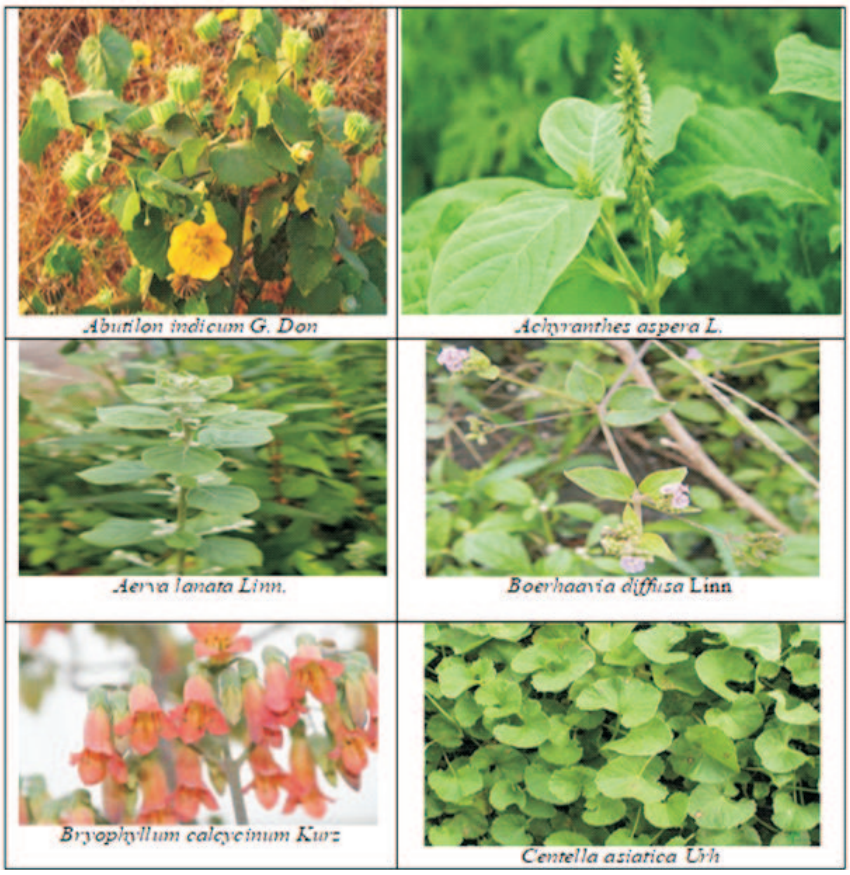
| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | + | + | + | + | + | + |
| 2. | Acetone (cold) | - | - | - | - | - | - |
| 3. | Acetone (hot) | + | - | - | - | + | - |
| 4. | Chloroform (cold) | - | + | + | - | - | + |
| 5. | Chloroform (hot) | - | - | - | - | - | - |
| 6. | Methanol (cold) | - | - | + | + | + | + |
| 7. | Methanol (hot) | + | + | + | + | + | + |
| 8. | Petroleum Ether (cold) | - | + | - | + | + | - |
| 9. | Petroleum Ether (hot) | + | - | - | - | + | - |

**Table 10: Quinine in the cold and hot leaf extracts of different plant samples**

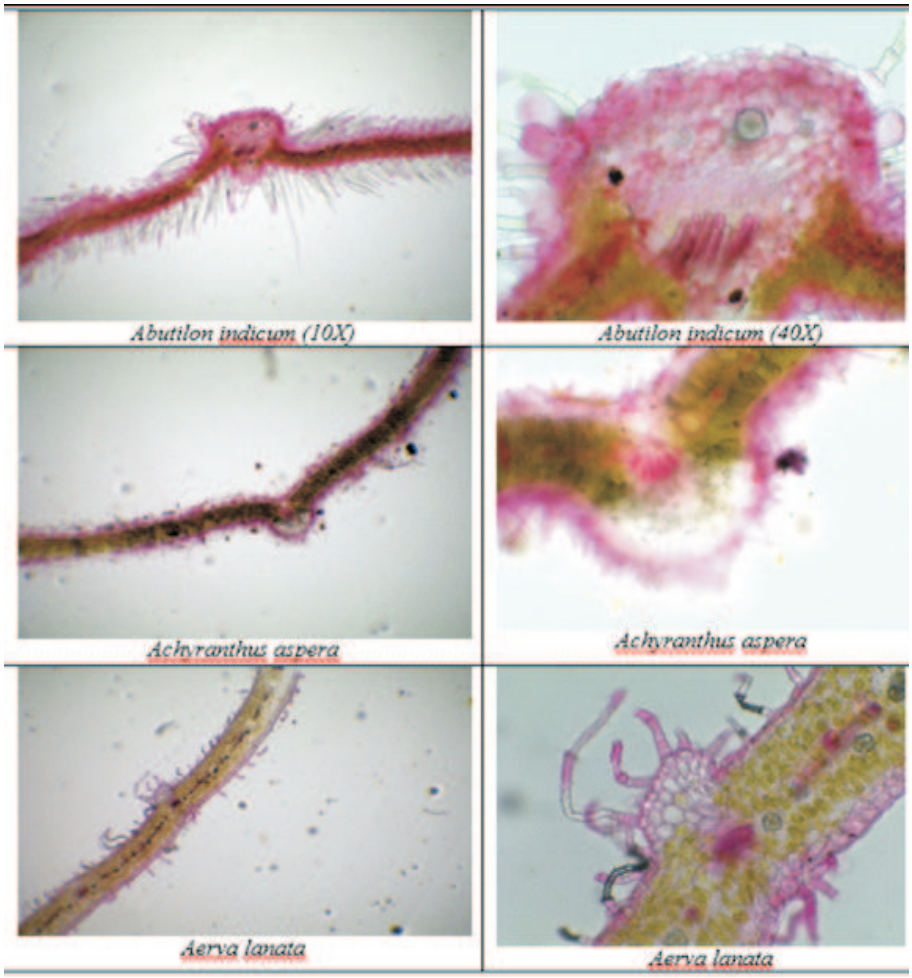
| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | - | + | + | - | - | - |
| 2. | Acetone (cold) | - | - | - | - | - | - |
| 3. | Acetone (hot) | + | - | - | - | - | - |
| 4. | Chloroform (cold) | - | - | - | - | - | - |
| 5. | Chloroform (hot) | - | - | - | - | - | - |
| 6. | Methanol (cold) | - | - | - | - | - | - |
| 7. | Methanol (hot) | + | - | - | - | - | - |
| 8. | Petroleum Ether (cold) | - | + | - | - | - | - |
| 9. | Petroleum Ether (hot) | - | - | - | - | - | - |

**Table 11: Phenols in the cold and hot leaf extracts of different plant samples**

| Sl.No | Solvent | Plant 1 | Plant 2 | Plant 3 | Plant 4 | Plant 5 | Plant 6 |
|---|---|---|---|---|---|---|---|
| 1 | Aqueous Solution (hot) | + | + | + | + | + | + |
| 2. | Acetone (cold) | - | - | - | - | - | - |
| 3. | Acetone (hot) | - | - | - | - | + | - |
| 4. | Chloroform (cold) | + | - | - | + | + | - |
| 5. | Chloroform (hot) | - | - | - | - | - | - |
| 6. | Methanol (cold) | + | + | - | - | + | + |
| 7. | Methanol (hot) | - | - | - | + | - | - |
| 8. | Petroleum Ether (cold) | + | + | - | - | - | - |
| 9. | Petroleum Ether (hot) | - | - | - | - | - | + |

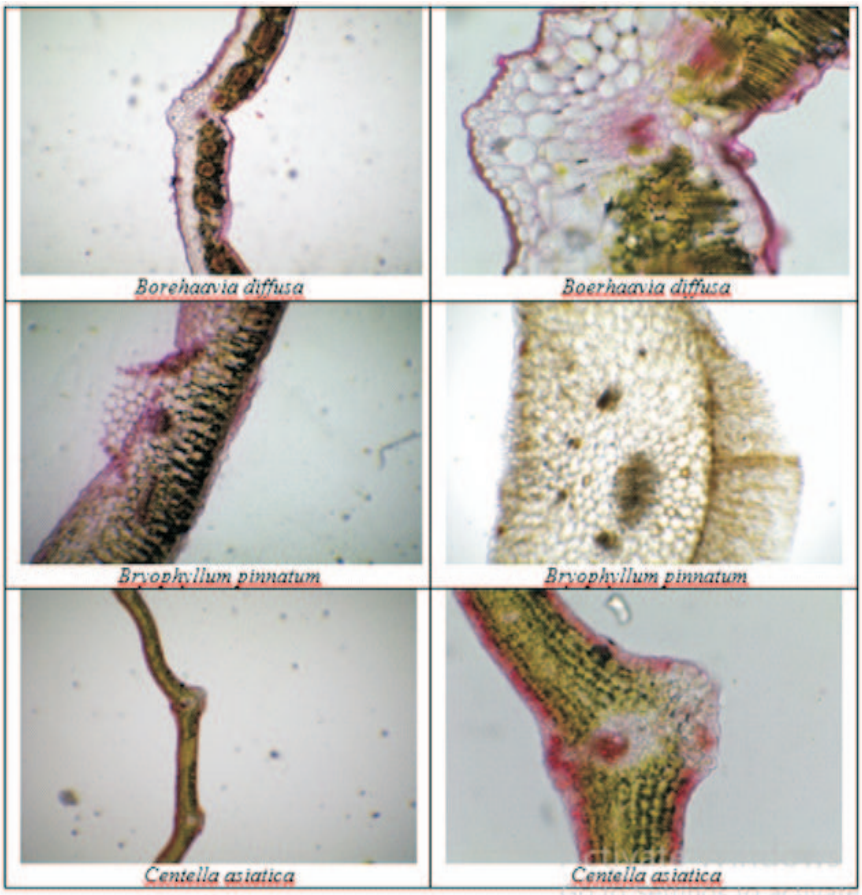

Abutilon indicum G. Don
Achyranthes aspera L.
Aerva lanata Linn.
Boerhaavia diffusa Linn
Bryophyllum calycinum Kurz
Centella asiatica Urb

**(A)**

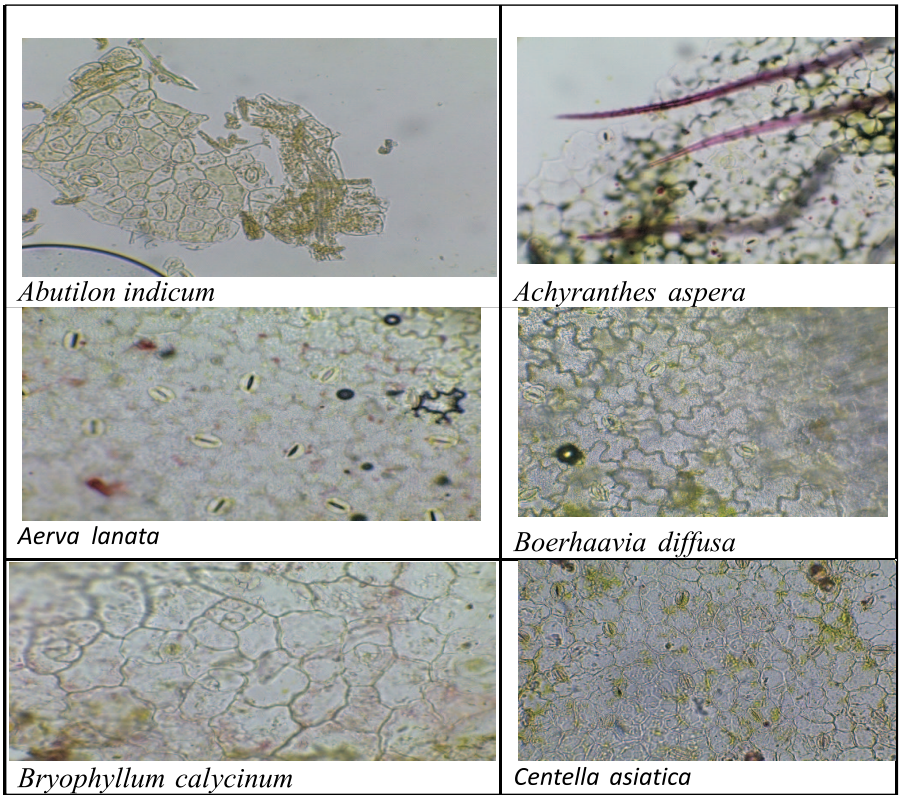



**(B)**

**Figure 1: Habit and Transverse Section of leaf. A. Habit of *Abutilon indicum, Achyranthus aspera, Aerva lanata, Boerhavia diffusa, Bryophyllum calycinum,* and *Centella asiatica*. B. T.S of leaf under 4X (whole T.S. of leaf) and 40X (midrib region) of *A. Indicum, A. aspera* and *A. lanata respectively*.**


Borehaavia diffusa
Boerhaavia diffusa
Bryophyllum pinnatum
Bryophyllum pinnatum
Centella asiatica
Centella asiatica

**(A)**




**(B)**

**Figure 2: Transverse section and stomata peel. T.S. of *Boerhavia diffusa, Bryophyllum calycinum,* and *Centella asiatica*.B. Leaf epidermal peel showing stomata in *Abutilon indicum, Achyranthus aspera, Aerva lanata, Boerhavia diffusa, Bryophyllum calycinum,* and *Centella asiatica***

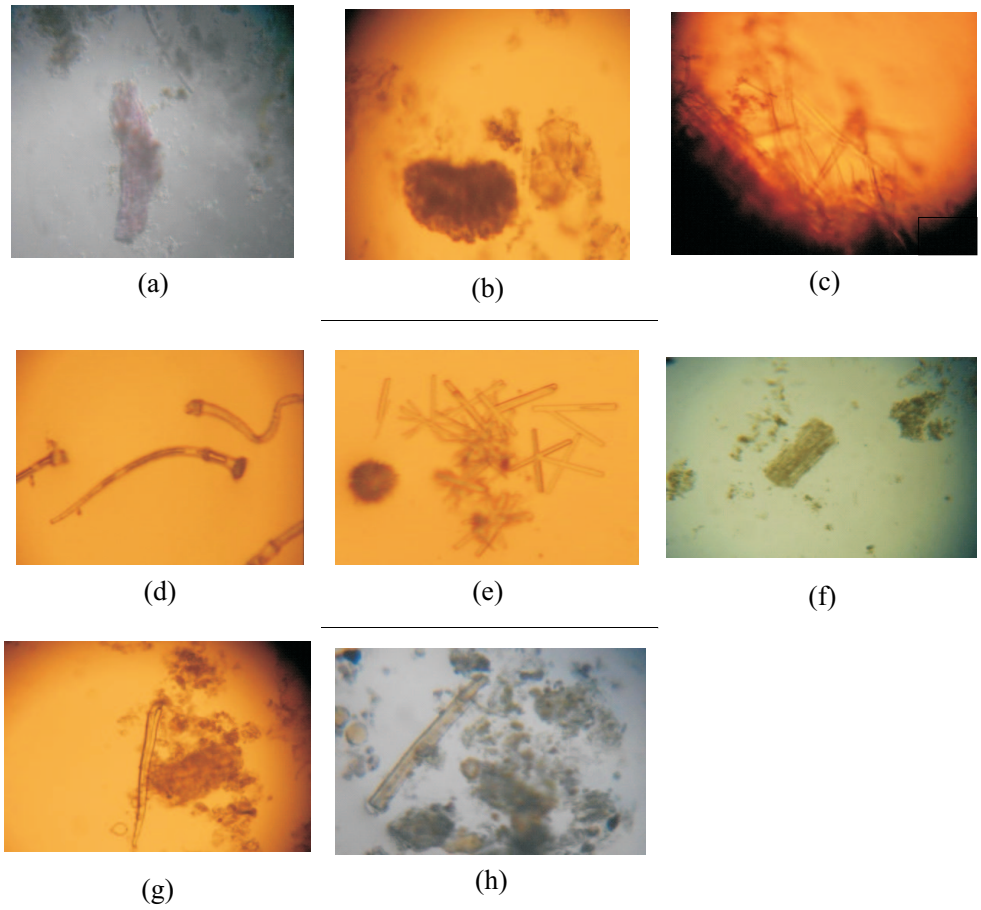



**Figure 3: Powder microscopy. *Abutilon indicum* leaf powder shows (a) xylaryfibers and (b) cortical cells; *Achyranthes aspera* leaf powder shows (c, d) long multicellular warty trichomes, (e) Stellate hair; *Boerhaavia diffusa* leaf powder shows (f) xylem vessel; *Bryophyllum calycinum* leaf powder shows (g) trichome; *Centella asiatica* leaf powder shows (h) tracheids**

## Discussion

An integral part of establishing the correct identification and consistency of a crude drug is the establishment of standards. These criteria must be defined before any drug can be included in pharmacopeia. It is possible to obtain much of the knowledge about the identity, purity, and consistency of the plant material from its macroscopy, microscopy, and phytochemical parameters[18,19].

The phytochemical investigation showed the presence of carbohydrates, protein, alkaloids, terpenoids, coumarin, tannins, saponin, flavonoids, quinine, and phenols in both aqueous and alcoholic extracts. The presence of these constituents is responsible for the wide medicinal use of these selected weeds leaves[20]. Leaf morphology, leaf anatomy and powder microscopy investigations are helpful in the preparation of monograph. The leaf powder showed the fibers, cortical cells, xylem vessels, tracheid cells, trichomes and stellate hairs[21,22]. stomata are anomocytic. Starch grains and calcium oxalate crystals were found in the *Aerva* leaf[23].

## Conclusion

It is concluded that the phytochemicals present in this plant extracts may also be useful for the treatment of different type of acute and chronic disease, which are correlated with the ethanobotanical data on the use of this plant in Indian Folklore and Ayurveda. The microscopic characters of the leaf powder of herbal drug of *Abutilon indicum* G. Don. *Achyranthus aspera* L. *Aerva lanataL.Boerhaavia diffusa* L. *Bryophyllum calycinum Salisb*.and *Centella asiatica* (L) Urban. To create the correct identification and consistency of the crude medication, these quality requirements may be integrated into quality management monographs. The plant can be exploited to discover bioactive products that can contribute to the production of new pharmaceuticals that meet clinical needs.

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# Isolation and Screening of Microalgae from Natural Habitats in The Thoothukudi Coast for Antibacterial Sources

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## Abstract

Marine phytoplankton is composed of floating microalgae. These microalgae may number several thousand in the sea and they vary in size from about 1 ?m to few millimeters. These microscopic, single-celled organisms are found in the greatest abundance in near-shore coastal areas, typically within the upper 50 m (160 ft) of the water column. Phytoplankton exhibit a tremendous variety of cell shapes, many with intricate designs and ornamentations. The scientific studies on microalgae are significant because of their dynamic growth characteristics, potent reproducibility, easily available in *vitro* culture method, distinguished taxonomy, hidden medicinal properties, and possible gene recombination ability. The present work details the microalgae isolation from natural habits in Thoothukudi sea, culture, and identification of their antibacterial potentiality. The isolated microalgae were studied under a microscope and were used as inoculum for the culture. Only one microalga identified based on morphology is *Chlorella* sp. In our study, algae biomass growth assessed based on the optical density (OD680 nm). Antibacterial activity of the aqueous and the methanol extracts of microalgae isolated was studied against two bacterial strains (E. *coli* and *Bacillus* sp.). In the present screening, methanolic extract of microalgae isolated showed the highest antibacterial activity against both the strains.

***Keywords:*** *Chlorella*, Algalculture, Antibacterial activity

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## Introduction

Marine microalgae are the microscopic unicellular plants of the sea. They are generally free-living pelagic forms and their size ranges from 2 to 20 m. The important components of the microalgae are the diatoms and dinoflagellates. The microalgae play a critical role in the coastal and marine aquaculture of fish, mollusks, shrimps and oysters.

Microalgae have been suggested as a potential feedstock for fuel production due to a number of advantages including higher photosynthetic efficiency, higher biomass production, and higher growth rates when compared to other energy crops. Microalgae represent an exceptionally diverse but highly specialized group of microorganisms adapted to various ecological habitats. Microalgae produce large amounts (e.g. 20-50% dry cell weight) of tri acylglycerols (TAG) for storage as lipids when under stress or other adverse environmental conditions. Microalgae with high oil productivities are desired for producing bio-diesel. Depending on the species, microalgae produce many different kinds of lipids, hydrocarbons and other complex oils.

Algae are the oxygen producers in aquatic environments especially planktonic microalgae. These microorganisms are widely distributed in nature and have adapted to different environments with great diversity in size, morphology, life cycle, pigments and metabolism. The production of many useful materials in cosmetic and medical fields depends on microorganisms. Hence there is a lot of research in the area.

The microalgae thrive in a wide range of habitats including fresh and saltwater, brackish, marine and soil environments. They have tolerance for a wide range of temperatures, salinities and pH values, different light intensities, and conditions. They can grow alone or in symbiosis with other organisms. They are the most efficient primarily biomass producers, accounting for the fixation of 30-50% of the inorganic carbon from the atmosphere[1]. They play an important role in $CO_2$ recycling through photosynthesis, which is similar to higher plants in $O_2$ evolved systems (PSI and PSII).

Considering the above facts the present work concentrated on isolation, identification, and culture of microalgae collected from the seawater of Thoothukudi coast and their potential in antimicrobial activity.

## Materials and Methods

#### Isolation of inoculums:

The seawater samples were collected from shallow regions of Thoothukudi coast. Then the water samples were analysed for microalgae under the microscope using 1 ml of water sample.

## Culture of microalgae

#### Culture of microalgae by using liquid media:

The culture of micro algae was carried out by following the procedure of Longhurst, A.R., 2017[2]. For culture, 10 ml of water sample containing microalgae was blended with 1000 ml of marine water and this mixture is mixed well. To this solution, TMRL nutrient medium was added to the culture flask and fix the air inlet pump inside the flask for better aeration. This solution mixture is fatherly kept for growth for a week time and the growth is recorded every day. Standardization based on the OD680 of isolate stocks was carried out previous to growth media, temperature, and pH and salinity tests. First, all isolates were centrifuged and the supernatant as discarded. Next, 45 ml of autoclaved and filter sterilized seawater was added to all stocks and the pellet was resuspended. Then, 500 $\mu$l of each were taken and placed in a cuvette to measure their OD in a Spectrophotometer, being seawater the blank in all cases.

#### Preparation of TMRL Medium:

TMRL medium was prepared according to the standard protocol of Tung Kang Marine Res. Lab. Potassium nitrate -10gm/100ml of Distilled Water (DW); Sodium orthophosphate -1gm/100ml of DW; Ferric chloride -0.3gm/100 ml of DW; and Sodium silicate -0.1gm/100 ml of DW were prepared. The chemicals were kept separately in a 100ml reagent bottle.1ml each to 1liter of sterilized seawater was added. This medium can be used for the mass culture of diatoms.

#### Culture of microalgae by using Agar plate:

The method was followed after Brand L. E, 1990[3]. For the agar plate study, 2.8 g of Nutrient agar medium is measured and poured into 1000 ml of distilled water. This agar mixture is kept for sterilization for obtaining a stable and microbe-free solution. After the sterilization process, the room-cooled medium was poured into the agar plates and kept under room temperature for incubation. After the medium plates come to hand-bearable condition, 1 ml of inoculum was introduced into the medium plates by the spread plate method.

#### Morphological identification:

Microalgal cultures were initially separated based on morphological examination. This general classification method was only used to distinguish isolates on the most basic level. Identification of these isolates to the genus level was based on the morphology of the individual cells following microscopic examination. The strains were identified using the method similar to those reported by Wehr and Sheath[4]. Each isolate in the collection was labeled and photographed at three magnifications

(20×, 40×, 60×) using the Nikon Eclipse E800 microscope (Nikon Inc., Tokyo, Japan) with the DXM1200 digital camera and the ACT-1 software program.

#### Cultivation and harvesting:

Each algal culture sample was monitored every day for cellular growth rates by measuring optical density at 680 nm. The cultures were continuously aerated using air pumps with air stones, and the specified media was added to each culture at the end of every week. Constant mixing of the algal culture in the tank was provided by the aeration. The temperature of the mass culture of algae in the tank remained between 21°C and 32°C.

#### Antibacterial activity of microalgae by Disc diffusion method:

The protocol standardized by Prakash J.W. *et al.* 2011 was used for testing the antibacterial activity[4]. Disc diffusion method was used for antibacterial activity. A stock solution of micro algal extract was prepared by dissolving 0.1 g of the extract with 10 mL of their respective solvents (distilled water and absolute ethanol) to produce a final concentration of 10 mg/mL. 20 $\mu$L of each dilution was impregnated into sterile, blank discs 6 mm in diameter. 5 $\mu$L of extract was spotted alternately on both sides of the discs and allowed to dry before the next 5 $\mu$L was spotted to ensure precise impregnation. Distilled water and ethanol-loaded discs were used as negative controls for aqueous and ethanol extracts, respectively. All discs were fully dried before the application on the bacterial lawn. The positive control used is ampicillin antibiotic discs for all E. *coli* and *Bacillus* sp. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (IZ) around the discs. The assay was repeated thrice. Antibacterial activity was expressed as the mean of inhibition zone diameters (mm) produced by the algal extract.

## Result and Discussion

Native species of microalgae were isolated from natural water bodies in Thoothukudi District, Tamil Nadu and were screened for their potential as antibacterial sources. In agar plate culture, two weeks after initial incubation, most of the plates showed bacterial colonial formations on top of the filter membrane; interestingly, some plates also showed strains growing beneath the filter surface. In some instances, fungal growth was observed throughout the filter surface. In some cases the fungal growth was so dense that no colonies could be rescued and those plates were discarded.

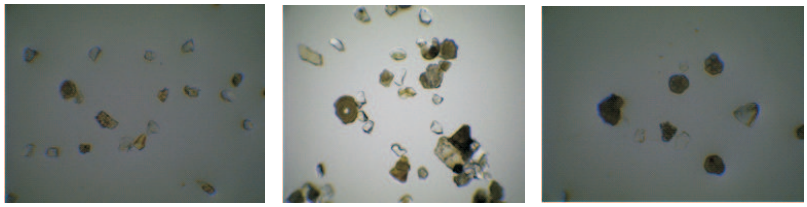
The isolated microalgae from seawater sample are considered as inoculum and were cultured into liquid TMRL media for biomass production. After 4 weeks the culture beakers showed decent/high biomass production. The green and yellow microorganisms from the medium were used for the identification. The microalgae

obtained from the sampling location are shown in Figure 1. Because of time duration and the pandemic situation, only one microalga is identified based on morphology is *Chlorella sp.* (Figure 1).

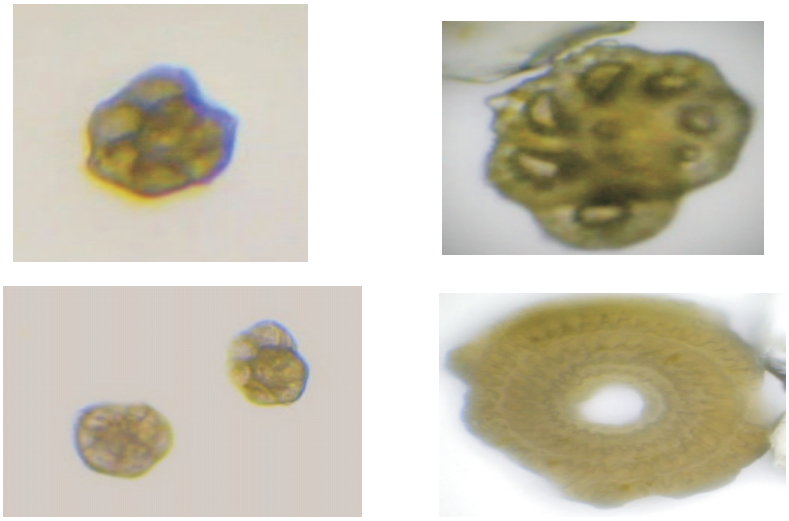
Global growth in energy, demand led to the exploitation of non-conventional renewable energy sources. One of these examples of a renewable energy source is the biomass of algae[5]. Its cells live in aquatic environments or in humid spaces, either in salt or freshwater. Since they are autotrophic organisms, they function as producers of organic matter. They are able to efficiently use less fertile areas more than energetic plants such as rapeseed or soya[6]. They are also capable of growing in waste water. Algae can assimilate carbon dioxide as well as phosphorous and nitrogen derived from air pollutants, and municipal and industrial sewage. For the proper growth of algae, not only is nonorganic carbon required but also nitrogen and phosphorous[7].

In our study, the growth of algae biomass was assessed based on the optical density OD 680, and results were presented in Fig.1. It was found that at the initial stage of the culture algae cells were being exposed to the new environmental conditions, although they earlier stayed in the anabiosis state. This led to inhibition of the cell division, which was associated with photoinhibition, particularly during the first three days. At the time, the optical density, as well as nitrogen and phosphorous contents, varied very slightly. The next step involved the acceleration of metabolic processes. The increase in optical density until the 3rd and 4th day of the culture was relatively low, but the microalgae assimilated the highest amount of nutrient media. This resulted in a significant change in optical density in the following days. The value increased three-fold (from 0.11 to 0.34) and the nutrient content decreased to 7th and 8th days so it showed again a decrease in growth rate. Changes in optical density and nutrient contents were statistically significant.

Antibacterial activity of the aqueous and methanol extracts Chlorella sps isolated from natural seawater in Thoothukudi were studied against two bacterial strains (E. *coli* and *Bacillus* sp). In the present screening, the methanolic extract of Chlorella showed the highest antibacterial activity against both strains (Table 1). The ability to produce antimicrobial activity may be significant not only for the defense of the algae but also as a source of novel bioactive compounds. Several lead compounds with antimicrobial properties have been identified due to screening in microalgae. Some of the substances identified include Chlorellin[8], Parsiguine[9], Nostocyclyne A[10], and Nostofungicidine[11]. This study confirmed the presence of antimicrobial activity of the supernatant and methanolic extract of microalgae.



**(A)**



**(B)**

**Figure 1: Micro algae isolated from the Thoothukudi sea A. Mixture of Microalgae isolated from the sea water under light microscope. B. Single micro algae isolated from the culture under light microscope (*Chlorella* sp.).**

**Table 1: Antibacterial activity of Chlorella extracts against E. *coli* and *Bacillus* sp.**

| **Extracts** | ***E. Coli*** | ***Bacillus sp*** | **Ampicillin (control)** |
|---|---|---|---|
| | **Diameter of Inhibition zone (mm)** | | |
| **Methanol** | 10 | 14 | 15 |
| **Aqueous** | 7 | 9 | 10 |

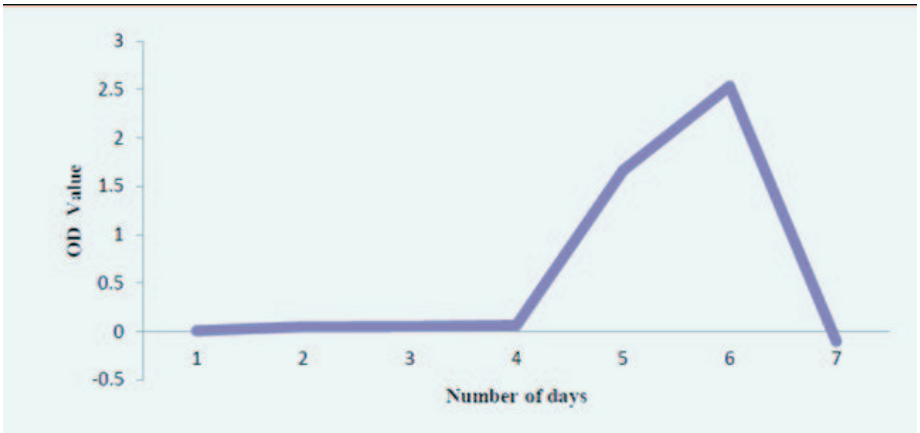



**Figure 2: Growth curve of Micro algae isolated from the Thoothukudi Sea in TMRL nutrient medium**

## Conclusion

Microalgae are increasingly used as a potential renewable energy source. One marine *Chlorella* sp. was isolated from the water samples collected from the shallow coastal region of Thoothukudi and cultured in TMRL media for biomass production. The method standardized in this work can be further exploited in growing the specific *Chlorella* sps in huge bioreactors for its industrial production. Further, the negligible or no bacterial growth in *Chlorella* cultured vessels hints its possible application in antibiotic industry. The present work has to be extended to develop refined findings in this area.

## Acknowledgement

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# Phytochemical Screening and Antioxidant Activity of *Tecoma Stans* (L.) Juss. Ex Kunth

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## Abstract

*Tecoma stans* (L.) Juss. Ex Kunth (Family:Bignoniaceae) was collected from near Government Polytechnic College, Thoothukudi, Tamil Nadu which is one of the common ornamental shrub. In the present study petroleum ether, acetone and ethanol extracts of stem, leaves and flower of *Tecoma stans* were subjected to phytochemical analysis and antioxidant activity assay.The qualitative analysis revealed the presence of alkaloids, carbohydrate, coumarins, flavones,phenols, protein, quinones, starch and terpenoid in petroleum ether, acetone and ethanol extract of stem, leaves and flowers of T. stans. All the extracts showed dose dependent DPPH radical scavenging activity and ethanol extract exhibited highest DPPH radical scavenging activity compared to other solvents.

***Keywords:*** *Tecoma stans*, phytochemical analysis, antioxidant activity assay, DPPH radical scavenging activity

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## Introduction

Herbal medicines are far superior to the synthetic drugs because they are naturally occurring, easily available without cost and have minimum side effects. Medicinal plants are the major sources of conventional medicine, which means medicinal plants are used by more than 3.3 billion people in the developing countries regularly[1]. There is a promising future of medicinal plants as there are about half million plants around the world, and most of them are not investigated yet for their medical activities

and their hidden potential of medical activities could be decisive in the treatment of present and future studies[2].

Almost all the parts of *Tecoma stans* are of medicinal importance and used traditionally for the cure of various diseases. The leaves,barks and roots of *T. stans* have been used for a variety of purposes in the field of herbal medicine.Bark shows smooth muscle relaxant, mild cardio tonic and chlorotic activity. It has various applications include the experimental treatment of diabetes, digestive problems, control of yeast infections and other medicinal applications. It contains several compounds that are known for their catnip like effects on felines. It is stated that the root of the plant is a powerful diuretic, vermifuge and tonic. A grinding of the root of *T. stans* and lemon juice is reportedly used as an external application and also taken internally in small quantities as a remedy for snake and rat bites[3]. The current study was carried out to examine the plant *T. stans* for its phytochemicals and antioxidant activity.

## Materials and Methods

#### Collection and Processing

The stems, leaves and flowers of *Tecoma stans* were collected near Government Polytechnic College, Thoothukudi, Tamil Nadu. The collected fragments were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant material was granulated or powdered by using a blender and sieved to get uniformed particles by using sieve No. 60. The chief constituents of the plant were extracted by using the final uniform powder.

### Preparation of extracts of Phytochemical screening:

#### Hot maceration method:

The coarse powder of sample (100 g) was extracted successively with petroleum ether, acetone and ethanol using Soxhlet apparatus. Whatman No.41 filter paper was used for the filtration of all the extracts. All the extracts (petroleum ether, acetone and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures[4–6].

### Qualitative phytochemical analysis of different extracts

The chemical test for various phytoconstituents in the extracts were carried out as described below:

#### Test for carbohydrates

1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added with 2ml of plant extract. Presence of purple or reddish color indicates the presence of carbohydrates.

#### Test for tannins

To 1ml of plant extract, 2ml of 0.7M NaOH and few drops of Folin-Denis reagent was added. Formation of dark blue or greenish black indicates the presence of tannins.

#### Test for saponins

2ml of distilled water was added to 2ml of plant extract and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1cm layer of foam indicates the presence of saponins.

#### Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Appearance of yellow color indicates the presence of flavonoids.

#### Test for carbohydrates

1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added with 2ml of plant extract. Presence of purple or reddish color indicates the presence of carbohydrates.

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To 1ml of plant extract, 2ml of 0.7M NaOH and few drops of Folin-Denis reagent was added. Formation of dark blue or greenish black indicates the presence of tannins.

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#### Test for flavonoids

To 2ml of plant extract, 1ml of 2N sodium hydroxide was added. Appearance of yellow color indicates the presence of flavonoids.

#### Test for protein

**Ninhydrin test**

To 1 ml of the sample add few drops of 0.2% ninhydrin solution. Appearance of pink or purple colour indicates the presence of protein.

#### Test for anthraquinones

To 1ml of plant extract few drops of 10% ammonia solution was added, appearance pink color precipitate indicates the presence of anthraquinones.

## Antioxidant Assays

#### DPPH free radical scavenging assay

The ability of the samples to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Shen [7]. Stock solution of compound was prepared to the concentration of 10 mg/ml. Different concentration of the extract (100, 250 & 500 g) of sample were added, at an equal volume to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min in dark at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. Ascorbic acid was used as standard control. Blank is pure methanol and control sample is methanol along with DPPH solution. The radical scavenging activity of sample was determined in percentage inhibition using the following formula

$$\% \text{ of Inhibition} = (\text{A of control} - \text{A of Test})/\text{A of control} * 100$$

## Result

#### Preliminary phytochemical analysis

Phytochemicals are non-nutritive plant chemicals that have either defensive or disease protective properties. They are non-essential nutrients and mainly produced by plants to provide them protection. These phytochemicals, either alone and or in combination, have tremendous therapeutic potential in curing various ailments and they offer protection against numerous diseases and disorders[8].

Therefore, the current study is attempted to find out the presence of preliminary phytochemicals present in petroleum ether, acetone and ethanol extract of stem, leaves and flowers of *Tecoma stans* and the results are depicted in Table 1-3.

The result of preliminary phytochemical screening revealed the presence of alkaloids, carbohydrate, coumarins, flavones, phenols, protein, quinones, starch and terpenoid in petroleum ether, acetone and ethanol extract of stem, leaves and flowers of *T. stans*. Anthroquinones present in the different extracts of leaf and stem of *T. stans* whereas saponin was present only in petroleum ether, acetone and ethanol extract of leaves of *T. stans*. Steroids were present in ethanol extract of stem and all the three extracts of flower. Presence of tannin was observed in ethanol extracts of stem and flowers.

## Antioxidant Activity:

### DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, acetone and ethanol extracts of stem, leaves and flowers of *T. stans* are shown in figure 1-3. Among the solvent tested, ethanol extract exhibited highest DPPH radical scavenging activity. At 10% concentration, ethanol extract of *T. stans* flower possessed 98.94% scavenging activity on DPPH which was higher than the standard ascorbic acid (96.3%). The result revealed that the DPPH scavenging effect of the extracts increased with concentration of extract samples.

**Table 1: Preliminary phytochemical screening of *Tecoma stans* Stem**

| Phyto components | Different extracts | | |
|---|---|---|---|
| | Petroleum Ether | Acetone | Ethanol |
| Alkaloids | + | + | + |
| Anthroquinones | - | - | - |
| Carbohydrates | + | + | + |
| Coumarin | + | + | + |
| Fixed oils | - | - | - |
| Flavones | + | + | + |
| Gum | - | - | - |
| Phenols | + | + | + |
| Proteins | + | + | - |
| Quinones | + | + | + |
| Saponin | - | - | - |
| Starch | + | + | + |
| Steroids | - | - | + |
| Tannins | - | - | + |
| Terpenoids | + | + | + |

**Table 2: Preliminary Phytochemical Screening of *Tecoma stans* Leaf**

| Phyto components | Different extracts | | |
|---|---|---|---|
| | Petroleum Ether | Acetone | Ethanol |
| Alkaloids | + | + | + |
| Anthroquinones | + | + | + |
| Carbohydrates | + | + | + |

| Phyto components | Different extracts | | |
|---|---|---|---|
| | **Petroleum Ether** | **Acetone** | **Ethanol** |
| Coumarin | + | + | + |
| Fixed oils | - | - | - |
| Flavones | + | + | + |
| Gum | - | - | - |
| Phenols | + | + | + |
| Proteins | + | + | + |
| Quinones | + | + | + |
| Saponin | + | + | + |
| Starch | + | + | + |
| Steroids | - | - | - |
| Tannins | - | - | - |
| Terpenoids | + | + | + |

**Table 3: Preliminary Phytochemical Screening of *Tecoma stans* Flower**

| Phyto components | Different extracts | | |
|---|---|---|---|
| | **Petroleum Ether** | **Acetone** | **Ethanol** |
| Alkaloids | + | + | + |
| Anthroquinones | + | + | + |
| Carbohydrates | + | + | + |
| Coumarin | + | + | + |
| Fixed oils | - | - | - |
| Flavones | + | + | + |
| Gum | - | - | - |
| Phenols | + | + | + |
| Proteins | - | - | - |
| Quinones | + | + | + |
| Saponin | - | - | - |
| Starch | + | + | + |
| Steroids | + | + | + |
| Tannins | - | - | + |
| Terpenoids | + | + | + |

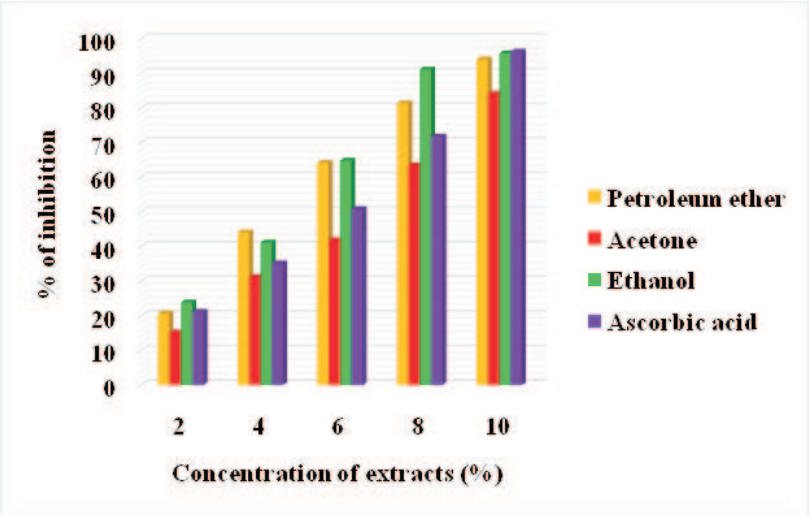


**Figure 1: DPPH radical scavenging activity of different extracts of *Tecoma stans* stem**

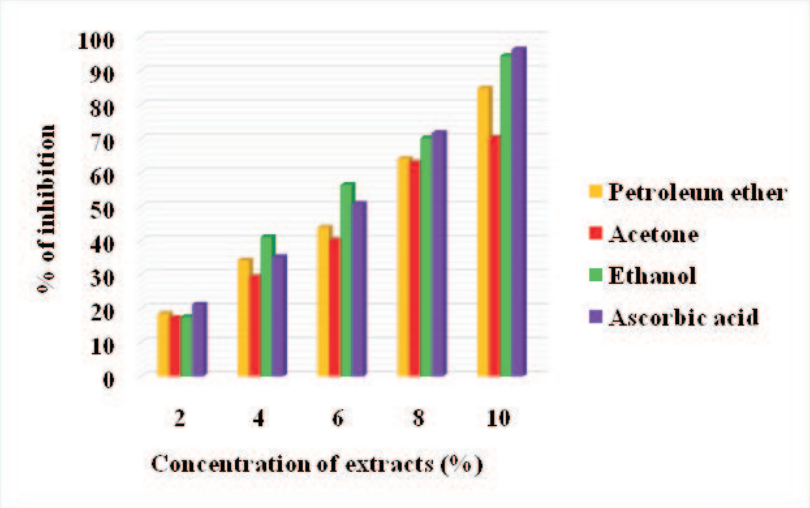


**Figure 2: DPPH radical scavenging activity of different extracts of *Tecoma stans* leaf**

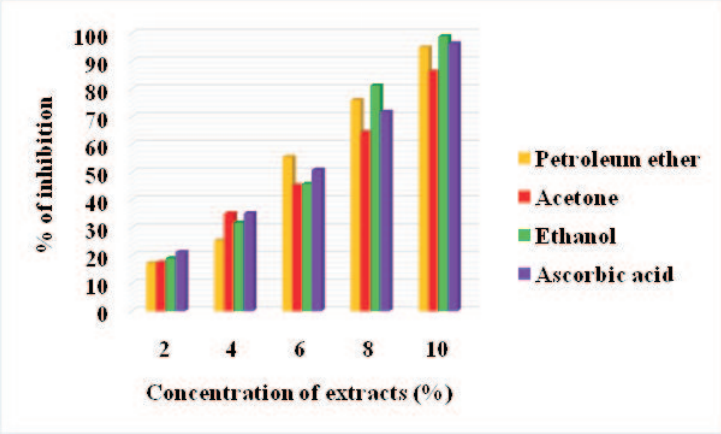


**Figure 3: DPPH radical scavenging activity of different extracts of *Tecoma stans* flower**

## Discussion

Phytochemical analysis is one of the important parameters to be evaluated in pharmacognostic study for standardization and authentication of medicinal plants and with help of which adulteration and substitution can be prevented[9]. Existence of certain significant compounds in an extract is discovered by colour reactions of the compounds with particular chemicals which act as dyes. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation[10]. Various tests had been conducted qualitatively to find out the presence or absence of bioactive compounds. Different chemical compounds were detected in the stem, leaf and flower extracts of *T. stans* extracts which could make the plant useful for treating different ailments as having a potential of providing useful drugs of human use. The pharmacological activity of any plant is attributed to the phytocompounds present.

The 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical is widely used as a model system to investigate the free radical scavenging activities of several plant extracts. DPPH is a nitrogen centered compound and a stable free radical which creates violet color in ethanol solution. It was reduced to a yellow colored product, dipheny lpicryl hydrazine, with the addition of the extracts[11]. The reduction in the number of DPPH molecules can be calculated with the number of available hydroxyl groups[12]. The result indicated that the different extract of *T. stans* showed concentration dependent scavenging effect against DPPH radical. This showed that the extract with their proton donating ability, could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants[13].

Phenolic compounds are the major antioxidant constitutes of natural plant goods, that are comprised of phenolic acid and flavonoids.These compounds are potent radical terminations by donating a hydrogen atom to the radical and preventing lipid oxidation at the initial step. The high ability of polyphenols to eliminate free radical may be due to their many phenolic hydroxyl groups. The polyphenolic compounds commonly found in plants have been reported to have multiple biological effects like anticancer, antiproliferative, antimicrobial, wound healing and antibacterial activities including antioxidant activity[14,15].

## Conclusion

This study has confirmed the presence of phytochemicals and antioxidant activities of petroleum ether, acetone and ethanol extracts of stem, leaves and flowers of *Tecoma stans*. It also revealed that *T. stans* contain a considerable quantity of phenolic and flavonoid compounds that were found to be the major contributor for their antioxidant activities. Ethanol is better solvent for extraction of antioxidant substances compared to the other solvents. Thus, the *T. stans* can be considered as an easily accessible source of natural antioxidants agents. It may be considered in future to replace synthetic preservatives in food and pharmaceutical products due to its potent antioxidant activity.

## Acknowledgement

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# Extraction of Natural Dye and its Application on Selected Fabrics

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## Abstract

Natural dyes satisfy the world's present demand for eco-friendliness. Natural dyes are unsophisticated and are in accordance with nature. From the present work, it is shown that the dye extracted from *Zinnia elegans* Jacq. with different medium showed different coloured dyes. They are mostly in the shades of orange to reddish-brown. The different fabrics showed various dye absorbing properties too. When the clothes were directly dyed, the dyeing was not shown properly or it was shown less absorption. When they were treated with various mordants like Copper sulphate, Ferrous sulphate and Potassium dichromate, it showed significant changes in the absorption. The maximum absorption was shown in the silk material with mordant potassium dichromate with ethanol and alkaline extract. For the cotton cloth, the maximum dyeing effect was shown in the aqueous and ethanol-treated with copper sulphate and potassium dichromate mordant. The colour fastness of the fabrics also showed varied changes. When the dyed clothes were washed and exposed to sunlight, they showed a very fair amount of dye retaining capacity. The maximum colour was retained in the silk cloth treated with copper sulphate and potassium dichromate mordant. The antimicrobial activity of the dyes extracted from *Zinnia elegans* Jacq. haven't shown any significant changes. The inhibition capacity was very limited compared with control. Only in the ethanol extract, the inhibition activity was considerable.

***Keywords:*** Natural dye, mordant, colour fastness, dye absorption, fabrics

***Abbreviations:*** M:L - Material to liquid, OD - Optical Density, $CuSO_4$ - Copper Sulphate, HCl- Hydrochloric acid, $K_2Cr_2O_7$ - Potassium dichromate. $FeSO_4$- Ferrous Sulphate, NaOH- Sodium hydroxide, PDA - Potato Dextrose Agar medium

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## Introduction

India harbours a wealth of useful germplasm resources and there is no doubt that the plant kingdom is a treasure-house of diverse natural products. One such product from nature is the dye[1]. Ancient Indians used natural dyes for dyeing textile goods. The art of dyeing was known in India as early as the Indus valley period (3500 B.C.)[2]. Textile processing is focused predominantly on synthetic dyeing materials. The textile industries thus produce poisonous and hazardous chemical waste. That constitutes a threat to human health and the environment[3]. Natural dyes are having the following advantages. They are obtained from renewable resources, no health hazards, sometimes they act as health care, practically no (or) mild chemical reactions are involved in their preparation, no disposal problems, they are unsophisticated and harmonized with nature and lot of creativity is required to use these dyes judiciously[4]. The production and use of natural textile dyes is both an art and science synonymous with human culture. To achieve a perfect performance the process requires both skills and experience. In addition to dyeing textiles, natural dyes are used in the form of pigments in paintings. By keeping the above scope in mind the flower of *Zinnia elegans* Jacq. was selected for the present study to carry out the following objectives.Extraction of dyes from *Zinnia elegans* flower using aqueous, acidic, alkaline and ethanol medium, dyeing of cotton, polyester and silk using above dye extracts, dyeing of extracted dye with different mordants, assessment of light fastness and washing fastness for each dyed sample and antimicrobial activities of each extracted dyes.

## Materials and Methods

#### Selection of Dyeing Material:

The yellow variant of *Zinnia elegans* is chosen and brought from the Flower market of Thoothukudi. The flowers are carefully bought in fresh and disease-free condition.

#### Selection of fabrics:

Three different fabrics like cotton, polyester and silk are chosen for the present study. The fabrics were bought from the local shop Kanna Silks, Thoothukudi. Since the clothes contain impurity and possible chemicals, they are later washed and stored for further studies.

#### Scouring:

2 g/1 sodium carbonate and 1 g/1 non-ionic (synthetic detergents were dissolved in soft water and 1:40 of the material-to-liquor ratio (MLR) medium were dissolved.The

cloth samples were kept in the scouring solution at 100°C for 30 minutes. Then the samples were taken out, rinsed thoroughly in soft water and dried[5].

**Extraction of dye from petals:**

10 gm fresh petals were boiled in 100 ml distilled water, 1% Sodium hydroxide, 1% acidic solution and in 50% alcohol respectively at 1000±C for 30 minutes. The decolorized petals were separated from the solvent for extraction. Finally, refine and use the solution for further analysis[6].

**Mordant:**

2%, 4% and 6% of mordants such as potassium dichromate, copper sulphate and ferrous sulphate were dissolved in 100 ml of distilled water in different 250 ml beakers. Wetted samples were then dipped into the mordant solution and then raised up to 80°C for 30 minutes at the heating temperature of the dye bath. It was then cooled for 15 minutes and washed with dry water and air.

**Dyeing:**

The dye extract was prepared by adding 10ml of natural dye extract in 20ml water (M:L - 1:2). The samples are kept in the boiling water bath at 75°C for 45 minutes. Dyeing was done by the conventional dyeing method. The dyed material was washed with cold water after dyeing and dried at room temperature.

**Determination of absorption (%):**

The absorbance of the dye solution was recorded before and after dyeing of the samples at particular wavelength in each case.

% dye absorption

$$= \frac{(\text{OD of the dye liquor before dyeing} - \text{OD of the dye liquor after dyeing})}{(\text{OD of the dye liquor before dyeing })} \times 100$$

**Solid dye content (%) of extracted dye solution:**

A measured quantity of extracts was taken in a pre-weighted petridish and the contents were dried in an oven at $100 \pm 5$°C till completely dried residue was obtained.The solid content of the extracted dye solution was obtained as follows.

$$\%\ \text{of solid content} = \frac{(\text{W2} - \text{W1})}{(\text{wt of the solution})} \times 100$$

W1 = Weight of the petridish, W2 = Weight of the petridish + solid, Wt = Initial weight of the solution

#### Wavelength scan for the selected dyes:

The diluted solution was then subjected to wavelength scanning and the wavelength at which maximum optical density obtained was noted for each dye.

## Determination of fastness properties of dyed fabrics

#### Wash fastness test:

The dyed clothes were washed with cold water for 30 minutes. Excess water was removed and-dried at room temperature after 30 minutes. The fastness was observed.

#### Light fastness test:

The dyed clothes were washed with distilled water for 30minutes. Excess water was removed after 30 minutes, and dried for 2 hours in direct sunlight. The dyed samples were taken and washed in soap solution in the next experiment, holding liquor for 30 minutes at a 1:50 content ratio.After that time, excess water was removed and washed with cold water then dried in direct sunlight for 2 hours.

#### Antibacterial assay:

Antibacterial assay was conducted as per Awoyinka O Aet al, 2007[7]. The culture of *Escherchia coli, Bacillus subtilis, Proteus vulgaris* and *Klebsiella pneumoniae* obtained from the Botany department laboratory, were used for evaluating antibacterial activity using the disc diffusion method. Petri Figures were prepared by pouring 30 mL of PDA medium for bacteria to grow. Briefly, inoculums containing *Escherchia coli, Bacillus subtilis, Proteus vulgaris* and *Klebsiella pneumoniae* species of bacteria were spread on Nutrient agar. The were incubated at 37°C for 24 hours. After 24 hours, sterile discs were prepared out of Whatmann No 1 filter paper, dipped in the prepared dye solutions and were inoculated in the bacterium containing petridishes. The were incubated at 37 for 24 hours for the inhibition zone to develop.

## Results

The extract of the *Zinnia elegans* flower dye was in different colours based on the extraction medium. The colour of *Zinnia elegans* flower dye in alkaline medium is light orange, in alcoholic medium the dye was pale yellow colour, in acidic condition the dye was red colour and aqueous medium it was a golden yellow colour. It is given in Figure1.

The absorption percentage of various dye extracts of *Zinnia elegans* flower is given in Table 1. It was observed that the maximum absorption of cotton fabric was shown in the aqueous extract followed by ethanol extract, alkaline and acidic extracts. In the same way, the maximum absorption by polyester fabric was noted in acidic

extract followed by aqueous, alkaline and ethanol extracts. Maximum absorption of silk fabric was observed in ethanol extract followed by aqueous, alkaline and acidic dye extracts.

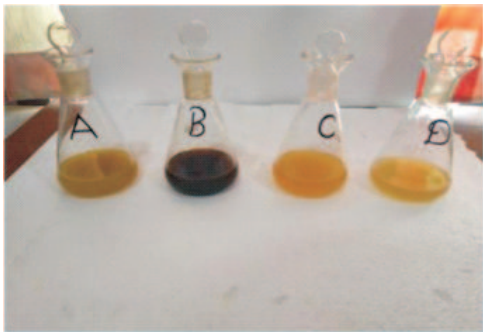


**Figure 1: A - Aqueous dye extract, B - Acidic dye extract, C - Alkaline dye extract, D - Ethanol dye extract**

**Table 1: Absorption percentage of Dye extracts on various fabrics**

| Sl. No | Extracts | Absorption Percentage | | |
|---|---|---|---|---|
| | | Cotton | Polyester | Silk |
| 1 | Aqueous | 11.08 | 10.75 | 9.35 |
| 2 | Acidic | 7.56 | 11.25 | 10.58 |
| 3 | Alkaline | 9.62 | 8.75 | 7.32 |
| 4 | Ethanol | 10.26 | 6.23 | 5.75 |

Aqueous, acid and alkali extraction media were tried to obtain solid dye content (%) of extracted dye solution and it is shown in Table 2. It was interesting to note that the dyes showed the maximum percent of solid dye content in alkali extraction media and the minimum in aqueous extraction. It was observed that maximum solid content in the alkaline extract is 26.2% followed by an acidic solution with 11.4%, ethanol extract with 10.8% and aqueous solution with 9.5%.

**Table 2: Solid Dye content (%) of extracted dye solution**

| Sl.No | Dye Extraction | Solid Dye Content (%) |
|---|---|---|
| 1 | Aqueous | 9.5 |
| 2 | Acid | 11.4 |
| 3 | Alkaline | 26.2 |
| 4 | Ethanol | 10.8 |

### Effect of Mordant

The comparative effect cotton, polyester and silk fabrics with copper sulphate were studied. Treated fabrics were first treated with extracts and then subjected to treatment with Copper Sulphate. It was observed that treated fabrics showing good staining property and upon treatment with Copper Sulphate. It is shown in Figure 2.

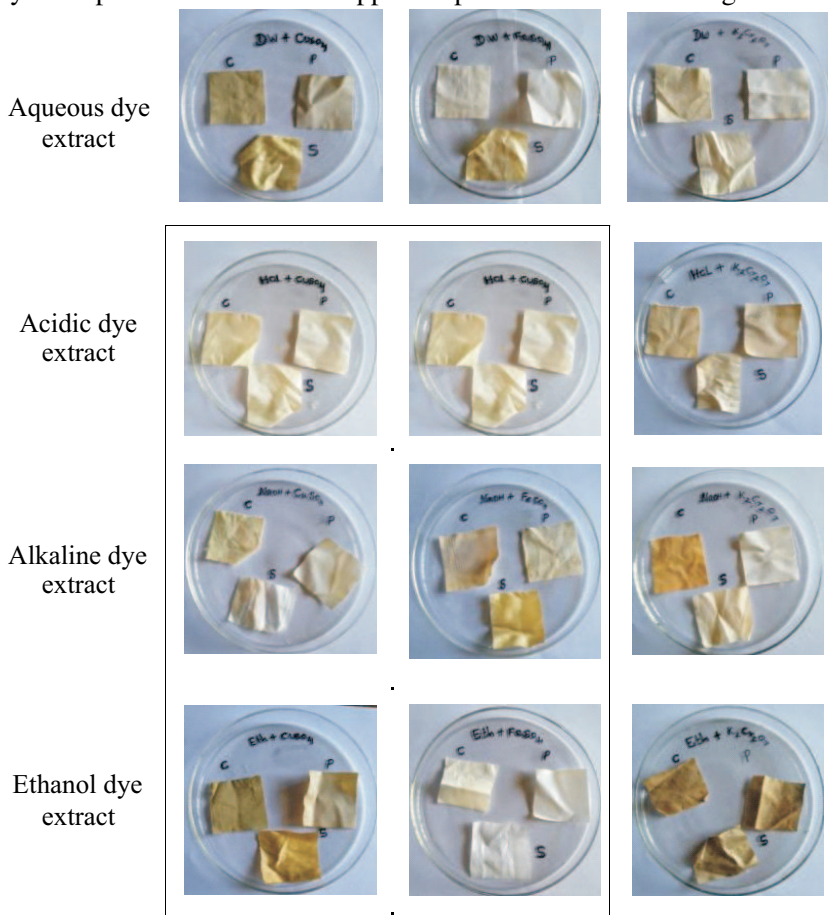


**Figure 2: Mordant treated fabrics, C - Cotton, P - Polyester, S - Silk**

## UV Visible Spectral Analysis

The absorbance pattern of the *Zinnia elegans* was dye extracted with different solvents were presented in Fig. 3. From the extracted dye liquor, it was observed that all the dyes exhibited a visible colour as yellow. It was seen that alkaline and ethanol dyes showed a maximum peak in the 300nm region and the acidic and distilled water dyes showed in the 350nm region.

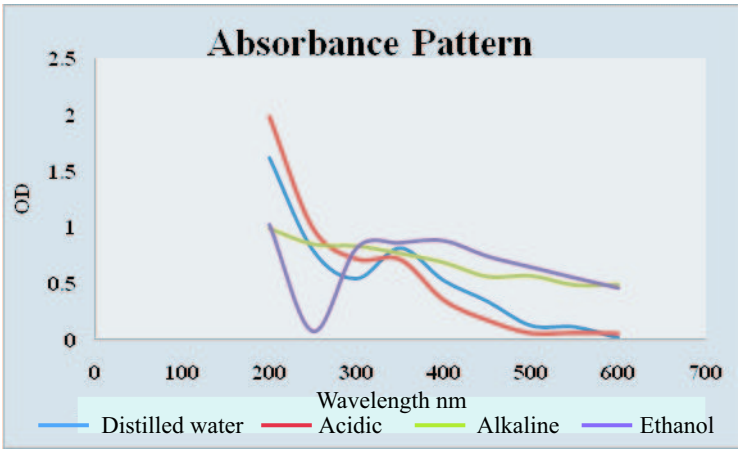


**Figure 3: Absorbance pattern of the dyes extracted with four different solvents using Zinnia**

Colour fastness grade of aqueous extracted dye treated cotton at optimum dyeing conditions with different mordants, mordanting methods as shown in Table 4. From that, it was inferred that for the cotton fabric, the alkaline and ethanol dye extract with potassium dichromate mordant show very fair results. In the polyester fabric the alkaline and ethanol extracts with ferrous sulphate and potassium dichromate mordants showed good results respectively. In silk, all the mordants exhibited relatively fair results.

**Table 4: Colour fastness of different dye treated fabrics.Fastness grade (1: Very poor, 2: Poor, 3: Fair, 4: Very fair, 5: Good; 6: Very good)**

| Sl.No | Dye Extract | Mordant | Colour Fastness Grade | | | |
|---|---|---|---|---|---|---|
| | | | Aqueous | Acidic | Alkaline | Ethanol |
| 1 | Cotton | $CuSO_4$ | 3 | 3 | 3 | 3 |
| | | $FeSO_4$ | 2 | 2 | 1 | 1 |
| | | $K_2Cr_2O_7$ | 1 | 3 | 4 | 4 |
| 2 | Polyester | $CuSO_4$ | 2 | 2 | 1 | 4 |
| | | $FeSO_4$ | 2 | 2 | 4 | 1 |
| | | $K_2Cr_2O_7$ | 1 | 3 | 1 | 4 |
| 3 | Silk | $CuSO_4$ | 4 | 2 | 1 | 4 |
| | | $FeSO_4$ | 2 | 4 | 4 | 1 |
| | | $K_2Cr_2O_7$ | 2 | 4 | 1 | 4 |

#### Antibacterial assay

The results of the antibacterial study are shown in Table 5. The inhibition zone developed stated the activity of the dyes against the selected bacterial strains, though the result was not significant.

**Table 5: Antibacterial activity of dye extracts**

| Sl.No | Bacteria | Zone of Inhibition (mm) | | | | |
|---|---|---|---|---|---|---|
| | | Control | Aqueous | Acidic | Alkaline | Ethanol |
| 1 | *Escherchia coli* | 4.5 | 2 | 1 | 2 | 3 |
| 2 | *Bacilulssubtilis* | 5 | 1 | 1.5 | 2.5 | 4 |
| 3 | *Proteus vulgaris* | 5 | 1 | 1 | 1 | 3.5 |
| 4 | *Klebsiella pneumonia* | 5.5 | 1.5 | 1.5 | 2 | 3 |

## Discussion

The extraction of dye using same the flower with different solvents gave different colours based on their solubility in the solvents. This was being observed in *Syzygium cumini* seed endosperm dye extraction with different extracts[8]. The absorption percentage of dyes helps to understand the required method of extraction for the dyeing process of specific fibres[9]. Determination of solid dye content was in agreement with the findings on "Natural dyes from some Indian plants"[10]. This report also recorded that with alkaline media, the highest solid dye content (percentage) was possible (29.0%) and the lowest amount of solid dye content i.e. 10.6%, followed by acid (12.5%), was reported with water extraction.In UV visible spectral analysis the yellow dye obtained from annatto seeds had also been studied at 350 nm wavelength[11].

## Conclusion

From the study, it is concluded that though *Zinnia elegans* Jacq. could be used to extract natural dyes, it needs certain extra care and methodology in the extraction process. Since we conducted only preliminary level tests here, a more advanced method of dye extraction and determination of fabric nature and microscopic studies are recommended for the further works.

## Acknowledgement

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# Assessment of Water Quality Parameters Along The Selected Coastal Areas of Thoothukudi

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## Abstract

The rapid growth in population and increasing industrial activities including marine activities have resulted in increasing water pollution. This is one of the primary issues related to environmental pollution in the coastal region of developing countries. The seawater samples were collected from six different sampling points in Thoothukudi coastal area from the vicinity of Thermal Power Plant to study Physico-chemical parameters using various analytical techniques. The results of the study revealed that the physical and chemical composition of all the six water samples collected from the sites nearby the thermal Power Plant mainly depends on the source of pollutants from the thermal power plant.

***Keywords:*** Industrial activities, Pollution, Thermal Power Plant, Physico-chemical parameters

***Abbreviations:*** TTPS - Thermal Power Station

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## Introduction

The ocean covers approximately 70% of the earth's total surface area. In the total water content of the earth, 97% is present in the oceans. Oceans are a rich source of biodiversity in which the population may exceed in trillions[1]. Oceans are the main regulatory agent of the earth's climate. The coastal region plays a key role in the country's economy due to its availability of resources, productive habits, and wealthy biodiversity.

India has about 7,500 km of coastline. The Tamil Nadu coastline is about 10% of the length of the total coastal length of the Arabian Sea, Bay of Bengal and the Indian Ocean[2]. Seawater is an extremely complex solution, its composition is determined by an equilibrium between rates of addition and loss of solutes, evaporation and the addition of freshwater[3]. Environmental pollution of the coast, inshore water, and deep ocean is one of the important topical issues in the context of human health and global warming[4]. The contamination of seawater, including trace metal concentration, affects marine organisms and then people consuming them causing some carcinogenic and non-carcinogenic impacts in their body[1].

The coastal areas of Thoothukudi are assuming greater importance owing to the increasing human population, urbanization, and accelerated industrial activities. These anthropogenic activities have put tremendous pressure on fragile coastal environments. Coastal pollution in Thoothukudi has seriously affected the exploitable living resources, recreational and commercial uses of coastal areas, and the overall integrity of the marine and coastal ecosystems. Effective planning for controlling and combating coastal pollution requires knowledge about the magnitude of the pollution, the entry, transport, and the state of pollutants in the marine environment and their effects on marine ecosystems[5]. Hence protection of the coastal marine regions from continuous exposure to pollution becomes the most essential in coastal resource management.

## Materials and Methods

#### Study area:

Thoothukudi Thermal Power Station (TTPS) is located along the Thoothukudi coast. Environmental Impact Assessment study was carried out by collecting water samples from six stations fixed around 300 km point of the power plant during December (2019) (Figure 1 and 2).

#### Method for sample collection:

The samples were collected as per the standard method[6]. The sample containers were cleaned by 1.0 mol/L of nitric acid and left for 2 days followed by thorough rinsing with distilled water. The samples were collected without any air bubbles in clean polythene bottles. The collected samples were tightly sealed after collection and labeled in the field itself.


**Figure 1: Location map of Thoothukudi district**

**Figure 2: Effluent discharge from Thermal power plant**

**Collection of the water samples and analysis:**

The water samples were collected from six different stations fixed from the entry point of the salt pan to analyse the different physical and chemical parameters also to understand the nature and extent of pollution using "Standard methods for the examination of water and waste water"[6]. Surface water temperature was measured using thermometer and pH was measured using Elico pH meter respectively[7]. The collected samples were analyzed for the parameters like dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, acidity, alkalinity, total hardness, and salinity using titrimetric methods[6,7]. Nitrite and phosphate were analyzed by spectrophotometric methods[7]. The elements like copper, lead, zinc and cadmium were estimated under GBC702 atomic absorption spectrophotometry[6].

## Results

Pollutants that originate far inland are being transported to the ocean via rivers. Petroleum, nutrients from fertilizers, debris, and industrial contaminants are the major pollutants of particular concern. Physical-chemical parameters are one of the most significant characteristics that have the ability to impact the marine ecosystem and show wider progressive and spatial variations. The results of the physicochemical parameters of the water samples studied were recorded and presented in Table 1.

**Table 1: Surface water quality for the selected sampling sites of Thoothukudi harbour area**

| Sl. No | Parameters | Station I | Station II | Station III | Station IV | Station V | Station VI |
|---|---|---|---|---|---|---|---|
| 1. | **Temperature** | 29 | 28 | 31 | 31 | 30 | 30 |
| 2. | **pH** | 8.24 | 7.62 | 8.60 | 8 | 7.78 | 7.73 |
| 3. | **DO (mg/L)** | 3.5 | 4.6 | 3.1 | 2.3 | 2.5 | 3.3 |
| 4. | BOD (mg/L) | 1.7 | 0.3 | 2.5 | 2.8 | 3.2 | 2.2 |
| 5. | **COD (mg/L)** | 10 | 7.2 | 10.6 | 8 | 9.3 | 4.8 |
| 6. | **Acidity (mg/L)** | 16 | 1.04 | 2.16 | 2.28 | 1.9 | 0.84 |
| 7. | **Alkalinity (mg/L)** | 183 | 213 | 349 | 454 | 434 | 166 |
| 8. | **Total hardness (mg/L)** | 316 | 316 | 78 | 78 | 342 | 342 |
| 9. | **Salinity (mg/L)** | 0.84 | 0.84 | 344 | 3.44 | 5.28 | 0.02 |
| 10. | **Nitrite (mg/L)** | 168 | 181 | 162 | 160 | 123 | 144 |
| 11. | **Phosphate (mg/L)** | 105 | 123 | 107 | 110 | 130 | 125 |
| 12. | **Coppper (mg/L)** | 6.325 | 4.833 | 48 | 0 | 0 | 483 |
| 13. | **Lead (mg/L)** | 14.24 | 0 | 14.3 | 14.3 | 14.3 | 0 |
| 14. | **Zinc (mg/L)** | 0 | 0 | 3 | 3 | 3 | 3 |
| 15. | **Cadmiun (mg/L)** | 0 | 0 | 3.8 | 0 | 3.8 | 0 |

Temperature is one of the important factors in an aquatic environment for its effects on the chemistry and biological reactions in the organisms. The changes in water temperature are due to change in atmospheric temperature and in season. The average variation in temperature of marine water samples in the study area varied from 29 to 31°C. The minimum temperature was observed in station IV and maximum temperature in stations V and VI. pH varied from 7 to 8 in the water samples studied. Dissolved oxygen content varied from 2.3 to 4.6 mg/L. The maximum value was observed in station I. Biological oxygen demand was high (3.2 mg/L) in station V followed by station IV, III, VI, I and II. Maximum COD was noticed in station III (10.6 mg/L). The water sample collected from station IV exhibited high acidity and alkalinity values. The hardness of water was above 300 mg/L in the station I, II, V and VI. Salinity values of the water samples varied from

0.02 mg/L to 344 mg/L. The nitrite content of the samples studied varied from 123 mg/L to 181 mg/L. Variation in phosphate content is very little in the samples studied. The copper content of the sample varied from 6.325 mg/L to 483 mg/L and samples from the site IV and VI had no copper. The lead was absent in samples from station II and VI. A trace of zinc was noticed in station III and IV. Cadmium was observed only in station III and V.

## Discussion

The results of the present study were closely related to the results of Muthuraman *et. al.*, 2019[8]. The disposal of fly ash slurry causes the high temperature in station III and IV. Sea surface temperature in station III and IV was high (31) during the study period. The temperature has been decreasing with increasing the distance from the surface water temperature is influenced by the intensity of solar radiation evaporation, freshwater influx, cooling and mix up with the ebb and flow from adjoining neritic water[9]. From the earlier reports, it was studied that the temperature 27-34°C was recorded in the surface waters of Thoothukudi, a part of the Gulf of Mannar[10]. The temperature has been decreasing with increasing distance[11].

The pH is a measure of the intensity of acidity or alkalinity and measures the concentration of hydrogen ions in water[12]. In the present study, the fluctuation of pH in the samples was from 7.62 to 8.60. The pH below 7 indicated that the sample water was slightly acidic may be due to the presence of minerals in the water. The variations in pH might be due to the minerals present and the condition of the earth. High alkalinity in marine water along Thoothukudi harbour coastal area[1]. High pH values observed may cause seawater deprivation and high density phytoplankton effect[13]. Fluctuations in the hydrogen-ion concentration of water, the dissolved oxygen content was high during the monsoon season[14].

Dissolved Oxygen content in water reflects the physical and biological processes prevailing in water and is influenced by aquatic vegetation and plankton population apart from the temperature and organic matters present. The presence of low oxygen content in water is an indication of organic pollution. DO content ranged from 2.3 - 4.6 mg/L in the study area. The variation of the DO is estimated by the oxygen consumption by bacteria when disintegrating organic matter in aerobic conditions. This is due to a large amount of drainage and waste water released into the marine environment[15]. The levels of DO recorded in the present study were compared with the previous work of Santhanam *et al*, 1994[16] and it was clear that the water at all the stations studied was polluted by the effluent discharge from the surrounding industries.

BOD is the measurement of dissolved oxygen used by microorganisms in the biochemical oxidation of organic matter. The low BOD value in water samples

showed the good sanitary conditions of the water. It varied from 0.3 to 3.2 mg/L in the study areas. COD is the oxygen required by the organic substances in the water to oxidize them by a strong chemical oxidant. COD values ranged from 4.8 to 10.6 mg/L. High acidity might due to the fact that industrial wastes that contain mineral acidity are let out into the sea.

Alkalinity in water is used to measure its ability to neutralize the acids. Alkalinity provides guidance in applying proper doses of chemicals to be used in water and waste water treatment processes. In natural water, the alkalinity is caused by bicarbonates, carbonates and hydroxides. In the present study phenolphthalein alkalinity was absent in all samples and methyl orange alkalinity ranged from 166.0 mg/L to 454 mg/L, these indicated the absence of hydroxyl and carbonate alkalinity and presence of bicarbonate.

Hardness in water is due to multivalent metallic cations and divalent calcium, magnesium, strontium, ferrous and manganese ions. The hardness in water is influenced by soil and rock formations. Total hardness was less in station III and IV (78 mg/L), station I and II, station V and VI showed similar results. The amount of dissolved material irrespective of the constituents is quantified as salinity. In station VI minimum salinity was recorded and maximum salinity was recorded in station V. Previous studies[8] revealed that the salinity is very high than the results of the current study.

Due to the dumping of various wastes from the Thermal power plant, which contains some heavy metals, there is the possibility of leaching of heavy metals into the soil and thereby contaminating the marine water. The copper concentration of the study area varies from 4.8 to 6.3 mg/L. The water samples at stations I, III, IV and V indicated an alarming figure of lead. The concentration of zinc as obtained from the analysis of water sample collected was 3.0 mg/L at station III, IV, V and VI. The results of the present study confirmed the presence of zinc in the wastes dumped. The concentration of lead varied from 0 to 14.3 mg/L. Such high values of lead in those areas might have been caused due to the leaching from industrial waste dumped. The concentration of cadmium varied from 0.0 to 3.8 mg/L.

The Nitrite amount ranged from 123 to 181 mg/L. The high nitrite content in the station I is an index of the balance of active biological oxidation by nitrifying bacteria and phytoplankton[17]. The amount of nitrogenous compounds from industrial wastes also contributes to the increase of nitrites in seawater. The amount of phosphate ranges from 105 to 130 mg/L.

## Conclusion

The present study concluded that both surface and subsurface water in the study area in and around thermal power station, is polluted and the concentration of pollution are unacceptable. The practice of effluent discharge into the coastal environment without any pretreatment affects the sea water and hence the health of the biota. Amidst the humble success seen today in reversing the toxic effects of the pollution, still much effort needs to be taken to protect marine environment for the future generation.

## Acknowledgement

The support provided by DBT Star College Scheme, Govt. of India, is greatly acknowledged.

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# GALLERY

## EQUIPMENTS PURCHASED UNDER STAR COLLEGE SCHEME

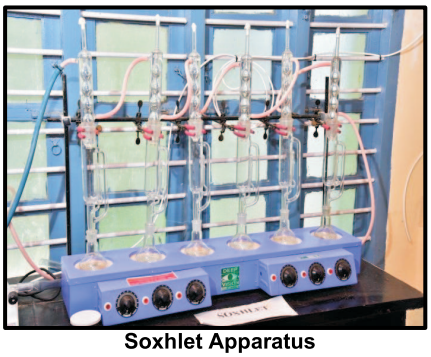
## (2019-2020)

## DEPARTMENT OF BOTANY

**Seed Germinator**


**Flame Photometer**

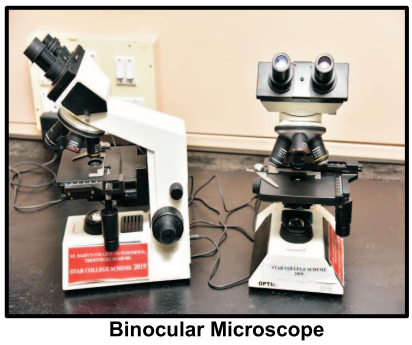
**Trinocular Microscope, Camera with Monitor and Computer System**



**Soxhlet Apparatus**

**Rotary Vacuum Evaporator**


**High Speed Centrifuge**

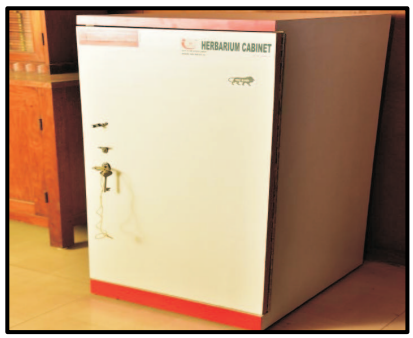


Binocular Microscope

Tissue Culture Hood

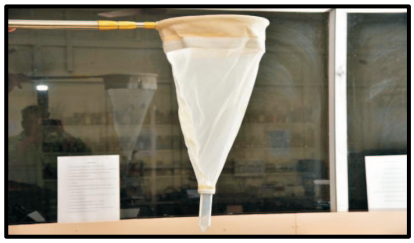

Incubator


Flat Bed Scanner




Herbarium Cabinet

Water bath



Plankton net

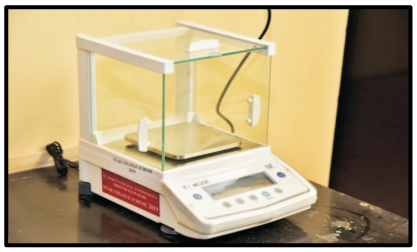
Muffle Furnace

Steam Distillation

Flourimeter

Suction Pump

Multipurpose Extractor (Mixie)



Electronic Balance

Magnetic Stirrer

# DEPARTMENT OF CHEMISTRY

# MESSAGE FROM THE HOD

**Dr. J. Martin Rathi**
**Head and Associate Professor**
**Department of Chemistry**
**St.Mary's College (Autonomous)**
**Thoothukudi - 628 001**

Research and development form the backbone of our curriculum. The staff and students are engaged in various path-breaking innovative research activities all throughout the year using various facilities availed from DBT Star College Scheme, along with existing facilities at St. Mary's College, Thoothukudi. The Department of Chemistry, right from its inception, has been active in multidisciplinary and interdisciplinary research and innovation and has setup an ambient academic environment for its undergraduate students. Nearly 50 students involved in group projects on 'Survey on Soil and Water Quality Analyses and Biogenic Synthesis of Copper Nanoparticles Using Seaweeds'. Students were also involved in interdisciplinary research on 'Biogenic Synthesis of Copper, Silver and Gold Nanoparticles Using Plant Materials, Corrosive Inhibitive Effects, Degradation of Microplastics and Polythene,' along with students from the Department of Botany, Physics and Zoology. DBT Star College Scheme has created research culture on contemporary and relevant topics. It has facilitated research in those areas which will lead to necessary metamorphosis in the academia as well. It has also enhanced the skills and development of our studies. The findings will benefit the society at large. Along with my colleagues I congratulate and wish our students for their research aptitude and involvement.

J Martin Rathi

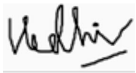
**Dr. J. MARTIN RATHI**

# MESSAGE FROM THE REVIEWER

**Dr. C. Vedhi,**
**Assistant Professor of Chemistry,**
**V.O.Chidambaram College, Thoothukudi.**

I am happy that the Department of Chemistry shows a keen interest to update the knowledge of the current research field. Physical Participation in the projects will create new networking of research. I believe that this project work under DBT star college Scheme will really enhance the knowledge of students and this will create a good platform for young researchers in this society. This chemical team work enhances the chemistry knowledge and good bonding of friendship. Topics discussed in these projects such as Nanoparticles, Soil analysis, water analysis are the real areas to be discussed in the current scenario. The department has taken effective steps to identify active areas to do research. I congratulate all the staff members and the students who worked hard to bring out this fruitful work.

I wish the team a happy success in their future endeavours.



**Dr. C. VEDHI**

# A Survey and Analysis on the Soil Quality Parameters in the Anthoniyarpuram Region

**J. Angel Selciya, T. Anto Threshinal, M. Arumuga Lingammal, S. Jesline Jenitha, M. Sri Devi, A. Judy Auxline, R. Maria Rasmitha, D. Sasirekha, C. Subbu Lakshmi, *Dr. Irudaya Antonat Sophia,**

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Received on 11 November 2020

## Abstract

Fertile soil provides essential nutrients to help the growth of plants.In this article a survey is done on the soil quality parameters from Anthoniyarpuram region, Thoothukudi, Tamilnadu. Soil quality parameters like electrical conductivity, pH, organic matter, nitrogen, phosphorous, copper , iron and manganese were measured using *Mridaparikshaka* minilab developed by ICAR-Indian Institute of Soil Science (IISS), Bhopal, an institute that comes under the Division of Natural Resource Management of Indian Council of Agricultural Research.

***Keywords:*** soil fertility, organic matter, nutrients, pH, conductivity

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## Introduction

A healthy, high-quality soil is fertile, has good soil structure, is biologically active and provides essential nutrients to plants needed by crop plants to grow. The primary nutrients plants taken up from soils include nitrogen, phosphorus, potassium, calcium and magnesium. Frequently, addition of soil nutrients by adding fertilizer, manure or compost, for good crop growth is needed.

Soil pH is one of the most important aspect of soil fertility. Most crops grow best when the soil pH falls between 6.2 and 6.8. This is the range in which plant roots can absorb most nutrients from the soil. Soil pH is a measure of the acidity or basicity of a soil.

The United States Department of Agriculture Natural Resources Conservation Service classifies soil pH ranges as follows:

| Denomination | pH range |
|---|---|
| Ultra acidic | $< 3.5$ |
| Extremely acidic | 3.5-4.4 |
| Very strongly acidic | 4.5-5.0 |
| Strongly acidic | 5.1-5.5 |
| Moderately acidic | 5.6-6.0 |
| Slightly acidic | 6.1-6.5 |
| Neutral | 6.6-7.3 |

Organic matter is composed of plant and animal residues, living and dead soil microorganisms, and substances produced through decomposition. Most agricultural soils contain only a small proportion of organic matter (usually less than 5%), but this small amount plays a very large role in soil quality. Soil organic matter tends to improve soil fertility, soil structure, and soil biological activity[1].

Soil is a major source of nutrients needed by plants growth. The three major nutrients are nitrogen (N), phosphorus (P) and potassium (K). Together they make up the trio known as NPK. Other important nutrients are calcium, magnesium and sulfur. The primary nutrients are nitrogen and potassium.The intermediate nutrients are sulfur, magnesium and calcium. The remaining essential elements are the micronutrients and are required in very small quantities. Calcium, Magnesium, Sulphur are essential plant nutrients. They are called “Secondary” nutrients because plants require them in smaller quantities than nitrogen, phosphorus, and potassium. Calcium and magnesium both increase soil pH , but sulfur from some sources reduces soil pH[2].

Some trace elements of potential concern as soil contaminants are: arsenic (As), boron (B), cadmium (Cd), chromium (Cr), copper (Cu), fluorine (F), lead (Pb), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), selenium (Se) and Zinc (Zn).

## Phosphorus Uptake by Plants

Plants take up phosphorus from the soil solution as orthophosphate ion either $HPO_4^{-2}$ or $H_2PO4^-$. The proportion in which these two forms are absorbed is determined by the soil pH, when at higher soil pH more $HPO_4^{-2}$ is taken up.The mobility of phosphorus in soil is very limited and therefore, plant roots can take up phosphorus only from their immediate surroundings.Since concentration of phosphorus in the soil solution is low, plants use mostly active uptake against the concentration gradient (i.e. concentration of phosphorus is higher in the roots compared with the soil solution). Active uptake is an energy consuming process, so conditions that inhibit root activity, such as low temperatures, excess of water etc., inhibit phosphorus uptake as well.

## Phosphorus Deficiency

Symptoms of phosphorus deficiency include stunted growth and dark purple color of older leaves, inhibition of flowering and root system development. In most plants these symptoms will appear when phosphorus concentration in the leaves is below 0.2%.

### Copper

Copper is a micronutrient in plants and an important constituent, in small amounts. Copper facilitates respiration and photosynthesis and is important for plant metabolism. It is a component of a variety of enzymes and plant cell walls so it is important for plant strength. Copper also affects the flavour, sugar content and storage life of fruits.

A variety of factors can affect the availability of copper including:

- Root growth - copper doesn't move through soil easily so anything that reduces root growth also prevents plants from taking up copper.
- pH - copper availability is higher in acidic soils and lower in alkaline soils.
- Organic matter - organic matter reduces the availability of copper
- Moisture - copper is less available in waterlogged soil.
- Zinc - excess zinc reduces copper availability.
- Nitrogen - excess nitrogen can prevent copper from being transported around plants and nitrogen deficiency can reduce the uptake of copper.
- Phosphorus - excess phosphorus can reduce copper uptake.

Ideally, for healthy and productive soil, the concentration of copper should be 2-50 mg/kg.

### Copper Deficiency and Symptoms

Copper deficiency is more likely to be seen in plants grown in sandy, alkaline soils. Copper deficiency in plants can result in poor growth, delayed flowering and sterility. Uniform chlorosis may also occur on new leaves and leaves may curl under and/or wilt.

### Copper Toxicity

Copper toxicity in plants can inhibit iron uptake and can also stunt growth. Excess soil copper can inhibit seed germination. High soil copper levels can occur as a result of excessive use of copper containing fungicides and industrial activity (such as mining).

### Iron

Iron is the fourth most abundant element found in soil though it is largely present in forms that cannot be taken up by plants. Iron, in small amounts, is essential for healthy plant growth and is classed as a micronutrient. It is important for the development and function of chlorophyll and a range of enzymes and proteins. It also plays a role in respiration, nitrogen fixation, energy transfer and metabolism. As with other nutrients, plants can have too much iron but this primarily affects the uptake of other nutrients rather than producing direct toxicity symptoms.

The amount of iron and its availability in soil is influenced by the following:

- pH - high pH reduces iron availability, low pH increases it
- Organic matter - organic matter provides iron and makes it more readily available
- Moisture - excess water in the soil, particularly in acidic soils, increases iron availability even to the point of toxicity
- Aeration and compaction - compacted and/or poorly aerated soils have an increased iron availability, particularly if the soil is acidic
- Phosphorus - excess phosphorus inhibits the uptake of iron
- Nitrogen - certain forms of nitrogen can reduce iron uptake
- Zinc - zinc deficiency can increase the uptake of iron in some plants, excess zinc decreases iron uptake
- Manganese - excess manganese inhibits the uptake of iron
- Potassium - potassium deficiency may increase iron uptake
- Molybdenum - excess molybdenum can reduce iron uptake especially in alkaline soils ”
- Nickel - excess nickel can reduce the uptake of iron
- Bicarbonate - bicarbonate in the soil can reduce iron uptake

### Ideal Soil Iron Levels

Some estimates suggest that soil should have at least 0.001 g of iron in every 100 g of soil (or 10 mg/kg).

### Iron Deficiency

Iron deficiency is most likely to occur in plants when the soil is alkaline or when the level of phosphorus, nitrogen, zinc, manganese or molybdenum in the soil is high. Heavy metal contamination can also lead to iron deficiency.

#### Symptoms of Iron Deficiency

Interveinalchlorosis is the most common symptom of iron deficiency. Symptoms are usually seen on young leaves first.

#### Iron Toxicity and Symptoms

Iron toxicity is most commonly associated with highly acidic soil though symptoms of iron toxicity are mostly symptoms of other nutrient deficiencies.

Symptoms of true iron toxicity usually include bronzing of the leaves and possibly also the formation of brown spots on leaves.

#### Manganese

Manganese is a plant micronutrient which is used in photosynthesis , synthesis of chlorophyll and nitrogen absorption as well as the synthesis of riboflavin, ascorbic acid and carotene.

A wide variety of factors can affect manganese availability including:

- pH - high pH reduces availability and low pH can increase availability to the point of toxicity if there is an excess in the soil
- Organic matter - the more organic matter in the soil the lower the availability of manganese
- Moisture - changes in soil moisture can convert available forms of manganese to unavailable forms and vice versa and rapid changes in soil moisture can cause deficiencies and toxicities
- Iron - excess iron reduces manganese uptake by plants
- Silicon - addition of silicon can reduce the likelihood of symptoms of toxicity in plants that uptake excess levels of manganese
- Nitrogen - low nitrogen levels can reduce manganese uptake by plants
- Anions (negatively charged ions such as nitrate, phosphate, sulfate) - excess anions can increase the uptake of manganese

Ideally, for healthy and productive soil the concentration of manganese should be 10-50 mg/kg. it would be worth conducting further investigations if your soil contains more than 3800 mg of manganese for every kg of soil.

#### Manganese Deficiency

Manganese deficiency is most common on alkaline and poorly drained soils as well as those high in available iron.

#### Symptoms of Manganese Deficiency

Symptoms of manganese deficiency include interveinalchlorosis of new leaves, necrotic spots and sometimes, small and/or irregularly shaped leaves.

#### Manganese Toxicity

Manganese toxicity is more common on very acidic soil. It can be toxic in it's own right but excess manganese can also cause iron deficiency.

#### Symptoms of Manganese

Toxicity Manganese toxicity may present as distorted leaves with dark specks. In severe cases, leaves will start to die from the outer edges in.

## Materials and Methods

#### Preparation of soil samples

Soil samples were collected from Anthoniyarpuram, Thoothukudi by digging the soil upto 1m from different areas. Five samples were collected and removed foreign materials like roots, stones, pebbles and gravels and finally labeled.

#### Instrument:

*Mridaparikshak* is a minilab developed by ICAR-Indian Institute of Soil Science (IISS), Bhopal, an institute that comes under the Division of Natural Resource Management of Indian Council of Agricultural Research. *Mridaparikshak* has been developed in technical collaboration with M/s Nagarjuna Agrochemicals Pvt. Ltd., Bhopal. With *Mridaparikshak* one can determine the available quantities of soil nutrients and prescribe fertilizer doses for nitrogen (N), phosphorus (P), potassium (K), sulphur (S), Iron (Fe), zinc (Zn), boron (B), copper (Cu) and Manganese (Mn) based on the measures soil test values.

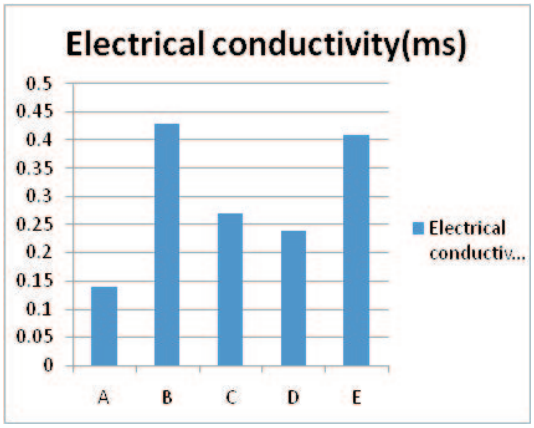
# Result

## Tables and Graphs

### 1. Electrical Conductivity of the soil samples:

**Table 1: Electrical Conductivity of the soil samples**

| Sample | Conductivity (mS/cm) |
|---|---|
| A | 0.14 |
| B | 0.43 |
| C | 0.27 |
| D | 0.24 |
| E | 0.41 |

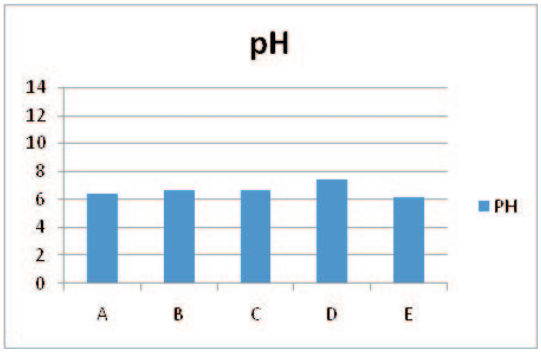



**Graph 1: Electrical Conductivity of the soil samples**

### 2. pH values of soil samples:

**Table 2: pH values of the soil samples**

| Sample | pH |
|---|---|
| A | 6.40 |
| B | 6.70 |
| C | 6.69 |
| D | 7.46 |
| E | 6.15 |

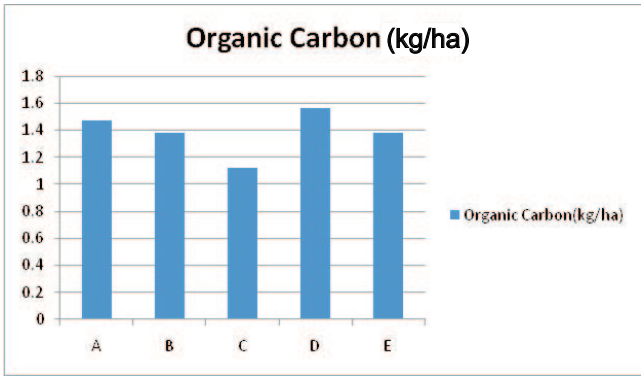



**Graph 2: pH values of the soil samples**

### 3. Organic Carbon, Phosphorous and Nitrogen content in the soil samples

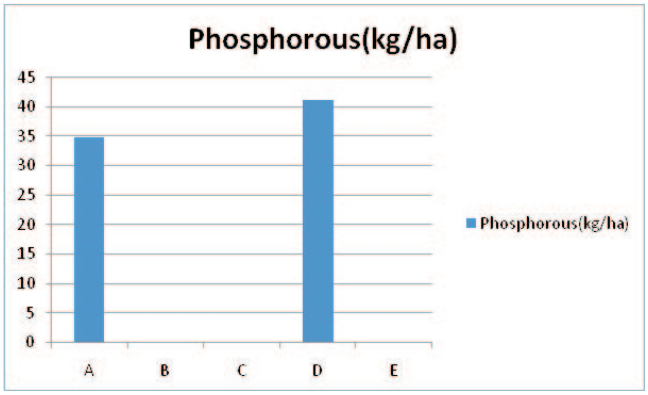
**Table3: Organic Carbon and Phosphorous Content in the soil samples**

| Sample | Organic Carbon (Kg/ha) | Phosphorous (Kg/ha) | Nitrogen (Kg/ha) |
|---|---|---|---|
| **A** | 1.47 | 34.85 | 414.1 |
| **B** | 1.38 | 0.00 | 409.4 |
| **C** | 1.12 | 0.00 | 337.9 |
| **D** | 1.56 | 41.11 | 399.9 |
| **E** | 1.38 | 0.00 | 371.3 |



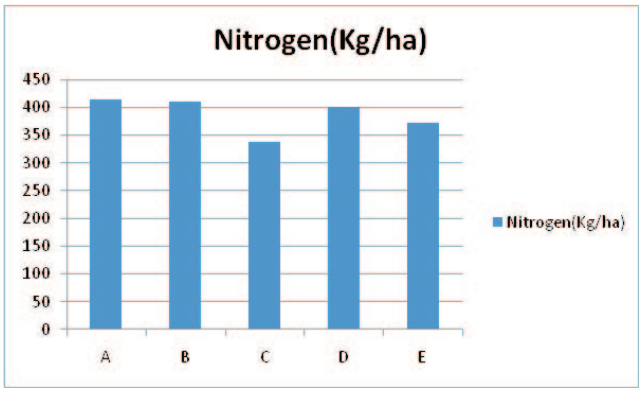

**Graph 3: Organic Carbon content in the soil samples**

**4. Phosphorous content in soil samples:**




**Graph 4: Phosphorous content in the soil samples**

**5. Nitrogen content in soil samples:**

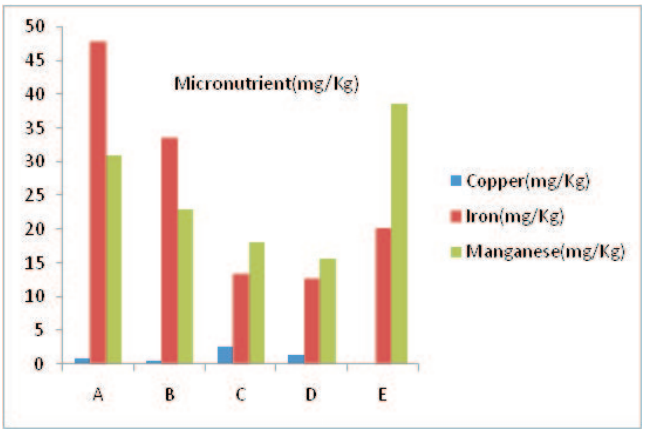



**Graph 5: Nitrogen content in the soil samples**

**6. Copper , Iron and Manganese content in soil samples:**

**Table 6: Copper , Iron and Manganese content in soil samples**

| Sample | Copper (mg/Kg) | Iron (mg/Kg) | Manganese (mg/Kg) |
|---|---|---|---|
| **A** | 0.84 | 47.8 | 31 |
| **B** | 0.46 | 33.5 | 22.9 |
| **C** | 2.52 | 13.3 | 18 |
| **D** | 1.4 | 12.7 | 15.7 |
| **E** | 0 | 20.2 | 38.6 |




**Graph 6: Copper , Iron and Manganese content in soil samples**

## Discussion

### Electrical Conductivity

Optimal electrical conductivity levels in the soil usually range from 110-570 milli Siemens per meter (mS/m). Too low electrical conductivity indicate low availability of nutrients, and too high electrical conductivity indicate an excess of nutrients.

From Table 1 it is clear that the electrical conductivity values range from 140-410 milli Siemens per meter.This indicates that sample A, C and D have low available nutrients and samples B and E have higher levels indicating the presence of excess nutrients.

Among the five samples B and E is rated good for growth of plants, C and D is average , A can be used for growing plants in the garden.

### Soil pH

The soil pH measures very useful for identification active soil acidity or alkalinity nature. A pH of 6.9 or less is acid. Soils with a pH of 7.0 are neutral, values higher

than 7.0 are alkaline. Under normal conditions, the most desirable pH range for mineral soil is 6.0 to 7.0 and 5.0 to 5.5 for organic soil.Having the right soil pH is key to growing a healthy garden.A pH of 6.5 is just about right for most home gardens, since most of the plants thrive in the 6.0 to 7.0 (slightly acidic to neutral) range. Some plants prefer more acidic soil, while a few do best in soil that is neutral to slightly alkaline.The level of acidity will specify the amount of soil amendment that is needed to bring it up or down to the appropriate level. Acidic ("sour") soil is counteracted by applying finely ground limestone or wood ash, and alkaline ("sweet") soil is typically treated with gypsum (calcium sulfate), ground sulfur, or compost.Table 2 clearly indicates that samples A,B,C and E have pH in the range of 6.15 to 6.7 thereby indicating acidic soil and sample D has pH of 7.46 indicating slightly alkaline soil. So it is clear that sample D is not much suitable for growing plants.

**Organic matter:**

Permissible level of nutrient

| Nutrient | Low | Medium |
|---|---|---|
| Organic carbon | $< 0.5$ % | 0.5 - 7.5% |
| Available nitrogen (N) | $<$ 240 Kg/ha | 240- 480 kg/ha |
| Available Phosphorus (P) | $<$ 11.0 Kg/ha | 11-22 Kg/ha |

From Table 3 it is clear that organic carbon is the medium level in all the five samples. Sample D has higher level of 1.56% followed by sample A with 1.47% , both samples B and E have 1.38% and sample C has a lower level of 1.12%.

Regarding phosphorous level Table 3 indicates maximum level for sample D (41.11 Kg/ha) and sample A (34.85 Kg/ha).Samples B, C and E have no phosphorous at all.

As far as nitrogen is concerned sample A has maximum level (414.1 Kg/ha) followed by sample B (409.4 Kg/ha), sample D (399.9 Kg/ha),sample E (371.3 Kg/ha) and sample C has the least level 337.9 Kg/ha.

Ideally, for healthy and productive soil, the concentration of copper should be 2-50 mg/kg. Table 4 shows only sample C contains 2.52 mg/Kg which is sufficient for healthy soil whereas sample A, sample B and sample D show copper levels less than 2 mg/Kg and sample E has no copper content all.

Some estimates suggest that soil should have at least 10 mg/kg of iron in soil. All the five samples contain higher than the minimum amount of iron required. Sample A has the highest level of Fe (47.8 mg/Kg), followed by sample B (33.5 mg/Kg), sample E (20.2 mg/Kg) and sample D has the minimum level of 12.7 mg/Kg.

Ideally, for healthy and productive soil the concentration of manganese should be 10-50 mg/kg. Samples A to E have manganese levels from 15.7 mg/kg to 38.6 mg/Kg, the highest level in sample E and lowest in sample D.

## Conclusion

Samples collected from Anthoniyarpuram ,Thoothukudi and tested using minilab revealed the following informations about the soil samples. Electrical Conductivity values range from 140-410 milli Siemens per meter. This indicates that sample A, C and D have low available nutrients and samples B and E have higher levels indicating the presence of excess nutrients. Samples A,B,C and E have pH in the range of 6.15 to 6.7 thereby indicating acidic soil and sample D has pH of 7.46 indicating slightly alkaline soil.Organic carbon is in the medium level in all the five samples. Sample A and D have maximum level of phosphorous whereas it is absent in Samples B, C and E. As far as nitrogen is concerned it is quite sufficient in all the samples. Only sample C contains the required quantity of copper. All the five samples contain higher than the minimum amount of iron required.Sufficient amount of manganese is also present in the samples. From the analysis of the collected samples it is clear that the soil is quite healthy for plant growth.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# An Analysis of Biological Oxygen Demand (BOD) of Water Samples Collected from Thoothukudi District

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## Abstract

The biochemical oxygen demand (BOD) test measures the oxygen consumed in the reaction. The standard test is carried out under controlled laboratory conditions, at a constant temperature and over a specified time. Since not all organic matter is biochemically decomposable, the test measures the oxygen equivalence of the degradable matter only. Compounds such as cellulose, lignin and many synthetic petrochemicals are very resistant to biological breakdown. The oxygen consumed during the process is differentiated from that required for the oxidation of organic matter. The unpolluted waters will have BOD value less than 2 mg/L. Since Thoothukudi district is surrounded by many fish processing and storage plants, emission of organic water pollutants leads to increase in BOD level. Thus it is important to monitor the BOD levels frequently in thoothukudi district. In the present study water samples were collected from various places in thoothukudi district and (BOD) 5 values were analyzed and found to be less than 2 mg/ml.

***Keywords:*** BOD, Water quality, Winkler method

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## Introduction

Water is a major natural resource, a basic human essential and a precious natural gift. It is indeed required in all aspect of life and health for producing food, agricultural activity,energy generation and maintenance of environment and a substance of life and development.Human activities such as urbanization, agricultural development, use of fertilizers, in adequate management of land use and sewage disposal affected

the quality of water and making it unfit for domestic purpose. Therefore freshwater has become a scare commodity due to over exploitation and pollution. Hence it is necessary to evaluate quality of water of that area in order to assess its suitability for various uses and to evolve the policies for the best use of water resources. The BOD test measures the strength of the waste water by measuring the amount of oxygen used by the bacteria as they stabilize the organic matter under controlled conditions of time and temperature. Neelam *et al.*, [1]developed a biosensor for the determination of BOD value of fermentation industry effluent. The authors fabricated the biosensor by immobilizing the microbial consortium on cellulose acetate (CA) membrane. AS Pujar et al., [2]reported the parameters such as pH, EC, Conductivity, total dissolved solid (TDS), chemical oxygen demand (COD), Biological oxygen demand (BOD), dissolved oxygen (DO), calcium, magnesium, alkalinity and chloride for the waste water from the sugar and cement industrial area situated in North Karnataka. Deepika *et al.*, investigated the potential pollution of textile industry effluent draining into Buddha Nallah stream located in Ludhiana, Punjab (India), and reported the seasonal variation in physicochemical parameters (pH, water temperature, total dissolved solids, total suspended solids,biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of Buddha Nallah water[3]. Amit Varale1 *et al.*, studied the BOD levels in the underground water samples near Halsiddhanath sugar factory located at Nipani and reported high BOD level than the desirable limit (30 mg/lit) suggested by Bureau of Indian Standards[4]. Md. Nuruzzaman1 *et al.*, experimentally determined the biochemical oxygen demand decay rates of Malaysian river water in a laboratory flume[5]. In the present study water samples were collected from various places in thoothukudi district and (BOD) 5 values were analyzed and reported.

## Materials and Methods

### Sample collection:

For BOD determination, water samples were collected in an air tight polythene bottle of 300 ml capacity. The containers were cleaned thoroughly with soap and water, and rinsed well with distilled water. In all the cases, containers were filled fully and stoppered tightly to avoid contact with air or to prevent agitation during transportation. The samples were collected from various areas in thoothukudi district and listed in Table.1

**Table 1: Water Collected areas in Thoothukudi district**

| Area | Sample No. |
|---|---|
| Tap water (Cruzpuram) | 1 |
| Municipal water ( Thoothukudi corporation) | 2 |
| Lake water (Kootampuli Village) | 3 |

| Area | Sample No. |
|---|---|
| RO water | 4 |
| Well water (Threspuram) | 5 |

#### Chemicals used

- Manganese sulphate
- Sodium thiosulphate
- NaOH
- Conc.$H_2SO_4$
- Potassium Hydroxide
- Potassium iodide
- Starch

#### Apparatus

- 300mL volume stoppered Erlenmeyer Flask
- BOD incubator

#### Reagents for BOD measurement:

- **Manganese sulphate solution:** 9.1 g $MnSO_4.H_2O$ is dissolved in distilled water, filter, and dilute to 1L.
- **Starch solution:** 2 g laboratory-grade soluble starch is dissolved in 100 mL hot distilled water.
- **Standard sodium thiosulphate titrant:** 6.205 g $Na_2S_2O_3$ .$5H_2O$ is dissolved along with 0.4g of NaOH and diluted to 1000 mL and standardized using potassium dichromate solution.
- **Conc.$H_2SO_4$:** 2 mL
- **Alkali iodide reagent:** 7g of KOH and 1.5g of KI dissolved in 10 ml of water. All the reagents were prepared using double distilled water.

## Methodology

#### The Winkler Method for DO Determination

When $MnSO_4$ and alkali-iodide reagent (NaOH+KI) are added to the sample, a pure white precipitate is formed if there is no oxygen is present in the sample,

$$Mn^{2+} + 2OH^- \rightarrow Mn(OH)_2 \qquad \text{(white precipitate)}$$

If sample contains some oxygen, $Mn^{2+}$ is oxidized to $Mn^{4+}$ and precipitates brown hydrated oxide.

$$Mn^{2+} + 2OH^- + 0.5O_2 \rightarrow MnO_2 \qquad \text{(brown hydrated precipitate)} + H_2O$$

The oxidation of $Mn^{2+}$ to $MnO_2$ is called fixation of the oxygen, occurs slowly at low temperature.

$$Mn(OH)_2 + 0.5O_2 \rightarrow MnO_2 + H_2O$$

The sample is shaken for a sufficient time to allow all oxygen to react, the floc is allowed to settle so to leave 5 cm of clear liquid below the stopper; then sulphuric acid is added. $MnO_2$ oxidizes to produce $I_2$ under the low pH conditions. $I_2$ is insoluble in water and forms complex is excess iodide ion is present in solution, thus preventing escape of iodine ions from solution.

$$MnO_2 + 2I^- + 4H^+ \rightarrow Mn^{2+} + I_2 + 2H_2O$$
$$I_2 + I^- \rightarrow I^{3-}$$

The method for determining Biochemical Oxygen Demand (BOD) consist of placing a sample in a full airtight 300 ml BOD bottle and then incubating the same at 20°C for 5 days. This is generally represented as BOD 520°C. Dissolved Oxygen was measured in the beginning and after incubation. BOD was calculated from the difference between initial and final dissolved oxygen (DO). BOD was calculated from the formula as given below.

$$BOD_5 \text{ (mg/1)} = (D1 - D2)/P$$

Where,

Dl = Dissolved oxygen of diluted sample immediately after preparation;

D2 = DO of oxygen diluted sample after five day incubation at 20°C.

P = Decimal volumetric fraction of sample used

**Table 2: BOD Level of water**

| BOD Level (in ppm) | Water Quality |
|---|---|
| 1 - 2 Very Good | Low organic waste present in the water supply. |
| 3 - 5 Fair: | Moderate |
| 6 - 9 Poor: | Fairly Polluted<br>Indicates the presence of organic matter |
| 100 or greater<br>Very Poor: Very Polluted | Contains organic waste. |

#### Procedure

300 mL of the sample was taken in BOD bottle. Two sets of the sample were prepared. One set is used for DO analysis for day 0 and another set of sample was kept in a BOD incubator for 5 days at 20°C.

**Figure 1: BOD incubator**

DO for day 0 is analyzed by adding 1 mL manganous sulfate solution and 2 ml of alkali iodide reagent. It was shaken well and the bottle was kept aside for 5 minutes to settle down the precipitate.

**Figure 2: Settling down of precipitate**

**Figure 3: Precipitate dissolved in Conc.$H_2SO_4$**

Then 2 mL of concentrated $H_2SO_4$ is added and shaken well so that all the precipitate formed is dissolved.

203 mL of sample was taken in a conical flask and titrated with standard sodium thiosulphate solution (0.025N) using starch as indicator and the titration is continued till the color of the solution becomes colorless. Volume of 0.025N sodium thiosulfate consumed was noted.

## Results and Discussion

DO analysis was carried out for the samples for day 5. The results were tabulated (Table.3)

Dissolved oxygen (DO) (in mg/L) = mL of sodium thiosulfate (0.025N) consumed

**Table 3: DO analysis of water samples**

| Sample No. | Area | Initial DO (mg/L) ($DO_0$) | DO at 5-day (mL) ($DO_5$) |
|---|---|---|---|
| 1 | Tap water (Cruzpuram) (Thoothukudi corporation) | 7.5 | 7.6 |
| 2 | Municipal water | 4.3 | 5.1 |
| 3 | Lake water (Kootampuli Village) | 7.5 | 8.1 |
| 4 | RO water | 10 | 10.5 |
| 5 | Well water (Threspuram) | 7 | 8.2 |

The concentration of dissolved oxygen (DO) is one of the most important factors of the overall physical condition of a body of water. Waters having high levels of DO ($>$ 6 mg/L) typically sustains most of the biological communities. Waters with low DO levels ($<$ 3 mg/L) may be almost devoid of aquatic life or may have only few species tailored to such conditions.

5-day BOD value of the sample at 20°C:

$$\mathbf{(BOD)_5 = [DO_t - DO_0]/(P)}$$

where P = Dilution factor = 300 mL/(sample volume in mL)

Sample volume taken = 300 mL. Substituting the values in above equation, $(BOD)_5$ values of the samples collected from various areas of thoothukudi district were calculated and listed below Table.4). The $(BOD)_5$ were compared using the chart and tap water collected from cruzpuram area have very low BOD and well water from Threspuram have comparatively high BOD value. The $(BOD)_5$ values calculated for the samples collected from various areas of thoothukudi district was found to be below 2 mg/L and its free from organic pollutants.

**Table 4: $(BOD)_5$ of water samples**

| Sample No. | Area | $(BOD)_5$ |
|---|---|---|
| 1 | Tap water (Cruzpuram) | 0.1 |
| 2 | Municipal water (Thoothukudi corporation) | 0.8 |
| 3 | Lake water (Kootampuli Village) | 0.6 |
| 4 | RO water | 0.5 |
| 5 | Well water (Threspuram) | 1.2 |

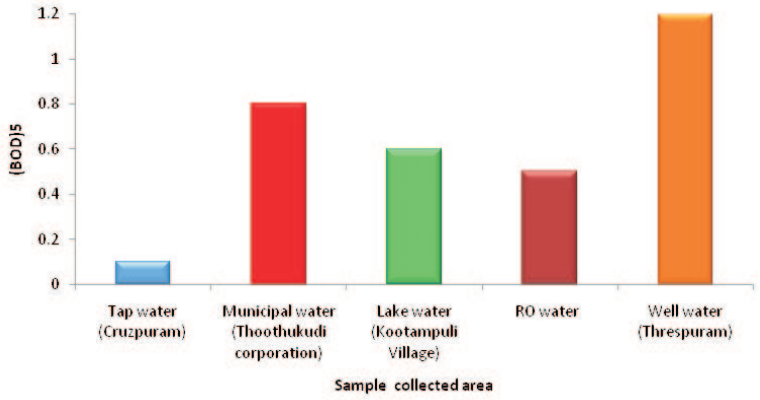


Chart 1.Comparitive analysis of (BOD)5 values calculated for the samples collected from various areas of thoothukudi district

## Conclusion

Micro-organisms utilize waste organic matter as food. In aerobic environment, the organic matter isbiochemically converted to carbon dioxide and water. Higher values indicate organic pollution from municipal or industrial wastes. The BOD of domestic and municipal wastes ranges between 150 and 400 mg/L. In slow moving streams, values greater than 8 mg/L indicate the possibility of onset of anaerobic conditions in the stream since the oxygen demand may exceed the supply of oxygen through atmospheric reaeration. The BOD test is used extensively in the modelling of oxygen concentration in rivers and streams subjected to pollution. The $(BOD)_5$ values calculated for the samples collected from various areas of thoothukudi district was found to be below 2 mg/L and its free from organic pollutants.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Green Synthesis of Copper Nanoparticles using Seaweeds

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## Abstract

Green synthesis of nanoparticles were carried out by using the aqueous extracts of the seaweed *Sargassum wightii* and aqueous solution of copper (II) nitrate. The conditions for the synthesis of nano particles were monitored by using potentiometer and colorimeter. EMF values were noted down during the course of the reaction and the volume of the extract and the copper (II) nitrate solution were optimized. Absorbance values were also noted during the reaction. UV-Vis and FTIR spectra were taken for the extract and the misture of extract with copper (II) nitrate.

***Keywords:*** Green synthesis, *Sargassum wightii*, nanoparticles.

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## Introduction

Nano particles are defined as having one structural dimension of less than 100nm. The nanoparticles are made up of a very few atoms. These nanoparticles find their applications in various fields such as biomedical, tissue engineering, environmental, drug delivery, optics and much more. Most of the metal nanoparticles possess remarkable antimicrobial effect[1]. Using plant extracts is much simple and easy process to produce nanoparticles at large scale relative to bacteria and fungi mediated synthesis. Normally aqueous plant extracts are used for the synthesis of nanoparticles[2]. Sargassum is one of the easily available and well known seaweed. Numerous species are distributed throughout the temperate and tropical oceans of the world. This species is used for the synthesis of nano particles like gold and silver[3,4]. Copper nanoparticles are very attractive due to their heat transfer properties such as high thermal conductivity. The main difficulty lies in their preparation and preservation as they oxidized immediately when exposed in air, so capping agents

have to be used. Some reducing and capping agents are very expensive and also have toxic effects[5].

## Materials and Methods

### Collection of samples

The brown colour seaweed *Sargassum wightii* was collected from the sea shores of Kanyakumari, Tamil Nadu, India in the month of January 2020. The seaweeds collected were cleaned and dried in sunshade for about a month.

### Preparation of the extract

About 10g of powdered seaweed was mixed with 100ml of distilled water in a beaker and boiled for 30 minutes. This was then filtered and was allowed to cool down to room temperature.

### Preparation of the Copper(II) nitrate solution

0.01M copper(II) nitrate solution was prepared by weighing exactly 0.24g of copper(II) nitrate crystals and was made up to 100ml in a standard measuring flask with distilled water.

### Measurement of potential using potentiometer

Exactly 40ml of extract was taken in 100mL beaker. The two electrodes one reference electrode and the working electrode were introduced into the extract. The electric potential of the extract shown in the potentiometer was noted. Then 1mL of copper(II) nitrate solution was added each time up to 10mL and the emf displayed in the potentiometer was noted each time.

### Measurement of absorbance using colorimeter

Five 100mL beakers were taken. About 40mL of the extract was added to each beaker. Then 5mL of the copper(II) nitrate solution was added to the first beaker and 10mL, 15mL, 20mL and 25mL of the copper(II) nitrate was added to the second, third, fourth and fifth beakers respectively. The solution was kept undisturbed for 10 minutes. Then the extract and the copper(II) nitrate mixture in the beakers were filled one by one in the cuvette and absorbance values were noted at 540nm.

### UV Spectral analysis

UV-Visible spectra were recorded using Jasco V-630 spectrophotometer for the extract and the mixture of extract with copper(II) nitrate solution in the range of 200-900 nm.

### FT-IR Spectral Analysis

FTIR spectra were recorded for the extract and the mixture of extract with copper(II) nitrate using Nicolet Si5 spectrometer using KBr pellets in the range of 4000-400 $cm^{-1}$

## Results and Discussion

### Measurement of potential EMF using potentiometer

EMF was measured using potentiometer for different concentrations of the copper(II) nitrate with the extract and the readings were tabulated.

The emf for the copper(II) nitrate solution is very high and the emf of the pure *Sargassum wightii* extract is very low. When copper(II) nitrate solution was slowly added to the extract the emf slowly increases steadily. A slow colour change is visible during the addition of the salt solution. After the addition of 18mL of the salt solution there is no increase in the potential. It remains unchanged. After all the nanoparticles are formed the rise in emf is stopped at a particular point where the formation of nanoparticles is completed. The constant emf is an indication of the completion of the reduction reaction[6]. For 40mL of the extract 17mL of the copper(II) nitrate is required for the complete reduction of copper (II) ions.

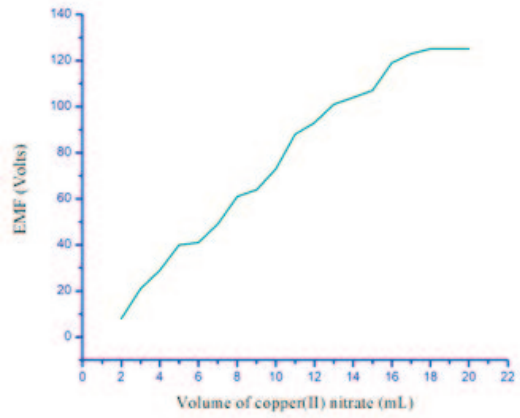


**Figure 1: Plot of EMF against volume of copper(II) nitrate solution (mL)**

The Plot of EMF against volume of copper(II) nitrate solution is given in figure 1. From the graph it is clearly seen that with increase in the concentration of copper(II) nitrate solution there is a steady increase in the emf values. The emf reaches a constant value at high concentration of the salt solution indicating the completion of the reduction reaction.

#### Measurements of absorbance using colorimeter

Absorbance for the pure extract and the extract with the slat solution were noted at 540nm. The absorbance value for the pure extract is 0.66. This is due to the various compounds present in the extract. Extracts of Sargassium species have carbohydrates, proteins, aminoacids, sterols and triterpenes[7].

| Volume of extract | Volume of Copper(II) nitrate (mL) | Absorbance values |
|---|---|---|
| 40 | 0 | 0.66 |
| 40 | 5 | 0.33 |
| 40 | 10 | 0.57 |
| 40 | 15 | 0.61 |
| 40 | 20 | 0.67 |
| 40 | 25 | 0.68 |

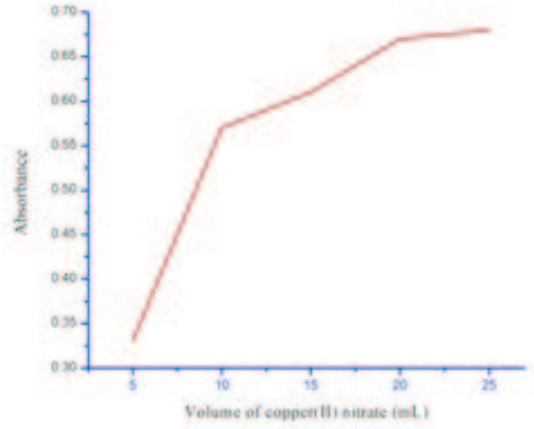


**Figure 2: Plot of Absorbance against the concentration of copper (II) nitrate solution (mL)**

Plot of absorbance against the concentration of copper(II) nitrate solution is given in figure 2. With the increase in the concentration of the copper(II) nitrate solution there is a visible change in the colour of the solution. First the absorbance values for the extract with 5mL amd 10mL copper nitrate are lower than the absorbance value of the extract. This is due to the formation of the nanoparticles since there is excess of extract present and the formation of nanoparticles will be more, since more capping, stabilizing and reducing agents are present.

With the increase in the concentration of copper(II) nitrate the absorbance values goes on increasing. Normally with the increase in the concentration of the metal ion the absorbance increases[5]. For 25mL of copper(II) nitrate there is no appreciable

difference in the absorbance value from the solution containing 20mL copper(II) nitrate and the absorbance values slightly exceeds the absorbance values of the pure extract. Also for the solutions with 20mL and 25mL copper(II) nitrate solutions, dark coloured visible particles are also present. This may be due to the aggregation of the particles making them large enough to be visible. The concentration of copper(II) nitrate required for the formation of nanoparticles lies before 20mL.

#### UV-Vis spectral studies

UV-Vis spectrum of the plant extract *Sargassum wightii* is given in figure 3. The spectrum shows peaks at 225nm, 276nm. These are due to the flavonoids present in the extract[8].

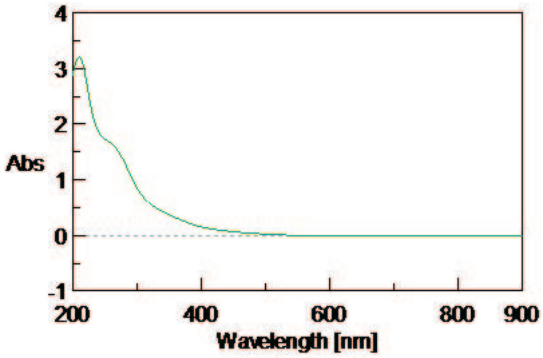


**Figure 3: UV-Vis spectrum of the extract**

The UV-Vis spectrum of the extract with copper(II) nitrate is given in figure 4. For the spectrum of the extract with copper(II) nitrate the two peaks due to flavonoids exhibit a red shift. And a peak in the range 290-310nm is much broadened. This shows that the interaction of the compounds present in the extract with copper(II) nitrate.

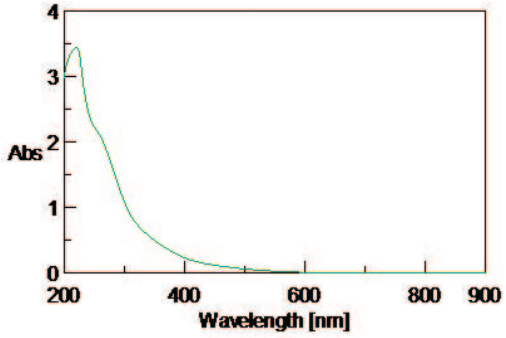


**Figure 4: UV-Vis spectrum of extract with copper(II) nitrate**

### FTIR studies

FTIR spectrum of the extract is given in figure. 5. It shows two sharp bands in the frequency range 1482 $cm^{-1}$ and also in 2165 $cm^{-1}$. The band at 1482 $cm^{-1}$ is due to the C-C stretch of aromatic ring[9].

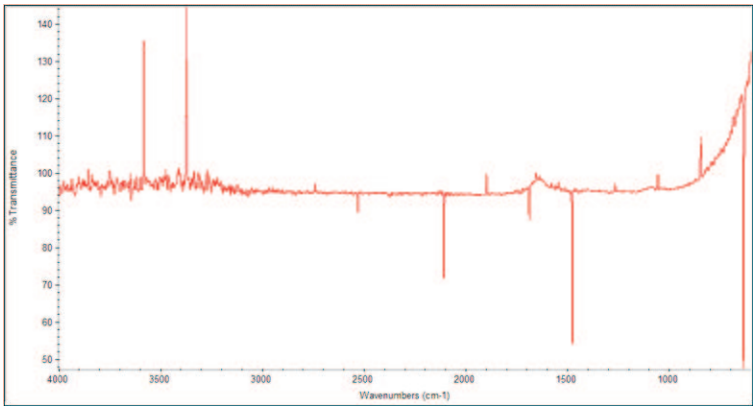


**Figure 5: FTIR spectrum of the extract**

FTIR spectrum of the extract with copper(II) nitrate is given in figure. 6. A new peak at 630 $cm^{-1}$ is present in the spectra of extract with coper(II) nitrate. This peak is due to the formation of nano particles.Similarly a new peak is also formed at the region 2320 $cm^{-1}$. The peak due to C-C stretch of aromatic ring is slightly shifted to a greater frequency.

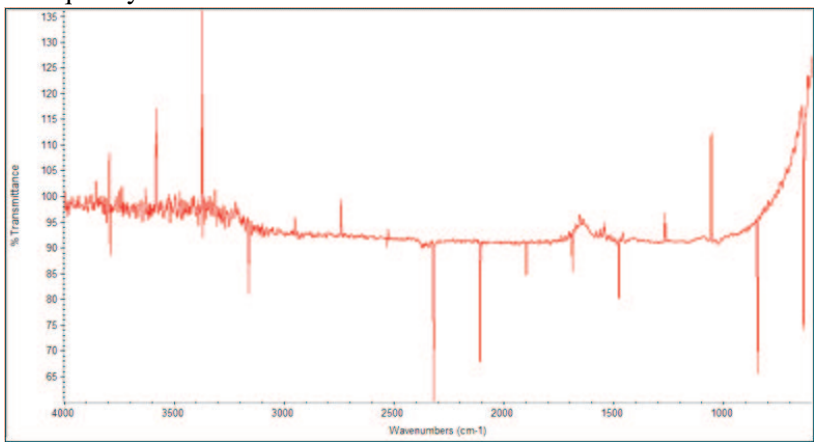


**Figure 6: FTIR spectrum of the extract with copper(II) nitrate**

## Conclusion

Copper nanoparticles are green synthesized using the plant extracts of Sargassum. The volume needed for the complete reduction of the copper(II) ions is found by the

emf values measured using potentiometer. Also colorimetric studies also carried out to study the absorbance values. The same results are obtained in the colorimetric studies also. The volume of the extract and the volume of the copper(II) nitrate solution required for the synthesis of copper nanoparticles are optimized.Further studies can be carried out on the properties and applications of the formed nanoparticles.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Algae Mediated Synthesis of Copper Nanoparticles using *Sargassum wightii*

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## Abstract

Nano technology is emerging effectively with good scope in all the fields. In view of this, we have suggested an eco-friendly method to synthesise nanoparticles. Nanoparticles can be synthesised by Green method than any other methods of synthesis. By the method of green synthesis, particle size and morphology can be studied very effectively. This method is also environment friendly. Copper nanoparticles received much attention due to its high electrical conductivity, high melting point, low electrochemical migration behavior, excellent solder ability and low material cost. Chemical methods of preparing copper nanoparticles are simple in operation, high exible and environment friendly. Plant -mediated synthesis of nanoparticles has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness. In the present study, we synthesized copper (Cu) nanoparticles using aqueous extract of dried leaves of *Sargassum wightii*. Sargassum species are tropical and sub-tropical brown macroalgae (seaweed) of shallow marine meadow. These are nutritious and rich source of bioactive compounds such as vitamins, carotenoids, dietary fibers, proteins, and minerals. Also, many biologically active compounds like terpenoids, flavonoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, pheophytine were isolated from different Sargassum species. These isolated compounds exhibit diverse biological activities like analgesic, anti-inflammatory, antioxidant, neuroprotective, anti-microbial, anti-tumor, fibrinolytic, immune-modulatory, anti-coagulant, hepatoprotective, anti-viral activity etc., Also it is used as bio fertilizers in agriculture, as natural plant growth stimulant, etc., Hence, Sargassum species have great potential to be used in pharmaceutical and neutralceuticalareas. This study covers the characterization of synthesized copper nanoparticles using Potentiometer, Photocolorimeter. This

method is cost economical as it does not involve temperature, pressure, energy, catalyst etc. This type of synthesis is easy and it it can also be suggested for various industrial and medical applications.

***Keywords:*** Copper Nanoparticle, Sargassum, Green synthesis

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## Introduction

A nanoparticle or ultrafine particle is usually defined as a particle of matter that is between 1 and 100 nanometers (nm) in diameter. Since 1990, scientific and technological research on the synthesis of inorganic nanoparticles has increased markedly due to their interesting physical properties and potential applications. This is mainly attributed because of their small size, shape, composition and high surface area to volume ratio. Particular attention has been given to metal particles, especially copper particles due to the fact that they exhibit unique physicochemical properties not seen in other materials and differ from their mass-counterparts in properties such as catalytic, optical, electrical, electronic, thermal, magnetic, and antimicrobial. Because of their attractive features nanoparticles of metallic copper can be used in different fields at laboratory- and industrial-scale production, for example, manufacture of electrical and electronic devices, preparation of thin films, high surface area catalysts in various chemical processes, application in cancer cells treatment, additives in germicides formulation, antibacterial materials, additive for lubricating oils to improve antiwear properties caused by friction, and conductive inks and coatings. Most of the preparation methods have several factors that limit their use in the synthesis of metal particles. Copper nanoparticles received much attention due to its high electrical conductivity, high melting point, low electrochemical migration behavior, excellent solderability and low material cost. The synthesis of nanoparticles has received considerable attention because of their unique physical and chemical properties that are distinct from the bulk materials. Several methods have been proposed for synthesis of copper nanoparticles by considering bottom-up and top-down methods. The majortechniques for preparing copper nanoparticles bychemical methods are Chemical reduction, sonochemical reduction, micro emulsion techniques, electrochemical, hydrothermal, sol gel synthesis, polyol process, biological synthesis and microwave assisted techniques. Chemical methods of preparing copper nanoparticles are simple in operation, high exible and environment friendly.

A plant extract is a substance or an active compound with desirable properties that is removed from the tissue of a plant usually by treating it with a solvent to be used for a particular purpose. Plant -mediated synthesis of nanoparticles has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness. In the present study, we synthesized copper (Cu) nanoparticles using aqueous extract of dried leaves of *Sargassum wightii*.

Synthesis of Silver Nanoparticles from the Aqueous extract of leaves of Ocimum Sanctum for Enhanced Antibacterial Activity was worked by Ram-Avatar Pandey *et al*. The synthesized AgNPs have been characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM) and X-ray diffractometry. Investigation on the antibacterial activity of AgNPs against E.coli and Staphylococcus aureus reveals high potential of Tulsi extract stabilized AgNPs to be used as antimicrobial agent in medical field as well as food and cosmetics[1]. Green synthesis of Silver Nanoparticles using Pinuseldanca Bark extract was worked by SiavashIravani *et al*. The effect of quantity of extract, substrate concentration, temperature and pH on the formation of silver nanoparticles were reported in this investigation[2]. Synthesis of Silver Nanoparticles using Aqueous Extract of medicinal Plants (Impatiens balsamina and Lantana camara) was worked by Henry F.Aritonang *et al*. It was shown that the aqueous extracts of fresh L. camara leaves containing Ag nanoparticles were comparable to ciprofloxacin in inhibiting bacterial growth[3]. Sargassumfulvellum protects HaCaT Cells and BALB/c Mice from UVB-Induced Proinflammatory Responses was worked by Chan Lee *et al*. From this work, it was suggested that SFE-EtOAc could be an effective anti-inflammatory agent protecting against UVB irradiation-induced skin damages[4]. Also, the synthesis of Gold Nanoparticles using various Plant Extract was worked by Shakeel Ahmed *et al*. In this research article, the various phytochemicals present in plant extract were used for the reducing and stabilisation of nanoparticles[5]. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property was worked by Peter Logeswari *et al*. The highest antimicrobial activity of silver nanoparticles synthesized by S. tricobatum, O. tenuiflorum extracts was found against S. aureus (30 mm) and E. coli (30 mm) respectively[6]. 'Green' synthesis of metals and their oxide nanoparticles for environmental remediation was worked by Jagpreet Singh *et al*. The synthesized products can be applied for environmental remediation in terms of antimicrobial activity, catalytic activity, removal of pollutants dyes, and heavy metal ion sensing[7]. Green Synthesis of silver nanoparticles using the plant extract of Salviaspinosa grown in vitro and their antibacterial activity assessment was worked by Saba Pirtarighat *et al*. Bactericidal activity assessment of the biosynthesized AgNPs showed their inhibitory function against both Gram-positive and Gram-negative bacteria. [8]Synthesis of Nanoparticles from Plant Extracts was worked by A. A. Haleemkhan *et al*. In this work, the metal nanoparticles synthesized from several plant extracts were applied to treat antimicrobial and anti-cancer activities was reported. [9]Green Synthesis of silver nanoparticles using plant extracts was worked by Rodica - Mariana Ion et al.Antimicrobial activity and antioxidant properties of the extracts were investigated[10].

## Materials and Methods

### Scientific Classification of Sargassum wightii:

Kingdom: Chromista; Phylum:Ochrophyta; Class: Phaeophyceae;
Order: Fucales; Family: Sargassaceae; Genus: Sargassum

**Figure 1: Sargassum**

*Sargassum* species are tropical and sub-tropical brown macroalgae (seaweed) of shallow marine meadow. These are nutritious and rich source of bioactive compounds such as vitamins, carotenoids, dietary fibers, proteins, and minerals. Also, many biologically active compounds like terpenoids, flavonoids, sterols, sulfated polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, pheophytine were isolated from different Sargassum species. These isolated compounds exhibit diverse biological activities like analgesic, anti-inflammatory, antioxidant, neuroprotective, anti-microbial, anti-tumor, fibrinolytic, immune-modulatory, anti-coagulant, hepatoprotective, anti-viral activity etc., Also it is used as bio fertilizers in agriculture, as natural plant growth stimulant, etc., Hence, Sargassum species have great potential to be used in pharmaceutical and neutralceutical areas.

### Extract Preparation:

Sargassum species is collected from Kanyakumari seashore,washed and dried in the absence of sunlight. 10 gm of dried plant sample is weighed in an electronic balance and boiled with water in the hot bath. It is then cooled and filtered to get the extract.

### Preparation of Cupric acetate Solution:

0.01M cupric acetate solution is prepared in a 100 ml SMF. 0.2g of cupric acetate is weighed using electronic balance and it was transferred to 100ml SMF to prepare 0.01M solution.

### Potentiometer:

Potentiometer is an instrument used for measuring the unknown voltage by comparing it with the known voltage. In other words, potentiometer is a three terminal device used for measuring the potential differences by manually varying the resistances. The known voltage is drawn by the cell or any other supply sources.

40 ml of the *Sargassum wightii* extract is taken in a small beaker. The platinum and calomel electrodes are dipped into the solution. Initial emf value is noted. 1ml of copper salt solution is now added using the burette to the extract. The solution in the beaker is stirred well and the potential values are noted. The addition of 1 ml of Copper salt solution is continued and the emf values are noted. Figure 2 shows the potentiometer used for the project work.

**Figure 2: Potentiometer**

**Figure.3 Photo Colorimeter**

#### Photo Colorimeter:

A colorimeter is a light-sensitive device used for measuring the transmittance and absorbance of light passing through a liquid sample. The device measures the intensity or concentration of the color that develops upon introducing a specific reagent into a solution.Intially the absorbance values of both pure extract and the copper salt solution are noted. Take 5 ml of the extract in five different 100ml beakers. Pipette out 5, 10, 15, 20 and 25mL of the copper salt solution in the beakers 1, 2, 3,4 and 5 respectively. Stir well. Keep the solution at room temperature for 10 minutes. Measure the optical density at 540 nm (green filter) using the reagent blank. Then pour the solution into the cuvette and note the absorbance values of each solution. Draw a standard graph using concentration along x-axis and absorbance along y-axis. Figure 3 shows the colorimeter used for this project work.

#### UV Spectral analysis

UV-Visible spectra were recorded using Jasco V-630 spectrophotometer for the extract and the mixture of extract with copper(II) acetate solution in the range of 200-900 nm.

#### FT-IR Spectral Analysis

FTIR spectra were recorded for the extract and the mixture of extract with copper(II) acetate using Nicolet Si5 spectrometer using KBr pellets in the range of 4000-400 $cm^{-1}$

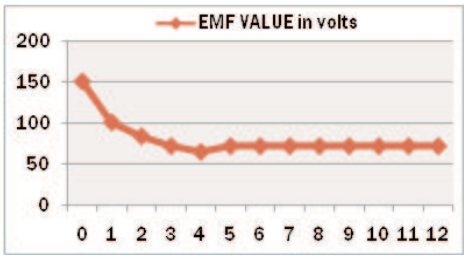
## Results and Discussion

### Confirmation of Nanoparticles using Potentiometer:

Using potentiometer, the formation of nanoparticles were confirmed from the emf values. EMF values noted from the potentiometer are plotted against the Volume of copper salt solution. Table 1.1 shows the potential values of the prepared extract.

**Table 1: Volume of Cupric Acetate Vs EMF Value & Graph:**

| VOLUME OF CUPRIC ACETATE in ml | EMF VALUE in volts |
|---|---|
| 0 | 151 |
| 1 | 101 |
| 2 | 84 |
| 3 | 72 |
| 4 | 65 |
| 5 | 72 |
| 6 | 72 |
| 7 | 72 |
| 8 | 72 |
| 9 | 72 |
| 10 | 72 |
| 11 | 72 |



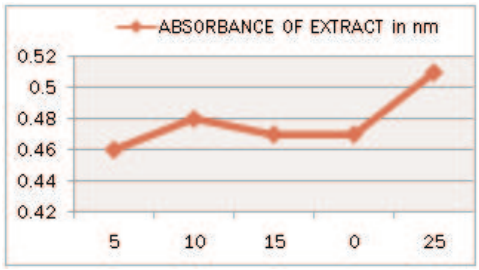

The potential values obtained above shows the formation of nanoparticles. As the concentration of the salt solution in the extract is increased, the nanoparticles are formed in the extract and it is confirmed by the constant emf values obtained from the potentiometer analysis.

### Confirmation of Nanoparticle using Photo Colorimeter:

The mixture containing $Cu^{2+}$ and extract was poured into the cuvette. The absorbance values are noted. The absorbance values noted from the colorimeter was plotted against the concentration of solution.The readings are tabulated and plotted in a graph below.

**Table 2: Concentration VS Absorbance of Extract & Graph:**

| CONCENTRATION in ml | ABSORBANCE in nm |
|---|---|
| 5 | 0.46 |
| 10 | 0.48 |
| 15 | 0.47 |
| 20 | 0.47 |
| 25 | 0.51 |




From the graph, it is clear that on increasing the concentration, the absorbance of the extract also increases.

### UV Vis Spectra Analysis

UV-Vis spectrum of the plant extract *Sargassum wightii* is given in figure 4. A peak was observed at 220 $cm^{-1}$. A peak was broadenend which shows the interaction of extract with Copper acetate (Figure 5).

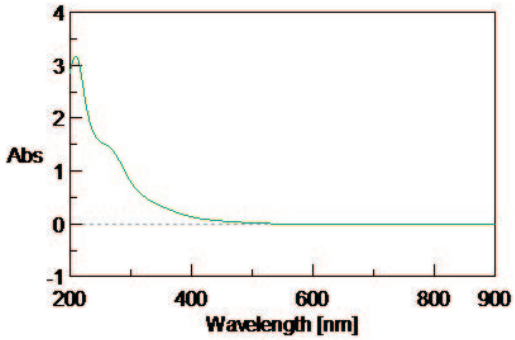



**Figure 4: UV-Visible analysis of plant extract**

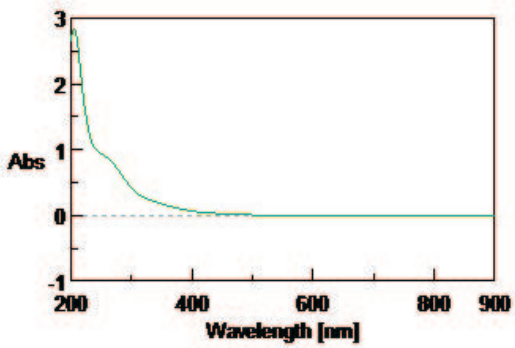



**Figure 5: UV-Visible analysis of plant extract with Copper (II) acetate**

**FTIR Analysis:**

Infrared spectroscopy is an technique to determine the nature of functional groups. FTIR was recorded for the plant extract (Fig.6) and for the extract with Copper acetate. A peak at 1500 indicates the C-C stretching frequency. A peak around 600 $cm^{-1}$ indicates the formation of Nanoparticles. A peak around 3300 to 3400 $cm^{-1}$ may be due to N-H stretching.

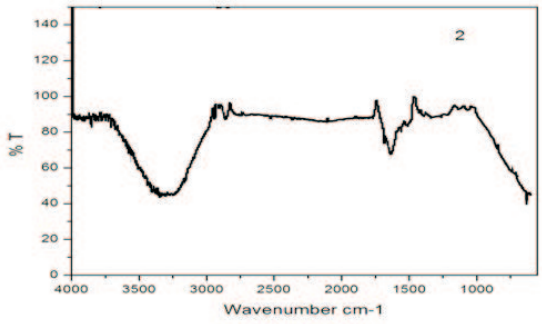



**Figure 6: FTIR analysis of extract**

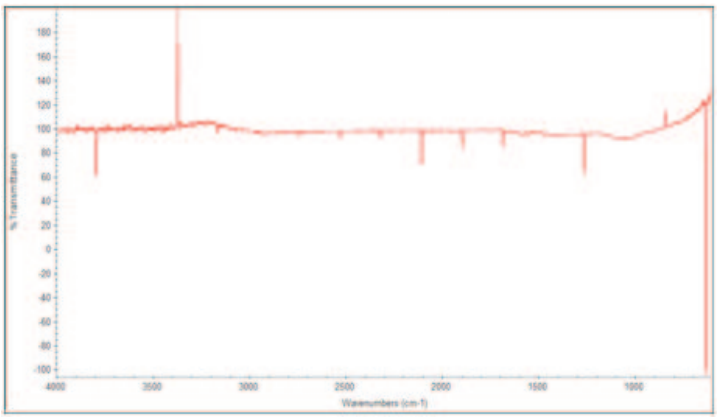


**Figure 7: FTIR analysis of extract with Copper (II) acetate**

## Conclusion:

Nano technology is emerging effectively with good scope in all the fields. In view of this, we have suggested an eco-friendly method to synthesise nanoparticles. This method is cost economical as it does not involve temperature, pressure , energy, catalyst.etc. This type of synthesis is easy and it it can also be suggested for various industrial and medical applications. On account of this we suggest the study of antibacterial applications of this nanoparticle as future research work. Also, the characterization of this nanoparticles using various techniques can be carried out in the future research work.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Biogenic Synthesis of Copper Nanoparticles using Sargassum

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## Abstract

Nanoparticles synthesis by green method is an eco-friendly method. In this method, sargassum was used to synthesis nano particles. This method of synthesis is simple and cost effective. Copper nanoparticles prepared by this green route exhibit improved properties. This method of investigation helps our environment to get free from toxic chemicals. In this method, sargassum was used to synthesise copper nanoparticles. This synthesis will helps us to get an efficient nanoparticles.

***Keywords:*** nanoparticles, sargassum, green synthesis

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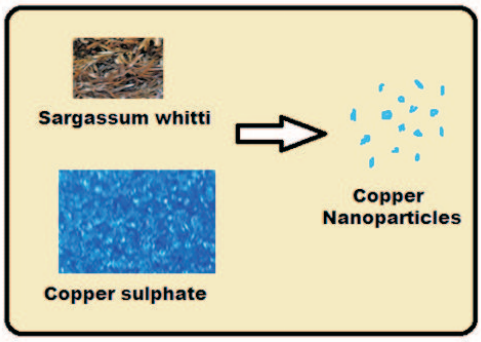


## Introduction

Synthesis of nanoparticles using toxic chemicals may cause environmental damage. Green synthesis is a best method to synthesise Nanoparticles. Sargassum is a genus of brown macroalgae in the order Fucales. Copper nanoparticles are of great importance

in medicinal field. It also play an important role in electrical industry, nanodevices. Green synthesis of nanoparticles from tectonagrandis[1], medicinal plant[2], saliva spinosa[3], Tulsi[4], alovera[5], mango[6], buchu[7], ocimum sanctum[8], *moringa olifera*[9], geraniol[10], *Aspergillus terreus*[11], *Penicillium autantiogriseum*, *Penicilliium citricum* and *Penicillium waksmanii*[12], *Achillea wilhelmsii*[13], *A. philippense*[14,15], *Asplenium scolopendrium*[16], *Actino pterisradiata*[17], *Azolla microphylla*[18] have been reported earlier.

## Materials and Methods

The precursor copper sulphate was purchased. The solutions were prepared using double distilled water. *Sargassum wightii* was chosen to study the synthesis of copper nanoparticles. Sargassum belongs to the family Sargassaceae and it belongs to the order Order Fucales.

**Figure 1:** ***Sargassum wightii***

Sargassum was collected from the seashores of Kanyakumari District (Fig.1). The plant species was cleaned in running tap water to remove the surface contamination and dried in shade upto15 days. After 15 days, it was finely powdered. Powdered materials are kept safely.

#### Preparation of plant extract:

10.0g of powdered plant species is weighed using electronic balance. It is then transferred to a 250ml conical flask containing 100ml of distilled water. It is then placed in a hot water bath for 30 minutes. After 30 minutes, it was cooled and filtered. The aqueous extract of the plant species was filtered in a 100 ml beaker using Whatman'sno.1 filter paper. The extract was further used for the synthesis of nanoparticles.

#### Preparation of copper sulphate solution:

0.01 M Copper Sulphatesolution is prepared in a 100 ml SMF. 0.25g of copper sulphate is weighed using electronic balance and it was transferred to 100ml SMF to prepare 0.01M solution.

#### Biogenic synthesis of copper nanoparticles

About 50ml of the extract was taken in a beaker and 50ml of the 0.01M copper sulphate is added drop wise. A colour change was observed in the reaction mixture. Potentiometer was used for measuring electric potential. Platinum and calomel were used as working and reference electrode for measuring emf.Colorimeter was used to determine the value of absorbance.

## Results and Discussion

$Cu^{2+}$ nano particles were synthesized using Sargassum extracts using green route method. The change of colour from blue to brown (Fig.2 ) indicates the formation of nanoparticles.

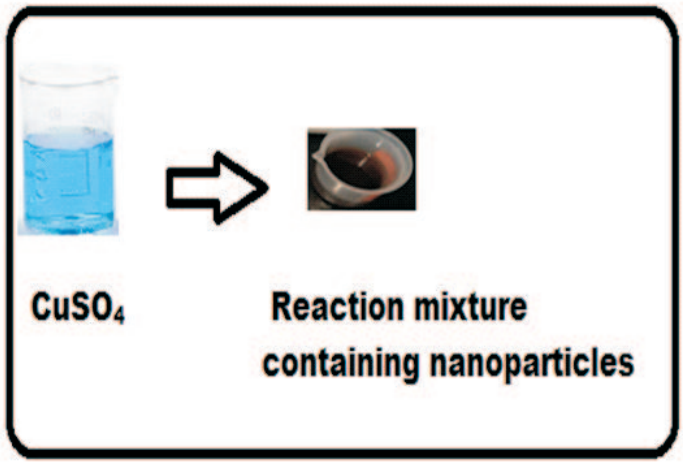


**Figure 2: Formation of Nanoparticles**

## Potentiometric Analysis

40 ml of extract is taken in a beaker. The burette is filled with the copper sulphate solution. The electrodes are dipped in the extract and the electric potential is measured for the pure extract. Then the extract is added with 5ml of $Cu^{2+}$ solution and the potential values are noted. Similar readings are taken by increasing the volume of 0.01M $CuSO_4$ solution by 1 ml till the reading becomes stable. The stable reading indicates the formation of nanoparticles. Table 1 represents the potentiometric data for the mixture containing Sargassum extract and $CuSO_4$. Fig.3 illustrates the plot of absorbance against the volume of 0.01M $CuSO_4$.

**Table 1: Volume of $CuSO_4$ versus EMF**

| Volume of 0.01M $CuSO_4$ added(ml) | Emf (volt) |
|---|---|
| 1 | 32 |
| 2 | 40 |
| 3 | 45 |
| 4 | 48 |
| 5 | 65 |
| 6 | 65 |
| 7 | 65 |
| 8 | 65 |
| 9 | 65 |
| 10 | 65 |
| 11 | 65 |
| 12 | 66 |

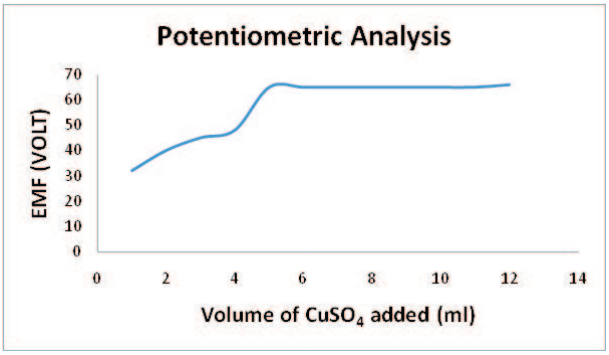


**Figure 3: Potentiometric Analysis of copper Nanoparticles**

#### Colorimeteric Analysis

Take 5 ml of the extract in a 100 ml beaker. Pipette out 5, 10, 15, 20 and 25mL of the copper salt solution into a separate 100ml beaker and label it as B1, B2, B3, B4 and B5. Stir well with a glass rod. Keep the solution at room temperature for 10 minutes. Switch on the instrument and keep the blank solution. Then measure the optical density of each solution by fixing the wavelength at 540nm (green filter). The absorbance values are noted for B1, B2, B3, B4 and B5. Now, draw the graph between concentration (x-axis) and optical density ( y-axis). Table 2 represents the colorimetric data of the synthesized nanoparticles. Fig.4 shows the plot of absorbance against the volume of Copper sulphate added. The results show that as the volume of the copper sulphate increases the value of absorbance decreased.

**Table 2: Volume of $CuSO_4$ versus EMF**

| Volume of 0.01M $CuSO_4$ added (ml) | ABSORBANCE (nm) |
|---|---|
| 5 | 0.52 |
| 10 | 0.41 |
| 15 | 0.38 |
| 20 | 0.35 |
| 25 | 0.22 |

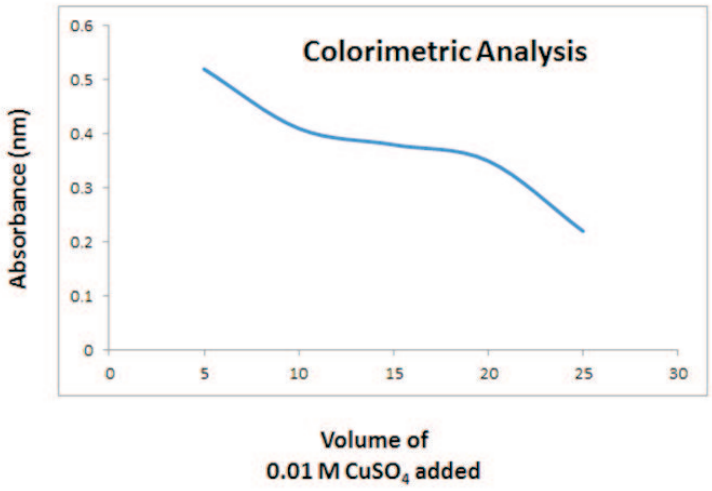


**Figure 4: Plot of Volume (ml) versus Absorbance (nm) for $Cu^{2+}$ Nanoparticles**

#### UV-Vis Analysis

UV-Visible spectra were recorded using Jasco V-630 spectrophotometer for the extract and the mixture of extract with copper(II) acetate solution in the range of 200-900 nm.

The optical absorbance properties of Nano Particles were investigated using UV-Visible diffuse reflectance spectrophotometer in the wavelength range 200-900 nm at ambient temperature. Figure 6 shows the different absorption spectra. There is a strong absorption peak between 218 to 240 nm which confirms formation of copper nanoparticles.

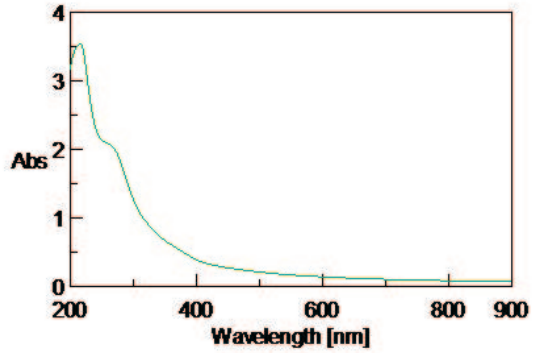


**Figure 5: UV Spectroscopy of extract**

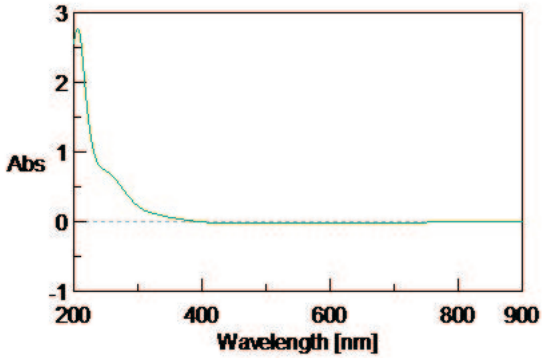


**Figure 6: UV-Vis spectrum of Copper Nanoparticles**

**FTIR Analysis of Copper nanoparticles**

FTIR spectra were recorded for the extract and the mixture of extract with copper(II) sulphate using Nicolet Si5 spectrometer using KBr pellets in the range of 4000-400 $cm^{-1}$

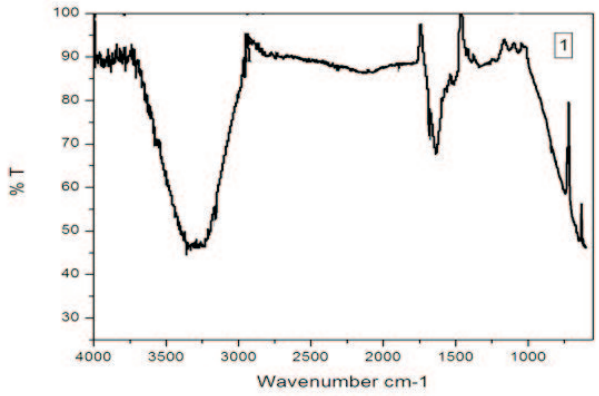


**Figure 7: FTIR Analysis of extract**

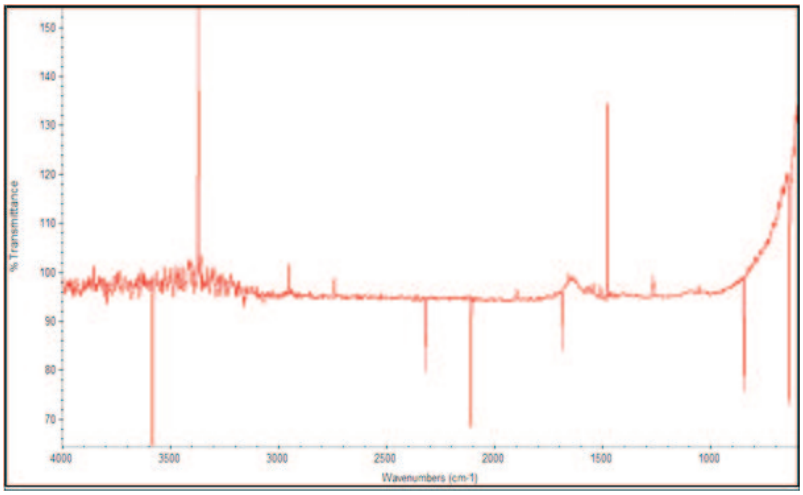


**Figure 8: FTIR Spectra of extract with Copper (II) Sulphate**

FTIR Spectroscopy is used to understand the nature of functional groups present. FTIR spectra of Copper nano particles are shown in Figure 5. The band at 1600 $cm^{-1}$ indicates the presence of carbonyl group. The brand and strong peak in the region 3300 $cm^{-1}$ is due to the -OH stretching vibrations which are very intense. The peaks around 620 $cm^{-1}$ indicate the formation of copper nano particles.

**Table 4: FTIR Data for Extract**

| **Extract** | |
|---|---|
| **Wave number ($cm^{-1}$)** | **Vibrations** |
| AROUND 600 | C-H out of plane bending |
| AROUND 1400 | aromatic C-H group |
| AROUND 1550 | C=O stretching vibration |
| AROUND 3400 | stretching vibration of hydroxyl groups (O-H stretching mode) |

**Table 5: FTIR Data for Extract and Precursor**

| **Extract + Precursor** | |
|---|---|
| **Wave number ($cm^{-1}$)** | **Vibrations** |
| AROUND 600 | C-H out of plane bending |
| AROUND 800 | Cu-O-H Vibration mode ($CuSO_4$) |
| AROUND 1600 | C=O stretching vibration / O-H bending |
| AROUND 2100 | C=N Stretch |
| AROUND 2300 | C=N Stretch |
| AROUND 3600 | stretching vibration of hydroxyl groups (O-H stretching mode ) |

## Conclusion

Biogenic Synthesis is the effective pathway for future research. The small size of the nano particle leads to the enhanced surface area. This leads to the availability of more reaction sites and enhanced activity. In this investigation, we came to a conclusion that the copper nano particles prepared by this green route was cost effective and it will exhibit improved properties. The size, distribution and morphology of the nano particles play a significant role in the research area. This way of synthesis is ecofriendly and it can be suggested for large scale synthesis. This will also helps our environment as there is no need of temperature, pressure, energy and toxic chemicals.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# GALLERY

## EQUIPMENTS PURCHASED UNDER STAR COLLEGE SCHEME

## (2019-2020)

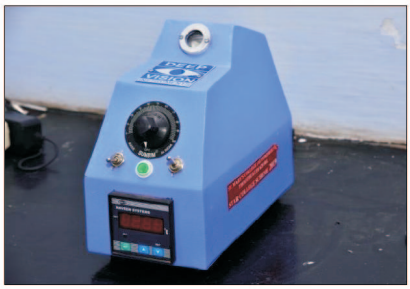
## DEPARTMENT OF CHEMISTRY

**UV -Vis Software, Computer and Printer**

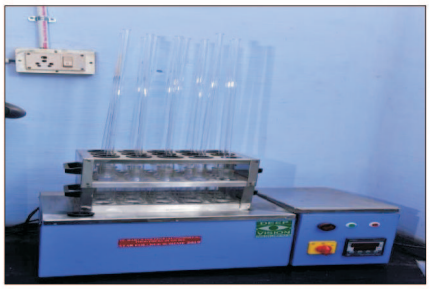
**Flame Photometer**



**MINILAB for Soil Analysis Set**



**Melting Point Apparatus**

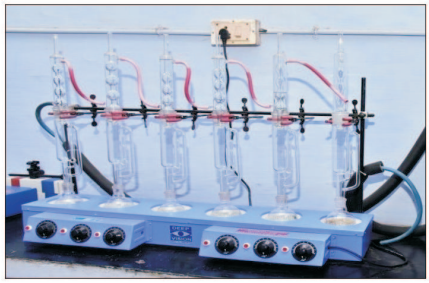


**COD Digester**

**UV- Fluorescent Cabinet**

**Digital Colorimeter**

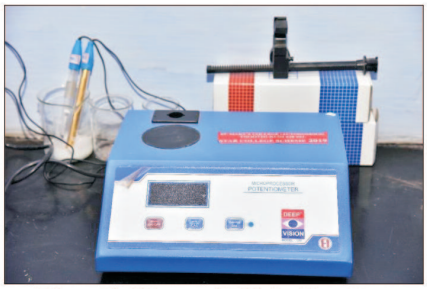
**Conductivity Meter**



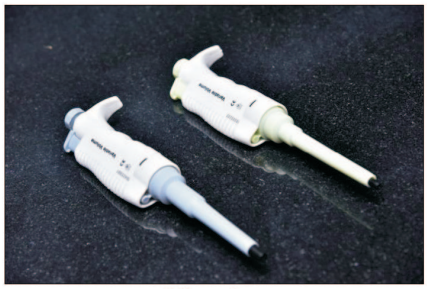
**Soxhlet Apparatus**

**pH Meter**

**Magnetic Stirrer with Hot Plate**



**Magnetic Stirrer with Potentiometer**

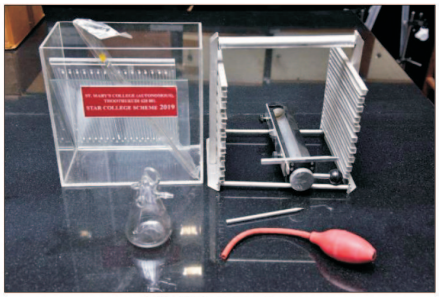


**Micropipettes**

**Hot Air Oven**

**BOD Incubator**

**Water Bath Circulator**



**TLC Apparatus**

# DEPARTMENT OF PHYSICS

# MESSAGE FROM THE HOD

**Sr. Dr. Jessie Fernando**
**Head, Department of Physics**
**St. Mary's College (Autonomous)**
**Thoothukudi.**

Warm greetings from the Department of Physics. Opting project work for UG students in the final year is very important as it enables the students to explore the frontiers of science and technology. It motivates them to have a hands-on training into research and to reap the benefits of doing research for knowing growing and sharing knowledge. I greatly appreciate the project mentors of my department for guiding the students to take up project work in the field of nano studies, ultrasonics, crystal growth and thermal studies which is the trend of applied research work in the current scenario. By doing the project, the students have learnt, formulating significant questions, developing investigating procedures, gathering and examining evidences, evaluating the results and sharing the inferences, conclusions with the research communities.The students also develop a healthy relationship with the faculty and both of them share the joy of discovering something new. It enables the staff to pick up capable and motivated students to help them in continuing further research activities. I thank DBT through Star College Scheme for all support rendered in the form of granting funds for furnishing the department with necessary research equipments. I also acknowledge with gratitude, the support and encouragement given by the administrators of St. Mary's College (Autonomous), Thoothukudi.

**Dr. Sr. JESSIE FERNANDO**

# MESSAGE FROM THE REVIEWER

**Dr. J. Poongodi**
**Associate Professor of Physics**
**Kamaraj College**
**Thoothukudi.**

I have reviewed the undergraduate research papers of the final year B.Sc Physics students of St. Mary's College (Autonomous) ,Thoothukudi. It is learnt that they have undertaken the research projects under DBT Star College Scheme. All the papers submitted for review kept good quality at par with degree level.

The quality of safety of agro-food products is a growing concern nowadays. Quality assessment of agricultural materials and food products by acoustic analysis has been one of the major research areas of physics. Acoustic properties of food can be used to indicate numerically important quality attributes such as crunchiness, crispness, firmness, water activity and other standardisation of procedures further widens its possibilities. The authors of *A Study of Acoustical Parameters of Fruit Extracts* did a preliminary study on the analysis of various fruits such as lime, coconut, orange, tomato, grape and pomegranate. The standardized values can be exploited in the quality analysis of commercially available fruit juices after testing against refined and sophisticated methods.

The authors of *Measurement of Thermal Conductivity by Forbe's Method* further measured the thermal conductivity of aluminium. The materials with high thermal conductivity exhibit high electrical conductivity' is explained and demonstrated using aluminium. The thermal conductivity study is important in heat sink applications. Though the work gives a chance of research exposure to the students, it has no novelty in technique or outcome point of view. Moreover it is one of the earliest methods for determination of thermal conductivity of a substance. The experiment is a tedious one.

*Synthesis and characterisation of SnO nanoparticles via Co-precipitation Method* demonstrates the characteristics and co-precipitation method for synthesis of tin oxide

nanoparticles synthesised using tin oxide nanoparticles tin chloride, triton x-100 and ammonia precipitators. The quality of the XRD study revealed the purity of the nanoparticles synthesised. Therefore the standardised procedure has potential application in industrial production of the SnO-nano particle.

The authors of *Synthesis and Characterization of Nickel Oxide Nanoparticles* by chemical precipitation method used nickel sulphate and sodium hydroxide for the synthesis of ni-nanoparticles. The authors have standardised ni-nanoparticles synthesis in a simple and cost-effective manner which can be further explored in its industrial production.

The last work, *Growth, Structural and Optical Properties of Pure kdp Single Crystals* by slow evaporation method, speaks the story of potassium dihydrogen phosphate (kdp) crystal growth and its basic property analysis. The authors have made significant achievement at their level in growing kdp crystal using a cost effective method.

To sum up, all the paper are of comparatively good quality and I do admire the students and the guiding faculty for following standard research methodology and writing. I am thankful to the college authorities for giving me an opportunity to review the student research papers generated under the DBT Star College Scheme.

**Dr. J. POONGODI**

# A Study of Acoustical Parameters of Fruit Extracts

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Received on 7 November 2020

## Abstract

Fruit extracts contain many health promoting nutrients such as vitamin *C*, polyphenols and carotenoids and thereby reducing heart diseases for better life expectancy of human beings. A knowledge about its acoustic parameters may help in the application of these extracts in medical field.This paper presents the result of a preliminary research using an ultrasonic technique to measure ultrasonic velocity, density and viscosity for different fruit extracts at room temperature. From these primary parameters, the derived parameters like adiabatic compressibility $(\beta_a)$, free length $(L_f)$, relaxation time $(\tau)$ and acoustic impedance $(Z)$ have been computed using standard relations and their correlation has been discussed.

***Keywords:*** Fruit extracts, ultrasonic velocity, adiabatic compressibility, free length.

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## Introduction

Ultrasonic study has gained momentum during the last two decades. The information derived from this study helps to gain knowledge about the state of any solution. The application of ultrasonics in the characterization of food stuffs helps in the understanding of its composition and used for carrying out different chemical process to improve their utility.In the testing of fruit juice, the percentage of sugar contents are determined[1−4].

Fruits, vegetables and their extracts help to fight against many deadly diseases as they possessed increased antioxidant, antiviral, and anticancer properties[5]. The presence of vitamin C, fibre content, arginine, potassium and folate has been proved positively for treatment of various health issues[6−8].

## Materials and Methods

Fresh fruits such as lime, orange, grape, pomegranate, tomato and tender coconut were purchased from the local market in Thoothukudi, Tamilnadu, South India. Before experimentation, juices were extracted using the extractor and it was filtered with the Whatmann's Filter paper No.1.

### Measurement of ultrasonic velocity:

The velocity of ultrasonic wave in the extracts of fruits was determined using a single frequency interferometer (Model F-81) with a high degree of accuracy operating at frequency 3 MHz supplied by Mittal enterprises, New Delhi.

Ultrasonic velocity is the speed at which ultrasonic sound waves travel through a medium. Ultrasonic velocity $(U)$ has been calculated from the expression

$$U = \lambda f(m/s)$$

where,

$\lambda = \dfrac{2d}{N}$ is the wavelength of the ultrasonic waves in the liquid mixture in metre

$f$ is the frequency of the generator (3 MHz) in hertz and

$d$ is the distance between two successive maxima or minima in metre

$N$ is the number of maxima or minima

### Measurement of density:

Density refers to quantity of matter packed in a substance. In liquids, density determines if a substance will float or sink in a particular liquid. The density $(\rho)$ of the fruit extract was determined using a specific gravity bottle of 5 ml capacity and the relation:

$$\rho_2 = \frac{w_2}{w_1}\rho_1 \ (kg\ m^{-3})$$

where $w_1$, $w_2$, $\rho_1$, and $\rho_2$ are masses of distilled water, mass of the fruit extract, density of distilled water and density of the fruit extract respectively.

### Measurement of viscosity:

The viscosity of a fluid is a measure of its resistance to deformation at a given rate. It was determined by an Ostwald viscometer containing the fruit extracts and the time flow was measured using a stop watch and calculated using the relation:

$$\eta = \rho t \times \frac{\eta_0}{\rho_0 t_0} \ (N.sm^{-2})$$

where $\eta_0$, $\eta$, $t_0$ and $t$ are coefficient of viscosity of distilled water, coefficient of viscosity of fruit extracts, time of flow of distilled water and time of flow of the fruit extracts respectively.

### Formulae:

The derived acoustical parameters such as adiabatic compressibility $(\beta_a)$, free length $(L_f)$, impedance $(Z)$ and relaxation time $(\tau)$ were calculated from the measured data. The formulae used[9] are listed below:

(i) Adiabatic compressibility $\beta_a = \dfrac{1}{\rho U^2}$ $(Nm^{-2})$

(ii) Specific acoustic impedance $Z = \rho U (Pa \cdot s/m^3)$

(iii) Intermolecular free length $L_f = K_T \sqrt{\beta}$ $(A^o)$

(iv) Relaxation time $\rho = 4/3\ \beta_a \eta$ $(s)$

## Result and Discussion

Velocity of 3MHz ultrasonic wave in fresh fruit extracts and their densities and viscosities were measured with pre-calibrated interferometer,density bottle and viscometer respectively to nearest mg in the room temperature and are tabulated in Table 1. The derived acoustical parameters such as adiabatic compressibility $(\beta)$, free length $(L_f)$, impedance $(Z)$ and relaxation time $(\tau)$ were calculated from ultrasonic velocity $(U)$, density $(\rho)$, viscosity $(\eta)$ and the results are tabulated in Table 2.

**Table 1: Ultrasonic velocity $(U)$, Density $(\rho)$ and Viscosity $(\eta)$ of fresh fruit extracts**

| Sample | Ultrasonic velocity | Density | Viscosity |
|---|---|---|---|
| | $U\ ms^{-1}$ | $\rho\ kg/m^3$ | $\eta\ 10^{-3}\ Nsm^{-2}$ |
| **Lime** | 1527 | 1071 | 1.071 |
| **Orange** | 1012 | 1012 | 1.136 |
| **Tender coconut** | 1530 | 1018 | 0.872 |
| **Grape** | 3132 | 1060 | 1.314 |
| **Pomegranate** | 3132 | 1031 | 1.061 |
| **Tomato** | 3114 | 995 | 1.109 |

Among the samples, ultrasonic velocity for grape, pomegranate and tomato were found to be more or less equal. The value of velocity for the extracts of lime and tender coconut matched with each other. The least value of ultrasonic velocity was observed for orange.

Density of all the fruit extracts except tomato were more or less equal and the value of viscosity was similar for all the extracts except tender coconut water.

**Table 2: Adiabatic compressibility ($\beta$), Free length ($L_f$), Acoustical impedance ($Z$) and Relaxation time ($\tau$) of fresh fruit extracts**

| Sample | Adiabatic Compressibility | Free length | Acoustical impedance | Relaxation Time |
|---|---|---|---|---|
| | $\beta$ $(10^{-10}m^2N^{-1})$ | $L_f$ $(10^{-10}m)$ | $Z$ $(10^6 kg\, m^{-2}s^{-1})$ | $(\eta\ 10^{-13}\ s)$ |
| **Lime** | 4.002 | 0.397 | 1.636 | 6.601 |
| **Orange** | 4.200 | 0.407 | 1.552 | 5.804 |
| **Tender Coconut** | 4.195 | 0.407 | 1.557 | 4.883 |
| **Grape** | 0.961 | 0.194 | 3.332 | 1.684 |
| **Pomegranate** | 0.988 | 0.197 | 3.229 | 1.398 |
| **Tomato** | 1.003 | 0.199 | 3.101 | 1.485 |

Adiabatic compressibility was found to be similar and higher for extracts of lime, orange and tender coconut. Similar and lower values of adiabatic compressibility were observed for extracts of grape, pomegranate and tomato.But the inverse trend was observed in the value of acoustical impedance. Free length was observed to be more or less equal for the extracts of lime, orange and tender coconut and similar values were found for extracts of grape, pomegranate and tomato. Maximum value of relaxation time was noticed for lime and there was a gradual decrease in the value for orange and tender coconut water. The relaxation time for the extracts of grape, pomegranate and tomato were observed to be more or less equal.

## Conclusion

The ultrasonic velocity $(U)$, density $(\rho)$ and viscosity $(\eta)$ have been measured for the fruit extracts at room temperature 304K. From these data, few acoustic parameters such as adiabatic compressibility $(\beta\alpha)$, free length $(L_f)$, relaxation time $(\tau)$ and acoustic impedance $(Z)$ have been computed using standard relations. The variations in ultrasonic velocity and other parameters play a significant role in understanding the properties of fruit extracts and hence can be utilized for medical applications.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

## References


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# Measurement of Thermal Conductivity by Forbe'S Method

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Received on 7 November 2020

## Abstract

Thermal conductivity is an intrinsic property of a material that expresses its ability to conduct heat. It relates the rate of heat loss per unit area of a material to its rate of change of temperature. In this project work, thermal conductivity of aluminum is measured using Forbe's method. The heat generated dissipates into the sample at a rate depending on the thermal properties of the material. By recording temperature vs. time response in the sensor, thermal conductivity, thermal diffusivity, thermal effusivity, and specific heat capacity of the material can be calculated. It is proved that the materials with a higher thermal conductivity are good conductors of thermal energy.

***Keywords:*** Thermal conductivity, intrinsic property, Forbe's method and Wiedemann-Franz Law.

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## Introduction

As a material property thermal conductivity occupies an important position in physical, biological and geological sciences. Materials of high thermal conductivity are widely used in heat sink applications and of low thermal conductivity are used in thermal insulation[1]. Thermal conductivity of materials is temperature dependent. Metals with high thermal conductivity e.g. copper, aluminum exhibit high electrical conductivity. In this Forbe's method at the steady state, the quantity of heat passing through any section of a bar will be equal to the quantity of heat lot by radiation by the remaining part of the bar[6].

## Materials and Methods

One of the methods of determining the thermal conductivity of metals is by Forbe's. Metals are good conductors of both heat and electricity. Their heat conduction ability is defined by thermal conductivity. Higher the value of thermal conductivity more is the heat conduction. For example, Copper is a very good conductor of heat (390 W/m K) followed by iron, aluminium etc...in decreasing order[4].

In order to calculate the thermal conductivity, a long rod of uniform cross section area, mercury thermometer, stopwatch, heater, Forbe's apparatus were used.

## Thermal Conductivity Related to Other Quantities

#### Thermal diffusivity:

It is the thermal inertia of the material. It represents how fast heat diffuses through the material. As, $k$ increases, thermal diffusivity also increases[7].

$$\alpha = k/\rho c_p$$

#### Thermal effusivity::

Thermal effusivity (sometimes called the heat penetration co-efficient) is the rate at which a material can absorb heat. It is the property that determines the contact temperature of two bodies that touch each other.

$$e = \sqrt{k\rho c_p}$$

#### Specific heat capacity:

It is defined as the amount of heat required for 1 kilogram of substance to increase the temperature by 1 kelvin. It is the heat storage capability of the material. It represents how much energy the material stores per unit volume.

As $k$ increases, $C_p$ value also increases[2].

#### Thermal resistivity:

It is the resistance to heat flow. It is the inverse of the thermal conductivity. Its unit is $K\ m\ W^{-1}$.

$$r = 1/k\ (mK/W)$$

#### Electrical conductivity:

By Wiedemann-Franz Law,

$$\frac{k}{\sigma} = LT$$

Therefore, As $k$ increases electrical conductivity also increases. But it is applicable only to metals.

#### Thermal resistance:

It is the inverse of the thermal conductance.

$$\text{Thermal resistance} = L/kA\ (K/W)$$

#### Heat Transfer Co-efficient

It is defined as the quantity of heat that passes per unit time through unit area of the plate of particular thickness, when it's opposite faces differs in temperature by 1K.

$$\text{Heat Transfer Co} - \text{efficient} = K/L\ (W/K/m^2)$$

#### Thermal insulance:

It is the reciprocal of heat transfer co-efficient.

$$\text{Thermal Insulance} = L/K\ (Km^2/W)$$

#### Thermal transmittance:

It quantifies the thermal conductance of a structure along with heat transfer due to convection and radiation[3].

## Formula:

**Thermal conductivity of the material**

$$K = \frac{\rho C}{\tan\alpha} \int_{x_1}^{L} \left(\frac{d\theta}{dt}\right) dx$$

Where,

$\rho$ = Density of the material

$C$ = Specific heat of the material

## Results and Discussion

### Observation:

**Table 1: Temperature recorded along the length of the rod after the steady state is reached**

| Time | Temperature along the length of the rod | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| | $X_1 = 0.267m$ | $X_2 = 0.318m$ | $X_3 = 0.368m$ | $X_4 = 0.418m$ | $X_5 = 0.468m$ | $X_6 = 0.518m$ |
| 01:00:00 | 70 | 60 | 52 | 45 | 42 | 38 |

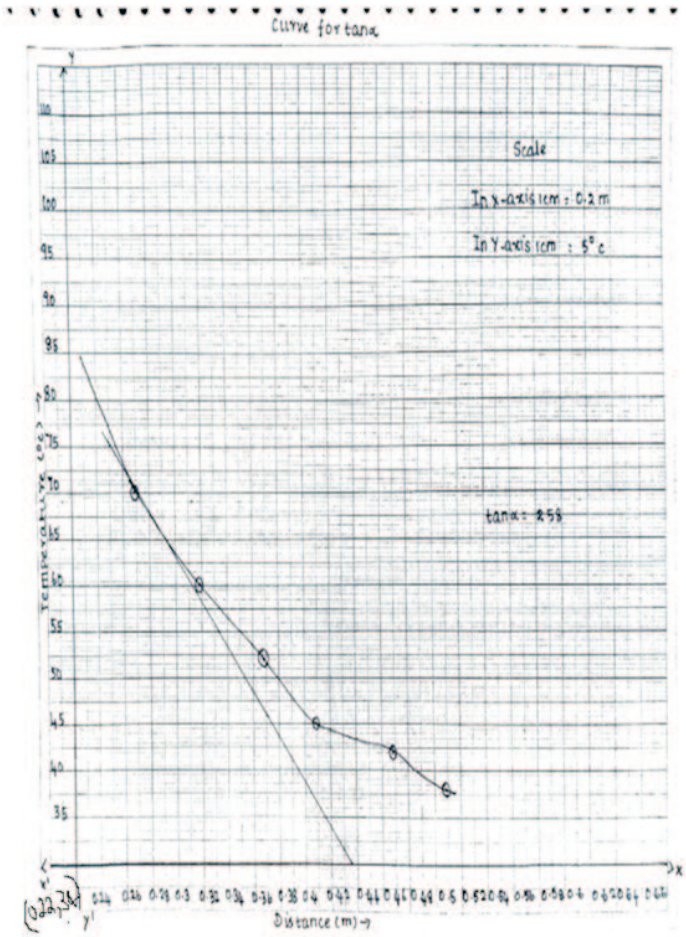
**Table 2: Temperature recorded during cooling of the rod at various distances from the heated end**

| Time | Temperature along the length of the rod | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ |
| | $X_1 = 0.267m$ | $X_2 = 0.318m$ | $X_3 = 0.368m$ | $X_4 = 0.418m$ | $X_5 = 0.468m$ | $X_6 = 0.518m$ |
| 01.00 | 70 | 60 | 52 | 45 | 42 | 38 |
| 02.00 | 69 | 60 | 52 | 45 | 42 | 38 |
| 03.00 | 68 | 60 | 51 | 44 | 42 | 37 |
| 04.00 | 68 | 60 | 51 | 44 | 42 | 37 |
| 05.00 | 67 | 59 | 51 | 44 | 42 | 37 |
| 06.00 | 65 | 59 | 50 | 43 | 41 | 36 |
| 07.00 | 65 | 59 | 50 | 43 | 41 | 36 |
| 08.00 | 64 | 56 | 50 | 43 | 41 | 35 |
| 09.00 | 63 | 55 | 49 | 42 | 40 | 35 |
| 10:00 | 62 | 55 | 49 | 42 | 40 | 35 |

**Table 3: Rate of cooling along the length of the rod: Total length of the rod $L = 61cm = 0.61m$**

| Distance $(m)$ | $(L-x_i)(m)$ | $d\theta/dt$ | $(L-x_i)d\theta/dt$ |
|---|---|---|---|
| 0.267 | 0.343 | 0.01666 | 0.00569 |
| 0.318 | 0.292 | 0.01724 | 0.00503 |
| 0.368 | 0.242 | 0.00722 | 0.00174 |
| 0.418 | 0.192 | 0.00680 | 0.00284 |
| 0.468 | 0.142 | 0.00570 | 0.00266 |
| 0.518 | 0.092 | 0.00637 | 0.00326 |
| | | | **0.02122** |

## Cooling Curve




## Conclusion

From these data,the thermal conductivity $(k)$ of aluminum is calculated and tabulated along with the values of Thermal effusivity $(e)$, Thermal resistivity $(r)$, Thermal diffusivity $(a)$, Density $(\rho)$ and specific heat capacity $(C_v)$[5]. The fact that the materials with high thermal conductivity exhibit high electrical conductivity is explained and demonstrated by this study.

| Material | Thermal conductivity $(k)$ $(W/mK)$ | Thermal Diffusivity $(a)$ $Mls$ | Thermal resistivity $(r)$ $(Km/W)$ | Thermal effusivity $(e)$ $W_s^{1'2}/m^2K$ | Density $\rho$ $(Kg/m^3)$ | Specific Heat $C_p$ $(J/kg-K)$ |
|---|---|---|---|---|---|---|
| Aluminium | 204 | 8.2306 | 4.9019 | 2253 | 2700 | 921 |

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Synthesis and Characterisation of SnO Nanoparticles via Co-Precipitation Method

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## Abstract

The present study illustrates the characteristics and co-precipitation method for the synthesis of tin oxide nanoparticles. The tin oxide nanoparticles were produced using tin chloride, Triton X-100 and ammonia precipitators. The Structure, size and surface morphology of the tin oxide were studied by X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). The results show sphere shaped tin oxide nanoparticles without chlorine contamination. The crystallite size determined by the Scherrer formula is about 23 nm. Lattice parameters calculated by Nelson-Riley equation show high quality of crystallization.

***Keywords:*** Co-precipitation; Tin oxide; Nanoparticles

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## Introduction

Tin oxide is one of the most important materials[1] due to its high degree of transparency in the visible spectrum, strong physical and chemical interaction with adsorbed species, low operating temperature and strong thermal stability in air (up to 500°C) . Tin presents two oxidation states +2 and +4, therefore two types of oxides are possible: stannous oxide (SnO - romarchite) and stannic oxide ($SnO_2$ - cassiterite), of which $SnO_2$ is more stable than SnO. $SnO_2$ is a n-type semiconductor with a wide direct band gap (3.6 eV at 300 K)[2]. It is widely used in optoelectronic devices , electrodes for lithium ion batteries , solar cells, transistors and gas sensors to detect the combustible gases such as $H_2S$, CO, liquid petroleum, NO, $NO_2$ and $C_2H_2OH$ . Tin oxide nanoparticles are suitable for gas sensing applications due to

high surface to volume ratio, compared to bulk tin oxide, which results in increased sensitivity and adsorption. Tin oxide nanoparticles have been prepared by physical and chemical methods. Chemical methods offer the advantage of being inexpensive and easy to perform[3]. Inexpensive methods for obtaining nanoparticles in low temperatures with no need of special atmosphere are the most important aims in synthesis of high quality nanoparticles. Co-precipitation is a suitable chemical method in nanoparticles synthesis because it does not require high pressure and temperature and impure materials are eliminated by filtration and washing[3]. In the present study tin oxide nanoparticles were synthesized by co-precipitation method and ultrasonic waves irradiation was used to homogenize nanoparticles.

## Materials and Methods

Two beakers containing 0.1M stannous chloride of 6.769g and 0.1M Sodium hydroxide of 2.397g were mixed with 300ml of distilled water each. The solutions were stirred and mixed to form a combined single solution of stannous oxide and its side products by dripping method. The resultant solution was left to precipitate. The precipitate was separated from the mother liquid which contained the side products by washing three times using distilled water. The cleansed precipitate was filtered using whatmann filter paper and was dried by natural method of evaporation. The dried product was then powdered and calcined at 800°C for one hour in a muffle furnace to get pure stannous oxide nanoparticles. The obtained stannous oxide nanoparticle was then sent for tests like XRD, UV Spectroscopy and FTIR for further characteristic and analysis.

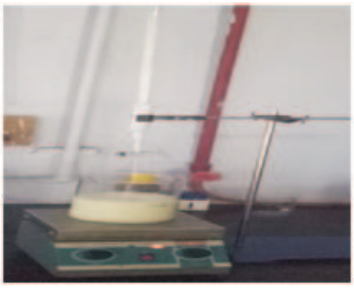

**Figure 1: Preparation of SnO nanoparticle by dripping method on constant stirring**

### Characterisation

The structural properties of SnO nano-powder were studied by X-ray diffraction measurements (Bruker D-8 advance diffractometer, Billerica, MA) using the Cu K$\alpha$ ($\lambda = 1.5406\mathring{A}$) as a radiation source, operated at 40kV and 30 mA with a scan rate of 0.02°/s over the range of 10°-80°. The average crystallite size d (h k l) of all

crystal planes for SnO powder was estimated from the classical Scherrer formula. The X-ray diffraction characterization is carried out for the prepared sample using XPERT-PRO diffraction system. FTIR spectrum is recorded in solid phase using KBr pellet technique .The analysis is recorded using an FTIR spectrometer in a range between 380-4000 $cm^{-1}$.

### Statistical analysis

The crystallite size D is calculated from the peak width using Debye Scherrer's formula,[4]

$$D = \frac{K\lambda}{\beta \cos\theta} \tag{1}$$

Where

$K$ -is the scherrer's constant.

$\beta$ - is the full width half maximum of the of a diffraction peak

$\lambda$ - is the wavelength of the incident wave.

$\theta$ - is the Bragg's diffraction angle.

The dislocation density is found out using the formula[5]

$$\delta = 1/D^2 \quad (\text{lines/sq.m}) \tag{2}$$

Where

$\delta$ - Dislocation Density

$D$ - Average grain size of the powder sample

The tetragonal lattice parameter $(a = b \neq c)$ for the tetragonal phase structure was determined by the equation,

$$\frac{1}{d^2} = \frac{h^2 + k^2}{a^2} + \frac{l^2}{c^2}$$

## Result and Discussion

Fig 2 shows that the XRD pattern of the SnO nanoparticles calcined at 800°C for 2 hours. The data were corrected for background, K$\alpha$2 stripped, fitted and refined.

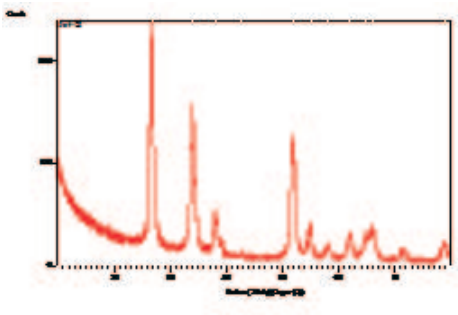

**Figure 2: XRD pattern of as-prepared SnO nanoparticles calcined at 800°C**

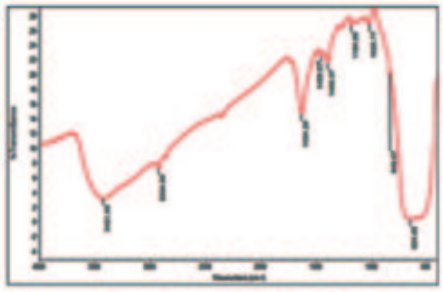

**Figure 3: The FTIR spectra of SnO nanoparticles with 0.1 M concentration calcined at 800°C**

**Table 1: Tabulation of d-spacing values observed for as-prepared SnO nanoparticles calcined at 800°C**

| S.No | $2\theta$ (degree) | Observed d-spacing values ($\AA$) | h k l | Dislocation Density ($10^{-4}$ lines/sq.m) | FWHM ($\beta$ value) | Average grain size (nm) |
|---|---|---|---|---|---|---|
| 1 | 26.7725 | 3.32997 | 110 | 4.0419 | 0.2342 | 49.7401 |
| 2 | 34.0045 | 2.63650 | 101 | 7.9397 | 0.2342 | 35.4892 |
| 3 | 51.8403 | 1.76368 | 211 | 0.5965 | 0.8699 | 12.9477 |
| 4 | 58.1069 | 1.58751 | 002 | 0.7797 | 0.8029 | 11.3245 |
| 5 | 62.0605 | 1.49554 | 310 | 0.2550 | 0.4684 | 19.8029 |

In XRD pattern, there are five strong peaks at $2\theta = 26.7725$°C, 34.0045°C, 51.8403°C, 58.1069°C, 62.0605°C, which can be attributed to the formation of the SnO nanoparticle. The average grain size of the SnO nanoparticles calculated from the FWHM values of the diffraction peaks using Debye-Scherrer formula.

Sharp diffraction peaks in the sample (calcined at 800°C) indicate the presence of long range order in these SnO nanoparticles. The JCPDS value of SnO nanoparticles is #41-1445.

The presence of vibration peaks attributed to Sn-O bond vibration confirms the formation of SnO. The bands have been assigned due to the absorption peaks of Sn-O, Sn-O-Sn, Sn-OH and C-O, bond vibrations. In which the strong absorption band at 3421.90 $cm^{-1}$ and the band at 1634.29 $cm^{-1}$ are due to the existence of OH on the adsorbed water and Sn-OH.

A sharp peak is appeared at 2924.09 $cm^{-1}$ due to carbon dioxide, which is incorporated from the atmospheric exposure. The absorption peak at 654.65 $cm^{-1}$ is assigned to Sn-O and Sn-O-Sn vibrations of SnO.

## Conclusion

In the present work, SnO are prepared using simple and economical co-precipitation method. The prepared sample is calcined at 800°C for 2 hours.

From XRD studies, it is found that SnO nanoparticle exhibit tetragonal structure as confirmed from JCPDS File number 41-1445. The absence of impurity peaks reveals that the SnO nanoparticle exhibit high crystalline quality. All the diffraction peaks of the prepared SnO nanoparticles obtained using the XRD analysis follows rutile tetragonal crystalline phase structure. The average grain size is 25.86088 nm.

From FTIR studies, the obtained peaks indicate the presence of SnO powders in nanometric scale. The presence of vibration peaks found in the given calcined SnO sample confirms the attributed Sn-O bond present in it.

The present study has given the insight that high quality SnO nanoparticles can be synthesized using different methods with varying reaction parameters to cater to the need of potential applications in future.Thus the aim of producing Tin oxide in nano scale done with precision has given the sweetest fruit to both our hard work and dedication and it can be used in further future products.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Synthesis and Characterization of Nickel Oxide Nanoparticles by Chemical Precipitation Method

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## Abstract

In the present study, Nickel oxide Nanoparticles have been synthesized by Chemical Precipitation method. Precursors used in this project are Nickel sulphate (as a basic material) and Sodium hydroxide (as a precipitator material). The synthesized NiO nanoparticles were annealed at 500°C and have been investigated by powder X-Ray Diffraction (XRD) and Fourier Transform Infrared Analysis (FT-IR). XRD analysis confirmed that the synthesized material is NiO nanoparticles of grain size around 12-17 nm. FT-IR spectra depicts a strong band at 420 $cm^{-1}$ which corresponds to the vibration of Ni-O band.

***Keywords:*** Nickel oxide, Sodium hydroxide, Nanoparticles, Precipitation.

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## Introduction

The word "nano" comes from the Greek word "nanos" meaning "dwarf". When quantifiable, it translates to one-billionth. Typically, nano means $10^{-9}$. The idea of nanoscience and technology was proposed in 1959 by the physicist Richard Feynman in his talk "There Plenty of Room at the Bottom". Nanoscience is an emerging area of science which concerns with the study of structure and phenomena of materials whose size is measured in nanometer scale (1-100 nm) at least in one dimension. Nanotechnology is the engineering of such nanoscale objects at molecular level using different techniques. In simple terms "the study of properties of materials at nanoscale is termed as nanoscience, whereas the fabrication and application of the nanostructures are termed as nanotechnology".

In recent years, there has been an increasing interest in the synthesis of nanosized crystal line metal oxides because of their large surface areas, unusual absorptive properties, surface defects and fast diffusivities[1]. NiO nanoparticles are used in alkaline batteries, electrochemical capacitors, smart windows and as an active layer for gas sensors. NiO became one of the most important transition metal oxides. NiO nanostructures are p-type semiconductors with particular magnetic behaviours such as super paramagnetic, super antiferromagnetic and ferromagnetic order depending upon the particle size, particle shape and synthesis route. We have synthesized NiO nanoparticles by chemical precipitation process in the presence of NaOH, which is a simple cost-effective method since the starting materials are few and inexpensive.

## Materials and Methods

The experiment was done with 4.642 g of Nickel sulphate and 2.439 g of Sodium hydroxide. Each of the substances were dissolved separately in distilled water and solutions of 300 ml were prepared. Nickel sulphate solution was taken in a burette and was allowed to drip into the NaOH solution that was at constant stirring. On completion of the dripping process, the solution mixture of Nickel sulphate and NaOH was kept at constant stirring for 30 minutes and left undisturbed for the precipitate to settle down. The colloidal precipitate thus formed was washed thrice with deionized water to remove the impurities present if any. The precipitate was filtered, dried for a week in atmospheric air and then powdered using a mortar and pestle. Further, the powdered sample was annealed at 500°C in the muffle furnace for an hour. During the process, a change in colour was observed. After annealing, the sample was powdered again and was used for characterization purposes.

**Figure 1: Pure NiO nanoparticles before annealing**

**Figure. 2 Pure NiO nanoparticles after annealing**

## Results and Discussion

### XRD Analysis:

Fig.3 shows that the XRD pattern of pure NiO nanoparticles annealed at 500°C with high intensity peak observed at $2\theta = 43.3418^{\circ}$ along the (200) hkl plane. The d-spacing values are identified with JCPDS file no. 89 - 5881[2].

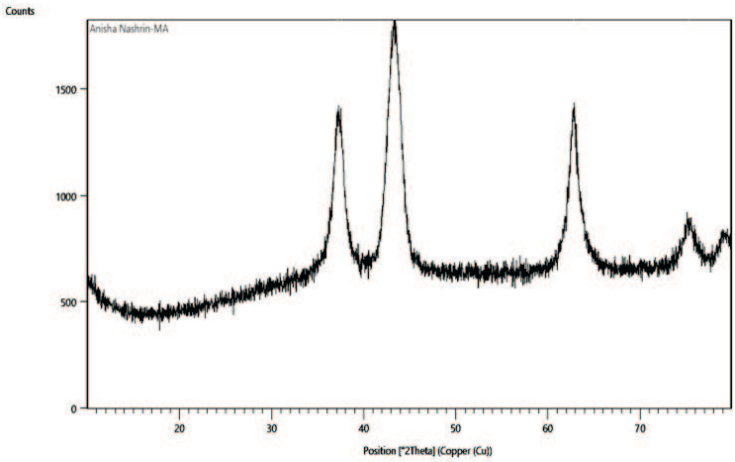


**Figure 3: XRD pattern of pure NiO nanoparticles annealed at 500°C**

**Table 1: Comparison of standard and observed d- spacing values of NiO nanoparticles annealed at 500°C**

| S.No | $2\theta$ (degree) | FWHM ($\beta$) (degree) | Observed d-spacing ($\AA$) | JCPDS d-spacing ($\AA$) | h k l | Relative intensity (%) |
|---|---|---|---|---|---|---|
| 1. | 37.1475 | 0.4684 | 2.42034 | 2.4113 | 111 | 69.14 |
| 2. | 43.3418 | 0.4684 | 2.08770 | 2.0883 | 200 | 100.00 |
| 3. | 62.7911 | 0.6691 | 1.47989 | 1.4776 | 220 | 67.29 |
| 4. | 75.3001 | 0.8029 | 1.26210 | 1.2592 | 311 | 15.60 |

**Table 2: Calculated grain size and dislocation density**

| $2\theta$ (degree) | FWHM ($\beta$) (degree) | Grain size (nm) | Dislocation density ($\delta$) ($10^{15}$ lines per sq.m) |
|---|---|---|---|
| 37.1475 | 0.4684 | 17.9014 | 3.1205 |
| 43.3418 | 0.4684 | 18.2591 | 2.9994 |
| 62.7911 | 0.6691 | 13.9185 | 5.1619 |
| 75.3001 | 0.8029 | 12.5032 | 6.3967 |

Thus, the average grain size of annealed NiO nanoparticles is calculated as 15.6456 nm and the average dislocation density is $4.4196 \times 10^{15}$ lines per sq.m.

## FTIR Analysis

The FTIR spectrum of the NiO nanoparticles annealed at 500°C was recorded in the range of 4000 $cm^{-1}$ to 400 $cm^{-1}$. Fig. 4 shows the FTIR spectrum of the annealed NiO nanoparticles.

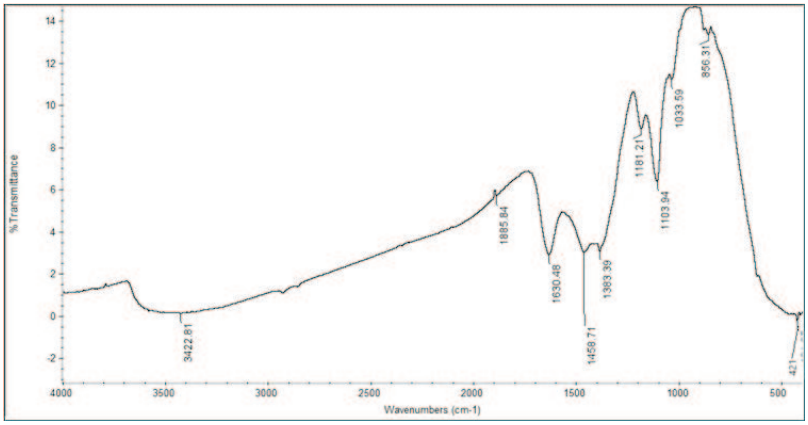


***Figure 4: FTIR spectrum of NiO nanoparticles annealed at 500°C***

The FTIR spectrum shows the characteristic peaks at 421 $cm^{-1}$, 1033.59 $cm^{-1}$, 1103.94 $cm^{-1}$, 1181.21 $cm^{-1}$, 1383.39 $cm^{-1}$, 1630.48 $cm^{-1}$ and 3422 $cm^{-1}$. The peak observed at 421 $cm^{-1}$ that corresponds to the vibration of Ni-O bond, reveals the presence of NiO nanoparticles. The broad band centered at 3422 $cm^{-1}$ is attributed to the O-H stretching vibration of the inter layer water molecules. The weak band near 1630.48 $cm^{-1}$ is attributed to H-O-H vibrations that are caused due to the adsorption of water in air[3]. The peaks at 1383.39 $cm^{-1}$, 1033.59 $cm^{-1}$, 1103.94 $cm^{-1}$ and 1181.21 $cm^{-1}$ are due to the O-C=O symmetric and C-O stretching vibrations originating from the adsorption of atmospheric $CO_2$, but the intensity of the bands have weakened which indicates that the ultra fine powder tends to physically adsorb water and $CO_2$[4].

## Conclusion

In this investigation, NiO nanoparticles are prepared by chemical precipitation method. NiO nanoparticles of small average grain size are obtained with minimal impurities. From the XRD studies, it is found that NiO nanoparticles exhibit cubic structure as confirmed from the JCPDS file no. 89 - 5881. The XRD peak of the NiO nanoparticles annealed at 500°C is very broad and sharp indicating that the particles are nano crystalline. The average grain size of the NiO nanoparticles annealed at 500°C is found to be 15.6456 nm and the dislocation density is found to be $4.4196 \times 10^{15}$ lines per sq.m. Further, the peak at 421 $cm^{-1}$ in the FTIR spectrum confirms the presence of Ni-O bond. This study is an evident proof that the NiO nanoparticles

can be prepared in a simple and cost-effective manner using chemical precipitation method to cater the needs of various applications in future.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Growth, Structural and Optical Properties of Pure KDP Single Crystals by Slow Evaporation Method

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## Abstract

Single crystal growth plays a prominent role in the present era of rapid technical and scientific advancement, where the application of crystals has unbounded limits. In this project KDP crystals were grown by Slow Evaporation Solution Technique (SEST) in the ratio of 3:1 and various properties of the grown crystals were studied. It is aimed at growing pure KDP crystals by Slow Evaporation Solution Technique (SEST) at room temperature and the crystal structure was identified by powder X-ray diffraction analysis (PXRD). The transmittance behavior of the crystals was also studied by UV-visible spectral analysis. Finally purity of the crystals were tested.

***Keywords:*** KDP crystal, Slow Evaporation Solution Technique, X-ray diffraction analysis, UV- visible spectral analysis

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## Introduction

A single crystal is a periodic array of atoms arranged in a three dimensional structure with equally repeated distance in a given direction. Since Single crystals are the fundamental building blocks for most modern technology their properties are extensively studied for the significant development of crystals to meet the ever growing applications in lasers, optical communication and data storage technology[1]. Hence, growth of single crystals has become inevitable resource for further research and technological development. The search of new materials is primarily focused on increasing the nonlinearity and microscopic homogeneity. Large size crystals are essential for device fabrication and efforts are taken to grow large crystals in short durations by fast growth techniques[3]. At present, the demand is at peak for large and high quality NLO, Ferro-electric and piezo-electric single crystals with minimum defects.

Organic nonlinear optical crystals have proved to be a unique for a number of applications: second harmonic generation, frequency mixing, electro-optic modulation, optical parametric oscillation, optical bistability etc[4]. Due to the technological importance of single crystals, the need for high quality organic crystals has grown dramatically over the last decade[5]. The above work has inspired to grow KDP single crystals due to its easy growth whereas having a wide optical application.

## Materials and Methods

Synthesis of the salt was done by slow evaporation technique in which 20 g of KDP salt was dissolved in double demineralized water and was left to stir in magnetic stirrer for few hours. The temperature was maintained at 50°C to prevent oxidation of the salt while heating. The solution was heated until the salt precipitated. The salt synthesized was grounded in a ceramic grinder. Pure KDP crystals were grown using a good quality seed crystals by slow evaporation technique at room temperature. Saturated solution of KDP salt was prepared by taking 20 gms of KDP (GR grade) salt and double demineralized water. The solution was stirred with the help of magnetic stirrer for about 5 hours to obtain homogeneous solution. For the preparation of seed crystals, a small quantity of saturated solution of pure KDP was poured in a petri dish and allowed to grow seed crystals within 2-3 days. The transparent and defect free seed crystals were gently dropped into the filtered saturated KDP solution which was taken in a borosil glass beaker. The PXRD patterns of KDP single crystal were recorded using X'PERT PRO powder diffractometer with Cu as the target and the Bragg reflected intensity pattern was recorded in $2\theta$ range of 10°-90° in steps of 0.04° as the interval[1].

## Results and Discussion

The beaker containing the saturated solution along with seeds was covered with porous paper as shown in the Fig.1 for controlled evaporation.


**Figure 1: Grown KDP crystals**

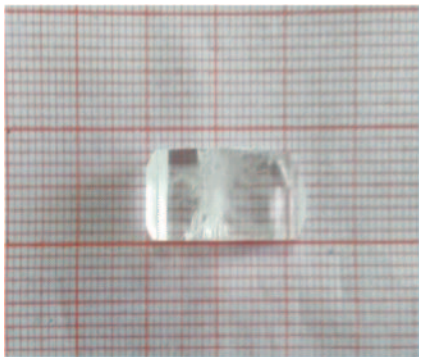

**Figure. 2 Harvested KDP crystal**

Pure KDP single crystal of size $23 \times 8 \times 4$ mm$^3$ was harvested within a period of 20 days. The grown crystals were found to be colorless and transparent. The experimental set is decided in figure and the harvested crystal is shown in Fig.2.

**Powder X-Ray Diffraction Analysis**

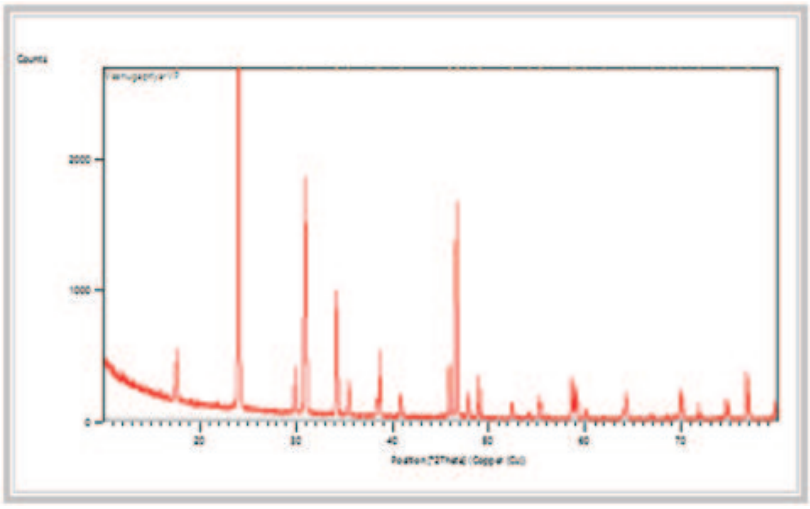

***Figure. 3 The obtained powder XRD pattern of SA sample***

Fig.3 shows that the XRD patterns of KDP single crystal with the concentration ratio 3:1 respectively. The crystallographic data (PXRD) of pure KDP is summarized in Fig.3. Using Bragg's relation and Debby Scherer's formula, the inter planer spacing and grain sizes are calculated and which are tabulated in Table 1 and Table 2.

**Table 1: Powder XRD data for pure KDP sample**

| $2\theta$ | FWHM | d-spacing ($\AA$) | Intensity | Grain size |
|---|---|---|---|---|
| 24.0336 | 0.1020 | 3.6998 | 100 | 13.8976 |
| 24.0968 | 0.0669 | 3.6933 | 97.28 | 21.1917 |
| 30.8847 | 0.0502 | 2.8953 | 71.14 | 28.6542 |
| 34.2002 | 0.0816 | 2.6197 | 40.61 | 17.7773 |
| 46.6745 | 0.1428 | 1.9445 | 70.13 | 10.5745 |
| 48.9650 | 0.0612 | 1.858 | 13.66 | 24.8934 |
| 58.6091 | 0.0816 | 1.5738 | 13.38 | 19.4843 |
| 59.0103 | 0.1428 | 1.5640 | 9.65 | 11.1562 |
| 64.1867 | 0.0816 | 1.4498 | 8.39 | 20.0563 |
| 69.8786 | 0.2448 | 1.3677 | 9.79 | 6.9091 |
| 76.7466 | 0.0612 | 1.2408 | 15.70 | 28.8976 |
| 79.6833 | 0.1632 | 1.2023 | 5.20 | 11.0647 |

**Table 2: Crystallographic data (PXRD) for pure KDP sample**

| Crystal Parameters | Pure KDP Sample |
|---|---|
| $a(\AA)$ | 7.4473 |
| $b(\AA)$ | 7.4473 |
| $c(\AA)$ | 6.8877 |
| Volume | 330.828 |
| System | Tetragonal |

#### FTIR Spectral Analysis

The Fourier Transform Infrared Spectra were recorded for powdered sample of KDP single crystals using SHIMADZU spectrometer by KBr pellet technique in the range 400 - 4000 $cm^{-1}$. FTIR spectrum in Fig.4 confirmed the functional groups present in the KDP single crystals and they are summarized in Table 3.

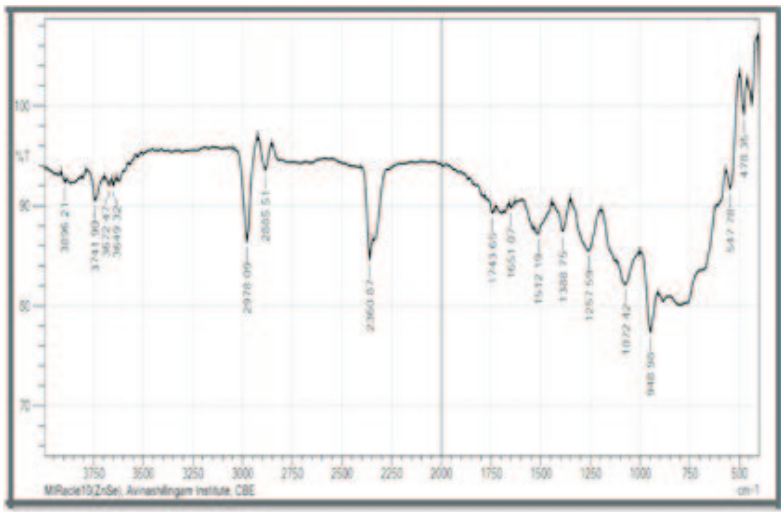


**Figure. 4 FTIR Spectrum of KDP single crystals for 3:1 concentration ratio**

**Table 3: FTIR assignments of KDP single crystal for 3:1 concentration ratio**

| Wavenumber ($cm^{-1}$) | Assignment |
|---|---|
| 3672 | O-H stretch |
| 3649 | O-H Alcohol |
| 2885 | C-H Alkane |
| 1743 | C=O stretch |
| 1651 | C=C stretch |
| 1388 | C-H bend |
| 1072 | C-O stretch |

### UV-Visible Spectral Analysis

The absorbance and transmittance spectra of KDP single crystal in powder form were recorded using UV-Vis Spectrometer (BERKIN ELMEP LAMBDA 35) in the wavelength range between 200 nm and 800 nm. The absorbance spectrum in fig. 5 shows the absorbance was not enrolled in the wavelength range starting from 200 nm to 800 nm and this is the advantage for material having NLO properties. This implies that the single crystal has the transmittance in UV-Vis region and Fig.6 shows the transmittance spectra. This is most desirable property of the materials possessing NLO devices and light emitting devices[2,6].

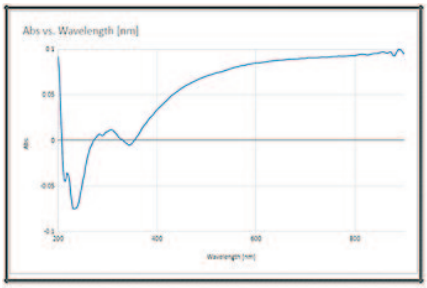


**Figure 5: Visible absorbance spectra of KDP crystals**

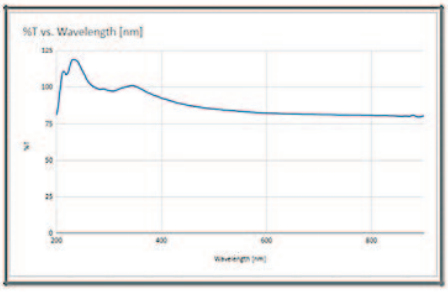


**Figure 6: Visible transmittance of spectra of KDP crystals**

The XRD patterns of KDP single crystals were compared with JCPDS file (35-0807) that confirmed the tetragonal crystal structure with cell parameters ($a = b = 7.43$, $c = 6.974$). Also no peaks of impurities was observed, suggested that the high purity of grown KDP single crystals. Here it is observed that the peak height and number of peaks are increased with the concentration ratio and this is due to the increase in their respective average grain sizes from 24.06 $\AA$ to 26.87 $\AA$.

FTIR spectrum in Fig.4 confirmed the functional groups present in the KDP single crystals.UV-Visible spectral analysis shows that the grown of 3:1 concentration ratio of KDP single crystals have no remarkable changes in their absorbance spectra as well as transmittance spectra.

## Conclusion

The project work was aimed to grow pure KDP crystals and to characterize the grown crystals by various studies. Pure KDP crystal was grown by Slow Evaporation Solution Technique (SEST) at room temperature. The crystal is found to be stable, colourless, transparent and of good quality. The grown crystals were subjected to powdered XRD analysis and all the prominent peaks were indexed. From the experimental data, it is observed that the KDP crystals. The XRD analysis confirms

the presence of hexagonal structure KDP compounds in grown crystals. The average grain size of KDP particles for 3:1 is observed. The observed sharp peak also confirms the crystalline nature.The UV visible transmission analysis shows KDP crystals have wide transparency window in the entire visible region.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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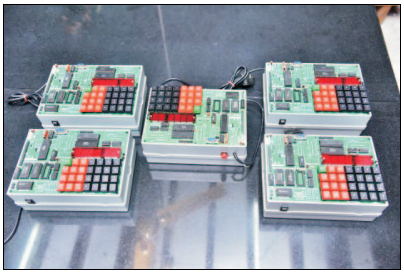
# GALLERY

## EQUIPMENTS PURCHASED UNDER STAR COLLEGE SCHEME

## (2019-2020)

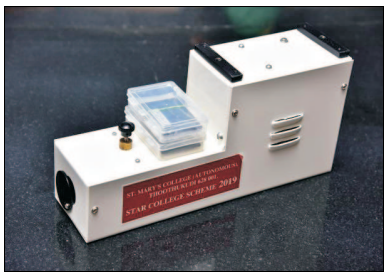
## DEPARTMENT OF PHYSICS



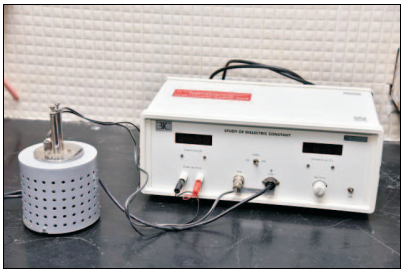
**8085 Microprocessor**

**Audio Frequency Generator**

**Cathode Ray Oscilloscope**



**Diode Laser, Lycopodium Powder Glas Plate**

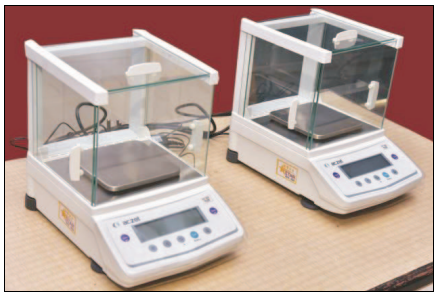


**Dielectric Constant Instrument**

**Forbe's Apparatus**

**Constant Temperarture Bath**

**Dual DC Regulated Power Supply**



**Digital Electronic Balance**

**Potentiometer**

**Glasswares**

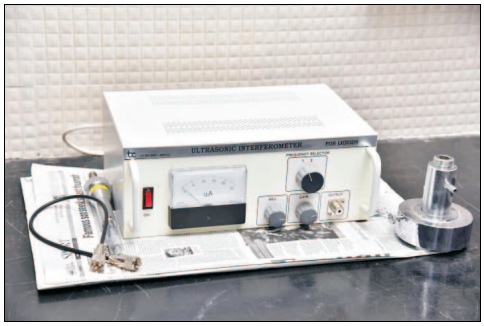
**Chemicals**

**Spherical Calorimeter**

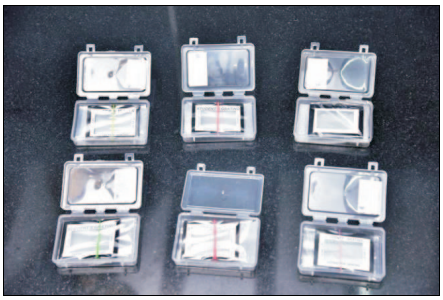

**Spot Reflecting Galvanometer**

**Hot Air Oven**


**Muffle Furnace**




**Ultrasonic Interferometer**



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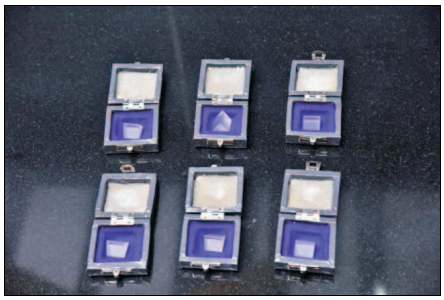


**Calcite Prism**

**IC Regulated Power Supply**

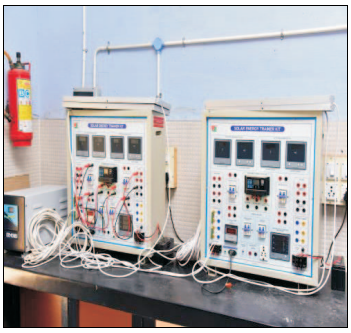
**DC Ammeter**

**DC Voltmeter**

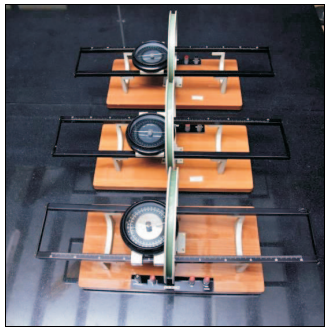
**DPDT Key**

**Magnetic Pellets**


**He-Ne Laser**

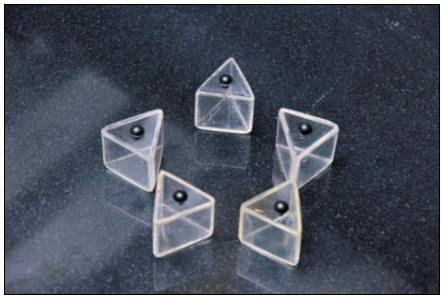


**Solar Cell Trainer Set**



**Axial Coil Apparatus**

**Head Phone**

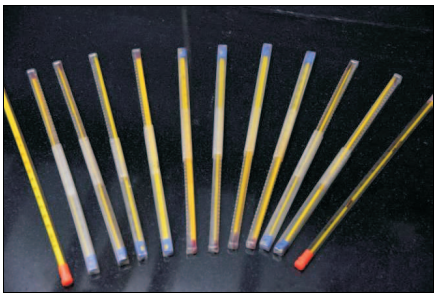


**Hollow Prism**


**Resistance Box**

**Screw Gauge and Vernier Caliper**

**Spectrometer Prism**



**Thermometer**

# DEPARTMENT OF ZOOLOGY

# MESSAGE FROM THE HOD

**Dr. Hermin Pasangha**
**Head and Associate Professor**
**Department of Zoology**
**St. Mary's College (Autonomous)**
**Thoothukudi**

Research and innovative approach is an important part of the college experience, especially as an undergraduate. It expands knowledge, understanding of the chosen field, foster critical thinking and analytical skills through hands on learning. Participation in an undergraduate research activity also benefits students in areas that can reach beyond academia. This helps the students, clarify a career path and is valuable not only for students, but for society at large. The Department of Biotechnology, New Delhi, recognizes the importance of science and research at the undergraduate level and has launched the Star College Scheme.

The Star College Programme is the one which has the favourable impact on valuable learning objectives, more intrinsic motivation to learn and prepare for their respective professions and clarification of a career path, through the financial support. I appreciate our students for their enthusiasm and hardwork in carrying out group projects in the department and their committed cooperation in participating in interdepartmental projects to obtain best outcomes. I congratulate the overall Coordinator and Member Secretary of Star College Scheme Dr. Sr. Arockia Jenecius Alphonse, Department Coordinator, Dr. Jemma Hermeline Jesy Diaz, other Coordinators and Research guides for their dedicated services, support and guidance for the successful completion of the proceeding.

I wish the success of the Programme in their future endeavours.

**DR. HERMIN PASANGHA**

# MESSAGE FROM THE REVIEWER

**Dr. R. D. Thilaga**
**Head and Associate Professor (Retd.)**
**Department of Zoology**
**St. Mary's College (Autonomous)**
**Thoothukudi.**

While reviewing the research papers from the Department of Zoology, St. Mary's College (Autonomous) I observed that the title of the papers are clear, catchy and simple and relevant. The key words accurately reflect the content. The well-written introductions summarise the recent research related to the topics. The methodology is crucial and appropriate to the objective of the study. The data provided are reliable and results seem plausible. All the papers are in appropriate length and the content is concise, accurate, clear and easy to read. The references quoted are recent and relevant.

The authors of the research paper Assessment of Biological Oxygen Demand as an Indicator of Threat to Species in Tuticorin (Gulf of Mannar) Tamil Nadu concludes that Threspuram and SPIC part of the Gulf of Mannar biosphere reserve calls immediate attention of the officials since they are highly polluted with domestic and industrial wastes. Bioactive components of the methanolic extract of skin and liver of puffer fish *Lagocephalus inermis* is investigated using GC-MS in the work Characterization of Bioactive Compounds in Methanolic Extract of *Lagocephalus inermis* from Thoothukudi Coast. The constituents revealed in GC-MS have potential applications in the pharmaceutical industry.

The research work entitled Isolation and Characterization of Metal Tolerant Bacteria from Industrial Area brings out new bacterial strain which can be applied as a bioremediation tool for the treatment of effluent containing copper. The toxicity analysis presented in Effect of Ethanolic Extract of *Phallusia nigra* and *Didemnum psammathodes* on *Artemia salina* brings out the importance of avoiding the concerned ascidian species in marine shrimp aquacultures. The anti-inflammatory study using the ethanolic fraction of *Didemnum psammathodes* depicted in In-Vitro Anti-Inflammatory Activity of Ethanolic Extract of *Didemnum psammathodes* discloses its efficacy as an anti-inflammatory drug. The work presented in Anti-Bacterial

Activity of *Didemnum psammathodes* against Human Pathogens depicts *Didemnum psammathodes* as a plausible natural antibiotic source against *Vibrio cholerae, Pseudomonas aerogenosa, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus*.

GC-MS and FTIR studies conducted by the authors of Analysis of Bioactive Constituents from the Marine Gastropod *Lambis lambis* (Linnaeus, 1758) discovers methanolic extract of *Lambis lambis* may have anticancer, anti-inflammatory, antioxidant, antibacterial, antidiuretics, antiviral, antifertility, wound healing and analgesics potential. The comparative study on the shell characteristics of various molluscs using XRD technique presented in the Evaluation of Calcium Content and XRD Studies in the Shells of Some Species of Gastropods depicts that *Lambis lambis* has the highest calcium content among the popular species studied. The authors of Isolation and Characterization of Polyhydroxybutyrate (PHB) Producing Bacteria from Contaminated Soil of Thoothukudi Region have isolated the biodegradable natural plastic polyhydroxybutyrate (PHBs) from five bacterial sources. Biodegradable plastics have potential role in replacing artificial plastics, and the work has great significance.

**DR. R. D. THILAGA**

# Assessment of Biological Oxygen Demand as an Indicator of Threat to Species in Tuticorin (Gulf of Mannar) Tamilnadu

**J. Malar, M. Sandhiya, A. SesilyPriya, S. Swetha Deva Dharshini, M. UmaMaheswari, *S.R.T. Sherly Cross**

PG and Research Department of Zoology, St.Mary's College (Autonomous), Thoothukudi, Tamilnadu, India.

(Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India)

Received on 5 November 2020

## Abstract

To determine the pollution strength of domestic and waste waters in terms of oxygen by biological oxygen demand as an indicator. Five water samples from different areas of Tuticorin were studied and the dissolved oxygen values before and after 5 days of incubation were recorded. The BOD values are calculated from the dissolved oxygen values and tabulated. The Seawater of Threspuram and SPIC stations showed high BOD values of 14.09mg/l and 8.46mg/l respectively. The higher BOD values indicate there is a threat to the species in Threspuram and SPIC coastal areas. It interpreted moderate pollution in those areas. Threspuram coastal area has a large number of drainages emptying into this part of the sea which contains many microorganisms which emit toxins and bad odour and consume oxygen from the water. SPIC station is highly polluted with industrial effluents, heavy metals, and pollution from salt pans.

***Keywords:*** BOD, dissolved oxygen, pollution, species threat.

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## Introduction

Biochemical oxygen demand (BOD) is an important index for monitoring organic pollutants in water[1]. It is the amount of dissolved oxygen needed (i.e. demanded) by aerobic biological organisms to breakdown organic materials present in a given water sample at a certain temperature over a specific period time[2]. Since BOD is directly connected with the oxygen status of any water body which is essential

for sustaining the biological lives in any aquatic systems, an understanding of the BOD values of different waste waters and the corresponding organic loads which are ultimately released to various aquatic bodies with or without any treatment appears to be essential.

## Materials and Methods

For the present study, five different stations from Tuticorin were selected. Water samples were collected from Threspuram landing centre, SPIC new harbour area, a well in Saveriyarpuram, a nearby pond and groundwater from our college campus. These stations were selected because of their variation in location and their usage. Biological oxygen demand value was determined by calculating the dissolved oxygen levels of water samples before and after 5 days of incubation.

$$\text{Biological Oxygen Demand, BOD} = \text{IDO - FDO} / (V_S/V_B)$$

Where,

IDO - Initial dissolved oxygen level of the sample, mg/L

FDO - Final dissolved oxygen level of the sample, mg/L

$V_S$ - Volume of sample, ml

$V_B$ - Volume of bottle, ml

Winkler's method is used to calculate the dissolved oxygen level of the water sample.

$$\text{Dissolved Oxygen, DO (mg/l)} = (V \times N(\text{Hypo}) \times 8 \times 1000) / \text{Volume of sample}$$

Where,

V - The volume of $Na_2S_2O_3$ used

N - Normality of $Na_2S_2O_3$ (Hypo) (0.025N)

8 - The equivalent weight of oxygen

The difference between the two DO levels represents the amount of oxygen used for the decomposition of any organic material in the sample and is a good approximation of the BOD level. It is confirmed by using the BOD Bottle[3].

## Result

In this study, five water samples and their BOD values are calculated and the results indicated the biological oxygen demand of the samples. The present study showed that the highly polluted water sample was the seawater sample from Threspuram with a value of 14.09 mg/l followed by SPIC station with a BOD value of 8.46 mg/l. The

other water samples from the nearby pond, well and groundwater from the college showed values of 2.82 mg/l, 1.69 mg/l and 1.12 mg/l respectively indicating that the pollution level in those places is very low.

**Table 1: Dissolved oxygen level of different water stations in Tuticorin (2020)**

| Study Area | Dissolved Oxygen (mg/l) Level In 1st Day | Dissolved Oxygen (mg/l) Level In 5th Day |
|---|---|---|
| THERESPURAM | 5.63984 | 2.81992 |
| SPIC | 3.383904 | 1.691952 |
| GROUNDWATER | 1.127968 | 0.9023744 |
| WELL WATER | 0.9023744 | 0.563984 |
| POND WATER | 1.127968 | 0.563984 |

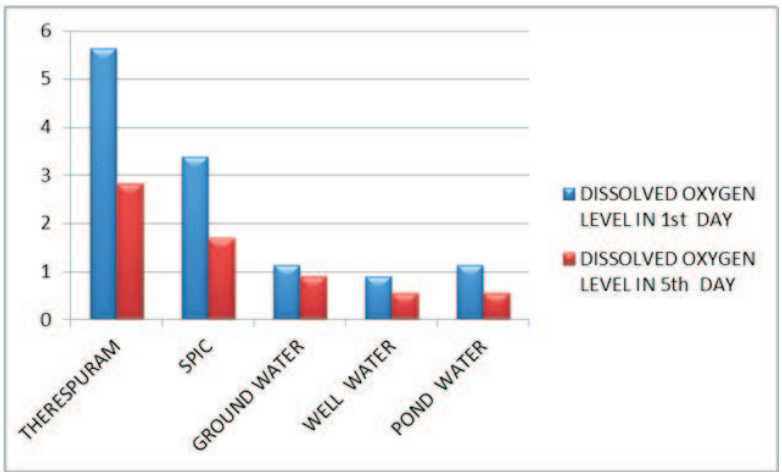


**Figure 1: Dissolved oxygen level of different water stations in Tuticorin (2020)**

**Table 2: BOD level of different water stations in Tuticorin (2020)**

| Stations | Values of BOD (mg/l) |
|---|---|
| Therespuram | 14.09 |
| SPIC water | 8.46 |
| Ground Water | 1.12 |
| Well Water | 1.69 |
| Pond Water | 2.82 |

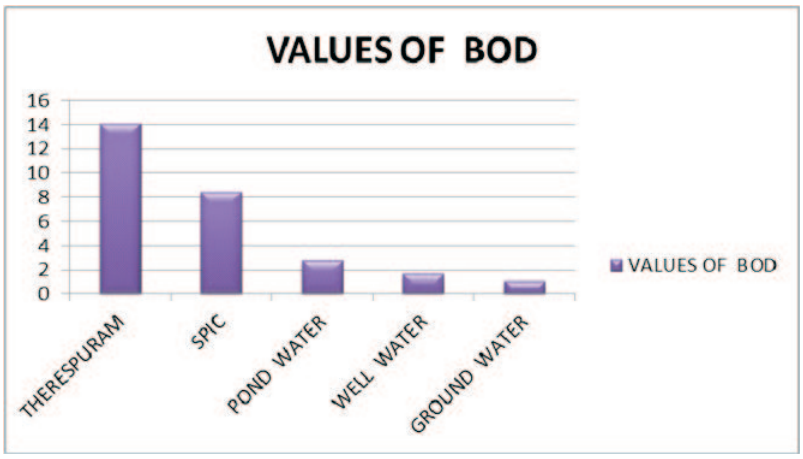


**Figure 2: BOD level of different water stations in Tuticorin (2020)**

## Discussion

The Biochemical Oxygen Demand (BOD) is one of the most widely used criteria for water quality assessment. It provides information about the ready biodegradable fraction of the organic load in the water.The presence of a sufficient concentration of dissolved oxygen is critical to maintaining the aquatic life and aesthetic quality of streams and lakes. Determining how organic matter affects the concentration of dissolved oxygen (DO) in a stream or lake is integral to water- quality management. The decay of organic matter in water is measured as biochemical or chemical oxygen demand. Oxygen demand is a measure of the number of oxidizable substances in a water sample that can lower DO concentrations.

A water sample showing a BOD level of 1-2 mg/l is considered very good. There will not be much organic waste present in the water supply. A water supply with a BOD level of 3-5 mg/l is considered moderately clean. In water with a BOD level of 6-9 mg/l, the water is considered somewhat polluted because there is usually organic matter present in this water which is decomposed by microorganisms which use the dissolved oxygen in the water. At BOD levels of 20mg/l or greater, the water supply is considered highly polluted with organic waste.

The present study showed that the highly polluted water sample was the seawater sample from Therespuram with a value of 14.09 mg/l. The reasons are that Therespuram receives a buckle canal which disposes of a huge amount of sewage and other domestic wastes. Threspuram is also a major fish landing centre which disposes of a huge amount of fishing wastes and machinery leakages.

Jalilzadeh Reza (2014) reported the dissolved oxygen content and biochemical oxygen demand of dez river by GIS software. This study showed the reason for

the low density of dissolved oxygen is due to the entrance of waste waters and the existence of microorganisms or algae.

The Study and Zoning of Dissolved Oxygen and Biochemical Oxygen Demand of Dez River by GIS Software reported the reason for the low density of DO in some stations is due to the entrance of waste waters, having stagnant water in some places, and the existence of microorganisms or algae.

BOD value of 8.46 mg/l was reported in the SPIC station. The main cause for the pollution of seawater in SPIC is by industrial effluents that are directed towards the sea; that mainly includes dirt, gravel, masonry, concrete, scrap metal, oil, scrap lumber, chemical waste disposals and waste disposals from the nearby port[1][3].

In the case of groundwater, biochemical oxygen demand showed minimum value (1.12 mg/l) compared to the other, water samples. Moreover, groundwater contains small microbes which are non-pathogenic and they live in water by consuming the oxygen present in the water[1]. Similarly, the well water which showed a BOD value of 1.69 mg/l is also considered not polluted. Water from these sources remain fresh and are not polluted and is in daily use.

In the case of pond water, the water remains stagnant after a few days of rain. At that time, the oxygen in the stagnant pond water is consumed by the microorganisms present in the water. The BOD value of pond water was recorded as 2.82 mg/l indicating that this pond water is moderately clean[2][6].

Polluted water reduces dissolved oxygen (DO) content, which, eliminates sensitive organisms like plankton, molluscs and fish etc. However, a few tolerant species like Tubifex (annelid worm) and some insect larvae may survive in highly polluted water with low DO content. Such species are recognized as indicator species for polluted water[7]. The consequences of high BOD values are, they affect the ecosystem by lowering the dissolved oxygen level where aquatic organisms become stressed, suffocate and later die. The ecosystem especially the aquatic environment is severely affected by water pollution due to the emission of toxic chemicals and has become the worldwide problem in recent years. So regular monitoring of the BOD is essential to protect the water sources against contamination in the near future.

## Conclusion

From this study, it was inferred that the seawater samples from Threspuram and SPIC stations have a prominent level of biological oxygen demand. Hence the study concluded that water was highly polluted with domestic and industrial wastes have a large amount of biochemical oxygen demand while the other three samples showed considerably fewer BOD values.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Characterization of Bioactive Compounds in Methanolic Extract of *Lagocephalus inermis* from Thoothukudi Coast

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(Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamilnadu, India)

Received on 5 November 2020

## Abstract

Bioactive components of the methanolic extract of skin and liver of puffer fish *Lagocephalus inermis* was investigated using Gas Chromatography-Mass Spectrometry. A total of 22 compounds were identified. Some of the major compounds are Cyclotrisiloxane (RT-16.213), Anthracene (RT-16.336), Pteridinone (RT-17.206), Cyclobarbital (RT-17.219), 2-Ethylacridine (RT- 15.948), Benzo(n) quinoline (RT-16.809), Benz(b)-1,4-oxazepine (RT - 16.336) and 1H-Indole (RT-17.178) showed antiasthmatics, antimicrobial, antioxidant, antibiotics, anticancer, antiviral, anti-infective, antiseptic, larvicidal, metallurgy and toxicological analysis.

***Keywords:*** *Lagocephalus inermis*, Bioactive compounds, methanol, GC- MS analysis.

*Correspondence: selvihenry76@gmail.com

## Introduction

The Marine ecosystem has drawn the attention of many researchers due to its rich and renewable source[1]. The bioresources present in the marine ecosystem have potent biomolecules which include many natural organic compounds. These compounds are reported to have biological activities like an anti-tumour, anti-viral, analgesic etc[2]. Fish is an excellent source for proteins, whereas in nutritional concerns fish protein is ranked above casein[3]. Puffer fishes are commonly distributed in the tropics but are relatively uncommon in temperate regions and completely absent from cold water. There are 189 species of puffer fishes and 28 genera in the family Tetraodontidae[4]. The skin and certain other internal organs of puffer fish are highly

toxic to humans. Puffer fish are known to carry tetrodotoxin (TTX)[5−7]. This is known as a non-protein organic compound and one of the strongest marine paralytic toxins today.

GC-MS is a highly efficient tool widely used to analyze semi-volatile and volatile organic personal care products as extremely low levels from environmental samples[8]. Bratu *et al.* (2013) studied quantitative determination of fatty acids from fish oil using GC-MS Method and H-NMR spectroscopy. Bhuvaneswari and Babu Rajendran (2012) studied GC-MS determination of organochlorine pesticides (OCPs) in fish from river Cauvery and Veeranam Lake.

The present study has been carried out to analyze the biochemical compounds in *Lagocephalus inermis* collected from Thoothukudi coast.

## Materials and Methods

#### Collection of specimen:

Specimens of the puffer fish *L. inermis* were collected from fish landing centre at fishing harbour Thoothukudi. They were kept in the ice box and transported to the laboratory. They were maintained in a deep freezer at $-20^{\circ}$C until use.

#### Preparation of methanol extract:

The preparation of methanol extract was followed by[11]. 10g of dry powdered tissue was soaked in methanol and kept in an orbital shaker for 72 hours. The extract was filtered through Whatman No.1 filter paper, centrifuged at 15,000 rpm for 30 minutes and the solvent was concentrated by rotary evaporator (VC 100 A Lack Rotavapor at 30°C) with reduced pressure to give a dark brown gummy mass. The resultant residue was stored at 4°C for further analysis.

#### GC-MS Analysis:

GC-MS analysis of methanol extracts of *L. inermis* was carried out by following the method of[12]. GC-MS method is a direct and fast analytical approach for the identification of chemical compounds. The importance of the study is to know the biological activity of these compounds. The analysis was performed by using a GC, Varian CP 3800 and MS, Saturn 2200 (VF 5ms 30 $\times$ 0.25 system) equipped with Elite-1, fused silica capillary column composed of 5% phenylArylene-95% Dimethyl polysiloxane. The system comprising a COMBIPAL autosampler set under the following conditions: helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 1$\mu$l EI was employed (split ratio of 1:10) injector temperature 250°C; the oven temperature was programmed from 100-270°C at the rate of 5°C; total GC running time was 63 minutes. Interpretation on the mass spectrum of GCMS was done by using the database of National Institute Standard

and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST, WILEY and FAME-8 library. The name, molecular weight and structure of the components of the test materials were ascertained.

## Results

GC-MS analysis of methanol extract of skin of *L. inermis* indicated the presence of ten compounds. They are Cyclotrisiloxane (RT-16.213), Octasiloxane (RT-16.213), Anthracene (RT-16.336), Hexahydropyridine (RT-16.336), Tris (RT-17.206), Methyl 3- bromo- adamantaneacetate (RT-17.206), Pteridinone (RT-17.206), Biphenylene (RT-17.219), 1-2 Benzisothiazol-3- (RT-17.219) and Cyclobarbital (RT-17.219) (Table 1, Fig 1-3).

The GC-MS analysis of methanol extract of muscle of L. inermis revealed the presence of twelve compounds. They are 2-Ethylacridine (RT- 15.948), 2,4,6-Cycloheptatrien (RT- 15.948), Benz(b)-1, 4-oxazepine (RT - 16.336), 5-Methyl- 2-phenylindolizine (RT-16.336), 2-Methyl-7-phenylindolizine (RT-16.733), 1,2-Benzisothiazol-3-amine (RT-16.733), Benzo(n) quinotine (RT-16.809), 2, 4-Cyclohexadine-1-one (RT-17.178), 1H-Indole(RT-17.178), 1,2-Bis(trimethylsilyl)-benzene (RT-17.263), biphenylindolizine (RT-17.064), Cyclotrisiloxane (RT- 17.064) (Table 2, Fig 4-6).

**Table 1: Activity of components identified in the skin sample (Methanol) of *Lagocephalus inermis* (CG-MS study)**

| S.No | RT | Compound Name | Molecular Formula | Molecular Weight (g/mol) | Peaks Area % | Activity |
|---|---|---|---|---|---|---|
| 1. | 16.213 | Cyclotrisiloxane | $C_{16}H_{18}O_3Si_3$ | 222.46 | 29.02 | Antiasthmatics, Bronchodilators Antitussive agent |
| 2. | 16.213 | Octasiloxane | $C_{16}H_{48}O_7Si_8$ | 577.2 | 29.02 | Antimicrobial, Antioxidnat |
| 3. | 16.213 | Anthracene | $C_{17}H_{18}$ | 222.32 | 25.01 | Specific therapeutics, Ophthalmic agent |
| 4. | 16.336 | Hexahydropyri-dine | $C_{12}H_{17}NO_2$ | 207.27 | 25.01 | Catalytic activity Specific therapeutic agents |
| 5. | 17.206 | Tris | $C_{18}H_4ASO_3Si_3$ | 468.7 | 23.70 | Antibiotics Antibacterial agent |

| S.No | RT | Compound Name | Molecular Formula | Molecular Weight (g/mol) | Peaks Area % | Activity |
|---|---|---|---|---|---|---|
| 6. | 17.206 | Methyl 3-bromo-l-Adamantaneace-tate | $C_{13}H_{19}BrO_2$ | 287.19 | 23.70 | Larvicidal Antibacterial agent |
| 7. | 17.206 | Pteridinone | $C_8H_9N_5O_2$ | 207.19 | 23.70 | Metallurgy |
| 8. | 17.291 | Biphenylene | $C_{17}H_{14}O_4$ | 282.29 | 22.27 | Antioxidant Antimicrobial Anticancer |
| 9. | 17.291 | 1, 2-Benziso-thiazol-3 | $C_{15}H_{20}N_2SSi$ | 264.46 | 22.27 | Specific therapeutic activity, Sexual disorders Osteoporosis |
| 10. | 17.291 | Cyclobarbital | $C_{12}H_{16}N_2O_3$ | 236.27 | 22.27 | Barbiturate poisoning, Homocid-suicide Toxicological Analysis |

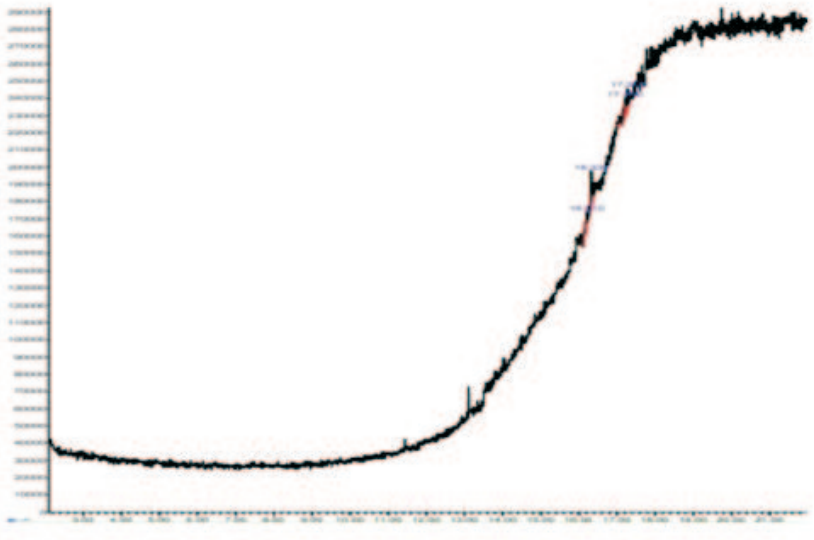

**Figure 1: Chromatogram-Methanol extract of skin of *Lagocephalus inermis***

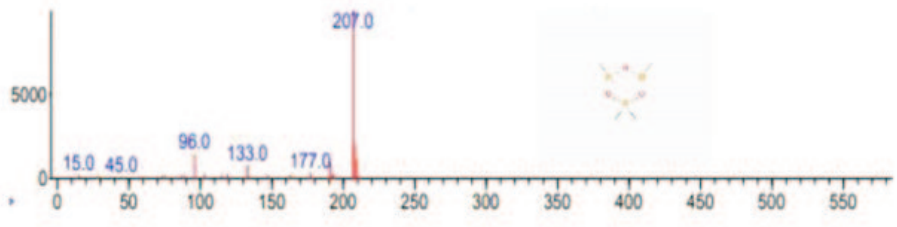


**Figure 2: Molecular Formula: $C_6H_{18}O_3Si_3$ Name: Cyclotrisoloxane Molecular weight: 222.46**

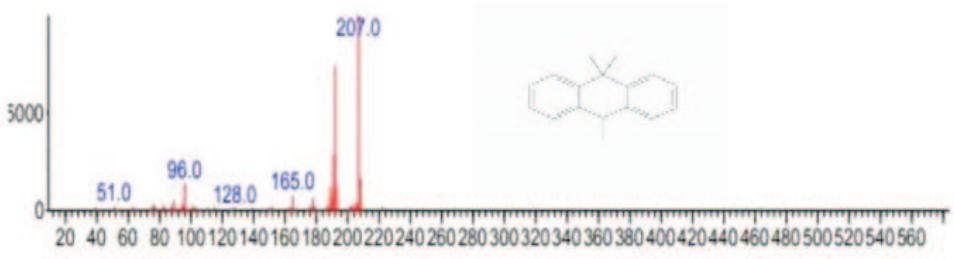


**Figure 3: Molecular Formula: $C_{17}H_{18}$ Name: Anthracene Molecular weight: 222.32**

**Table 2: Activity of components identified in the muscle sample (Methanol) of *Lagocephalus inermis* (CG-MS study)**

| S.No | RT | Compound Name | Molecular Formula | Molecular Weight (g/mol) | Peaks Area % | Activity |
|---|---|---|---|---|---|---|
| 1. | 15.948 | 2-Ethylacridine | $C_{15}H_{13}N$ | 207.27 | 6.67 | Metallurgy Natural gas |
| 2. | 15.948 | 2,4,6-Cycloheptatrien | $C_{13}H_{22}OSi_2$ | 250.48 | 6.67 | Antibacterial Activity |
| 3. | 16.336 | Benz(b) - 1, 4 - oxazepine | $C_{11}H_{13}NOS$ | 207.29 | 10.50 | Antioxidant Antibiotic Anti-inflammatory Antiviral |
| 4. | 16.336 | 5-Methyl-2-phenylindolizine | $C_{15}H_{13}N$ | 207.27 | 10.50 | Antimicotics Fungicides Anti-infective Antibiotics Antiseptics Thermography |

| S.No | RT | Compound Name | Molecular Formula | Molecular Weight (g/mol) | Peaks Area % | Activity |
|---|---|---|---|---|---|---|
| 5. | 16.733 | 2-Methyl-7-phenylindole | $C_{15}H_{13}N$ | 207.27 | 13.73 | Antipyretic Analgesic Anti-inflammatory Allergic disorder |
| 6. | 16.733 | 1,2-Benzisothiazole-3-amine tms | $C_{10}H_{14}N_2SSi$ | 222.38 | 13.73 | Anti-fungal infection Antiseptic Disinfectant Antibacterial |
| 7. | 16.809 | Benzo(h) quinolone | $C_{15}H_{13}N$ | 207.27 | 7.14 | Heterocyclic compound Metallurgy Edible oil |
| 8. | 17.178 | 2,4-Cyclohexadine-1 - one | $C_{14}H_{22}O_2$ | 222.32 | 6.91 | Antitumor activity Metallurgy Involving hydrolase |
| 9. | 17.178 | 1H-indole | $C_{15}H_{13}N$ | 207.27 | 6.91 | Bronchodilators Mucolytic Specific therapeutic Anti-Parkinson drugs Artificial tears Irrigation solutions |
| 10. | 17.263 | Biphenylindolizine | $C_{17}H_{14}O_4$ | 282.29 | 25.54 | Antioxidant activity Antimicrobial and Anticancer antivities |
| 11. | 17.064 | Biphenylindolizine | $C_{17}H_{14}O_4$ | 282.29 | 25.54 | Antioxidant activity Antimicrobial and Anticancer activities |
| 12. | 17.064 | Cyclotrisiloxane | $C_6H_{18}O_3Si_3$ | 222.46 | 25.54 | Antiasthmatics Bronchodilators Antitissive agent |

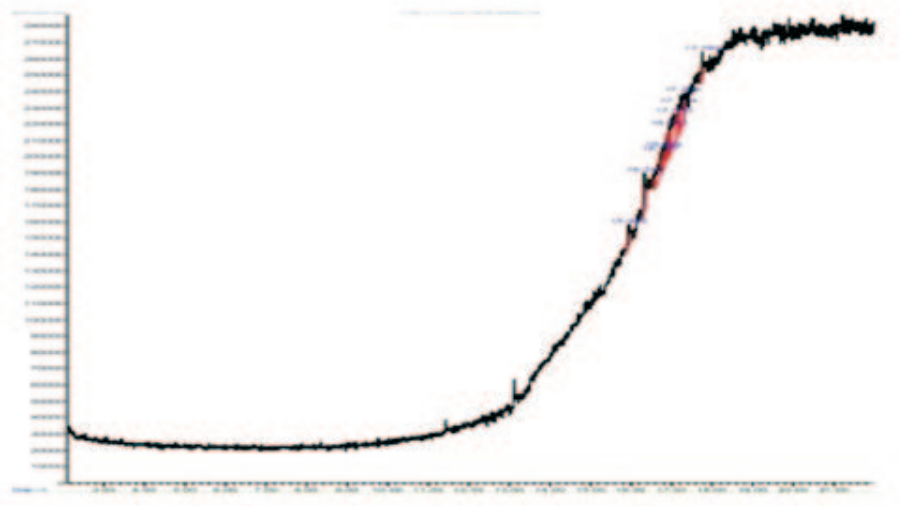

**Figure 4: Chromatogram - Methanol extract of muscle of *Lagocephalus inermis***

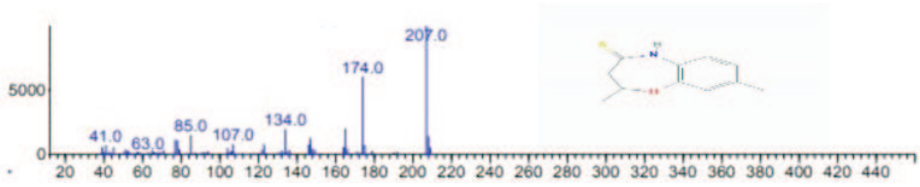


**Figure 5: Molecular Formula: $C_{11}H_{13}NO\,S$ Name: Benz (b)-1, 4-Oxazepine Molecular weight: 207.29**

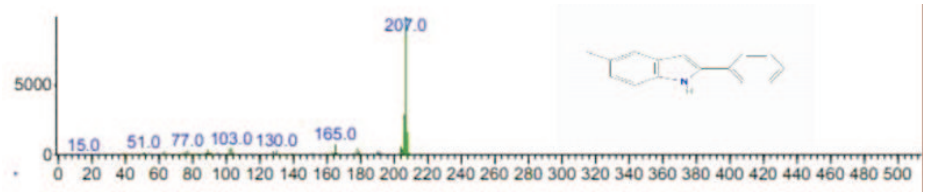


**Figure 6: Molecular Formula: $C_{15}H_{13}N$ Name: 1H-Indol, 1 methyl-2-phenyl Molecular weight: 207.27**

## Discussion

GC-MS analysis is an indirect method to detect TTX in a crude extract which is difficult to purify in other advanced analysis methods[13]. Tetrodotoxin (TTX) is a

toxin mainly occurring naturally in contaminated puffer fish. It is also detected in various marine organisms like globe fish, star fish, sun fish, stars, frogs, crabs, snails, Australian blue-ringed octopuses, and bivalve molluscs. TTX is produced by marine bacteria that are consumed mainly by fish of the *Tetraodontidae* family and other aquatic animals. This neurotoxin causes food intoxication and poses an acute risk to public health[14]. Kreimer *et al.* (2016), determined TTX and fatty acid contents of five specimens of *Lagocephalus inermis* by LC-MS/MS analysis in intestine, skin and muscle. Detection of tetrodotoxin in puffer fish using GC-MS was reported by[16]. Indumathi & Khora (2017) analysed the presence of tetrodotoxin in the puffer fish *Takifugu oblongus* through GC-MS study. GC-MS chromatogram of methanol extract of skin and muscle of puffer fish *Lagocephalus inermis* showed twenty-two peaks indicating the presence of twenty-two chemical components. *Lagocephalus spadiceous* was found to be non-toxic. However, *Arothron hispidus* and *Lagocephalus lunaris* are toxic for human consumption as the level was $> 2$ $\mu$g/g. In the present study, 22 compounds were identified in the skin and muscle extracts of *L. inermis*. These compounds exhibited the following biological activities such as antiasthmatics, antitussive, antimicrobial, antioxidant, antibiotics, antibacterial, anticancer, antiviral, antiinflammatory, antimycotics, anti-infective, antiseptic, antifungal, antitumor, drugs for the genital, gonadal disorder, larvicidal, metallurgy,ophthalmic agents, osteoporosis, specific therapeutics, sexual disorders, and toxicological analysis.

## Conclusion

Bioactive compounds from various marine sources have often been found to possess promising pharmaceutical agents. The tissue extracts of *L. inermis* showed varying degrees of chemical compounds. It is interesting to note that crude extracts of tissues showed prominent biological activity. Hence this information may help to develop potential bioactive compounds from *L. inermis* in the pharmaceutical industry for the development of drugs.

## Acknowledgement

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# Isolation and Characterization of Metal Tolerant Bacteria from Industrial Area

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## Abstract

One of the debilitating effects of increasing anthropogenic activity is pollution. Metals are major environmental pollutants affecting soil and water. Identification of metal tolerant microbes is important to study the metal tolerance of living organisms. Harnessing the molecular mechanism of metal tolerance is important for devising genetic engineering strategies as a tool for bioremediation. The present study throws light on the isolation of heavy metal tolerant microorganisms. Five bacterial strains were identified from probable metal contaminated areas of Thoothukudi city, Tamil Nadu near copper smelter unit. The biochemical characterization and tolerance to heavy metal (Cu) of these isolates was examined. Four among five isolates were found to grow at high concentration of $CuSo_4$(8mM).

***Keywords:*** Metal tolerance, soil microbes, soil pollution, copper resistant bacterial strains, biochemical analysis, metal tolerance test.

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## Introduction

Soil is the most biodiverse environment on earth. One gram of soil contains as many as $10^{10}$-$10^{11}$ bacterial cells[1] belonging to $10^3$-$10^4$ species[2]. There are currently estimated to be 61 distinct bacterial phyla, of which 31 have cultivable representatives. The soil microbial community is a gold mine for genes and pathways that code novel biocatalyst for biosynthetic or biodegradation processes, including degradation of pollutants, synthesis of biofuels, and production of novel drugs[3].

Maintenance of good soil quality is of prime importance for sustainable agriculture. Microorganisms play vital roles in soil fertility and primary production through organic matter decomposition and nutrient cycling. When some stress factors such as temperature, extreme pH or chemical pollution occur, soil biota is affected. Soil may become contaminated with metals from a variety of anthropogenic sources. Various potentially toxic elements, including heavy metals, are present in industrial effluents. Elevated concentrations of these compounds are known to affect soil microbial populations and their associated activities[4].

Many of them such as Zn, Cu, Co, Ni, Mn, and Fe are trace elements which are necessary for living organisms because they are required for biological activities and microbes have evolved mechanisms to tolerate and utilize these metals[5]. Other heavy metals such as Pb, Cd, Hg and As the non-essential heavy metals do not have any beneficial effect on organisms and thus are categorised as main threats since they are harmful to both plants and animals[4]. Heavy metals cannot be degraded or destroyed. Heavy metal pollution of the environment due to anthropogenic activity is a major threat to the aquatic ecosystem and human health[6]. They can cause damage to the kidney, lungs, liver and other vital organs apart from reducing the function of the Central Nervous System[7]. They influence the microbial population and result in decreased biomass as well as diversity by affecting the growth, morphology and biochemical activities. Therefore microbes have developed mechanisms to tolerate heavy metals through efflux, complexation, or reduction of metal ions or to use them as terminal electron acceptors in anaerobic respiration[5]. Most mechanisms reported involves the efflux of metal ions outside the cell, and genes for tolerance mechanisms have been found on both chromosomes and plasmids. Bacteria that are resistant to and grow on metals play an important role in the biogeochemical cycling of those metal ions.Bacteria that demonstrate the capacity of surviving in toxic heavy metal concentrations have been isolated from various sources[8].

In this study, we selected soil samples from an area with known pollution problems where heavy metals and other pollutants have been emitted by the industry for nearly 30 years. This area is particularly affected by the release of liquid effluents from Copper Smelter industries. This ecosystem is appropriate for studying microbial interactions because they are gradually contaminated by toxic concentrations of heavy metals. Here heavy metals are in equilibrium with the soil so they can be considered as a good model for examining the effects of heavy metals on soil microorganisms.

## Materials and Methods

### Sample collection

Soil samples for isolation of microbes were obtained from 10 cm to 1m below the soil surface near the industrial area where copper smelter is situated in Thoothukudi. It was collected in sterilized plastic bags on site and was used immediately for isolation of microbes.

### Isolation of microbes from the soil sample

Processing of the samples for the isolation of microbes was carried out within 24 hours of sample collection. Serial dilution method was used.1g of soil was mixed in 1ml of 0.9% NaCl solution. 10-fold dilutions of fresh soil (1g) were made in 0.9% NaCl solution. 100 $\mu$l from each of these dilutions were spread on L.B agar medium and incubated at 37°C for 24 hours.

### Isolation of pure culture

After the incubation period (24-48 hrs) the plates were observed for any kind of growth on the media and the microbial pure cultures were isolated using the streak plate method.

### Study of colony morphology

Isolated colonies of purified bacterial strain grown on solidified nutrient agar plates were observed and data was recorded regarding the Form (circular, filamentous, irregular), Elevation (flat, convex, and umbonate), Margin (entire, undulate, filamentous and erose), Optical feature (opaque, translucent and transparent) of the colonies.

### Gram staining

Gram staining was performed as per standard protocol[9].

### Biochemical characterization

Catalase test, Indole test, Methyl red test and citrate tests were performed as per standard protocols[10].

### Copper tolerance

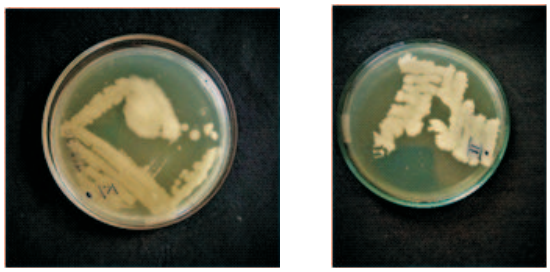
Nutrient agar with various concentration of $CuSO_4$ (0.5mM, 0.8mM, 10mM) was prepared. Each bacteria was patched on the plates to analyse the metal tolerance of the microbes.

## Results

### Strain isolation

Bacterial strains were isolated by serial dilution of soil samples in 0.9% NaCl solution. Dilutions till $10^{-4}$ dilution were plated on nutrient agar plates. Individual colonies were isolated and streaked in separate nutrient agar plates to obtain pure culture (Fig. 1). Five individual colonies were taken for further analysis and named as HS 1,2,3,4 and 5.

**HS1** **Hs2** **HS3**



**HS4** **Hs5**

**Figure 1. Pure cultures of isolated strains**

### Morphological characterization of isolated bacterial strains

Gram staining was done on the five bacterial strains isolated and the results are tabulated in Table. 1. The colony morphology was observed and the results are consolidated in Table 1.

**Table 1. Gram staining and Cellular Morphology of the Isolated Strains**

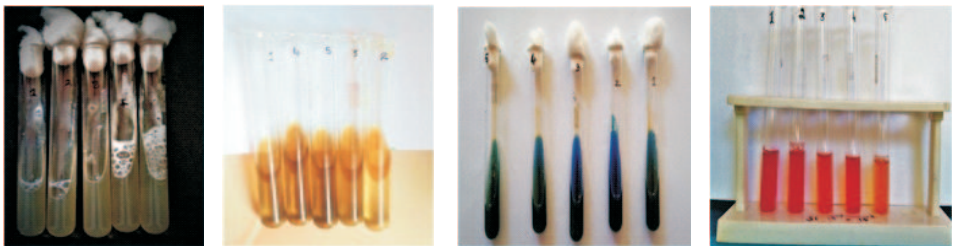
| Strain | Gram Staining | Form | Colour | Margin | Shape |
|---|---|---|---|---|---|
| **HS 1** | +ve | Circular | Pink | Undulate | Bacillus |
| **HS 2** | -ve | Irregular | Pale Yellow | Undulate | Coccus |
| **HS 3** | -ve | Circular | Yellow | Undulate | Coccus |
| **HS 4** | -ve | Filamentous | White | Filiform | Bacillus |
| **HS 5** | +ve | Irregular | White | Undulate | Bacillus |

**Biochemical tests:**

The catalase test was performed on the isolates to identify the aerobic organism. Colonies HS4 and HS5 were catalase-positive which indicate that the colonies may be (aerobic) *Staphylococci* species (Fig. 3 A). Indole test was done and the colony HS 2 was found to deaminate tryptophan and hence positive for the test, indicated that it may belong to members of the family *Enterobacteriaceae* (Fig. 3 B). Methyl red test was performed to identify the acid fermenters. Observation of red colour after the addition of the methyl red indicator represents the colonies to be acid fermenters. Colonies HS1, HS2, HS3, and HS4 remained red indicating that they can ferment glucose to acid products and they may belong to the family *Enterobacteriaceae* (Fig. 3 C). Citrate test was done to identify if the organisms can use citrate as an energy source. The colour change of the Simmon Citrate medium to blue indicated the utilization of Citrate by the organisms. Colonies HS1, HS2, and HS3 gave positive results. (Fig. 3D). The results are tabulated in Table 2.

**Table 2. Results of biochemical tests**

| Strain | Catalase Test | Indole Test | Methyl red Test | Citrate Test |
|---|---|---|---|---|
| **HS 1** | - | - | + | + |
| **HS 2** | - | + | + | + |
| **HS 3** | - | - | + | + |
| **HS 4** | + | - | + | - |
| **HS 5** | + | - | - | - |



**A.** **B.** **C.** **D.**

**Figure2. Biochemical tests of the isolated colonies. A. Catalase test; B. Indole test; C. Methyl red test D. Citrate test**

**Copper tolerance of isolated bacterial strains**

To assess the metal tolerance of the isolated microbial colonies, the colonies HS1, HS2 HS3, HS4 and HS5 were taken for analysis. The colonies were patched on plates with nutrient agar supplemented with various concentration of $CuSO_4$ (0.5mM,

0.8mM, 10mM ). The isolates HS 1, HS 2, HS 3 and HS 4 showed tolerance to high concentrations of $CuSO_4$ at 8mM. These isolates can be used for bioremediation (Fig.4).

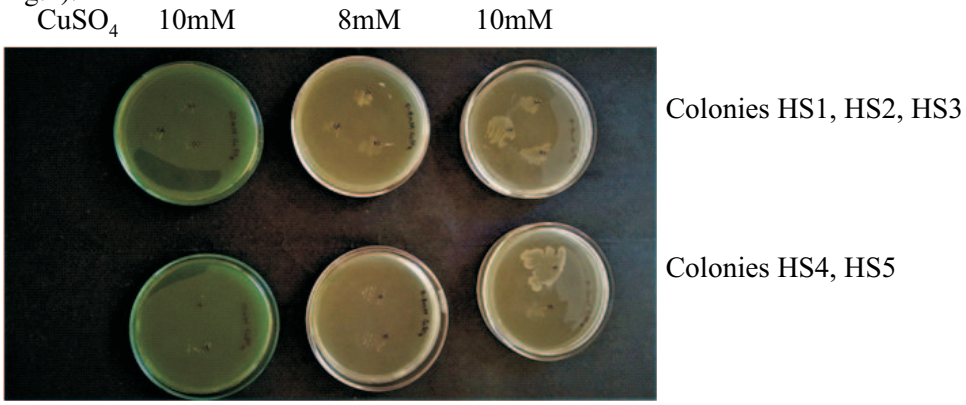



**Figure 3. Copper tolerance test**

## Discussion

The capability of bacterial resistance against different heavy metals may offer a beneficial tool for the simultaneous monitoring of many contaminants and pollutants in the environment. The toxic effects of domestic and industrial wastes are the reason for the emergence of resistant varieties of bacteria that are hazardous for human health and the environment. Therefore, the study is very useful to suggest that the possible impact of metal contaminated locations in human life may be greater than the direct consequence of the pollution.Based on the results obtained isolates HS1, HS2, were able to tolerate 0.8mM of CuSo4 while all isolates except HS 5 showed tolerance to 0.5mM of CuSo4. These showed that the bacterial species isolated can be used as a bioremediation tool for the treatment of effluent containing copper. Morphological and biochemical characterisation by catalase test revealed that the isolates belong to the genus *Enterococcus, Streptococcus* (catalase-negative) and *Staphylococcus* (catalase positive). Indole test was performed to determine the ability of the organism to convert tryptophan into indole.Most strains of *E.coli, P. vulgaris, M. morganii* and *Providencia* are generally indole positive. Methyl Red (MR) test determined whether the microbe performs mixed acids fermentation when supplied with glucose. It helped to differentiate various genera of Enteric bacteria. The citrate test detected the ability of the organism to use citrate as the sole source of carbon and energy. The morphological identification and biochemical tests were performed to identify the isolates and characterize them based on Bergey's manual. Further molecular characterization by 16s rDNA sequence of the isolates has to be performed to further confirm their specific identity.

## Conclusion

The soil samples collected from the area where probable metal pollution is envisaged, we could observe metal tolerant bacterial strains. We can attribute the presence of metal tolerant bacterial strains to be novel strains or common soil bacterial strains which have acquired this property by adaptive mutations due to continuous exposure to high concentrations of metal in their environment. Further experiments have to be done to measure the level of metals in the soil samples collected. The microbes have to be further characterized and identified using 16s rDNA sequence.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Effect of Ethanolic Extract of *Phallusia nigra* and *Didemnum psammathodes* on *Artemia salina*

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## Abstract

The present study was a preliminary toxicity screening of ethanolic extracts of the ascidian *Phallusia nigra* and *Didemnum psammathodes* against *Artemia salina*. Ascidians are the source of secondary metabolites. These species are common in Tuticorin coast. 85% mortality was observed in 100 $\mu$g/ml of ethanolic extract of *Phallusia nigra* and 90% mortality was observed in 100 $\mu$g/ml of ethanolic extract of *Didemnum psammathodes*. The results obtained at 100 $\mu$g/ml of the extract were near to the effect of standard drug Berberine hydrochloride.

***Keywords:*** *Phallusia nigra, Didemnum psammathodes,* cytotoxicity.

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## Introduction

Most of the organisms are a rich source of bioactive compounds, but they are largely unexplored[1]. Marine organisms especially ascidians are significant as the first marine natural product entering human clinical trials, didemnin B, is an ascidian metabolite[2]. Ascidians commonly called sea squirt, are an interesting group of marine sedentary organisms that produce secondary metabolites with the unique structural pattern, for their chemical defence which does not occur in terrestrial plants. They grow on all underwater marine structures and are considered as a nuisance and usually thrown away. This discards may have a wealth of natural products. Bioactive peptides with novel structures have also been shown in ascidians[3]. Marine organisms, especially those that are a nuisance to the environment like biofoulers can be used for the study. Ascidians are available in plenty along the Tuticorin coast and hence an attempt

has been made to assess their toxicity. This study is based on the ability of the extract of *Phallusia nigra* and *Didemnum psammathodes* to kill laboratory-cultured *Artemia* nauplii brine shrimp. This assay is considered as a useful tool for preliminary assessment of toxicity[4].

## Materials and Methods

### Specimen collection and identification

*Phallusia nigra* and *Didemnum psammathodes* were collected from Tuticorin harbour by SCUBA diving.Epibiontsand the fragments of shell attached to the specimens were removed and washed several times with sterile seawater. Identification up to the species level was carried out based on the key to identification of Indian ascidians[5].

### Systematic position

*Phallusia nigra* belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Suborder: Phlebobranchia; Family: Ascidiidae; Genus: *Phallusia;* Species: *nigra*

*Didemnum psammathodes* belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Family: *Didemnidae;* Genus: Didemnum; Species: *psammathodes*

### Preparation of powder and extract

Samples of *Phallusia nigra* and *Didemnum psammathodes* were dried at 45°C and powdered separately. The extract was taken using methanol by Soxhlet extraction method[6]. It was taken in a conical flask and the mouth of the flask was covered by porated aluminium foil. The separation of the extract from the solvent was made by a natural evaporation method using porated aluminium foil.The crude extracts were kept at $-20$°C until further processing.

### Hatching of brine shrimp

Three litres of water was taken using a measuring cylinder and pour into the rectangular jar. 27 g of table salt was weighed by a balance and added it into the jar containing water and mixed it with a spatula. The tip of an airline from an air pump was placed into the bottom of the jar to maintain proper aeration. 10 g of brine shrimp eggs were added at the top water level and mixed. A light was (60-100 Watt bulb) placed a few inches away from the jar and switched on. Nauplii hatched out after 20-24 hours and were collected for the experimental purpose using pipette[7].

#### Lethality assay of brine shrimp

Ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* were used for the determination of Brine Shrimp Lethality Assay[8]. Clean test tubes were taken and labelled. 10 mg of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* were weighed by an analytical balance. Then stock solutions were prepared by dissolving the extract in 1 mL of water. Five concentrations of each extract were prepared at 20, 40, 60, 80 and 100 g/ml. Then each concentration was taken in the embryo cup containing 20 nauplii and 1 mL of seawater. The number of dead nauplii was counted after 24 hours. The mortality of the nauplii was determined as the absence of controlled forward motion during 30 seconds of observation.Berberine produces toxicity which is time and concentration-dependent, it was used as standard and seawater as control[9].

## Results

Toxicity of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* was carried out against *Artemia salina* using the brine shrimp lethality bioassay method is shown in figure 1 and 2.

Ethanolic extract of *Phallusia nigra* was tested at 20, 40, 60, 80 and 100 $\mu$g/ml and showed significant toxicity, indicating the presence of cytotoxic compounds. The different concentration of the extract showed toxic properties against *Artemia salina nauplii*. The maximum toxicity (85%) was observed in 100 $\mu$g/ml of ethanolic extract of *Phallusia nigra*. The effect of toxicity was observed in a dose-dependent manner. No mortality was observed in the control. 35, 50, 70, 75 and 85% mortality was observed in 20, 40, 60, 80 and 100 $\mu$g/ml of ethanolic extract of *Phallusia nigra*. The maximum toxicity (90%) was observed in 100 $\mu$g/ml of ethanolic extract of *Didemnum psammathodes*. The effect of toxicity was observed in a dose-dependent manner. 45, 55, 75, 80, and 90% mortality was observed in 20, 40, 60, 80 and 100 $\mu$g/ml of ethanolic extract of *Didemnum psammathodes*. 100% mortality was noticed in the group which was treated with Berberine hydrochloride.

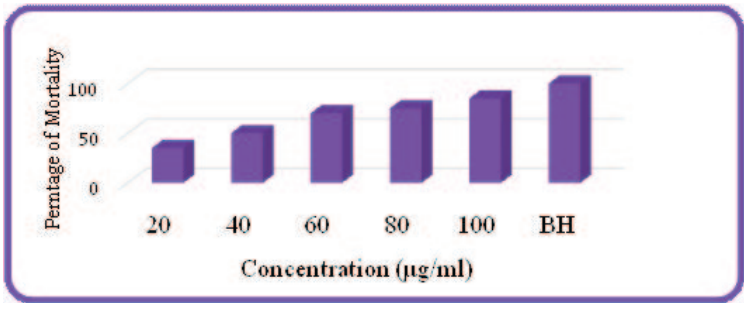


**Figure 1: Toxicity of ethanolic extract of *Phallusia nigra***

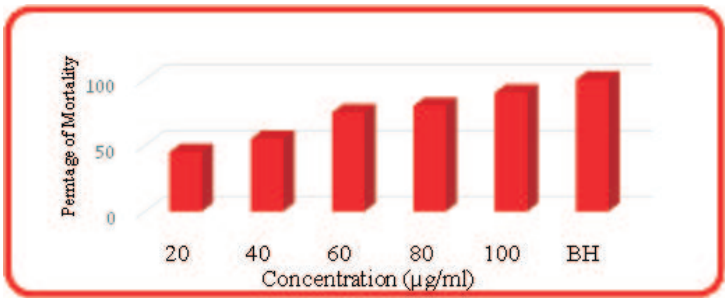


**Figure2: Toxicity of ethanolic extract of *Didemnum psammathodes***

## Discussion

Apyridoacridine alkaloid - Ascididemnin,derived from a *Didemnum* sp. showed cytotoxicity by inducing oxygen stress-related proteins and reactive oxygen species[10]. Ascidians are a rich source of compounds with cytotoxic properties[10]. GC-MS analysis of an ethanolic extract of *Phallusia nigra* has shown the presence of compounds like 2-Piperidinone, Benzeneacetamide, Tetradecanoic acid, n-Hexadecanoic acid, Phenol 3-pentadecyl, (Z, Z, Z)- phenylmethyl ester of 6, 9, 12-Octadecatrienoic acid, (z)-phenylmethyl ester of 9- Octadecenoic acid, Cholesterol, Cholestan-3-ol and 3-hydroxy-(3a, 17a)-Spiro [androst-5-ene17, l'-cyclobutan]-2'-one with antioxidant, cancer preventive and anticancer properties[11].

GC-MS chromatogram of the ethanolic extract of *Didemnum psammathodes* shows the presence of anti-cancer compounds such as 2,6,10,15,19,23-hexamethyl-, (all-E)-2,6,10,14,18,22- tetracosahexaene, [Synonyms: All-trans-Squalene]; (3*á*)-Cholesta-4, 6-dien-3-ol; (3*á*)-Cholest-5-en-3-ol carbonochloridate; 26-Nor-5-cholesten-3*á*-ol-25-one and Cholestan-3*á*-ol[12]. GC-MS analysis of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* contains anticancer compounds which might be the reason for the significant toxicity.

## Conclusion

In the present study, the ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* exhibited cytotoxicity significantly. 85% and 90% of cytotoxicity was observed in 100 µg/ml of ethanolic extract of *Phallusia nigra* and *Didemnum psammathodes* respectively. When compare the cytotoxicity of ethanolic extract of both species of ascidians, more significant activity was observed in *Didemnum psammathodes*. Studies on isolation, purification and structure determination are needed to determine the compound responsible for the activity.

## Acknowledgement

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# In-Vitro Anti-Inflammatory Activity of Ethanolic Extract of *Didemnum psammathodes*

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## Abstract

Rich secondary metabolites are present in marine tunicates. *Didemnum psammathodes* are common in Tuticorin coast. The present study was carried out to assess the anti-inflammatory activity of the ethanolic extract of *Didemnum psammathodes* on heat and hypotonicity induced haemolysis of erythrocytes. The ethanolic extract of *Didemnum psammathodes* at 200 $\mu$g/ml concentration showed 39% and 40% of inhibition of haemolysis of erythrocytes by heat-induced and hypotonicity induced method respectively. Anti-inflammatory activity was observed in a dose-dependent manner. The results proved that the ethanolic extract of *Didemnum psammathodes* contains the compounds responsible for anti-inflammatory activity.

***Keywords:*** *Didemnum psammathodes*, anti-inflammatory, haemolysis of erythrocytes

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## Introduction

Inflammation is a defence response of our body to hazardous stimuli such as allergens and injury to the tissues. It is part of the biological, protective response of body tissue involving immune cells, blood vessels and molecular mediators against harmful stimuli such as pathogens, damaged cells or irritants. The anti-inflammatory agents block substances that cause inflammation. The commercially available drugs used to treat inflammatory disorders cause adverse side effects. Drugs derived from natural products offer hope for the treatment of inflammatory disorders with little or no side effects.As fouling organisms however, they are significant and contribute largely to the problem of growth on the hulls of ships, wood, and metal pilings barrels, nets,

hard rocks, pebbles, cobbles, boulders, cement blocks, barges etc. It also grows on organisms such as sponges, macroalgae hydroids, anemones, bryozoans, mussels and oysters, and even other tunicates. Ascidians are ciliary and filter feeder which consume a large amount of water. Marine organisms, especially those that are a nuisance to the environment like biofoulers can be screened for anti-inflammatory activity. Ascidians rank second with a most promising source of drugs[1]. In India, studies on the anti-inflammatory property of ascidians especially in *Didemnum psammathodes* are lacking. As ascidians are available in plenty along the Tuticorin coast, an attempt has been made to assess the in vitroanti-inflammatory activity of *Didemnum psammathodes*.

## Materials and Methods

### Specimen collection and identification

*Didemnum psammathodes* were collected by handpicking in fresh condition from Gulf of Mannar region of Thoothukudi of Western Ghats, Tamilnadu. Identification up to the species level was carried out based on the key to identification of Indian ascidians[2].

### Systematic position

*Didemnum psammathodes* belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Family: Didemnidae; Genus: *Didemnum*; Species: *psammathodes*

### Preparation of powder and extract

Samples of *Didemnum psammathodes* were dried and powdered separately. The extract was taken using ethanol by Soxhlet extraction method to get valuable bioactive compounds[3]. The separation of the extract from the solvent is made by the natural evaporation method.It is taken in a conical flask and the mouth of the flask was covered by porated aluminium foil. The crude extracts were kept at $-20^{\circ}$C and used further for anti-inflammatory study.

## Membrane stabilization

### Preparation of RBCs suspension

The Blood was collected from the healthy human who has not taken any non-steroidal anti-inflammatory drugs for 2 weeks before the experiment. The blood was transferred to the centrifuge tubes, centrifuged at 3000 rpm for 10min and washed three times with equal volume of normal saline. The volume of blood was measured and reconstituted as 10% v/v suspension with normal saline[4,5].

#### Heat-induced haemolysis

2 ml of the reaction mixture consisted of 1 ml test sample of different concentrations (50, 100, 150 and 200 $\mu$g/ml) and 1 ml of 10% RBCs suspension, instead of test sample the only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30min. The tubes were kept under cool running tap water. The reaction mixture was centrifuged for 5 min at 2500 rpm and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples[4,6]. The Percentage inhibition of haemolysis was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Control absorbance} - \text{Sample absorbance}) \times 100}{\text{Control absorbance}}$$

#### Hypotonicity induced haemolysis

Different concentrations of extract (50, 100, 150 and 200 $\mu$g/ml), reference sample, and control were separately mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. Diclofenac sodium (100 $\mu$g/ml) was used as a standard drug. All the assay mixtures were incubated at 37°C for 30minutes and centrifuged at 3000rpm. The supernatant liquid was decanted and the haemoglobin content was estimated using a spectrophotometer at 560nm[7]. The percentage of hemolysis was estimated by assuming the haemolysis produced in the control as 100%

$$\text{Percentage protection} = 100 - (\text{OD sample}/\text{OD control}) \times 100$$

## Results

#### Effect of ethanolic extract of *Didemnum psammathodes* on heat -induced haemolysis of erythrocytes

The extract was effective in inhibiting the heat-induced haemolysis at different concentration. The results showed that ethanolic extract of *Didemnum psammathodes* at various concentrations protect significantly the erythrocyte membrane against lysis induced by heat (Figure - 1). Aspirin offered significant protection against the damaging effect. The extract at 200 g/ml concentration showed 39% of inhibition of haemolysis whereas, Aspirin (100 $\mu$g/ml) showed 50% of inhibition of haemolysis.

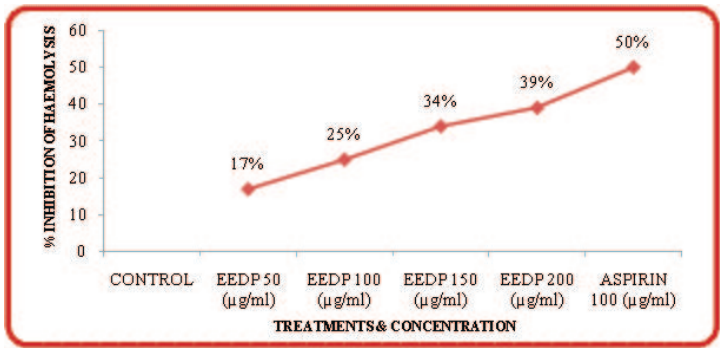


**Figure 1: Effect of ethanolic extract of *Didemnum psammathodes* on heat-induced haemolysis of erythrocytes**

**Effect of ethanolic extract of *Didemnum psammathodes* on hypotonicity induced haemolysis of erythrocytes**

Figure- 2 depicts the effect of ethanolic extract of *Didemnum psammathodes* on hypotonicity induced haemolysis of erythrocytes. The results showed that ethanolic extract of *Didemnum psammathodes* at concentrations of 50, 100, 150 and 200 µg/ml protect erythrocyte membrane against lysis induced by the hypotonic solution. Diclofenac sodium (100 µg/ml) offered significant protection against the damaging effects of the hypotonic solution. The extract at 200 µg/ml concentration showed 40% of inhibition of haemolysis whereas, Diclofenac Sodium (100 µg/ml) showed 48% of inhibition of haemolysis when compared to control.

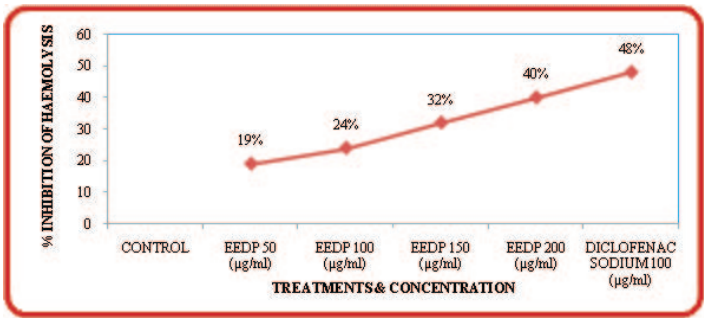


**Figure 2: Effect of ethanolic extract of *Didemnum psammathodes* on hypotonicity induced haemolysis of erythrocytes**

## Discussion

Anti-inflammation refers to the property of a substance that reduces inflammation. Alternately herbal medicines play a role in anti-inflammation apart from that marine-

derived bioactive compounds also showed anti-inflammatory action. Many steroids and non-steroidal anti-inflammatory drugs are widely used for the treatment of inflammation. Marine invertebrates gave a significant number of natural products and secondary metabolites with pharmacological properties. The natural products have a wide range of therapeutic properties including anti-inflammatory effects.

An alkaloid is a group of biological amine and cyclic compounds having nitrogen in the ring, naturally occurring in plant, microbes, animals and marine organisms. Indole alkaloids from marine invertebrates have reported being anti-inflammatory potentials and these include conicamin from tunicate, Lepadiformines A and B from ascidian[8,9]. Alkaloids Ascidiathia zone isolated from ascidian (Ascidian Aplidium) for anti-inflammatory activity in human neutrophils[10]. Synthesis of silver nanoparticles using *Didemnum psammathodes* can be used as a lead compound for designing a potent anti-inflammatory drug to cure inflammation[11].

The results obtained from the study on ethanolic extract of *Didemnum psammathodes* have shown a potential anti-inflammatory activity. Percentage of haemolysis depends on the bioactive compounds present in the ethanolic extract of *Didemnum psammathodes*. Aqueous extracts of *Gracilaria* species were effective in inhibiting the heat-induced haemolysis at different concentrations due to the presence of flavonoids and triterpenoids[12]. *Oroxylum indicum* has been found to scavenge DPPH, superoxide anion, hydroxyl, nitric oxide, and $Fe^{3+}$ radicals, which are major players in eliciting the inflammatory response[13]. *Oroxylum indicum* contains flavonoids and other phenolic compounds that may have contributed to its anti-inflammatory actions[14].

The methanolic extract of *Phallusia nigra* showed anti-inflammatory potential in both acute and chronic phases of inflammation due to the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds, tannins, etc[15]. Hence the anti-inflammatory activity may be due to the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds, tannins, etc. The extract fractions may serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat-induced albumin denaturation and stabilized the red blood cell membrane.

## Conclusion

This study gives an idea that the compound of ethanolic extract of *Didemnum psammathodes* can be used as a lead compound for designing a potent anti-inflammatory drug. Extensive study is needed to prove the exact mechanism of anti-inflammatory action of the extract of *Didemnum psammathodes*.

## Acknowledgement

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# Anti-Bacterial Activity of *Didemnum psammathodes* against Human Pathogens

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## Abstract

Human health is affected by various bacteria. The drugs which are used as anti-bacterial might have some side effects. Ascidians have been reported to be rich source of biologically active compounds. The present study was carried out to investigate the anti-bacterial activity of five bacteria - *Vibrio cholerae, Pseudomonas aeroginosa, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus* in crude extracts of ascidians collected from Tuticorin coast. Methanolic extract of *Didemnum psammathodes* revealed anti-bacterial activity against all bacteria indicated by zone of inhibition ranging from (5-18mm) in diameter and the highest activity was observed in *Vibrio cholerae*. The result obtained at 25 $\mu$l/ml of ethanolic extract against *Vibri cholerae* was better the effect of standard drug.

***Keywords:*** *Didemnum psammathodes*, methanol extract, anti-bacterial activity

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## Introduction

Human health is affected by various pathogens such as bacteria, virus, fungi and protozoans. Among them bacteria cause diseases in various systems of human body. Nowadays there are many drugs available in the market against these bacterial diseases. Though they are effective, they also produce some side effects because of their synthetic chemical composition. The drugs derived from natural products against the human bacterial diseases are harmless. Ascidians are marine, sessile prochordate filter feeders. In recent years a variety bio-active compounds have been extracted from various marine animals like tunicates, sponges, soft corals, sea horses, bryozoans,

sea slugs and some of them are currently used in clinical trials[1,2]. Sessile animals have secondary metabolites for their defence. Halocyamine A, an antimicrobial substance was isolated from haemocytes of the solitary ascidian *Halocynthia roretzi*[3]. Various species of ascidians have shown activities like antimicrobial, antiproliferative, antitumour, immunomodulatory, antiinflammatory, antifertility, wound healing, CNS depressant, cardioprotective etc[4−14].

The present study was carried out to investigate the anti-bacterial activity of methanolic extract of *Didemnum psammathodes* against human pathogens such as *Vibrio cholerae, Pseudomonas aeroginosa, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus*.

## Materials and Methods

### Specimen collection and identification

Samples of *Didemnum psammathodes* were collected from the under surface of the barges of Tuticorin harbor. Identification upto the species level was carried out based on the key to identification of Indian ascidians[15].

### Systematic position

*Didemnum psammathodes* belongs to Phylum: Chordata; Subphylum: Urochordata; Class: Ascidiacea; Order: Enterogona; Family: Didemnidae; Genus: *Didemnum*; Species: *psammathodes*

### Preparation of powder and extract

Samples of *Didemnum psammathodes* were dried, powdered and the extract was taken using methanol by Soxhlet extraction method[16]. The separation of the extract from the solvent is made by natural evaporation method. It is taken in a conical flask and the mouth of the flask was covered by porated aluminium foil. The crude extracts were kept at $-20^{\circ}$C until further processing.

### Microbial strains used

Anti-bacterial activity was determined against five different bacterial pathogens, four gram negative bacteria -*Vibrio cholerae, Pseudomonas aerogenosa, Salmonella typhi, Shigella flexneri* and one gram positive bacteria -*Staphylococcus aureus*.

## Preparation of test micro organisms

A loopful of the test bacterial pathogen was transferred to already sterilized 10 ml Nutrient agar and incubated overnight at 37°C. 25 ml of sterilized Muller- Hinton

Agar (MHA) was poured in petridishes and allowed to solidify at room temperature on which the test microbes were inoculated.

#### Anti-microbial assay

The anti-microbial activity was measured by Disc Diffusion method[17]. The sterile cotton swabs are used to spread the bacterial inoculum on already sterilized MHA plates. The sterile discs were impregnated with the three different concentrations of the methanolic extract of *Didemnum psammathodes* at 10 µl, 15 µl, 20 µl and standard drug Amphicillin at 25 µl. The discs were then placed on the already inoculated petridishes containing the inoculum of test microbes[18].

#### Incubation

The bacterial pathogens were maintained on MHA plates and incubated at 37°C for about 24 hrs. Zones of inhibition were measured in millimeter using a scale[18]. The experiment was repeated thrice to get the concordant value.

## Results

In the present investigation, methanolic extract of *Didemnum psammathodes* was tested against human pathogens such as *Vibrio cholerae, Pseudomonas aeruginosa, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus*. Plate: 1 and Figure: 1 to 5 depicts the anti-bacterial activity of *Didemnum psammathodes* against five human pathogens.

Figure-1 depicts anti-bacterial activity of methanolic extract of *Didemnum psammathodes* against *Vibrio cholerae*. While screening against *Vibrio cholerae*, the extract was more efficient at the concentration of 25 µl/ml and the inhibition zone was about 18mm. The concentrations at 10 µl, 15 µl, 20 µl, 25 µl showed about 5mm, 7mm, 5mm and 18mm of zone of inhibition. The standard drug amphicillin at the concentration of10 µl shows about 16mm diameter of zone of inhibition in *Vibrio cholerae* sp.which is lower than the extract of *Didemnum psammathodes*.

Anti-bacterial activity of methanolic extract of *Didemnum psammathodes* against *Pseudomonas aeruginosa* is shown in Figure-2. The methanolic extract of *Didemnum psammathodes* exhibited maximum zone of inhibition which is about 12mm at the concentration of 10 µl which was slightly lesser than the standard drug. The amphicillin showed about 15mm of inhibition zone.

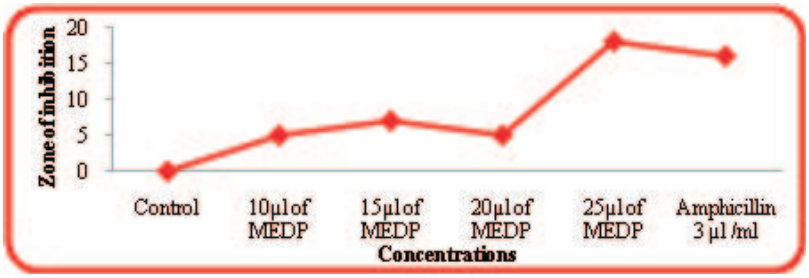



**Figure 1: Antibacterial activity of Methanolic extract of *Didemnum psammathodes* (MEDP) against *Vibrio cholerae***

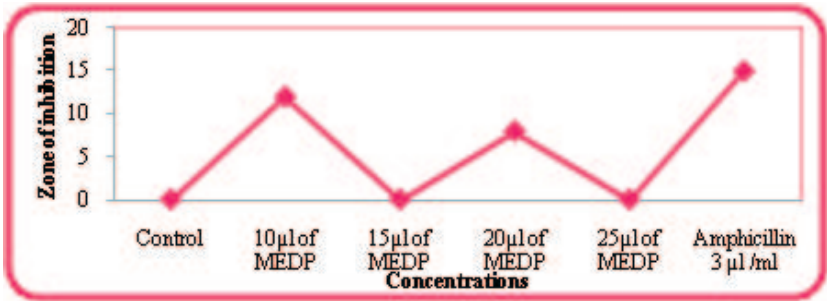



**Figure 2: Antibacterial activity of Methanolic extract of *Didemnum psammathodes* against *Pseudomonas aeroginosa***

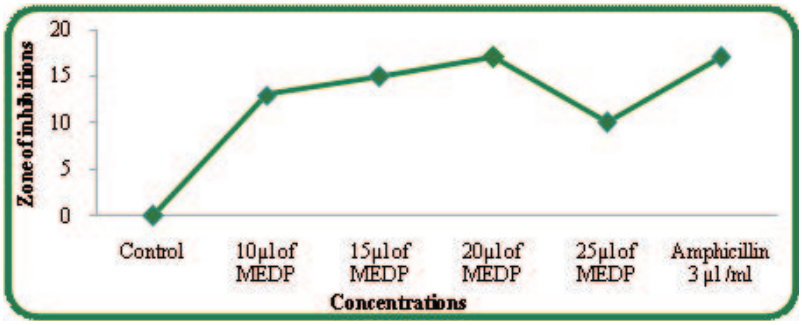



**Figure 3: Antibacterial activity of Methanolic extract of *Didemnum psammathodes* against *Salmonella typhi***

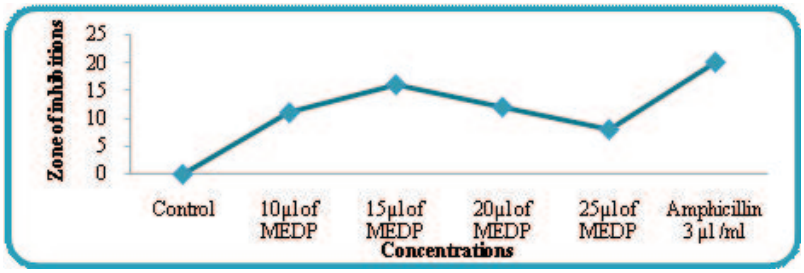



**Figure 4: Antibacterial activity of Methanolic extract of *Didemnum psammathodes* against *Shigella flexneri***

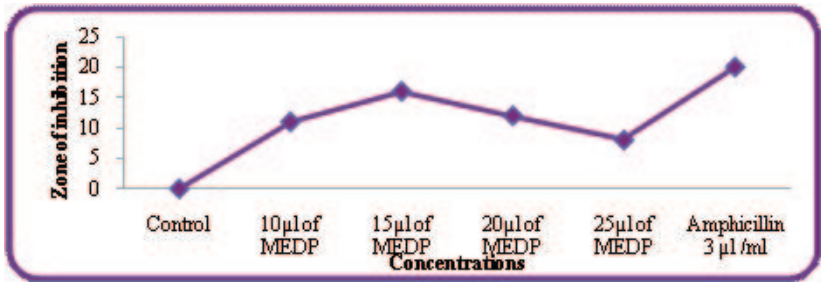



**Figure 5: Antibacterial activity of Methanolic extract of *Didemnum psammathodes* against *Staphylococcus aureus***

Figure-3 depicts anti-bacterial activity of methanolic extract of *Didemnum psammathodes* against *Salmonella typhi*. There was a well growth of *Salmonella typhi* around the control disc. *Salmonella typhi* exhibited a gradual rising in the clearzone formation along with theincrease of extract concentration. It reached the maximum zone of inhibition which was about 17mm at 20l concentration of methanolic extract of *Didemnum psammathodes*. The zone of inhibition for Amphicillin also 17 mm. The concentrations of the extract at 10 $\mu$l, 15 $\mu$l, 20 $\mu$l and 25 $\mu$l showed 13mm, 15mm, 17mm and 10 mm of zone of inhibitions respectively.

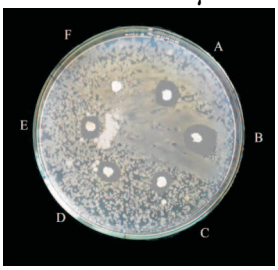
Figure-4 depicts anti-bacterial activity of methanolic extract of *Didemnum psammathodes* against *Shigella flexneri*. Water exhibited no inhibitory activity against *Shigella flexneri*. Peak of inhibitory activity was observed at 15 $\mu$l concentration in which the inhibitory zone was about 16mm. Amphicillin formed 20mm diameter of zone of inhibition.

Figure-5 depicts anti-bacterial activity of methanolic extract of *Didemnum psammathodes* against *Staphylococcus aureus*. The methanolic extract of *Didemnum psammathodes* exhibited about 16mm at the concentration of 20$\mu$l. The

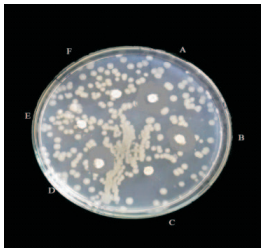
concentrations of the extract at 10 µl, 15µl and 20µl exhibited 12mm, 14mm, 16 mm and 9 mm of zone of inhibition respectively. The standard drug formed 23mm of inhibition zone.

Among the pathogens used in the present study, *Salmonella typhi* and *Staphylococcus aureus* showed considerable inhibition zone at the highest concentration of methanolic extract of *D.psammathodes*.

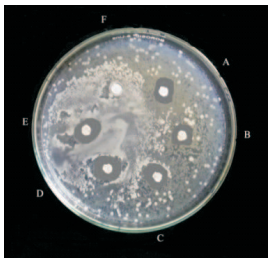
In the present study the maximum inhibition zone (18 mm) was observed against *Vibrio cholerae* by the methanolic extract of *D. psammathodesat* the concentration of 25 µl/ml. The maximum inhibition zone (12 mm) was observed in the methanolic extract of *D. psammathodes* at 25 µl/ml.



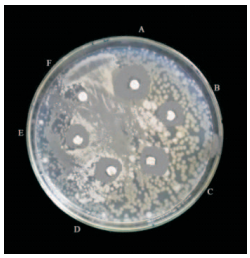

A. *Vibrio cholerae*



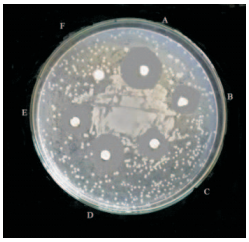
B. *Pesudomonas aerogenosa*



C. *Salmonella typhi*



D. *Shigella flexneri*



E. *Staphylococcus aureus*

**Plate – 1: Antibacterial Activity of *Didemnum psammathodes***

A. Control
B. 10µl of methanolic extract of *Didemnum psammathodes*
C. 15µl of methanolic extract of *Didemnum psammathodes*
D. 20µl of methanolic extract of *Didemnum psammathodes*
E. 25µl of methanolic extract of *Didemnum psammathodes*
F. Amphicillin (3 µl /ml)

At 20 µl/ml concentration of methanolic extract of *D.psammathodes*, maximum zone of inhibition (17 mm) against *Salmonella typhi* was observed. The maximum (16 mm) inhibition zone was observed against *Shigella flexneri* at 15 µl/ml concentration of methanolic extract of *D.psammathodes*. Maximum inhibition zone (16 mm) was observed against *Staphylococcus aureus* by the methanolic extract of *D. Psammathodes* at the concentration of 20 µl/ml (Plate-1).

## Discussion

Anti-biotic therapy can shorten the duration and severity of diarrhoea caused by *Vibrio cholera* but also aggravates the appearance of anti-biotic resistance[19]. The ascidian *D. psammathodes* is found to have remarkable antimicrobial activities against isolated microbes[20]. *Didemnum psammathodes* contain a high amount 69.07% of flavonoids[21]. Flavonoids are well known as anti-bacterial agents against a wide range of pathogenic microorganism[22]. Due to the presence of flavonoids, the present study may show significant anti-bacterial activity. As the extract of *Didemnum psammathodes* contains flavonoids this might be the reason for the anti-bacterial activity against *Vibrio cholerae*. Ascidians are the sedentary animals they contain flavonoids which are more or less similar to that of plants[23].

*S.typhi* strains were resistant to amphicillin, chloremphenical and trimethoprim-sulfamethoxazole[24]. Yet, these strains are multidrug resistant they may be treated with the methanolic extract of *D.psammathodes* as it showed equal inhibitory of zone as that of amphicillin.

Phenol is one amongst the oldest anti-bacterial agents. It acts as a bacteriostatic by inhibiting biological process of bacteria at concentrations of 0.1-1%[25]. Extract of *Didemnum psammathodes* exhibited broad-spectrum antibacterial activity[26]. The species *Didemnum psammathodes* has 86.13% of phenol content[27]. The anti-bacterial activity of the extract of the species may due to either the presence of phenol or flavonoids.Vanadyl sulfate and sodium vanadate were unpalatable to fish, although these salts do not accurately reflect the chelation environment or oxidation state of vanadium in living tunicates[28]. Vanadium compounds have been proposed for the treatment of diabetes, cancer and diseases caused by parasites[29]. The presence of vanadium compounds may be the reason for the anti-bacterial activity.

## Conclusion

It has been reported that thousands of natural products have been isolated from ascidians such as alkaloids, cyclic peptides and polypeptides etc. Most of these secondary metabolites possess diverse bioactivities such as anti-bacterial, anti-fungal and anti-tumor activities. Compounds found in the extract showed significant anti-

bacterial activity for discovery of novel marine drug. The animals which are considered as the nuisance and affect the economy by corrosion were used for this study. Such a natural product is good for health and devoid of side effects. A further study on isolation, purification, structure determination and subsequent recognition of the novel mechanism of action of the clinically effective agent is suggested.

## Acknowledgement

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# Analysis of Bioactive Constituents from the Marine Gastropod *Lambis lambis* (Linnaeus, 1758)

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## Abstract

The marine life constitutes almost 80% of the world's biota and is a source of unique natural products used as food, fragrances, pigments, insecticides, medicines etc. Marine organisms offer infinite source to discover useful therapeutics. We are still in need of safer, cheaper and effective drug products. The present study was aimed to investigate the bioactive components present in *Lambis lambis* by GC-MS analysis. The GC-MS analysis of methanolic extract confirmed the presence of 11 active compounds. The active compounds produced by marine organisms are used in traditional and complementary medicine.

***Keywords:*** *Lambis lambis*, methanol, FT-IR, GC-MS, bioactive compounds

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## Introduction

Ocean serves as a reservoir for newly discovering potential therapeutic agents. Numerous compounds with interesting pharmaceutical activities have been reported from marine organisms during the past decades. Hence, marine organisms are considered an important source of bioactive molecules to treat various diseases[1]. GC-MS helps to identify different substances within the test samples.

India is endowed with rich and diverse bio-resources and the molluscs are not an exception. Molluscs are a heterogeneous group of animals both in shape and diversity and are represented by amphineura, gastropods, bivalves, cephalopods and scaphopods. Molluscs have also proven to be the rich source of structurally diverse

bioactive compounds with valuable pharmaceutical and biomedical applications[2]. Nearly all of the molluscs are consumed by the coastal people in their normal diet[3].

Infrared spectral analysis of biological materials was utilized to investigate their chemical constituents. These are recognized even when the availability of material is less[4]. Molluscs are said to be the pharmacologically significant outlet. There are more than thousands of bioactive compounds discovered in molluscs. They are peptide, sterols, sesquiterpenes, terpenes, polypropionate, nitrogenous compounds, macrolides, prostaglandins miscellaneous compounds and alkaloids[5]. The secondary metabolites derived from the number of marine animals possess antibiotic, anti-parasitic, antiviral, antioxidant and anti-cancer activities[6].

In recent years, the pharmaceutical industry encourages lead compounds from a marine organism which paved the way for more collaborative efforts between academia and the pharmaceutical industry to translate the natural products into clinical trials[7]. However, the biologically active products from marine molluscs are largely unexplored in India[8]. Biomedical screening of solvent extracts of marine molluscs would provide a valuable base for new drug leads and ultimately used for the treatment of chronic diseases[9].

The present study has been planned to evaluate the bioactive principles found in the methanolic extract of marine gastropod *Lambis lambis* through FT-IR and GC-MS studies.

## Materials and Methods

### Collection of experimental animals

In the present study, the gastropod *Lambis lambis* were collected from the trawl nets used for crab fishing from the Gulf of Mannar coastal region. They were brought to the laboratory and maintained under laboratory conditions for further observations.

The freshly collected samples were cleaned and washed with fresh seawater to remove all impurities. The shells were broken, tissues were removed and then dried in a hot air oven at 56°C for 48 hours and used for further studies.

### Preparation of extract

One gram of the sample was dissolved in 20ml of the methanol solvent and mixed well in a conical flask, after mixing the sample was incubated at 40°C, 60-70 rpm in an orbital shaker for 24 hrs. The extract was filtered through Whatman No.1 filter paper and the extract residue was resuspended in 20 ml of 100% A.R grade methanol. The methanol soluble extracts were dried and solubilized in deionized water. Different concentrations of extracts were prepared and stored at 0°C for further use.

#### Fourier Transform Infra-Red spectrum analysis

The functional groups present in the methanolic extract of *Lambis lambis* were determined using FT-IR spectroscopy (Bio-read FT-IR 8400s models, USA) at Ayya Nadar Janaki Ammal College, Sivakasi.

#### GC-MS analysis

GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer System comprising an AOC 20i autosampler and gas chromatography interfaced to a mass spectrophotometer (GC-MS) instrument at Ayya Nadar Janaki Ammal College, Sivakasi.

#### Identification of compounds

Interpretation of the mass spectrum was conducted using the database of National Institute of Standard Technology (NIST Ver.21), WILEY 8 and FAME having more than 62,000 patterns. The unknown components found in the body tissues of *Lambis lambis* were matched with the spectrum of the known components stored in NIST, WILEY and FAME, the MS library and predicted from Duke's Ethno Botanical Database.

## Results

#### FT-IR spectral analysis

FTIR analysis of Lambis lambis confirmed the presence of alcohols, phenols, alkanes, carboxylic acid. In the present scrutiny of *Lambis lambis*, revealed the graph with wave number against the intensity of 15 peaks with frequencies ranging from 458.06 to 3446.56 $cm^{-1}$. Here, ten functional groups iodide (C-I), bromide (C-Br), chloride (C-Cl), phosphates, nitrates, ketones (C=O), cyanide (C-N) and alcohol (O-H) were found. The wavenumbers 3446.56, 2925.81, 2852.52 and 2360.71 distinctive of asymmetrical stretching of $CH_2$ and 1641.31, 1544.88, 1441.69, 1409.87, 1385.76, 1190.0, 1112.85, 1041.49, 873.69, 619.11 and 458.06 positions of the spectrum are the characteristics of C=O stretching, C-OH, C-H, C-O and skeletal stretch respectively (Figure 1).

#### GC-MS analysis

The sample was subjected to GC-MS analysis. GC-MS analysis from experimental organism *Lambis lambis* revealed the presence of 11 compounds. The identified compounds were n-Hexadecanoic acid, Octadecanoic acid, Cis-Vaccenic acid, 9-Octadecenoic acid, Oleic acid, 1- Octadecene, 1- Nonadecene, Erucic acid, 1-Pentadecene, Cyclotrisiloxane, hexamethyl -, 2 - Ethylacridine. The bioactive compounds with their retention time (RT), molecular formula, molecular weight (MW) and concentration (area) are presented in table 1. The mass spectrum was also

shown in Fig.2. The identified chemical components present in the crude methanolic extract of *Lambis lambis* could be responsible for various bioactivities such as antitumour, antiasthmatics, antidermatitis, antiulcer, antipsoriatics, surfactants, bronchodilators, insectifuge, antiandrogenic, wound healing, analgesic, antioxidant, cancer preventive, antifertility, antimicrobial, anti-inflammatory, antidiuretics, anti-influenza, and antiviral activities.

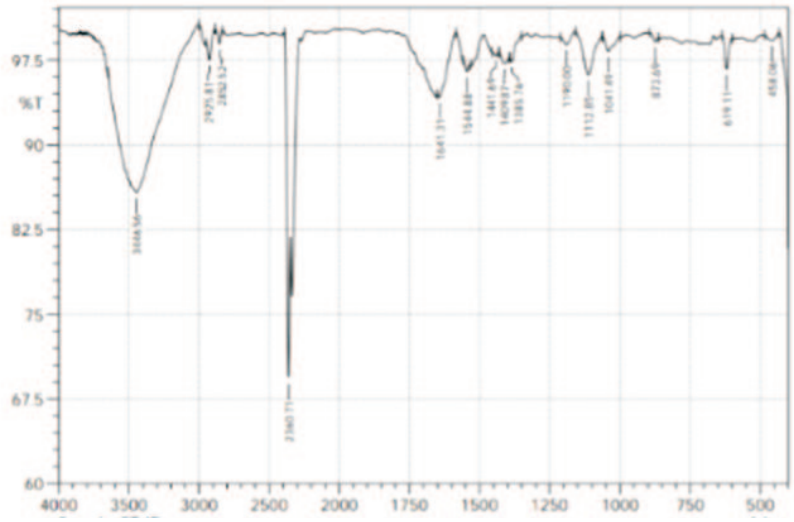


**Figure 1: FT-IR spectrum of *Lambis lambis***

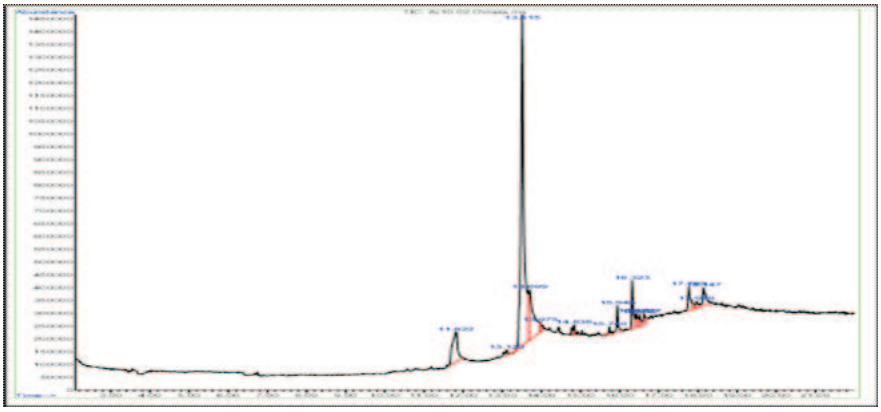

**Figure 2: GC-MS spectral analysis of *Lambis lambis***

**Table 1: Activity of components identified in the methanolic extract of *Lambis lambis* by GC-MS**

| S. No | RT Min | Name of Compound | Molecular Formula | MW g/mol | Area % | Compound Nature | Activity |
|---|---|---|---|---|---|---|---|
| 1 | 11.826 | n-Hexa-decanoic acid | $C_{16}H_{32}O_2$ | 256.42 | 8.83 | Palmitic acid | Antimicrobial activity, Antitumour activity, Antioxidant. |
| 2 | 11.826 | Octadecanoic acid | $C_{18}H_{36}O_2$ | 284.5 | 8.83 | Fatty acid ester | Antiasthmatics, Antidiuretics, Antidermatitis, Surfactants, Antioxidant |
| 3 | 13.121 | Cis-Vaccenic acid | $C_{18}H_{34}O_2$ | 282.5 | 0.58 | Fatty acid | Antibacterial, Antibiofilm, Antiulcer, Antipsoriatics, Telomerase reverse transcriptase |
| 4 | 13.121 | 9-Octadecenoic acid, (E)- | $C_{18}H_{34}O_2$ | 282.5 | 0.58 | Fatty acid ethyl ester | For throat disorders, Antiasthmatics, Bronchodilators, Expectorants, Mucolytics |
| 5 | 13.698 | Oleic acid | $C_{18}H_{34}O_2$ or $C_{18}H_{17}CH = CH(CH_2)COOH$ | 282.5 | 13.56 | Fatty acid | Cancer preventive, Anemiagemic, Insectifuge, Antiandrogenic, Dermatitigenic |
| 6 | 14.842 | 1-Octadecene | $C_{18}H_{36}$ | 282.5 | 0.98 | Alkene | Anticancer, Anti-inflammatory, Diuretic, Antihepatotoxic, Antifertility, Antiurolithiatic, Analgesic, Antioxidant, Antimicrobial |
| 7 | 15.740 | 1-Nonadecene | $C_{19}H_{38}$ | 266.5 | 0.41 | Alkene | Antiasthmatics, Bronchodilits, Urine acidifiers of the Kidneys, contraceptives |
| 8 | 16.421 | Erucic acid | $C_{22}H_{42}O_2$ | 338.6 | 1.57 | Fatty acid | Anticancer, Anti-influenza, Antiviral, Anti-inflammatory, Antioxidant, Wound healing |
| 9 | 16.629 | 1-Pentadecene | $C_{15}H_{30}$ | 210.4 | 1.16 | Alkene | Wound healing |
| 10 | 17.773 | Cyclotrisiloxane hexa methyl- | $C_6H_{18}O_3Si_3$ | 222.46 | 3.15 | Phenol | Antimicrobial, Antioxidant |
| 11 | 17.773 | 2-Ethylacridine | $C_{15}H_{13}N$ | 207.27 | 3.15 | - | Antitumour, Antioxidant, Antibacterial |

## Discussion

Thousands of bioactive compounds identified in marine organisms revealed that sea creatures constitute a large reservoir for pharmacologically active drug[9]. The presence of strong bonds above 3000 cm-1 indicates the presence of an aromatic ring. FTIR analysis revealed the presence of bioactive compounds signals at different ranges. The present study discusses and presents some selected applications of the extraction of target compounds with antioxidant and anticancer activities. The wave numbers 3446.56, 2925.81, 2852.52 and $cm^{-1}$ distinctive of asymmetrical stretching of $CH_2$ and 1641.31, 1544.88, 1441.69, 1409.87, 1385.76, 1190.0, 1112.85, 1041.49, 873.69, 619.11 and 458.06 $cm^{-1}$ positions of the spectrums are the characteristic of C=O stretching, C-OH, C-H, C-O and skeletal stretch respectively (Figure 1). *Pila virens* showed 3840, 3421, 3196, 2585, 2499 and 2310 distinctive of asymmetrical stretching of $CH_2$ and 1992, 1627, 1271, and 1016 and 682-403 positions of the spectrums are the characteristic C=O stretching, CoH, CH, C-O and skeletal stretch. The present study corroborates well with the above findings.

In the present study, the IR spectrum indicates the presence of iodine, bromide, chloride, phosphates, nitrates, ketones, arenes, cyanide and alcohol functional groups. Divya Dharan (2018) showed the presence of aliphatic bromo compounds, phenol, tertiary alcohols, carbonyl compounds and carboxylic acids in ascidian *Aplidium multiplicatum*. GC-MS chromatogram of the methanolic extract of *Aplidium multiplicatum* showed 21 peaks which indicated the presence of 21 chemical compounds with various biological activities like anti-microbial, anti-inflammatory, pesticide, chemoprevention, diuretic and antioxidant similar to the present findings.

An attempt has been made to outline the most important aspects of the empirical approach to find new lead compounds from natural resources such as animals. In the present study, 11 chemical constituents have been identified from methanol extract of the whole animal of *Lambis lambis* by gas chromatogram mass spectrometry (GC-MS) analysis (Table 1) respectively. The compounds from methanolic extract of *Lambis lambis* could be responsible for various activities like anticancer, anti-inflammatory, antioxidant, antibacterial, antidiuretics, antiviral, antifertility, wound healing and analgesics.

In the present study, GC-MS analysis confirmed the presence of 11 compounds that could be identified as n-Hexadecanoic acid, Octadecanoic acid, Cis-Vaccenic acid, 9-Octadecenoic acid, Oleic acid, 1- Octadecene, 1- Nonadecene, Erucic acid, 1- Pentadecene, Cyclotrisiloxane, hexamethyl -, 2 - Ethylacridine. The present study correlates well with the above findings. Chemical drugs may lead to adverse effects but the natural bioactive principles from animal sources will not exhibit much side effects. Pioneering research efforts of marine bio-prospecting may provide potent

and sustainable results on finding new bioactive molecules of marine origin in light of the high biodiversity of marine ecosystems.

## Conclusion

The findings of the present study suggest that gastropod extract could be a potential source of natural bioactive substances. More clinical trials are required to confirm this study. More research is required across all molluscan group.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Evaluation of Calcium Content and XRD Studies in the Shells of Some Species of Gastropods

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## Abstract

Molluscan shells are composed of more than 95% calcium carbonate and less than 5% of an organic matrix consisting mostly of proteins, glycoproteins and polysaccharides. The present study aimed to determine the calcium content in the five molluscan shells viz., *Lambis lambis, Chicoreus virgineus, Babylonia spirata, Murex tribulus* and *Conus betulinus*. The results confirm that *Lambis lambis* had the highest calcium content followed by *Murex tribulus, Chicoreus virgineus, Conus betulinus* and *Babylonia spirata* respectively. Thus, there is a considerable scope for utilization of shell calcium on a commercial basis in varied industries and the new applications are constantly being developed so that its use will continue to grow well into the next millennium.

***Keywords:*** Molluscan shell, Gastropods, Calcium Carbonate, XRD, Calcite, Aragonite

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## Introduction

Gastropod shells are rich in calcium carbonate which is the major source of raw material for the lime industries. The molluscan shell forms one of the most important raw materials for many calcium carbonate industries since 33 to 40% of the shell is calcium, 90 to 98% of which occurs as calcium carbonate. Calcium determination in the shell of the snails is an important parameter that is widely used as an indirect evaluation of metabolic alterations, the influence of the environment and conditions of stress on the physiology of molluscs[1,2 & 3]. The calcium present in the shell is predominantly found as calcium carbonate incorporated in the shell structure as

calcite and aragonite crystals associated with the organic matrix, composed basically by conchiolin.

Molluscan shells are mainly used as food and in the manufacture of lime, pharmaceuticals, ornaments, poultry, fish feed etc. They are also used for domestic purposes such as washing utensils as an abrasive material. Also, the shell body and its by-products are being studied and used in many areas of modern medicinal applications. One of the most abundant biominerals in the molluscan shell is calcium carbonate ($CaCO_3$). $CaCO_3$ which is one of the most important components in hard tissues such as teeth and bones also. The composite materials created by biological organisms from calcium salts and proteins are architecturally complex and functionally diverse[4]. Shell calcium as burnt lime has been widely used in agriculture and aquaculture. Keeping in view of the significance of calcium in molluscan shells, the present study was aimed to assess the calcium content of five molluscan shells which are abundant along the Gulf of Mannar Thoothukudi coastal region through XRD.

## Materials and Methods

### Collection of experimental animals

In the present study the gastropod *Lambis lambis, Chicoreus virgineus, Babylonia spirata, Murex tribulus* and *Conus betulinus* were collected from the trawl nets used for crab fishing from the Gulf of Mannar coastal region. The freshly collected samples were brought to the laboratory, cleaned and washed with fresh seawater to remove all impurities. The shells were broken, tissues were removed and then dried in a hot air oven at 56°C for 48 hours and used for further studies.

### Calcium Determination

Shells of each species were collected in separate Ziploc bags and were carried to the laboratory for calcium analysis. The shells were cleaned, dried at room temperature and weighed. Calcium content was estimated by following the technique described by[1]. The amount of calcium was expressed as milligram (mg) of $CaCO_3$ per gram of dry shell weight.

### XRD Analysis of Gastropod Shells

The major compound analysis and biomineralization property studies were done by X-ray diffraction (XRD) methods. The crystal structure was characterized by a powder X-ray diffractometer. Powder diffraction data were recorded at room temperature using a powder diffractometer [Bruker AXS D8 Advance (Cu K (Ni filtered) with scintillation detector, $2\theta$ range from 20 - 800 and step size of 0.02°)].

## Results

### Calcium Content of Gastropod Shells

In the present study, calcium carbonate content in the shells of five different gastropod species *Lambis lambis, Chicoreus virgineus, Babylonia spirata, Murex tribulus* and *Conus betulinus* were evaluated. The calcium carbonate content was observed as 903mg $CaCO_3$/g in *Lambis lambis*, 753mg $CaCO_3$/g in *Murex tribulus*, 620mg $CaCO_3$/g in *Chicoreus virgineus*, 567mg $CaCO_3$/g in *Conus betulinus* and 418 mg $CaCO_3$/g in *Babylonia spirata*. The calcium content of five gastropods shells is depicted in figure 1. The above results indicated that *Lambis lambis* had highest calcium content followed by *Murex tribulus, Chicoreus virgineus, Conus betulinus* and *Babylonia spirata* respectively.

### Powder XRD Pattern of Gastropod Samples

In the present study, five gastropod samples viz., *Lambis lambis, Chicoreus virgineus, Babylonia spirata, Murex tribulus* and *Conus betulinus* showed a mixture phase of aragonite and calcite, the common form of $CaCO_3$ minerals as shown in figures 2-6. The X-ray diffraction patterns of five gastropods samples exhibits peak characteristics of $2\theta$ = *Lambis lambis*, $2\theta$ = 26.26, 27.26 in *Chicoreus virgineus*, $2\theta$ = 26.16, 27.15 in *Babylonia spirata*, $2\theta$ = 26.52, 27.49 in *Murex tribulus* and $2\theta$ = 26.18, 26.66 in *Conus betulinus* the intensities corresponding to the calcite appear and the appearance aragonite peak at $2\theta$ = 33.19, 36.10 in *Lambis lambis*, *Lambis lambis*, $2\theta$ = 33.19, 36.16 in *Chicoreus virgineus*, $2\theta$ = 33.06, 33.46 in *Babylonia spirata*, $2\theta$ = 33.4, 36.43 in *Murex tribulus* and $2\theta$ = 33.1, 36.04 in *Conus betulinus*. For all refinements, lattice parameters and phase fractions of the samples are in good agreement.

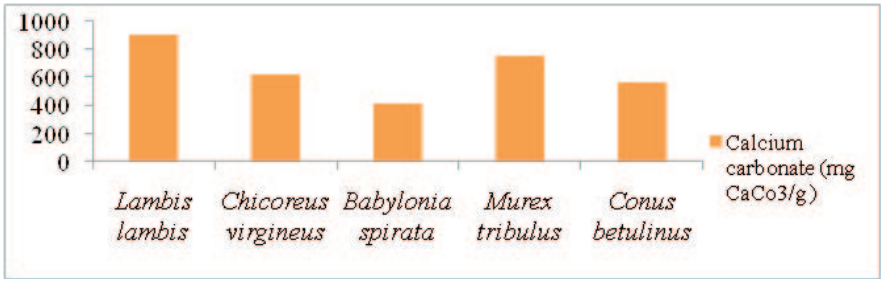


**Figure 1: Calcium Content of Gastropod Shells**

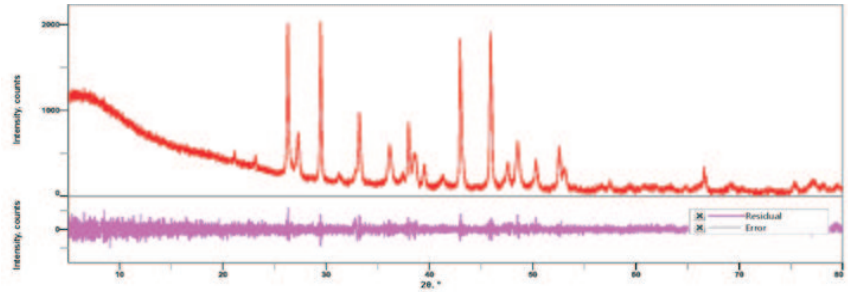


**Figure 2: Diffraction patterns of the shell powder of *Lambis lambis***

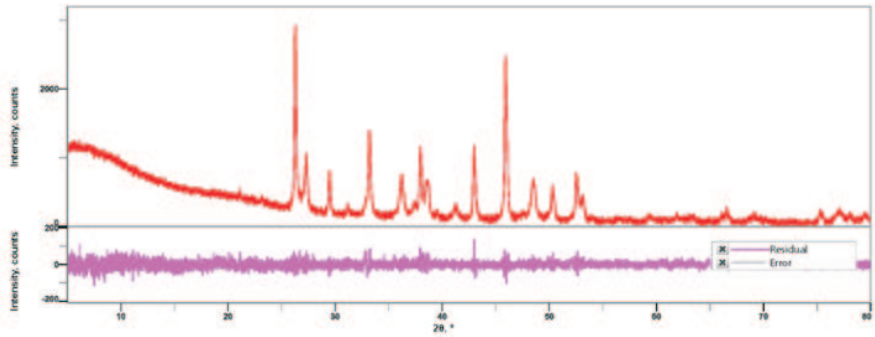


**Figure 3: Diffraction patterns of the shell powder of *Chicoreus virgineus***

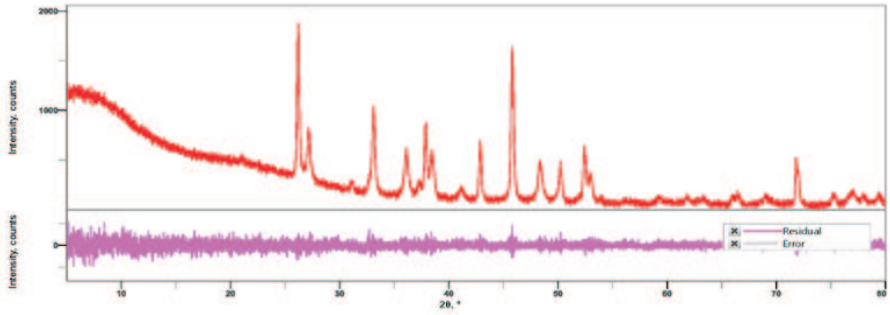


**Figure 4: Diffraction patterns of the shell powder of *Babylonia spirata***

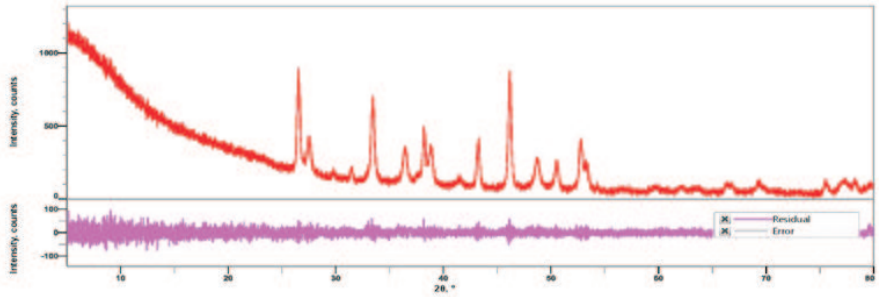


**Figure 5: Diffraction patterns of the shell powder of *Murex tribulus***

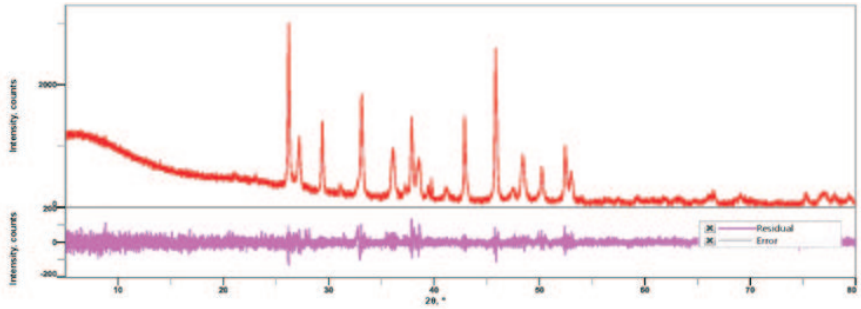


**Figure 6: Diffraction patterns of the shell powder of *Conus betulinus***

## Discussion

Calcium is now used in a wide range of products that touch people's everyday lives. Among many other applications of molluscan shells, preparation of lime is one of the attractive incomes for the local people and is used on a large scale in agriculture and aquaculture management. Calcium, the rich source provided by the molluscan shells, has been used as medicine for the cure of several ailments such as rheumatism, cardiac disease, controlling blood pressure, asthma, rickets, calcium metabolism, nervousness, giddiness, in treating infections like cough, measles and tuberculosis and also providing missing vitamins and minerals[5]. It is used for healing wounds and some believe that it can be used for magico-religious purposes. In Nigeria, the shells burnt into ashes, ground into a fine powder and mixed with other ingredients to form herbal concoction are used[6].

The present result confirms that *Lambis lambis* had the highest calcium content followed by *Murex tribulus, Chicoreus virgineus, Conus betulinus* and *Babylonia spirata* respectively. The present results were in conformity with those of[7,8]. Anima Panda and Mishra (2007) reported that mollusc shells such as *Anadora granosa, Meretrix meretrix, Meretrix casta* and *Pirenella cingulata* were used for lime production. Joseph Uday Ranjan and Ramesh Babu (2015) evaluated the calcium carbonate contents in the shells of Gastropoda and Bivalvia The calcium carbonate

content was found to be 920±42.4 mg$CaCO_3$/gm in *Anadora granosa*, 896±33.4 mg$CaCO_3$/gm in *Crassostrea madrasensis*, 909±45.3 mg $CaCO_3$/gm in *Meretrix meretrix*, 758±36.9 mg $CaCO_3$/gm in *Perna virdis*, 589±26.3 mg $CaCO_3$/gm in *Telescopium telescopium*, 420±26.8 mg $CaCO_3$/gm in *Cerethidea cingulata*, 655± 15.9 mg $CaCO_3$/gm in *Cerethidea obtuse*. The present study agrees well with the above findings.

Polymorphism of $CaCO_3$ and microstructure of *Limnoperna fortune* was reported by[11]. Similar, to the present study intensities corresponding to the aragonite appear, at the intensity of $2\theta = 31$ greater than $2\theta = 33.5$. The appearance of the calcite peak ($2\theta$=29.5) can be seen, albeit with lesser intensity and the presence of the two peaks of the aragonite, which are the same as the internal region of the shell, albeit with lower intensities, which is related to the concentration of this crystalline phase in that region. The phase identification of the molluscan shell by using the X-ray diffraction (XRD) technique was reported by[12].

In the present study, the X-ray diffraction patterns of five gastropods samples exhibits peak characteristics of $2\theta$ = 26.25, 27.25 in *Lambis lambis*, $2\theta$ = 26.26, 27.26 in *Chicoreus virgineus*, $2\theta$ = 26.16, 27.15 in *Babylonia spirata*, $2\theta$ = 26.52, 27.49 in *Murex tribulus* and $2\theta$ = 26.18, 26.66 in *Conus betulinus* the intensities corresponding to the calcite appear and the appearance of aragonite peak at $2\theta$ = 33.19, 36.10 in *ambis*, $2\theta$ = 33.19, 36.16 in *Chicoreus virgineus*, *Chicoreus virgineus*, $2\theta$ = 33.06, 33.46 in *Babylonia spirata*, $2\theta$ = 33.4, 36.43 in *Murex tribulusand* $2\theta$ = 33.1, 36.04 in *Conus betulinus* (Fig. 2-6).

## Conclusion

Shellfish can absorb minerals directly from the aquatic environment through gills and body surface. In the present study, the shell of *Lambis lambis, Chicoreus virgineus, Babylonia spirata, Murex tribulus* and *Conus betulinus* showed a higher level of calcium. The calcium concentration varied in different gastropod shells. These shells can be used for the preparation of calcium for the medicinal purpose. To date, there is still a lack of data on gastropod shells composition that potentially could highlight the superiority of the material. Therefore,this study was carried out to add the currently existing data's on gastropod shell materials.

## Acknowledgement

We sincerely acknowledge the financial assistance funded by DBT, New Delhi for the successful completion of the project work.

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# Isolation and Characterization of Polyhydroxybutyrate (PHB) Producing Bacteria from Contaminated Soil of Thoothukudi Region

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## Abstract

Polyhydroxybutyrates (PHBs) are energy reserves synthesized by different micro-organisms such as Alcaligenes, Pseudomonas, Staphylococcus, Algae, in excess of carbon and limitation of nutrients like nitrogen. These biopolymers are a suitable alternative to synthetic carbon-based polymers. The present study was carried out to isolate the PHB producing bacteria from the contaminated soil. The PHB was found to be 108.54% at the concentration of 2.54 $\mu$g/ml of selected isolates respectively.

***Keywords:*** Biopolymer, Polyhydroxybutyrates, Microorganism, Citrate Utilization Test

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## Introduction

Polyhydroxyalkanoates (PHAs) is a biodegradable polymer possesses a criterion of plastics and produced by microbes to serve as a food stock which could serve as an alternative that will not be affected by the depleting fossil fuels, rise in crude oil prices.To find alternative materials, researchers have developed fully biodegradable plastics, such as polyhydroxybutyrate (PHB). PHBs extracted from bacterial cells show material properties that are similar to polypropylene. More than 80 different forms of PHBs have been detected in bacteria[1].

PHB based plastics made by combining PHB with other biocompatible polymers (like 3-hydroxyvalerate)[2] find many applications in agriculture, packaging and medical field including drug delivery and tissue engineering. Despite these interesting properties, industrial production of PHB is still not well established due to its high

production cost. This has made it unable to compete with conventional plastics in the commercial market. Therefore, much research is needed to discover and identify novel species with vastly superior production capacity and optimization of conditions for the maximal synthesis of PHB. So, the present study focuses on isolation and characterization of PHB producing bacteria from the easy and convenient source i.e. from the contaminated soil sample.

## Materials and Methods

### Collection of soil sample

The Soil sample was collected from a local industrial area of Milavittan region, Thoothukudi (8.8193°N latitude and 78.0909°E longitude). The sample was taken from 0-10 cm depth from the surface area and collected inside sterile sample bottles and transported to the laboratory for analysis.

### Isolation of Bacterial Strains

Serial dilution of the collected soil samples was conducted according to the method of Nehra *et al.* (2017)[3].

### Isolation of Polyhydroxybutyrate (PHBs) Producing Bacteria

For the isolation of PHB producing bacterium contaminated soil was chosen and it was done according to the method of Olutiola *et al.* (2000)[4]. Detection for PHB production was employed by using lipophilic stain Sudan Black B. Stain was prepared by dissolution of 0.3 g powdered stain in 100 ml of 70% ethanol. For microscopic studies, smears of colonies were heat-fixed on clean, grease-free glass slides, followed by staining with 0.3% solution of the Sudan Black B. After leaving the slides undisturbed for 15 minutes, immersion in xylene and counterstaining with safranin was performed. Cells appearing blue-black under a microscope were accredited as PHB positive stains.

### Extraction and Quantitative Analysis of PHB

Bacterial cells containing polymer were collected after centrifugation at 4000 rpm for 10 min. Then pellet was resuspended in an equal volume of 4% sodium hypochlorite and incubated at 37°C for 24 hours. Pellet was washed with acetone, ethanol and water to remove the unwanted materials. The whole mixture was centrifuged again and the supernatant was discarded. Finally, polymer granules were dissolved in hot chloroform. The PHB production was calculated according to the method of Juan *et al.* (1998) and Law and Slepecky (1961)[5,6].

#### Quantifications of Bacterial Growth and Dry Weight

Cell growth was monitored by measuring the optical density (O.D) at 600 nm using a spectrophotometer. Ten-milliliter culture medium was centrifuged at 10,000 rpm, 4°C for 15 min and the cell pellet was washed with 10 mL distilled water. The cell pellet was harvested by centrifugation and dried at 105°C for 48h or till constant weight was obtained.

$$\text{PHB accumulation } (\%) = \frac{\text{Dry weight of extracted PHB } (gl)}{\text{DCW } (gl)} \times 100$$

#### Identification of bacterial isolates

The selected, most efficient PHB producing bacterial isolates were further identified by morpho-physiological and biochemical characterization (Hydrogen Sulphide Test, Citrate Utilization Test, Indole Production Test, Methyl Red and VogesProskauer Test) using Bergey's Manual of Determinative Bacteriology[7].

## Results

#### Isolation and screening of PHB producing bacteria

Totally, 15 distinct colonies, were chosen based on their shapes and colours. After 24-48 hours culture period, Sudan Black B staining was done to confirm the presence of PHB granules. Among 15 isolates, 5 were found to be Sudan positive, i.e. they were capable of producing lipid granules which could have the presence of PHB (Table 1).

The PHB was found to be 108.54% at the concentration of 2.54 g/ml of selected isolates respectively (Figure 1). PHB was produced from five bacterial isolates that were isolated from contaminated soil. By performing various biochemical and morphological tests and comparing their results with Bergey's Manual of systematic bacteriology, the isolates were identified as *Bacillus, Klebsiella, Pseudomonas, Staphylococcus* and *Enterobacter*.

#### Morphological and biochemical characterization

The growth characteristics such as size, shape, colour, margin, consistency and Gram-staining of the bacterial colonies were observed to characterize the bacterial colonies. Various biochemical tests namely indole production test, methyl red and Voges-Proskauer, citrate utilization test, $H_2S$ production were observed for their biochemical characterization were done. The biochemical characterization of the isolates was also done using the standard microbiological and biochemical procedure. The biochemical characteristics of selected isolates were given in table 2 and figure 2-6 respectively.

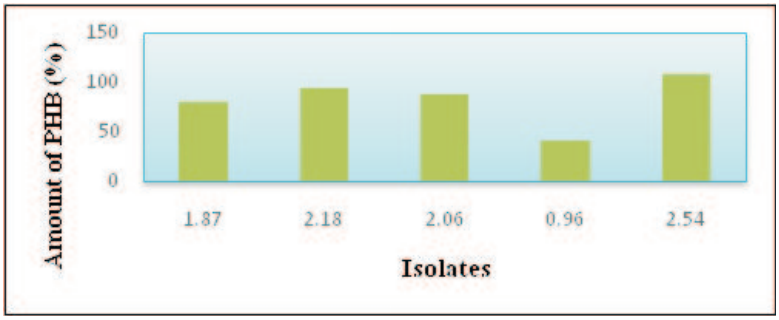


**Figure 1: Extracted PHB from selected isolates**

**Table 1: Morphological characters of selected isolate**

| S.No. | Character | Isolate 1 | Isolate 2 | Isolate 3 | Isolate 4 | Isolate 5 |
|---|---|---|---|---|---|---|
| 1. | Size | 2.5mm | 1.5mm | 2mm | 3mm | 4mm |
| 2. | Shape | Circular | Circular | Round | Helical | Round |
| 3. | Margin | Entire | Entire | Lobate | Entire | Serrate |
| 4. | Consistency | Smooth | Smooth | Irregular | Smooth | Smooth |
| 5. | Colour | White | White | Yellow | White | White |
| 6. | Gram Staining | Positive | Negative | Positive | Negative | Positive |

**Table 2: Biochemical characteristics of selected isolates**

| S.No. | Biochemical Test | Isolate 1 | Isolate 2 | Isolate 3 | Isolate 4 | Isolate 5 |
|---|---|---|---|---|---|---|
| 1. | $H_2S$ Production Test | Negative | Negative | Negative | Negative | Negative |
| 2. | Citrate Utilization Test | Negative | Negative | Positive | Negative | Positive |
| 3. | Indole Production Test | Negative | Positive | Negative | Negative | Negative |
| 4. | Methyl Red Test | Positive | Positive | Positive | Positive | Positive |
| 5. | Voges -Proskauer Test | Negative | Negative | Negative | Negative | Negative |

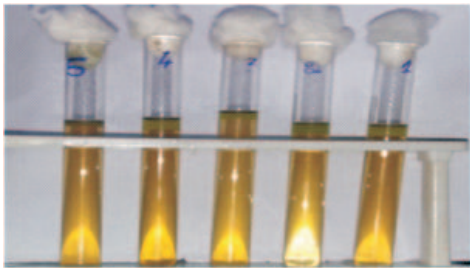

**Figure 2: $H_2S$ Production Test**

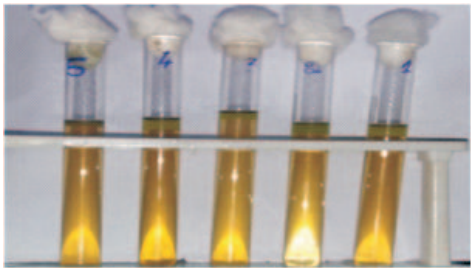

**Figure 3: Citrate Utilization Test**

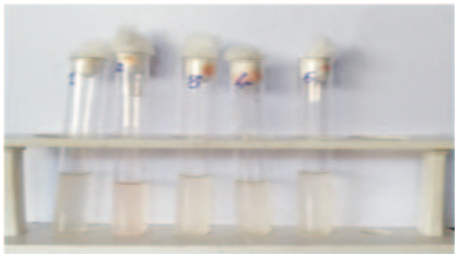

**Figure 4: Indole Production Test**

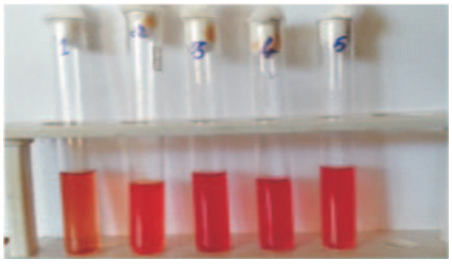

**Figure 5: Methyl Red Test**

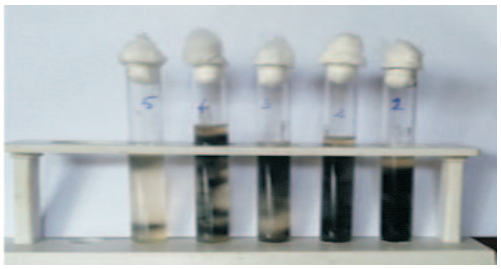

**Figure 6: Voges - Proskauer Test**

## Discussion

There are many types of biodegradable plastics with different degrees of biodegradability are available. Among them, polyhydroxybutyrate (PHBs) are the only 100% biodegradable ones. PHBs are macromolecules synthesized by bacteria and are inclusion bodies accumulated as reserve material when the bacteria grow under different stress conditions[8]. Potential PHB accumulating bacteria were isolated from diverse sources and potential strains were selected for further studies *Bacillus* sp are reported to be ideal PHB producers in many previous studies[9]. The comparable result was reported by[10] from *R. sphaeroides* $N_2O$ and [11] from *Alcaligenes latus* using glucose as carbon source. Glucose is an easily assimilable carbon source that encourages bacteria to produce more PHB[12,13 & 14].

Paramjeet *et al.* (2012) obtained 60% PHB from sugarcane bagasse by *Pseudomonas aeruginosa*. On the other hand, straw and banana peel resulted in less biomass and PHB production. Noha *et al.* (2013) also reported maximum cell density and PHB accumulation at 37°C after 48 h. The alteration in the PHB content by temperature variance can be because of extreme temperatures slow down the metabolic activity (enzyme activity) of microorganisms that ultimately reduces their ability to produce PHB. This result is in line with the reports by [17]in which optimum microbial growth and PHB production occurred in the pH range of 6.0-7.5.

The least cell dry weight and PHB content were achieved with ammonium sulphate. It has been reported by [15]that, the nitrogen concentration in bacteriological media highly influences the production of intracellular PHB.

Desouky *et al.* (2014) reported PHA extraction and optimization from *Bacillus thuringiensis*. It was clear that, after optimization of growth factors, the productivity of PHA was enhanced from 2.5 g/L to 4.1 g/L. In general, PHA polymer is synthesized by the bacterial cells under limiting growth conditions, when the carbon source is in excess and nitrogen, phosphorus, magnesium, sulfur or oxygen is present in a limiting concentration[19]. Fatimah Alshehrei (2019) isolated polyhydroxy butyrate (PHB) producing bacteria from soil of Saudi Arabia. Watsana Penkhrue *et al.* (2020) reported response surface method for polyhydroxybutyrate (PHB) bioplastic accumulation in *Bacillus drentensis* BP17 using pineapple peel. In the present study, the PHB was found to be 108.54% at the concentration of 2.54 $\mu$g/ml respectively.

## Conclusion

From the present study, it was concluded that the five bacterial isolates were used to obtain biopolymers. This PHBs serve as a potential candidate for some application in packaging and biomedical material production due to their purity, non-toxic behaviour, biocompatibility, degradability and obtain ability in the form of drug carriers. It was concluded that five isolates can be considered as good candidates for industrial production of PHB from the soil.

## Acknowledgement

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# GALLERY

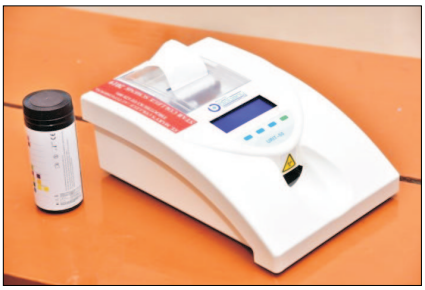
## EQUIPMENTS PURCHASED UNDER STAR COLLEGE SCHEME

## (2019-2020)

## DEPARTMENT OF ZOOLOGY

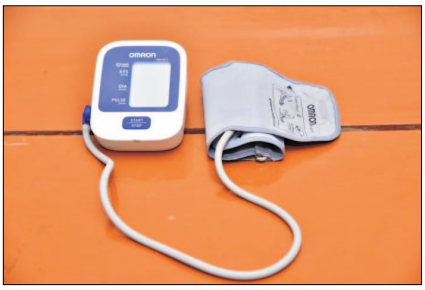
**BOD Analyzer**

**Colony Counter**



**Urine Analyzer**

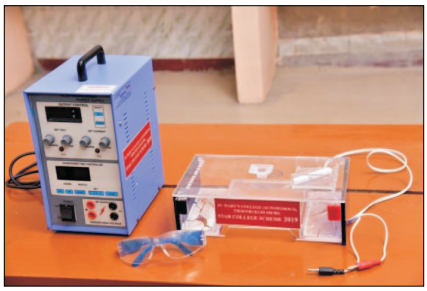
**Digital Glucometer**




**Digital Sphygmomanometer**

**Digital Thermometer**


**Egg Incubator**



**Gel Electrophoresis Unit with Power Pack**

**Hemocytometer**

**Microscopic HD Camera**

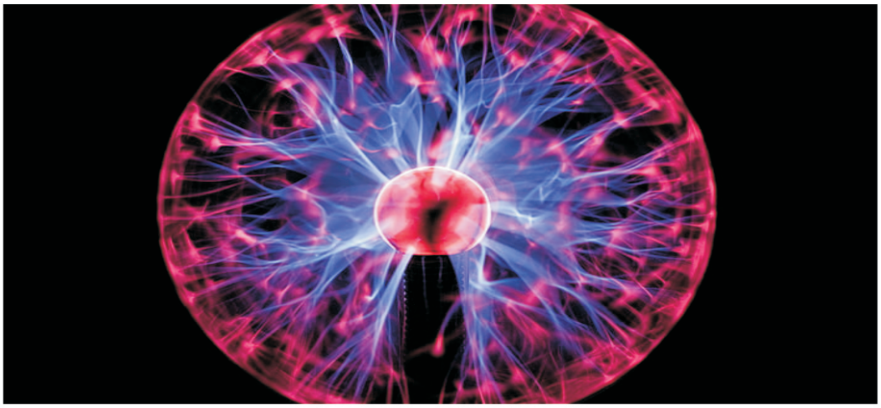
**Plankton Net (Regular model)**

**Ocular and Stage Micrometer**

**Refractometer**

**Student Microscope**

**Computer System**



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