PROCEEDINGS

SECOND INTERNATIONAL CONFERENCE ON
PLANT FUNCTIONAL BIOLOGY

(ICPFB-22)

Editors:

Dr. Shackira A.M., Dr. Abdussalam A.K. & Dr. K.N. Ajoy Kumar

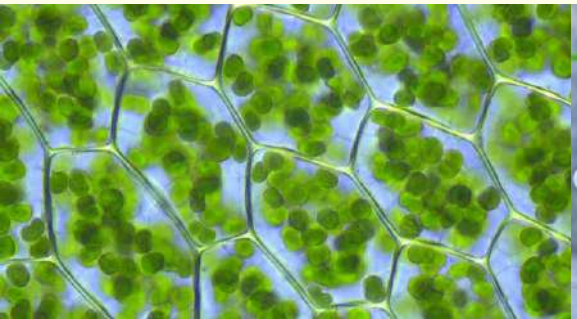
Jointly Organized by

Department of Botany, Sir Syed College and Kannur University

In association with **Kerala State Higher Education Council (KSHEC), IQAC Kannur University and Sir Syed College**

25 & 26 October, 2022

Cherussery Auditorium, Kannur University



PLANT FUNCTIONAL BIOLOGY

EDITORIAL BOARD

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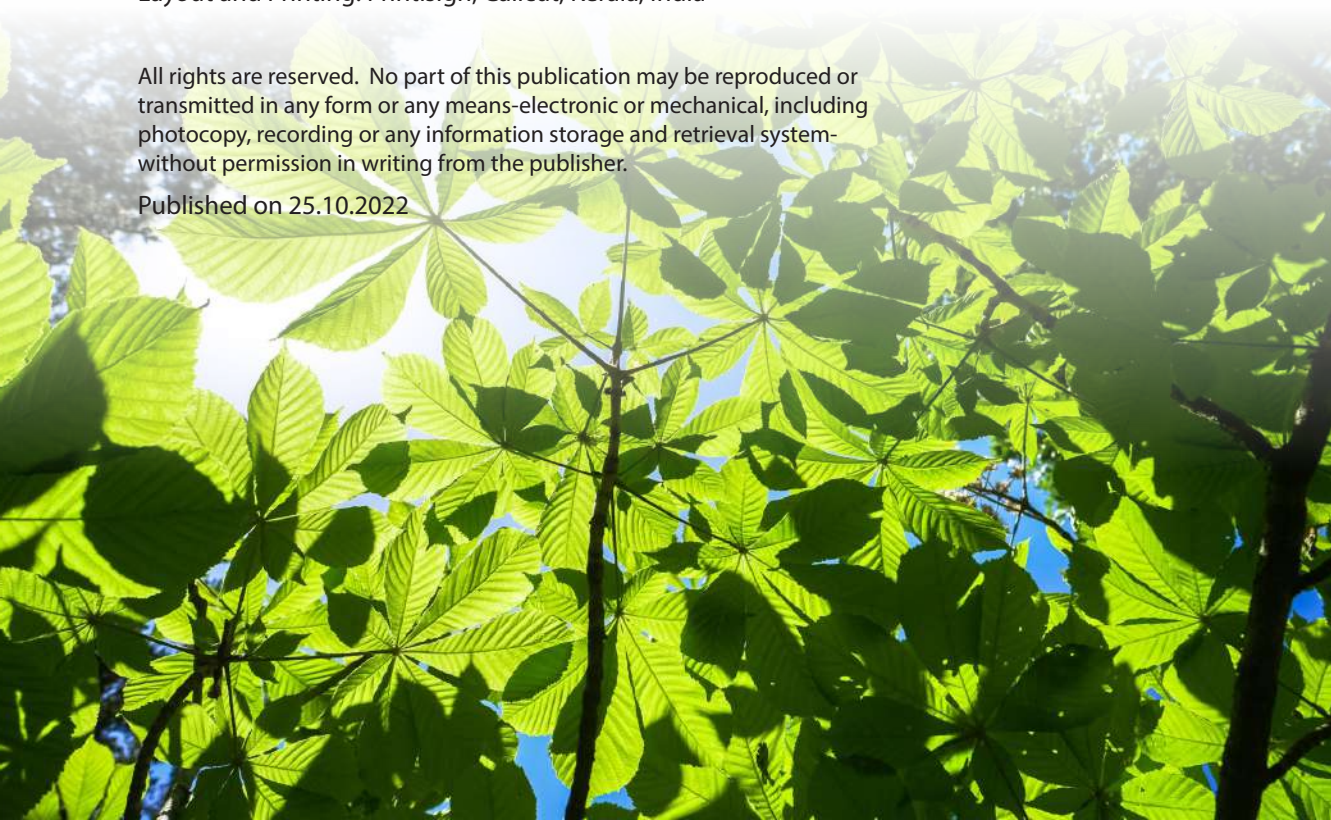

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SIR SYED COLLEGE PUBLICATION DIVISION
SIR SYED COLLEGE
(Affiliated to Kannur University | Re-accredited by NAAC with A Grade)
Taliparamba, Kannur, Kerala, India-670142

About the Conference

The Second International Conference on 'Plant Functional Biology' (ICPFB-22) encourages discourses on emerging concepts and findings in plant biology and studies on the functional areas encompassing work from the molecular field. 'Plant Functional Biology' as a discipline faces various issues such as lack of adequate exposure to the direct experience in research, effective sharing of knowledge, unawareness of practices and technologies in support of the implementation of the research programmes carried out in the field. This programme will be a means to connect the debates in 'Functional Biology' world over to the Indian scenario and to bring together a significant number of academics in India. The conference also discusses new and significant informations about plant functions and their regulation, especially in relation to changing environments since plants are surprisingly able to adapt with extreme environmental conditions such as high and low temperatures, drought, flooding, salinity, pathogens, and other major abiotic and biotic stress factors. Moreover, the new and significant information regarding the functional biology of plants at all scales from the molecular through whole plant to community will also be detailed and conversed.

IDis-22 (Intellectual Discourses)

IDis offers a platform for exchanging ideas, dismantling preconceived notions and constructing new pathways in the intellectual terrains. IDis offers you an opportunity to interact with the best talent in the educational arena of the nation. Over a period of one year, a series of seminars will be organized in Sir Syed College campus. Scholars and experts from different parts India and abroad will be invited. They will exchange their vision and innovation in their respective fields of knowledge to their peers in our campus. Teachers and academicians have a chance to present their own ideas and academic initiatives. The IDis platform will help our teachers to update their academic skills and also give them an opportunity to publish their papers in academic and research journals.

. . . . MESSAGE . . . .

പ്രൊഫ. ഗോപിനാഥ് രവീന്ദ്രൻ
വൈസ് ചാൻസലർ
Prof. Gopinath Ravindran
Vice-Chancellor

കണ്ണൂർ സർവകലാശാല
KANNUR UNIVERSITY
(Established under Act 22 (1996) of Kerala Legislative Assembly)

Vice Chancellor's Message

It is our privilege to host the two-day international conference on plant functional biology jointly organised by the Dept. of Botany Kannur University and Sir Syed College.

It is hoped that this international conference on Plant Functional Biology will discuss emerging concepts and findings in the functional areas of plant science. As a discipline, Plant Functional Biology deals with many of the central areas of basic research in plant science including Physiological, Biochemical and Molecular mechanisms of plants. As in some other research areas, plant science also faces a lack of adequate exposure to new research. This international conference will help us in the effective sharing of knowledge, especially with modern technologies which are inevitable for our research programmes.

It is hoped that the knowledge sharing at this conference would increase the exposure of the students, teachers and researchers of new trends in plant biology. I assure that this conference will be very productive and informative.

22.10.2022

Vice-Chancellor

. . . . MESSAGE . . . .

Adv. P. Mahamood
Manager, Sir Syed College

I would like to congratulate the entire faculty of the Department of Botany for conducting two day international conference on "Plant Functional Biology" in association with Department of Botany, Kannur University under the auspicious of IDis-22. It makes me proud as Manager of Sir Syed College to say that this program is being run by the Kerala State Higher Education Council under the banner of KSHEC-ERUDITE scheme. It is a matter of great pleasure and pride that this program is jointly organized by Sir Syed College and Kannur University at the University heard quarters. Indeed, this is an excellent example of an integrated academic gathering. We can use this occasion as an opportunity to acquire some important knowledge and research skills to accelerate career growth as far as research is concerned. Information is the most valuable asset of the time. Knowledge is generated only when online learners comprehend the subject matter and are able to put the components together. Dear friends, every seminar, interactions and discussions provided valuable opportunities for students, researchers and teachers.

As a manager of sir Syed College, I am grateful and very much happy for this novel endeavor, undertaken by the Department of Botany and college IQAC. The programme is more relevant in the post covid period. I extend my warm welcome to all the representatives of teachers and scholars from various institutions and from various states. I wish you everything good for this conference. I hereby express my warm regards and thanks to Prof. Om Parkash Dhankher, College of Natural Sciences, Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA for delivering the Erudite Lecture under the title 'Feeding and Fueling the Future: Climate Resilient Crops for Enhanced Production of Food and Fuels' in this two day International Conference.

Thank you once again.

. . . . MESSAGE . . . .

Dr. Ismail Olayikkara
Principal, Sir Syed College, Taliparamba

Respected Professors, Research Scholars, Colleagues and Friends,

For a long time, the seminars have become webinar due to Covid-19, pandemic around the globe, but today is the most happiest day since the conference is being held here as offline, really this is the most preference days for the academic society. It is for the first time that one of the Department in Sir Syed College is jointly organizing an International Conference at the academic centre of the University in collaboration with Department of Botany, Kannur University. A new era has come and research is becoming more and more important for integration. This is the proudest moment for me as the Principal of Sir Syed college. Higher Education is, of course, a knowledge-producing centre, and it is only becoming reality when each educational institution changes from acquisition of knowledge to production of knowledge.

I would like to congratulate my team Botany for organizing such a relevant programme on this days. The two day International Conference on "Plant Functional Biology" under the auspicious of KSHEC-ERUDITE Scheme, IDis-22 and IQACs. I would like to place on record our sincere gratitude to Kerala State Higher Education Council for their support and encouragement. We are fortunate to have, among all other dignitaries; Prof. Om Parkash Dhankher, College of Natural Sciences, Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA, Dr. Babu Valliyodan, Assistant Professor of Molecular Biology and Genomics, Department of Agriculture and Environmental Science, Lincoln University, USA, Dr. Sujith Puthiyaveetil, Associate Professor, Department of Biochemistry and Purdue Center for Plant Biology, Purdue University, USA and Prof. Manish Kumar P.R., Former Head & Coordinator, Dept. of Biotechnology, University of Calicut, Kerala. The conference is enriched by the presence of 4 eminent resource persons and around 150 participants from different institutions, many of them will have a chance for paper presentation. Probably, a new experience for many of us. Hope you will enjoy and benefit it.

Thank you all. Good day.

. . . . EDITORIAL . . . .

Second International Conference on Plant Functional Biology

Prof. K.N. Ajoy Kumar
Course Coordinator, Kannur University

Plant functional biology is one of the most vital areas of fundamental research which envisions a broad area of the functions of plants ranging from molecular through the whole plant. The international conference on plant functional Biology is historic as it covers many of the central areas of basic research in plant sciences. The conference will address Plant Genomics, physiological and molecular mechanisms of plant development and the metabolic engineering of plants. The conference is more inspiring as it is a collaborative, effort by two Institutions; the Botany Departments of Sir Syed college and Kannur University Campus. This would encourage coordination between the researchers, institutions, organizations of plant sciences and communities. In one way it would help to popularise plant science research. On the other hand, It gives a great opportunity to plant science researchers and scholars to interact with internationally renowned academics. This effort will lead us to do collective research in future. The association is important when Universities and colleges face serious challenges in research.

The inattention to basic research is a foremost challenge now. It is a fact that most research backing, including funding, channelised through universities and colleges primarily aimed at applied research; basic science research is being neglected. Many of the basic research areas of Plant physiology and biochemistry are overlooked. It is a fact that Basic science expands the knowledge base needed for breakthrough scientific signs of progress. This conference and further research challenges will help us in focussing basic and applied research in plant functional biology and moving towards a new area of scientific endeavour. This conference proceedings deal with multitudes of areas in functional biology- no article in this can subtract from plant functions. The articles in this proceeding here are of wide-ranging areas of research. One may find wide differences between articles accessible here. That itself shows the scope of heterogeneous research areas in plant functional biology.

Our task at Plant functional biology is to help scientists expose their discoveries. We expect it's good that scientific publishing is not monopolized by only some publishers so that there are options for authors to share their findings. The conference will help us in sharing the research papers as well as to seek forward a new method of publication when the publishing sector is highly monopolised. Plant functional Biology needs support from all plant biologists. Working together as authors and reviewers we can make Plant functional biology one of the best platforms for scientists to share their work and advance their careers. The Editorial Board looks forward to more publications from the contributors of this proceedings. The articles presented here are much worthy and it can be reemphasized that the effectiveness of science can be measured in terms of the articles published. Of course, Plant functional Biology is a highly disciplined and easily quantifiable discipline.

Applied science makes reforms, Basic science makes revolution'-JBS Haldane

. . . . FOREWORD . . . .

Dr. Tajo Abraham
Head, Department of Botany
Sir Syed College

Plants and the biological systems that surround them are critical to the planet's and its inhabitants' long-term health. Humanity poses significant issues in the areas of food, health, energy, and the environment, which are aggravated by climate change. Plant systems (the bacteria, microbes, fungi, insects, and other organisms that live on, in, or around plants) are critical to addressing these issues because they are the foundation of climate change sentinels, healthy ecosystems and environments.

This conference is intended to help inspire biologists, biotechnologists, plant science experts, botanists, researchers, scientists, academicians, young graduates and research fellows and research communities. This conference will help us to define our abilities to do critical and far-reaching plant systems science research, which is critical in today's world. Through this summit we strive to lay out a broad strategy for solving some of the world's most serious issues through research, practical applications, and education whilst emphasizing on the potential of plants for a sustainable, green and healthy future.

2nd International Conference of Plant Functional Biology will focus on the plant physiology and related areas to develop technologies and to discuss new strategies to increase plant and planet life. The program of ICPFB will cover all the essential topics extending from plant physiology to plant molecular biology, genomics and plant enzymes to plant metabolic engineering. The meeting will offer an exclusive platform to talk over the research developments, technological advancements, and major challenges in plant science & research.

The proceeding of International conference on 'Plant Functional Biology' organized by Department of Post Graduate Studies and Research in Botany, Sir Syed College, Taliparamba includes various lectures by eminent researchers from the field of plant functional physiology and allied areas. I acknowledge well the financial support from Department of Higher Education, Govt. of Kerala and I vehemently appreciate the members of faculty of the Department of Botany, Kannur University in organizing the seminar.

We hope that this program will further stimulate research in area of plant functional biology and we feel honored and privileged to serve the recent developments in the field plant science.

. . . . PREFACE . . . .

Dr. Shackira A.M.
Convener, Second International Conference on Plant Functional Biology

The two day International Conference on 'Plant Functional Biology' (ICPFB-22) is jointly organized by the Department of Botany, Kannur University and Sir Syed College, Taliparamba during 25th and 26th October 2022 at Kannur University. The prime focus of this International conference is to ensure the sharing and collaborative aptitude of researchers and students of various disciplines which may bring significant input in the field of Plant Physiology. This platform will help to bridge and enlighten the minds of young and vibrant researchers with eminent and experienced scientists, and this could help to discuss and find solutions to the intensity of global climatic change and the applied aspects of engineering climate resilient crops. We are fortunate to have the following eminent scientists as invited speakers for enriching the academic sessions in the conference. Prof. Om Parkash Dhankher, College of Natural Sciences, Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA, Dr. Babu Valliyodan, Assistant Professor of Molecular Biology and Genomics, Department of Agriculture and Environmental Science, Lincoln University, USA, Dr. Sujith Puthiyaveetil, Associate Professor, Department of Biochemistry and Purdue Center for Plant Biology, Purdue University, USA and Prof. Manish Kumar P.R., Former Head & Coordinator, Dept. of Biotechnology, University of Calicut, Kerala. This conference proceeding contains contributions from 4 invited speakers and 18 researchers under various thematic sessions like Strategies for abiotic stress management, Molecular stress physiology, Climate Change and crop productivity, Anthropogenic induced stress, Ecophysiology of Plants, Biotechnology and crop improvement.

The timely support and encouragement rendered by the Hon. Vice Chancellor of Kannur University, Prof. Gopinath Ravindran is greatly acknowledged. I also express my deep sense of gratitude to the Principal of Sir Syed College, Dr. Ismail Olayikkara and Manager, Adv. P Mahamood for their sincere effort and guidance to make this conference a grant success. I record my profound gratitude to all my colleagues (Dr. Tajo Abraham, HOD, Dr. Sreeja P., Dr. Abdussalam AK., Dr. Gayathri R. Nambiar, Mr. Jazeel K. and Dr. Jasna T.K.) and faculty members of Botany Department of Kannur University (Dr. K.N. Ajoykumar, Coordinator., Dr. Ratheesh Chandra, Dr. Sangeeth T., Ms. Arya Sasidharan and Ms. Anju Ramachandran). I am grateful to the scholars, students and other supporting staff of the department for the timely help rendered for the execution of the event. We are proud to acknowledge the financial assistance from Kerala State Higher Education Council under ERUDITE Scheme and IQAC of Kannur University. I acknowledge IDis, funded by PTA and Golden Jubilee programme for the financial assistance. I wish you all a rewarding academic and research experience and memorable moments in the two day International Conference on 'Plant Functional Biology' (ICPFB-22).

Contents

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KANNUR UNIVERSITY

Kannur University was established by the Act 22 of 1996 of Kerala Legislative Assembly. The University by the name "Malabar University" had come into existence earlier by the promulgation of an Ordinance by the Governor of Kerala, on 9th November 1995. The University was inaugurated on 2nd March 1996 by the then Chief Minister of Kerala, Sri. A.K Antony. The objective of the Kannur University Act 1996 is to establish in the Kerala state, a teaching, residential and affiliating University, promoting the development of higher education in Kasargod and Kannur revenue Districts and the Mananthavady Taluk of Wayanad District. Kannur University is unique in the sense that it is a multi- campus university with campuses spread over at various locations under its jurisdiction. The Act envisages that the University shall establish, maintain, manage and develop campuses at Kannur, Kasaragod, Nileswaram, Mangattuparamba, Mananthavady, Payyannur, Thalassery and such other places as are necessary for providing study and research facilities to promote advanced knowledge in Science and Technology and other relevant disciplines.

BOTANY DEPARTMENT - KANNUR UNIVERSITY

This is a newly - started department. It has been a long demand to start a course on plant science at Kannur University. MSc Plant Science with a specialisation in Ethnobotany programme was started in 2021. The course was started as a part of the new-generation courses sanctioned by the Kerala Government. The first batch was started in March 2021. The curriculum for the MSc. plant science Programme with a specialisation in Ethno Botany has been designed to encourage broad instructional goals. At present, there are five faculties in the department. Since this is a newly-started Department, presently all the attention, time and effort are devoted to building up basic infrastructure and facilities, including the laboratory and library.

Course Design

The design of the course,framing of the syllabus etc. have been done with the help of experts in the field. The objective is to impart skills in frontier areas of plant science with a special emphasis on Ethno botanical studies. The design of the course is different from the conventional postgraduate Biology courses.The course is designed to prepare, the learner for the History and methodology of Biological Sciences to understand the development and evolution of knowledge systems in the field. Philosophical problems generated by the study of complexity are a thrust area of the MSc Plant science programme. The programme acclaims that traditional or indigenous knowledge is to be used not only to find new drugs but also to derive new concepts about conservation.

Programme Objectives

The M.Sc plant Science programme is designed to equip students with essential knowledge and technical skills to study plants holistically. Students would be trained in all areas of plant biology using a unique combination of core and elective papers with significant interdisciplinary components. Students would be exposed to cutting-edge technologies that are currently used in the study of plant life forms, their evolution and interactions with other organisms and with the ecosystem. Students would also become aware of the social and environmental significance of plants and their relevance to the national economy. In addition to academic rigour and training in subject-specific areas students will also become well-trained in ethical issues, critical thinking, reasoning and analytical skills, , laboratory safety, biodiversity, sensitivity to the environment and sustainable living. Then the student is expected to acquire a scientific temper and attitude which is a prerequisite for a building up of a democratic society.

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THE KANNUR UNIVERSITY MANANTHAVADY CAMPUS

Kannur University has planned for a multi-campus system which is an central step towards the decentralisation of Educational campuses. This will help in the dissemination of knowledge to a wider geographical area. Wynad is the prominent part of the Western Ghats with its immensely rich Biodiversity. Mannathavady is famous for its wildlife and its spice plantations. Wayanad is part of a forest reserve, located on the border of Tamil Nadu and Kerala. Because of its wild green coverage, it offers great scenic beauty with very diverse flora along with the experience of seeing wildlife. The Plant Science program proposes to utilise the rich biodiversity of the Nilgiri biosphere (World heritage site of UNESCO), which is identified as a hotspot of biodiversity in the Western Ghats. This area is an abode of endemic and endangered species, that also include medicinal plants commonly used by tribal communities, which remain largely unexplored. a very rich diversity of flora, and genetic diversity with a very high level of endemism and several ethnic groups with diverse cultural background.

SIR SYED COLLEGE

Sir Syed College, was established in 1967 by a handful of educational visionaries of Kannur under CDMEA (Cannanore District Muslim Educational Association) with a vision to impart moral and liberal education to the public, especially socio politically backward minority communities of North Malabar region. The college, named after Sir Syed Ahmed Khan, the founder of Aligarh Muslim University, was started in 1967 as a junior college under the University of Kerala. It came under the University of Calicut in 1968 and under the Kannur University in 1996. The institution was re-accredited by NAAC with A grade in 2017. Today Sir Syed College is one of the biggest post graduate institutions under Kannur University. The college at present offers Under Graduate programmes in Botany, Chemistry, Forestry, Physics, Mathematics, Statistics, Zoology, Arabic, Economics, Functional English, History, Malayalam, Commerce and Mutimedia and Communication; Post Graduate Programmes in Arabic, Botany, Chemistry, Physics and Commerce. Apart from that the departments of Botany and Chemistry are research centres as well.

The college has a strength of 2161 students, 71 Faculty Members and 36 non teaching staff. 15 of the Faculties are Ph.D holders and 24 have M.Phil degree. There are 13 research guides attached to the research departments of Chemistry and Botany and 34 Research Scholars and one post doctoral fellow are working under them. Thirty scholars have been awarded Ph.D from these research centres so far (19 Botany, 7 Chemistry and 4 Hindi). Many Faculties are engaged in minor and major research projects funded by various agencies. The members of the faculty have contributed extensively to literatures in their respective fields. 9 books have been

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published by the faculties out of which one has attained national award. About 400 research papers of faculties and students have been published in various international and national journals. The Publication division of the college publishes the research journal, SEARCH. The college boasts of an extensive library with 42000 books, 86 journals and INFLIBNET facility. The other facilities offered by the college include Sir Syed IT Centre, UGC aided Coaching Centre, Career Guidance and Counselling Cell, Photostat Centre, Gymnasium, Health Club, Sports Pavilion, Ladies Retreat room etc. There are separate hostels for men and women. An indoor stadium funded by UGC with four court. An ambitious project of the institution, the PG and Research block as GOLDEN JUBILEE BUILDING with four floors, is under construction.

The students are provided ample opportunities for co-curricular and extra curricular activities through National Cadet Corps, National Service Scheme and different clubs like Language Club, Farm Club, Forestry Club, Hindi Club. ED Club, Film Club, Music Club, Tourism Club, Literary Club, Media Club Cultural Forum, Arts Forum, Science Forum etc. The Internal Quality Assurance Cell, Tutorial Scheme, Grievance Redressal Cell, Parent Teachers Association, College Co-Operative Society etc. ensure that the students get an ideal environment congenial to bring out the best in them. The college office ensures that maximum number of students get scholarships and endowments. Presently more than half the strength of students are enjoying the benefits of different scholarships and endowments.

Hortus Sir Syedicus, the district medicinal plants demonstration garden developed by the Garden Club and NSS with the financial aid of State Medicinal Plants Board is one of a kind under the Kannur University. Sir Syed College is a front runner in academics with the maximum number of students becoming toppers year after year. The college has also left its mark on account of its envious success, excellent performance and participation in the arts festivals and many a time students from this institution have represented the University and state at sports meets across the country.

The institution is extremely proud that in its 55 years of glorious existence it has stood true to its mission of changing the social fabric of North Malabar region. The role played by Sir Syed College is undeniable and pivotal in the cultural, political, economic and social mapping and development of the region.

DEPARTMENT OF BOTANY

The Department of Botany at Sir Syed College is one of the leading departments sponsored by Department of Science and Technology(DST) FIST Programme, dedicated to teaching and research with specialization in Angiosperm Taxonomy, Plant Physiology and Biochemistry, Plant Tissue culture and Molecular Biology, Fungal Taxonomy, Environmental Science, Ecophysiology, Microbiology, etc. The Department offers post graduate and undergraduate programmes in Botany. Two elective papers such as Techniques and Instrumentation and Crop Improvement are studying under M.Sc. programme. The department is well equipped with sophisticated research equipments funded by DST and UGC. The major instruments are GC-MS, UV-Visible Spectrophotometer, Oxygen Electrode etc. In addition to the Central library books (2543) we keep a Library with collections of 372 reference books and periodicals. The department also houses a herbarium with about 4000 specimens. The botanical Garden - Hortus Sirsyedicus is well equipped with large collection of medicinal plants. Green Hose with Orchidarium, Aroid home, Herbal Garden, Nakshathrvanam, Bio Path, Aranyakam etc. are the major attractions of the garden. Aroid Home is established with conservation of Indian Aroids. Department has published more than four hundred research articles in different journals having good impact factor. Department has already completed different project work funded by DST, UGC, KSCSETE, BRNS, MoEF in different topic. The northern Kerala is blessed with mangrove forest, most of the major project was focused on the same.

Faculty (Past)

1. Mrs. Vani Devi C.K., MSc., MPhil. (1967-2000)
2. Mrs. Thankam P.V., MSc. (1969-2002)
3. Mrs. Fathima KS., MSc. (1976-2008)
4. Mr. Abdul Kareem N.P., M.Sc., M.Phil. (1976-2004)
5. Dr. Raveendran K., M.Sc., Ph.D. (1976-2007)
6. Dr. Beebi Razeena P.M., M.Sc., M.Phil., Ph.D (1982-2009)
7. Dr. Fathima PA, M.Sc., M.Phil., Ph.D (Relieved on 30-06-2008)
8. Dr. K.M. Khaleel, M.Sc., Ph.D. (1982-2015)
9. Mrs. Valsamma Thomas, M.Sc., B.Ed. (1985-2015)
10. Mrs. Abidal Beevi, M.Sc. M. Phil. (1985-2016)
11. Dr. Abdul Jaleel V, M.Sc., M. Phil., M.Ed., Ph.D (2011-2019)

Faculty (Present)

1. Dr. Tajo Abraham, MSc, M.Phil., Ph.D., Assistant Professor and Head
2. Dr. Sreeja P., M.Sc., B.Ed., Ph.D., Assistant Professor
3. Dr. Abdussalam A.K., M.Sc., M.B.A., M.Phil., Ph.D., Assistant Professor

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4. Dr. Gayatri R. Nambiar, M.Sc., Ph.D., Assistant Professor
5. Mr. Jazeel, K., M.Sc., B.Ed., Professor
6. Dr. Shackira A.M, M.Sc., M.Phil., Ph.D, Assistant Professor
7. Dr. Jasna T.K. (M.Sc., B.Ed., Ph.D., Assistant Professor

Laboratory

Angiosperm Taxonomy

Plant Physiology And Biochemistry

Molecular Biology And Tissue Culture

Mycology And Microbiology

Environmental Science

Instrumentation Room

Equipments

Gas Chromatography Mass Spectrometry (GC-MS)

UV-Visible Spectrophotometer

Leica M 80 Stereo Microscope

PCR-Thermal Cycler

Electrophoresis Unit

Magcam Camera System

Cooling Centrifuge

Milli Pore And Milli-Q Water Purification System

Oxygraph- Oxygen Monitoring System

GC-MS

UV-Visible Spectrophotometer

Oxygraph

INAUGURAL SESSION

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Cherussery Auditorium, Kannur University

INAUGURATION

Prof. Gopinath Ravindran
Hon. Vice Chancellor,
Kannur University

ERUDITE LECTURE

Prof. Om Parkash Dhankher
College of Natural Sciences
Stockbridge School of Agriculture
University of Massachusetts, Amherst, USA.

INVITED LECTURES

Dr. Babu Valliyodan
Assistant Professor of Molecular Biology and Genomics
Department of Agriculture and Environmental Science
Lincoln University, USA.

Dr. Sujith Puthiyaveetil
Associate Professor
Dept. of Biochemistry & Purdue Centre for Plant Biology
Purdue University, USA

Prof. (Dr) Manish Kumar P.R.
Former Head & Coordinator
Dept. of Biotechnology
University of Calicut
Malappuram, Kerala 673 635

SIR SYED COLLEGE
Taliparamba, Kannur, Kerala, India

DEPARTMENT OF BOTANY
Mananthavadi Campus,
Kannur University, Kannur, Kerala, India

ALL ARE INVITED

PROGRAMME

Day 1 - Inaugural Session

Registration : **8.30-9.30 am**

Inauguration : **9.30-10.45 am**

Welcome speech : **Dr. K.N. Ajoykumar**, Course Coordinator, Department of Botany, Mananthavady Campus, Kannur University

Presidential Address : **Dr. Ismail Olayikkara**, Principal, Sir Syed College

Inauguration : **Prof. Gopinath Ravindran**, Hon. Vice Chancellor, Kannur University

'Releasing of Conference Proceedings

Distribution of Prof. Govindjee Endowment Award-2022'

Felicitation : **Adv. P Mahamood**, Manager, Sir Syed College

: **Dr. Ashraf T.P.**, Syndicate Member, Kannur University

: **Dr. Nafeesa Baby T.P.**, DSS, Kannur University

Vote of Thanks : **Dr. Tajo Abraham**, IQAC Coordinator and HoD of Botany, Sir Syed College

Technical Session I (11.00-12.30pm)

ERUDITE Lecture : **Prof. Om Parkash Dhankher**, College of Natural Sciences, Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA.

'Feeding and Fueling the Future: Climate Resilient Crops for Enhanced Production of Food and Fuels'

Technical Session II (1.30-3.00pm)

Invited Talk 1 : **Dr. Babu Valliyodan**, Assistant Professor of Molecular Biology and Genomics Department of Agriculture and Environmental Science, Lincoln University, USA.

'Genetic and Genomics Tools for Legume Crop Improvement'

Technical Session III (3.15-5.00pm)

Paper Presentations - OP01 to OP09

Day 2

Technical Session IV (9.30pm-10.30pm)

Invited Talk-2 : **Dr. Sujith Puthiyaveetil**, Associate Professor, Department of Biochemistry and Purdue Center for Plant Biology, Purdue University, USA

'Ironing out diatom bloom and bust: physiological and molecular mechanisms'

Technical Session V (10.30-11.30am)

Invited Talk-3 : **Prof. (Dr) Manish Kumar P.R.**, Former Head & Coordinator, Dept. of Biotechnology, University of Calicut, Kerala

'Bioassays for Plant-Drug Evaluation'

Technical Session VI (11.30-12.30pm)

Paper Presentations : OP10 to OP19

Valedictory Function: 2.00-3.00pm

Welcome speech : Dr. Sreeja P., Asst. Professor, Dept. of Botany, Sir Syed College

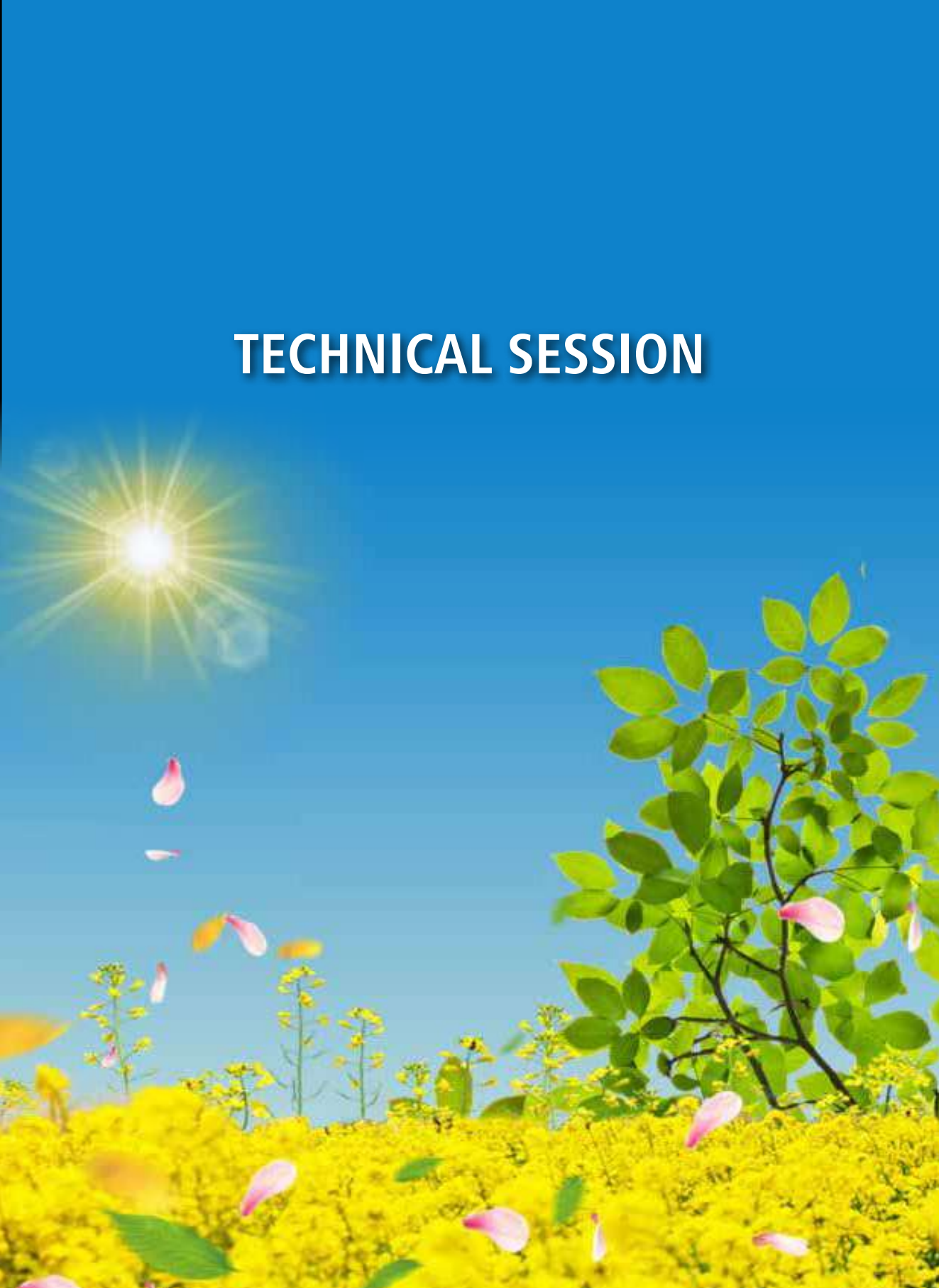
Valedictory Speech : Dr. K.T. Chandramohanan, Syndicate Member, Kannur University

'Best Paper Award Distribution'

Feedback session

Vote of thanks : **Dr. Gayatri R. Nambiar**, Asst. Professor, Department of Botany, Sir Syed College

TECHNICAL SESSION



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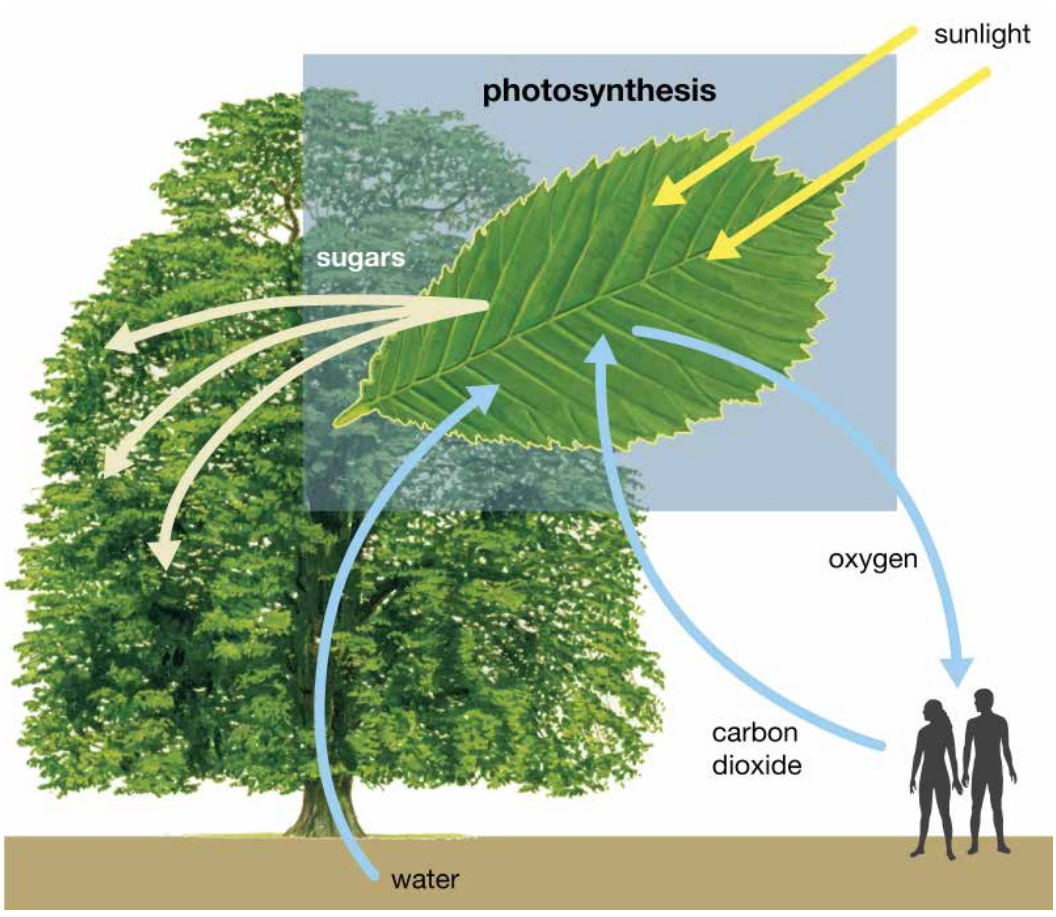
ERUDITE LECTURE



ERUDITE SCHOLAR

Prof. Om Parkash Dhankher
Stockbridge School of Agriculture,
University of Massachusetts,
Amherst, USA
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Professor Om Parkash Dhankher is a leading scientist in the field of Crop Biotechnology and has contributed much to the scientific community for the last few decades. Prof. Dhankher completed his M. Sc. and M. Phil degree from Kurukshetra University, Haryana and was awarded Ph. D. in Plant Molecular Biology from Durham University, England (U.K.) in 1998. He dedicated his research career towards understanding the molecular and biochemical mechanisms of abiotic stress tolerance in plants (including heavy metals, drought, high temperature, salinity and nutrient stress) for developing 'climate resilient crops'. He was instrumental in engineering non-food crops for phytoremediation of heavy metals and metalloids and also dealt with metabolic engineering of oil seed crops for increasing oil contents for food, biofuels and value-added bioproducts. He elucidated the role of glutathione homeostasis for enhanced oxidative stress tolerance in plants. Prof. Dhankher has also exploited the applied aspects of nanomaterial as nano-fertilizers and nano-pesticides for sustainable agriculture and food safety. He has explored the molecular and physiological strategies for developing arsenic free rice. He was honored with several prestigious awards of various scientific bodies. Prof. Dhankher received Outstanding Agriculture Research Scientist Award from the Association of Agriculture Scientist of Indian Origin (AASIO) in 2021, Haryana Gaurav Samman Award from the Govt. of Haryana State, India, Healey Endowment Research Award, UMass Amherst, Award of Excellence for outstanding Research, American Chemical Society and Commonwealth Scholarship award. Prof. Dhankher is a Fellow of the Crop Science Society of America (CSSA), Agronomy Society of America (ASA), International Society of Environmental Botanists (ISEB), Indian Society for Plant Physiology and is also serving as an adjunct Professor, Haryana Agriculture University Hisar, India. He served as the Vice President of the International Phytotechnology Society (IPS), Chair of Faculty Senate Council for University Relations and Advancement, Member of International Award Committee, Agronomy Society and Crop Science Society of America (ASA and CSSA) and Dean's Research Advisory Committee, College of Natural Sciences, UMass Amherst. He is the editor of various reputed scientific journals like Plant Cell Reports, Plant Physiology Reports, International J. of Phytoremediation, The Plant Genome, Crop Science and Food & Energy Security. He had published 120 research publications in reputed international journals and has 5 international patents. He has achieved total 6500 citations and an h-index 44.

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ERUDITE LECTURE

Feeding and fueling the future: Climate resilient crops for enhanced production of food and fuels

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Abstract

Renewable transportation fuels (biodiesel and green diesel) from plant seed oils are considered as environmentally and economically feasible alternatives to petroleum-derived fuels. *Camelina sativa*, due to its unique seed and oil attributes, has attracted much interest as an emerging crop dedicated for biodiesel and jet fuel production. To increase oil yield, we engineered Camelina by co-expressing *Arabidopsis DGAT1*and yeast *GPD1* genes under the control of seed-specific promoters. Transgenic lines exhibited up to 13% higher seed oil content and 52% increase in seed mass compared to wild-type plants. Further, DGAT1- and GDP1 co-expressing lines produced almost double seed and oil yields per plant basis compared to wild-type or plants expressing DGAT1 and GPD1 alone. To identify the bottlenecks for further improving the seed and oil yield in Camelina, we utilized metabolomic and transcriptomic profiling approaches in developing seeds in Camelina overexpressing TAG related genes. Our approach revealed several key genes/gene networks associated with significant changes especially in the TCA cycle and storage/retention of lipids in seeds. Overexpression of candidate genes showed further increase in oil and seed yield. We are now translating this strategy in edible oilseed crops such as Indian mustard and soybean for increasing edible oil contents and seed yields.

Further, to enhance plants productivity under the adverse conditions, we over expressed novel stress related genes including wax synthase gene (WS) for increasing the tolerance to multiple abiotic stresses. WS transgenic plants, when exposed to drought, salinity and heavy metals stresses, exhibited strong tolerance phenotype and had reduced water loss and cuticle permeability due to increased deposition of epicuticular leaf and stem wax loading. Ultimately, our aim is to stack the genes/gene networks responsible for increasing seed and oil yields as well as abiotic stress tolerance to enable these cultivars to growth on marginal lands and under extreme environmental conditions.

Key words: *Camelina sativa*, triacylglycerols, biofuels, transgenic plants, metabolic engineering, RNA Seq, metabolome, abiotic stresses.

INVITED SPEAKER

Dr. Babu Valliyodan
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Dr. Babu Valliyodan is placed as the Chair, LUHI and Director of the Center for Climate Smart Commodities at the Lincoln University. In addition, he is an Adjunct faculty at the Division of Plant Sciences, University of Missouri, Columbia, MO, USA. Prior to the current position, he was a Senior Research Scientist in Genetics & Genomics at the National Center for Soybean Biotechnology and Division of Plant Sciences, University of Missouri. He completed his PhD in Plant Physiology and Biochemistry from the University of Calicut, Kerala, India. Dr. Valliyodan's research focuses on the development and deployment of genetic and genomic tools for crop resilience, climate smart and sustainable agriculture. Dr. Valliyodan coordinated large scale genome sequencing project for soybean and providing legume genome sequence information and haplotype maps to the public breeding programs worldwide. Current research focuses of Dr. Valliyodan laboratory are application of genetic and genomic technologies to study genome variations and complex trait improvement in legumes and major crops. This includes dissecting the molecular/ biochemical regulation of stress tolerance traits, carbon sequestration traits, studying the role of plant-microbiome interactions, and genome editing for water and nutrient use efficiency towards yield improvement. Dr. Valliyodan is collaborating with various research institutions including University of Western Australia, Nanjing Agricultural University, University of Calicut, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), and the Indian Council for Agricultural Research (ICAR) on biotechnology and genomics assisted crop improvement. Dr. Valliyodan is serving in the editorial board of several scientific journals including Corp Science, Plants, Plant Physiology and Frontiers in Genetics. He published more than 80 peer reviewed publications including in the journal Nature, Nature Communications, Nature Scientific Data and 12 book chapters. He has two Patents and U.S. patent applications pending. Dr. Valliyodan achieved more than 12,000 Citations, h-index: 41 and the i10-index 70.

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INVITED LECTURE

Genetic and genomic tools for legume crop improvement

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Abstract

Legumes are the major source of food, feed and fuel. In view of climate change and human population increase, acceleration of yield genetic gain and resilience to stress environments are needed to ensure legume production and global food security. Identification and utilization of untapped genetic resources including the wild species is an essential component to achieve this goal. Investigation of the natural genetic variation for major traits associated with stress tolerance and yield will help to achieve higher genetic gains. Availability of large-scale genomic resources for major legume crops and cost effective genotyping technologies are enhancing the breeding programs for sustainable food production. Development of pan genomes for cultivated and wild type of legumes significantly impact upon the identification of haplotypes and rare alleles associated with major traits. The application of genome assisted breeding approaches has resulted in the development of improved lines in legume crops including soybean, chickpea, and groundnut. Integration of crop specific genomic resources, genotype-phenotype-environment interaction data with artificial intelligence focused on machine and deep learning will help develop smart breeding schemes towards designing crop varieties with improved genetic gain.

INVITED SPEAKER

Dr. Sujith is very active in photosynthetic research and is currently working as Associate Professor in the Department of Biochemistry and Center for Plant Biology, at Purdue University. He has completed his masters degree at Sir Syed College during 1997-99 and doctoral degree from Queen Mary University of London, in 2008. Dr. Sujith's professional achievements include his service as Reviewer for Trainee Early-Career Award for Mentoring in Unexplored Problems (TEAM-UP) Postdoctoral Award, at Department of Biochemistry and Molecular Biology in Michigan State University, during 2020. Dr. Sujith has been a part of EXploring College Emerging Leaders (EXCEL) outreach activity which was aimed to encourage and promote higher education for Native American students, Washington State University. Dr. Sujith and team is pioneering in studying the molecular and evolutionary aspects of photosynthesis research and has contributed much to unravel the mechanism behind it.

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INVITED LECTURE

Ironing out Diatom Bloom and Bust: Physiological and Molecular Mechanisms

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Department of Biochemistry and Center for Plant Biology, Purdue University,
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Abstract

Diatoms are a diverse group of mostly aquatic unicellular eukaryotic algae. Diatoms form the basis of the oceanic food web by fixing as much carbon as all rain forests combined. Iron is an important micronutrient for photosynthesis as the photosynthetic electron transport chain has a high iron-load. The planktonic diatoms however successfully inhabit chronically iron-poor polar waters and form large blooms when the ocean is fertilized with iron. The genetic and molecular control mechanisms that enable diatom survival under low iron and diatom blooms under excess iron are not fully understood. Using physiological, proteomic and genetic analyses, here we show that diatom photosynthesis is profoundly reconfigured under changing iron levels. Under low iron, iron acquisition proteins and non-iron-containing small electron carriers are significantly upregulated. Acclimation of the photosynthetic machinery to iron-sufficient condition involves a marked increase in the relative abundance of photosystem I and cytochrome b 6 f complex, in turn improving their stoichiometric relationship with photosystem II. This reconfiguration of the photosynthetic electron transport chain results in a higher operating efficiency of photosystem II and linear electron flow from water to NADP + . Furthermore, proteins involved in photoprotective thermal dissipation of absorbed light energy are downregulated under iron-sufficient condition, allowing efficient use of light energy in photosynthesis. Under iron sufficiency, transcription of

plastid genes encoding core subunits of photosystem I and cytochrome b 6 f complex increases. We further show that the redox state of the photosynthetic electron carrier plastoquinone likely functions as a regulatory signal under changing iron levels and that the plastoquinone redox signals are transduced to plastid gene expression machinery through an iron-sulfur containing. Chloroplast Sensor Kinase. Two Chloroplast Response Regulators bind plastid genes and likely act as functional partners of the Chloroplast Sensor Kinase for iron-responsive plastid gene expression. Our results thus provide crucial insights into physiological, molecular, and genetic mechanisms of iron-acclimation of diatom photosynthesis.

INVITED SPEAKER

Prof. P. R. Manish Kumar (Rtd)
Formerly, Co-ordinator &
Head of the Department
Dept. of Biotechnology
University of Calicut

Prof. Manish Kumar has obtained his ph. D degree from University of Calicut in 1991. Prof. Manish Kumar was awarded Biotechnology Research Associateship (1992-'94) at Indian Institute of Science, Bangalore. He started his carrier as college Lecturer and then affiliated to Univ. of Calicut, joined Dept. of Biotechnology University of Calicut as teaching faculty in 1996. His area of teaching includes Cell & Developmental Biology, Molecular Biology and Recombinant DNA Technology for the M.Sc. Biotechnology Program. Several students have completed M.Phil. and Ph.D. Program at University of Calicut under his supervision. Prof. Manish Kumar has undergone Specialized Training in Electron Microscopy (AIIMS, N. Delhi); Radiotracer techniques (BARC, Mumbai); Basic molecular biology and recombinant DNA techniques (Univ. of Calicut; IISc Bangalore); Animal cell culture (Reliance Life Sciences, Navi Mumbai);Advances in Biotechnology (TERI, N.Delhi), etc. He was awarded 'Best Subject Expert for the year 2000' by University Grants Commission for the Educational video on DNA Fingerprinting entitled 'DNA evidence' produced by Audiovisual Research Centre (AVRC). He serves as Co- Principal Investigator in DBT (Govt. of India) sponsored research project (2012-2015) on 'Screening for antiproliferative and anticancer drugs from endemic species of Zingiberaceae on human cancer cell lines'. He served as Chairman, Board of studies (Single Board) in Genetics, Member – Board of Studies in Biotechnology, Univ. of Calicut; DBT (Govt. of India) nominee – Institutional Biosafety Committee - External subject Expert nominee– Research Admission Committee – IISR, Calicut;; Department of Biotechnology, VIT, Vellore; Member – Board of Studies (Marine Biotechnology) KUFOS –Kerala University of Fisheries and Ocean Studies, Panangad, Kochi.

Coordinator of DBT M.Sc. program and Bioinformatics facility & Head of the Dept - 2008 - 2010 & 2012 – 2014. Coordinator : UGC-ASC / HRDC Refresher courses – Biotechnology (2 courses) and Life Sciences (1 course)

Research Interests : Molecular Biology of Cell cycle regulation; DNA cassettes involved in bacterial drug resistance; novel anticancer drugs from plant; human cancer cell lines; DNA damaging agents ;biogenic nanoparticle production and their cellular effects

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INVITED LECTURE

Bioassays for Plant-Drug Evaluation

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Abstract

Plant-based natural products continue to serve as a valuable source of molecular diversity in drug-discovery programs. The efficacy of new candidate drugs or those based on time-tested ancient wisdom practiced as traditional medicine needs to be scrutinized and validated in the light of modern biology and medicine. Bioassays serve as a starting point for multi-disciplinary efforts involving botanists, pharmacognosy / pharmacology experts, molecular biologists, toxicologists, bioinformaticians and clinicians. The presentation intends to give an overview of some of the popular bioassays.

OP-01

The profile diversity of species under the family Linderniaceae selected areas along Kannur district Kerala

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Abstract

Vast stretches of laterite capped hillocks are the characteristics of Kannur district. We analysed the distribution of species under the family Linderniaceae in different ecological condition along laterite stetches and paddy fields of the Kannur district during pre-monsoon, monsoon and post monsoon. Associated plants are *Utriclaria cecilii, Limnophilia repens, Cyperus rotundus, Justicia ekakusuma, Desmodium trifolium, Eriocaulon eurypeplon, Centranthera.* In the monsoon season mostly *Lindernia ciliata, Lindernia crustacea* was observed in the laterite stretches from Cheemeni, Ponnurikkipara, Karakund, Madyipara, Mattanur, Chenglayi, Chudala, Keezhara, Ammuparamb. During post monsoon more *Lindernia* species such as *Lindernia crustcea, Lindernia antipoda, Lindernia anagallis, Lindernia caesopitosa, Lindernia hyssopioides, Lindernia rotundifolia, Lindernia tenuifolia* was observed in the paddy fields of Bakklam, Pariyaram, Pazhayangadi, Pappinisseri, Vaaram. Likewise it was found that the some of the species are surviving in water deficit condition, and disturbed sites along the roadsides. To conclude *Lindernia* species might be promising candidate for stress tolerance study, but more investigation is needed to reveal the phytochemicals involved in the stress response.

Keywords: Distribution, Diversity, Linderniaceae, Laterite stretches, Phytochemicals.

1. Introduction

Kerala is divided according to its geomorphological condition into three regions: coastal plain, midland hills and highland hills. Vast stretches laterite hillocks are one of the characteristic features of the midland hills which include Kannur district. Besides the fact that it provides a good watershed area, it also serves as a major ecosystem for many plants. Laterite plateaus can be considered as an amphibious ecosystem where there is an unusual ecosystem that supports a unique biota due to the alteration of very wet and dry conditions. So, to tolerate the shift of environmental conditions they grow and reproduce in a short time. Many species forms endemics to such geographic areas (Balakrishnan *et al*., 2010). Linderniaceae, a herbaceous angiosperm flourishing in these geographic areas predominantly occurs in laterite stretches, paddy field and also thrives in disturbed sites along the edges of roadside, damp places, marshy and wetlands (Randall, 2012).There are huge reports and case studies from different regions

of India on the ethnomedicinal aspects of plants under the family Linderniaceae, especially in the genus *Lindernia* (Bhatt and Kunwar, 2020; Sen and Bhakat, 2020; Swargiary and Roy, 2021) *interviews, and field excursions involving herbalists and taxonomists. A semistructured questionnaire was used to interview a total of 120 informants. By using different quantitative indices, results were analyzed for fidelity level (FL.*

Scrophulariaceae was studied by the molecular phylogenetic studies revealed to be polyphyletic and the family Linderniaceae emerged as separate lineages including *Lindernia* as a relative genera (Albach et al., 2005; Olmstead and Reeves, 1995; Rahmanzadeh et al., 2005)2005; Olmstead and Reeves, 1995; Rahmanzadeh et al., 2005. *Lindernia* species are herbaceous angiosperm occurs along the banks of occasional ponds flooded by monsoon rain in peninsular India. There are 30 taxa of the genus *Lindernia* reported from India (28 species 1 subspecies and 1 variety) and among them 25 are from south India. In the checklist of flowering plants of Kerala 18 species of *Lindernia* were reported in Kerala (Nayar et al., 2008). The floristic exploration from India reported a few new species, *viz*. *Lindernia dubai, Lindernia tamilnadensis, Lindernia nelliampathiensis, Lindernia madayiparense* (Patel et al., 2021; Prasad and Sunojkumar, 2014).

Lindernia occurs in moist shady habitat, erect or prostrate annual herbs with serrate margins. The flowers are solitary, axillary in position, often seen in racemes; bracts and bracteoles are absent in these genus. Calyx five, corolla caducous having five lobes and are lipped. Stamens are seen two or four, mostly the lower pair reduced to staminoides. Ovary globose or oblong and stigma two lamellate. Flowers in every season. The anatomical and taxonomical study on genus *Lindernia* occurring in Thrissur district was done through repeated analysis of specimen of *Lindernia* species such as *Lindernia hyssopiodes* (L.) Haines, *Lindernia rotundifolia* (L.), *Lindernia antipoda* (L.), *Lindernia Crustaceae* (L.), *Lindernia caespitosa* (Blume) Panigrahi, *Lindernia viscosa* (Hornem.) Merr (Subramanian and Varghese, 2017).

2. Materials and Methods

For the studies intensive field exploration in different seasonsare done in areas where occurrence of Linderniaceae isobserved and reported in the past research reports. The required specimens for the work are collected during the flowering stages. For each field survey was conducted 1m x 1m quadrat analysis and observations are recorded regarding the distribution, the associated plants in the premonsoon, monsoon and post monsoon.

Study area

Laterite stretches in Kannur such as Cheemeni, Ponnurikkipara, Karakund, Madyipara, Mattanur, Chenglayi, Chudala, Keezhara, Ammuparamb and agricultural field including the paddy fields of Bakklam, Pariyaram, Pazhayangadi, Pappinisseri, Vaaram was selected for the analysis.

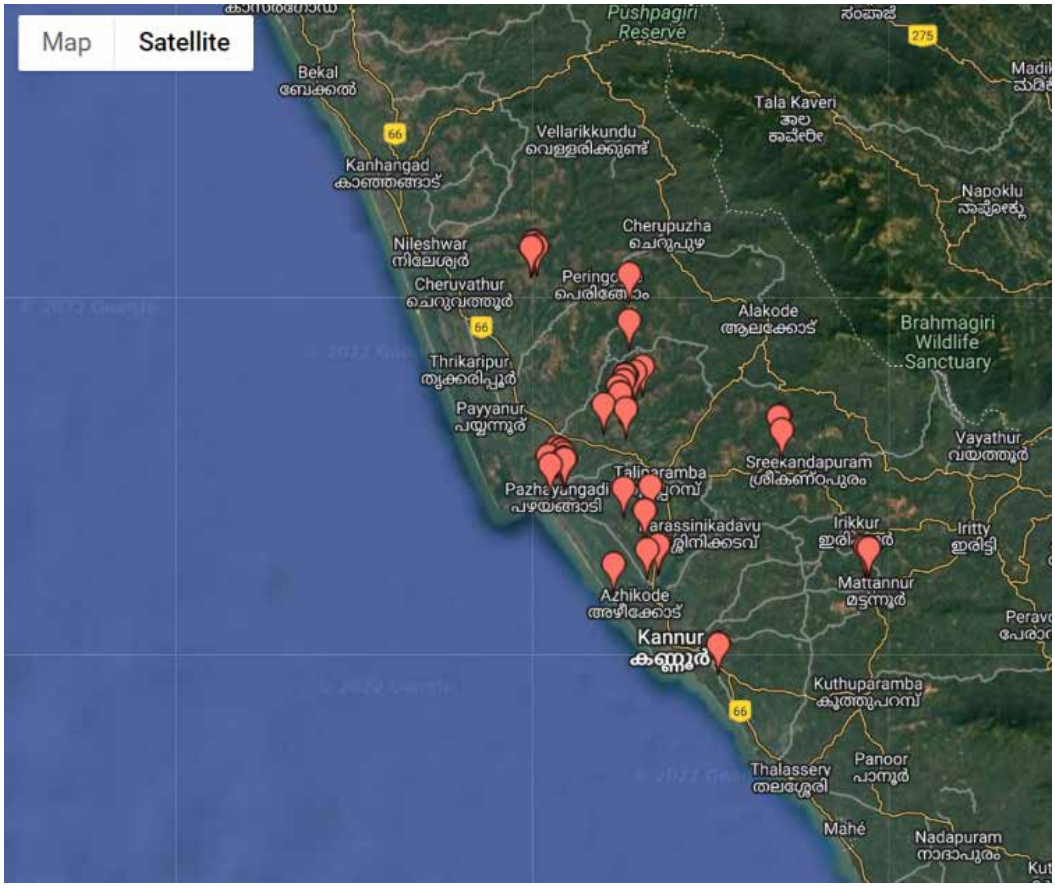

Figure 1: Study area. Laterite stretch of Kannur district. Source of satellite image: downloaded from GPS essential

3. Results

In our study it was analysed the distribution of species of Linderniaceae in different ecological condition along laterite stetches and paddy fields of the Kannur district during pre-monsoon, monsoon and post monsoon season (Fig 1). *Lindernia ciliata, Lindernia crustacea* were seen abundant in all the season, observed flourishing in laterite stretches especially during monsoon and post monsoon. *Lindernia antipoda* were also seen in all season more in Post monsoon in agricultural field, and damp marshy areas. Aquatic species such as *Lindernia anagallis and Lindernia hyssopioides* wereobserved more in the agricultural field and laterite stretches during monsoon and post monsoon. *Lindernia tenuifolia* were also observed in the agricultural field during post monsoon. *Lindernia rotundifolia* were seen in the agricultural fields during post monsoon and pre monsoon (Fig 2).

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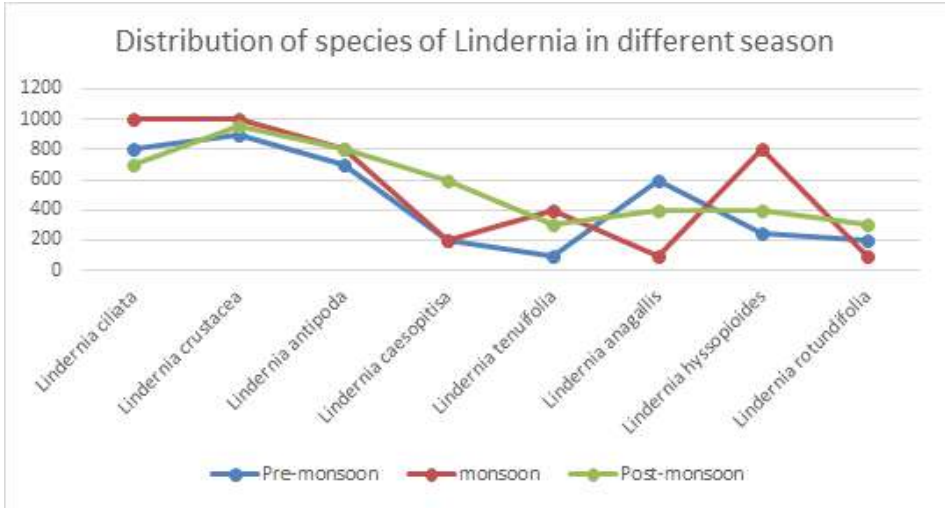

Figure 2. Distribution of the species of Linderniaceae in the premonsoon, monsoon and post monsoon.

Lindernia cilata and *Lindernia crustcea* were observed in the laterite stretches of Cheemeni, Ponnurikkipara, Karakund, Madyipara, Mattanur, Chenglayi, Chudala, Keezhara, Ammuparamb in the Kannur district during monsoon. *Lindernia rotundifolia, Lindernia anagallis, Lindernia antipoda, Lindernia hyssopioides, Lindernia tenuifolia* were seen in the agricultural paddy field near Bakkalam, Pariyaram, Vaaram, pappinisseri during post monsoon.

Likewise it was found that the some of the species are surviving in water deficit condition, and disturbed sites along the roadsides especially *Linderniacrustcea and Lindernia antipoda*. Interestingly it was observed that presence of *Lindernia crustacea* in disturbed sites such as in the edges of the roadside, temple stones, fence made of laterite stone also in newly made land fill areas.

4. Discussion

Even though it is considered as a weed, Linderniaceae, is reported to have high medicinal values and is distributed throughout the world. Among the Linderniaceae family, owing to the traditional knowledge, pharmacological experiments, genus *Lindernia* has been studied extensively regarding ethnopharmacological as well as phytochemical search (Umakrithika et al., 2017). *Lindernia antipoda* and *Lindernia crustacea* are seen in every physiograph (Subramanian and Varghese, 2017).

In this context this study evaluated the diversity of Lindernia species in Kannur district in different ecological sites. It was observed *Lindernia hyssopioides* and *Lindernia tenuifolia* are semi aquatic and found from paddy fields, among these *Lindernia hyssopioides* were collected from both laterite stretches and paddy fields. But it was disappeared from the locality during post monsoon. *Lindernia crustacean* were seen in all the season in the laterite as well as agricultural field. Moreover it shows significant morphological variation in different sites as colour of the plant were observed reddish brown in some of the laterite area, also the size of the plants are comparatively short in theses laterite areas compared to the plants collected from the field.

5. Conclusion

Linderniaceae is a family which is reported to have high medicinal value in traditional medicine used worldwide. There should be detailed phytopharmacological studies to explore the active compounds in the plants. Some of the species are surviving in water deficit condition, and disturbed sites along the roadsides. To conclude *Lindernia*species might be promising candidate for stress tolerance study, but more investigation is needed to reveal the phytochemicals involved in the stress response.

6. Acknowledgement

The first author is grateful to the University Grants Commission (UGC), New Delhi for the award of Junior Research fellowship to carry out the study. The third author is grateful to Counsil of Scientific Research (CSIR), New Delhi for the research fellowship.

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OP-02

Screening of cytotoxicity in *hemiparasite Dendrophthoe* falcata (L.f.) Ettingsh growing on four different host plants

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Abstract

The ethanol extracts of whole plant of *Dendrophthoe falcata* growing on *Anacardium occidentale* (DFA), *Lagerstroemia speciosa* (DFL), *Mangifera indica* (DFM) and *Strychnos nux-vomica* (DFS) were screened for cytotoxicity. *Dendrophthoe falcata* (L.f.) Ettingsh is a hemiparasitic medicinal plant which is one of the most commonly occurring mistletoes of the family Loranthaceae. The plant grows on around 401 host plants. The plant's chemical constituents determine the medicinal value of *D. falcata* that may vary according to the host specificity. MTT assay is conducted on L929 fibroblast cells using five different concentrations of each sample to analyse the cytotoxic effect of *Dendrophthoe falcata* growing on selected host plants. The cytotoxic effect is assessed based on the percentage of viable cells retained after 24 hours of incubation and LC50 values. The four samples of *Dendrophthoe falcata* showed marked variation in cytotoxic effect on MTT assay. The cell viability percentage of DFA extract is 66.64% at 100 μg/ml concentration, with the lowest LC50 value of 145.93 μg/ml and that of DFL extract is 86.23%, with the highest LC50 value of 396.43μg/ml. The cell viability percentage for DFM is 72.89% and for DFS is 67.91%. The notable variations in cytotoxicity in *D. falcata* samples points out the need for detailed screening, while using the plant for a therapeutic purpose.

Keywords: *Dendrophthoe falcata*, Cytotoxicity, MTT Assay, LC50

1. Introduction

Dendrophthoe falcata (L.f.) Ettingsh is a hemiparasitic medicinal plant which is one of the frequently occurring mistletoes of the family Loranthaceae that grows on around 401 host plants [1]. The plant contains therapeutically important chemical constituents such as alkaloids, phytosterols, fixed oils, phenolic compounds, gallic acid, ellagic acid, triterpenes, quertcetin, quercetrin, rutin, chebulinic acid, beta-amyrin acetate, beta sitosterol, stigmasterol etc [2]. Scientific studies conducted in *D. falcata* proved its potentiality having Antidiabetic [3-4], Antioxidant [5], Antinociceptive [6], Antimicrobial [7-8] Diuretic and Antilithiatic [9], Anticancerous [10-11], Anxiolytic [12], Anthelmintic [13], Hepatoprotective [14], Immunomodulatory [15], Contraceptive [16], Estrogen receptor binding [17], Cytotoxic [18],

Analgesic and Antiinflammatory [19], Anticonvulsant and Muscle relaxant [20] activities. The plant's chemical constituents determine the medicinal value of *D. falcata* that vary according to the host specificity. The various medicinal uses of the plant and good chemical composition signifies the need for cytotoxicity screening in selected samples of *D. falcata.*

Cytotoxic effect of herbal drugs can be utilized in the treatment of cancer cells. MTT assay is a reliable method that helps to determine cell toxicity or antiproliferative activity of any of the test compounds [21-22]. The MTT assay is an *in vitro* cytotoxicity method that is based on the ability of mitochondrial dehydrogenase enzymes in living cells to convert the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyl tetrazolium bromide (MTT) into a dark purple formazan product which is insoluble in water. Viable cells alone possess the capacity to reduce the yellow MTT under tetrazolium ring cleavage to a water-insoluble purple product which precipitates in the cellular cytosol and can be dissolved after cell lysis, whereas dead cells cannot transform MTT. The quantity of formazan product is directly proportional to the viable cell number and inversely proportional to the degree of cytotoxicity. The dehydrogenase enzymes associated with the endoplasmic reticulum and the mitochondria mediates the reaction mechanism [23]. The aim of the present work is to determine the cytotoxic effect of *Dendrophthoe falcata* samples grown on different host plants through MTT assay using L929 Fibroblast cells.

2. Materials and Methods

Plant Material and Identification

The whole plant of *Dendrophthoe falcata* (L.f.) Ettingsh growing on *Anacardium occidentale L.* (DFA)*, Lagerstroemia speciosa* (L.) Pers*.* (DFL)*, Mangifera indica* L*.* (DFM) and *Strychnos nux-vomica* L*.* (DFS) were collected from different places of Malabar region. The plants were identified with the help of taxonomic characters by Scientists of Botany division, Centre for Medicinal Plants Research, Kottakkal Arya Vaidya Sala.

Extract Preparation

Entire plant of each sample cut into small pieces, were dried in shade for three weeks and powdered in electric mill. 150g of each powdered drug is extracted in 600ml ethanol in Soxhlet apparatus for 5 hours. Extract is filtered and concentrated to dryness in a water bath.

Cell lines

L929 (Fibroblast) cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco's modified Eagles medium - DMEM (Sigma-Aldrich, USA).

Subculture of cell lines

The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% Fetal Bovine Serum, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37^{o}C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

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Cells seeding in 96 well plate:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO_2 incubator.

Preparation of compound stock:

1mg of sample was weighed and dissolved in 1ml DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

Cytotoxicity Evaluation

After 24 hours the growth medium was removed, freshly prepared compounds in DMEM were five times serially diluted (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM) and100µl at each concentration were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO_2 incubator. Non treated control cells were also maintained.

Cytotoxicity Assay by Direct Microscopic observation

Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Cytotoxicity Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide: DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm [24].

The percentage of growth inhibition was calculated using the formula:

$$\text{Percentage of viability} = \frac{\text{Mean OD of Samples} \times 100}{\text{Mean OD of control}}$$

Statistical Analysis

The assays of all samples and the standard were done in triplicate. The results were indicated in terms of mean values and their standard deviations.

3. Results and Discussion

Cytotoxicity Assay by Direct Microscopic Observation

Notable changes were observed in the morphology of the cells, such as rounding and shrinking

of cells, membrane blebbing with increasing concentration of the extract. Formation of granules and vacuoles in the cytoplasm of the cells were detected at high concentration of extracts, which indicates the cytotoxic effect as shown in figure 1.

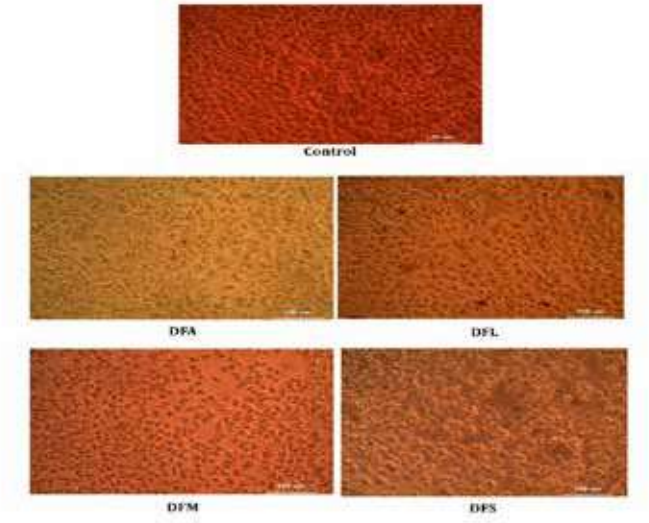

Figure 1 : Comparative Microscopic images showing the cytotoxic effect of Control and Samples on L929 fibroblast cells.

MTT Assay Results

The four samples of *D. falcata* parasitic on *Anacardium occidentale* (DFA), *Lagerstroemia speciosa* (DFL), *Mangifera indica* (DFM) and *Strychnos nux-vomica* (DFS) showed notable variation in cytotoxic effect in L929 fibroblast cells on MTT assay. The cytotoxic effect is evaluated by calculating the percentage of cell viability retained after 24 hours of incubation and LC50 values (Table 1-5).

Table1 : MTT assay details of Control		
Control	Optical Density (OD)	Cell Viability (%)
	1.05 ± 0.01	100

Table 2 : MTT Assay Result of DFA on L929 fibroblasts			
Concentration (µg/ml)	Optical density (OD)	Cell Viability (%)	LC50Value (µg/ml)
6.25	1.00 ± 0.00	96.80	145.93
12.5	0.98 ± 0.01	94.93	
25	0.95 ± 0.01	91.16	
50	0.81 ± 0.00	78.30	
100	0.69 ± 0.01	66.64	

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Table 3 : MTT Assay Result of DFL on L929 fibroblasts			
Concentration (µg/ml)	Optical density (OD)	Cell Viability (%)	LC50Value (µg/ml)
6.25	1.01 ± 0.01	97.76	396.43
12.5	1.00 ± 0.00	96.60	
25	0.98 ± 0.01	94.40	
50	0.95 ± 0.01	91.25	
100	0.89 ± 0.01	86.23	

Table 4 : MTT Assay Result of DFM on L929 fibroblasts			
Concentration (µg/ml)	Optical density (OD)	Cell Viability (%)	LC50Value (µg/ml)
6.25	1.01 ± 0.01	97.54	188.39
12.5	0.99 ± 0.00	96.12	
25	0.95 ± 0.01	91.17	
50	0.90 ± 0.01	86.50	
100	0.76 ± 0.01	72.89	
Table 5 : MTT Assay Result of DFS on L929 fibroblasts			
Concentration (µg/ml)	Optical density (OD)	Cell Viability (%)	LC50 Value(µg/ml)
6.25	0.98 ± 0.01	94.73	161.39
12.5	0.93 ± 0.02	89.93	
25	0.89 ± 0.00	86.11	
50	0.81 ± 0.00	78.31	
100	0.70 ± 0.01	67.91	

*Each OD value is presented as Mean ± Standard Deviation (n=3)

Percentage of cell viability at 100 µg/ml concentration of DFA extract is 66.64% and it has the lowest LC50 value of 145.93 µg/ml. Percentage of cell viability at concentration 100 µg/ml of DFL is 86.23% and it has the highest LC50 value of 396.43µg/ml. Percentage of cell viability for DFM is 72.89% and for DFS is 67.91% at 100 µg/ml concentration. DFL having highest LC50 value is observed as the safe drug among the samples with lowest cytotoxic effect on selected cell lines. DFA with lowest LC50 value is slightly cytotoxic, causing notable reduction in cell viability percentage to 66.64% at 100 µg/ml of the extract.

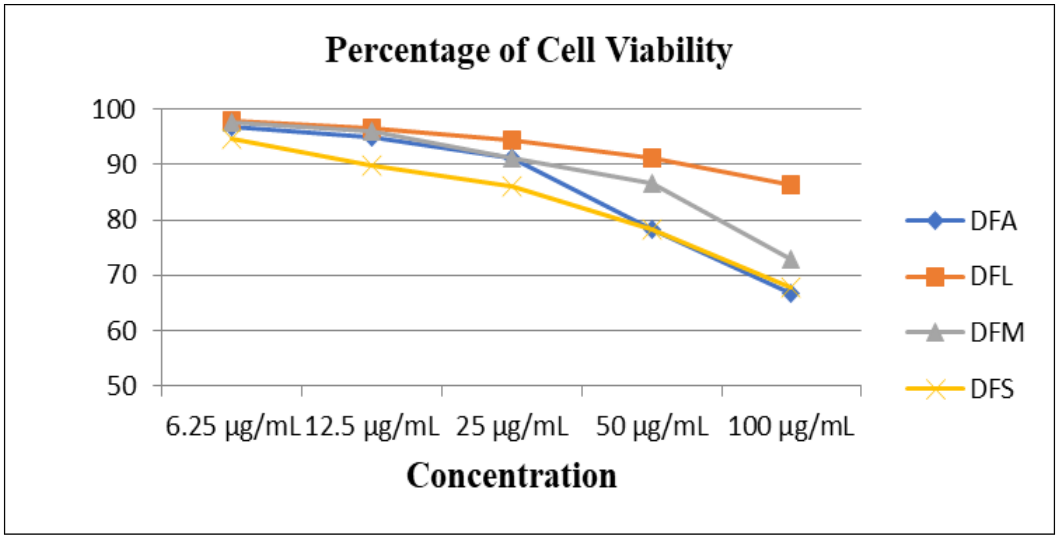

Figure 2 : Change in percentage of cell viability on MTT assay with four samples of *D. falcata*

The graphical illustration (figure 2) shows that the cytotoxic effect of all selected samples strengthens progressively as evident from the reduction in cell viability, upon increasing concentration of the extract. The significant differences in cytotoxic effect between the samples might be correlated to the qualitative and quantitative variations in the phytochemical contents in the extracts that arise due to host - hemiparasite interactions. Significant variations in the quantity of important phytochemicals such as phenolics, flavonoids and tannins were observed in case of four samples under study.

The extracts that showed LC50 values greater than 100 µg/ml are considered to be non-toxic as per health standards [25]. The LC50 values of four samples of *Dendrophthoe falcata* were above 100 µg/ml as plotted in figure 3.

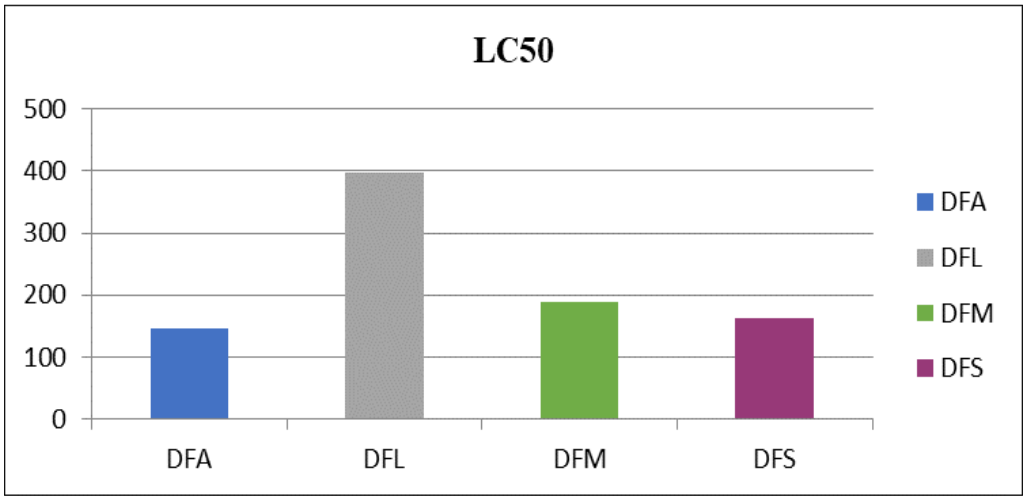

Figure 3 : LC50 values of four samples of *Dendrophthoe falcata*

4. Conclusion

Even though ethanol extract of *D. falcata* parasitic on four different hosts exhibited marked variations in cytotoxic effect against L929 fibroblast cells, all four samples were observed to be safe as per the toxicity standards. The specific phytochemical constituents responsible for the cytotoxic effect need to be identified and isolated from each sample of *D. falcata*. The present study points out the need for considering selection of host while using *D. falcata* for a medicinal purpose. However the samples of *D. falcata* screened for cytotoxicity in the present study can be effectively used as therapeutic agent in limited doses from the scientific point of view.

5. Acknowledgement

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6. Conflict of Interest

The authors declared that they have no conflict of interest.

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OP-03

Pollen morphological variation of selected species of Kannur District

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Abstract

The morphological analysis of pollen grains serves as a taxonomical key for analysis of different plants. In the present study morphological variation of 14 species were analysed. Erdtman Acetolysis technique was performed and variations was noticed in trinocular research microscope with 45x & 100x magnifications. Morphological variation of *Acanthus ilicifolius*, *Caesalpinia Pulcherrima*, *Centrosema molle*, *Carica papaya*, *Catharanthus roseus*, *Cosmos sulphureus*, *Tithonia diversifolia*, *Bougainvillea spectabilis*, *Hibiscus rosa- sinensis*, *Ixora coccinea*, *Mullugo oppositifolia*, *Kandelia kandel*, *Portulaca grandiflora*&*Turnera subulata* were investigated. Pollen grains were classified on the basis of size, shape, ornamentation of pollen surface, spine, length, number and aperture. Colpate type of pollen is mostly observed when compared to porate type of pollen. Results revealed that these morphological variations may help in identifying the members of a taxon, determine plant community structure & also establishing evolutionary relationships among the plants.

Key words: Acetolysis, Morphology, Pollen.

1. Introduction

Pollen grain is a minute body, of varying shape and structure, formed in the male structures of seed-bearing plants. Pollen morphological characters are used for solving taxonomic problems, [1] reposition of several disputed genera and interpret problems related to the origin and evolution of many taxa& also provide classification systems of angiosperms [2]. The structure of a pollen grain is so distinctive that, in some cases species may be identified by pollen grains alone. Characteristics such as the exine sculpturing and the size and number of apertures through which the pollen tubes grow are useful as taxonomic tools [3] [4] [5] [6]. Pollen characters are therefore useful tools in the identification, characterization and delineation of taxa, especially at the generic and specific levels [7] [8] [9] [10].

In the present study, the Pollen Morphological analysis of 14 species belongs to 12 families were evaluated.Pollen Morphology of 14 species representing *Acanthus ilicifolius* of the family Acanthaceae, *Caesalpinia pulcherrima* and *Centrosema molle* of the family Fabaceae , *Carica papaya* of the family Caricaceae, *Catharanthus roseus* of the family Apocynaceae , *Cosmos sulphureus* and *Tithonia diversifolia* of the family Asteraceae, *Bougainvillea spectabilis* of

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Nyctaginaceae family, *Hibiscus rosasinensis* of Malvaceae, *Ixora coccinea* of the family Rubiaceae, *Kandelia kandel* of Rhizophoraceae, *Mullugo oppositifolia* of the family Aizoaceae, *portulaca grandiflora* of portulacaceae and *Turnera subulata* of the family passifloraceae were examined from the different localities of Kannur district. Studies revealed the pollen morphological variations which act as a promising source for identification of several plant species.

2. Materials and methods

Collection and preservation

Anthers were collected from matured flower &fixed in 70% ethyl alcohol for an approximately one and half hours and then crush with a glass road.

Treatment

Fixed materials were squeezed by fine mesh and collected in centrifuge tube. The mixture was centrifuged for 10 minutes and liquid part was decanted. The residue was carefully rinsed to eliminate remaining chemicals. 5 ml acetic acid was added and again centrifuged for 10 minutes (C.×2000 rpm). Liquid fraction was decanted and collected the residue.

Acetolysis

Preserved pollen grains were mixed with freshly prepared acetolysed mixture. 1. 1 ml acetolysis mixture (1 ml sulphuric acid added drop by drop to 9 ml acetic anhydride {9:1}) were added to the residue and heat the mixture in a boiling water for 3- 5 minutes.

Centrifugation

The mixture was centrifuged for 10 minutes (C.×2000rpm) and decanted.Again5 ml glacial acetic acid were added to residue and centrifuged for 10 minutes. Then the residuewas collected. Distilled water was added to the residue and centrifuged again for 10 minutes. Liquid fraction was decanted off. Repeat the process.

Mounting

Residue were collected on glassslide. Then specimens were mounted by using glycerine jelly or Canada balsam. Acetolysed pollen was mounted by using a cover glass. Unwanted portion were removed by using tissue paper. Then mounted material was sealed with nail polish or paraffin wax.

Microscopic analysis

Then specimen was examined under Mag camtrinocular microscope (45x – 1500x). Theimage of pollen grains was observed and take pictures of pollen on 45 x. The size, shape, and exine ornamentation of eachspecimenwere recorded.

3. Results and discussion

Pollen grains of 14 species belongs to 12 families collected from different locations showed considerable morphological variations.Based on this study all pollen grains are monad type. Among these 14 species, pollen grains may vary in size such as large, medium and small. In Hibiscus rosa-sinensis pollen grains are relatively larger in size as compared to other species. *Tithonia diversifolia, Turnera subulata, Centrosema molle, Acanthus ilicifolius, Catharanthus roseus*

are medium in size. Remaining species are smaller in size. Ornamentation pattern of pollen surface is important in the study of plant systematic taxonomy. According to the morphology of *Bougainvillea spectabilis, Centrosema molle, Acanthus ilicifolius* having reticulate type of exine. Among 14 species majority of the pollen grains are in spherical in shape. *Acanthus ilicifolius* in Prolate shape. The Prolate -spheroidal shape of the pollen grains is found in *Carica papaya*. *Catharanthus roseus, Centrosema molle, Mollugo oppositifolia* are seen in Subprolate shape. *Caesalpinia pulcherrima* and *Centrosema molle* are coming under same family but still it showsslightmorphologicaldifference.

In most of the plant species we have studied in this research pollen grains are colpate. They are *Acanthus ilicifolius, Carica papaya, Catharanthus roseus, Cosmos sulphureus, Bougainville spectabilis, Kandelia kandel* and *Turnera subulata*. Other four species have colporate pollen which include *Caesalpinia pulcherrima, Centrosema molle, Ixora coccinea* and *Mollugooppositifolia.* Remaining two species are devoid of colpus, they are pantoporate.

In the present investigation, most frequently noticed morphological variations include:

1) Tricolpatepollen

It is most frequently noticed type of pollen. Among 14 species, pollen aperture of four species is tricolpate and three species shows tricolporate pollen grains. Tricolpate pollens found in *Bougainvillea spectabilis* (Fig.6), *Caesalpinia pulcherrima* (Fig.3), *Carica papaya* (Fig. 2), and*Catharanthusroseus* (Fig.5).

2) Pantocolpatepollen

Pollen which consists of more than three apertures and it is distributed over the entire surface. Pantocolpate pollen grains noticed in both *Portulaca grandiflora*(Fig.13) and *Mollugo oppositifolia* (Fig.12) in the present study.

3) Pantoporate pollen

Asteraceaefamily and*Hibiscusrosasinensis* (Fig. 9) shows Pantoporate pollens.

4) Zonocolpatepollen

It is mainly observed in *Kandelia kandel* (Fig. 11) and *Acanthus ilicifolus*(Fig. 1)

5) Tricolporatepollen

Tricolporate pollen is mainly seen in species such as *Cosmos sulphureus* (Fig. 2&3),*Turnera subulata* (Fig. 14) and Centrosema molle (Fig.4)

Based on shape, observed pollen variations include:

Spheroidal pollen

Pollen grains which are spherical in shape, and is mainly observed in the case of *Hibiscus rosasinensis* (Fig.9), *Portulaca grandiflora* (Fig. 13), *Cosmos sulphureus* (Fig. 7) and *Turnera subulata* (Fig.14).

Prolate pollen

Pollen grains which are usually bilaterally symmetrical and a grain with polar axis that is

greater than the equatorial diameter. Only *Acanthus ilicifolius* are in prolate shape ie (Fig.1).

Subprolatepollen

Subprolate shape are seen in *Carica papaya* (Fig. 2), *Ixora coccinea* (Fig.10) and *Mullugo oppositifolia* (Fig.12).

Oblatepollen

Thesepollens are somewhat rounded but slightly flattened at top and bottom. These are mainly seen in the case of *Caesalpinia pulcherrima* (Fig.3), *Carica papaya* (Fig.2) and*Bougainvillea spectabilis*(Fig.6).

Spiny pollen

Asteraceae (Fig.7, 8) members & Malvaceae members shows spiny pollengrains.

4. Conclusion

A considerable variation in pollen morphology was observed among the investigated species. The similarities in their structure showed inter species relationships and the reason for them to be the same genus, while differences in their pollen characters gives information about their existence in distinct genus. The result indicates that morphological analysis of pollen grains serves as a taxonomical key for analysis of different plants, and also which may use as a powerful tool in the field of embryology, pollination ecology and forensic science.

Each species is unique in pollen morphology, species belonging to same family also differ in pollen morphology. Pollen characters such as pollen size, shape, aperture, were evaluated. These details are important in establishing evolutionary relationships among the plants, and also which may help in identifying the members of a taxon, plant community structure in a place.

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Table 1

Sl. No	Name of the species	Pollen Type and Character
1	*Acanthusilicifolus*	Grains are 3-zonocolpate, prolate, exine reticulate, operculum present.
2	*Caesalpiniapulcherrima*	Oblate to spheroidal shape, Isopolar, tricolpate, colporate, sunkenaperture.
3	Caricapapaya	Tricolpate, oblate spheroidal to prolate, bireticulateexine
4	*Catharanthusroseus*	Subprolate, aperture colporus, tricolpate, exine stratificationobscure.
5	*Centrosemamolle*	Grains are spheroidal, tricolporate, exine reticulate,tectate.
6	*Cosmossulphureus*	Spherical, tricolporate, exine tectate, conspicous spines, echinate ornamentation,polyporous
7	*Bougainvilleaspectabilis*	Isopolar, tricolpate, zonocolpate, oblate, sculpturingreticulate.
8	*Hibiscus rosa-sinensis*	Grains spherical, apopolar, periporate, pantoporate, baculate, polyporous
9	*Ixora coccinea*	Subprolate shape ,Colpate, Isopolar , colporous.
10	*Kandelia candel*	Rounded triangular, grains are 3 zono-colpate, exine punctate.
11	*Mollugo oppositifolia*	Subprolate, triporate, colpate. exine punctate.
12	*Portulaca grandiflora*	Spheroidal, aperture sunken, colpus, colpate, pantocolpate.
13	*Tithonia diversifolia*	Polypantoporate, spheroidal, spines are fairly long with pointed end, pores are densely situated, polyporous.
14	*Turnera subulata*	Spheroidal shape, tricolporate, polyporous.

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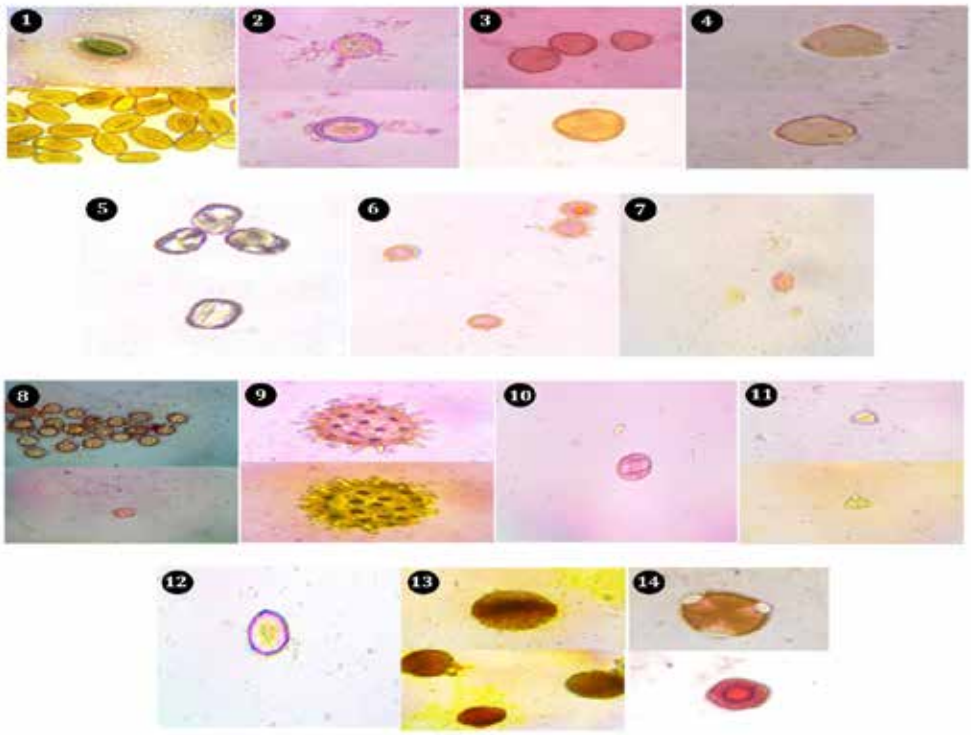

1) Acanthus ilicifolius 2) Carica papaya 3) Caesalpinia pulcherrima 4) Centrosema molle 5) Catharanthus roseus 6) Bougainvillea spectabilis 7)Cosmos sulphureus 8) Tithoniadiversifolia 9) Hibiscus rosa- sinensis 10) Ixora coccinea 11) Kandelia kandel 12) Mollugooppositifolia 13) Portulaca grandiflora 14) Turnera subulate

OP-04

Molecular docking studies of some bio-active components of cinnamon against SARS-CoV-2

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Abstract

The novel Corona Virus Disease (COVID-19) outbreak affected all over the world. WHO declared COVID-19 as a pandemic since it is transmitting exponentially. It is caused by an RNA virus with a positive sense single strand. In this study, we explored the binding interaction of some bioactive compounds of 'Cinnamon', a medicinal plant with COVID-19 main protease by molecular modelling and molecular docking studies to check whether it is a potential candidate in anti-viral drug design against SARS-Co-V-2. The 3D structure of COVID-19 main protease (PDB ID: 6LU7) is retrieved from the Protein Data Bank (PDB). The bioactive compounds Gallic acid, Caffeic acid, Cinnamic acid, Protocatechuic acid, Coumarin, Ferulic acid and Vanillic acid are identified from the literature survey and the respective 3D structures were taken from PubChem. The modelling and docking doneon AutoDock software. The binding energies for all the above molecules are calculated and all bind to 6LU7 with a favourable negative binding energy and gallic acid showing more affinity.

Key words: Bioactive molecules, Molecular modelling, SARS-CoV-2.

1. Introduction

The human being on the earth faced very difficult situation and passed through a critical stage due to the recent outbreak of COVID-19[1]. The daily life of people affected too much and the world economy facing challenges[2]. The governments take so many measures to reduce the transmission of disease and flatten the curve [3,4]. The COVID-19 disease affected all aspects of the human being on the blue planet[5]. Up to this time there is no antiviral drug available against SARS CoV-2. So there is an urgency to discover a new drug against COVID-19, but developing an antiviral drug is not too easy. The researchers and scientists all around the world are seriously engaging to develop drug candidate or vaccine against COVID-19. The research is going in two directions first upon repurposing the existing drug candidates and secondly discovering a new drug candidate. The repurposing of existing drugs will shorten

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the time of drug discovery and if it became success the urgency of drug against COVID-19 will become easy[6]. In the history so far reported many viral diseases including malaria, HIV/AIDS, Sever Acute Respiratory Syndrome (SARS), Middle East Respiratory Syndrome (MERS), etc. and some drugs against these diseases were developed and we are interesting to study the molecular level understanding of the action of these drugs against SARS CoV-2 by a wide range of methods including invitro, Insilco, crystallographic, computational, pharmacodynamics and pharmacokinetics[7,8]. The homology modelling, molecular modelling, and molecular dynamics simulation studies will shorten the time and we will get an atomic level information about the action of drug molecule[9,10]. Our country is blessed with many medicinal plants and it consisting of so many small molecules[11,12.] Searching for an efficient lead molecule against SARSCoV-2 from the mother nature is very important[13]. In this study, we explored the binding interaction of some bioactive compounds of 'Cinnamon', a medicinal plant with COVID-19 main protease by molecular modelling and molecular docking studies to check whether it is a potential candidate in anti-viral drug design against SARS-Co-V-2.

Kerala, the land of "God's own country", is famous for spices and herbs[14]. Cinnamon (figure.1.) a popular spice used in many ayurvedic medicines and as aromatic condiment in foods and beverages[15]. Cinnamon consists of many bioactive components like vanillic acid, gallic acid, protocatechuic acid, caffeic acid, ferulic acid, cinnamic acid, coumarin etc[16,17]. The medicinal properties of cinnamon is due to the presence of this bioactive components. Cinnamon has many medicinal properties including ant-oxidant, anti-inflammatory, anti-diabetic, anti-microbial, ant-obesity and ant-cancer properties[18-22].

Figure 1. *Cinnamomum verum*: Dried bark strips, bark powder and bark flowers of the tree.

2. Materials and Methods

Vanillic acid

Vanillic acid (4-Hydroxy-3-methoxybenzoic acid) used as a flavouring agent (Fig.2.). Other names of vanillic acids are 4-Hydroxy-m-anisic acid and vanillate. It has many medicinal properties[23, 24]. The 3D structure of vanillic acid is downloaded from PubChem and optimized using Gaussian 03 software.

Gallic acid

Gallic acid (Fig.3) is a trihydroxybenzoic acid. It has many medicinal properties[25, 26]. The 3D structure of vanillic acid is downloaded from PubChem and optimized using Gaussian 03 software.

Protocatechuic acid

Protocatechuic acid (PCA) is a phenolic acid and has structural similarity with other phenolic acids (Fig.4). The IUPAC name is 3, 4-Dihydroxybenzoic acid. Protocatechuic acid has many medicinal properties including ant-oxidant, anti-inflammatory, and anticancer effects[27,28]. The 3D structure of Protocatechuic acid is downloaded from PubChem and optimized using Gaussian 03 software.

Caffeic acid

Caffeic acid (Fig.5.) is found in many plants and is present in foodssuch as coffee, wine, and tea. Caffeic acid has many medicinal effects like ant-oxidant, anti-inflammatory, and anticancer properties[29,30]. The 3D structure of caffeic acid is downloaded from PubChem and optimized using Gaussian 03 software.

Ferulic acid

Ferulic acid (Fig.6) is abundant phytochemical found in plant cell walls. Ferulic acid has many medicinal effects like anti-microbial, ant-oxidant, anti-inflammatory, and anticancer properties[31-33]. The 3D structure of ferulic acid is downloaded from PubChem and optimized using Gaussian 03 software.

Cinnamic acid

Cinnamic acid (Fig.7)is naturally occurs in a number of plants. Cinnamic acid has anti-microbial, ant-oxidant, anti-inflammatory, and anticancer properties[34-36]. The 3D structure of cinnamic acid is downloaded from PubChem and optimized using Gaussian 03 software.

Coumarin

Coumarins (2H-1-benzopyran-2-one plants and is known as 1, 2-benzopyrone or less commonly as o-hydroxycinnamic acid[37]. Coumarins has anti-microbial, ant-oxidant, anti-inflammatory, ant-viral and anticancer properties[38-40]. The 3D structure of cinnamic acid is downloaded from PubChem and optimized using Gaussian 03 software.

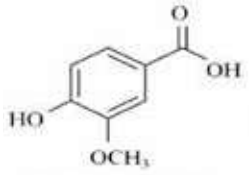

Figure 2: Structure of Vanillic acid

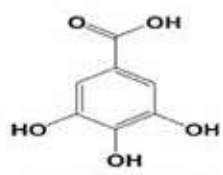

Figure.3. 2D Structure of Gallic acid

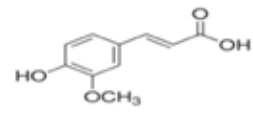

Figure.6. Structure of Ferulic acid

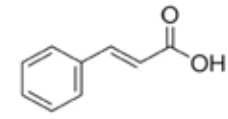

Figure.7. Structure of Cinnamic acid

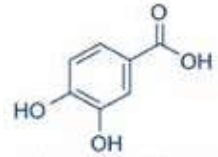

Figure.4. 2D Structure of Protocatechuic acid

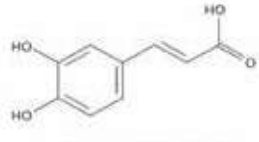

Figure.5. 2D Structure of Caffeic acid

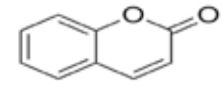

Figure.8. 2D structure of coumarin

COVID-19 main protease

The crystal structure of Covid-19 protease (Fig.9) downloaded from Protein Data Bank(PDB ID: 6LU7) and prepared for docking studies as per Auto Dock protocol[41].

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AutoDock

AutoDock, the molecular docking and modelling software widely using in the field of drug design and development and is freely available on the web downloaded and installed on the computer[42]. The bioactive small molecules and the macromolecule were prepared as per the docking protocols. The methods and protocols followed from our previous publications[14, 43-45].

3. Result and Discussion

The mode of binding and affinity of 6LU7 with small molecules were analysed using Autodock.

Binding analysis of vanillic acid with COVID-19 main protease

The biomolecular recognition of vanillic acid with 6LU7 was studied by Molecular Docking using AutoDock software. The hydrophobic interactions and hydrogen bond force play a major role in the biomolecular recognition of Vanillic acidwith 6LU7. Total 50 docking conformationswere obtained. The lowest binding energy is obtained for the 7th conformation and it is selected for the further analysis. The lowest binding energy in this 6LU7-Vanillic acid interaction is -7.30kcal/mol. The rmsd from reference structure is 66.858 A. Figure.10 shows the recognition of 6LU7 with Vanillic acid.

Table.1 shows the atoms in H bonds when the protein 6LU7 is binding with the ligand vanillic acid.

Table.1. Details of hydrogen bonding between Vanillic acid and 6LU7

RESIDUES IN H BONDS
6LU7: A: GLY143:HN
6LU7: A: CYS145:HN

Figure.11. illustrates the details of the vanillic acid binding with the near amino acid residues of 6LU7. LEU4, VAL3, ASN142, GLY143, CYS145, SER144, HIS163, and GLU166 are the various amino acid residue bind with the ligand (vanillic acid) molecule.

The statistical mechanical calculations were done for the binding process of vanillic acid with 6LU7 and tabulated as shown in table 3.2. The partition function, free energy, internal energy, entropy and temperature of the interaction were given in the table. The binding process between Vanillic acid and 6LU7 was spontaneous and entropy driven.

Table.2. The statistical mechanical analysis results of Vanillic acid with 6LU7

STATISTICAL MECHANICAL ANALYSIS						
Receptor	Ligand	Temperature(T) Kelvin(K)	Partition Function(Q) kcal/mol	Free energy(A) kcal/mol	Internal Energy(U) kcal/mol	Entropy(S) kcal/mol/K
6LU7	Vanillic acid	298.15K	50.50	-2323.69	-5.89	7.77

The clustering analysis were done for the docked conformations of Vanillic acid with 6LU7 as shown in figure.12. with rms deviation 2.0A^{O}.

Binding analysis of Gallicacid with COVID-19 main protease

The biomolecular recognition of gallic acid with 6LU7 was studied by Molecular Docking using AutoDock software. Thedocking studies give an insight into the hydrophobic interactions and hydrogen bond forcesin the recognition of 6LU7 with Gallic acid. From 50 docking conformations, the lowest binding energy is obtained for the 7th conformation. The lowest binding energy in this 6LU7-Gallic acid interaction is -8.07kcal/mol. The RMSD from reference structure is 66.381 A. Fig.13 shows the binding site of 6LU7 with Gallic acid.

Table. 3 shows the atoms in H bonds when the protein 6LU7 is binding with the ligand Gallic acid.

Table. 3. Atoms in h bonds.

ATOMS IN H BONDS
Gallicacid: : UNL1:H
6LU7: A: HIS163:HE2

Figure.14. illustrates the details of the binding forces Gallic acid with the surrounding amino acid residues. CYS145, GLY143, ASN142, SER144, LEU141, HIS163, LEU4, HIS172, GLU166 and PHE140 are the various amino acid residue bind with the ligand (Gallic acid) molecule.

The statistical mechanical calculations were done for the binding process of Gallic acid with 6LU7 and tabulated as shown in Table.4. The partition function, free energy, internal energy, entropy and temperature of the interaction were given in the table. The binding process between Gallic acid and 6LU7 was spontaneous and entropy driven.

Table. 4. The statistical mechanical analysis results of Gallic acid with 6LU7

STATISTICAL MECHANICAL ANALYSIS						
Receptor	Ligand	Temperature (T) Kelvin(K)	Partition Function(Q) kcal/mol	Free energy(A) kcal/mol	Internal Energy(U) kcal/mol	Entropy(S) kcal/mol/K
6LU7	Gallic acid	298.15K	50.50	-2323.73	-5.93	7.77

The clustering analysis were done for the docked conformations of Gallic acid with 6LU7 as shown in Figure.15 with rms deviation 2.0A^{O}.

3.3. Binding analysis of Protocatechuic acid with COVID-19 main protease

The biomolecular recognition of Protocatechuic acid with 6LU7 was studied by Molecular Docking using AutoDock software. The hydrophobic interactions and hydrogen bond forces are the main factors involving the binding mechanism of 6LU7 with Protocatechuic acid. The conformation with the lowest free binding energy was selected from out of the 50 50 obtained conformations and analyzed. The lowest binding energy is obtained for the 3rd conformation. The lowest binding energy in this 6LU7-Protocatechuic acid interaction is -6.65kcal/mol.

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The RMSD from reference structure is 54.495 A. Figure.16. illustrates the recognition of 6LU7 with Protocatechuic acid.

Figure.9. crystal structure of6LU7

Figure.10. Binding site of 6LU7 with Vanillic acid

Figure. 11. Binding mechanism of Vanillic acid with the 6LU7.

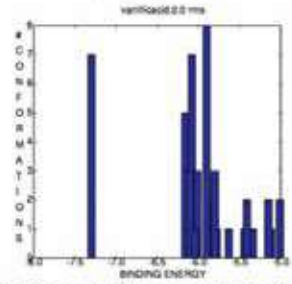

Figure. 12. Representative examples for clustering analysis of docked poses for Vanillic acid drug during binding to 6LU7

Figure.13. Binding site of 6LU7 with Gallic acid

Figure.14.Binding mechanism of Gallic acid with 6LU7

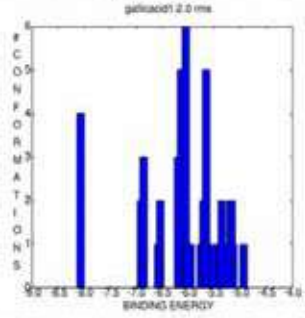

Figure.15. Representative examples for clustering analysis of docked poses for Gallic acid drug binding to 6LU7.

Figure. 16. Binding site of 6LU7 with Protocatechuic acid

Table.5 shows the atoms in H bonds when the protein 6LU7 is binding with the ligand Protocatechuic acid.

Table. 5. Atoms in h bonds.

ATOMS IN H BONDS
6LU7: A: ARG217:HH12
6LU7: A: GLN256:HE22
6LU7:A:THR304:HG1

Figure. 17. illustrates the details of the binding forces Protocatechuic acid with the surrounding amino acid residues. VAL303, GLN256, THR257, THR304, GLN306, ARG217 and ILE213 are the various amino acid residue bind with the ligand (Protocatechuic acid) molecule.

The statistical mechanical calculations were done for the binding process of Protocatechuic acid with 6LU7 and tabulated as shown in Table.6. The partition function, free energy, internal energy, entropy and temperature of the interaction were given in the table. The binding process between Protocatechuic acid and 6LU7 was spontaneous and entropy driven.

Table.6. The statistical mechanical analysis results of Protocatechuic acid with 6LU7

STATISTICAL MECHANICAL ANALYSIS						
Receptor	Ligand	Temperature (T) Kelvin(K)	Partition Function (Q) kcal/mol	Free energy (A) kcal/mol	Internal Energy(U) kcal/mol	Entropy(S) kcal/mol/K
6LU7	Protocatechuic acid	298.15K	50.48	-2323.46	-5.66	7.77

The clustering analysis were done for the docked conformations of Protocatechuic acid with 6LU7 as shown in figure 3.9 with rms deviation 2.0A^{O}.

3.4 Binding analysis of Caffeic acid with COVID-19 main protease

The biomolecular recognition of Caffeic acid with 6LU7 was studied by Molecular Docking using AutoDock software. The docking suggests hydrophobic interactions and hydrogen bond force dominated the recognition of 6LU7 with Caffeic acid. The lowest binding energy is obtained for the 1st conformation out of 50 conformations. The lowest binding energy in this 6LU7-Vanillic acid interaction is-6.59kcal/mol. The RMSD from reference structure is 65.852 A. Figure.19 shows the binding of Caffeic acid with 6LU7.

Table.7 shows the atoms in H bonds when the protein 6LU7 is binding with the ligand Caffeic acid.

Table.7. Atoms in h bonds.

ATOMS IN H BONDS
Caffeicacid::UNL1:H

Figure.20. illustrates the details of the binding forces Caffeic acid with the surrounding amino

acid residues. LYS97, ASN95, TRP31, MET17, VAL18, GLY120, ALA70, GLY71,GLN59and GLN19 are the various amino acid residue bind with the ligand (Caffeic acid) molecule.

The statistical mechanical calculations were done for the binding process of Caffeic acid with 6LU7 and tabulated as shown in table.8. The partition function, free energy, internal energy, entropy and temperature of the interaction were given in the table. The binding process between Caffeic acid and 6LU7 was spontaneous and entropy driven.

Table.8. The statistical mechanical analysis results of Caffeic acid with 6LU7.

STATISTICAL MECHANICAL ANALYSIS						
Receptor	Ligand	Temperature (T) Kelvin(K)	Partition Function(Q) kcal/mol	Free energy(A) kcal/mol	Internal Energy(U) kcal/mol	Entropy(S) kcal/mol/K
6LU7	Caffeic acid	298.15K	50.49	-2323.59	-5.79	7.77

The clustering analysis were done for the docked conformations of Caffeic acid with 6LU7 as shown in figure.21 with rms deviation 2.0A^{O}.

3.5.Binding analysis of Ferulic acid with COVID-19 main protease

The biomolecular recognition of Ferulic acid with 6LU7 was studied by Molecular Docking using AutoDock software. The lowest binding energy is obtained for the 1st conformation. The lowest binding energy in this 6LU7-Ferulic acid interaction is -6.63kcal/mol. The RMSD from reference structure is 68.706 A. Figure.22 shows the binding site of 6LU7 with Ferulic acid.

Table.9 shows the atoms in H bonds when the protein 6LU7 is binding with the ligand Ferulic acid.

ATOMS IN H BONDS
Ferulicacid::UNL1:H
6LU7: A: VAL202:HN

Figure.23 illustrates the details of the binding forces Ferulic acid with the surrounding amino acid residues PRO241, GLU240, PRO108, GLY109, HIS246, VAL202, GLN110, SN203, ILE200 and THR201 are the various amino acid residue bind with the ligand (Ferulic acid) molecule.

The statistical mechanical calculations were done for the binding process of Ferulic acid with 6LU7 and tabulated as shown in Table.10. The partition function, free energy, internal energy, entropy and temperature of the interaction were given in the table. The binding process between Ferulic acid and 6LU7 was spontaneous and entropy driven.

Table.10: The statistical mechanical analysis results of Ferulic acid with 6LU7

STATISTICAL MECHANICAL ANALYSIS						
Receptor	Ligand	Temperature (T) Kelvin(K)	Partition Function(Q) kcal/mol	Free energy(A) kcal/mol	Internal Energy(U) kcal/mol	Entropy(S) kcal/mol/K
6LU7	Ferlic acid	298.15K	50.50	-2323.69	-5.89	7.77

The clustering analysis were done for the docked conformations of Ferulic acid with 6LU7 as shown in figure.24 with rms deviation 2.0A^{O}.

3.6.Binding analysis of Cinnamic acid with COVID-19 main protease

The biomolecular recognition of Cinnamic acid with 6LU7 was studied by Molecular Docking using AutoDock software. The lowest binding energy in this 6LU7-Cinnamic acid interaction is-5.96kcal/mol. The RMSD from reference structure is 80.458 A. Figure.25 shows the binding site of 6LU7 with Cinnamic acid.

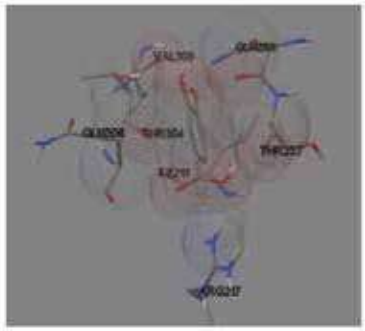

Figure. 17. Binding mechanism of Protocatechuic acid with 6LU7.

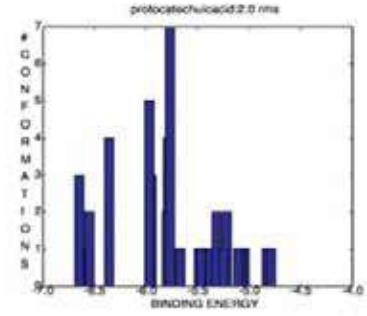

Figure.18. Representative example of clustering analysis of docked poses of Protocatechuic drug

Figure. 19. Binding site of 6LU7 with Caffeic acid.

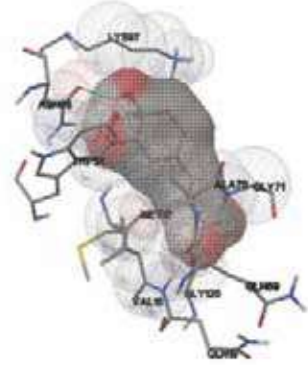

Figure. 20. Binding mechanism ofCaffeic acid with 6LU7.

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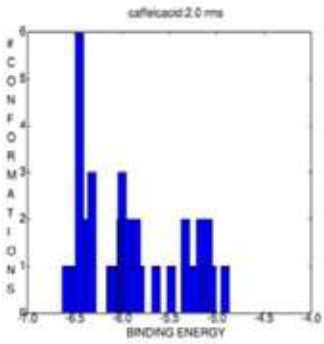

Figure. 21. Representative examples for clustering nalysis of docked poses for Caffeic acid drug binding to 6LU7.

Figure.22. Binding site of 6LU7 with Ferulic acid

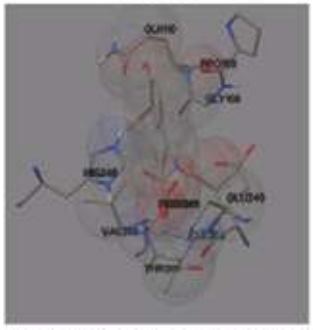

Figure.23. Binding mechanism of Ferulic acid with 6LU7

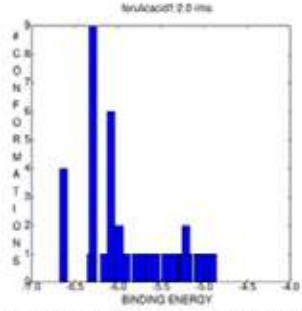

Figure.24. Representative examples for clustering analysis of docked poses for Ferulic acid drug binding to 6LU7.

Table 3.11 shows the atoms in H bonds when the protein 6LU7 is binding with the ligand Cinnamic acid.

Table 3.11: Atoms in h bonds.

ATOMS IN H BONDS
6LU7: A:PHE181:HN

Figure.26 illustrates the details of the binding forces Cinnamic acid with the surrounding amino acid residues. GLY179, CYS85, ASN180, PHE181, VAL186, PHE185, PRO184 and GLY183 are the various amino acid residue bind with the ligand (cinnamic acid) molecule.

The statistical mechanical calculations were done for the binding process of Cinnamic acid with 6LU7 and tabulated as shown in Table.12. The partition function, free energy, internal energy, entropy and temperature of the interaction were given in the table. The binding process between Cinnamic acid and 6LU7 was spontaneous and entropy driven.

Table. 12: The statistical mechanical analysis results of Cinnamic acid with 6LU7

STATISTICAL MECHANICAL ANALYSIS						
Receptor	Ligand	Temperature (T) Kelvin(K)	Partition Function (Q) kcal/mol	Free energy(A) kcal/mol	Internal Energy(U) kcal/mol	Entropy(S) kcal/mol/K
6LU7	Cinnamic acid	298.15K	50.44	-2323.97	-5.17	7.77

The clustering analysis were done for the docked conformations of Cinnamic acid with 6LU7 as shown in figure.27 with rms deviation 2.0A^{O}.

3.7 Binding analysis of Coumarin with COVID-19 main protease

The biomolecular recognition of Coumarin with 6LU7 was studied by Molecular Docking using AutoDock software. The lowest binding energy in this 6LU7-Coumarin interaction is -6.26kcal/mol. The RMSD from reference structure is 54.388 A. Figure.28 shows the binding site of 6LU7 with Coumarin.

Table.13 shows the atoms in H bonds when the protein 6LU7 is binding with the ligand Coumarin.

Table 3.13: Atoms in h bonds.

ATOMS IN H BONDS
6LU7: A: GLN256:HE22

Figure. 29 illustrates the details of the binding forces Coumarin with the surrounding amino acid residues. THR257, GLN256, ARG217, THR304, GLN306, VAL303, ILE213 and VAL 212 are the various amino acid residue bind with the ligand (Coumarin acid) molecule.

The statistical mechanical calculations were done for the binding process of coumarin with 6LU7 and tabulated as shown in Table.14. The partition function, free energy, internal energy, entropy and temperature of the interaction were given in the table. The binding process between Coumarin acid and 6LU7 was spontaneous and entropy driven.

Table.14: The statistical mechanical analysis results of Coumarin with 6LU7

STATISTICAL MECHANICAL ANALYSIS						
Receptor	Ligand	Temperature (T) Kelvin(K)	Partition Function (Q) kcal/mol	Free energy(A) kcal/mol	Internal Energy(U) kcal/mol	Entropy(S) kcal/mol/K
6LU7	Coumarin	298.15K	50.47	-2323.33	-5.53	7.77

The clustering analysis were done for the docked conformations of Coumarin with 6LU7 as shown in figure. 30 with rms deviation 2.0A^{O}.

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Figure.25. Binding site of 6LU7 with Cinnamic acid

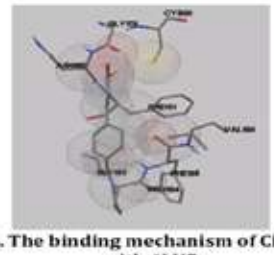

Figure.26. The binding mechanism of Cinnamic acid with 6LU7

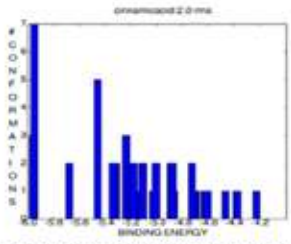

Figure. 27. Representative examples for clustering analysis of docked poses for Cinnamic acid drug binding to 6LU7.

Figure.28. Binding site of 6LU7 with Coumarin

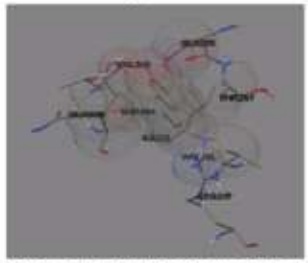

Figure.29. The detailed binding mechanism of Coumarin with 6LU7.

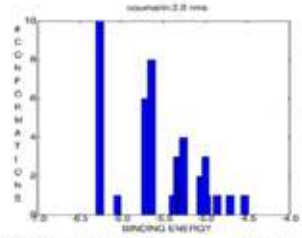

Figure 30. Representative examples for clustering analysis of docked poses for Coumarin drug binding to 6LU7.

The overall result of statical mechanical analysis of the seven studied ligand molecules with 6LU7 are given in the Table15. From the table it is clear that the negative binding energy is maximum for Gallic acid(-8.07kcal/mol) and least for Cinnamic acid (-5.96kcal/mol) at 298.15K.

Table15: Overall result of statistical mechanical analysis of the studied ligands with 6LU7.

Sl. No	Recep-tor	Ligand	Tempe rature (T) K	Partition Function (Q) kcal/mol	Free Energy (A) kcal/mol	Internal Energy (U) kcal/mol	Entropy (S) kcal/mol/K	Binding Energy Kcal/ Mol
1	6LU7	vanillic acid	298.15	50.50	-2323.69	-5.89	7.77	-7.30
2	6LU7	Gallic acid	298.15	50.50	-2323.73	-5.93	7.77	-8.07
3	6LU7	Protocatechuic acid	298.15	50.48	-2323.46	-5.66	7.77	-6.65
4	6LU7	Caffeic acid	298.15	50.49	-2323.59	-5.79	7.77	-6.59
5	6LU7	Ferulic acid	298.15	50.49	-2323.56	-5.76	7.77	-6.63
6	6LU7	Cinnamic acid	298.15	50.44	-2323.97	-5.17	7.77	-5.96
7	6LU7	Coumarin	298.15	50.47	-2323.33	-5.53	7.77	-6.26

4. Conclusion

The molecular docking studies of some bio-active component of cinnamon against the corona virus, SARS-CoV-2 has been done using AutoDock Software. The interaction between the bioactive molecules- Ferulic acid, Vanillic acid, Caffeic acid, Gallic acid, Protocatechuic acid, Cinnamic acid and Coumarin with the protein 6LU7 are explored. Docking was done for 50 conformations, the lowest free binding energy conformation selected and further analysis carried out. The binding energy of each protein-ligand interaction is analyzed. If the binding energy is more negative, then the affinity to bind ligand with protein is more. The interactions of 6LU7 with different ligands are studied. From the study, it is clear that 6LU7 binding with Gallic acid shows more negative binding energy (-8.07kcal/mol) than other ligands. Therefore, binding affinity is more for Gallic acid with 6LU7. The cluster analysis of conformation and macromolecule drug interaction for lowest binding energy is also analyzed. The various amino acid residues that bind with ligand molecules are noted. The results suggest that the studied molecules can use further studies for the development of ant-viral drugs against SARS-CoV2.

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OP-05

The impact of salinity on the distribution of aquatic cyanobacteria

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Abstract

Cyanobacteria, the primitive photoautotrophic prokaryotes, are found distributed in almost all habitats. Several species of cyanobacterium are found distributed in freshwater, estuarine & marine environments. It is observed that certain species are present in all three aquatic ecosystems in spite of their wide salinity variations. The hypersaline condition affects the growth, photosynthetic efficiency and alters several biochemical reactions in cyanobacteria. High salinity causes both osmotic and ionic stress, which causes oxidative damage to the cells. In this review, the influence of salinity on the distribution and adaptation of aquatic cyanobacteria in various saline environments is discussed.

Key words: Cyanobacteria, salinity, stress, distribution, adaptation.

1. Introduction

Cyanobacteria or blue-green algae, one of the largest groups of photosynthetic prokaryotes, occur in a wide range of terrestrial and aquatic environments all over the world. They form dominant members of freshwater, marine, and brackish algae throughout the world. It mostly found on rocks, soils, and in symbioses with plants and fungi. These prokaryotes represent some of the most ancient life form on earth. Cyanobacteria are often called "blue-green algae" and are named after the dominant blue pigment phycocyanin, which, together with chlorophyll a and other pigments, is used to capture light for photosynthesis (Fogg *et al*., 1973). These organisms have different types of adaptations which allow them for persistence, optimal growth, and support them by the ability to outcompete algae during good conditions. In general, many species produce hidden cells or components that remain inactive until the condition become favourable. Some species have specific cells which are able to convert nitrogen gas into nitrogen fixation forms (Awatef Saad & Ahmed Atia, 2014).

Salinity is considered to be a controlling factor of cyanobacteria in general (Kononen, 1992; Lehtimäki*et al*., 1994), affecting photosynthesis, the functioning of the plasma membrane (Singh et al., 2002, Schubert et al., 1993) and therefore, resulting in oxidative

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stress and programmed cell death caused by changes in gene expression and protein synthesis (Moisander et al., 2002, Srivastava et al., 2005). Salt stress or salinity stress is one type of osmotic/ ionic stress, which results in a dramatic increase in the concentration of inorganic salts. Exposure of cells to high salt concentrations in the surrounding environment puts the organism under two major threats -the water potential becomes lower while osmotic pressure increases and increased amount of ion concentrations which are toxic to the cells. The cyanobacterial cell maintains an equilibrium of water influx necessary for keeping the cells turgid, and to achieve this the osmotically active compound in the cells should be higher in concentration than that of the surroundings (Ladas & Papageorgiou, 2000).

In addition, the cyanobacterial morphology and life cycle vary in response to fluctuations in salinity (Iranshahi et al., 2014), as well as the turgor pressure on the cell (Ladas and Papageorgiou, 2000). It has been demonstrated that *Nodulariaspumigena* tolerates a large variation in water salinity (0–35 ppm) (Blackburn et al., 1996).Salinity has been suggested as controlling factor for blooms also, especially for N2-fixing cyanobacteria in estuaries. Moisander *et. al* (2002) studied the effect of salinity on the growth, N2 fixation, and photosynthetic activities of estuarine and freshwater isolates of heterocystous bloom-forming cyanobacteria.

Cyanobacteria have the remarkable tolerance potential to adjust to several environmental stresses such as heat, salinity and osmotic changes which are frequently encountered in nature.The understanding of the complex physiology and molecular biology of cyanobacterial acclimation to salt stress becomes increasingly important because cyanobacteria will be used for the biotechnological production of fuels and chemical feedstocks in the future.In this review, the influence of salinity on the distribution and adaptation of aquatic cyanobacteria in various saline environments is discussed.

Distribution of Cyanobacteria

Tropical aquatic ecosystems are characterized by the diversity and magnitude of photosynthetic microflora of which the role of cyanobacteria is very significant in initiating and supporting the food chain. These blue-greens of nature occur in all the habitats freshwater and marine and even withstand the stress caused by the fluctuation of environmental parameters such as light, temperature, pH and salinity. In the range of habitats which they occupy, the cyanobacteria are rivalled only by the bacteria (Fogg *et al*., 1973). Physiologically, cyanobacteria are well adapted to tie over physicochemical stresses such as hypersalinity, dessication, excessive irradiance and extreme temperature fluctuations.Elevated salinity was found to be one of the several constrains tocyanobacterial growth and expansion(Thomas *et al*, 1988).

Palaniselvan et al, 1998 studied the salinity and age induced changes in pigments and biomass production in the marine cyanobacterium, *Phormidium tenue* and observed that higher salinity induced higher mass production.Kaushik and Sharma (1997) studied the effect of salinity stress on the halotolerant form *Nostoc linckia*, *Westiellopsis prolifica* and *Tolypothrix ceylonica* and found that there was a 3-5% reduction in total protein content when these forms were grown in 100 mM NaCl. Many cyanobacteria appear to have an innate flexibility to adapt themselves to freshwater, estuarine and marine habitats (Subramanian, 1998).

Gotto *et. al* (1979) studied the effect of varying concentration of NaCl on the various strains of the genus *Anabaena sp.* and one of the marine strains did not show requirement for NaCl.

Atrie, V (1998) studied the response of a freshwater cyanobacterium *Lyngbya birgei* Smith to salinity. It was found that the growth of these organisms in the presence of NaCl depends on the availability of nutrients and the rate and mode of carbon fixation. Growth of cyanobacteria in response to salt stress showed contrary results (Atrie, 1998). In *Lyngbya*, low salt concentrations stimulated growth and photosynthesis but decreased at high comcentrations which was directly proportional to the chlorophyll content and the rate of photosynthesis.

Prabaharan (1988) reported that the marine Cyanobacterium *Phormidium valderianum* BDU 30501 was capable of growing in the salinities ranging from 0-90 ppt. The study conducted by Nagasathya and N. Thajuddin, (2008) showed a decline in the biodiversity was noticed in the higher salinities. The species richness was higher in lower salinities (48, 50, 62, 91 and 98 ppt) when compared with that of higher salinities (150 and 185 ppt). Thajuddin and Subramanian (1992, 1995) reported 50 species of 19 genera of cyanobacteria in saltpans with salinity over 50 ppt.

A study investigating the effect of salinity fluctuation on three submerged cyanobacterial mats at a transect on intertidal flat of the Arabian Gulf showed that in response to varying salinities, the cyanobacterial diversity decreased from lower to the upper tidal mat while other bacterial species exhibited opposite pattern. This shows that the microbial mats survive the salinity fluctuations by adjusting the diversity and functions of their microbial communities (Abed et al., 2007)rates of gross photosynthesis (GP.

Sellner et. al., 1988 attempted to understand the limitations of horizontal distributions of cyanobacterial blooms in Potomac River estuary in response to increasing salinity. In the three laborotary experiments conducted with daily salinity increase of 1-2 p.p.t. (thorugh adding NaCL or a complement of full sea salts) they observed that, in comparison to the control (no salt), species diversity varies in the cyanobacterial blooms. For instance, densities of Microcyslis spp. reduced while an increased growth has been observed for Aphanizomenon spp. (Sellner et al., 1988)

A recent study was reported on the distribution of cyanobacterial diversity from Rann of Kachchh (RoK), which is a large and understudied hypersaline transitional marsh between terrestrial and marine ecosystems, located in Gujarat, India. Through high throughput sequencing and metagenomic data it has been shown that the dominant cyanobacterial communities colonized in this region were majorly from Pseudanabaenales, and Oscillatoriales, followed by Nostocales and Chroococcales in mat sample. Therefore, interestingly in the underexplored and unique desert environment, the filamentous cyanobacteria were present in majority, specifically nitrogen fixers along with stress survivor Chroococcales(Patel et al., 2019)which is exposed to dynamic environmental changes such as salinity, temperature, and nutrients throughout the year. In this study, the pooled mat sample was examined for the cyanobacterial community structure using culture-dependent and culture-independent approaches. Taxonomic profiling was studied using amplicon sequencing that revealed the enrichment of Pseudanabaenales and Oscillatoriales by QIIME and MG-RAST, respectively. Other abundant orders were represented by Chroococcales, Nostocales, and unclassified cyanobacteria by both approaches. Nine cyanobacterial cultures were isolated from mat samples showing 90–98% similarities with available sequences in GenBank. The culture-dependent study suggested that mat was dominated by cyanobacterial orders such as

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Oscillatoriales—filamentous and Chroococcales—unicellular. Our results from the culture-dependent approach also indicated that despite high similarities in gene sequences, six cyanobacteria fall into the separate clade in the phylogenetic analysis that could be signs of evolution due to an extreme environment. Cultured isolates are correlated well with abundant taxa from amplicon sequencing. Further, protein profiling was done specifically for phycobiliproteins which will be helpful to elucidate their roles in light harvesting and energy transfer mechanism in the unique environment of RoK."","author":[{"dropping-particle":"","family":"Patel","given":"Hiral M.","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Rastogi","given":"Rajesh P.","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Trivedi","given":"Ujjval","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Madamwar","given":"Datta","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"3 Biotech","id":"ITEM-1","issue":"8","issued":{"date-parts":[["2019","8","1"]]},"page":"1-13","publisher":"Springer Verlag","title":"Cyanobacterial diversity in mat sample obtained from hypersaline desert, Rann of Kachchh","type":"article-journal","volume":"9"},"uris":["http://www.mendeley.com/documents/?uuid=d1100056-1406-36fc-8124-87c114b54f91"]}],"mendeley":{"formattedCitation":"(Patel et al., 2019. A similar study on cyanobacterial mats from hypersaline continental pools near the Empty Quarter desert, Oman showed a diversity coccoid and filamentous forms similar to those normally found in salt ponds and in intertidal flats (Abed et al., 2011).

A very recent report on diversity of heterocystous cyanobacteria growing at different salinities from freshwater mats of Giblin River (Tasmania) to metahaline and hypersaline mats of Shark Bay (Western Australia) exposed an unexpectedly large number and diversity of heterocystous cyanobacteria. Salinity plays an important role in the distribution and diversity of these blue-green algae, low salinity induced the growth of hetercystous cyanobacteria in freshwater mats while those mats in higher salinities favoures the growth of filamentous and unicellular non-heterocystous ones (Campbell et al., 2022).

Cyanobacterial adaptation to salinity

One of the major abiotic stress factors, that affect the growth of microbes and plants from time immemorial would be the high and changing concentrations of salt in the living environment. Especially for aquatic organisms mostly cyanobacteria, salinity - the total concentration of inorganic salts is a crucial environmental factor. During the evolution these microbes have developed numerous strategies to adapt and acclimatize to the changing salinity (Joset et al., 1996; Shu & Huang, 2021; Tandeau de Marsac & Houmard, 1993)cyanobacteria have a long history of adaptation to the Earth's environment. By evolving oxygen via photosynthetic reactions similar to those of plants and green algae, these prokaryotes were essential to the evolution of the present biosphere. They continue to make a large contribution to the equilibrium of the Earth's atmosphere by production oxygen and removing carbon dioxide. To survive in extreme or variable environments, cyanobacteria have developed specific regulatory systems, in addition to more general mechanisms equivalent to those of other prokaryotes or photosynthesis eukaryotes. Specific regulatory systems control the differentiation of specialized nitrogen-fixing cells and of cell types facilitating the dispersion of species. In the past decade, considerable progress has been made towards understanding

the expression of the cyanobacterial genome in response to variations in the intensity and spectral quality of incident light and in response to nutritional conditions, especially carbon, nitrogen and sulphur sources. These studies have provided insights into the relationships between carbon and nitrogen intermediary metabolism, and a start towards understanding of the interconnected pathways which lead from the perception of environmental signals to the regulation of enzyme activities and gene expression. Cyanobacterial regulatory mechanisms share common features with those of other prokaryotes, but are unique since these essentially photo-autotrophic organisms must maintain a proper cellular C/N balance, in spite of dailty variations in incident light. Thus an appropriate coordination between photosynthesis and other metabolic processes must be achieved through control of the catalytic activity of key enzymes by reducing equivalents and ATP produced by photosynthetic or respiratory electron transport. Recently discovered kinases/phosphatases act by post-translational modification of specific proteins which probably act as signal transducers or modulators of gene expression in a manner similar to the well-known two-component regulatory systems described in other bacteria. In this overview, we present our current knowledge on the molecular aspects of the biology of cyanobacteria, as well as on their mechanisms of resistance to metal ions and their responses to metabolic stress.","author":[{"dropping-particle":"","family":"Tandeau de Marsac","given":"Nicole","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Houmard","given":"Jean","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"FEMS Microbiology Reviews","id":"ITEM-1","issue":"1-2","issued":{"date-parts":[["1993","1","1"]]},"page":"119-189","publisher":"Oxford Academic","title":"Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms","type":"article-journal","volume":"10"},"uris":["http://www.mendeley.com/documents/?uuid=c355257e-425e-3dbe-b4d3-b28a6316a716"]},{"id":"ITEM-2","itemData":{"DOI":"10.1111/J.1399-3054.1996.TB00251.X","ISSN":"00319317","abstract":"Cyanobacteria, the only prokaryotes performing oxygenic photosynthesis and probable ancestors of chloroplasts, constitute valuable models for the study of the molecular mechanisms involved in tolerance to high salinity, or to its corollary, drought, a major agricultural problem. The critical demands of cyanobacteria exposed to high salinity, i.e., accumulation of osmoprotectors and extrusion of sodium ions, are met through immediate activation and/or long term (protein synthesis-dependent. In the recent years, with the development of new cytological, molecular and genome- wide techniques a better understanding of salt stress adaptations in cyanobacteria has been achieved. In this section we aim to address these in the context of adaptation strategies acquired by cyanobacterial cells in the molecular and biochemical levels.

It has been shown that sensing and signalling of salt stress in cyanobacterium is performed by a two- component signal transduction system comprising of a histidine protein kinase (HIK) with a response-regulator protein (RRE).Another pathway concerned with salt stress signalling is identified in *Synechocystis*were the extracellular signals were perceived by plasma membrane components and passed onto a signalling cascade consisting of salt over- sensitive (SOS) proteins (Huang et al., 2006).

Most of the microbes has acclimatized two strategies to compact highsalt stress – the "salt-in" and the "salt- out" strategies. The "salt-in" strategy works by incorporating large number of inorganic ions into the cytoplasm in order to ensure water influx into cells and turgor

pressure. While this requires very less energy expenditure only a few organisms- mostly true halophytes- incorporated "salt-in" strategy. Most microbes including cyanobacteria perform the "salt-out" strategy to alleviate high or changing salt concentrations. There are mainly three mechanisms used by cyanobacterial cells to achieve this – ion homeostasis, production of compatible solutes and physiological responses (Pade et al., 2015).

Cyanobacterial cells establish ion homeostasis by maintaining a high Na+ / K+ ratio as observed in many cyanobacteria such as *A. halophytica, Nodulariaharveyana*, and *Synechocystis* PCC 6803. As high cytoplasmic concentrations of Na+ are toxic, cyanobacteria has developed mechanisms to check the influx of Na+ as well as to remove excess Na+ entered the cell during a hypersaline condition (Hagemann, 2011)cyanobacteria have adapted to aquatic habitats with various salt concentrations. High salt concentrations in the medium challenge the cell with reduced water availability and high contents of inorganic ions. The basic mechanism of salt acclimation involves the active extrusion of toxic inorganic ions and the accumulation of compatible solutes, including sucrose, trehalose, glucosylglycerol, and glycine betaine. The kinetics of these physiological processes has been exceptionally well studied in the model Synechocystis 6803, leading to the definition of five subsequent phases in reaching a new salt acclimation steady state. Recent '-omics' technologies using the advanced model Synechocystis 6803 have revealed a comprehensive picture of the dynamic process of salt acclimation involving the differential expression of hundreds of genes. However, the mechanisms involved in sensing specific salt stress signals are not well resolved. In the future, analysis of cyanobacterial salt acclimation will be directed toward defining the functions of the many unknown proteins upregulated in salt-stressed cells, identifying specific salt-sensing mechanisms, using salt-resistant strains of cyanobacteria for the production of bioenergy, and applying cyanobacterial stress genes to improve the salt tolerance of sensitive organisms. © 2010 Federation of European Microbiological Societies.""author":[{"dropping-particle":"""fam ily":"Hagemann""given":"Martin""non-dropping-particle":"""parse-names":false"suffix":""}]"- container-title":"FEMS microbiology reviews""id":"ITEM-1""issue":"1""issued":{"date-parts ":[["2011""1"]]}"page":"87-123""publisher":"FEMS Microbiol Rev""title":"Molecular biology of cyanobacterial salt acclimation""type":"article-journal""volume":"35"}"uris":["http://www. mendeley.com/documents/?uuid=0b3ffeb7-e997-34e0-b8fe-b2f5b82d63e9"]}]"mendeley":{" formattedCitation":"(Hagemann, 2011.Sheathed cyanobacteria have been shown to produce exopolysaccharides (EPS), that enables chelation of a high percentage of

Na+ and there by immobilizing the excessive Na+ in the saline environmentand preventing their entry into the cell(Pathak et al., 2022). Along with Na+, K+ and Cl- influx/efflux is also regulated to keep the ionic balance in cyanobacterial cells.

Biosynthesis of compatible solutes which are low-molecular mass organic compounds, with no net charge and can be accumulated in high (molar) amounts without affection the cell metabolism, is another pathway adapted by cyanobacterial cells. In a salt stressed cell, accumulation of these compounds aid the organism by increasing the water potential to take up water, establishing or maintaining turgor pressure.Different types of compatible solutes have been identified and characterised in many cyanobacterial species including – sucrose and trehalose (in *Nostoc muscorum* strain 7119, *Synechococcus* 6301and *Anabaena variabilis* strain Lefevre 305), Glucosylglycerol (GG) (in *Synechococcus*, *Microcystis firma*strain Gromov

398, *Spirulina platensis*), and Glycine betaine (in *Aphanothece* sp. and *Synechococcus*sp. WH 8102)(Hagemann, 2011; Liang et al., 2020)cyanobacteria have adapted to aquatic habitats with various salt concentrations. High salt concentrations in the medium challenge the cell with reduced water availability and high contents of inorganic ions. The basic mechanism of salt acclimation involves the active extrusion of toxic inorganic ions and the accumulation of compatible solutes, including sucrose, trehalose, glucosylglycerol, and glycine betaine. The kinetics of these physiological processes has been exceptionally well studied in the model Synechocystis 6803, leading to the definition of five subsequent phases in reaching a new salt acclimation steady state. Recent '-omics' technologies using the advanced model Synechocystis 6803 have revealed a comprehensive picture of the dynamic process of salt acclimation involving the differential expression of hundreds of genes. However, the mechanisms involved in sensing specific salt stress signals are not well resolved. In the future, analysis of cyanobacterial salt acclimation will be directed toward defining the functions of the many unknown proteins upregulated in salt-stressed cells, identifying specific salt-sensing mechanisms, using salt-resistant strains of cyanobacteria for the production of bioenergy, and applying cyanobacterial stress genes to improve the salt tolerance of sensitive organisms. © 2010 Federation of European Microbiological Societies.","author":[{"dropping-particle":"","family":"Hagemann","given":"Martin","non-dropping-particle":"","parse-names":false,"suffix":""}],"container-title":"FEMS microbiology reviews","id":"ITEM-1","issue":"1","issued":{"date-parts":[["2011","1"]]},"page":"87-123","publisher":"FEMS Microbiol Rev","title":"Molecular biology of cyanobacterial salt acclimation","type":"article-journal","volume":"35"},"uris":["http://www.mendeley.com/documents/?uuid=0b3ffeb7-e997-34e0-b8fe-b2f5b82d63e9"]},{"id":"ITEM-2","itemData":{"DOI":"10.1128/AEM.02904-19","ISSN":"10985336","PMID":"31953341","abstract":"Salinity is one of the most important abiotic factors in various natural habitats of microbes. Cyanobacteria are the most widely distributed family of photosynthetic microorganisms in environments with fluctuating salinity. In response to salt stress, many cyanobacteria de novo synthesize compatible solutes to maintain osmotic balance in the cell. However, the regulation of intracellular accumulation of these compounds is still not well understood. The freshwater cyanobacterium Synechococcus elongatus PCC 7942 (Syn7942.

In *Anabaena* sp. During high salt conditions, expression of many stress-induced proteins occurred by the combined effect of transcriptional activation of genes and protein synthesis programme.Transcriptome analysis in salt stressed cells has revealed co-regulation of genes encoding subunits of protein complexes belonging to the photosystem (PS) I and II, phycobilisomes, ribosomes, or enzymes of the tricarboxylic acid (TCA). High salinity on the cell surface of *Synechocystis* 6803 cells induced proteins falling under the „Murein sacculus and peptidoglycan" category and indicates that salt stress induces a reorganization of cell wall structures, which possibly decrease its permeability for inorganic ions. These results shows that cyanobacteria adapt to salinity by modulating basic cell physiology(Klähn et al., 2021).

Increased salt stress also affects the nitrogen fixing capacity of cyanobacteria. It has been shown that Nitratereductase and glutamine synthetase are two major enzymes regulating salinitystress tolerance and cyanobacterial cell growth. In Nostoc calcicolea maximum inhibitionof nitrate reductase and glutamine synthetase was shown after 48 and 24 h under 2000 mM NaCl concentrations. Enhanced nitrate uptake has shown beneficial effect in tolerating salt stress in species like *Anabaena torulosa*, *Anabaena doliolum* and *Synechocystis*

sp. PCC 6803(Singh et al., 2022; Sun et al., 2021)which are found in all types of habitat. Due to their evolutionary history, a cyanobacterium with a cosmopolitan distribution ranges from saline soils to coastal swamps. Both the heterocystous and nonheterocystous cyanobacteria differently feature under high salinity stress. High salt concentration during stress reduces the water availability inside cytosol and increases the amount of inorganic ions. The hypersaline condition affects the cyanobacterial growth, photosynthesis, plasma membrane composition, and alteration of several other biochemical reactions. High salinity causes both osmotic and ionic stress, which causes oxidative damages. The generation of an antioxidative defense system could mitigate this, and cyanobacterial cell also has several defense mechanisms to acclimate under high salinity stress. Most of the work has focused on several specific salinity-sensing mechanisms and the identification of numerous proteins forming under salt stress. However, this chapter summarizes the adaptive mechanism of the cyanobacterial cell under salt tolerance and their bioreclamation properties for salt-affected soil will help in remediation of reconstructing green agriculture and promote the sustainable development of human society.","author":[{"dropping-particle":"","family":"Sin gh","given":"Rahul Prasad","non-dropping-particle":"","parse-names":false,"suffix":""},{"dro pping-particle":"","family":"Yadav","given":"Priya","non-dropping-particle":"","parse-name s":false,"suffix":""},{"dropping-particle":"","family":"Kujur","given":"Reena","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","family":"Pandey","given":"Ka pil Deo","non-dropping-particle":"","parse-names":false,"suffix":""},{"dropping-particle":"","fam ily":"Gupta","given":"Rajan Kumar","non-dropping-particle":"","parse-names":false,"suffix":""}], "container-title":"Cyanobacterial Lifestyle and its Applications in Biotechnology","id":"ITEM-1","issued":{"date-parts":[["2022","1","1"]]},"page":"253-280","publisher":"Academic Press","title":"Cyanobacteria and salinity stress tolerance","type":"article-journal"},"uris":["http://www.mendeley.com/documents/?uuid=7fc8ac20-fdac-317d-8bb9-713de6566540"]},{"id":"ITEM-2","itemData":{"DOI":"10.1038/s41396-021-01079-6","ISSN":"1751-7370","PMID":"34389794","abstract":"Ammonia oxidizers are key players in the global nitrogen cycle, yet little is known about their ecological performances and adaptation strategies for growth in saline terrestrial ecosystems. This study combined 13C-DNA stable-isotope probing (SIP

As a secondary response of ionic stress and water stress induced by salinity, the cyanobacterial cells undergo oxidative stress which results in the increases production of enzymes involved in oxidative stress responses(Klähn et al., 2021; Latifi et al., 2009). A recent study in cyanobacterium *A. sphaerica* shows oxidative bursts encountered by salt stressed cells. This resulted in increased SOD (Superoxide dismutase) and CAT (Catalase) activities and elevated biosynthesis of glutathione, proline, and sucrose in response to increasing exogenous NaCl. This suggested a robust and effective antioxidative defense mechanism in *A. sphaerica*to compact salt stress. In addition, this study also showed that the salt tolerance property of *A. sphaerica*coupled with nitrogen fixing ability makes it an excellent candidate for improving the microbial community structure in salt-affected paddy fields (Kharwar et al., 2022).

2. Conclusion

This review is aimed to provide an insight to the distribution of cyanobacteria in response to salinity stress and overview of adaptations in cyanobacteria. Fluctuations in salinity and high

salt content has been shown to affect the cyanobacterial diversity in diverse environments, including deserts and estuaries. This depicts the resilience shown by the cyanobacterial community in establishing at harsh environments and thereby balancing the concerned ecosystems.

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OP-06

Biomolecular recognition of Some Bioactive compounds of Jack Fruit with Tumour Necrosis Factor Alpha (TnF-α)

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Abstract

Jackfruit (*Artocarpus Heterophyllus*) is a very common fruit found in various parts of the world including Asian, African and South American countries. Jackfruit trees are of great utility, their different components being used for a variety of purposes. The jackfruit in itself is used as a food item in its different stages of growth. The ripened jackfruit consists of multiple layers, including the spiky outermost layer, peel, pulp, flakes, axis and seeds. It is rich in many bioactive components, carbohydrates, lectins, proteins, and dietary fibres. The jackfruit is known for its immense medicinal value, as it has anti-diabetic, anti-cancer, anti-inflammatory, and, antioxidant properties among several others. It is beneficial in managing heart disease, ulcers, dermatological issues and macular degeneration. In this work interaction of some important bioactive compounds presented in jack fruits such as Valtratum, Hallacridone, Garlic acid, and Carnosine with Tumour Necrosis Factor Alpha (TnF-α) were explored through AutoDock software. We identified the various bioactive components present in Jackfruit by literature survey. The 3D structures of bioactive components were downloaded from the PubChem database and optimized using Gaussian03 and GaussView software. The ligands were prepared for docking using Chimera software. The molecular docking studies of the selected ligand molecules with TnF-α were carried out using AutoDock software. The results were analyzed and the respective figures and graphs were plotted. Moreover, the binding affinities of different compounds were compared. From the study it is clear that TNFα binding with Hallacridone shows more negative binding energy (-7.56kcal/mol) than other ligands.

Keywords: Jack fruit, Biomolecular recognition, Tumour Necrosis Factor Alpha (TnF-α).

1. Introduction

Jackfruit (*Artocarpus Heteroph*yllus), very common fruit growing in various parts of the world including Asian, African and South American countries[1]. Jackfruit trees are of great utility, their different components being used for a variety of purposes. The jackfruit in itself is used as a food item in its different stages of growth. The ripened jackfruit consists of multiple layers, including the spiky outermost layer, peel, pulp, flakes, axis and seeds[2]. It is rich in many bioactive components, minerals, carbohydrates, proteins, lectins, and dietary fibres[3]. The jackfruit is known for its immense medicinal value, as it has antioxidant, anti-inflammatory,

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anti-diabetic, and anti-cancer properties among several others[4, 5]. It is beneficial in managing heart disease, ulcers, dermatological issues and macular degeneration[4]. The jack fruit on a tree is given in Figure.1 and the cross-sectional view of ripen jackfruit is given in Figure.2.

Fig 1. Jack fruit on tree

Fig. 2. The cross-sectional view of jack fruit

Jack fruit consist of many bioactive compounds and Phyto chemicals. In this work, the interaction of some important bioactive compounds presented in jack fruits such as Valtratum, Hallacridone, Garlic acid, and Carnosine with Tumour Necrosis Factor Alpha (TnF-α) were explored through AutoDock software. We identified the various bioactive components present in Jackfruit by literature survey. Tumour necrosis factor alpha (TNFα), the cytokine has very importance in medicine and drug designing. TNFα has role in regulation of immune cells. TNFα is a major target in addressing various diseases like cancers, Alzheimer's, inflammation, fever, etc.[6-11].

2. Materials

The three-dimensional crystal structure of (TNFα) was taken from the Protein Data Bank (PDB, ID: 2AZ5)(https://www.rcsb.org/pdb)as shown in Figure.3[11]. The molecules bind with TNFα, water molecules and other unnecessary molecules were removed and prepared for docking study. The 3D structure of ligands taken from PubChem and Cambridge Crystallographic Data Centre (CCDC) data basis[12].The following ligands are used for the computational studies

Gallic acid was downloaded from PubChem database. The structure is optimized using Gaussian 03 software. The optimized structure is given in Fig.4

Carnosine downloaded from PubChem database. The structure is optimized using Gaussian 03 software. The optimized structure is given in Fig.5

Hallacridonewas downloaded from PubChem data base. The structure is optimized using Gaussian 03 software. The optimized structure is given in Fig.6

The 3D structure of Valtratum downloaded from PubChem data base. The structure is optimized using Gaussian 03 software and is given on Fig.7.

Molecular Docking and Modeling Studies:

The mode of binding and interaction of gallic acid, carnosine, hallacridone, and valtratum with TnF-α were explored by molecular modelling and docking studies by using AutoDock software. The docking process was carried out as per our previous papers[13-16].

3. Result and Discussion

3.1 Binding analysis of Valtratum- TnF-α

The biomolecular recognition of Valtratum with TNF was studied by Molecular Docking using AutoDock software. From the total 25 conformations, one with the least binding energy was selected and analysed. The results reveal hydrogen bonds and hydrophobic interactions are the major factors of the binding mechanism of TNF with Valtratum. The lowest binding energy is obtained for the 10^{th} conformation. The lowest binding energy in this TNF-Valtratum interaction is -6.86kcal/mol. As shown in Figure 8, Valtratumwas inserted into the TnF-α with favourable binding energy.

Figure.9 illustrates the dtailed binding forces involved in Valtratum- TnF-αrecognition. ARG82, GLN125, LEU83, ASN92, PHE124, GLN126, GLU127, etc., are the various amino acid residue bind with the ligand (Valtratum) molecule.

3.2 Hallacridone– TnF-α

The biomolecular recognition of Hallacridone with TNF was studied by Molecular Docking using AutoDock software. From the total 25 conformations, one with the least binding energy was selected and analysed. The results reveal hydrogen bonds and hydrophobic interactions are the major factors of the binding mechanism of TNF with Hallacridone.The lowest binding energy is obtained for the 22^{nd} conformation. The lowest binding energy in this TNF-Hallacridon interaction is -7.56kcal/mol. As shown in Figure 10, Hallacridonwas inserted into the TnF-α.

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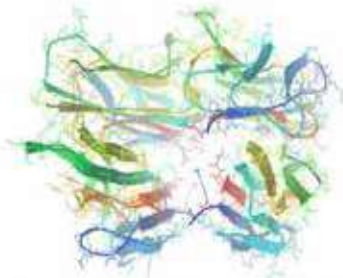

Figure 3. 3D structure of TnF-α(PDB Id:2AZ5)

Fig.4 Optimized structure of Gallic acid

Fig.5 Optimized structure of Carnosine

Fig.6. Optimized structure of Hallacridone

Fig.7.Valtratum

Fig.8. Binding site of TNFα with Valtratum.

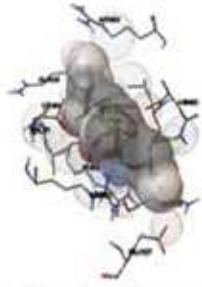

Figure 9: Detailed binding of Valtratum with TNF-alpha.

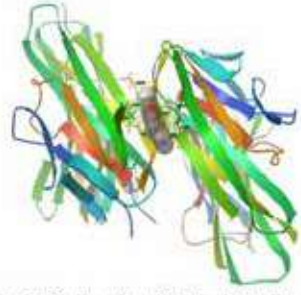

Fig.10. Binding site of TNFα with Hallacridone.

Figure.11. illustrates the detailed binding of hallacridone with TnF-α residues. GLN125, LEU93, ASN92, PHE124, VAL91, etc., are the various amino acid residue that binds with the ligand (Hallacridone) molecule.

3.3 Gallic acid - TnF-α

The biomolecular recognition of Gallic acid with TNF was studied by Molecular Docking using AutoDock software. From the total 25 conformations, one with the least binding energy was selected and analysed. The results reveal hydrogen bonds and hydrophobic interactions are the major factors of the binding mechanism of TNF with Gallic acid. The lowest binding energy is obtained for the 7th conformation. The lowest binding energy in this TNF- Gallic acid interaction is -5.54kcal/mol. As shown in Figure 12, Gallic acid was inserted into the TNF-α.

Figure 13. illustrates the details of the binding interaction of Gallic Acid with the nearby residues of TnF-α. ALA18, GLN149, GLU146, GLY148, PHE144, PRO20, SER147 VAL150, VAL17etc., are the various amino acid residue bound with the ligand (Gallic Acid) molecule.

3.4 Carnosine–TnF-α

The biomolecular recognition of Carnosine with TNF was studied by Molecular Docking using AutoDock software. From the total 25 conformations, one with the least binding energy was selected and analysed. The results reveal hydrogen bonds and hydrophobic interactions are the major factors of the binding mechanism of TNF with Carnosine. The lowest binding energy is obtained for the 25th conformation. The lowest binding energy in this TNF- Carnosine interaction is -5.11kcal/mol. As shown in Figure 14, Carnosine was inserted into the TNF-α.

Figure 15.illustrates the detailed binding of Carnosine with amino acid residues of TNFα. GLU116, LYS98, PRO100, SER99, etc., are the various amino acid residue bind with the ligand (Carnosine) molecule.

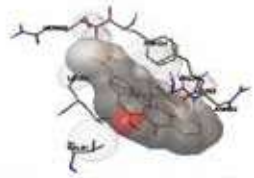

Figure 11. Detailed binding ofHallacridone with near residues of TnFα.

Fig.12.Binding site of TNFα with Gallic Acid.

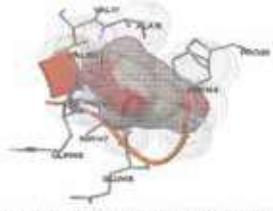

Figure 13. Detailed binding of Gallic acid with near residues of TnFα.

Fig.14.Binding site of TNFα with Carnosine.

Figure 15. Detailed binding of Carnosine with near residues of TnFα.

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The statistical mechanical calculations were done for the binding process of the ligands with TNFα and tabulated as shown in table 1.0. The partition function, free energy, internal energy, entropy and binding energy of the interaction were given in the table. The binding process between the ligands and TNFα was spontaneous and entropy-driven.

Table 1.The statistical mechanical analysis results of ligands with TNFα

Sl No	Receptor	Ligand	Partition Function (Q) Kcal/Mol	Free Energy (A)Kcal/Mol	Internal Energy(U) Kcal/Mol	Entropy (S)Kcal/ Mol/K	Binding Energy Kcal/Mol
1	TNFα	Valtratum	25.22	-1912.41	-5.29	6.40	-6.86
2		Hallacridone	25.30	-1914.12	-7.00	6.40	-7.56
3		Gallic Acid	25.20	-1911.79	-4.67	6.40	-5.54
4		Carnosine	25.15	-1910.69	-3.56	6.40	-5.11

*At temperature 298.15k

The overall result of the statistical mechanical analysis of the four studied ligand molecules with TNFα is given in Table 1. From the table, it is clear that the negative binding free energy is maximum for Hallacridone (-7.56 Kcal/mol) and least for Carnosine(-5.11 Kcal/mol) at 298.15K

4. Conclusion

The Jack fruit is a very large fruit available in Asian countries and some other parts of the World. It has many nutritional and medicinal properties. In the state of Kerala of India, jackfruit is available in every nuke and corner. The jack fruits constitute proteins, vitamins, minerals, fibres, carbohydrates, sugars, and different bioactive components. We identified some bioactive components contained in jack fruit by literature survey. The understanding of the molecular recognition of bioactive small molecules with bio-macromolecules like protein, DNA, and RNA has great importance in the area of drug discovery and drug design. In this work interaction of some important bioactive compounds presented in jack fruits such as Valtratum, Hallacridone, Gallic acid, and Carnosine with TNFα were explored through AutoDock software. The molecular modelling and docking reveal the hydrophobic interactions of TNFα with all the molecules. The hydrogen bonds have a great role in the recognition of studied bioactive components of jackfruit with TnF-α. From the total 25 conformations, one with the least binding energy was selected and analysed. If the binding energy is more negative, then the affinity to bind ligand with protein is more. The interactions of TNFα with different ligands are studied. From the study, it is clear that TNFα binding with Hallacridone shows more negative binding energy (-7.56kcal/mol) than other ligands. The molecular docking studies of the important bioactive components shows the promising result for future drug design and development.

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OP 07

Extraction and applications of natural dyes from *Sphagneticola trilobata* (L.) Pruski

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Abstract

Natural dyes are those that come from plants, invertebrates, or minerals. The bulk of natural dyes come from plant sources such as roots, berries, bark, leaves, and wood, as well as other biological sources like fungi. Recent years have seen a rise in the use of natural dyes in textile dyeing because many synthetic dyes are mutagenic, allergenic, and carcinogenic. In view of the ever-increasing health hazards due to synthetic dyes, research studies are now focusing on finding alternate methods for production of cheaper, environment-friendly and non-toxic dyes. This study aims to identify and extract the natural dyes from a weed from Kerala, standardization of procedure of natural dye extraction and application of natural dyes to the cotton fabrics. The dyes were extracted from the flowers of *Sphagneticolatrilobata*with different extracting media. The standardized procedures for extracting natural dyes from the above plants and its applications were described. So, the present study indicates that natural colorants from weeds could have great value in dyeing of textile fabrics. Moreover, the weed plants can be controlled by the over exploitation of these weeds from our surroundings for natural dye preparation.

Key words: Natural dyes, extraction, *Sphagneticola trilobata*, weeds

1. Introduction

The natural dyes from plants were traced long time ago. In India 450 plants are found to be good source of natural dye. For the extraction of natural dye different plant parts are used such as seeds, flowers, leaves and barks. From recent past, the use of synthetic dye exponentially increases in many important industries, such as textile, pharmaceutical, food processing etc. The synthetic dyes are easily available and show superior fastness properties over natural dye. However, though synthetic dye exhibits superior fastness properties, it produces many side effects on human body causing allergic reaction. Synthetic dye is not easily degradable and bio-accumulated in natural environment. It has been estimated that, nearly 10, 00,000 tons of synthetic dye were used per annum. The synthetic dye may cause pollution, skin diseases, health hazards to human and other important organisms. Hence the use of ecofriendly and biodegradable dye has main concern in worldwide (Patil et al., 2016; Prabhavathi et al., 2014). From recent past years, the use of synthetic dye exponentially increases in many important

industries, such as textile, pharmaceutical, food processing etc. The synthetic dye is easily available and show superior fastness properties over natural dye. However, though synthetic dye exhibits superior fastness properties, it produces many side effects on human body causing allergic reaction. Synthetic dye is not easily degradable and bio accumulated in natural environment. It has been estimated that, nearly 10, 00,000 tones of synthetic dye were used per annum (Meena Devi et al., 2013). The synthetic dye may cause pollution, skin diseases, health hazards to human and other important organisms (Samanta& Agrawal, 2009; Patil et al., 2016). Environmental pollution has become a major concern due to its deleterious effect on climate and living habitat. Though industrialization has led to the progress of mankind, it has also affected the environment due to use of chemicals and improper waste management (Das et al., 2016). Synthetic dyes are widely used in industries like textile, leather and also as commercial indicators. The production and use of these dyes have been reported to have toxic effects on the environment and living habitat (Divya et al., 2013; Pervaiz et al., 2016).

In view of the ever-increasing health hazards due to synthetic dyes, research studies are now focusing on finding alternate methods for production of cheaper, environment-friendly and non-toxic dyes (Satyanarayana & Chandra, 2013). Dyeing with white onion peel or turmeric gave very good UV protection functionalization to cotton fabrics (Karabulut&Atav, 2020).The dye from Chinese tallow tree is very promising and considered as the potential alternative candidate of synthetic dyes in the textile industry (Mia et al., 2022). A weed is any plant that grows uninvited. The many reasons for controlling weeds become more complex with the increasing development of technology. Plants become weeds as a function of time and place. Tall weeds on roadsides presumably were not problematic prior to the invention of the automobile. Modern weed control can be classified as mechanical, chemical, or biological. As a result, extracting natural colours from weeds aids in the elimination of such pests and benefits humans by lowering the usage of synthetic dyes and their associated risks.

2. Materials And Methods

1.Selection of materials

1A.Selection of plant materials for the extraction of dyes: Flowers of *Sphagneticolatrilobata*(L.) Pruski(Asteraceae) was selected.

1B. Selection of fabric: Light weight cotton fabric woven was purchased from local shop and cut into square pieces with the dimension of 5 × 5 cm

1C. Selection of scouring agent: 10% NaOH solution was used for bleaching of cotton fabric clothes in all the experiments.

1D. Selection of mordant: The mordants such as Potash alum (KAI $(SO_4)_2.12H_2O$), Stannous chloride ($SnCl_2.2H_2O$) and Copper sulphate ($CuSO_4$) purchased from Merck Life Science Pvt. Ltd. A natural mordant, Myrobalan (*Teminaliachebula*) powder purchased from local ayurvedic shop was also used.

1E. Selection of eco-friendly fixing agents:Lime juice was selected as eco-friendly fixing agent for all the experiments.

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2. Preparation of cotton fabrics for dyeing:

2A. Cotton scouring:The cotton fabric contains dust, oil and other impurities that interfere with the absorption of the dye. So, cotton fabrics were boiled in 10% NaOH solution for 10 min followed by drying.

2B. Pretreatment of cotton fabrics with myrobalan:Pre-treatment was required for cotton fabric as it had no affinity towards natural dyes. Myrobalan powder at different concentration were selected to enhance the dye uptake on the fabrics in various time intervals in the preliminary experiments.

2C. Pretreatment of cotton fabrics with mordants:Clothes after scouring and pretreatment with myrobalan were kept in mordant solution. 10% Copper sulphate, Potassium alum, Stannous chloride were used as mordant for preliminary experiments.

3. Optimization of Dye Extraction Methods:

Dye extraction methods such as acidic, alkaline, aqueous and acetone were tried for extraction of dye from the selected sources.

4. Optimization of concentration of source materials:

4A. Acidic extraction method: In this method, 5, 10, 20, 30,40, 50 g of crushed leaves and flowers of the above-mentioned plants were extracted separately in 100 mL of acidic solution (1% HCl solution) and the extraction was carried out by boiled at 100°C for 15 minutes. This extract collected was filtered by muslin cloth and used for dyeing.

4B. Alkaline extraction method: In this method, 5, 10, 20, 30,40, 50 g of crushed leaves and flowers of the above-mentioned plants were extracted separately in 100 mL of alkaline solution (1% NaOH solution) and the extraction was carried out by boiled at 100°C for 15 minutes. This extract collected was filtered by muslin cloth and used for dyeing.

4C. Aqueous extraction method: In this method, 5, 10, 20, 30,40, 50 g of crushed leaves and flowers of the above-mentioned plants were extracted separately in 100 mL of distilled water and the extraction was carried out by boiled at 100°C for 15 minutes. This extract collected was filtered by muslin cloth and used for dyeing.

4D. Acetone extraction method: In this method, 5, 10, 20, 30,40, 50 g of crushed leaves and flowers of the above-mentioned plants were crushed with 100ml acetone using mortar and pestle. The supernatant is collected and filtered using muslin cloth and used for dyeing.

5. Optimization of mordanting procedure:

5A. Optimization of the concentration of myrobalan:Pre-treatment was required for cotton fabric as it had no affinity towards natural dyes. Myrobalan powder at 5%,10%,20%,40% were selected to enhance the dye uptake.

5B. Optimization of the mordanting time with myrobalan:Myrobalan powder at different concentrations were selected to enhance the dye uptake on the fabrics for 15 min, 30 min, 60 min, 120 min in the preliminary experiments.

5C. Selection of mordants: Clothes after scouring were kept in 10% of copper sulphate, Potassium alum, Stannous chloride solutions for 30 minutes in preliminary studies. Stannous

chloride solution was used as the mordant for further studies due to its high mordanting capability.

5D. Optimization of mordant concentration: To optimize the concentration of mordants, four concentrations (1, 5, 10 and 20%) of Stannous chloride were tried with each material.

6. Optimization of Dyeing Method:

Optimization of Dyeing time for different plant extracts:To optimize the dyeing time, 50% acidic extract of *Sphagneticolatrilobata* were taken in five separate containers. The cotton clothes scoured with 10% NaOH solution followed by pretreatment with 5 % myrobalan and 5% stannous chloride were soaked in acidic extract at different time intervals as follows 15min, 30min, 60min, 120min, 24 hrs.

7. Post-treatment with fixing agents:

This is a post-treatment given to dyed fabrics to aid to enhance colour uptake. A Natural fixing agent, lime juice was selected for the treatment for 30 min in all the experiment. The cotton fabrics were allowed to shade dry.

8. Soaping-Off:

The dyed cotton cloths after post-treatment with lime juice were washed in 2% Teepol solution for 10 min to remove the loose dye on the fabric and then rinsed thoroughly in water and dried.

9. Colour matching:

The colour of different extracts and dyed cotton cloths were compared with standard RAL colour chart.

3. Results and discussion

The dye extracted from the fresh petals of *Sphagneticolatrilobata* with four different extraction solutions. Melon yellow dye was obtained by acid extraction method and Maize yellow dye was obtained by Alkaline extraction method. Light ivory dye was obtained by aqueous Extraction method, yellow orange dye was obtained by acetone method.

Table 1. Colour of dyes obtained by different extraction methods

Sl. No	Extractionmethods	Colourofthedye
1	Acid	Melon yellow
2	Alkali	Maize yellow
3	Aqueous	Light ivory
4	Acetone	Yellow orange

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Fig.1. Dyes obtained from *Sphagneticolatrilobata* by different extraction methods

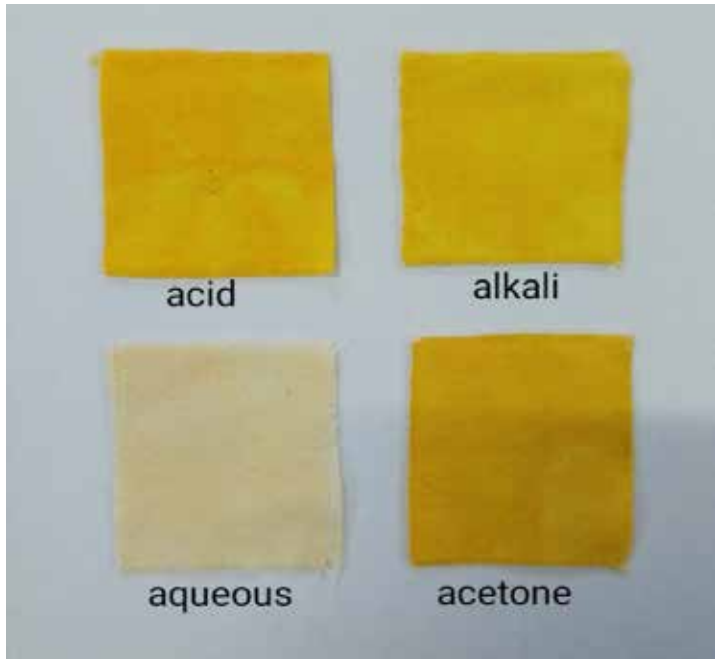

Fig. 2. Cotton clothes stained with dyes obtained by different extraction methods

Optimization of the concentration of myrobalan:

The maximum colour intensity was found at 5 % of myrobalan and no change in colour intensity was observed at higher concentrations. Hence 5 % of myrobalan was selected.

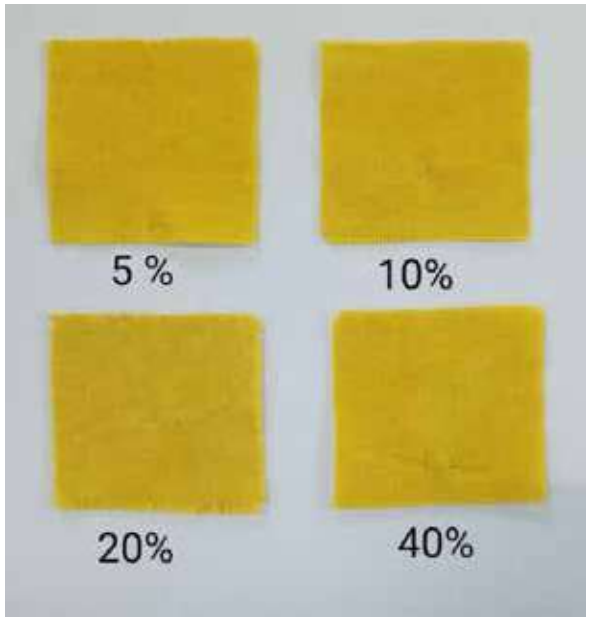

Fig. 3. Cotton clothes stained with acidic extraction at different concentration of myrobalan

Optimization of the mordanting time with myrobalan:

It was founded that the maximum colour intensity was at 5% of myrobalan for 30 mins. There is no increase in colour intensity thereafter. Hence 30 min was selected.

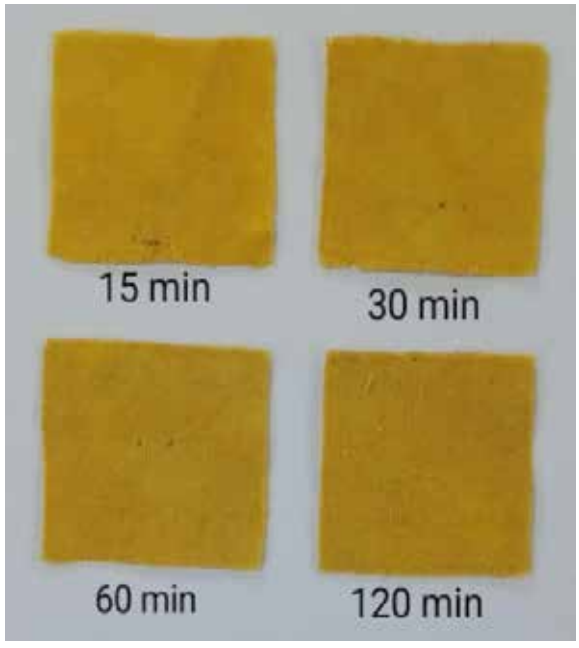

Fig. 4. Cotton clothes stained with 5% myrobalan in different time intervals

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Optimization of mordant concentration:

The maximum colour intensity was found at 5 % of Stannous chloride and no change in colour intensity was observed at higher concentrations. Hence 5 % Stannous chloride was selected as mordant.

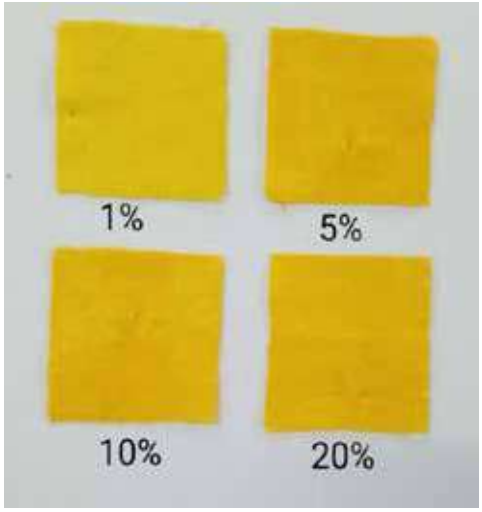

Fig. 5. Cotton clothes stained with acid extraction for different mordant concentrations

Optimization of dye material concentration:

The suitable concentration of material for the extraction of dye from *Sphagneticolatrilobata.*was found that 50g/100ml of 1 % acidic solution (50%).

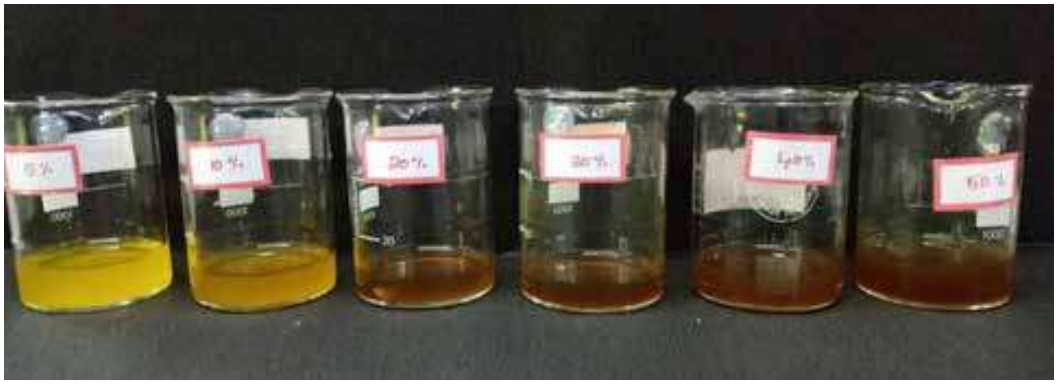

Fig. 6. Dye obtained by different concentration of materials

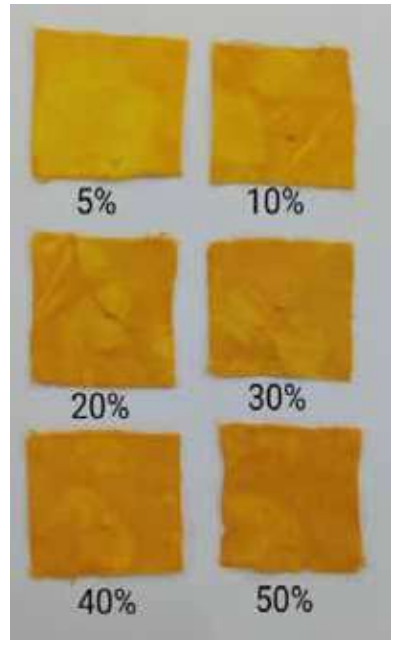

Fig. 7. Cotton clothes stained with dye obtained by different concentrations of materials

Optimization of Dyeing time for different plant extracts:

It was founded that the maximum colour intensity was observed for 1 hrs of dyeing time. There is no increase in colour intensity noticed thereafter. Hence 1 hrs was selected.

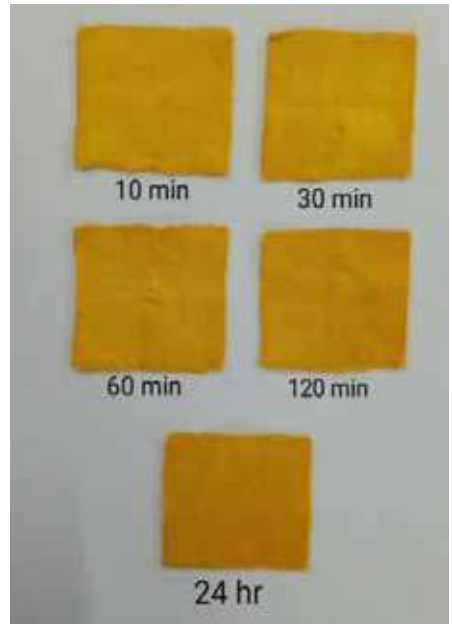

Fig. 8. Cotton clothes stained with dye at different dyeing time

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The cotton clothes stained with dyes obtained by four different methods and standardized its extraction concentration, pre-treatment concentration, mordant, dyeing time. It was found that clothes after scouring with 10% NaOH and pre-treatment with 5 % Myrobalan for 30 min., mordanting with 5 % $SnCl_2$, and dyeing time for 1 hr. in 50% aqueous extract, fixing with lime juice for 30 minutes gave Bright red orange after washing with Teepol. It was also found that clothes without scouring and mordanting gave poor colouration. Dyed cloths obtained without pretreatment and fixation were washed off when they treated with Teepol solution. Mordanting was carried out with application of a different metal salt to bring about an improvement in wash fastness or a change in shade of the dyeing especially with the mordant is $SnCl_2$ (Blackburn, 2017). Lime juice is regarded as freshening agent to restore the original brightness of the shades in home laundering. Post-treatment with lime juice had brightened and darkened the orange shade of annatto on cotton. Alum mordanted samples displayed dark orange shades with reddish tinge on cotton. When mordant concentration was increased, the depth of the shade was also increased. Slight reddish tinge was added to bright brown shade obtained on ferrous sulphate mordanted samples treated with lime juice (Prabhavathi et al., 2014). It was found that acidic and aqueous extraction procedures yield superior to alkali and acetone methods for extracting natural dyes. Therefore, it is suggested that cotton clothing can be coloured using dyes derived through acidic and aqueous extraction.

Table 2. Standardized parameters for the extraction of dyes from *Sphagneticolatrilobata*

Sl. No	Parameters	*Sphagneticolatrilobata*
1	Extraction method	Acid
2	Material concentration	50%
3	Myrobalan concentration	5%
4	Myrobalan timing	30 min
5	Mordant concentration	5%
6	Dyeing time	hr

4. Summary and conclusion

The dye was extracted from the flowers of *Sphagneticolatrilobata* with four different extraction solutions. Melon yellow dye was obtained by acidic method and light ivory was obtained by aqueous methods. Maize yellow was obtained by alkaline method, yellow orange dye was obtained by acetone method. Out of which, dye obtained by acid extraction method showed intense coloration on pretreated dyeing cloths. This dye fixed on the cotton by post treatment with lime juice. Finally bright red orange colour was obtained after washing.

It was founded that acidic and aqueous extraction methods of natural dye extraction provide better result compared to alkali and acetone methods in *Sphagneticolatrilobata*. So, it is proposed that the dyes obtained by acidic and aqueous extraction can be utilized for coloring the cotton clothes. A standardized procedure for extracting natural dyes from flowers of *Sphagneticolatrilobata* with acid extracting medium, bleaching in 10% NaOH solution,

mordanting with 5% myrobalan, 5% Stannous chloride, and dyeing for 1 hour, and post treatment with lime juice, etc. was proposed in the present study.

The present study results strongly indicate that pigments and natural colorants from weeds could have great value in dyeing of textile fabrics. Moreover, the weed plants can be controlled by the over exploitation of these weeds from our surroundings for natural dye preparation.

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OP-08

Allelopathic effect of Lagerstroemia speciosav L. & Simarouba glauca Dc. on seed germination & seedling growth of Vigna radiata &Amaranthus cruentus

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Abstract

Most of the locally available plants shows reduction in their growth & finally leads to the complete elimination due to the inhibition of other plants. Allelopathy is an ecological phenomenon by which plants release organic chemicals (allelochemicals) in to the environment influencing the growth and survival of other organisms. Allelopathic potential can be measured by the effect of allelochemicals on seed germination and seedling growth of plants. *S. glauca*, "The tree of Paradise" are widely cultivated for their medicinal uses and *L. speciosa*, "Pride of India" which are grown as ornamental tree can cause their allelopathic effect to neighboring local plants which thus affect the biodiversity of the locality. In the present investigation, allelopathic effects of leaf extracts (crude, 1:1, 1:2 & 1:4) of tree species such as *Lagerstroemia speciosa* L. (Crape myrtle) &*Simarouba glauca*Dc. (Lakshmitaru) on seed germination and seedling growth of *Vigna radiata* and *Amaranthuscruentus* were evaluated. The results revealed that rate of germination and seedling growth shows decrease with increase in concentration of extract.Seed germination inhibition was greatly noticed in the seeds that are treated in *S. glauca* extract than *L. speciosa* extract. Crude treatment of *S. glauca* reduce the rate of seed germination and shows stunted growth while in the case of *L. speciosa,* delayed germination, reduction in root & shoot length are observed. Results revealed that the local plants that grow near to the selected plants will eliminate completely due to their strong allelopathic effect especially plants that grow near to *S. glauca*.

Key words: Allelopathy, *Lagerstroemia speciosa*, *Simarouba glauca.*

1. Introduction

In nature many plants secrete secondary metabolites or phytotoxins that may constrain the growth of other plant. If the metabolite or phytotoxin produces inhibitory effect, they are known as allelochemicals[1][2][3] . Allelopathic compounds generally occurs in natural plant communities and are suggested to be one mechanism by which weeds interfere with crop growth.Allelochemicals canstimulate or inhibit the germination orgrowthof plants, andincrease the resistance of crops to biotic and abiotic stress. Allelochemicals may

causeimbalances to the several phytohormones or change the plant growth regulator contents,which reduce the plant growth and development including the germination of seed and growthof seedling[4][5] [6][7][8]. The phytochemicals directly release into the surrounding environment whichinhibit the seed germination and growth of established neighbouring species [9].In the present study, extract of *Lagerstroemia speciosa* L. (Crape myrtle) and*Simarouba glauca* Dc. (Lakshmitaru) is analysed, for its allelopathic effect on seed germination and seedling growth of *Vigna radiata* and *Amaranthus cruentus.*

Lagerstroemia speciosa is a deciduous shrub or small tree seen in tropical areas. It is one of only a few trees or a shrub to offer brilliant colour in late summer through autumn, at a time when many flowering plants have exhausted their blooms. It is an oftenmulti-stemmed, deciduous tree with a wide spreading, flat topped, rounded, or even spikeshaped open habit. The risk of introduction of *L. speciosa* is very high. This species is one of the most common ornamentals commercialized in the nursery and landscaping trade around the world. Many cultivars resistant to drought, freezing conditions, and pests have been developed by private individuals, nurseries, and public institutions thus the likelihood of colonizing new habitats remains high. *Simarouba glauca* Dc. Popularly known as "Lakshmitaru" is a medium sized evergreen tree belongs to the family Simaroubaceae. The trees are polygamodioecious and because of its many benefits and uses, this nature's wonder herb is also called as the 'Tree of paradise'. All parts of the plant are used in herbal medicine. An infusion of leaves or bark is considered as astringent, digestive, antihelminthic and emmenagogue. The main active groups of chemicals in *S. glauca* are called quassinoids, which belong to the triterpene chemical family [10].

Most of the tree species such *as Lagerstroemia, simarouba* used for its medicinal value, ornamental purposes and grown as shade trees. Due to its extensive habitat, it can influence the other plant species seed germination, growth and development [11][12][13][14][15][16]. Local people cultivate these plants as an alternative herbal medicine, can be grown as shade trees and used for its ornamental value. Locally available plants are disturbed and totally eliminated by the allelopathic effect of these tree species. So, the purpose of present study investigates the allelopathic potential of plant extracts of *L. speciosa* and *S. glauca* on seed germination and seedling growth of *Vigna radiata* and *Amaranthus cruentus*.

2. Materials & methods

In order to study the allelopathic effects of tree species, Seed germination and seedling development were evaluated.

2.1 Collection & preparation of plant extracts

Fresh leaves of *L. speciosa* & *M. citrifolia* were collected from different parts of Kannur district, kerala. Crude extract was prepared by grinding the raw materials with mortar and pestle. This was then filtered to remove particulate matter. Distilled water was used as medium for dilution. From this extract crude, 1:1, 1:2 and 1:4 solutions were prepared.

2.2 Seed preparation and treatment

Seeds of *Vigna radiata* (mung bean) and *Amaranthus cruentus* (Indian spinach) were collected& determine the seed viability by calculating germination percentage. Seeds were stored in a sterilized container and then rinsed with distilled water before planting.

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2.3 Laboratory bioassay

Allelopathic effect of the test plant extract was screened using seed germination and seedling growth analysis. Seeds of *Vigna radiata* and *Amaranthus cruentus* were germinated over moist cotton and plant extract with different concentrations are provided. Crude, 1:1, 1:2, 1:4 and control solution are prepared and added to each of the plate with seeds. Petridish containing seeds were moistened once a day with different concentration of extracts. Each treatment was replicated three times.

2.4 Observation

Seeds were observed every day and the number of germinated seeds were recorded and counted for 10days for *Vigna radiata* and 14 days for *Amaranthus cruentus*. Thereafter, seedling root length and shoot length were observed.

2.5 Data analysis

Data on allelopathic effects of *L. speciosa* and *S. glauca* on seed germination and seedling growth (shoot length, root length,) of *V. radiata* and *A. cruentus* were compared. Germination percentage, time of germination, root length, shoot length, formation of leaves are the major parameters used for the present study.

Equations:

Germination % = Number of seeds germinated/ Total no. of seeds in the sample * 100

3. Results & discussion

In the present study, allelopathic impact of leaf extracts of *L. speciosa* and *S. glauca* on seed germination and seedling growth of*V. radiata* and *A. cruentus* were evaluated. Drastic changes occur in seed germination and seedling growth of plant species when treated with extract. Rate of germination and seedling growth shows significant decrease with increase in the concentration of extract.

A wide range of abnormal growth of seedlings are observed when seed treated with plant extract. Seed germination inhibition (fig. 1 &5), stunted growth (3 & 9), reduction in shoot and root length (fig. 2,3,6 & 9), absence in production of leaves , are the abnormalities noticed during the course of treatment.

There is positive correlation occur between concentration of extract and frequency of abnormalities. Seed germination inhibition was greatly noticed when the seeds treated with *S. glauca* extract than the *L. speciosa* extract. There is a delay of time is observed in germination of seeds when treated with *L. speciosa* (Table 1). But Seed germination is strictly inhibited when seeds treated with *S. glauca* extract. Major abnormalities noticed during the treatment include:

Root length reduction

It is the common phenomenon observed in seedlings when treated with *L. speciosa* (Table 3&4) and *S. glauca* extract (table 3&4). It is commonly observed in *S. glauca* 1:1, 1:2extract and *L. speciosa* crude, 1:1 & 1:2 plant extracts.

Shoot length reduction

Sample treated with *S. glauca* shows reduction inshoot, but allelochemicals of **S. glauca** is much more severe, hence it totally stops the growth of plant. (fig.3&6).

Absence of leaves

Sample when treated with control produce the leaves, but samples treated with extracts mainly shows defect in the appearance of leaves.

Delay in splitting of leaves

Splitting of leaves is delayed in samples treated with *S. glauca* than *L. speciosa* extract (fig.8). Seeds of *Vigna* is tolerant than *Amaranthus*species, that is leaf splitting is earlier in the case of *Vigna* species than the *Amaranthus* species.

Curly growth

It is clearly noticed in the samples treated with plant extracts. The toxicchemicals may inhibit shoot/root growth, they may inhibit nutrient uptake, or they may attack a naturally occurring symbiotic relationship thereby destroying the plant's usable source of anutrient. It disturbs the normal growth of plant.

Fade coloration

Fade coloration is mainly observed in the case of leaves. Particularly, allelochemicals can reduce chlorophyll content by enhancing stimulation ofChlorophyll degradation or inhibition of Chlorophyll synthesis. It results in fade coloration and gradually decrease the growth of seedlings.

4. Conclusion

In the present investigation, allelopathic effects of leaf extracts of tree species such as *Lagerstroemia speciosa L.* (Crape myrtle) and *Simarouba glauca Dc.* (Lakshmitaru) on seed germination and seedling growth of *Vigna radiata* and *Amaranthus cruentus* were evaluated. Allelopathic effect on germination and seedling growth was found to be increasing with increasing the concentration of extract. *S. glauca* is considered to be more allelopathic than *L. speciosa*. The decrease in the rate of seed germination is mainly noticed in the sample treating with the crude extracts of *S. glauca*. Delay of seed germination is seen in the case of samples treating with *L. speciosa* extract. Most of the people, plant the trees for shade purpose, ornamental purpose, source of medicine and for household purposes. This can seriously affect the growth of our natural plant species specially the endemic species. Allelochemicals can totally destroy the natural habitat of these species and even cause the invasion of foreign species. So, in the present study revealed the allelopathic effect of an ornamental tree *Lagerstroemia speciosa* and medicinal tree *Simarouba glauca*, "The tree of paradise".

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Table 1: No. of *V. radiata* seeds germinated on *L. speciosa*&*S. glauca* plant extracts

Days	No. of seeds germinated in different concentrations of plant extracts									
	Crude		**1:1**		**1:2**		**1:4**		**control**	
	L. speciosa	***S. glauca***	***L. speciosa***	***s. glauca***	***L. speciosa***	***S. glauca***	***L. speciosa***	***S. glauca***	***L. speciosa***	***S. glauca***
1	-	-	-	-	-	-	-	-	-	-
2	3	0	5	0	7	1	9	2	10	10
3	5	0	6	0	8	2	9	4	10	10
4	7	0	8	1	10	2	10	5	10	10
5	7	1	9	3	10	3	10	5	10	10
6	9	3	9	4	10	4	10	7	10	10
7	9	4	9	4	10	5	10	7	10	10

Table 2: No. of *A. cruentus* seeds germinated on *L. speciosa*&*S. glauca* plant extracts

Days	No. of seeds germinated in different concentrations of plant extracts									
	Crude		**1:1**		**1:2**		**1:4**		**control**	
	L. speciosa	***S. glauca***	***L. speciosa***	***s. glauca***	***L. speciosa***	***S. glauca***	***L. speciosa***	***S. glauca***	***L. speciosa***	***S. glauca***
1	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-
3	2	0	2	0	3	1	5	2	8	8
4	4	0	5	0	9	2	9	3	10	9
5	6	0	5	2	9	2	10	4	10	10
6	7	1	9	2	10	4	10	4	10	10
7	8	3	8	3	10	4	10	5	10	10

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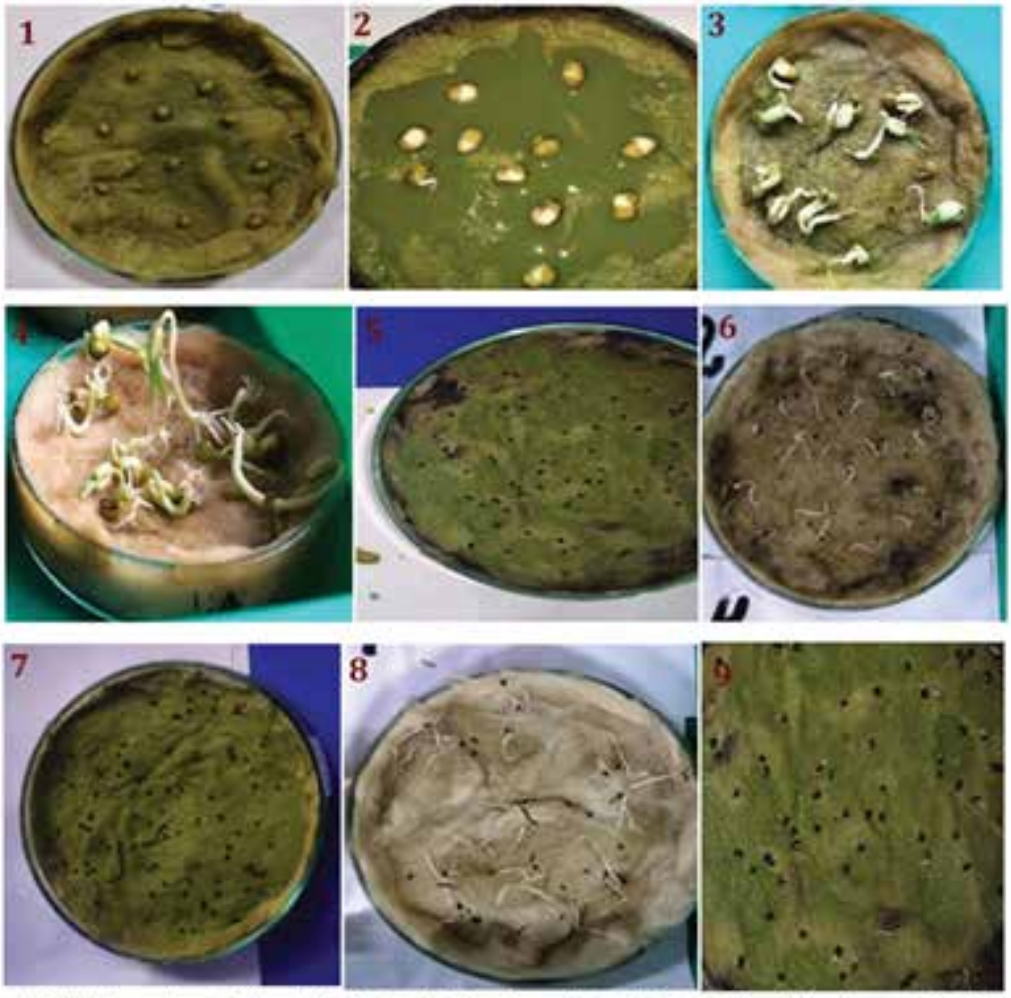
Table 3: Root & shoot length of *V. radiata* treating with *L. speciosa*&*S. glauca* extracts

Days	Seedling growth																			
	Root length										Shoot length									
	L. speciosa					*S. Glauca*					*L. speciosa*					*S. glauca*				
	Crude	1:1	1:2	1:4	control	Crude	1:1	1:2	1:4	control	Crude	1:1	1:2	1:4	control	Crude	1:1	1:2	1:4	control
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	0.2	0.3	0.3	0.5	0.7	-	-	-	0.3	0.5	-	-	-	-	-	-	-	-	-	-
3	0.4	0.8	1	1.5	2.2	-	-	0.5	1.5	2						-	-	-	-	-
4	0.6	1	1.5	2	3	-	0.3	1	2	2.2	0	0.5	0.5	0.7	1.5	0	0	0.2	0.5	1.5
5	1	1.5	1.7	3	4	1	1.5	1.2	2.5	3	0.5	1	1.5	2	3	0	0	0.8	1	1.9
6	1	1.5	2	3	5.2	1	1.5	2	2.5	4	0.5	1	1.7	3	3.2	0	0	1	2	3.2
7	1	1.7	2.2	3.2	5.5	1	1.6	2.2	2.6	4	0.5	1	1.8	3.4	3.4	0	0	1	2.2	3.5

Table 4: Root & shoot length of *A. cruentus* treating with *L. speciosa*&*S. glauca* extracts

Days	Seedling growth																			
	Root length										Shoot length									
	L. speciosa					*S. Glauca*					*L. speciosa*					*S. glauca*				
	Crude	1:1	1:2	1:4	control	Crude	1:1	1:2	1:4	control	Crude	1:1	1:2	1:4	control	Crude	1:1	1:2	1:4	control
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	0.2	0.3	0.4	0.4	0.6	0	0	0.2	0.4	0.4	-	-	-	-	-	-	-	-	-	-
4	0.3	0.5	0.7	0.8	2	0	0	0.4	0.6	2	-	-	0.4	0.6	1	-	-	-	0.2	1
5	0.6	0.9	1.1	1	3	0.4	0.7	1	1		0.5	1	1.5	2	2	-	-	-	0.8	2
6	0.7	1.5	2	2	3.5	0.4	1	1.3	1.5	3.2	0.5	1	1.7	2.8	3.2	-	-	-	1	2.8
7	0.7	1.6	2.2	2.3	3.6	0.4	1.2	1.3	1.5	3.3	0.5	1	1.8	2.9	3.5	-	-	-	1	3



1)Inhibited seed germination in V. radiata 2) Delayed growth of radicle 3) Reduced shoot length 4) Delay in splitting of leaves 5)Inhibited seed germination on A. cruentus 6) Delayed appearance of shoot system 7) Fade coloration 8) Delay in appearance of leaves 9) Reduced root length

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OP-09

Study on distribution of *Rotala L.* species in Northern Malabar region of Kerala.

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Abstract

The genus *Rotala* L. is found throughout the world's tropical and subtropical regions, with the majority of the species found in South Asia. It is usually found in laterite shallow ponds as well as in paddy fields. A brief survey on distribution of *Rotala* L. and its associated plant species in North Malabar region of Kerala is carried out. Fifteen species of *Rotala* L. have been identified from Northern Malabar region, of which *Rotalatulunadensis , R. malabarica , R. malampuzhensis , R.baileyana , R.cheruchakkiensis , R. meenkulamensis , R.kasargodensis and R.khaleeliana*are endemic while *R. indica , R. rosea , R. rotundifolia , Rotaladensiflora, , R.macrandra , R.mexicana ,R.occultiflora* are indigenous. Most common associated plants of *Rotala*in laterite shallow ponds include *Eriocolon, Blyxa, Dopatrium, Shoenoplectiella* and *Weisneria*. There are about thirty two new distributional sites but most of these areas are under threat due to anthropogenic activities. These areas must be protected for the conservation of *Rotala* L. along with its associated plants.

Keywords: *Rotala*, associated plants, laterite, shallow ponds.

1. Introduction

The genus *Rotala* L. belongs to the family Lythraceae found on laterite soil and is now represented by more than 55 species distributed in tropical and subtropical regions of the world. It shows greatest diversity in tropical Asia (Cook, C.D.K. 1979). In India, 66 percent of the globally recognized species have represented the genus, and 83 percent of them are strikingly endemic, indicating their evolutionary significance (Rijuraj, M.P. et al. 2017). Several researchers (Bamps,1989; Beesley, 1990; Lu, 1979; Mathew, and Lakshminarasimhan,. 1990; Pradeep, *et al.* 1990; Prasad, *et al.* 2012; Prasad and Raveendran 2013; Sunil,*et al*. 2013; Yadav,*et al*. 2010) introduced 11 species to the genus after Cook's revision in 1979. *Rotala* L. is characterised by 29 species with an overall morphological diversity of 26 species, including 18 endemic taxa, in Peninsular India. In Peninsular India, Kerala is the largest genus distribution

centre with 21 species, of which 14 species are endemic, including 9 exclusive endemics. This highest degree of genus endemism is mainly known from the laterite areas of northern Kerala with a very restricted range of distribution. Most species of *Rotala* L. in the Peninsular Indian regions are distributed mainly in lateritic plateau amphibious ecosystems, especially in northern Kerala. Based on the existence of habitats, the species of the *Rotala* genus falls into two classes, namely aquatics that develop in shallow waters and semi-aquatic or terrestrial species that flourish in marshy lands. Lateritic plateau edaphic matrix provides habitats on a smaller scale with hydro-geomorphological anomalies that support significant plant taxa, particularly endemics in restricted areas. The restriction of endemic plant species to nutritionally imbalanced substratum (laterite) is a wide spread phenomenon in endemic rich areas. The current study is a preliminary work for analysing the distribution and associated plants of the genus *Rotala* in North Malabar region of Kerala.

2. Materials and methods

Several field visits were conducted in North Malabar regions of Kerala from January 2021 – September 2022. The geological coordinates were recorded with the help of GPSEssentials. Thespecimen of*Rotala* L. along with associated plants were collected in polythene bags and were carried to laboratory for further study. The plants were identified by using regional floras, taxonomic bulletins and further confirmed by taxonomic experts.

3. Results and discussion

In Kerala low altitude plateaus are distributed in Kasargod, Kannur, Kozhikode, Malappuram, Palakkad and Thrissurdistricts. Several researches are going on in these laterite plateaus, plants on these plateaus are adapted to different microhabitats. These microhabitats are unique in their edaphic properties, water availability and composition of the plant. Pramod, (2015) investigated the floristic and ecological aspects of the Madayippara lateritic plateau in northern Kerala. He described the vegetation of the Madayippara lateritic plateau, including the associated scared groove and plateau edges. Ansari, *et al.* (2014) studied Kerala's aquatic and wetland plants and described 699 flowering plant species.Sreejith, *et al.* (2016) compiled a checklist of flowering plants from the low elevation lateritic hills of northern Kerala, listing 535 species in 364 genera. Lemiya, (2017) collected species of *Rotala* like *Rotala anamika, Rotala cheruchakkiensis, Rotala densiflora, Rotala fimbriata, Rotala indica, Rotala juniperina, Rotala macrandra, Rotala kasaragodensis, Rotala malampuzhensis, Rotala malabarica, Rotala Mexicana, Rotala occultiflora, Rotala rosea, Rotala rotundifolia, Rotala tulunadensis* and *Rotala verticillaris* from different sites in India. But comprehensive study on the distribution of *Rotala* is not conducted yet. Here we focused the study on distribution of *Rotala,*revealed that there are fifty four sites with different species of *Rotala* in North Malabar Region of Kerala (Table1). Fifteen species of *Rotala* are identified from the study area in which eight species are endemic and rest are indigenous in distribution (Table 2). *Rotalamala barica* and *Rotala malampuzhensis are* present together in most of the sites. There are thirty two new distributional sites of *Rotala*were identified in which Kannur and Kasargod districts have maximum number of distribution.

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Table 1. List of distribution of *Rotala* species in North Malabar region.

Sl. No	Distribution	District	Geological coordinates	Botanical name
1	Madaippara	Kannur	12°01′ .74.6″N, 075°15′.95.8″E	*R. indica*
		Kannur	12°01′ 66.5″ N,075°15′.84.1″E	*R. malabarica*
		Kannur	12°03′ 19.83″ N,075°26′.27.35″E	*R. malampuzhensis*
		Kannur	12°02′ 72.28″ N,075°25′.54.8″E	*R. rosea*
2	Ramapuram*	Kannur	12°05′ 05.4″ N,075°26′.52.7″E	*R. malabarica*
		Kannur	12°05′ 05.0″ N,075°26′.52.8″E	*R. malampuzhensis*
3	Cherukunnu*	Kannur	11°98′ 703″ N,075°30′.180″E	*R. malampuzhensis*
4	Kannapuram*	Kannur	11°98′ 80.3″ N,075°30′.28.0″E	*R. malampuzhensis*
		Kannur	11°98′ 80.3″ N,075°30′.28.1″E	*R. rosea*
5	Meenkulam	Kannur	12°10′ 37.0″ N,075°18′.53.0″E	*R. meenkulamensis*
		Kannur	12°10′ 36.0″ N,075°18′.52.0″E	*R. malabarica*
		Kannur	12°10′ 37.0″ N,075°18′.52.8″E	*R. malampuzhensis*
		Kannur	12°10′ 36.8″ N,075°18′.52.8″E	*R. khaleeliana*
6	Peringome*	Kannur	12°12′ 29.7″ N,075°16′.27.5″E	*R. malabarica*
		Kannur	12°12′ 29.7″ N,075°16′.27.5″E	*R. malampuzhensis*
7	Eramam	Kannur	12°09′ 48.1″ N,075°16′.39.9″E	*R. malabarica*
		Kannur	12°09′ 48.1″ N,075°16′.39.9″E	*R. malampuzhensis*
8	Karakundu*	Kannur	12°07′ 02.6″ N,075°21′.02.5″E	*R. malabarica*
		Kannur	12°07′ 02.6″ N,075°21′.02.5″E	*R. malampuzhensis*
9	Ezhumvayal*	Kannur	12°07′ 32.7″ N,075°22′.12.3″E	*R. malampuzhensis*
10	Kirathppara*	Kannur	12°07′ 07.2″ N,075°21′.07.2″E	*R. malampuzhensis*
11	Nidiyenga*	Kannur	12°04′ 48.1″ N,075°29′.98.9″E	*R. malabarica*
		Kannur	12°04′ 48.1″ N,075°29′.98.9″E	*R. malampuzhensis*
12	Chengalay*	Kannur	12°04′ 44.1″ N,075°48′.98.9″E	*R. malabarica* *R. malampuzhensis*
13	Kakkakkananppara*	Kannur	12°00′ 80.2″ N,075°34′.14.2″E	*R. malabarica*
		Kannur	12°00′ 77.1″ N,075°35′.14.0″E	*R. malampuzhensis*
14	Kalliad	Kannur	11°59′ 77.4″ N,075°35′.07.5″E	*R. malabarica*
		Kannur	11°59′ 77.2″ N,075°35′.07.5″E	*R. malampuzhensis*
15	Blathoor	Kannur	12°00′ 82.5″ N,075°34′.08.0″E	*R. rosea*
		Kannur	12°00′ 82.3″ N,075°34′.08.5″E	*R.indica*
		Kannur	12°00′ 82.5″ N,075°34′.08.5″E	*R. malampuzhensis*
		Kannur	12°00′ 40.4″ N,075°34′.71.7″E	*R. malabarica*
		Kannur	12°00′ 54.2″ N,075°34′.52.0″E	*R. rotundifolia*
16	Valiyavelicham*	Kannur	11°49′ 40.2″ N,075°36′.63.4″E	*R. malampuzhensis*
17	Navodaya*	Kannur	11°56′ 18″ N,075°63′.85″E	*R. malampuzhensis*
18	Panoor*	Kannur	11°76′ 76.1″ N,075°57′.65.5″E	*R. rotundifolia*

	Poyiloor*	Kannur	11°46′ 38.4″ N,075°38′.07.4″E	*R. rosea*
		Kannur	11°46′ 38.4″ N,075°38′.06.9″E	*R. indica*
			11°46′ 38.4″ N,075°38′.07.9″E	*R. occultiflora*
	Ayithara*	Kannur	11°86′ 97″ N,075°60′.38″E	*R. malampuzhensis*
	Mayyil*	Kannur	11°59′ 49.9″ N,075°25′.73.0″E	*R. rosea*
		Kannur	11°59′ 499″ N,075°25′.730″E	*R. indica*
	Thottada*	Kannur	11°51′ 47.1″ N,075°24′.64.5″E	*R. malampuzhensis*
	Mulool*	Kannur	12°01' 58" N,075°32'.52"E	*R. rosea*
		Kannur	12°01' 58" N,075°32'.52"E	*R. indica*
		Kannur	12°01' 58" N,075°32'.52"E	*R. malampuzhensis*
	Kanay	Kannur	12°08′66.1″ N,075°14′.67.5″E	*R. malampuzhensis*
		Kannur	12°08' 69.6" N,075°14'.07.6"E	*R. malabarica*
		Kannur	12°08' 696" N,075°15'.076"E	*R. thulunadensis*
	Perinthatta*	Kannur	12°17′ 25.7″ N,075°30′.88.0″E	*R. malabarica*
		Kannur	12°17′ 25.7″ N,075°30′.88.0″E	
	Ulliyeri	Kannur	11°26' 05.6" N,075°46'.04.7"E	*R. malabarica*
		Kannur	11°26' 05.6" N,075°46'.04.7"E	*R. malampuzhensis*
	Naduvil*	Kannur	12°06' 25.3" N,075°27'.14.6"E	*R. malabarica*
		Kannur	12°06' 25.3" N,075°27'.14.6"E	*R. malampuzhensis*
	Urathoor*	Kannur	12°06′ 54.2″ N,075°27′.01.6″E	*R. malampuzhensis*
		Kannur	12°06' 54.2" N,075°27'.01.6"E	*R. malabarica*
	Paravoor*	Kannur	12°0′8 15.1″ N,075°19′.44.5″E	*R. malampuzhensis*
		Kannur	12°0'8 15.1" N,075°19'.44.5"E	*R. malabarica*
	Mattanur*		11°93′ 92″ N,075°56′.75.5″E	*R. malampuzhensis*
				R. malabarica
	Palvally*	Kannur	12°08′ 27.1″ N,075°20′.52.5″E	*R. malampuzhensis*
		Kannur	12°08' 27.1" N,075°20'.52.5"E	*R. malabarica*
	Kannadippoyil*	Kannur	12°50′ 23.1″ N,075°00′.12.5″E	*R. malampuzhensis*
	Bakkalam*	Kannur	11°99′ 38″ N,075°36′.75.5″E	*R.rotundifolia*
	Valiyanoor*	Kannur	11°54′ 66.5″ N,075°25′.85.5″E	*R. rosea*
	Mananthery*		11°84′ 28.1″ N,075°61′.50.5″E	*R. rosea*
			11°84′ 28.1″ N,075°61′.50.5″E	*R. indica*
	Irikkur		11°98′ 69″ N,075°55′.39.5″E	*R. densiflora*
	Padayottuchal*	Kannur	12°16′ 17.5″ N,075°11′.08.3″E	*R. rotundifolia*
		Kannur	12°16' 17.0" N,075°11'.08.1"E	*R. malampuzhensis*
	Velichamthode*	Kasargode	12°13′ 62.3″ N,075°16′.49.5″E	*R. cheruchakkiensis*
		Kasargode	12°13' 62.3" N,075°16'.49.5"E	*R. malabarica*
		Kasargode	12°13' 62.3" N,075°16'.49.5"E	*R. malampuzhensis*
		Kasargode	12°13' 62.3" N,075°16'.49.5"E	*R. mexicana*

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	Pothamkandam	Kasargode	12°22′ 90.3″ N,075°27′.42.5″E	*R. malabarica*
		Kasargode	12°22' 90.3" N,075°27'.48.0"E	*R. malampuzhensis*
	Ariyittappara	Kasargode	12°14′ 29.8″ N,075°16′.43.3″E	*R. malabarica*
		Kasargode	12°14' 29.3" N,075°16'.43.1"E	*R. malampuzhensis*
	Kayyur	Kasargode	12°16′ 17.6″ N,75°11′.08.3″E	*R. malabarica*
		Kasargode	12°16' 17.6" N,75°11'.08.3"E	*R. malampuzhensis*
		Kasargode	12°15' 79.3" N,75°10'.91.8"E	*R. thulunadensis*
		Kasargode	12°16' 17.6" N,75°11'.08.2"E	*R. occultiflora*
		Kasargode	12°16' 17.6" N,75°11'.08.3"E	*R. mexicana*
		Kasargode	12°16' 17.6" N,75°11'.08.2"E	*R. rosea*
		Kasargode	12°16' 17.6" N,75°11'.07.8"E	*R. indica*
		Kasargode	12°16' 17.6" N,75°11'.07.2"E	*R. macrandra*
		Kasargode	12°15' 78.9" N,75°10'.89.8"E	*R. baileyana*
		Kasargode	12°14′ 73.1″ N,075°14′.22.5″E	*R. occultiflora*
	Cheemeni	Kasargode	12°14' 73.1" N,075°14'.22.5"E	*R. occultiflora*
		Kasargode	12°14' 71.1" N,075°14'.26.5"E	*R. rosea*
		Kasargode	12°14' 72.1" N,075°14'.29.5"E	*R. indica*
		Kasargode	12°14' 70.1" N,075°14'.21.5"E	*R. rotundifolia*
	Angakalari, Pattena	Kasargode	12°16′ 51.7″ N,75°08′.27.2″E	*R. malabarica*
		Kasargode	12°16' 89.7" N,75°08'.58.3"E	*R. malampuzhensis*
		Kasargode	12°16′ 89.7″ N,75°08′.58.3″E	*R.thulunadensis*
	Karindalam*	Kasargode	12°16′ 30.3″ N,75°14′.09.2″E	*R. malabarica*
		Kasargode	12°16′ 30.3″ N,75°14′.09.2″E	R. malampuzhensis
		Kasargode	12°16′ 30.3″ N,75°14′.09.2″E	R. occultiflora
	Permude	Kasargode	12°39′33.9″ N,75°00′.41.1″E	*R. thulunadensis*
		Kasargode	12°39′33.9″ N,75°00′.41.1″E	R. malabarica
		Kasargode	12°39′33.9″ N,75°00′.41.1″E	R. malampuzhensis
	Periya	Kasargode	12°23′ 48.1″ N,75°06′.45.6″E	*R. malabarica*
		Kasargode	12°23′ 48.1″ N,75°06′.45.6″E	R. malampuzhensis
			12°31′ 00.8″ N,75°08′.55.4″E	*R. malabarica*
	Kanathoor	Kasargode	12°31′ 00.8″ N,75°08′.55.4″E	R. malampuzhensis
	Ummeripoyil	Kasargode	12°16′ 23.2″ N,75°19′.29.1″E	*R. malabarica*
		Kasargode	12°16′ 23.2″ N,75°19′.29.1″E	R. malampuzhensis
	Bovikkanam	Kasargode	12°29′ 59.2″ N,75°05′.07.5″E	*R. malabarica*
		Kasargode	12°29′ 59.2″ N,75°05′.07.5″E	R. malampuzhensis

	Kottappara	Kasargode	12°20′ 31.6″ N,75°13′.39.8″E	*R. malabarica*
		Kasargode	12°20′ 31.6″ N,75°13′.39.8″E	*R. malampuzhensis*
	Seethangoli	Kasargode	12°35′ 36.3″ N,74°01′.27.9″E	*R. malabarica*
			12°35′ 36.3″ N,74°01′.27.9″E	*R. malampuzhensis*
	Mugu	Kasargode	12°60′ 51″ N,75°01′.00″E	*R. malabarica*
		Kasargode	12°60′ 51″ N,75°01′.00″E	*R. malampuzhensis*
		Kasargode	12°60′ 51″ N,75°01′.00″E	*R. kasargodensis*
	Kinaloor*	Kozhikode	11°46′ 96″ N,075°86′.21″E	*R. densiflora*
	Poovattuparamb	Kozhikode	11°26′ 47″ N,075°89′.29″E	*R. malampuzhensis*
		Kozhikode	11°26′ 47″ N,075°89′.29″E	*R. mexicana*
		Kozhikode	11°26′ 47″ N,075°89′.29″E	*R. macrandra*
		Kozhikode	11°26′ 47″ N,075°89′.29″E	*R. indica*

*Indicates new distribution sites

Table 2. Species of *Rotala* with distribution/Status

Sl. No	Botanical Name	Distribution /Status
	Rotala malabarica K.T. Joseph &Sivar.	Endemic
	Rotala malampuzhensis R. V. Nair ex C. D. K. Cook	Endemic
	Rotala meenkulamensis K. S. Prasad &Raveendran	Endemic
	Rotala occultiflora Koehne	Indigenous
	Rotala rosea (Poir) C. D. K. Cook.	Indigenous
	Rotala rotundifolia (Buch.-Ham. Ex Roxb.) Koehne	Indigenous
	Rotala indica (Willd.) Koehne	Indigenous
	Rotala densiflora (Roth) Koehne	Indigenous
	Rotala baileyana Rogi, Joby, Rameshan, Nisha & I. Antony	Endemic
	R. cheruchakkiensis Anto, Devikrishna, Pulickal, C.D.Varghese & I Antony	Endemic
	R. kasargodensis K.S.Prasad&Raveendran	Endemic
	R. khaleeliana Sunil, Ratheesh&Nandakumar	Endemic
	R. macrandra Koehne	Indigenous
	R. mexicana Cham. &Schltr.	Indigenous
	Rotala tulunadensis K. S. Prasad, P. Biju. Raveendran & K.G.Bhat	Endemic

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Rotala malabarica and *R.malampuzhensis* are usually present in shallow ponds in association with the plants like *Eriocaulon cinereum*, *Eriocaulon cuspidatum*, *Blyxa octandra*, *Dopatrium junceum*, *Shoenoplectiella lateriflora*, *Eriocaulon fysonii*, *Oryza rufipogon* and *Wiesneria triandra*.

Rotala thulunadensis, *R.baileyana* and *R. malampuzhensis* are also seen in shallow ponds with the association on *Blyxa octandra*, *Eriocaulon cinereum*/ *Eriocaulon cuspidatum*, *Nymphoides indica* / *Nymphoides krishnakesara var. krishnakesara./Nymphoides krishnakesara var.bispinosa* and *Oryza rufipogon*.

Rotala indica and *Rotala rosea* are commonly found in the paddy fields and also they are considered as weed. *Rotala rotundifolia* was found to be dominated at marshy land along with Salvinia and also found dominated in a stream. Even though there were many distributional sites for *Rotala*most of the areas are under threat due to anthropogenic activities. Most of the laterite areas are private lands. These lands are changing into housing areas and also laterite mining is going on. Tourists are also attracted to the laterite areas leading to plastic pollution. Washing of clothes and vehicles also pollute shallow ponds. Since most of the species are endemic, these areas must be protected.

4. Conclusion

In the present study it was found that Kannur, Kasargod district have greatest number of distribution of *Rotala*species .Almost fifty four distributional sites were identified from North Malabar region. There were thirty two new distributional sites. *Eriocolon, Blyxa, Dopatrium, Shoenoplectiella* and *Weisneria* were the common associated plants in laterite shallow ponds. Thehabitats of *Rotala* are facing serious threats of extinction due to laterite mining. These areas are considered as wastelands and hence these plateaus are destroyed by anthropogenic activities. Conservation of laterite plateaus are very much necessary for saving the microhabitats of both plants and animals. It can only be achieved by creating awareness to the people as well as implementing laws by govt. for conservation of laterite biodiversity.

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OP-10

HPTLC analysis of Phenolic acids and Antimicrobial Activities of Methanol extract obtained from *Cyphomandra betacea* Cav. Fruits.

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Abstract

Fruits are an important part of our daily diet, as they provide potential health benefits, being a rich source of proteins, vitamins, minerals and antioxidants. Wild food plants are categorized as underutilized or neglected crops which grow in the wild. Cyphomandra betacea is one among such wild edible plants. The main objective of the study was to find out the phenolic acid composition and antimicrobial properties of ripe fruits of Cyphomandra betacea. The plant belongs to the family solanaceae and commonly known as 'marathakkali' in Malayalam. The methanol fruit extract was subjected to HPTLC analysis of phenolic acids as well as antibacterial and antifungal test. Phenolic compounds such as Caffeic acid, Ferulic acid and Gallic acid were detected in the sample. Methanol fruit extract of Cyphomandra betacea showed good antifungal activity against fungal strain Aspergillus niger. Antibacterial activity of methanol fruit extracts tested against three bacterial strains such as Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae. The extract exhibits good antibacterial activity also. The results of the present study revealed that Cyphomandra betacea fruit extract is a potential source of natural bioactive components. These fruits could be included in our daily diet due to many health benefits.

Key words: Phenolic acid, Cyphomandra betacea, Solanaceae, HPTLC, Antimicrobial

1. Introduction

In recent times considerable attention has been devoted to wild edible fruits with antimicrobial properties.The phenolic compounds are one of the most widely occurring group of phytochemicals which are of significant morphological and physiological importance in plants (Kumar and Goel, 2019). Phenolic compounds are known to possess antimicrobial, anticancerous and antioxidant activities (Sharma *et al*., 2018). *Cyphomandra betacea*is a nutrient rich fruit, which is a cheap source of carbohydrates, dietary fibres, proteins, vitamins, minerals and fatty acids. The antimicrobial activities of wild fruits were assessed. Wild food plants are generally categorized as underutilized or neglected crops which grow in the wild.*Cyphomandra*

*betacea*is one among such wild edible plants.The plant *Cyphomandra betacea* belongs to the family solanaceae and commonly known as 'Marathakkali' in Malayalam.The main objective of the study was to find out the phenolic composition and antimicrobial properties of ripe fruits.

2. Materials and methods

Collection of plant material

Mature fruits of *Cyphomandra betacea* were collected from various localities such as Meppadi, Pulpalli, Batheri, Puthoorvayal, Thirunelly of Wayanad district, Kerala. The plantwas identified based on authentic literature and with the help of taxonomists. Herbarium specimenswas also prepared.

Preparation of extract

The fruit pulp was dried and powdered using mechanical grinder. From this powder 30g was weighed and extracted with 180 ml of methanol using Soxhlet extractor for 8-10 hrs. Methanol extract was concentrated and subjected to HPTLC analysis of Phenolic acids and antimicrobial studies using standard methods.

HPTLC analysis

HPTLC analysis was performed by CAMAG HPTLC system (Switzerland) at Centre for Medicinal Plants Research, Kottakal, Malappuram. The stationary phase aluminium backed pre-coated silica gel plates Merck 60 F254 (0.2 mm thickness). 10 µl of Samples were applied to the plate as bands at 10 mm from the bottom of the plate by using CAMAG ATS 4. The plate was developed up to 80 mm in ascending mode with solvent system Toluene: ethyl acetate: formic acid (5:5:0.5) at room temperature (28 ± 2°C) in a Twin Trough Chamber (Camag, Switzerland) which previously saturated with mobile phase. After development the air-dried plate scanned at 254 nm, 366 nmand 550 nm after derivatizing with anisaldehyde sulphuric acid reagent in CAMAG TLC SCANNER 3 with win CATS software (Deepak *et al*., 2019)

Antimicrobial studies

Microbial strains

The extract of *Cyphomandra betacea* fruits individually tested against three bacterial strains such as *E.Coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumonia* (ATCC13883) and two fungal strains like *Aspergillus niger* (ATCC 16404), and *Candida albicans* (ATCC 10231) by following agar well diffusion method (Perez *et al*., 1990).

3. Results and discussion

The results of the HPTLC analysis proved that *Cyphomandra betacea* fruit extract is an important source of Caffeic acid, Ferulic acid and Gallic acid. The area percentage for Caffeic acid, Ferulic acid and Gallic acid were 35.34%, 7.06% and 7.98% respectively. The fruit extract showed good antibacterial and antifungal properties. Phenolic compounds are beneficial to human as they are potential antioxidants and also used in therapeutic, cosmetic and food industries (Kumar and Goel, 2019). HPTLC analysis of *Solanum melongena* was done bySatam*et al*. (2013). The results confirmed the presence of good amount of phenolic acids in the fruit extract. Sharma *et al*. (2018) Carried out HPTLC fingerprinting evaluation of phenolic compounds in unripe and ripe fruits of mango. The ripened stage had more amount of phenolics.

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Methanol fruit extract of *Cyphomandra betacea* showed good antibacterial activity against *E. coli*and antifungal activity against *Aspergillus niger*. Many phenolic compounds exhibit antimicrobial activity. Invitro antibacterial activity against pathogenic bacteria of phenolic acid was already reported in literatures (Maddox *et al*., 2010). Antimicrobial screening of natural phenolic compounds like caffeic acid, gallic acid and coumaric acid were carried out against microbial species (Tyagi *et al*., 2015).

Table 1: HPTLC analysis of Phenolic acids

Phenolic acids	Start position (Rf)	Start height (AU)	Max position (Rf)	Max Height (AU)	Max %	End Position (Rf)	End Height (AU)	Area (AU)	Area %
Caffeic acid	0.43	13.8	0.47	155.3	29.12	0.51	24.6	5393.7	35.34
Ferulic acid	0.57	20.1	0.61	37.6	7.05	0.63	28.2	1077.5	7.06
Gallic acid	0.29	10.7	0.34	47	8.81	0.37	0.3	1218.4	7.98

Table 2: Antifungal activity

		Diameter of inhibition zone(mm)	
	Conc. of extracts (µg/ml)	*Aspergillus niger*	*Candida albicans*
Cyphomandrabetacea	250	10	ND
	500	12	
	1000	18	

Table 3: Antibacterial activity

		Diameter of inhibition zone(mm)		
	Conc. of extracts (µg/ml)	*E.coli*	*Staphylococcus aureus*	*Klebsiella pneumoniae*
Cyphomandrabetacea	250	9	ND	ND
	500	10		
	1000	12		

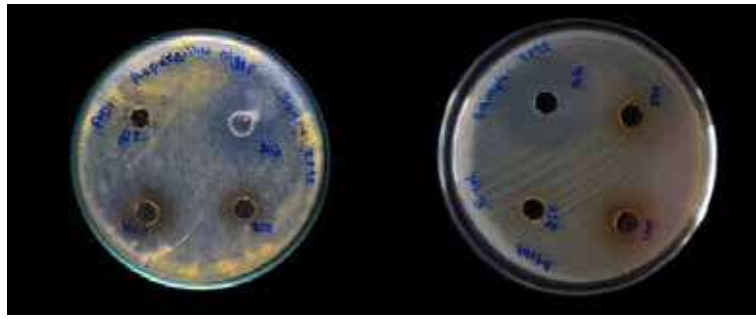

Plate I Plate II

Agar well diffusion method of *Cyphomandrabetacea* fruit extract using *Aspergillus niger*and *E. coli* as test microorganisms. Plates showing inhibition zone formed around the well(Plate I and II).

4. Conclusions

The methanol fruit extract of *Cyphomandrabetacea* has strong antimicrobial activities, as it contains much amount of phenolic acids such as Caffeic acid, Ferulic acid and Gallic acid. Phenolic compounds are considered to be a vital dietary component. These fruits could be included in our daily diet due to many health benefits.

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OP-11

Preliminary phytochemical analysis and FTIR studies in *Brachystelma ariyittaparensis*, an endemic plant in North Kerala

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Abstract

Brachystelma ariyittaparensis P Biju et al. belong to the family Asclepiadaceae and belongs to endemic and critically endangered categories in IUCN.Many of the tuberous *Brachystelma* are known to be used medicinally as well as food by local people. The present paper deals with the preliminary phytochemical analysis of the tuber extracts of *B.ariyittaparensis* in different solvents and FTIR study of the functional groups.The results revealed the presence of carbohydrates, amino acids, protein,glycosides, tannins, alkaloids, saponins, monosaccharides, and phenolic compounds are present in different extracts. While FTIR analysis depicted different functional groups in the tubers of *B. ariyittaparensis*. Hence the species can be studied further for their medicinal and food properties.

Key words: *Phytochemicals, FTIR spectra, Bioacive compounds, Brachystelma*

1. Introduction

Brachystelma R.Br. (Apocynaceae: Asclepioideae) is one of the poorly studied and little-known genera of geophytic plants in India. The plant groups that consist of short-lived ephemerals or geophytes with underground storage organs such as bulbs, corms, tubers or rhizomes are inadequately explored and studied due to their narrow seasonality and highly restricted distribution ranges. The genus *Brachystelma* R.Br. is the second largest genus in the tribe Ceropigiae of the subfamily Asclepiadoideae (Apocynaceae) and consists of about 160 species, distributed mainly in the old-World tropics, particularly in sub-Saharan Africa, India, Sri Lanka, South East Asia and Northern Australia (Prasad *et al*,2016). After the recent revision the genus is now represented by 38 taxa in India, which includes 34 species and four varieties. Among them, five new species and one new combination available (Prasad and Venu, 2021). Among the Indian species, four are from the foothills of the Himalaya in northern India and the remainder are from peninsular India, where they are known from the extreme south in Kerala and Tamil Nadu to Maharashtra in the west and are particularly associated with the foothills of the Western Ghats. A total of 21 are endemic to India (Venu and Prasad, 2015). About 10% of the species of the genus *Brachystelma* occur in India with high intra specific diversity.

Among the Ceropegieae, *Brachystelma* is the least evaluated in terms of phytochemical use and thus poorly documented. This may be due to their small size and inconspicuous nature accompanied by general rarity. They only appear above ground during the rainy season (Masinde, 2007; Smith, 1991). *B. ariyittaparensis* is an endemic critically endangered plant, which are identified recently. They are found to be very rare in distribution.

Members of the genus *Brachystelma* are well-known for their diverse uses, especially their medicinal and nutritional values. The tubers of several *Brachystelma* species are eaten raw or prepared by some indigenous groups in Africa, Asia and Australia (Moteetee, 2011; Deshmukh and Rathod, 2013; Pare *et al*, 2016). Some *Brachystelma* species have also been reported as medicinal herbs against different disease conditions (Pare *et al*, 2016; Ramachandan *et al*, 2005). Hence the present study aims to analyse the phytochemicals and study the functional groups by Fourier -Tranform infrared spectroscopy (FTIR) of the unexplored *Brachystelma* species, *B. ariyittaparensis* from Kasaragod district, Kerala.

2. Materials and Methods

1. Collection and preparation of tubers

The tubers were collected from the type locality which is not reported from any others, laterite plateaus, Kasaragode during the rainy season June to August. Tubers were washed thoroughly with tap water first and then with distilled water to remove all specks of dirt. The collected materials were weighed and cut into thin slices and dried until constant weight under shade to remove all moisture contents. The dried samples were finely powdered using an electric grinder and packed into airtight sample bottles and stored in airtight containers.

2. Preparation of extracts

The shade-dried and powdered tubers were dissolved separately in the following solvents; petroleum ether, methanoland distilled water. 1gm of white powder is accurately weighed in a weighing balance and dissolved in 100 ml solvent and subjected to extraction using an ultrasonicator. The extracts were then filtered using Whatman no: 1 filter paper. Preliminary phytochemical tests are carried out in the filtrate obtained (Chitravadivu *et al*, 2009).

1. Test for Alkaloids

a. Mayer' s test

To a few ml of plant sample extract, two drops of Mayer's reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

b. Wagner's test

A few drops of Wagner's reagent are added to few ml of plant extract along the sides of test tube. A reddish- brown precipitate confirms the test as positive.

2. Test for Amino acids

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for amino acids.

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a. Ninhydrin test

Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) are added to 2 ml of aqueous filtrate. Appearance of purple colour indicates the presence of amino acids.

3. Glycosides

For 50 mg of extract is hydrolysed with concentrated HCl for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following test.

a. Borntrager' test

2 ml of filtered hydrolysate, 3 ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia solution is added to it. Pink colour indicates the presence of glycosides.

4. Saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water bath and filtered. The 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a suitable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, and then the formation of emulsion was observed.

5. Test for Carbohydrates

a. Molish' s test

To 2 ml of plant sample extract, two drops of alcoholic solution of α- naphthol are added. The mixture is shaken well and few drops of concentrated sulphuric acid is added slowly along the sides of test tube. A violet ring indicates the presence of carbohydrates.

b. Benedict's test

To 0.5 ml of filtrate, 0.5 ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes. A characteristic-coloured precipitate indicates the presence of sugar.

6. Test for Fixed oils

a. Spot test

A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

7. Test for Proteins

The extract (100 mg) is dissolved in 10 ml of distilled water and filtered through Whatman No. 1 filter paper and the filtrate is subjected to test for proteins.

a. Millon's test

To 2 ml of filtrate few drops of Millon's reagent are added. A white precipitate indicates the presence of proteins.

b. Biuret test

2 ml of filtrate is treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) is added, followed by an excess of potassium hydroxide pellets. Pink colour ethanolic layer indicates the presence of protein.

8. Test for monosaccharides

a. Barfode's test

Take 1ml of a given sample in a clean dry test tube. Add about 2-3 drops of Barfode's reagent to the test tube and mix them. Keep the test tube in a water bath for 1-2 minutes. Red-coloured precipitate indicates the presence of monosaccharide.

9. Tannins and Phenolic compound

About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration. A few drops of alcohol and ferric chloride solution was mixed with the plant extract. A blue green or red colour indicates the presence of phenol.

Fourier Transform Infrared spectrophotometer (FTIR)

The tubersof *B.ariyittaparensis*, were ground into fine powder by using an electric grinder. Dried powdered samples of the tubers were used for FTIR analysis in the range of 400-4000cm-1 by employing the standard kb pellet technique. Fourier Transform Infrared Spectrophotometer (FTIR) is the most powerful tool for identifying the types of chemical bonds (Functional groups) present in compounds.

3. Results and Discussion

Preliminary phytochemical analysis of tuber extract of *B.ariyittaparensis* were analysed qualitatively and results showed the presence of carbohydrates, alkaloids, amino acid, protein, glycosides, saponins, monosaccharide, tannins and phenolics in different solvents. The results of the preliminary phytochemical analysis are given in table no:1. The results reveal that all bioactive compounds are not extractablein the same solvents. So that it's proven that distilled water is the best solvent over methanol and petroleum ether.

The results of FTIR studies revealed the presence of different functional groups present in the tuber extracts of *B.ariyittaparensis*.The FTIR spectrum was used to identify the functional groups of the active components present in the extract based on the peak values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on the ratio of its peak. The results of FTIR analysis confirmed the presence of N-H, O-H, C-C, C=C, C-H, C-O and CH3 functional groups (Figure 1 and Table 2). FTIR spectroscopy is proved to be a reliable and sensitive method for the detection of biomolecular composition.

The traditional healers or practitioners make use of water primarily as a solvent, our studies have proven that distilled water extracts of these plants were better than any other extract. This may be due to the better solubility of the active components in a polar solvent. The presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines**.** For

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instance, some alkaloids are known to be used as antimalarial agents (Yusuf and Kabir, 1999). The presence of tannins could also show that it is an astringent, help in wound healing and is anti-parasitic. The presence of terpenes suggests it is possible to use them as an anti-tumour and anti-viral agent as some terpenes are known to be cytotoxic to tumour cells. Tannins bind to proline-rich proteins and interfere with protein synthesis. The antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell (Shimada, 2006). The preliminary studies on this plant support the traditional knowledge of local users and it is a preliminary, scientific, validation for the use of these plants as medicine and to promote proper conservation and sustainable use of such plant resources.

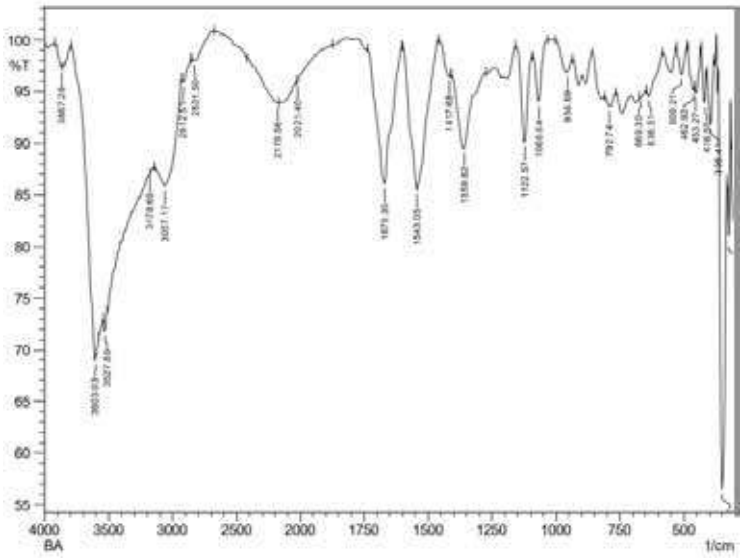

Figure 1: FTIR analysis

Table 1: Preliminary Phytochemical analysis

Tests	Solvents		
	Distilled Water	Methanol	Petroleum ether
Alkaloid	+ +	_ _	_ _
Amino acids	+ +	+ +	_ _
Glycosides	+ +	+ +	_ _
Saponins	+ +	_ _	_ _
Carbohydrates	+ +	+ +	_ _
Proteins	++	+ +	_ _
Fixed Oil	_ _	_ _	_ _
Monosaccharides	++	_ _	_ _
Tannins	+ +	_ _	+ +
Phenolics	+ +	_ _	_ _

+ + -Indicates presence of Phytochemicals
_ _ -Indicates absence of Phytochemicals

Table 2: FTIR values and functional groups

Sl. No	Wave number	Functional group
1.	3603.03	OH free alcohol
2.	3527.8	N-H Amines and amides
3.	3178.69	C=O Carboxylic acids
4.	3057.17	C=C Aromatic alkenes
5.	2912.51	C-C Alkanes
6.	2831.5	C-C Alkanes
7.	1670	C=N Imine and Oxime
8.	1543.05	N-H Amines and amides
9.	1417.68	CH_3 Alkane
10.	1359.82	CH_3 Alkane
11.	1122.57	C-N Amine
12.	1066.64	C-O Alcohol, Carboxylic acids, ester, ether,anhydride

4. Conclusion

The present work has been performed to establish the various Phytochemical, and FTIR parameters, which could serve as important information to facilitate further studies on the discovery of bioactive constituents, resolution of their efficacy by in vivo studies and demonstration of their safety and efficacy in clinical trials.

5. Acknowledgement

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OP-12

Preliminary investigation on genetic diversity and phytochemical composition of a marginalised legume genus from Northern Kerala

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Abstract

The primary objective of this study is to look into the nutritional quality and diversity of the genus Canavalia. The 'Paniya' and 'Kattunaikka' indigenous peoples of northern Kerala provided ethnobotanical data. Standard techniques were used to assess nutritional and antinutritional components using a UV-visible spectrophotometer. All of the plants used for the study are perennial creepers or climbers with compound leathery leaves that are about round in shape. Flowers are brilliant pink-purple and appear in racemes, with the exception of *Canavalia gladiata* (which has white flowers). These bear large fruits, up to 8-12 cm in length, with large seeds inside. Wild habitats of Kunnapuram, Kannur district, and Chembra peak, Wayanad district were explored to harvest *Canavalia gladiata* and *Canavalia cathartica* respectively. *Canavalia rosea* was found growing along the banks of the 'Kabani' River (Panamaram). The costal species *Canavalia maritima* was found abundantly thriving in the sand dunes of Elathur beach, Kozhikkode district. *Canavalia ensiformis*, a cultivated species, was obtained from a private homestead garden in Taliparamba, Kannur. Moisture content, dry weight content, crude fibre, pectin, ash content, total carbohydrate, total starch, reducing sugar, soluble sugar, total protein, free amino acid content, total antioxidant activity and anti-nutritional components were all quantified. The current study provides information on the diversity, place of collection, ethnobotanical information, nutritional and antinutritional properties of four underutilised indigenous legumes and a cultivated species of the genus Canavalia. Then the outcome of this comparative profile of phytochemical properties suggest that these seeds need to be highly recommended for man, as each of them can help us to satisfy our nutritional demands.

Key words: Legume, Phytochemicals, Genetic diversity, Canavalia.

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1. Introduction

Canavalia is a legume genus with so many edible specieses bearing edible pods and seeds, found profusely growing in the study area. It is divided into four subgenera, comprising 51 species (Smartt, 1990). *Canavalia cathartica*, *Canavalia ensiformis, Canavalia gladiata, Canavalia maritima* and *Canavalia rosea* are the five species chosen for the present comparative study (Fig. 1). Industrialization, deforestation, pollution, and other human activities have had a negative impact on the distribution of pantropical legumes such as *C. rosea*, *C. maritima*, and *C. cathartica* (D' Cunha and Sridhar, 2011). *C. rosea* and *C. cathartica* are perennial creeping legumes, are dominant sand binders associated with rhizobia, endophytic fungi, and arbuscularmycorrhizal fungi found in abundance on Indian sand dunes (Chen et al, 2000, Seena and Sreedhar, 2006). *C. rosea* is a pioneer plant on sand dunes and is ecologically significant in coastal habitats (D'Arcy, 1980). *C. rosea* grows on the beach and on the backshore above the high tide mark, but it can also climb over rocks and grow near the shores of coastal lagoons and roadsides (Tijani et al, 2019).

Fig. 1. Habit of*Canavalia cathartica*, *Canavalia ensiformis, Canavalia gladiata, Canavalia maritima* and *Canavalia rosea.*

Ethnobotanical inferences are provided for the use of root infusion, plant decoction, seed powder and leaf paste of *C. rosea* to alleviate pain and discomfort (Bhagya and Srighar, 2009). Forest dwellers in the Wayanad area of northern Kerala used the immature pods and seeds for culinary purposes on occasion. They only eat the seed meal with their regular diet after decanting it numerous times and boiling it thoroughly (Thangadurai *et al*., 2001). The in vitro protein digestibility of *C. gladiata* and *C. ensiformis* seeds are lower than *C. cathartica* and comparableto different cultivars of *Phaseolus vulgaris* (Siddhuraju and Becker, 2001). As a result, it's critical to look in to the nutritional value and calorific value of this wild legume.

2. Materials and methods

1) Field survey and ethnobotanic investigation

The Study areas are Kozhikkode, Wayanad, Kannur districts of Northern Kerala. Intensive field visits were conducted to explore the genetic diversity of the selected species during November 2017 to March 2018. The study camp will be concentrated at two important places especially MSSRF's Community Agrobiodiversity Centre in Wayanad since the area is blessed with diverse plant groups; and the second is Sir Syed College, Taliparamba, Kannur.

The collected plants were identified taxonomically by using the flora of Presidency of Madras (Gamble, 1936) and handbook of flowering plants published by KFRI (Sasidharan, 2004). The identified plant specimens were then confirmed with the herbaria of Calicut University and MSSRF - CAbC, Kalpetta. Web based taxonomic database 'The Plant List' (www.theplantlist.

org), Kew Botanical Garden (www.mpns.kew.org) and keralaplants.in (http://keralaplants.in/) were also referred for proper and correct identification and updated nomenclature. The herbarium specimens were deposited in the Herbarium, Research Department of Botany, Sir Syed College, Taliparamba, Kannur, Kerala.

C. gladiata and *C.cathartica* were collected from the wild habitats of Kunnapuram, Kannur district, and Chembra peak, Wayanad district, respectively. *C.rosea* was spotted growing along the banks of 'Kabani' River (Panamaram). In the sand dunes of Elathur beach, Kozhikkode district, the costal species *C. maritima* was abundantly growing. Another cultivated species of *C. ensiformis* was obtained from a private homestead garden in Taliparamba, Kannur.

2) Methods of Phytochemical Analysis

Dry matter and moisture of the material were determined by the method of AOAC (FAO, 2016). Then the seeds were tested for the percentage composition of Ash (Seena and Sridhar, 2004), fat (Sadasivam, S. and Manickam, A. (1996), crude fiber (Maynard, A. J. (1970) and pectic substance (Ranganna, S. (1979). Then these were quantitatively estimated for total starch (Sadasivam, S. and Manickam, A. (1996), total carbohydrates (Hedge, J. E. and Hofreiter, B. T. (1962), reducing sugar (Miller, G. L. (1972), total soluble sugar (Dubois *et al*.,1956), total protein (Lowry *et al.*, 1951)total anti-oxidant activity (Halliwell, B. (1996); and total free amino acid (Lee, Y. P. and Takahashi, T. (1966).

Statistical analysis

Microsoft Excel was used to conduct the statistical analysis. Each set of data is the average of triplicates and reflects the mean ± standard error.

3. Results and discussion

The nutritional and anti-nutritional components of *C. ensiformis* have been widely investigated (Agbede JO, Aletor VA, 2006), it contains relatively significant levels of tannin (Belmar *et al*., 1999), saponin (Bressani and Sosa, 1990), phytic acid (Mello and Walker, 1991), and poly phenols (Babar et al, 1988 and D Mello) as well as other nutritional and anti-nutritional components (Agbede JO, Aletor VA, 2006). Several studies on *C. gladiata* seeds have produced quantitative and qualitative secondary metabolite data (Siddhuraju and Becker K 2001 &Bressani et al, 1987). The majority of phytochemical components of *C. cathartica* have also been studied (Mohan VR, Janardhanan, 1994 &Arun*et al*., 2003). In contrast, *C. maritima* is a common mat-forming wild legume found in coastal sand dunes. *C. maritima* seeds were previously studied for their proximate composition, minerals, protein fractions, amino acids, fatty acids, and anti-nutritional factors (Seena et al., 2005). However, like many other wild relatives of this family, *C. rosea*is a relatively unknown species, like many other wild relatives of this genus.

The present investigation includes the percentage analysis of moisture content, dry weight, crude fat, ash content, crude fiber content and pectic substance in legumes (table. 1). *C. enciformis*, the cultivated species is having highest value in moisture content (85.77 %). The weight of tissue left after the removal of moisture is called as the dry weight content, which is found highest in *C. cathartica* (22.88 %). The amount of crude fat and ash content were highest in *C. gladiata*. This infers *C. gladiata* is having moderately high levels of mineral nutrients

among the five species selected. Highest crude fiber content is observed in seeds of *C. rosea* and highest pectic substance was obtained from the samples of *C. cathartica.*

Because of the presence of high amount of globulins, antinutritional agents, and secondary metabolites in Canavalia seeds, protein digestibility is poor. In which globulins are the major seed proteins. Antinutritional elements such as phytic acid, tannin, saponin, and others are usually seen abundant in the legume seed meal (Bressani *et al*., 1987, Bressani and Sosa, 1990).

Plant name	Moisture content (%)	Dry weight (%)	Crude fat (%)	Ash content (%)	Crude fiber content (%)	Pectic substance (%)
Canavaliacathartica	77.12	22.88	3.22	3.76	8.62	2.26
Canavaliaenciformis	85.77	14.23	4.18	3.06	8.84	1.68
Canavaliagladiata	83.59	16.41	5.23	4.27	7.99	1.92
Canavaliamaritima	79.05	20.95	4.06	4.21	7.93	1.64
Canavaliarosea	83.66	16.34	2.64	2.98	9.17	1.92

Table. 1. Results of percentage analysis of moisture content, dry weight, crude fat, ash content, crude fiber content and pectic substance in legumes.

Carbohydrate, starch, protein, reducing sugar, soluble sugar, total free amino acid and total antioxidant activity were estimated quantitatively to determine the nutritional quality (Table. 2). *C. maritima* and *C. enciformis* had the best results for starch and soluble sugar, while seeds of *C. rosea*got the best results for carbohydrate and soluble sugar. *C. cathartica* is proven to be superior in terms of protein, total free amino acid, and total antioxidant activity.

Plant name	Carbohydrate (mg/g DW)	Starch (mg/g FW)	Reducing sugar (mg/ gDW)	Soluble sugar (mg/ gDW)	Total Free Amino acid (mg/g DW)	Protein (mg/g DW)	Total Antioxidant activity (mg GAE/ g DW)
Canavaliacathartica	635.7±1.03	325.4±2.01	5.219±1.22	22.95±1.24	2.513±0.11	396.4±1.03	3.899±1.25
Canavaliaenciformis	613.7±1.25	327.3±1.03	5.701±0.05	26.71±2.01	2.314±0.23	314.3±0.01	3.295±0.03
Canavaliagladiata	551.6±1.22	183.7±0.07	5.694±2.08	26.09±0.06	2.025±0.05	371.4±0.23	3.599±0.59
Canavaliamaritima	614.8±1.24	341.5±2.41	5.778±1.34	20.16±0.04	1.832±0.26	382.1±1.07	3.023±0.93
Canavaliarosea	658.9±1.08	299.7±0.04	7.184±0.05	25.67±2.41	2.017±0.38	327.7±1.35	2.954±1.16

Table. 2. Amount of carbohydrate, starch, reducing sugar, soluble sugar, protein, total free amino acid and total antioxidant activity in legumes.

During qualitative tests, each genus will undoubtedly have its own distinct chemical signature. Saponin, tannin, flavonoid, alkaloid, phytic acid and total phenol are common chemical substances found in Canavalia (Table. 3). Many of these chemicals have antimicrobial properties that could be useful against human diseases (Kim *et al*., 2003). Tannins have the ability to inhibit digestive enzymes (Jambunathan and Singh 1981), whereas saponin has the ability to limit nutritional intake (Cheeke*et al*., 1971). Phenolicsand flavonoids are extensively

dispersed in legumes (Fang *et al*.,2002 &Kar2007), however most of the phenolic content was lost after cooking and steaming (Babar *et al*.,1988). The presence of glycoside moieties such as saponins, cardiac glycosides, and flavonoids, which are known to suppress or act against gastrointestinal illnesses, is pharmacologically significant and supports the plant's usage in ethnomedicine (Prabhu *et al*., 2010).

Comparatively high levels of phytic acid are observed in *C. maritima* (28.57±0.39 mg/g DW) and high level of tannin is found in *C. cathartica* (38.65±0.19 mg/g DW). Alkaloid (6.624±0.08 mg/ gDW) and Saponin (5.245±0.17 mg/ g DW) were highest in *C. enciformis*. While Flavanoid (0.969±2.08 mg QE/g DW), and Phenolic content (19.71±3.05 mg GAE/ g DW) were found highest in *C. rosea*and *C. cathartica*respectively.

Plant name	Phytic acid (mg/g DW)	Tannin (mg/g DW)	Alkaloid (mg/ gDW)	Flavanoid (mg QE/g DW)	Saponin (mg/ g DW)	Phenolic content (mg GAE/g DW)
Canavalia cathartica	21.34±0.16	38.65±0.19	6.205±1.13	0.352±0.43	3.342±0.67	19.71±3.05
Canavalia enciformis	9.849±1.38	37.99±1.76	6.624±0.08	0.766±2.09	5.245±0.17	15.63±0.43
Canavalia gladiata	22.23±2.06	31.43±0.46	6.101±3.03	0.552±1.04	4.162±2.08	17.52±0.59
Canavalia maritima	28.57±0.39	31.04±0.24	5.541±2.67	0.575±0.61	4.112±2.56	18.83±4.01
Canavalia rosea	25.38±0.21	34.71±2.37	6.093±0.55	0.969±2.08	4.006±1.69	19.68±2.65

Table.3. Amount of phytic acid, tannin, alkaloid, flavonoid, saponins and phenolic content in legumes.

4. Conclusion

This research study led us to the conclusion that wild legumes, particularly *C. cathartica*, are high in protein and should be used more as a protenaceous vegetable. Proteins are not only components of legumes; they can also serve as a rich source of other nutrients such as sugar, carbs, and dietary fibres. The high ash content in *C. gladiata* indicates the superior mineral nutrient composition. This can also be utilised as a component in the formulation of neutraceutical products for human and veterinary use. Because bioactive secondary metabolites such as phytic acid, tannin, alkaloid, flavonoid, saponinsetc were plentiful in all of these five seeds. *In vivo* antioxidant properties, as well as characteristics of nutritional quality including food efficiency ratio, net protein retention, protein retention efficiency, true digestibility, biological value, and so on, need to be explored further for these advantageous wild beans.

5. Acknowledgment

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6. Conflict of interest

The authors have no conflict of interest to report.

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OP-13

Elucidation of antioxidant activity and bioactive potential of *Hygrophila ringens* in glycophytic and halophytic conditions

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Abstract

Salinity stress induces several changes in the concentration of phytochemicals and antioxidants, which in turn affect the bioactive potential of plants. These changes are genetically governed and even remain stable over years. *Hygrophila ringens* (L.) R. Br. ex Steud.(family-Acanthaceae) is a mangrove associated plant growing in saline as well as non saline habitat. It is locally known as "neerchulli". Its leaves are used together with salt as a depurative and also to cure swelling of male organs. The present study investigates some of the antioxidant activities and changes in bioactive components of *H.ringens* grown hydroponically in glycophytic and halophytic conditions. Plants cultivated without salt stress served as the control. The phytochemical analysis was done with the help of GC-MS and antioxidants were analyzed following standard procedures using UV-Visible Spectrophotometer. Significant changes were observed in the presence of antioxidants like SOD, guaiacol peroxidase, ascorbate peroxidase, total polyphenols and ascorbic acid. Guaiacol peroxidase proved to be the major enzymatic antioxidant in providing salinity stress tolerance. Polyphenol content showed significant increase in its production during saline stress conditions. The study also found presence of 9 major compounds in the control and 13 major bioactive components in the treatment. Terpenes and steroids, which play a major role in the anti-inflammation process, were found in greater amount in the methanol leaf extracts of salt treated plants compared to the control. Both enzymatic and non-enzymatic antioxidants play vital role in tolerating the detrimental effects of salinity stress. The phytochemical investigations exposed that the plant contains several bioactive components which prove it as a promising plant for developing potential drugs. The present study also highlights the fact that halophytic conditions are ideal sites for collection of this particular plant so as to explore its full therapeutic properties.

Keywords: *Hygrophila ringens*, Acanthaceae, Antioxidants, GC-MS, Glycophytic, Halophytic.

1. Introduction

Medicinal plants form an integral part of treatment in various indigenous systems of medicine[1]. Such herbal medicines have even become the trademark of Ayurvedic science[2]. Many traditional healing herbs and their parts have proved its medicinal value and used to prevent, alleviate or cure many human diseases[3]. *Hygrophila ringens* (family-Acanthaceae) is an important plant which form an integral part of traditional medicine. People used its leaves together with salt as a depurative[4].The leaf paste is used to cure boils and tumor[5]. It is devoid of toxicity principles and even possess anti-diabetic property[6]. Environmental stresses impose severe threat to the growth and distribution ofmedicinal plants. Abiotic stresses like salinity leads to numerous changes in the concentration of phytochemicals and antioxidants, which in turn affect the bioactive potential of plants. Salinity stress leads to excessive production of reactive oxygen species. Antioxidants play vital role in detoxifying such harmful reactive oxygen species and hence, increase in antioxidant production prove to be the best alternative in adapting plants to stresses like salinity[7]. The unrestricted commercialization of herbal products has led to hysterical ways of collection of plant material even without knowing the proper place of collection of plants[8]. Hence, detailed information regarding the place of collection of medicinal plants is essential to extract out the complete medicinal potential of plants. Gas Chromatography- Mass Spectrometry (GC-MS) is an important tool to analyze the volatile and semi-volatile compounds present in biological samples. It also serves as the major option to investigate the tolerance mechanisms of plants under conditions of nutrient deficiencies, abiotic stresses and mineral toxicities.Inspiteof the use of this plant by traditional healers for anti-inflammation purposes, the scientific proof of presence of anti-inflammation compounds or the best suited regions to extract out the immense potential of the plant still remains unknown. The present study investigates some of the antioxidant activities and bioactive potentials of *H.ringens* hydroponically grown in glycophytic and halophytic conditions.

2. Materials and methods

Plant Material

Plants were collected from the coastal regions of Northern Kerala, washed with water and then grown in Hoagland solution for cultivation. Modified Hoagland solution[9] prepared as described by Taiz and Zeiger[10] was used for the hydroponic study. The concentration in which the plants survived but exhibited approximately 50% growth retardation was selected as the treatment. The treatment used for the present study was 250millimolar (mM) of Sodium chloride (NaCl). Plants cultivated in Hoagland solution without any salt stress served as the control. The plants harvested after 20 days of growth in culture conditions was used throughout the study.

Antioxidant analysis

Enzymatic antioxidants

Enzyme extracts preparation and assay of enzyme activity

Fresh plant tissues (0.5 g) were weighed and homogenized in 5 ml of ice-cold 50 mM potassium phosphate buffer (pH 7.0) using a pre-chilled mortar and pestle. The homogenized

extract was filtered using muslin cloth and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatants were collected and used for the enzyme assay[11].

Superoxide dismutase (SOD, EC: 1.15.1.1) activity was assayed by method of Giannopolitis and Ries[12]. SOD activity was monitored fordetermining the ability of SOD to inhibit the photochemical reduction of nitrobluetetrazolium (NBT). The formazan accumulation was quantified using Shimadzu UV–VIS spectrophotometer, by recording the absorbance at 560 nm against the blank.

Guaiacol peroxidase (GPX, EC: 1.11.1.7) activity was measured according to Chance and Maehly[13]. The increase in absorbance due to oxidation of guaiacol was measured at 420 nm using Shimadzu UV–VIS spectrophotometer for 3 min at intervals of 30 s.

Ascorbate peroxidase (APX, EC: 1.11.1.11) activity was assayed as described by Nakano and Asada[14]. The absorbance was read at 290 nm at interval of 15 sec up to 60 seconds. One unit of the enzyme was defined as μ moles of ascorbate oxidized per minute per mg protein.

Non-enzymatic antioxidants

Ascorbic acid estimation : Ascorbic acid content wasmeasured by the method of Mukherjee and Choudhari[15].The estimation was based on the reduction of dinitrophenylhydrazine to phenyl hydrazone.The sample was extracted using 6% Trichloroacetic acid (TCA) and the concentration of ascorbic acid in the sample was calculated from a standard curve of known concentration of ascorbic acid in 6 % TCA.

Total phenolic content estimation: The total phenolics of the plant extract was determined by the method described by Makkar[16] using Folin – Ciocalteau Phenol reagent and the results were expressed in terms of Gallic acid equivalents (GAE).

GC-MS analysis

The GC-MS analysis of the methanol leaf extract of the selected plant was performed using Thermo Scientific Trace 1300 Gas chromatograph with TG- 5MS Column (30m x 0.25mm ID x 0.25μM) interfaced to an ISQ – QD Mass Specrophotometer (Perkin-Elmer GC Clarus 500 system) at Research Division, Sir Syed College, Taliparamba, Kannur. For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1mL/min and an injection volume of 1μL was employed. The injection port temperature was set at 280° C and ion source temperature at 200° C. The oven temperature was programmed from 60° C for 3min with an increase of 5° C/min to 240° C with a hold time of 5min. The scan interval was programmed for 0.2sec with a mass range of 40 – 550 amu. The total GC running time was 35 min. The components were identified based on the comparison of their relative retention time and mass spectra with those of the Wiley NIST 7N Library data. The results were also confirmed by comparing the compounds of elution and their respective indices on non-polar phases with other available literatures.

Statistical Analysis

Each set of data is an average of three independent experiments. The data represents mean± standard error.Analysis of variance (ANOVA) was performed using SPSS software 18.0. Means were compared using theDuncan'smultiple range test at 5 % probability level.

3. Results

Significant changes were observed in the concentration of antioxidants in *H.ringens*when grown in saline and non-saline conditions. Both enzymatic and non-enzymatic antioxidants showed an increasing trend upon exposure to saline conditions. Fig. I shows the amount of SOD activity in case of both control and treatment. The leaves exhibited more SOD activity than stem and root tissues. Compared to the control, the treatment showed an increase (P≤ 0.05) of 1.47 times in the leaf tissues, 1.22 times in stem tissues and 1.34 times in the root tissues.Under treatment conditions, APX activity was higher (P≤ 0.05) in the leafstem and root tissues (Fig. III). The rise in APX activity on the 20th day was found to be 3.5 fold in stem tissues, 1.7 fold in leaf tissues and 1.49 times in the root tissues.Out of the analyzed enzymatic antioxidants, guaiacol peroxidaseproved to be the major antioxidant (P≤0.05) that provides salinity stress tolerance. The stem tissues exhibited 3fold increase in the amount of guaiacol peroxidase activity while the leaf tissues showed a two fold increase in the GPX activity in case of treatment when compared to the control (Fig. II).

The non-enzymatic antioxidants also showed significant increase in their activity upon exposure to saline conditions. All the tissues (leaf, stem and root) exhibited a significantly higher (P≤ 0.05) concentration of ascorbic acid(Fig. IV) and total polyphenol content (Fig. V). The leaf tissues showed significant increase in ascorbic acid content compared to root and stem tissues.Compared to the control, all plant parts of the treatment showed significant increase in total phenol content.

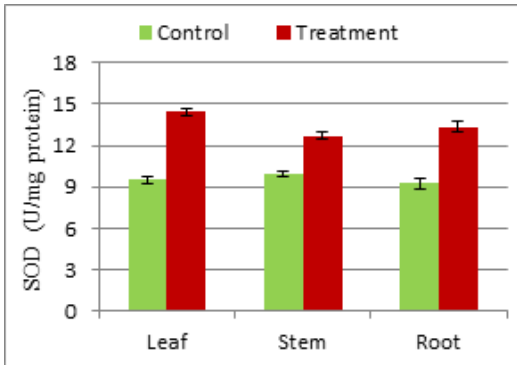

Fig. I.Superoxide dismutase activity

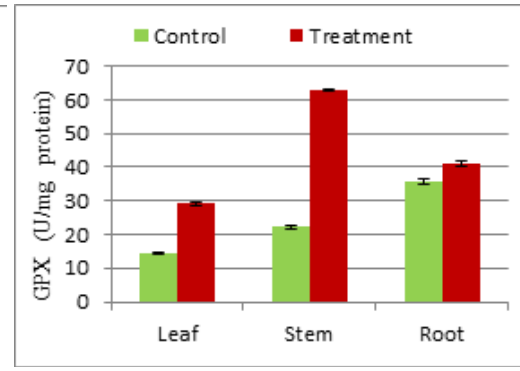

Fig. II.Guaiacol peroxidase activity

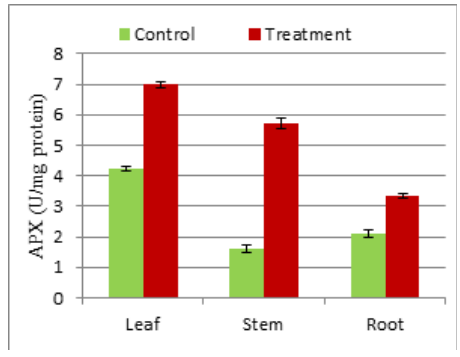

Fig. III. Ascorbate peroxidase activity

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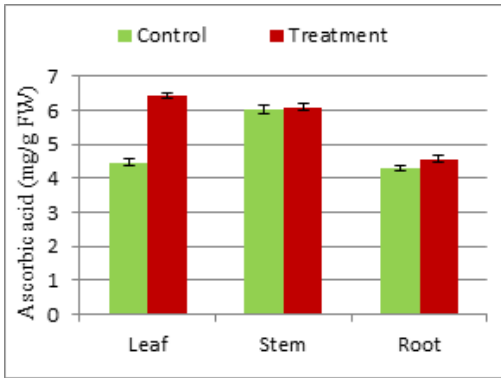

Fig. IV. Ascorbic acid content

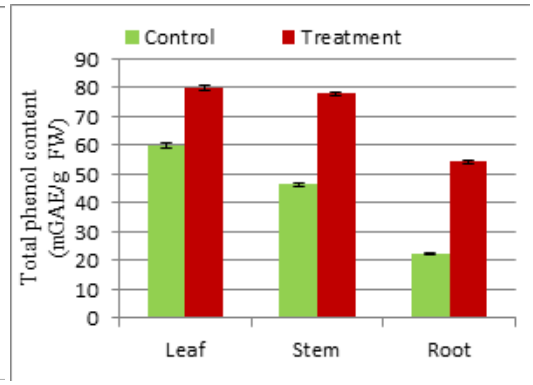

Fig. V. Total Phenolic content

The GC-MS analysis of methanol leaf extract of the control contained 9 compounds whereas the treatment contained 13 bioactive components. Hexatriacontylpentafluoropropionate (10.56 %) and Tritetracontane (10.02%) were the major compounds present in the control (Table I; Fig. VI). The other bioactive components in the control include à-Pinene (0.35%), Phytol (2.77%), Neophytadiene (5.17%), Tricyclo [20.8.0.0 (7,16)]triacontane , 1(22),7(16)-diepoxy- (1.87%), 1-Heptatriacotanol (3.27%), Thunbergol (3.32%) and ç-Sitosterol (9.58%).

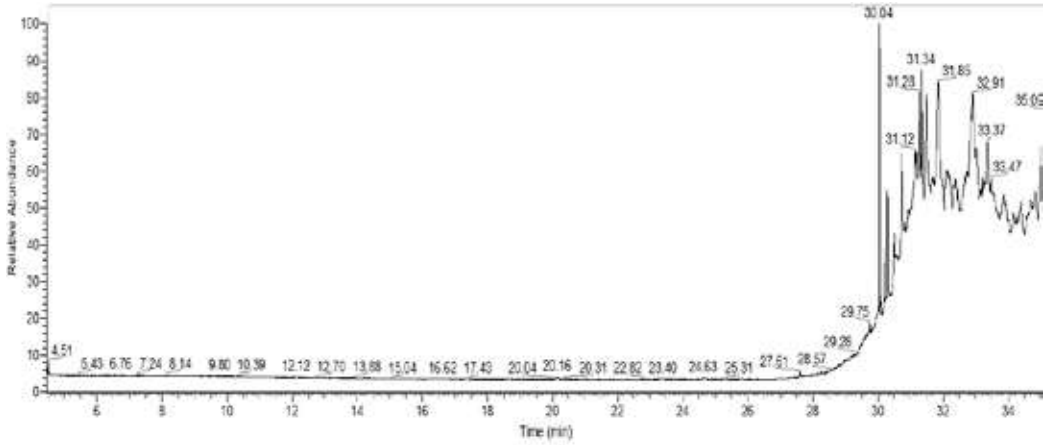

Fig. VI. GC-MS chromatogram of methanol extract of *H.ringens* (Control)

Table I :List of bioactive compounds in Methanol extract of *Hygrophilaringens*(Control)				
Sl. No.	Name of the compound	Molecular formula	Retention time	Peak Area %
i) Monoterpenes				
1	à-Pinene	C10H16	4.51	0.35
ii) Sesquiterpenes				
2	Neophytadiene	C20H38	30.04	5.17

iii) Hydrocarbons				
3	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-	C30H52O2	31.12	1.87
4	Tritetracontane	C43H88	31.85	10.02
5	1-Heptatriacotanol	C37H76O	33.37	3.27
6	Hexatriacontylpentafluoropropionate	C39H73F5O2	35.09	10.56
iv) Diterpenes				
7	Phytol	C20H40O	31.34	2.77
8	Thunbergol	C20H34O	35.6	3.32
v) Steroids				
9	ç-Sitosterol	C29H50O	32.91	9.58

Phytol (13.5%) was the major compound present in the treatment (Table II; Fig. VII). The other bioactive components in the treatment include 1-Heptatriacotanol (0.16%), Tetradecane (0.43%), Hexadecane (0.55%), Neophytadiene (7.19%), Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy- (3.65%), 17- Pentatriacontene (4.93%), 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester (4.1%), Lup-20(29)-en-3-ol, acetate, (3á)- (6.65%), á-Amyrin (5.39%),Stigmasta-3,5-diene (5.76%),Lupeol (0.94%) and ç-Sitosterol (5.8%).

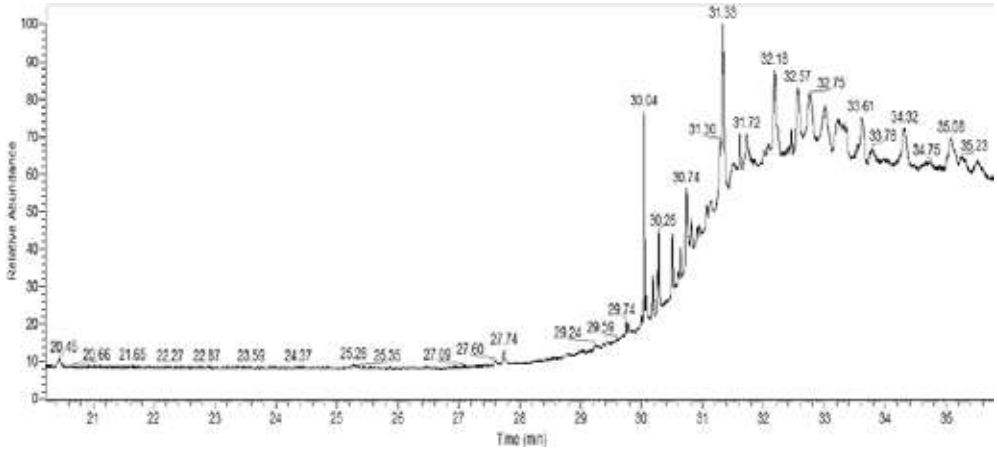

Fig. VII. GC-MS chromatogram of methanol extract of *H.ringens* (Treatment)

Table II :List of bioactive compounds in Methanol extract of *Hygrophilaringens* (Treatment)				
Sl. No.	Name of the compound	Molecular formula	Retention time	Peak Area %
i) Hydrocarbons				
1	1-Heptatriacotanol	C37H76O	3.79	0.16
2	Tetradecane	C14H30	20.45	0.43
3	Hexadecane	C16H34	27.74	0.55
4	Tricyclo [20.8.0.0 (7,16)] triacontane, 1(22), 7(16)-diepoxy-	C30H52O2	30.28	3.65
5	17-Pentatriacontene	C35H70	34.32	4.93
ii) Sesquiterpenes				
6	Neophytadiene	C20H38	30.04	7.19
iii) Dicarboxylic acid esters				
7	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	C20H30O4	30.74	4.1
iv) Diterpenes				
8	Phytol	C20H40O	31.33	13.5
v) Triterpenes				
9	Lup-20(29)-en-3-ol, acetate, (3á)-	C32H52O2	32.18	6.65
10	á-Amyrin	C30H50O	32.57	5.39
vi) Steroids				
11	ç-Sitosterol	C29H50O	32.75	5.8
12	Stigmasta-3,5-diene	C29H48	33.61	5.76
13	Lupeol	C30H50O	35.23	0.94

4. Discussion

Reactive oxygen species (ROS) are normally produced during various physiological processes in plants. However, under stress conditions such as biotic or abiotic stresses, there is an increased production of reactive oxygen species which causes unrestricted oxidative damage to the cells[17]. The increased production of antioxidants during stress conditions was studied by Parida*et al*[18]. SOD aids in converting superoxide radicals to H_2O_2 and molecular oxygen[19]. It was found that diminution in APX activity made plants more vulnerable to oxidative damage induced by salinity[20]. Increased GPX activity plays vital role in consuming the excess H_2O_2 and was reported to prevent oxidative damage in the leaves of *Carthamustinctorius* cultivars[21].

Ascorbic acid and polyphenols plays vital role in neutralizing reactive oxygen species, thereby imparting salinity tolerance[22]. The present study also found increased production of enzymatic as well as non-enzymatic antioxidants in *H.ringens*, which might be one of the strategy adopted by the plant to withstand salinity stress.

Hydrocarbons, steroids and terpenoids were the major groups of compounds present in the control while the treatment contained hydrocarbons, steroids, terpenoids and dicarboxylic acid esters.The present study reported the presence of steroids like Stigmasterol, stigmastan-3,5-diene, ç-sitosterol and lupeol as well as terpenoids like Lupeoltrifluoroacetate, á-Amyrin, phytol, á-Pinene, Neophytadiene, Thunbergol and Lup-20(29)-en-3-ol,acetate, (3á) in *H.ringens*. These phytosterols play major role in reducing the damages caused due to the production of reactive oxygen species during stress conditions and also possess anti-inflammatory properties[23].Most of the identified steroids possess chemical structure ideal for anti-inflammatory activities and also show various pharmacological properties like anti-tumor, anti-bacterial, anti-helminthic, hepatoprotective, immunosuppressive and cardiotonicactivities[24]. Terpenoids are another group of bioactive compounds which are widely used as anti-inflammatory drugs and also provide defence against environmental stresses[25]. The acetate-mevalonic pathway in mitochondria and cytosol results in the production of ubiquinones, sterols and sesquiterpenes, while mevalonic acid pathway in the plastids helps in the synthesis of mono-, sesqui-, hemi- and diterpenes[26]. The sesquiterpenes observed in the present study include Neophytadiene. The diterpenes include Thunbergol and Phytol. Sesquiterpenes was reported to possess anti-bacterial, anti–tumor, anti-inflammatory, sedative and analgesic properties. Similarly, diterpenes possess antimicrobial properties[27].

The anti-diabetic potency and absence of toxicity principles in the leaf extracts of *H.ringens* was confirmed experimentally [6]. This plant itself is known as 'neerchulli' by local people, as it is used by them to cure swellings and inflammations. The available literature also throws light on the fact that this plant is used by ethno-botanically to cure swelling of male organs, but the phytochemicals responsible for such anti-inflammatory property still remains unexplored. Also, there is still no data regarding the place of collection of this plant in order to explore such therapeutic properties. The increase in number of bioactive compounds as well as the amount of anti-inflammatory compounds confirmed by GC-MS study is a notable finding. The present study thus highlights the fact that halophytic conditions are ideal sites for collection of this particular plant so as to explore its full therapeutic properties.

5. Acknowledgement

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6. Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this work.

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OP-14

Assessment of biomass and carbon sequestration in mangroves of selected sites in Kannur district, Kerala

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Abstract

Mangrove vegetations provide a valuable service to the global population by storing vast amounts of carbon and are one of the most efficient and cost-effective solution for offsetting carbon emissions. The preliminary aim of the study includes estimation of biomass and carbon storage potential of mangrove species distributed in the selected study stations of Kannur district, Kerala. The selected stations of Kannur district for the study include Cherukunnu (S 1) and Nadal (S 2). Quadrant study was carried out for the non-destructive estimation of biomass of mangrove species. Existing general allometric equations were applied for computing biomass and carbon content of mangrove species. Study station 1 (S 1) consists of 7 true mangrove species. The total biomass and carbon content of mangrove trees in ten study plots from S 1 was found to be higher in *A. officinalis* (9274.53 Kg and 6058.64 Kg C) and lower in K. candel (94.65 Kg and 46.8 Kg C). Study station 2 (S 2) consists of 6 true mangrove species. Total biomass and carbon content of *A. officinalis* in ten study plots from S 2 had greater values (6563.8 Kg and 3562. 51 Kg C), whereas *K. candel* had lower values (117.5 Kg and 58.3 Kg C). The total biomass of all true mangrove trees from the study plots was estimated to be 19951.42 Kg in S 1 and 14336.79 Kg in S 2. The total carbon content (AGC + BGC) of all true mangrove trees from the study plots was estimated to be higher in S1 (10584.21 Kg C) compared to S 2 (7231.15 Kg C) which is equivalent to the weight of 38844.04 CO 2 and 26538.32 CO_2. Mangrove species distributed in the undisturbed area of the study stations (S 1) exhibit high biomass and carbon content. It signifies the potential of mangrove species in carbon sequestration and thereby plays a vital role in climate change mitigation by reducing atmospheric CO_2 emissions.

Key words: biomass, carbon sequestration , mangroves.

1. Introduction

Mangroves are one of the world's richest storehouses of biological and genetic diversity[2]. Mangrove vegetations provide a valuable service to the global population by storing vast amounts of carbon and are one of the most efficient and cost-effective solution for offsetting carbon emissions. Mangroves are important tropical carbon sinks, and their role in mitigatingclimate change is well documented across the globe. However, the biomass and carbonstocks in the mangroves of India have not been studied comprehensively. Data fromthis region is very limited for providing sufficient insights and authentic evaluation ofcarbon stocks on a regional scale.The preliminary aim of the study includes estimation of biomass and carbon storage potential of mangrove species (*Aegiceras corniculatum, Avicennia marina, Avicennia officinalis, Bruguiera cylindrica, Exoecaria agallocha, Kandelia candel* and *Rhizophora mucronata*) distributed in the selected study stations of Kannur district, Kerala.

2. Methodology

The study was conducted in Kannur district, situated in the state of Kerala. The total extent of mangroves in Kerala was estimated to be 19.531 km^2. Kannur district has the most mangrove cover (7.465 km^2), representing about 38.22% of the total mangrove area of the Kerala state[6]. The selected three study stations within Kannur district are as follows:

Study Station 1 (S_1) Cherukunnu (11.99497^0 N & 75.2887^0 E): Near Govt. Welfare HSS, Cherukunnu. A portion of the mangrove forest in this station is owned and protected by Mathrubhumi. It is a well conserved site with the presence of 7 true mangrove species.

Study Station 2 (S_2) Nadal (11.81798^0 N & 75.43247^0 E): The mangrove area present in Nadal is under private ownership. Human disturbances and other developmental activities are a major threat to mangrove diversity in this area which calls for its urgent conservation initiatives. The station consists of 5 true mangrove species (*Avicennia officinalis, Bruguiera cylindrica, Excoecaria agallocha, Kandelia candal, Rhizophora mucronata*).

Biomass and carbon content estimation

Quadrant study was carried out for the non-destructive estimation of biomass of mangrove species. At each study station, a total of 10 plots (10×10m^2) were taken for data collection.

All mangrove trees ≥ 3 cm in girth was measured at Breast height to the nearest centimetre and identified to the species level[4]. The diameters of the trees were calculated by dividing the girth by π. Allometric equations developed by Komiyama *et al*., (2005) for mangrove species in South-east Asia were used for the estimation of aboveground biomass (AGB) and belowground biomass (BGB).

The values of aboveground biomass and below ground biomass was summed to get the total biomass, and this biomass value was averaged to get mean total biomass (Kg). The carbon content was calculated by multiplying the individual tree biomass with the conversion factor 0.5[1]. Since understorey vegetation (seedlings and herbs) is negligible in mangrove systems, they were not considered for ecosystem carbon stock estimations[3& 7].Average biomass and carbon content of individual mangrove species was calculated by dividing the total biomass of each mangrove speciesfrom 10 study plots by total number of mangrove species. To estimate the weight of carbon dioxide sequestrated in the mangrove tree, multiply the total carbon

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content of mangrove tree by 3.67 (ratio of CO_2 to carbon). Mean total carbon content of research plots was multiplied with the total area of the study site gives the carbon sequestration by mangrove species distributed in the study station.

3. Results and discussion

Study station 1 (S_1) consists of 7 true mangrove species, which includes *Aegiceras corniculatum, Avicennia marina, Avicennia officinalis, Bruguiera cylindrica, Exoecaria agallocha, Kandelia candel* and *Rhizophora mucronata.* Population density was highest for *Avicennia* species and lowest for *A. corniculatum*.

Total Biomass in Kg (AGB + BGB), Total Carbon Content in Kg C (AGC + BGC) and W_{CO2} of each mangrove trees counted and measured from the ten study plots (10 × 10 m^2) of S_1 and S_2were given in table 1.1.

The total biomassand carbon content of mangrove trees counted and calculated from ten study plots from S_1 was found to be higher in *A. officinalis* (9274.53 Kg and 6058.64 Kg C) and lower in *K. candel* (94.65 Kg and 46.8 Kg C). Average biomass/ tree was also estimated to be higher in *A. officinalis* (171.75 Kg) followed by *A. marina* (148.79 Kg) and *R. mucronata* (118.65 Kg). Average carbon content/ tree was calculated to be higher in *A. officinalis* (112.20 Kg C) and lower in *K. candel* (3.34 Kg C).

Mangrove species	S_1			S_2		
	Total Biomass/ plot m^2 (Kg)	Total Carbon Content/ plot m^2 (KgC)	W_{CO2}	Total Biomass/ plot m^2 (Kg)	Total Carbon Content/ plot m^2 (Kg C)	W_{CO2}
Aegicerascorniculatum	115.8	56.9	208.82	-	-	-
Avicennia marina	2380.6	997.23	3659.83	631.56	378.5	1389.09
Avicennia officinalis	9274.53	6058.64	22235.21	6563.8	3562.51	13074.41
Bruguiera cylindrica	1347.9	674.35	2474.86	828.5	419	1537.73
Exoecariaagallocha	567.85	236.72	868.76	346.71	145.8	535.09
Kandeliacandel	94.65	46.8	171.76	117.5	58.3	213.96
Rhizophoramucronata	6170.09	2513.57	9224.8	5848.72	2667.04	9788.04
Total	19951.42	10584.21	38844.04	14336.79	7231.15	26538.32

Table 1.1 Total Biomass in Kg (AGB + BGB), Total Carbon Content in Kg C (AGC+BGC) and W_{CO2} of each mangrove trees counted and measured from the ten study plots (10 × 10 m^2) of S_1 and S_2

Study station 2 (S_2) consists of 6 true mangrove species. Population density was highest for *R. mucronata*and lowest for *A. marina* followed by *K. candel* and *E. agallocha*. Total number of mangrove trees in 10 study plots, Average Biomass/ tree (Kg) and Average Carbon Content/ tree (Kg C) from S_1 and S_2 were given in table 1.2.

It was discovered that total biomass and carbon content of *A. officinalis* in ten study plots from S_2had greater values (6563.8 Kg and 3562. 51 Kg C), whereas *K. candel* had lower values (117.5 Kg and 58.3 Kg C). *A. officinalis* (172.73 Kg) had the highest average biomass/ tree, followed

by *A. marina* (90.22 Kg) *R. mucronata* (89.98 Kg). *A. officinalis* had a higher average carbon content/ tree (93.75 Kg C), while *K. candel* had a lower average carbon content (3.43 Kg C).

Mangrove species	Total number of trees from 10 study plots($10\times10m^2$)		Average Biomass/tree (Kg)		Average Carbon content/ tree (Kg C)	
	S_1	S_2	S_1	S_2	S_1	S_2
A.corniculatum	7	0	16.54	-	8.13	-
A.marina	16	7	148.79	90.22	62.33	54.07
A.officinalis	54	38	171.75	172.73	112.20	93.75
B.cylindrica	34	27	39.64	30.68	19.83	15.52
E. agallocha	25	18	22.71	19.26	9.47	8.1
K. candel	14	17	6.76	6.91	3.34	3.43
R. mucronata	52	65	118.65	89.98	48.34	41.03

Table 1.2 Total number of mangrove trees in 10 study plots, Average Biomass/ tree(Kg) and Average Carbon Content/ tree(Kg C) from S_1 and S_2.

The total biomass of all true mangrove trees from the study plots was estimated to be 19951.42 Kg in S_1 and 14336.79 Kg in S_2. The total carbon content (AGC + BGC) of all true mangrove trees from the study plots was estimated to be higher in S1 (10584.21 Kg C) compared to S_2(7231.15 Kg C) which is equivalent to the weight of 38844.04 CO_2 and 26538.32 CO_2. Comparison of average carbon content/ tree of different true mangroves from S1 and S2 were given in Figure 1.1.

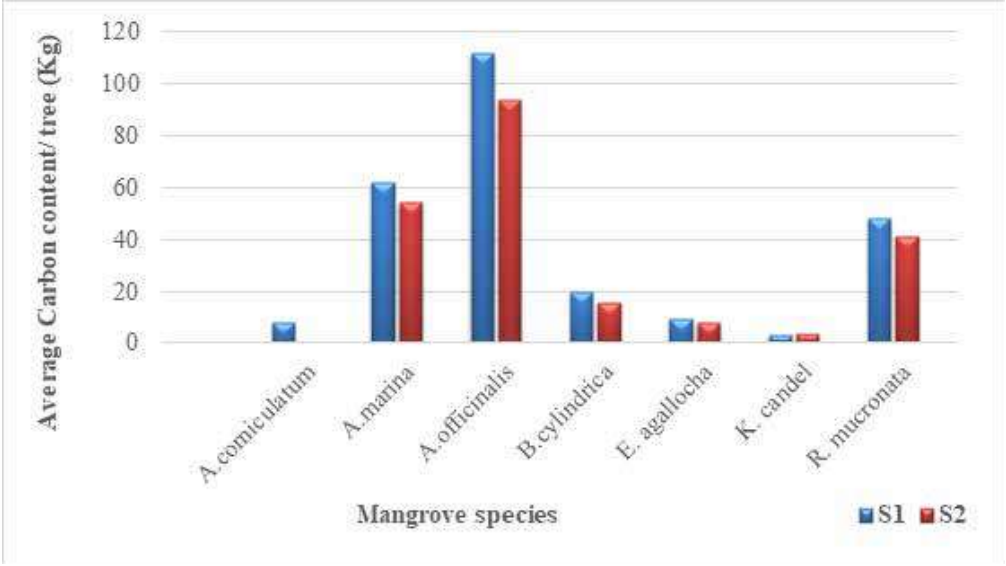

Figure 1.1 Comparison of average Carbon content/ tree of different true mangroves from S_1 and S_2

4. Conclusion

Anthropogenic disturbances including exploitation for firewood and timber, land reclamation for urbanisation and industrialisation and waste dumping have causes a significant reduction in the biomass and carbon content of mangrove species, thereby it affects the total carbon stock of mangrove vegetations. According to this study total biomass and carbon content of mangrove species were found to be higher in S_1. Compared to S_2, S_1 is a well conserved area. This emphasises the critical need for the conservation of mangrove vegetation in order to reduce carbon emissions and counteract global warming and climate change.

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OP-15

Competent cell preparation and heat shock transformation of uropathogenic *Escherichia coli* with plasmid pCAS for CRISPR-Cas9 gene editing

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Abstract

Infectious diseases are a common cause of death worldwide and there will be a future risk of increased mortality and global economic burden due to ineffective treatments. The antibiotic resistance shown by most of infectious bacteria and the emergence of superbugs is a major threat. Urinary Tract Infections (UTIs) caused by uropathogenic *E. coli* (UPEC) are one of the most common bacterial infections in humans, especially in females. If left untreated, the infection can spread up to the kidneys and bloodstream and become life-threatening. Antibiotic resistance shown by UPEC are the major limiting factors for UPEC treatments. In this situation, genome editing using the highly efficient and target specific CRISPR-Cas9 method is relevant. Simple and cheap method for delivering CRISPR components to the UPEC cell is a crucial step for this purpose. Heat shock transformation method which is simple and less expensive is used as a transformation procedure in this study.

Key words: Uropathogenic *E. coli* (UPEC), Transformation, CRISPR.

1. Introduction

Urinary tract infections (UTI) are an important medical problem and one of the common bacterial infections in human. It affects more than 150 million people each year worldwide. Most UTIs are caused by specialized *E. coli* strains referred to as uropathogenic *E. coli* (UPEC). If left untreated, the infection can spread up to the kidneys and blood stream and become life threatening. UPEC possess distinctive bacterial properties, products and a variety of virulence factors (VFs) which help the organism overcome host defences and colonize or invade the urinary tract (Samie, 2017). Antibiotics are considered the first-line of treatment for UTI. The antibiotic resistance shown by the UPEC is a limiting factor in the treatment of UTI. There exists the importance to develop effective and widely usable therapeutic strategies to manage *E. coli*-caused UTIs. Gene editing will be an alternative strategy against UPEC.

A recent breakthrough in gene editing technology is based on the class of RNA-guided endonucleases, known as Clustered Regularly Interspaced Short Palindromic Repeats

(CRISPR)-associated Cas9. This is the natural protection system of bacteria and archaea from foreign genetic elements such as plasmids or bacteriophages. The CRISPR-Cas9 system is developed from Type II CRISPR-Cas systems by which bacteria degrade targeted nucleic acids (Yang, 2015). The targeting of CRISPR- associated nuclease (Cas9) to specific DNA sequences facilitates genome editing. The Cas9 is guided to the target site by a small RNA molecule called CRISPR RNA (crRNA) or the guide RNA (gRNA). The crRNA is the transcript developed from the spacer regions present within the clustered repeats. The approach involves re-programming Cas9 by using a crRNA complementary to a target chromosomal locus and introducing a template DNA harbouring a desired mutation and an altered crRNA recognition site for recombination with the target locus. Multiple crRNA can be used to modify several loci simultaneously (Jiang et al., 2013). The simplicity of CRISPR-Cas9 programming, together with a unique DNA cleaving mechanism, the capacity for multiplexed target recognition, and the existence of many natural type II CRISPR-Cas system variants, has enabled remarkable developments using this technology to precise and efficiently target, edit, modify, regulate and mark genomic loci of a wide array of cells and organisms (Doudna and Charpentier, 2014). When the CRISPR system is programmed to selectively kill virulent bacteria by targeting virulence gene, it leaves other bacteria unaffected.

Cas9 or its variant endonucleases and gRNA are the two major requirements for CRISPR-Cas9 gene editing. gRNA which is complementary to the target region guide the Cas9 endonuclease to the target site to be cleaved and edited. Upon reaching the target site, cas9 make a cut in the target site followed by the repair mechanism by cell. The cells repair cleaved DNA area through non-homologous end joining or homology repair which make target site modifications (gene editing). Mostly the gRNA and Cas9 packed in vectors and deliver to the target cells for editing purpose. pCas is the plasmid packed with cas9. In this study, we use simple and cheap heat shock transformation method for the effective transfer of cas9 to UPEC cells.

2. Materials

UPEC Strain

The UPEC strain was obtained from a hospital in the North Malabar region of Kerala, India. It was isolated from a female patient with UTI by the hospital. The sample was cultured overnight at 37˚C on Luria Bertani (LB) media (Himedia M1245) and stored in 50% glycerol stocks at -20˚C. The cultures are revived at a regular interval of 30 days.

pCas plasmid

The vector packed with Cas9 (Fig.1) endonuclease was selected from Addgene, a non-profit plasmid repository. The selection was based on the expression system, gene insert and purpose. The features of the plasmid are given in table1.

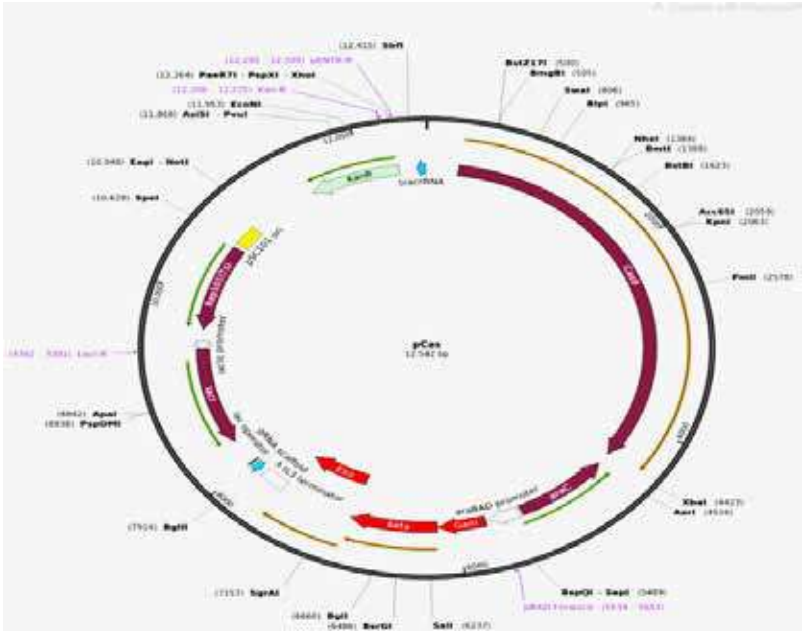

Fig1: Vector map of pCas

Addgene ID	62225
Backbone size (bp)	6300
Vector type	Bacterial Expression, CRISPR
Bacterial Resistance	Kanamycin
Growth Temperature	30°C
Growth Strain	DH5alpha
Gene/Insert name	Cas9
Promoter	Native Cas9 promoter
Purpose	Constitutive expression of cas9

Table 1: Features of pCas vector

3. Methods:

Competent cell preparation

For the preparation of cells, 2ml of UPEC glycerol stock (50%) was transferred into 250 ml of conical flask which had Luria Bertani broth (LB). To culture bacteria, the conical flask was kept in shaking incubator for overnight. From the well-developed overnight bacterial culture, 5ml was transferred into a 50 ml of LB broth and subcultured for 3-4 hours and harvested when OD600≈1.The subcultured bacterial cells were transferred to sterile, disposable ice cold

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50 ml polypropylene tubes. To cool the cultures, the tubes were stored at 0° C for 10 minutes. The cells were recovered by centrifugation at 2700 g (4100 rpm) for 10 minutes at 4 °C.After centrifugation, the medium was decanted from the cell pellets. Then the tubes were kept in an inverted position on a pad of paper towels for 1 minute to allow the last traces of media to drain away. Each obtained pellets was resuspended by swirling or gentle vortexing in 30 ml of ice cold MgCl2 - CaCl2 solution (80 Mm MgCl2, 20 Mm CaCl2). The cells were then recovered by centrifugation at 2700 g (4100 rpm) for 10 minutes at 4 °C. The medium was decanted from the cell pellets and the tubes were kept in an inverted position on a pad of paper towels for 1 minute to drain away the last traces of media. The pellets were resuspended by swirling or gentle vortexing in 2 ml of ice cold 0.1M CaCl2 for each 50 ml of original culture and the culture was stored at -20 °C.

Transformation

From each suspension of competent cells, 200 µl was transferred into a sterile, chilled 17×100 mm polypropylene tube using a chilled micropipette tip. To each tube DNA was added (50 mg in a volume of 10 µl). The contents of the tubes were mixed gently by swirling. The tubes were stored on ice for 30 minutes. The tubes were transferred to a rack placed in a preheated 42 °C circulating water bath. The tubes were stored in the rack for exactly 90 seconds without shaking the tubes. The tubes were rapidly transferred to an ice bath. Then cells were allowed to chill for 1-2 minutes. For each tube 800µl of LB medium was added. The culture was incubated for 45 minutes in a water bath which was set at 37 °C; the bacteria were then allowed to recover and to express the antibiotic resistance marker encoded by the plasmid. Appropriate volume of transformed competent cells was transferred on to LA medium containing 50mg/ml kanamycin. The plates were then inverted and incubated at 37 °C for 16 hours (Sambrook&Russel, 2001).

Transformed colonies on the agar plates were cultured on LB medium followed by plasmid extraction by alkaline lysis with sodium dodecyl sulphateand agarose gel electrophoresis for confirmation purpose.

4. Results and discussion

Transformation is the process by which an organism acquires exogenous DNA. The experiment was conducted with positive and negative controls.The negative control includes Kanamycin in LA medium with non-transformed competence cells. It showed no growth in the medium in the presence of antibiotic Kanamycin (Figure 2a). The positive control includes transformed colony in LA medium without Kanamycin, which was showed very large number of colonies (Figure2b). The third plate includes Kanamycin in LA medium with transformed *E. coli*, it was observed that small amount of large sized colony (Figure 2c). The optimum growth of bacterial cells was found in optimum temperature 37 °C.

The success of transformation depends on the competence of the host cell. Competence is the ability of a cell to incorporate naked DNA in the process of transformation. Here in heat shock method, the UPEC cells are made competent by treatment with calcium chloride. This procedure lower considerably outer membrane fluidity of cells. The decrease in fluidity was caused by release of lipids from cell surface to extra-cellular medium. A subsequent cold-shock (42°C→0°C) to the cells raised the fluidity further to its original value and this was

caused by release of membrane proteins to extra-cellular medium. When the cycle of heat-pulse and cold-shock steps was repeated, more release of lipids and proteins respectively had taken place, which ultimately enhanced transformation efficiency gradually up to third cycle. Study of competent cell surface by atomic force microscope showed release of lipids had formed pores on cell surface. Moreover, the heat-pulse step almost depolarized cellular inner membrane. They proposed that heat-pulse step had two important roles on DNA entry i.e. release of lipids and consequent formation of pores on cell surface, which helped DNA to cross outer membrane barrier, and lowering of membrane potential, which facilitated DNA to cross inner membrane of UPEC.

Transformation efficiency is the effectiveness by which cells can take up extracellular DNA and express genes encoded by it. This is based on the competence of the cells. It can be calculated by dividing the number of successful transformants by the amount of DNA used during a transformation procedure. A total of 71 transformed colonies obtained in the LB agar plate with kanamycin. Efficiency is calculated by the following formula, it showed that 1.7×10^4 cells transformed per μg of plasmid.

$$\frac{\text{Number of transformants}}{\mu\text{g of DNA}} \times \frac{\text{final vol at recovery (mL)}}{\text{vol plated (mL)}} = \text{Number of transformants per } \mu\text{g}$$

The transformed colonies were further confirmed by plasmid extraction and agarose gel electrophoresis. Four colonies on the LA plates were selected for this purpose. The DNA band on agarose gel were confirmed as pCas plasmids by their size, 12542bp (Fig 2d).

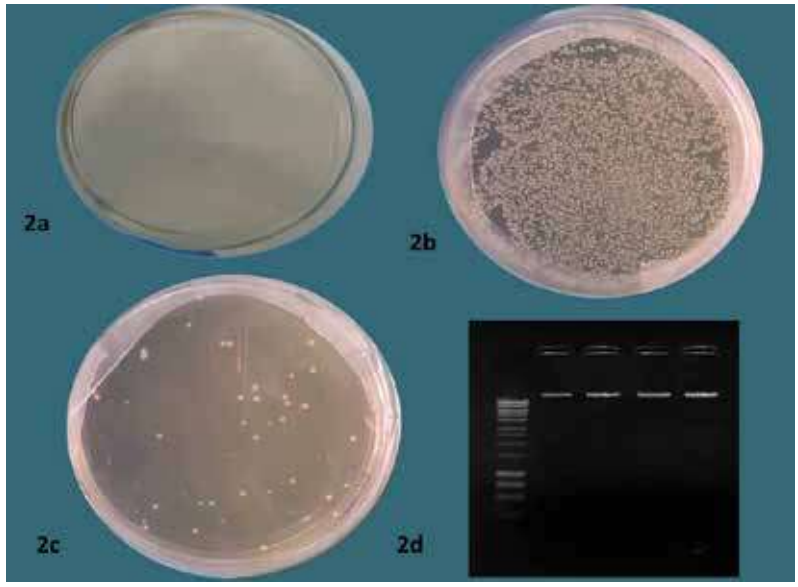

Fig 2: a): Kanamycin LA plate with no colony growth, b): Non-transformed & transformed colonies on LA plate without kanamycin, c): Transformed colonies on LA plate with kanamycin, d): Agarose gel image of isolated pCas

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The results showed that the methodology followed is suitable for transformation studies of pCas plasmid with DH5 UPEC competent cells. This suggests that CRISPR- Cas9 gene editing machinery can be successfully transformed to UPEC using simple, fast and cheap heat shock method.

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OP-16

Study on target genes involved in tomato virus infection for CRISPR-cas9 mediated gene editing

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Abstract

Viruses pose a serious threat to agriculture worldwide. The viral diseases negatively affect tomatoes (*Solanum lycoprsicum)* by drastically reducing yield and fruit quality. Under these circumstances, conventional strategies can fail to control rapidly evolving and emerging plant viruses. Genome-engineering strategies have recently emerged as promising tools to introduce desirable traits in many eukaryotic species, including plants. Among these genome engineering technologies, the CRISPR (clustered regularly interspaced short palindromic repeats)/CRISPR-associated 9 (Cas9) system has received special interest because of its simplicity, ef□ciency, and reproducibility. Recent studies have used CRISPR/Cas9 to engineer virus resistance in plants, by modifying the host plant genome to introduce viral immunity. Here, we brie□y describe the biology of the plant viruses that seriously affect the tomatoes and the characters of host genes that seem to be required for the viral infection and their silencing delay or suppress viral infections.

Key words- CRISPR-Cas9, Virus resistance, Tomato, *Solanum lycopersicum*

1. Introduction

Tomato (*Solanum lycopersicum*) is one of the major agronomically important food crops consumed worldwide. Virus diseases are considered one of the most important problems affecting tomato production in many countries. Viral infections must be controlled in order to keep up the standard and abundance of food, fibre and feed produced by farmers around the globe. In the past decade, the introduction of the concept of genome editing (GE)/modi□cation in crop plants revolutionized every aspect of plant science. Developing reliable and reproducible tools for GE in plants will have signi□cant e□ects on basic as well as applied plant research. GE technologies accelerate functional analyses of genes and the introduction of novel traits into important crop plants.A recently developed gene editing technique is CRISPR (Clustered Regularly Interspaced Short PalindromicRepeats); it is named after the curious arrays of tandem repeated sequences found in abouthalf of the bacterial genomes that have been sequenced. The arrays of repeated sequences areflanked by a conserved set of genes known as Cas (CRISPR associated) genes

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that showsimilarity to genes encoding nucleases. The targeting ofCRISPR- associated nuclease (Cas9) to specific DNA sequences facilitates genome editing.The Cas9 is guided to the target site by a small RNA molecule called CRISPR RNA(crRNA). The crRNA is the transcript developed from the spacer regions present within theclustered repeats. The approach involves re-programming Cas9 by using a crRNAcomplementary to a target chromosomal locus and introducing a template DNA harboring adesired mutation and an altered crRNA recognition site for recombination with the targetlocus. The main advantage of CRISPR-Cas9 technology is its inherent ability to cleavemultiple sites of the genome in parallel. Another important feature is its specificity. Thesystem is highly specific and it effectively cleaves the target sites in recognition to Watson and Crick base pairing. Genome-engineering strategies have recently emerged as promising tools to introduce desirable traitsin many eukaryotic species, including plants. Among these genome engineering technologies,the CRISPR system has received special interest because of its simplicity, efficiency, andreproducibility. Recent studies have used CRISPR-Cas9 to engineer virus resistance in plants, either by directly targeting and cleaving the viral genome, or by modifying the host plantgenome to introduce viral immunity.

There are many viruses that seriously affect the tomato plant. *Tomato yellow leaf curlvirus* (TYLCV), *Tomato leaf curl virus (ToLCV)* and *Tomato mosaic virus* (ToMV) are the important viruses. These areeconomically relevant viral threats to tomato plants.Many plant virologists consider it as the most vital and serious plant viruses.Plants have developed a variety of resistance mechanisms, either ready to meet incoming pathogens or induced by them. High-throughput technologies allow following changes in geneexpression upon virus infection at the genome level and evaluating the functions of these genes duringinfection in susceptible as well as resistant plants. It is very important that the identification of plant genes whose function or expression is altered as a consequence of pathogen attacks. It is essential to identify the host genes affected by infection and to determine their role in susceptible and resistant plants.

2. Results and discussion

Virus host genes interactions

Members of the *Tomato yellow leaf curlvirus* (TYLCV) complex are among the most important pathogens impairing production of agriculture crops in nature, the TYLCVs are exclusively transmitted bythe whitefly Bemisiatabaci. Members of the TYLCV complex have a single 2,700-2,800 nucleotide(n) circular ssDNA genome encapsulated in a geminate particle.*Tomato yellow leaf curl virus* is associated with modi□cations of the expression patterns of many genes, as well as changes in the protein and metabolite contents of both host plant and insect vector. All these changes are thought to facilitate host invasion, virus genome replication and expression, and to resisthost defenses. The Heat shock protein cognate 70 (Hsp70) family is actively participating in the biology of geminiviruses.The map position of Hsp70 is given in figure1.The viruses depend on host chaperones/heat stress proteins (HSPs) for folding, protein quality control (PQC) and maintenance of proteostasis. HSPs affect virus expression, replication, and assembly and counter the plant responses to infection HSPs are involved in the assembly of the large virus-induced protein aggregates sheltering the virus, promoting their activity and their multiplication. TYLCV CP can form complexes with HSPs in tomato. In the infected host cell HSP70 participates in the translocation of CP from the cytoplasm into the nucleus. Viral amounts decrease when HSP70 is inhibited.

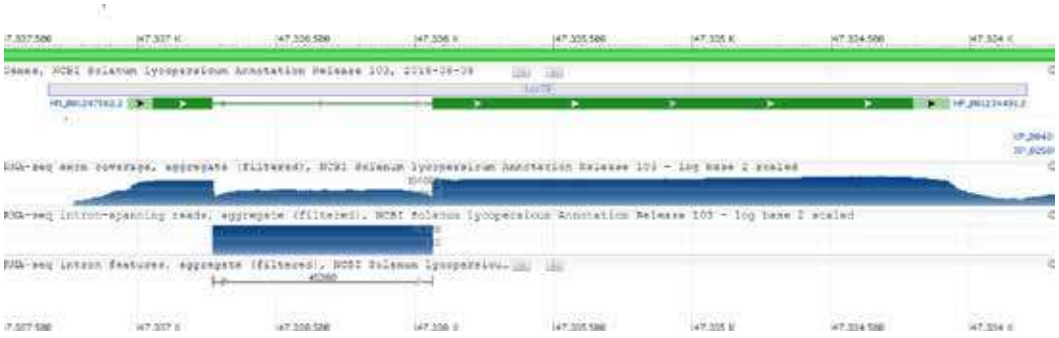

Figure 1. Showing map position of Hsp70 (NCBI)

Dcl2b (Endoribonuclease Dicer2b) gene involved in Tomato mosaic virus (ToMV) .ToMV belongs to Tobamoviruses family. ToMV particles are rigid rods of 300 x 18 nm that contain single-stranded RNA (2000 kDa) and a coat protein of a single polypeptide.Tomato encode four DCL family proteins, that are DCL1, DCL3, DCL4 and DCL2 was considered as a substitute for DCL4 for defense against viruses. The DCL2 subfamily members are DCL2a, DCL2b, and DCL2c. When infected by tomato mosaic virus (ToMV), however, the dcl2b mutants displayed more severe developmental defects consisting of strange narrow patterns on the leaves, □owers and fruits compared to the wild type. The map position of Dcl2b is given in figure2.Even DCL4 was still functional, indicating that DCL2b played a major role in the defense against ToMV**.** Genome-wide small RNA expression pro□ling revealed that DCL2b was required for the processing 22-nt small RNAs, including a few species of miRNAs. Interestingly, these DCL2b-dependent 22-nt miRNAs functioned similarly to the DCL1-produced 22-nt miRNAs in Arabidopsis and could serve as triggers to generate a class of secondary siRNAs. In particular, the majority of secondary siRNAs were derived from plant defense genes when the plants were challenged with viruses. And differentially expressed genes in dcl2b through RNA-seq and observed that numerous genes were associated with mitochondrial metabolism and hormone signaling under virus-free conditions. Notably, when the loss-of-function dcl2b mutant was challenged with tomato mosaic virus, a group of defense response genes was activated, whereas the genes related to lipid metabolism were suppressed. The loss of tomato DCL2b would increase a plant's susceptibility to ToMV infection in a natural environment.

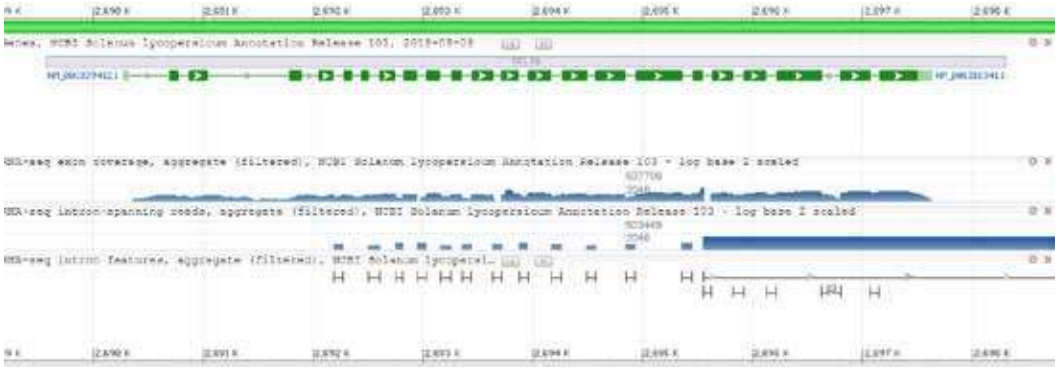

Figure.2: Showing Map position of Dcl2b (NCBI)

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Amongst various geminiviruses, the Begomovirus (Tomato leaf curl virus; ToLCV) causes devastating disease in tomato. The ToLCVs are known to be transmitted from one host to another host by white fly (*Bemisia tabaci*) insect vector and are either monopartite or bipartite in nature. Amongst Indian isolates of ToLCVs, a monopartite Tomato leaf curl Joydebpurvirus (ToLCJoV) is prevalent in the eastern part of India.CTR1 (CONSTITUTIVE TRIPLE RESPONSE) is known to be a negative regulator of ethylene signaling and actively suppresses the ethylene signaling cascade.CTR1 is upregulated during ToLCV infections in tomato.

Name of the virus	Host gene	NCBI ID	Location
Tomato yellow leaf curl virus(TYLCV)	Hsp70-1	100736433	Chromosome 6
Tomato leaf curl virus (ToLCV)	Dcl2b	101256737	Chromosome 11
Tomato mosaic virus (ToMV)	CTR1	544127	Chromosome 10

Table 1: showing characters of genes.

CTR1is an important negative regulator of ethylene signalling that was first identified by CTR1is an important negative regulator of ethylene signalling that was first identified by the isolation of a constitutive ethylene-response mutant in *Arabidopsis*. In the absence of ethylene treatment, *ctr1* mutants exhibit the same phenotypes as ethylene-treated wild-type plants. Three CTR1 homologues are involved in ethylene signalling and display differential gene expression patterns that might reflect specific function in tomato (*Solanum lycopersicon*). CTR1 encodes a Raf-like serine/threonine (Ser/Thr) protein kinase with an N-terminal regulatory domain and a C-terminal kinase domain. CTR1 acts downstream of the ethylene receptors and upstream of EIN2. When the receptors perceive ethylene, CTR1 kinase activity is shut off, thereby leading to responses. CTR1 has been shown to physically associate with the ethylene receptors at the endoplasmic reticulum membrane, but the biochemical mechanisms of CTR1 regulation remain unclear at this point. The downstream substrate(s) of CTR1 are unknown as well. The map position of CTR1is given in figure3. CTR1 has the highest sequence similarity to Raf protein kinases, so it has been long assumed that CTR1 functions in a mitogen-activated protein kinase (MAPK) cascade. The existence of a MAPK cascade in ethylene signalling has been controversial however, and functional similarities between CTR1 and MAP kinase kinasekinase (MAPKKKs) remain speculative.

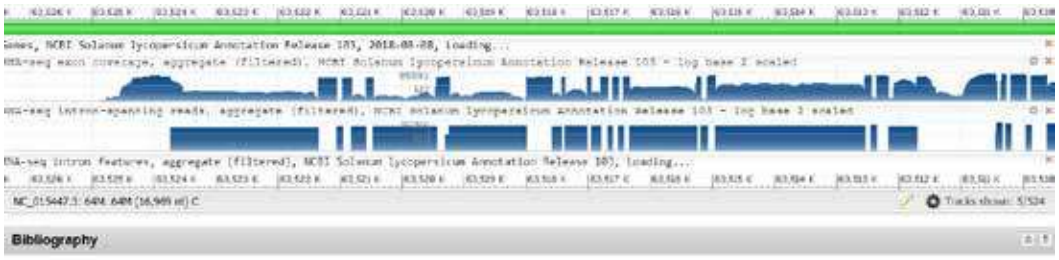

Figure 3 showing map position of CTR1gene(NCBI)

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OP-17

Assessment of total carbon storage in pneumatophores of *Avicennia species* in selected mangrove wetlands of Kannur district, Kerala

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Abstract

Mangrove swamps are coastal wetlands found in tropical and subtropical regions. They have different types of special root systems which supports epiphytic algal flora on them especially on the pneumatophores. Presence of this epiphytic algae enhances the productivity of the ecosystem as well as the carbon sequestration capacity of pneumatophores. The present study aims to find out the epiphytic algal biomass and carbon sequestration capacity of pneumatophores from the selected study stations such as Kunhimangalam, Valapattanam and Patuvam of Kannur District.

Key words: *Avicennia*, Pneumatophores, Carbon storage

1. Introduction

Mangrove ecosystem comprises unique salt tolerant trees, shrubs and other plants which are tropical and subtropical in distribution. The total area of mangroves in India is estimated to be 6740 sq.kms, which covers about 7% of the world's mangrove area (Krishnamurthy,1987) and 8% of Indian coastline (Untawale,1987). The mangrove vegetation covers only 1,095 ha in Kerala (Kurien et. al, 1994). A recent study by Radhakrishnan et.al (2006) showed that mangrove vegetation in four districts of Kerala i.e.Kasargod, Kannur, Kozhikode and Malappuram is approximately 3,500ha, which represents about 83% of mangrove cover in the state. Mangroves have different types of special root systems which provides a substratum for the attachment of epiphytic algae which increase the productivity of the ecosystem.The epiphytic algal biomass on the pneumatophores of grey mangrove *Avicennia marina* (Forssk). Vierh. were estimated for the first time from the Indus Delta region by Saifullah and Ahammed (2007). The mangroves are the most carbon rich ecosystems of the world. They are the highly efficient carbon sinks.Algae contribute significantly to the carbon pool by sequestering and cycling carbon.

STUDY SITES

Kunhimangalam (S1): Located at 12°15′N and 75°30′E. This place is known as 'KandalGramam' meaning "Mangrove village" as the area has luxuriant mangroves.

Valapattanam (S2): Located 11.93358 N and 75.28894 E. The area is highly polluted by industrial wastes and slaughterwastesand showed degraded root systems.

Pattuvam (S3):locatedat12.039°N75.321°E. The area were also polluted but the intensity low.

2. Materials and methods

The field visits were conducted in the study areas during post monsoon season (2021 November to 2022 January).Quadrate study of pneumatophores of *Avicennia species*were done, which were then collected, brought to the laboratory. The epiphytic algal biomass surrounding the pneumatophores were separated and fresh weight taken which then placed in an oven, left to dry at 80celcious for a constant dry weight for one day. The fresh weight and dryweight of pneumatophores with attached epiphytic algae, pneumatophores devoid of algae,andepiphyticalgaealonewereweighedwithaweighingbalance.From the biomass estimation carbon content in the pneumatopheres were calculated using the carbon conversion factor (0.39) value (Kauffman & Donato 2011).

3. Results and discussion

Assessment of total biomass and carbon sequestration potential of pneumatophores were done in selected study stations such as Kunhimangalam, Valapattanam and Pattuvam of Kannur District. This result signifies the role of mangroves in carbonsequestration through their special roots and associated algal forms. This is an importantvalue of mangroves when discussing global issues like climate change and global warming.

Table 1. shows the average number, average length and breadth of roots from the study sites. The average length of pneumatophorescollected from Kunhimangalam, Pattuvam and Valapattanam are 28, 26, 23 cm whereas the average breadth of pneumatophores from these study sites are 0.748, 0.577, 0.656cm respectively. The average number of pneumatophores from the above study sites also shows differences. Kunhimangalam, one of the mangrove conservation zone has more number of pneumatophores when compared to the other two sites. Valapattanam which ishighly polluted with city's immense industrial wastes and municipal wastes, have degradedroot system. They show less number of pneumatophores compared to other sites.

Table1-Averagelengthand breadthof pneumatophores fromthestudysites

Sl. No.	Sitename	Average Number of pneumatophores	Averagelength (Cm)	Average breadth (Cm)
1.	Kunhimangalam	520	28	0.748
2.	Pattuvam	480	26	0.577
3.	Valapattanam	366	23	0.656

Table 2. Shows fresh weight and dry weight and thereby the total biomass for all pneumatophores.The average fresh weight of pneumatophores with attached epiphytic algae of Kunhimangalam study site is 10.905 gm. Pattuvam with an average fresh weight of 8.0157gm and Valapattanam have an average of 5.105 gm. The total dry weight of sampledpneumatophores along with attached epiphytic algae of Kunhimangalam, Pattuvam,

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andValapattanam study stations were 5.53, 1.76, and 1.7 gm respectively. The total biomass ofall pneumatophores from the study sites Kunhimangalam, Pattuvam and Valapattanam are 2.876, 0.844 and 0.622kg respectively. Kunhimangalam shows the highest biomass, followed by Pattuvam, and then Valapattanam. From these results we can see that there is a considerable variation in the epiphytic biomass of algae on pneumatophores. Algae play a major role in increasing the productivity of mangrove ecosystem.

Table 2 Biomass estimation from pneumatophores

SITE	Average number of pneumatophores	Average Fresh weight of sampled roots. (gm)			Average Dry weight of sampled roots. (gm)		Total dry weight of sampled roots.(gm)	Total biomass of allpneumatophores.(kg)
		Pneumatophores with algae	Pneumatophore (devoid of algae)	Algae only	Pneumatophores	Algae only		
Kunhimangalam	520	10.905	10.272	0.355	5.44	0.091	5.531	2.876
Pattuvam	480	8.157	5.497	0.463	1.68	0.08	1.76	0.844
Valapattanam	366	5.105	4.806	0.705	1.60	0.1	1.7	0.622

Table 3. shows the total carbon content at the three study sites. The average carbon content from the selected Quadrate study sites of Kunhimangalam, Pattuvam, and Valapattanam are 224.33, 65.89, 48.53 C/m^2.The total carbon content of the three study sites Kunhimangalam, Pattuvam and Valapattanam were1243.68 kg carbon per 5.54 hectare, 198.85 kg carbon per 3.018 hectare and 182.65 kg carbon per 3.76 hectare respectively.

Table 3. Total carbon content study from the three selected wetlands

Site	Average Number of Pneumatophores	Biomass of all sampled roots. (kg)	Average carbon content (C/m^2)	Total carboncontent in thewholestudy area.(kg/ha)
Kunhimangalam	520	2.876	224.33	1243.68
Pattuvam	480	0.844	65.89	198.85
Valapattanam	366	0.622	48.53	182.65

Bio

Mangroves serve as a natural carbon reservoir. They are able to store and stock carbon from the atmosphere. The studies conducted on the above three sites have shown that mangroves can store much amount of carbon within a small area. So mangroves present all over the world can sequester large amounts of carbon and their value is much greater than tropical forests. The presence of associated epiphytic algae adds the carbon sequestration capacity of the mangrove ecosystem in addition to productivity of mangrove ecosystem.

Figure. shows Average biomass of selected sites

4. Conclusion

In the present study the biomass and carbon sequestration capacity of pneumatophores were assessd, especially the role of epiphytic algae in productivity and carbon storage. Highest biomass observed at Kunhimangalam followed by Pattuvam then the Valapattanam. Higher the biomass, higher the carbon content. Mangroves serve as natural carbon reservoir and the epiphytic algae enhances the carbon storage capacity of mangrove wetlands.

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OP-18

The antioxidants and overall antioxidant potential of some traditional food plants; A strategy for diversifying the diet with stress tolerant plants

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Abstract

Crops are subjected to environmental stresses due to global warming and climate change. It resulted in significant crop loss and demanded the development of stress-tolerant, climatic-resilient crop varieties to feed burgeoning populations all over the world. It is necessary to diversify the diet of humans with nutritionally rich and stress-tolerant plants other than staples. Exposure to various abiotic stresses results in forming free radicals which cause severe damage to the plants. The balance between the generation of free radicals and scavenging by antioxidants plays a crucial role in the stress-tolerant mechanism of the plant. The study explores the antioxidant potential of four traditional food plants namely *Canavalia ensiformis* L. (DC.), *Celosia argentea* L., *Cnidoscolus aconitifolius* I. M. Johnst. and *Moringa oleifera* Lam. The polyphenol and flavonoid content and the DPPH antioxidant activity in methanol, ethanol and aqueous extracts were studied to comprehend their antioxidant potential. The Overall Phytochemical Composite Index was used to rank the plants based on their antioxidant composition. Along with that with the aid of ICP-OES the elements manganese, zinc and copper were quantified which act as a cofactor in antioxidative enzymes. The higher quantity of minerals, polyphenols, flavonoids and highest antioxidant activity and OPCI score in methanol, ethanol and aqueous extracts were recorded by *C. argentea.* But more studies are required in future to understand their stress-tolerant mechanism. Beyond the antioxidant potential, the nutritional values of the plants were also studied through proximate composition analysis, mineral quantification and Fourier-transform Infrared spectroscopy.

Key words: antioxidants, zinc, copper

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