



RESEARCH AND ACTIVITY REPORT 2020-21

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Regional Plant Resource Centre
Bhubaneswar, Odisha

Research and Activity Report 2020-21

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CONTENTS

MESSAGES

FROM CHIEF EXECUTIVE'S DESK

1. INTRODUCTION

2. MANDATE

3. EXECUTIVE SUMMARY

4. RESEARCH ACHIEVEMENTS

4.1. Development of bioinoculant for growth enhancement of *Dalbergia latifolia* and *Desmodium oojeinense* using native microflora

4.2. Morphotaxonomic characterization and documentation of Fungi of Odisha

4.3. Production, purification and evaluation of anticancer properties of extracellular secondary metabolite from *Colletotrichum* sp.

4.4. Harnessing the potential of endophyte against root-knot nematode *Meloidogyne incognita* in banana.

4.5. Developing micro-propagation methods for some endangered forest trees species of Odisha

4.6. Deciphering molecular regulation of fruit ripening in banana through quantitative proteome analysis following advanced (iTRAQ) isobaric tags for relative and absolute quantitation

4.7. Omics-approach to regulate ripening and enhance fruit shelf-life in banana: an important fruit crop for food security

4.8. Pharmacological profiling of *Geophila repens* and *Piper trioicum* and evaluation of their therapeutic potential against Alzheimer's disease

4.9. Therapeutic validation of *Pterocarpus marsupium* and *Piper trioicum* as a cholinesterase inhibitor for the treatment of Alzheimer's disease (AD).

4.10. Ameliorative effects of *Homalium zeylanicum* on diabetes-induced oxidative stress and inflammation in Wistar rats.

4.11. Collection and characterization of germplasm and nutritional analysis of different wild species and cultivated varieties of *Musa* in Odisha

4.12. Mass Propagation of Local *Musa* varieties of Odisha, Commercialization using Tissue Culture Techniques

4.13. Standardization of various macropropagation methods of useful wild edible fruits of Odisha (State Plan 2020-21)

4.14. Evaluation and characterization of pectin in selected wild edible fruits of Odisha.

4.15.Re-introduction of rare and endangered mangrove spp. *Heritiera fomes* and *H. littoralis* in protected areas of Odisha coast.

4.16.Assessment of antifungal activity of *Indigofera astragalina* and *Stachytarpheta indica* solvent extracts against *Aspergillus flavus*

4.17.Selection of suitable protocols for propagation of vulnerable medicinal plants *Mesua ferrea* and *Paederia foetida* & their biological evaluation.

4.18.Propagation and reintroduction of endangered species of Odisha
Taxonomic revision of the genus *Physalis*, *Datura*, *Capsicum* and *Nicotiana* (*Solanaceae*) in Eastern Ghats of India

5. RESEARCH PROJECTS (LIST)

6. PUBLICATIONS

7. TRAINING AND EDUCATION

8. LIBRARY

9. HERBARIUM

10. EX-SITU CONSERVATION & GERMPLASM COLLECTION

11. ESTABLISHMENT OF ORCHID RESEARCH LABORATORY

12. ESTABLISHMENT OF OMICS RESEARCH LABORATORY

13. ESTABLISHMENT OF ORCHID PROPAGATION FACILITY

14. ESTABLISHMENT OF VISITORS SHED IN BOTANICAL GARDEN

15. FLOWER SHOW 2021

16. INAUGURATION OF RESEARCH LABORATORY AND NURSERY COMPLEX

17. RELEASE OF BOOKS AND OTHER PUBLICATIONS

18. OTHER MAJOR ACTIVITIES CARRIED OUT DURING 2020-21

19. BALANCE SHEET



SHRI BIKRAM KESHARI ARUKHA

Minister

Forest, Environment & Climate Change Department
Government of Odisha



I am pleased to learn that RPRC, a reputed center for fundamental and applied scientific research, has been implementing various research projects while achieving its goal in the field of plant taxonomy and conservation, biotechnology, biochemistry, microbiology, horticulture, medicinal and aromatic plants. Apart from intensive research on plant biodiversity assessment, microbial applications, wild edible fruits, mushrooms, mangroves, orchids and phyto-chemicals of selected medicinal plants, few other aspects like bio-fuel production, viral indexing of economically important crops including tissue culture of banana are noteworthy as important thrust and frontier areas of plant sciences. It has consistently maintained and added to its rich collections of rare and endangered plants, orchids, bamboos, palms, cacti and succulents, mangroves, medicinal & aromatic plants, for developing a repository of bio-resources for research and crop improvement. I wish that the outcomes of the work carried out in RPRC would find its way to benefit all stakeholders of Odisha.

I am hopeful that the Research & Activity Report (2020-21) published by the center would be useful for students, teachers, researchers and conservationists. I appreciate the effort of the staff of the center in this regard and convey them my best wishes.

(Bikram Keshari Arukha)



DR. MONA SHARMA, IAS
Additional Chief Secretary

Forest, Environment & Climate Change Department
Government of Odisha



The Regional Plant Resource Centre (RPRC), a Research & Development organization, under the Forest, Environment and Climate Change Department, in Odisha has been implementing several innovative research projects for bio prospecting indigenous macro and microflora for wider use through fundamental and applied research. The centre has strengthened and augmented its research and development programmes for conserving, propagating, and documenting the rich biological wealth of the region. Production of quality planting materials on commercial scale, germplasm conservation & analysis of wild edible fruits and re-introduction of rare and endangered plants including mangroves & orchids are some of the significant activities of the organization.

I am happy to learn that RPRC has also successfully completed several research projects funded by different Departments of Government of India and Odisha. I hope the Institute would continue to endeavor in finding solutions to meet recent challenges in conserving the biological diversity of the State.

I express my appreciation for bringing out this research & activity report (2020-21) which would be a positive source of information to disseminate the findings of various research activities being undertaken by the Institute.

(Dr. Mona Sharma)



SMT. PUSAZULE MEKRO, IFS
Chief Executive

This Research & Activity Report 2020-21 presents an synoptic appraisal on implementation of various research programmes which are prioritized to address issues pertaining to conservation and bio-resource utilization relevant to the eastern ghats in general and Odisha state in particular. In continuation to our effort to establish germplasm banks of various plant groups for conservation and scientific enquiry, the center initiated and maintained research activities focusing the prioritized areas such as germplasm conservation and re-introduction of RET and other important special group of plants including mangroves and orchids, screening of wild edible fruits and medicinal plants for active bio-molecules, nutraceuticals, antioxidants, microbial applications (bioinoculant) for benefiting forest species, viral indexing of crop plants, and micro-propagation of plantation crops and endangered plants.

Research funds have been made available to the Scientists of RPRC from state Forest, Environment & Climate Change Department under state plan budget after rigorous evaluation by the Research Advisory Committee (RAC) headed by the PCCF & HoFF in the Government. The centre has implemented several such research projects covering various thrust areas of research relevant to the sate as per recommendation of the RAC.

RPRC has been encouraging and nurturing academic intellect by guiding Ph.D. and M.Sc. students. A six month Project training progarmme for M.Sc. (Biotech) students from various organizations is being organized to provide hands on training to fulfill the requirement of their M.Sc. degree. Several research papers in national and international journals have been brought out by the Centre and many new processes and technologies have been developed.

All the research group, administrative group and supporting staff of RPRC made sustained effort and contributed to growth of the institute, and I extend my sincere thanks to them for their endeavour. Financial support received from various agencies of Govt. of India and Odisha is gratefully acknowledged. We are grateful to Additional Chief Seceretary, Forest, Environment and Climate Change Department, Government of Odisha for providing the research grant under state plan budget and support provided by Director, (Environment)-cum-Special Secretary is thankfully acknowledged.



(Pusazule Mekro)

INTRODUCTION

Regional Plant Resource Centre (RPRC), Bhubaneswar, an autonomous R&D institute of Forest, Environment and Climate Change Department, Govt. of Odisha, has been taking all efforts to showcase its various R&D activities primarily through implementation of various research projects relevant to the state and establishment of different State of the Art facilities to produce and conserve plant genetic resources such as Modern Tissue Culture Laboratory, Orchidarium, Threatened Plants (RET) Garden: Wild Edible Fruits Garden, Medicinal Plants Garden, Cacti and other Succulents, Jagannath Vatika, Fragrant Flower Garden, Morning Health Walk etc. RPRC has implemented various research programmes under financial support from Forest, Environment & Climate Change Dept., Government of Odisha, RKVY, Science & Technology Dept., Govt. of Odisha, other apex central funding agency like DBT, NMPB, Govt. of India, which were prioritized to address issues pertaining to conservation and bio-resource utilization relevant to Odisha state.

During the year 2020-21, the Center initiated and maintained its core research activities focusing the prioritized areas such as re-introduction of RET and other important special group of plants including mangroves and orchids, domestication and evaluation of wild edible fruits and medicinal plants for active bio-molecules, nutraceuticals, antioxidants, application of bioinoculant for forest species, useful secondary metabolites from fungi, micro-propagation of forest species, plantation crops and endangered plants. A total 8 external funded, 16 state plan funded projects have been implemented during the year 2020-21, engaged around 40 research fellows, published 33 research papers, 3 books, 4 book chapters, 1 Research & Activity Report (2019-20), trained 5 M.Sc. Biotech students for their PG degrees.

MANDATE

The center has a mandate of promoting bioresource conservation, research and to augment plant resources for sustainable development in the following areas;



Germplasm collection of selected plant groups (living collections) for long term conservation and research.



Survey, evaluation, propagation and conservation of medicinal, aromatic, oil-yielding and other economic plants including rare/endangered species.



Genetic manipulation of plants through cell, tissue and organ culture, somatic embryogenesis, transformation techniques and other biotechnological approaches.



Studies on production, conversion and utilization of biomass especially of fuel-wood species.



Provide necessary expertise and assistance in landscaping, garden lay out, green belt development, plant identification and impart training on plant propagation and nursery technologies.



Dissemination of information through publication of scientific and popular articles.



Co-operate and collaborate with other national and international institutions to promote the cause of conservation of biological diversity of plants and exchange of seed and plant materials.

EXECUTIVE SUMMARY

Research activities carried out during 2020-21 in Regional Plant Resource Centre under various research and developmental projects are summarized below:

Development of bioinoculant for growth and development of important forest species using native microflora

The microbial bioinoculants /biofertilizers would reduce the cost of chemical fertilizers involved in plantation programs, improve the native soil fertility and sustainability thereby provide economic benefits. Experiments on native microflora have been carried out that exhibited positive effect on growth and establishment of *Dalbergia latifolia* under pot culture conditions. Application of native microbes and the development of microbial consortium will be useful for the development of nursery technology for this tree species.

Morphotaxonomic characterization and documentation of fungi of Odisha

Odisha is endowed with wealth of diversified resources including heavy metal mine, mangroves, good and huge forest cover, very long sea coasts which develop a most suitable habitat for fungi and attained opportunity for multifarious diversity. More than 300 no. of fungi have been segregated to prepare a "Compendium of fungi of Odisha"

Production, purification and evaluation of useful Endophytic fungi

Endophytic fungi have been reported as potential candidate for the production of various secondary metabolites and exploited in different agriculture, food, pharmaceutical and health care industries. A fungal endophyte has been studied for the production of secondary metabolite and further evaluation for its anticancer properties. Besides, endophytes isolated from different banana cultivars were tested against *Meloidogyne incognita* (nematode) and two bacterial strains having nematocidal properties were under process for the field trial.

Mass production of selected medicinal plants of Odisha through tissue culture and other conventional methods

Many of the medicinal plants of the state of Odisha exhibit some sorts of problems in their natural propagation and require an alternative method such as tissue culture. To overcome this, tissue culture methods are being developed for *Celastrus paniculatus*, *Litsea glutinosa*, *Mesua ferrea*, *Paederia foetida*, *Saraca asoca*. Meanwhile, a reliable method has been developed for the production of planting materials of *Paederia foetida*.

In another research programme, 6000 Amrita "*Tinospora cordifolia*" saplings have been produced through stem cuttings and delivered the same to Sambhav (NGO) of Kendrapara district for distribution to farmers. Besides, *Paedaria foetida*(1000nos), *Piper longum*(100) and *Bacopa monnieri*(300) plants were also raised.

Harnessing the potential of traditional knowledge based medicinal plants for identifying SARS- CoV2 compounds

Twenty medicinal plants growing in Odisha have been selected, which could possibly be used for the treatment of COVID-19. Extracts from leaves and/or stems of these plants are being processed for testing against the SARS – CoV-2 virus.

Studies on therapeutic validation of phytochemicals from rare and important medicinal plants useful to treat Alzheimer's disease

A study has been conducted towards findings of phytochemicals useful for treatment of Alzheimer's disease using various medicinal plants like *Geophila repens*, *Pterocarpus marsupium* and *Piper trioicum*. Strategic approach has been initiated to establish the lead findings such as cholinesterase inhibitor for the treatment of Alzheimer's disease through therapeutic validation.

Regulation on banana fruit ripening process to enhance its shelf life

The protein-protein interaction studies were performed for annotated proteins from four different samples, considered in this study. Based on the results, several proteins such as regulatory, signalling, sugar, hormonal regulation and cell wall metabolism related proteins were tightly interacting in a network mode during the ripening process in banana. The knowledge obtained through this study would be highly useful for selecting the right candidates and utilize them in controlling ripening and also to enhance fruit shelf-life in banana.

Developing micro-propagation methods for endangered forest trees species

Since conventional regeneration process is difficult, tissue culture has been opted as alternative method of mass propagation. Various vegetative explant sources were considered for the micro-propagation of both *Santalum album* and *Desmodium oojeinense*. In both the species, shoot bud induction was seen after 10-days of inoculation with nodal segments on basal medium, basal medium supplemented with 1.0 and 1.5mg/l BAP.

Mass production and evaluation of local banana varieties of Odisha.

In order to carry out various studies on tissue culture banana, a banana mother block has been established with Champa, Patakpara ,Bantala and Yagambi. Besides, suckers of Champa, Patakpara and Bantala were collected from Puri, Khurdha, Jagatsinghpur and Keonjhar of Odisha for initial tissue culture. Experiments are being carried out for development of suitable protocol for mass propagation of banana and also to study the effect of antioxidants during different stages of banana Tissue culture for the varieties Champa and Patakpara to avoid lethal browning. Plantlets of different varieties were transferred to Nursery from Banana Tissue Culture Laboratory for experimental work as well as sale purposes. Banana cv. Gaja Bantal fruit and pseudo-stem are being subjected to nutrient analysis.

Standardization of various macropropagation methods of useful wild edible fruits of Odisha

Propagation trial has been initiated with 10 no of target species viz. *Antidesma ghaesembilla*, *Carissa carandas*, *Carissa spinarum*, *Ehretia microphylla*, *Cordia dichotoma*, *Eugenia roxburghii*, *Polyalthia suberosa*, *Glycosmis pentaphylla*, *Toddalia asiatica*, *Ziziphus oenoplia*. Out of 10, 9 species were propagated following different methods of vegetative propagation like girdled stem cuttings (black taping), micro stem cutting, juvenile stump cutting, root cuttings and wildling. So far, all together more than 600 saplings have been raised and studied for their hardening and adaptation for suitable domestication.

Evaluation and characterization of pectin in selected wild edible fruits of Odisha.

Optimization and characterization of pectin in target wild edible fruit species viz. *Ficus auriculata*, *Citrus medica*, *Limonia acidissima*, *Phyllanthus embelica*, *Diospyros malabarica* and *Syzygium cumini* have been carried out. Pectin yield ranged from 5% to 27%. The highest pectin yield was recorded in *Citrus medica* i.e 27% where as lowest pectin yield was recorded in *Limonia acidissima* i.e 5%. Further analysis of pectin components with reference to galacturonic acid and uronic acid are being carried out using advance instrumentation technique i.e. through FTIR spectroscopy.

Re-introduction of rare and endangered mangrove spp. *Heritiera fomes* and *H. littoralis* in protected areas of Odisha coast.

To create and enhance awareness amongst stakeholders on mangrove biodiversity conservation, promote social, ecological & economical benefits of the state, very important rare and endangered mangrove species *Heritiera fomes* (*sundari*) and *Heritiera littoralis* (*dhala sundari*) are being propagated, hardened for re-introduce in protected coastal areas of Odisha. So far, more than 1500 saplings of *H. fomes* and 100 saplings of *H. littoralis* have been raised and studied (morpho-physico-chemical) for their hardening process against varied salt-stress under captive shade-net house condition. Currently, hardened saplings are being subjected to re-introduce in different suitable sites of Bhitarkanika for their adaptation studies.

Propagation and reintroduction of endangered species of Odisha

In order to achieve mass production and subsequent reintroduction of endangered plants of Odisha, different propagation methods are being adopted for *Oroxylum indicum*, *Cordia macleodi* and *Symplocos racemosa*. Seed propagation for *O. indicum* vegetative propagation for *C. macleodi* and *S. racemosa* have been achieved and seedlings/saplings are being raised for conservation.

Taxonomic revision of the genus *Physalis*, *Datura*, *Capsicum* and *Nicotiana* (Solanaceae) in Eastern Ghats of India

Systematic, morphology, taxonomy and ethnobotany of *Physalis*, *Datura*, *Capsicum*, *Withania*, *Lycianthes*, *Nicotiana*, *Nicandra* and *Cestrum* from the Eastern Ghats were carried out on the basis of fresh samples collected from the field trips and analysis of existing relevant herbarium specimens. So far, *Lycianthes neesiana*, *Withania coagulance* and *P. angulata* var. *pendula*, are newly reported from Odisha as well as Eastern Ghats of India and also described two more new species from the genus *Cestrum*.

RESEARCH ACHIEVEMENT

Development of bioinoculant for growth enhancement of Dalbergia latifolia and Desmodium oojinense using native microflora

PI: Dr.(Mrs.) Nibha Gupta, Principal Scientist

Application of Mineral solubilizers to the forest tree seedlings could be an effective method to achieve higher growth and establishment of tree species on afforestation sites. The microbial bioinoculants /biofertilizers would reduce the cost of chemical fertilizers involved In plantation programs, improve the native soil fertility and sustainability thereby provide economic benefits. During preliminary study carried out on some selected fungi with few tree species has opened avenue towards more detailed experiments for the development of microbial consortium for the growth and development of plants having problems of seed germination , establishment during transplantation and growth under adverse conditions. Dalbergia latifolia and Desmodium oojinense are growing naturally but their population in Odisha forests has been gradually declined. The drastic depletion of this species in the natural forests, underlines the need to prioritize the establishment of large-scale plantations.

With this view, 26 fungi (including 1 bacteria) and 22 fungi (including 2 bacteria) were isolated from Dalbergia latifolia and Desmodium oojinense, respectively. All fungi morphologically characterized and segregated at genus level. All microbial isolates were screened for mineral solubilisation and four of them were selected for pot experiments. Experiments on native microflora have been carried out that exhibited positive effect on growth and establishment of Dalbergia latifolia under pot culture conditions. Application of native microbes and the development of microbial consortium will be useful for the development of nursery technology for this tree species.

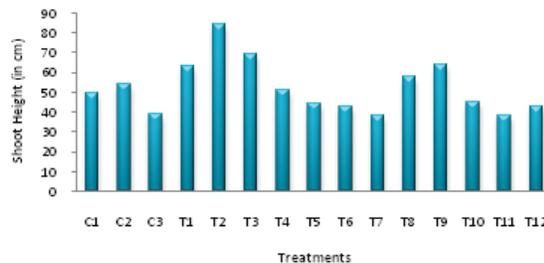
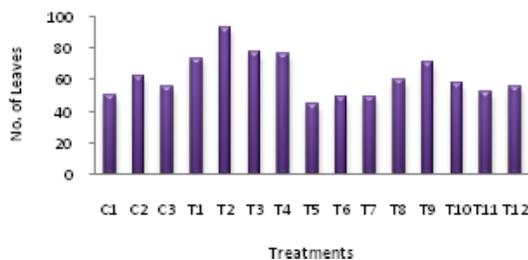
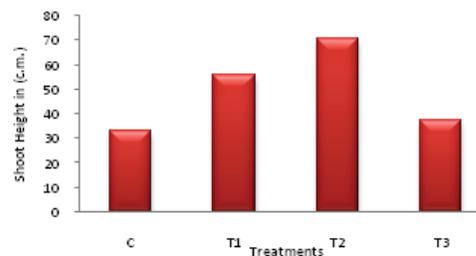
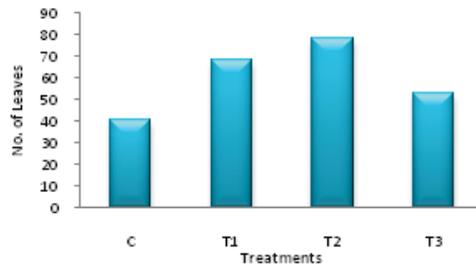




Fig. 1: Microbial applications and growth and development of *Dalbergia latifolia* under protected climatic conditions

Morphotaxonomic characterization and documentation of fungi of Odisha

PI: Dr.(Mrs.) Nibha Gupta, Principal Scientist

Odisha endowed with wealth of diversified resources including heavy metal mine, mangroves, good and huge forest cover, very long sea coasts which develop a most suitable habitat for fungi and attained opportunity for multifarious diversity. We have collected fungi from different ecological habitats of Odisha like mangroves, mines, endophytic environment of orchids, medicinal plants etc. A systematic documentation is required to prepare checklist of fungi of Odisha. Presently, more than 200 no. of fungi have been segregated at genus level and will be identified at species level in future. Mostly it includes species of *Penicillium*, *Aspergillus*, *Fusarium*, *Colletotrichum*, *Curvularia*, *Paecilomyces*. A comprehensive list of fungi isolated by RPRC from different sources of Odisha will be documented.

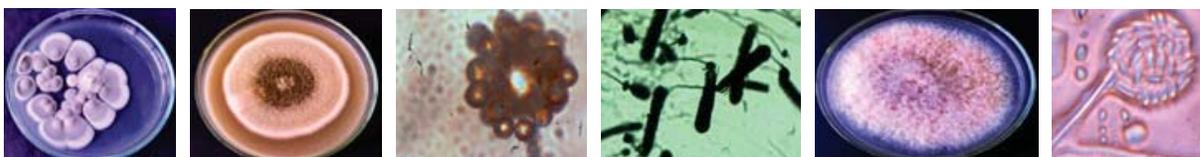


Fig. 2: Different morphological types of fungi of Odisha

Production, purification and evaluation of anticancer properties of extracellular secondary metabolite from *Colletotrichum* sp.

PI: Dr.(Mrs.) Nibha Gupta, Principal Scientist

Endophytic fungi have been reported as potential candidate for the production of various secondary metabolites and exploited in different agriculture, food, pharmaceutical and health care industries . On the other hand the huge requirement of potent drugs for various diseases induced researchers to explore and investigate alternative avenues for searching novel bioactive molecules. In this regard, endophytes appear to be most possible candidate for drug discovery and formulations. A fungal endophyte was studied for the production of secondary metabolite and further evaluated for anticancer properties. The positive bioactive potential and its exhibition through inhibition of cancer cell lines has lead an avenue to work in details for the purification of different secondary metabolites present in the crude sample , characterization and evaluation further for their anticancer properties.

Harnessing the potential of endophyte against root-knot nematode *Meloidogyne incognita* in banana.

PI: Dr.(Mrs.) Nibha Gupta, Principal Scientist

A NETWorking project (DBT, Govt. of India) on North Eastern Region Banana has been carried out in Collaboration with Tamil Nadu Agricultural University (TNAU), Coimbatore and Assam Agricultural University (AAU), Jorhat, Assam. Endophytes isolated from NER regions were screened for secondary metabolites , enzymes and mineral solubilization (organic acid, antibiotics, phenolic content, plant growth hormones, polysaccharides , extracellular enzymes etc.). Endophytes isolated from different banana cultivars were tested against *Meloidogyne incognita* (nematode) and two bacterial strains having nematocidal properties were under process for the field trial by collaborative centres. Liquid and granulated form of Bioformulation of these bacterial strains have been tested for shelf life through plate culture and population count technique. Standardized liquid and soil formulations may be exploited for commercialization through various biofertiliser and biopesticide industries.



Nematicidal Bioformulations (powder, granulated and liquid)

Production of planting materials of Orchids

PI: Dr.Nihar Ranjan Nayak, Senior Scientist

Orchids require the tissue culture technology for the production of planting materials. During the year about 20,000 Tissue culture plantlets of Dendrobium, Cattleya and Cymbidium orchids have been raised. All of these have been successfully acclimatized in the greenhouse. The planting materials produced through the tissue culture are being grown on the coconut husk pieces for further growth and development.



Dendrobium



Cymbidium



Cattleya



Orchids acclimatization in the polyhouse

Production of the medicinal plants using tissue culture technology

PI: Dr.Nihar Ranjan Nayak, Senior Scientist

Efforts have been made to grow planting materials of medicinal plants mentioned below using tissue culture technology.

- *Celastrus paniculatus* Willd
- *Paederia foetida* L.
- *Litsea glutinosa* (Lour.) C.B. Rob.
- *Saraca asoca* (Roxb.) Willd.
- *Mesua ferrea* L.

Different explants like shoot tips, nodal explants, hypocotyls etc. have been used as the explants for the production of new shoots. Different concentrations and combinations growth regulators were applied in the nutrient medium for the production and multiplication of new shoots. Of the five species selected, successful protocol has been developed for the mass production of planting materials of *Paederia foetida* L. Experiments are under development for other four species.



Figure 1: Different stages of production of plants of *Paederia foetida*: (a) Mother plant, (b) nodal explant inoculated, (c) initiation of new shoots, (d) multiplication, (e) rooting

Developing micro-propagation methods for *Pterocarpus santalinus*, *Anogeissus latifolia*, *Santalum album* and *Desmodium oojeinense*, some endangered forest trees species of Odisha

PI: Giridara Kumar Surabhi, Senior Scientist

The seeds of *Desmodium oogeinense* (sandan) retain their viability for 12 months only. Heavy weed competition can kill seedlings. This tree is susceptible to defoliators and borers. Timber exploitation has degraded the natural stands of this species in the wild. To reverse this condition and to conserve the species in natural forest, micro-propagation is needed for production planting material. Another tree species, Sandalwood (*Santalum album* L.) is recalcitrant to in vivo and in vitro propagation, for which only limited success has been achieved so far. Hemi-parasitism, poor seed viability, threats like-fire, grazing and most importantly exploitation of the wood for the furniture and oil purposes, this species has become vulnerable to extinction.

The project is aimed to develop efficient micro-propagation protocol for important and endangered forest trees. Different explant source was considered for the micro-propagation of *Santalum album* and *Desmodium oojeinense* for the experiment. Both the species, *Santalum album* and *Desmodium oojeinense*, shoot bud induction was seen after 10-days of inoculation with nodal segments on basal medium, basal medium supplemented with 1.0 and 1.5mg/l BAP.

Explants establishment, shoot induction and multiplication: Various types of explants (shoot tips and nodal segments) were cultured on MS media containing different concentrations of BAP to evaluate its effects on shoot initiation or induction for species i.e., *Santalum album* and *Desmodium oojeinense*. Explants grown on plant growth regulator-containing media showed new growth in shoot initiation depending on the type of explants and different concentration of BAP added in it. The nodal segments of *Santalum album* showed better results instead of shoot tip. The explants of *Santalum album* grow rapidly on the MS basal media containing plant growth regulators, where it showed slow growth on the MS basal media without any plant growth regulators.

The nodal explants of *Santalum album* showed shoot bud initiation in basal medium having different combinations of plant growth regulators (1.0-1.5 mg/L BAP). Early shoot initiation can be observed in 1.0 mg/L BAP supplemented culture medium after two weeks. The explants showed multiple shoot induction of *Santalum album* (more than 10 per explant) were produced on MS media having 1.0 and 1.5 mg/L BAP after 8-10 weeks of inoculation. Sub-culturing of newly growing shoots was done and transferred to the new culture bottles having same media for further growth and development.

The nodal explants of *Desmodium oojeinense* showed shoot bud initiation in MS basal media supplemented with different combinations of plant growth regulators (1.0 and 1.5 mg/L BAP). Early shoot initiation is observed in MS media supplemented with 1.0 mg/L BAP after 2 weeks of inoculation.



(1) (A-E): Different stages of in vitro micro-propagation by nodal segments of mature *Santalum album* L. (Indian Sandalwood). (A) Observed multiple shoot induction after 31-days of inoculation on MS media supplemented with 1.5 mg/L BAP. (B) Observed multiple shoot induction after 62-days of inoculation on MS supplemented with 1.0 mg/L BAP. (C) Multiple shoots were observed after 60- days of inoculation on 1.0 mg/L BAP. (D) Multiple shoots were observed after 67-days of inoculation on MS supplemented with 1.0 mg/L BAP. (E) showed multiple shoots after 67-days of inoculation on MS supplemented with 1.5 mg/L BAP.

Deciphering molecular regulation of fruit ripening in banana through quantitative proteome analysis following advanced (iTRAQ) isobaric tags for relative and absolute quantitation

PI: Giridara Kumar Surabhi, Senior Scientist

In this study, proteins were phenol extracted from banana peel and pulp tissues at 90-DAF (days after flowering) and 12-DAR (days after ripening). Proteins were quantified by using 1X quick start Bradford assay and by using BSA as a standard. 150µg of proteins were resolved on 12% SDS-PAGE and separated proteins were visualized by using colloidal Coomassie blue stain. Modulation of several protein bands were observed in 12 DAR samples when compared to 90 DAF fruit protein samples of pulp and peel tissues (Fig.2). Further, quantitative proteome analysis using advanced isobaric tags for relative and absolute quantitation (iTRAQ/TMT) was performed for the identification of proteins to decipher the molecular regulation of fruit ripening process in banana at pre-climacteric (90DAF) and climacteric stage (12 DAR) and followed by mass spectrometry-based protein identification.

Transcriptomic analysis alone may not adequately predict exact protein abundance and activities. By contrast, gel-free proteomics can be used to obtain quantitative and structural modification information about functional proteins and the dynamic state of the cell during ripening. In this regard, Tandem mass tag (TMT) has been proven to be a robust quantitative proteomics approaches based on mass spectrometry (MS) stable isotopic labelling which leads to a better coverage and accuracy with regard to changes in protein levels during fruit ripening. In this study, Proteins were phenol extracted from banana peel and pulp tissues at 40, 60 and 90-DAF (days after flowering) and 12-DAR (days after ripening). Proteins were quantified by using 1X quick start Bradford assay and by using BSA as a standard. 150µg of proteins were resolved on 12% SDS-PAGE for quality check purpose and separated proteins were visualized by using colloidal coomassie blue stain (Fig. 1).

Further, the peptides (100µg each) were labelled with TMT 6-plex in two reactions (Table 1). In the first reaction, peptides were labelled with different isobaric tags i.e. 126-tag/S1 (40-DAF pulp), 127-tag/S2 (60-DAF pulp), 128-tag/S5 (40-DAF peel) and 129-tag/S6 (60-DAF peel). In the second reaction, peptides were labelled with 126-tag/S3 (90-DAF pulp), 127-tag/S4 (12-DAR pulp), 128-tag/S7 (90-DAF peel) and 129-tag/S8 (12-DAR peel). Proteins were identified through high-resolution Orbitrap fusion mass spectrometry (Quadrupole tribrid LC-MS/MS) (Fig. 2-3).

Figure 1. SDS-PAGE profile of banana peel and pulp tissues at 40, 60 and 90-DAF and 12-DAR for quality check.

Figure 1. Depicted image represents the SDS-PAGE profiles of 40, 60 and 90-DAF (pulp and peel) and 12-DAR (pulp and peel). Lane 1 represents the marker protein ranging from 14.4-200 kDa. 150 µg of protein samples were loaded in each lane and proteins were visualized by using colloidal coomassie blue stain. Difference in banding patterns of SDS-PAGE profiles can be clearly observed between peel and pulp samples.

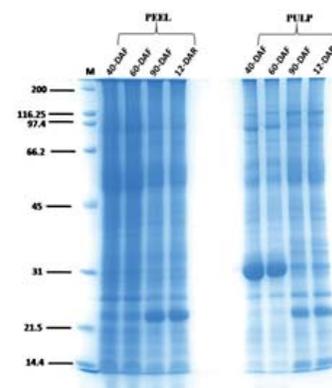


Table 1. Sample details and experimental plan for TMT 6-plex.

Sample details	TMT reaction	TMT 126	TMT 127	TMT 128	TMT 129
Group 1	Reaction 1	S1 (40-DAF pulp)	S2 (60-DAF pulp)	S5 (40-DAF peel)	S6 (60-DAF peel)
Group 2	Reaction 2	S3 (90-DAF pulp)	S4 (12-DAR pulp)	S7 (90-DAF peel)	S8 (12-DAR peel)

Figure 2: Mass spectra profiles of proteins from 40, 60 and 90-DAF and 12-DAR generated through orbitrap fusion mass spectrometry.

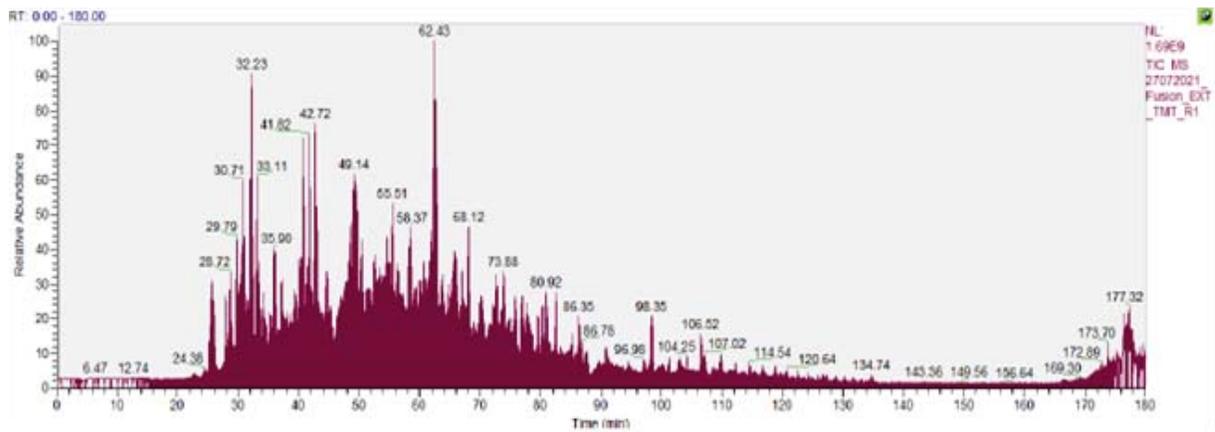
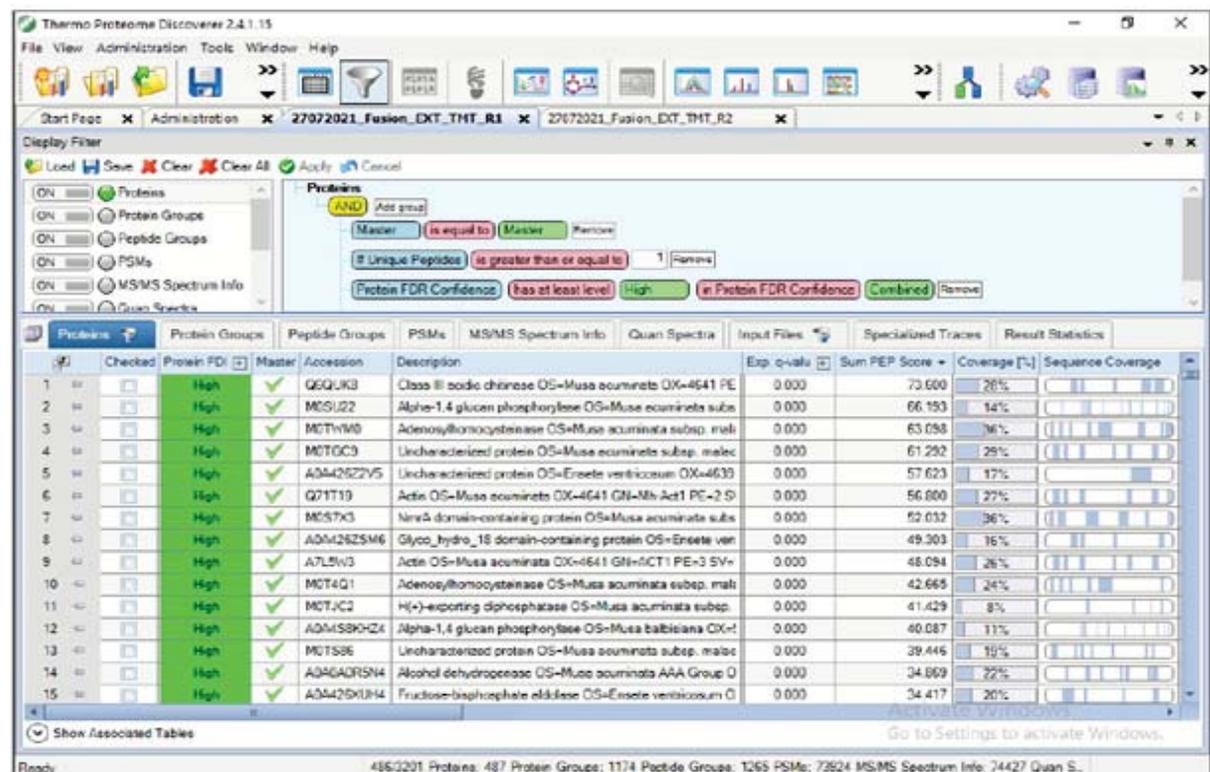


Figure 3: Proteins identified through Orbitrap fusion mass spectrometry and Thermo Proteome discoverer version 2.4.1.15.



Omics-approach to regulate ripening and enhance fruit shelf-life in banana: an important fruit crop for food security

PI: Giridara Kumar Surabhi, Senior Scientist

Banana (*Musa* spp.) is one of the most important crop plants grown in the tropics and sub-tropics. It is a climacteric fruit and undergoes ethylene dependent ripening. Once ripening is initiated, it proceeds at a fast rate making post harvest life short, which can result in heavy economic losses. During the fruit ripening process a number of physiological and biochemical changes take place and thousands of genes from various metabolic pathways are recruited to produce a ripe and edible fruit. In this investigation, the project work utilizing the high throughput omics approach to identify candidate genes and proteins and utilizing them to improve fruit shelf-life.

A. TRANSCRIPTOME SEQUENCING:

Differential Expression Analysis: Gene level Expression Analysis

The input data for differential gene expression analysis are read counts from gene expression level analysis. The differential gene expression analysis contains three steps: (1) Read counts Normalization, (2) Model dependent p-value estimation, (3) FDR value estimation based on multiple hypothesis testing

We have used DESeq2 for differential expression analyses. The DESeq2 package is designed for normalization, visualization, and differential analysis of high- dimensional count data. It makes use of empirical Bayes techniques to estimate priors for log fold change and dispersion, and to calculate posterior estimates for these quantities' details. Genes have been prioritized with $|\log_2FC| > 1$. The results can be seen in (Fig.1-A: Volcano plots).

DEGs Cluster: Cluster Analysis of differential expressed genes DEGs

Cluster analysis is used to find genes with similar expression patterns under various experimental conditions. By clustering genes with similar expression patterns, it is possible to discern unknown functions of previously characterised genes or functions of unknown genes. In hierarchical clustering, areas of different colours denote different groups (clusters) of genes, and genes within each cluster may have similar functions or take part in the same biological process. The analysis is shown in the form of heatmap (Fig.1-B: Venn diagram).

DEG_GO Enrichment Analysis of DEGs

We used top GO, an R- bio conductor package for enrichment analysis. Thereafter, the individual, enriched annotation terms passing the enrichment P-value threshold are reported in a tabular format ordered by the enrichment probability (enrichment P-value). The enrichment P-value calculation, i.e. number of genes in the list that hit a given biological class as compared to pure random chance, have been performed with the aid of statistical-multi test adjustment methods. Finally, the terms are arranged on the basis of top GO Fisher Ranking and a maximum of 5 terms for the sake of clarity is represented in terms of DAGs. DAG (Directed Acyclic Graph, DAG) visualizes the enriched GO term of differentially expressed genes and its hierarchy. The graphical result is a GO hierarchical image containing all statistically significant terms. The smaller of the terms adjusted p-value, the more significant statistically, and the node's colour is darker and redder (Note: adjusted p-value here means that the value of the multiple-test adjusted p-value). Inside the box of the significant terms, the information

includes: GO term, adjusted p-value, GO description, item number mapping the GO in the query list and background, and total number of query list and background. In this project, DAG of biological process and molecular function are drawn respectively (DAG plot)(Fig.1- C).

DEG_KEGG(Kyoto Encyclopedia of Genes and Genomes) Enrichment: KEGG Pathway Enrichment Analysis of DEGs

Pathway enrichment analysis was performed to gain insights into the underlying biology of differentially expressed genes and proteins. Analysing high-throughput molecular measurements at the functional level is very appealing for two reasons. First, grouping thousands of genes, proteins, and/or other biological molecules by the pathways they are involved in reduces the complexity to just several hundred pathways for the experiment. Second, identifying active pathways that differ between two conditions can have more explanatory power than a simple list of different genes or proteins. Cluster Profiler was used for the pathway enrichment at a probability value-cut off of 0.05. Cluster Profiler an R Bioconductor package was used for KEGG pathway analysis using the information of reference genome (DAG-KEGG) (Fig. 1-D).

Protein-protein network analysis

In this study PPI network analysis was performed using STRING database. The STRING database contains information from numerous sources, including experimental data, computational prediction methods and public text collections. It is freely accessible and it is regularly updated. The resource also serves to highlight functional enrichments in user-provided lists of proteins, using a number of functional classification systems such as GO, P fam and KEGG (Fig.1- E).

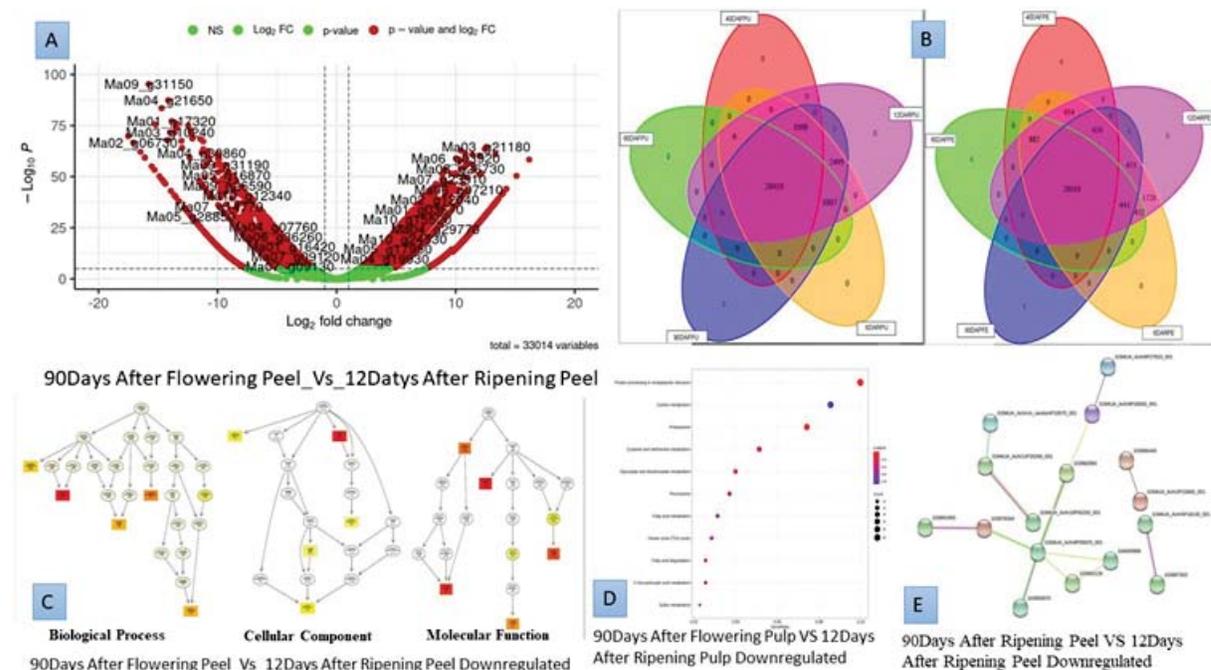


Figure: 1 (A-E): (A)Volcano plots, (B) Venn Diagram, (C) GO Enrichment pathway, (D) KEGG pathway, and (E) Protein-protein interaction.

B. SMALL RNA SEQUENCING

Differential Expression Analysis: Gene level Expression Analysis

Data quality assessment and quality control (i.e. the removal of insufficiently good data) are essential steps of any data analysis. These steps should typically be performed very early in the analysis of a new data set, preceding or in parallel to the differential expression testing. We define the term quality as fitness for purpose. Our purpose is the detection of differentially expressed genes, and we are looking in particular for samples whose experimental treatment suffered from an abnormality that renders the data points obtained from these particular samples detrimental to our purpose the input data is the readcount value from the miRNA expression level analysis. For samples with biological replicates, TMM was used to normalize the read count value, and the edgeR was used to do the differential micro RNA analysis. Differentially expressed miRNAs were filtered out using a cut-off of adjusted p -value < 0.05 and $|\log_2 \text{fold change}| > 1$ for known miRNA and p -value < 0.05 and $|\log_2 \text{fold change}| > 1$ for novel miRNA (Heat map, Volcanoplots, Venn diagram).

DEG_GO Enrichment Analysis of DEGs

Gene Ontology (GO) is an international standardized classification system for gene function, which supplies a set of controlled vocabulary to comprehensively describe the property of genes and gene products. There are 3 ontologies in GO: molecular function, cellular component and biological process. The basic unit of GO is GO-term, each of which belongs to one type of ontology. GO enrichment analysis is used on predicted target gene candidates of known and novel miRNAs ("target gene candidates" in the following). It will provide all GO terms significantly enriched in the predicted target gene candidates of known and novel miRNAs compared to the reference gene background, as well as the genes corresponding to certain biological function. The result could reveal the functions significantly related with predicted target gene candidates of known and novel miRNAs. This method (Young et al, 2010) firstly maps all target gene candidates to GO terms in the database (<http://www.geneontology.org/>), calculating gene numbers for each term, then using Wallenius non-central hyper-geometric distribution to find significantly enriched GO terms in target gene candidates comparing to the reference gene background. DAG (Directed Acyclic Graph, DAG) visualizes the enriched GO term of differentially expressed genes and its hierarchy. The graphical result is a GO hierarchical image containing all statistically significant terms. The smaller of the terms adjusted p -value, the more significant statistically, and the node's colour is darker and redder (Note: adjusted p -value here means that the value of the multiple-test adjusted p -value). Inside the box of the significant terms, the information includes: GO term, adjusted p -value, GO description, item number mapping the GO in the query list and background, and total number of query list and background. In this study, DAG of biological process and molecular function are drawn respectively.

DEG_KEGG Enrichment: KEGG Pathway Enrichment Analysis of DEGs

Pathway Enrichment analysis insights into the underlying biology of differentially expressed genes and proteins. Analyzing high-throughput molecular measurements at the functional level is very appealing for two reasons. First, grouping thousands of genes, proteins, and/or other biological molecules by the pathways they are involved in reduces the complexity to just several hundred pathways for the experiment. Second, identifying active pathways that differ between two conditions can have more explanatory power than a simple list of different genes or proteins. GProfiler was used for the pathway enrichment at a p -value-Cut-off of 0.05.

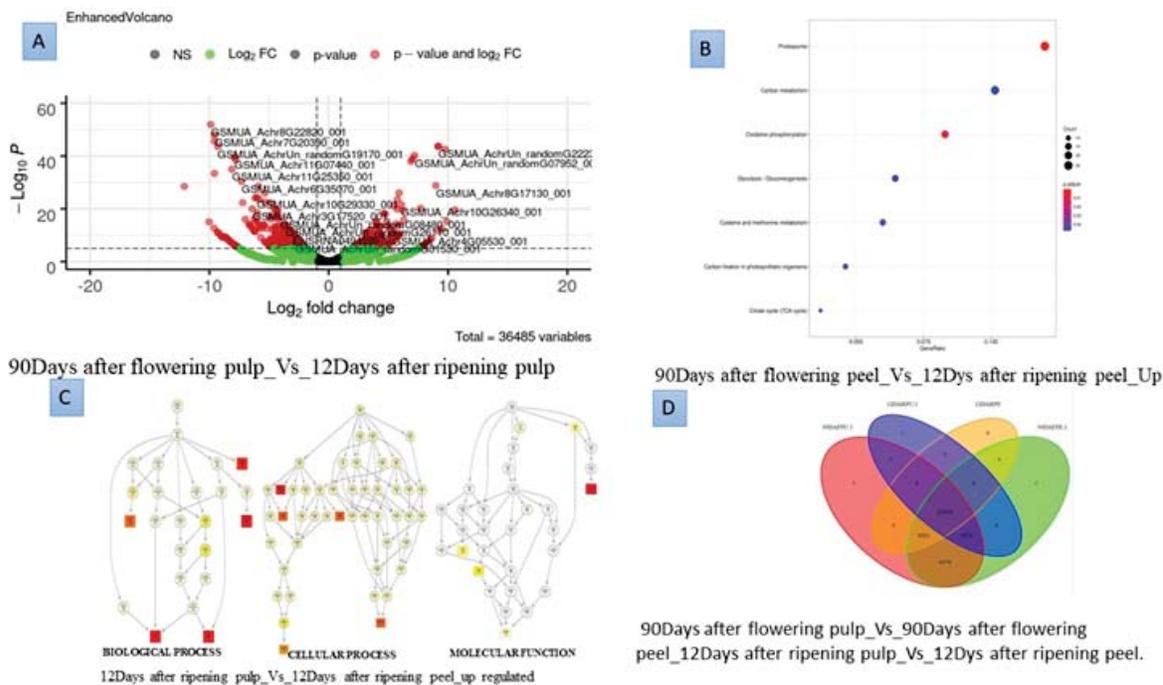


Figure 2)- (A-D): (A) Volcano plot, (B) KEGG pathway, (C) GO Enrichment pathway, and (D) Venn Diagram.

Pharmacological profiling of *Geophila repens* and *Piper trioicum* and evaluation of their therapeutic potential against Alzheimer’s disease

PI: Dr. Atish Kumar Sahoo, Senior Scientist

Rational: Introduction and significance:

Alzheimer’s disease (AD) is a type of incurable and progressive neurodegenerative disorder characterised by formation, aggregation and accumulation of amyloid (Aβ)-plaque and neurofibrillary tangles (NFTs), production of inflammatory cytokines, impaired calcium homeostasis leading to cognitive decline and senile dementia. It is an acquired cognitive and behavioural impairment disorder associated with memory impairment, thinking abilities, judgement, limited social skills and daily functioning. AD is the 6th leading reason of fatality and the total number of people living with dementia is estimated to reach 82 million by 2030. There is no potential drug for treatment of AD, however Food and Drug Administration (FDA) approved synthetic (rivastigmine, velnacrine), or natural compounds (galanthamine from *Galanthus* spp.) are the partial inhibitors of enzyme activities in brain). Because of complex pathogenic features, the treatment of AD has failed, and unfortunately; synthetic drugs have been linked with numerous adverse effects such as anorexia, vomiting, insomnia and nausea due to nonspecific interaction with non-targeted organ. Recent studies have focused on multi-target therapeutic strategy of bioactive extract of botanicals to block the progression and disease-inducing mechanisms in AD. Traditionally, *Geophila repens* is used for various therapeutic purposes

including memory enhancer in India. So far, the scientific validation of their ethnomedicinal uses have been evaluated by in vitro antioxidant studies and found that the hydroalcohol extract of leaves of *G. repens* (GRHA) revealed significant antioxidant activities. We further validated the ex-vivo cell-based antioxidant protection in erythrocytes (CAP-e) of GRHA at the cellular level to measure reducing oxidative damage within the cell. Based on our previous findings, additional research is needed to examine the efficacy of hydroalcohol fraction of *Geophila repens* (L.) I.M. (GRHA) for memory enhancing properties in in vivo models. We investigated the modulatory role of prime identified compounds in GRHA in mitigating scopolamine-induced neurotoxicity in experimental rats of Alzheimer's disease (AD) via attenuation of cholinesterase, β -secretase, MAPt levels and inhibition of oxidative stress imparts inflammation. Among the reported 31 compounds by GC-MS/MS analysis, 8 major bioactive compounds possess antioxidant, neuroinflammation, neuroprotective activities, and were considered for docking analysis towards cholinesterase, β -secretase activities in AD. GRHA-400 significantly improved learning and memory impairment with the improvement of oxidative stress (MDA, SOD, GSH, CAT), DNA damage (8-OHdG), neurochemical (AChE, BuChE, BACE1, BACE2, MAPt), neuroinflammation (IL-6, TNF- α) markers in neurotoxic rats.

Objectives:

1. To determine the therapeutic potential of *Geophila repens* as an anticholinesterase property in experimental rat model.
 - Estimation of oxidative markers in serum and brain: Study of Malondialdehyde (MDA), Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH).
 - Estimation of neuroinflammatory markers in serum and brain: Study of Interleukin 6 (IL-6) and Tumor necrosis factor (TNF- α)
 - Estimation of neurochemical markers in brain: Study of Acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE)
2. Histopathology studies of brain and estimation of brain markers level of *G. repens* treated rats towards Alzheimer's disease.

Significant achievements/ Tangible outcome:

The study highlights the modulatory role of prime identified compounds in the bioactive fraction of *Geophila repens* in mitigating scopolamine-induced neurotoxicity in experimental rats of Alzheimer's disease via attenuation of cholinesterase, β -secretase, MAPt levels and inhibition of oxidative stress imparts inflammation. Our previous reports on in vitro antioxidant, ex-vivo cell-based antioxidant protection in erythrocytes (CAP-e) of GRHA with the present implication of *Geophila repens* (GRHA) in mitigating scopolamine-induced neurotoxicity in experimental rats of Alzheimer's disease (AD) via attenuation of cholinesterase, β -secretase, MAPt levels and inhibition of oxidative stress imparts inflammation scientifically validated the ethnomedicinal claim of the plant as a memory enhancer in AD. Furthermore, to determine the neuroprotection of GRHA at the cellular level, histopathological analysis of the brain's cortex was also performed.

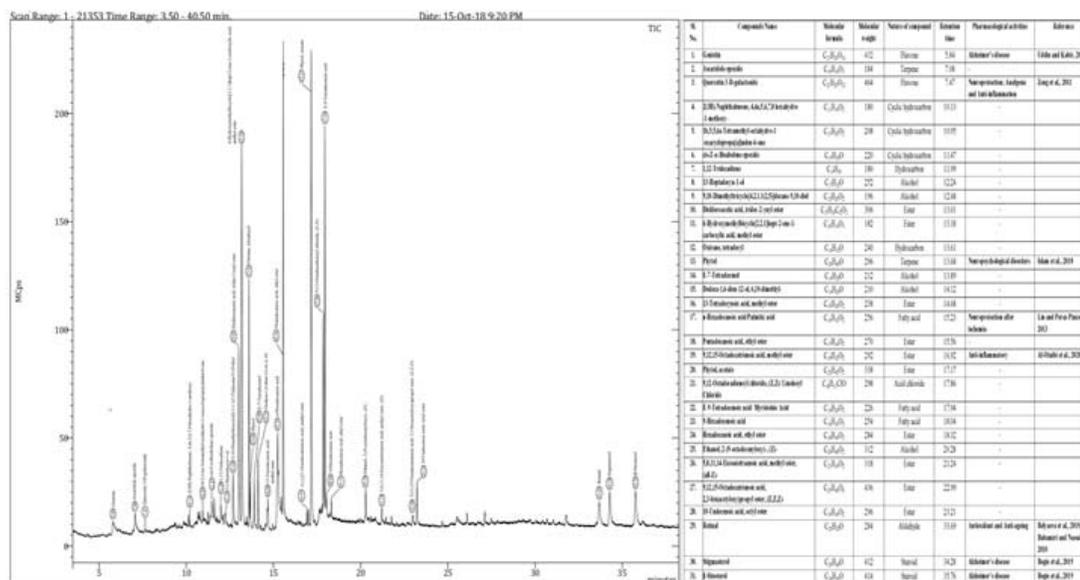


Fig. 1. GC-MS/MS analysis of hydroalcohol fraction of *G. repens* (GRHA) and reported bioactive molecules.

The prime identified compounds in GRHA were reported by GC-MS analysis identified and found the presence of 31 no. of compounds of different class such as flavone, terpene, ester, steroid and aldehydes in GRHA (Fig. 1). The chromatograms of identified compounds were compared with NIST mass spectral library and reported with their respective molecular formula, molecular weight, peak number, retention time (RT), nature of compound, and biological activities. The major identified compounds were reported (Fig. 1). Based on the potential therapeutic interest to cure neurological disorders, only 8 no. of bioactive compounds e.g., genistein, quercetin-3-D-galactoside or hyperoside, phytol, stigmasterol, n-hexadecanoic acid, 9,12,15-octadecatrienoic acid methyl ester, retinal, and β-sitosterol possess antioxidant, anti-inflammatory, and neuroprotective activities.

In the animal study, the experimental procedures were followed as specified in the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Rats were divided into 5 groups and different dosage were given to respective groups. The group I and II rats were treated with normal saline daily (n.s.; p.o.) and scopolamine (SCP; 3 mg/kg, i.p.), respectively. Group III, IV, and V were treated with donepezil (DPZ; 2 mg/kg, p.o.), a low dose of GRHA (300 mg/kg, p.o.), and a high dose of GRHA (400 mg/kg, p.o.) at every 24 h interval for 14 consecutive days. The behavioural analysis of all groups was carried out 30 min before dose administration during experimental days. After completion of the behavioural tests, on the 15th day, rats were sacrificed. Blood serum and the whole brain of each experimental rat were collected. Oxidative stress markers (MDA, SOD, GSH, and CAT), and inflammatory markers (IL-6, TNF-α) of serum and brain were estimated. Further, the neurochemical estimation (AChE, BuChE, BACE1, BACE2, and MAPt) and histopathological studies of brain tissues were performed.

Oxidative markers (MDA, SOD, GSH and CAT) play a crucial role in various cellular activities and immune system functioning. In normal cellular processes, oxygen radicals are generated as by-products and affect amino acids to cause a loss in protein functions or enzymatic activities. In the present study, the induction of neurotoxicant scopolamine (SCP) triggers the generation of free radicals and play key roles in memory deficits and brain ageing by affecting biomolecules (lipids, nucleic acids, proteins, and carbohydrates) in cells. Furthermore, ROS-

induced lipid peroxidation causes overproduction of MDA and altered antioxidant defence enzymes (SOD, CAT, and GSH). Hence, MDA is a prime marker that indicates lipid peroxidation in oxidative damage in brain cells.

As shown in Figure 2 and 3, the serum (GRHA: 18.01 ± 0.93 nM/mg protein) and brain (GRHA: 22.48 ± 2.13 nM/mg protein) MDA levels were significantly increased in SCP-induced group as compared to normal. But, the pre-treatment of GRHA-400 significantly reduced the MDA level in serum (GRHA: 12.61 ± 0.78 nM/mg protein) and brain (GRHA: 9.97 ± 0.52 nM/mg protein), and were found comparable with standard drug (donepezil/DPZ) treated group (Fig. 2 and 3). SOD is the first line antioxidant defence which catalyses superoxide anion to O₂ and H₂O₂. These free radicals cause the level of SOD depletion in the cell. In the present study, SOD level in the SCP-induced group significantly (p < 0.001) decreased in serum (GRHA: 5.38 ± 0.73 U/mg protein) and brain (GRHA: 4.83 ± 0.82 U/mg protein) as compared to the normal group. But, in the case of GRHA-400 treated rats significantly increased (p < 0.001) the level of SOD in serum (GRHA: 8.01 ± 0.85 U/mg protein) and brain (GRHA: 6.69 ± 0.56 U/mg protein) and found comparable to standard DPZ treated group (Fig. 2 and 3).

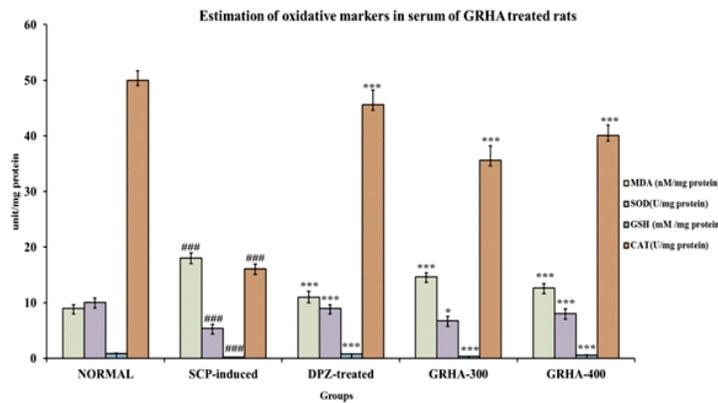


Fig. 2. Depicts the estimation of oxidative markers in serum of GRHA treated (300 and 400 mg/kg) experimental rats, in which donepezil (DPZ) as standard was administrated to positive control group. ###p < 0.001 represents the significant values when toxic control (SCP-induced) group were compared with normal control group. *p < 0.05, **p < 0.01, and ***p < 0.001 represents average, moderate and most statistical significant values when experimental groups were compared with SCP-induced (toxic control) group.

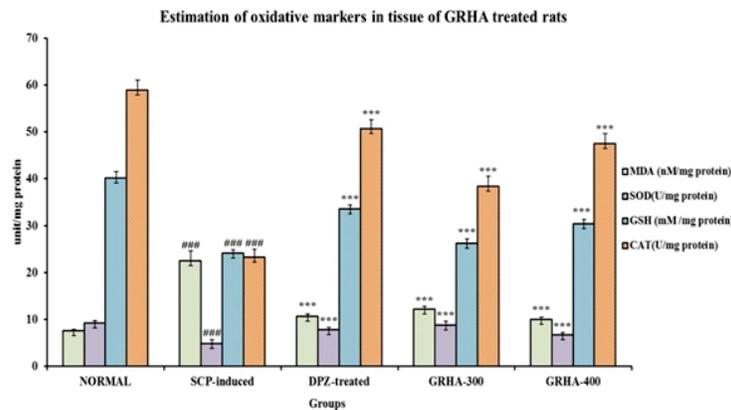


Fig. 3. Represents the estimation of oxidative markers in tissue (brain) of GRHA treated (300 and 400 mg/kg) experimental rats, in which donepezil (DPZ) as standard was administrated to positive control group. ###p < 0.001 represents the significant values when toxic control (SCP-induced) group were compared with normal control group. *p < 0.05, **p < 0.01, and ***p < 0.001 represents average, moderate and most statistical significant values when experimental groups were compared with SCP-induced (toxic control) group.

The antioxidant GSH is essential for the cellular detoxification of xenobiotic compounds and ROS in brain cells. A compromised GSH system in the brain has been connected with the oxidative stress occurring in neurological disorders like AD. In the present study, the reactive metabolites of SCP interacted with GSH and decreased its level in serum (GRHA: 0.19 ± 0.01 mM/mg protein) and brain (GRHA: 24.11 ± 0.71 mM/mg protein) significantly ($p < 0.001$) in SCP-induced group than normal control group, whereas GRHA-400 neutralised the overproduction of H₂O₂ by increasing GSH level in serum (GRHA: 0.59 ± 0.03 mM/mg protein) and brain (GRHA: 30.34 ± 1.04 mM/mg protein). The results were comparable to the standard (donepezil/DPZ) treated group (Fig. 2 and 3). Catalase (CAT) is an enzyme that mops up excess oxidants and helps to prevent the kind of damage seen in the brains of patients with AD, and in some cases, the catalase proteins deposited within amyloid plaques. So, the CAT estimation in SCP-induced group were performed and the CAT level was decreased significantly ($p < 0.001$) in serum (GRHA: 16.04 ± 0.92 U/mg protein) and brain (GRHA: 23.20 ± 1.77 U/mg protein) as compared to the normal group. But, GRHA-400 showed significant ($p < 0.001$) increase in CAT activities in serum (GRHA: 40.07 ± 1.83 U/mg protein) and brain (GRHA: 47.49 ± 2.14 U/mg protein) and found comparable to standard DPZ treated group (Fig. 2 and 3). The current finding signified that GRHA is potent intracellular radical scavengers that scavenge ROS and their precursors by ameliorating neurological disorders like AD. The balancing of oxidative stress markers in different groups of experimental rats occurred due to the synergistic effect of radical scavengers and neuroprotective bioactive molecules in GRHA as genistin, quercetin 3-D-galactoside, phytol, 9,12,15-octadecatrienoic acid, methyl ester, retinal, stigmasterol and β -sitosterol.

Further, to correlate the results of oxidative markers, we estimated the neuroinflammatory markers (IL-6 and TNF- α) in the brain of different group of experimental rats. Cytokines (IL-6 and TNF- α) are pro-inflammatory proteins produced by astrocytes, neurons, and microglial cells in the brain, and they have a role in the pathological condition that occurs during pain. In addition, activated microglia cells in the brain boost the expression of cerebral neuroinflammation in AD. Likewise, the IL-6 is involved in immune response and synaptic dysfunction in physiological systems in the brain. In the present study, the anti-inflammatory role of GRHA was established by estimating the level of TNF- α and IL-6 in serum and brain. The study of pro-inflammatory markers showed a significant ($p < 0.001$) increase in the level of IL-6 in serum (GRHA: 61.67 ± 4.18 pg/mL protein) and brain (GRHA: 711.03 ± 16.04 pg/mL protein) and TNF- α in serum (GRHA: 82.97 ± 4.05 pg/mL protein) and brain (GRHA: 97.41 ± 5.2 pg/mL protein) in SCP-induced group.

The elevated level of IL-6 and TNF- α after SCP-induction leads to increase the amyloid- β (A β) production, decrease A β clearance, increase neuronal loss and cell death; and are linked to cognitive impairment in Alzheimer's disease. However, the GRHA-400 treated rats showed significant improvement in the level of IL-6 in serum (GRHA: 42.63 ± 2.36 pg/mL protein) and brain (GRHA: 483.81 ± 9.98 pg/mL protein); and TNF- α in serum (GRHA: 60.63 ± 2.95 pg/mL protein) and brain (GRHA: 76.81 ± 2.61 pg/mL protein), which found similar to standard drug DPZ treated groups (Fig. 4 and 5). The decreased pro-inflammatory cytokines are due to the presence of potent anti-inflammatory and antioxidant molecules in GRHA as genistin, quercetin 3-D-galactoside, phytol, retinal, stigmasterol and β -sitosterol.

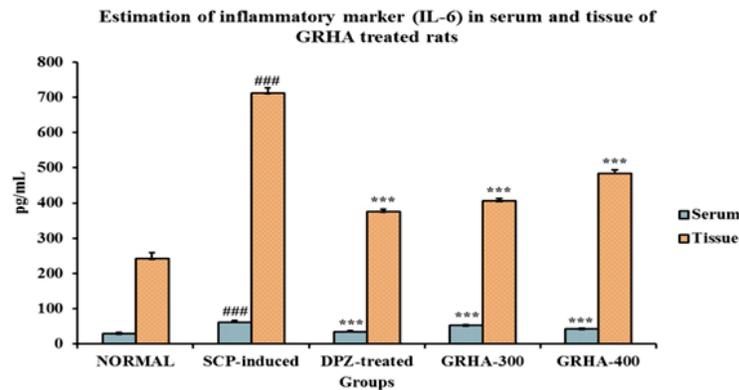


Fig. 4. Represents the estimation of inflammatory marker (IL-6) in serum and tissue (brain) of GRHA treated (300 and 400 mg/kg) experimental rats, in which donepezil (DPZ) as standard was administered to positive control group. ### $p < 0.001$ represents the significant values when toxic control (SCP-induced) group were compared with normal control group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ represents average, moderate and most statistically significant values when experimental groups were compared with SCP-induced (toxic control) group.

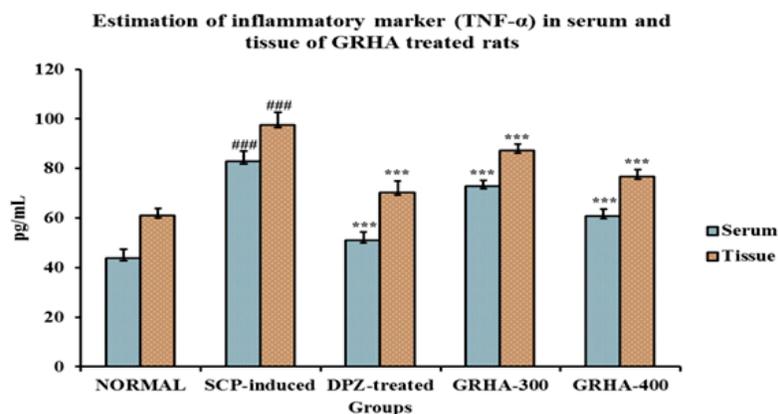


Fig. 5. Depicts the estimation of inflammatory marker (TNF- α) in serum and tissue (brain) of GRHA treated (300 and 400 mg/kg) experimental rats, in which donepezil (DPZ) as standard was administered to positive control group. ### $p < 0.001$ represents the significant values when toxic control (SCP-induced) group were compared with normal control group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ represents average, moderate and most statistically significant values when experimental groups were compared with SCP-induced (toxic control) group.

Cholinesterase enzymes (AChE and BuChE) are found in glial cells and neurons. The key role of AChE and BuChE are to hydrolyse acetylcholine (ACh) to acetate and choline. Any disruption in these pathways leads to neurodegenerative diseases like AD. So, the major strategies to achieve the therapeutic target in the AD treatment are the inhibition of brain cholinesterase level. In the present study, the rate of cholinesterase activities in brain tissue was estimated (Fig. 6) and found that the level of cholinesterase significantly ($p < 0.001$) increased for AChE (GRHA: 39.19 ± 1.86 nM/min/gm protein) and BuChE (GRHA: 25.53 ± 1.16 nM/min/gm protein) in SCP-induced group as compared to normal group. But, GRHA-400 treated rats were significantly ($p < 0.001$) improved and normalised the level of cortical AChE (GRHA: 31.71 ± 1.46 nM/min/gm protein) and BuChE (GRHA: 20.61 ± 1.46 nM/min/gm protein). The results were comparable to the DPZ-treated group (Fig. 6). The cholinesterase inhibitory effect of GRHA was due to the positive synergism in antioxidant action of bioactive molecules in GRHA as genistin, quercetin-3-D-galactoside, phytol, stigmasterol and β -sitosterol towards the SCP-induced neuropathological alterations.

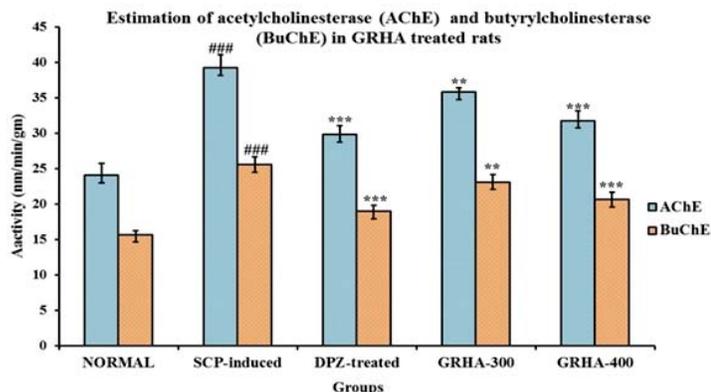


Fig. 6. Rate of cholinesterase (AChE and BuChE) estimation in GRHA treated experimental rats, in which standard drug donepezil (DPZ) was administered to positive control group. ### $p < 0.001$ represents the significant values when toxic control (SCP-induced) group was compared with normal control group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ represents average, moderate and most statistically significant values when experimental groups were compared with SCP-induced (toxic control) group.

BACE1 is a type I integral membrane protein and primarily localises to the Golgi apparatus and late endosomes, highly expressed in the brain and pancreas. Like BACE 1, the second integral membrane protein is BACE2 and found in deficient levels at peripheral tissues of astrocytic cells. Microtubule-associated protein Tau (MAP τ) plays a crucial role in stabilising and forming microtubules in neural cells to maintain its integrity and function. Though the complete pathogenesis of β -secretase and MAP τ in AD is not yet established, the level of β -secretase and phosphorylated MAP τ is higher in AD patients with unknown reasons. In the present study, the level of β -secretase and MAP τ were estimated (Fig. 7), and found that the level of BACE1 and BACE2 significantly ($p < 0.001$) increased in BACE1 (GRHA: 119.31 ± 1.88 ng/mL protein) and BACE2 (GRHA: 14.45 ± 0.82 ng/mL protein) in SCP-induced group as compared to the normal group. But, oral administration of GRHA-400 significantly ($p < 0.001$) decreased the level of BACE1 (GRHA: 80.89 ± 1.82 ng/mL) and BACE2 (GRHA: 7.68 ± 0.45 ng/mL) and found comparable to standard DPZ-treated group. Due to the hyper-phosphorylation of MAP τ , the level was decreased in the SCP-induced group (GRHA: 7.94 ± 0.28 ng/mL) as compared to the normal group. GRHA-400 significantly neutralised ($p < 0.001$) the MAP τ level (GRHA: 10.79 ± 0.2 ng/mL) as compared to DPZ treated group (Fig. 7). The level of BACE1 and BACE2 were counterbalanced by the activation of the PKC/MAPK/ERK 1,2 signalling pathway which can be correlated with the cumulative mode of action of bioactive compounds in GRHA i.e., genistin, quercetin-3-D-galactoside, phytol, stigmasterol and β -sitosterol.

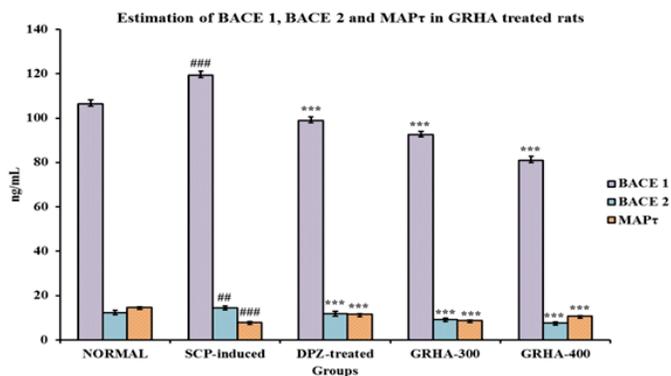


Fig. 7. Depicts the estimation of β -secretase (BACE 1 and BACE 2) and MAP τ in GRHA treated experimental rats, in which standard drug donepezil (DPZ) was administered to positive control group. ### $p < 0.001$ represents the significant values when toxic control (SCP-induced) group was compared with normal control group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ represents average, moderate and most statistically significant values when experimental groups were compared with SCP-induced (toxic control) group.

In histopathological observation, we examined the histological alterations in brain cortex of different groups of experimental rats. The histological sections were stained with haematoxylin and eosin (H&E) to study the pathological hallmarks of AD at the cellular level. The normal control groups were showing intact glial cells (GC), neural cells (NC) and eosinophil cells (E) (Fig. 8A). In the SCP-induced group, distorted necrotic cells, shrunken glial, neural and eosinophil cells were found in a scattered form, leading to apoptotic changes (Fig. 8B). Although GRHA-300 showed moderate cellular improvement of glial, neural and eosinophil cells (Fig. 8D) but, a closer look to GRHA-400 treated groups displayed regenerative efficiency with normal appearance of glial, neural and eosinophil cells (Fig. 8E), and found similar cellular architecture like DPZ-treated groups (Fig. 8C).

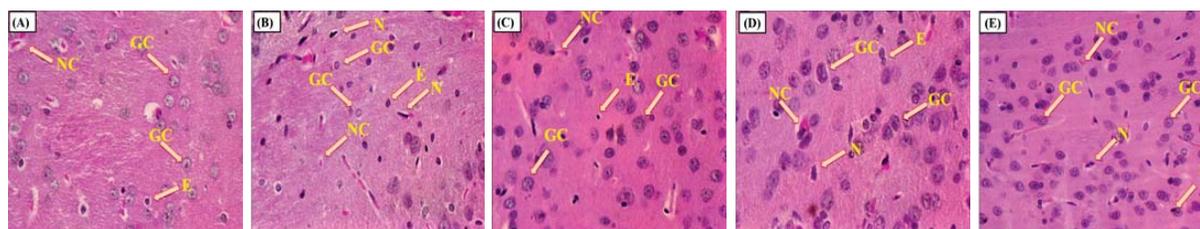


Fig. 8. Histopathological observations of brain cortex (T.S.) in scopolamine (SCP)-induced AD rats (40X, LYNX, Lawrence & Mayo, India). A. Brain tissue of normal control group showed normal histological findings with healthy cortical appearance with glial cells (GC), Neural cells (NC), Eosinophil (E) and no Necrotic cells (N). B. Toxicant control (SCP-induced) group revealed severe damage in normal cellular architecture as appearance of necrotic cells, shrunken glial cells and eosinophil along with the damaged neural cells and displayed marked neuronal degeneration (arrow) with neurophagia and gliosis. C. Positive control (Donepezil/DPZ treated) revealed the healing of eosinophils, glial, neural cells compared to the SCP-induced group. D. GRHA-300 treated group showed moderate recovery of cellular architecture. E. GRHA-400 treated group recovered the cortex to normal status with the appearance of healthy eosinophil, glial cells, and neural cells with less necrotic cells against SCP-induced neurotoxic rats. GRHA, hydroalcohol extracted fraction of *G. repens*.

Therapeutic validation of *Pterocarpus marsupium* and *Piper trioicum* as a cholinesterase inhibitor for the treatment of Alzheimer's disease (AD).

PI: Dr. Atish Kumar Sahoo, Senior Scientist

Rational: Introduction and significance:

Pterocarpus marsupium is a multipurpose leguminous tree. Based on the preliminary scientific claim, *P. marsupium* rhizomes, heartwood and stem has been used in neurodegenerative diseases associated with oxidative stress. Heart wood is astringent, bitter, acrid, cooling, anti-inflammatory, depurative, haemostatic, revulsive, anthelmintic, constipating, rejuvenating memory learning and neurological disorders. The paste of seed and wood is useful in anaemia, body pain and diabetes.

Piper is an economically and ecologically important genus in the family Piperaceae. This family comprises more than 2,000 species of shrubs, herbs, and lianas. *Piper trioicum* (Piperaceae) is distributed in South Asian countries and the whole plant is used as rubefacient, diuretic, hepatoprotective and used for treatment of diabetes, muscular pains, headache, and toothache, internal remedy for cholera in folk medicine, and the root is used as diuretic (Madhava et al., 2008). Due to its highly medicinal value and based on the ethno botanical uses of Indian System of Medicine (ISM), *Piper* species are used as a memory enhancer. Till date there is no scientific evidence of *P. trioicum* to prove its neuroprotective activities in AD. By so far, the scientific validation of the lead finding in *P. marsupium* and *P. trioicum* towards cholinesterase inhibition study is limited and there is no

literature evidence to get insight mechanism of certain bioactive principles as the inhibitor of oxidants in AD. So, this project proposes the therapeutic validation of *P. marsupium* and *P. trioicum* with the strategic approach to establish the lead finding as a cholinesterase inhibitor for the treatment of Alzheimer's disease (AD).

Objectives:

To establish *P. marsupium* and *P. trioicum* as forestry species of Odisha and exploring its bioactive lead molecules cholinesterase inhibitor for the treatment of Alzheimer's disease (AD).

- To establish the radical scavenger mechanism to counter cellular oxidants ORAC and CAPE.
- Study of key markers for Alzheimer's disease e.g., Acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and BACE1.
- Detection and quantitation of bioactive lead molecules in *P. marsupium* and *P. trioicum*.
- Neurodegenerative properties analysis by in vivo model
- Evaluation of tissue cholinesterase activity
- Biochemical estimation to assess the change in biomarkers e.g., MDA, SOD, CAT, GSH.

Significant achievements/ Tangible outcome:

The percentage of yield in extraction is high in hydroalcohol extract of *Pterocarpus marsupium* (leaf, Bark, heart wood) and *P. trioicum* as 11.2%, 12.67% and, 10.43% for *P. marsupium* while, *P. trioicum* hydroalcohol fraction has 9.32% of yield (Table 1). The plant samples are repository of biologically active components with various chemical structure and protective properties. The phytochemicals are secondary metabolites which are present in high amount some of these phytochemicals include alkaloids, glycosides, flavonoids, tannins, terpenoids etc. In primary screening of all the fraction extract of *Pterocarpus marsupium* including hydroalcohol fraction extract of *P. trioicum* indicated presence of various secondary metabolites which have already been reported for their therapeutic activities for different chronic disease (Table 2). To understand the ethnomedicinal claim and biological activities of both the plants, various secondary metabolites qualitative analysis has been performed. Hydroalcohol fraction of both the plant showed high content of Total Phenolic Content (TPC) (*Pterocarpus marsupium*→: leaf 201.25; bark 98.33; heart wood 144.70 mg of GA/gm of extract), Total Flavonoid Content (TFC) (*Pterocarpus marsupium*→: leaf 622.83; bark 414.63; heart wood 372.70 mg of QE/gm of extract), and Total Alkaloid Content (TAC) (*Pterocarpus marsupium*→: leaf 76.30; bark 232.51; heart wood 420.74 mg of AE/gm of extract) as compared to other fraction of extracted materials (Table 3). Likewise, the hydro alcohol fraction of *P. trioicum* leaves showed significant amount of TPC, TFC and TAC (Table 3). To understand the antioxidant nature of both plants, DPPH freeradical scavenging activity and ORAC assay have been conducted, and we observed that hydroalcohol fraction extract of both *P. marsupium* and *P. trioicum* demonstrated potent free radical scavenging activity of DPPH (*P. marsupium*: leaf 188.7 ± 1.23 ; bark 140.74 ± 1.56 ; heart wood 182.316 ± 2.01 $\mu\text{g/mL}$), and *P. trioicum* leaf 48.56 ± 0.98 $\mu\text{g/mL}$ and the results were found comparable with the reference drug ascorbic acid with IC50 value of 20.5 ± 0.45 $\mu\text{g/mL}$ (Table 4 and Fig. 1). The potent hydroalcohol fraction of both plants were considered for the ORAC assay to validate the Total Antioxidant Capacity (TAC) of it. ORAC assay depends on assessing the effect of presumed antioxidants by measuring the fluorescence quenching and based on in situ production of peroxy free radicals generated via AAPH. Peroxy radical reacts with oxidizable substrate and increases the rate of fluorescence decay. Antioxidant

potential of extract was expressed in term of net AUC. Net AUC for *P. marsupium* (leaf, bark, and heart wood) and *P. trioicum* (hydroalcohol) with thereference drug (trolox) were calculated to be 15.67, 12.14, 15.57, 11.14, and 17.05 respectively (Fig. 2). For the neuro degenerative evaluation AChE and BChE inhibition activity of both plants were conducted with hydroalcohol fraction and found *P. marsupium* heart wood showed most significant cholinesterase inhibitor activity with IC_{50} 37.23 μ g/mL among other fractions of both plants (Table 5 and Fig. 3). Likewise, in BChE study, among all; the hydroalcohol fraction *P. marsupium*, heart wood demonstrated significant inhibitory effect with having IC_{50} 94.33 μ g/mL compared to other fractions of both plants (Table 5 and Fig. 4). Results suggest that, both plants metabolites are played a role of first-line cholinesterase inhibitor which may be relevant in slowing AD progression due to its effective radical scavenging activities. GC-MS analysis of *P. trioicum* was conducted to identified the bioactive compounds responsible for anticholinesterase and antioxidant activities (Fig. 5).

The initial test results obtained from in vitro studies, both plants have some promises in inhibiting cholinesterase activities as a neuroprotective agent. Further studies are needed to prove their efficacy in in vivo test model. So, the bioactive extracted fractions such as hydroalcohol extract of bark of *P. marsupium* (PMBHA), hydroalcohol extract of haertwood of *P. marsupium* (PMHHA), and hydroalcohol extract of leaves of *P. trioicum* (PTHA) were evaluated for its acute oral toxicity with a dose ranging from 500-4000 mg/kg b.w. Among all treated groups, group-I and II showed no mortality, sign of any toxicity, or behavioural changes during experimental period. Rats in group-III, IV and V exhibited mortality rate of 1/6, 2/6 and 2/6, respectively and the survived animals showed symptoms of restlessness, salivation, tremor, increased urination and higher degree of irritation (Table 6). As the death rate of rats in group-V of all the three extracted fraction was found to be 50%, LD50 were recorded 4000 mg/kg b.w. The effective doses of the entire three fraction PMBHA, PMHHA and PTHA were calculated to be 300 and 400 mg/kg b.w. respectively for each extract.

Table 1. Percentage of yield of different extract of *P. marsupium* and *P. trioicum*

Plant Material	Extract	% yield (gm)
<i>P. marsupium (Leaf)</i>	Hexane	2.01
	Chloroform	8.12
	Ethyl acetate	6.54
	Methanol	7.42
	Hydroalcohol	11.2
<i>P. marsupium (Bark)</i>	Hexane	0.67
	Chloroform	9.34
	Ethyl acetate	5.55
	Methanol	7.56
	Hydroalcohol	12.67
<i>P. marsupium (heart wood)</i>	Hexane	0.98
	Chloroform	1.24
	Ethyl acetate	5.21
	Methanol	6.87
	Hydroalcohol	10.43
<i>P. trioicum</i>	Hydroalcohol	9.32

In vivo antioxidant assay of PTHA was conducted in Scopolamine-induced Alzheimer's model and found it having potent antioxidant property in cellular level. Result in Table 9 showed that SCP-induced group exhibited significant increase ($p < 0.001$) in MDA level (25.34 ± 2.12 nM/mg protein) as compared to normal group (13.45 ± 1.59 nM/mg protein). But, pre-treatment of PTHA-400 showed significant decrease in level of MDA (17.12 ± 1.17 nM/mg protein) and found comparable to DPZ treated rats 15.55 ± 2.35 nM/mg protein). Administration of PTHA significantly inhibited oxidative stress by balancing antioxidant enzymes and reduced the production of MDA in SCP-induced groups. SOD level in SCP-induced group significantly decreased ($p < 0.001$: 9.35 ± 1.89 U/mg protein) as compared to normal group (14.27 ± 1.41 U/mg protein). But, pre-treatment of PTHA-400 significantly increased ($p < 0.001$) the level of SOD 12.56 ± 1.32 U/mg protein) and found comparable to DPZ

treated group (13.37 ± 1.31 U/mg protein). In SCP-induced group, the CAT level was decreased significantly ($p < 0.001$: 13.68 ± 1.12 U/mg protein) as compared to normal group (61.05 ± 1.04 U/mg protein). But, PTHA-400 showed significant increase in CAT activities ($p < 0.001$: 38.58 ± 2.28 U/mg protein) and found comparable to DPZ treated group (49.47 ± 1.88 U/mg protein). The current finding signified that PTHA is a potent intracellular radical scavenger that scavenge ROS and their precursors and ameliorate neurological disorders in AD (Table 7)

Table 2. Preliminary Phytochemical Screening of *P. marsupium* and *P. trioicum*

Plant material	Extract Fractions	Preliminary Phytochemical Screening Results							
		Glycoside	Alkaloid	Flavonoid	Phenol	Terpenoid	Anthraquinones	Steroid	Tannin
<i>P. marsupium</i> Leaf	Hydroalcohol	-	-	+	+	-	-	-	-
	Chloroform	-	+	-	+	-	-	-	+
	Ethyl acetate	+	-	+	-	-	-	+	-
	Methanol	-	-	+	-	-	-	-	-
<i>P. marsupium</i> Bark	Hydroalcohol	-	-	+	+	-	-	-	-
	Hexane	+	-	-	-	-	-	+	-
	Chloroform	-	-	+	-	-	-	+	-
	Ethyl acetate	+	-	+	+	-	-	+	-
	Methanol	-	-	+	+	-	-	-	-
<i>P. marsupium</i> Heartwood	Hydroalcohol	-	-	+	+	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-
	Ethyl acetate	-	-	+	+	-	-	-	-
	Methanol ext.	-	-	+	+	-	+	+	-
<i>P. trioicum</i> Leaf	Hydroalcohol	-	+	+	+	-	-	-	-

(+) Denote presence i of phytochemicals, (-) Denotes absence of phytochemicals

Table 3. Biochemical estimation of *P. marsupium* and *P. trioicum*.

Plant materials	Extract Fractions	Experiment Results		
		TPC ($\mu\text{g/g}$ eqv. of GA)	TFC ($\mu\text{g/g}$ eqv. of Quercetin)	TAC ($\mu\text{g/g}$ eqv. of Atropine)
<i>P. marsupium</i> Leaf	Hydroalcohol	201.25	622.83	76.30
	Chloroform	28.7	370.28	252.52
	Ethyl acetate	28.33	200.12	16.47
	Methanol	116.25	436.63	84.22
<i>P. marsupium</i> Bark	Hydroalcohol	98.33	414.63	232.51
	Chloroform	15.83	160.33	120.49
	Ethyl acetate	74.16	604.56	152.7
	Methanol	211.25	792.48	259.7
<i>P. marsupium</i> Heartwood	Hydroalcohol	144.70	372.70	420.74
	Chloroform	55.91	306.42	63.04
	Ethyl acetate	87.08	232.84	188.01
	Methanol	139.66	444.30	112.11
<i>P. trioicum</i> Leaf	Hydroalcohol	211.42	171.3	15.46

Table 4. DPPH free radical scavenging activity of various extracted fraction of *P. marsupium* and *P. trioicum* with IC_{50} ($\mu\text{g/mL}$) values.

Plant Materials	Extract Fractions	DPPH Assay IC_{50} ($\mu\text{g/mL}$)
<i>P. marsupium</i> Leaf	Hydroalcohol	188.7 ± 1.23
	Chloroform	387.518 ± 0.98
	Ethyl acetate	780.01 ± 1.24
	Methanol	274.632 ± 1.09
<i>P. marsupium</i> Bark	Hydroalcohol	140.74 ± 1.56
	Chloroform	1160.89 ± 0.89
	Ethyl acetate	240.129 ± 0.56
	Methanol	63.79 ± 1.45
<i>P. marsupium</i> Heartwood	Hydroalcohol	182.316 ± 2.01
	Chloroform	498.788 ± 2.04
	Ethyl acetate	399.262 ± 2.05
	Methanol	184.882 ± 1.98
<i>P. trioicum</i> Leaf	Hydroalcohol	48.56 ± 0.98
Standard drug	Ascorbic acid	20.5 ± 0.45

Table 5. Acetylcholinesterase and butyrylcholinesterase inhibitory activities in *P. marsupium* and *P. trioicum* with IC₅₀ values.

Extracts	Acetylcholinesterase activity IC ₅₀ (µg/mL)	Butyrylcholinesterase activity IC ₅₀ (µg/mL)
PMLHA	61.26	137.9
PMBHA	47.79	101.99
PMHHA	37.23	94.33
PTHA	89.75	102.54

Table 6. Acute toxicity study of *Pterocarpus marsupium* and *P. trioicum* in Swiss albino rats

<i>Acute toxicity study of hydroalcohol fraction of Pterocarpus marsupium and P. trioicum</i>				
Groups	Fraction	Dose (mg/kg)	D/T	Symptoms
I	PMBHA	500	0/6	-
II		1000	0/6	-
III		2000	1/6	Defecation
IV		3000	2/6	urination and irritation
V		4000	2/6	Death
I	PMHHA	500	0/6	-
II		1000	0/6	-
III		2000	1/6	Defecation
IV		3000	2/6	Urination and irritation
V		4000	2/6	Death
I	PTHA	500	0/6	-
II		1000	0/6	-
III		2000	1/6	Defecation
IV		3000	2/6	Urination and irritation Death
V		4000	2/6	

Table 7. Effects of PTHA on SCP-induced AD model rats

Groups	MDA (nM/mg protein)	SOD (U/mg protein)	SOD (U/mg protein)
	Serum	Serum	Serum
Normal control	13.45 ± 1.59	14.27 ± 1.41	61.05 ± 1.04
Negative control (SCP induced)	25.34 ± 2.12 ###	9.35 ± 1.89 ###	13.68 ± 1.12###
Positive control (DPZ-treated)	15.55 ± 2.35 ***	13.37 ± 1.31***	49.47 ± 1.88***
PTHA-300	19.86 ± 0.38***	8.07 ± 0.17***	33.02 ± 2.90***
PTHA-400	17.12 ± 1.17***	12.56 ± 1.32***	38.58 ± 2.28***

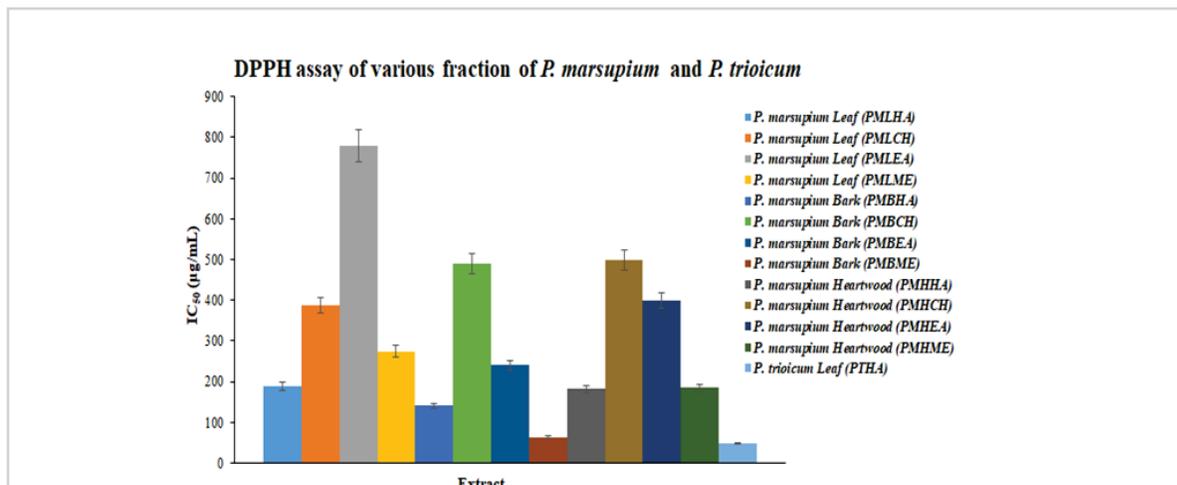


Fig. 1. DPPH assay of various fractions of *P. marsupium* and *P. trioicum*

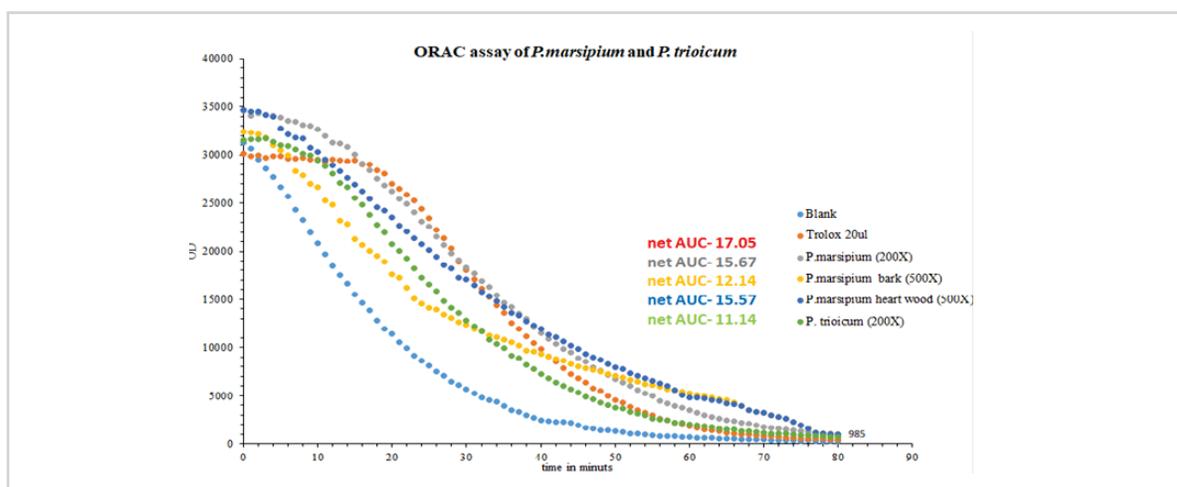


Fig. 2. ORAC assay of *P. marsupium* and *P. trioicum*.

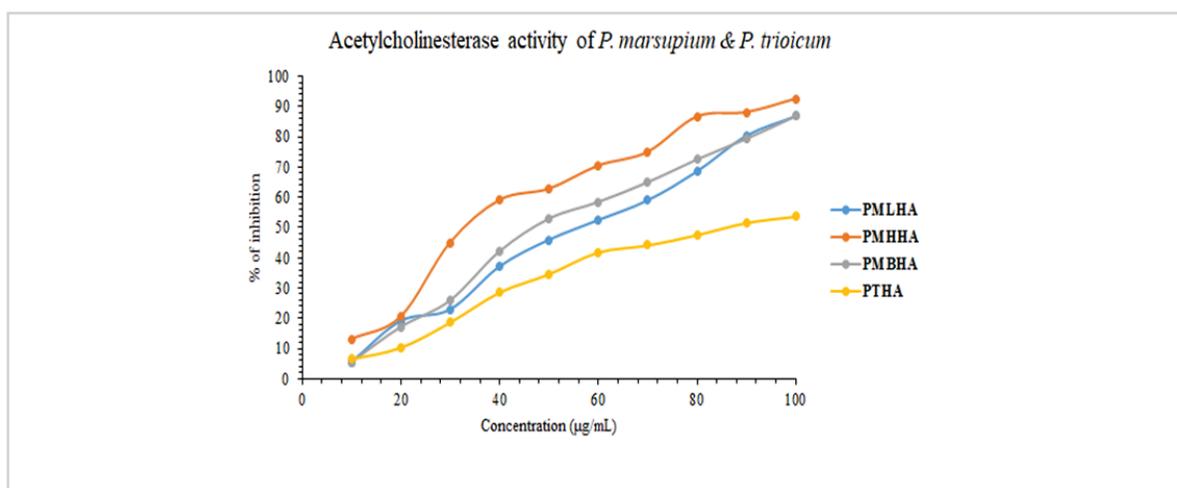


Fig. 3. Acetylcholinesterase inhibitory activities in *P. marsupium* and *P. trioicum*

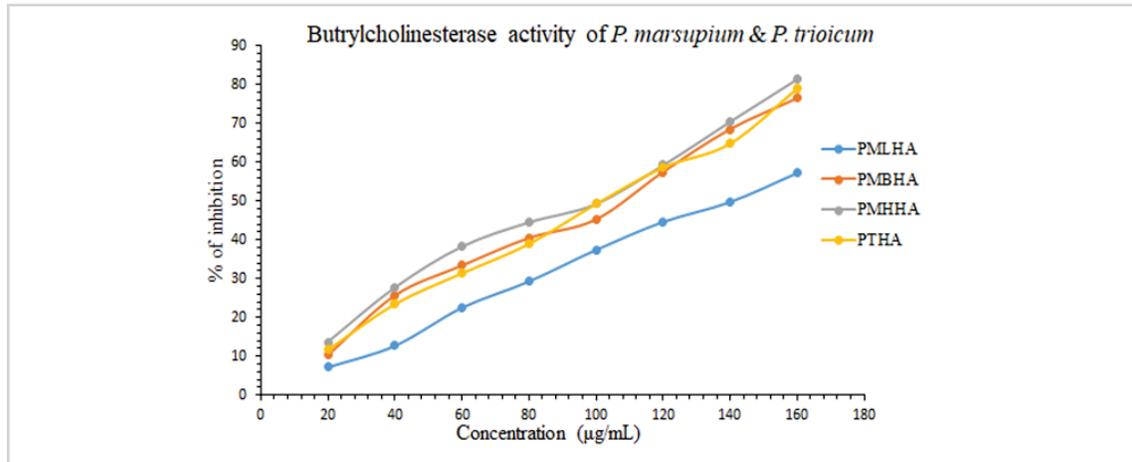
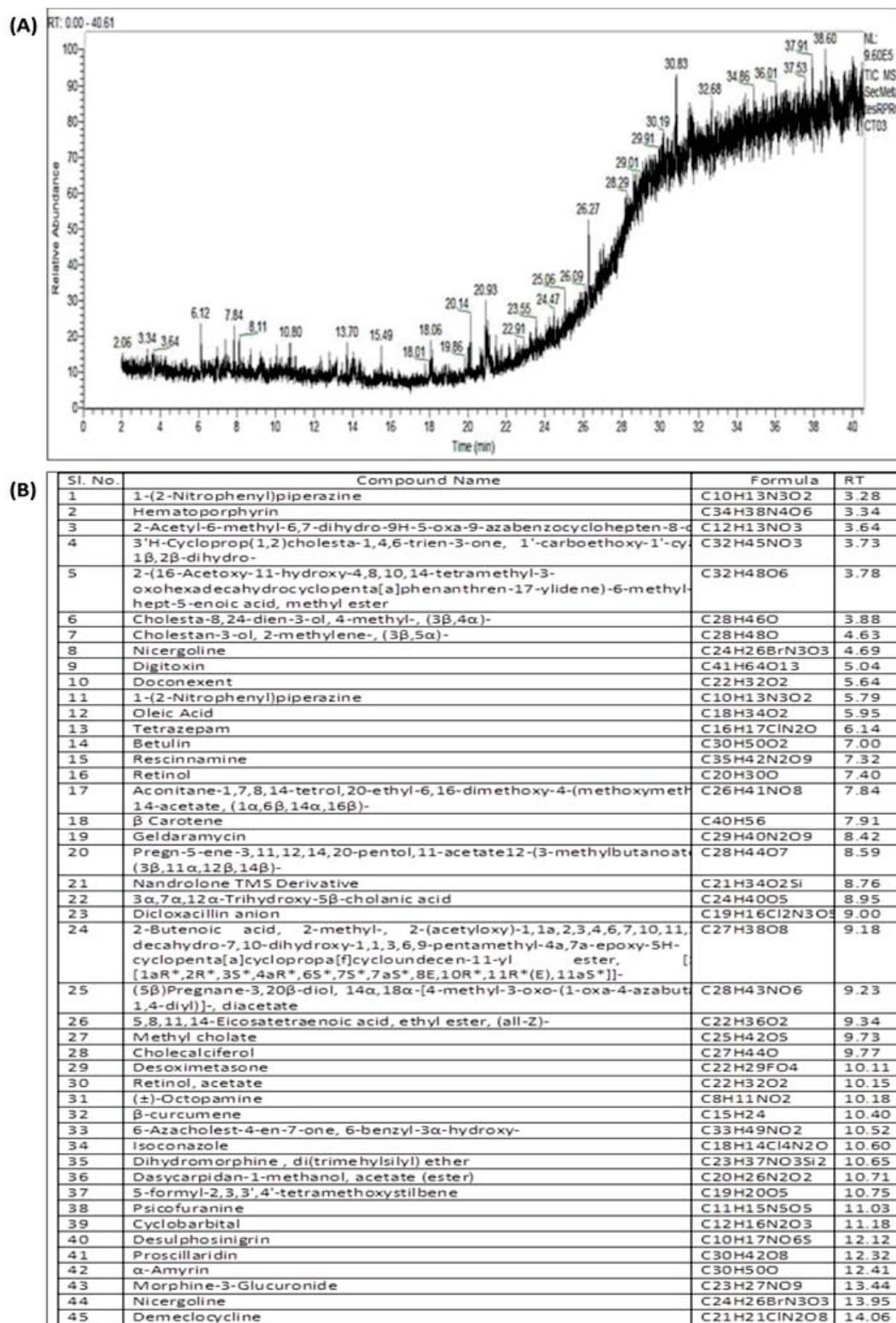


Fig. 4. Butyryl cholinesterase activity inhibitory activities in *P. marsupium* and *P. trioicum*

Fig. 5. GC-MS/MS analysis of hydroalcohol extract of *Piper trioicum* (PTHA).

Ameliorative effects of *Homalium zeylanicum* on diabetes-induced oxidative stress and inflammation in Wistar rats.

PI: Dr. Atish Kumar Sahoo, Senior Scientist

Rationale: Introduction and significance

Homalium zeylanicum (Gardner) Benth. (Salicaceae), commonly known as 'Kalladamba', is distributed in Western Ghats, Andhra Pradesh, Tamil Nadu, and Kerala of India. Ethnobotanically, the bark and leaf of this plant are traditionally used in diabetes, rheumatism, malaria and wound healing by the isolated section of the society in Rayalaseema region of Andhra Pradesh, India. So, further investigation on this plant for the presence of active constituents needs to be validated. Hence this project has been undertaken to establish scientifically the efficacy of this plant in combating diabetes-induced oxidative stress and inflammation in experimental Wistar rat model.

Objectives

To identify the potential bioactive compounds in HAHZB and HAHZL by GC-MS/MS analysis.

1. Toxicity profiling of HAHZB and HAHZL in Wistar rats.
2. In vivo antioxidant, antidiabetic and anti-inflammatory activity of HAHZB and HAHZL in experimental rats.
3. In vivo oxidative DNA damage study of HAHZB and HAHZL in experimental rats.
4. Histopathological study of liver and pancreas of experimental rats.

Significant achievements/ Tangible outcome

The hydroalcohol fraction of bark (HAHZB) and leaves (HAHZL) of *H. zeylanicum* were subjected to GC-MS/MS analysis for the identification of bioactive compounds. Toxicity profiling of both fractions has been done according to OECD, 2001 guideline. Doses have been fixed and both the fractions were subjected to in vivo animal experimentation to determine its efficacy in Wistar rat model.

GC-MS/MS analysis of HAHZB and HAHZL

GC-MS/MS analysed the secondary metabolites of both fractions e.g., HAHZB and HAHZL, and validated by comparing the mass spectra of compounds with the standard mass spectra of NIST library (Version-11). A total of 28 no. of compounds were identified in each of the fractions which belongs to glycoside, carboxylic acid, hydrocarbon, fatty acid, diterpene, ester, acid chloride, alcohol, phenolic, flavone and steroid groups were represented/described with peak number, retention time (RT), compound name, molecular formula, molecular wt. and peak area (Fig.1 a and b).

GC-MS/MS analysis of HAHZB detected 15 dominant compounds e.g. ethyl β -D-ribose (8.72%), ethyl α -D-glucopyranoside (20.23%), 5,7-dodecadiyn-1,12-diol (12.05%), 17-octadecynoic acid (1.54%), 10-methyl-8-tetradecen-1-ol acetate (1.49%), oleic acid (1.03%), ethyl tridecanoate (11.77%), 5-heptenoic acid, 6-methyl-4-[(4-methylphenyl)sulfonyl] (5.70%), phytol (1.29%), 9,12-octadecadienoic acid, methyl ester, (E,E)- (5.97%), 9,12-octadecadienoyl chloride, (Z,Z)- (10.96%), pentadecanoic acid, ethyl ester (1.98), ergosta-5,22-dien-3-ol, acetate, (3 β ,22E)- (7.36%), stigmasterol (1.30%), β -sitosterol (2.86%) (Fig.1). Out of 15 dominant compounds, 6 compounds such as oleic acid, ethyl tridecanoate, phytol, 9,12-Octadecadienoic acid, methyl ester, (E,E)-, stigmasterol and β -sitosterol have antioxidant, anti-inflammatory and anti-diabetic activities (Fig.1a).

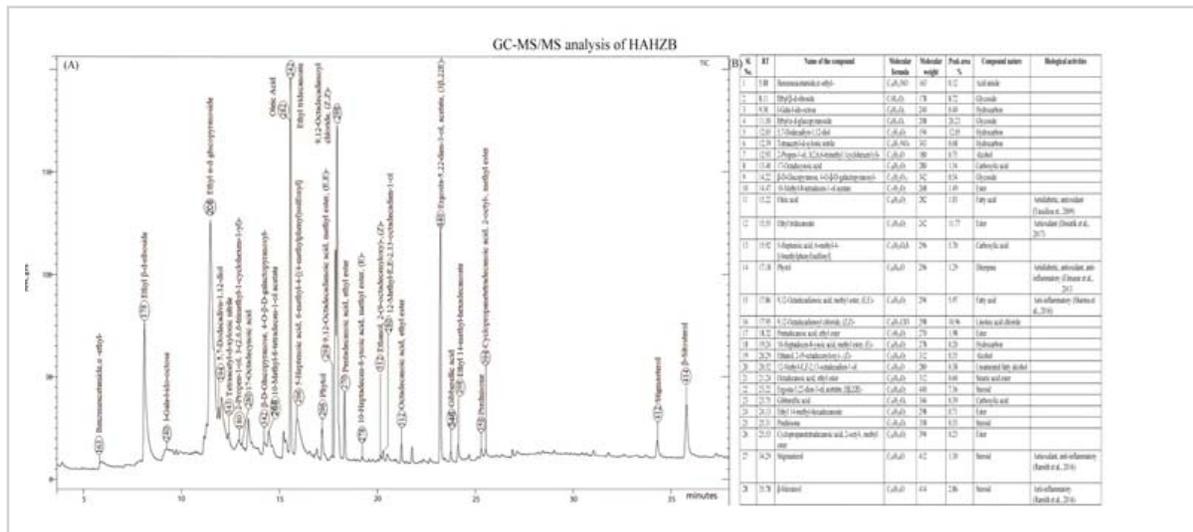


Fig. 1a. GC-MS/MS chromatogram and list of identified compounds of HAHZB. A: GC-MS/MS chromatogram of HAHZB in which encircled peaks represent identified compounds. B: List of identified antioxidants, antidiabetic and anti-inflammatory compounds that are reported in HAHZB by GC-MS/MS study. HAHZB: Hydroalcohol fraction of bark of *H. zeylanicum*.

Eighteen major compounds of HAHZL that were identified with their % peak area by GC-MS e.g. 1,4-Cyclohexanediol, trans- (1.44%), Benzoic acid (2.11%), Phenol, 4-ethenyl-,acetate (28.68%), Hydroquinone (9.10%), Formamide, N-methyl-N-4-[1-(pyrrolidinyl)-2 butynyl]- (4.28%), 1,3-Propanediol, 2-ethyl-2 (hydroxymethyl)- (10.56%), 2,7-Dioxa tricyclo[4.4.0.0(3,8)] deca-4,9-diene (1.43%), Tetraacetyl-d-xylonic nitrile (4.57%), β-D-Glucopyranose, 4-O-β-D-galactopyranosyl- (8.70%), 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol (2.24%), n-Hexadecanoic acid (0.55%), Hexadecanoic acid, ethyl ester (0.72%), Phytol (0.57%), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (0.83%), E-2-Hexenyl benzoate (1.10%), Arbutin (17.65%), Vitamin E (1.04%), β-Sitosterol (1.54%) (Fig.1B). Out of which, 7 compounds such as Phenol, 4-ethenyl-,acetate, Hydroquinone, n-Hexadecanoic acid, Phytol, Arbutin, Vitamin E, β-Sitosterol have antioxidant, anti-inflammatory and anti-diabetic activities.

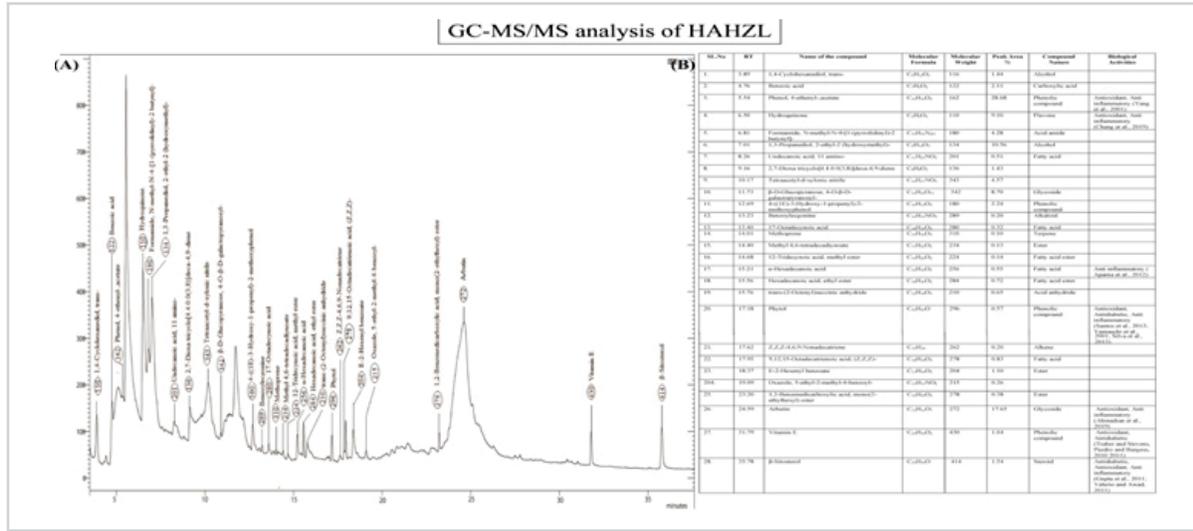


Fig. 1b. GC-MS/MS chromatogram and list of identified compounds of HAHZL. A: GC-MS/MS chromatogram of HAHZL where encircled peaks represent identified compounds. B: List of identified antioxidants, antidiabetic and anti-inflammatory compounds reported in HAHZL. HAHZL- hydroalcohol fraction of leaf of *H. zeylanicum*.

Acute toxicity study for HAHZB and HAHZL has been conducted according to the Organization for Economic Cooperation and Development (OECD) guideline 423 (OECD, 2001). Animals were divided randomly into five experimental groups (n = 6). HAHZB and HAHZL were dissolved in distilled water and administered in a single dose of 500, 1000, 2000, 3000 and 4000 mg/kg b.w., respectively by oral gavage to overnight fasted animals. All animals were observed individually for mortality and general changes. Toxicity sign and symptoms such as alertness, restlessness, irritability, fearfulness, touch response, gait, defecation, and urination were observed for 72 h. No mortality, or any difference in sign and symptoms were found in the HAHZB, and HAHZL treated group (I, II), while the ratio of death/treatment in group III, IV and V was 1/6, 2/6 and 3/6, respectively with symptoms of salivation, tremor, higher degree of irritation, restlessness, and increased urination. As 50% rats were died at 4000 mg/kg b.w., LD50 was recorded as 4000 mg/kg b.w. and effective doses of HAHZB and HAHZL were calculated to be 300 and 400 mg/kg b.w.

Experimental design

Wistar albino rats of either sex were fed with high fat diet (HFD) for 14 days except normal control rats. STZ at a dose of 40 mg/kg b.w. has been administered for the induction of experimental diabetes in rat. On 10th day after STZ administration, rats with blood glucose level ≥ 250 mg/dL were considered as diabetic rats for the experiment. The treatment with standard drug (pioglitazone-HCl), low and high dose of HAHZB were started from the 11th day and continued till 28 days.

The animals were randomly allocated into 7 groups (n= 6) as follows:

- Group-I (Non diabetic rats): administered 0.9% normal saline (1 mL/kg)
- Group-II (Diabetic rats): administered 0.9% normal saline (1 mL/kg)
- Group-III (Positive control): administered pioglitazone-HCl (4 mg/kg, p.o.)
- Group-IV (HAHZB-300): administered lower dose of HAHZB (300 mg/kg, p.o.)
- Group-V (HAHZB-400): administered higher dose of HAHZB (400 mg/kg, p.o.)
- Group-IV (HAHZL-300): administered lower dose of HAHZL (300 mg/kg, p.o.)
- Group-V (HAHZL-400): administered higher dose of HAHZL (400 mg/kg, p.o.)

Effect of HAHZB and HAHZL on serum antidiabetic, lipoprotein, renal and thyroid functional markers

Serum anti-diabetic markers such as serum glucose (SG), serum insulin (SI), HbA1C and HOMA-IR have been estimated in experimental rats. Elevated levels of SG, HbA1C, HOMA-IR and decreased level of SI has been observed in toxic control rats. Upon treatment with HAHZB and HAHZL at 400 mg/kg significant ($p < 0.001$) normalization (SG, 209.62 ± 1.05 mg/dL, HAHZB; 173.98 ± 4.21 mg/dL, HAHZL; HbA1C, $8.93 \pm 0.03\%$, HAHZB; $7.68 \pm 0.14\%$, HAHZL; HOMA-IR, $7.79 \pm 0.04\%$, HAHZB; $5.94 \pm 0.22\%$, HAHZL; SI, 15.07 ± 0.11 μ U/mL, HAHZB; 13.83 ± 0.25 μ U/mL, HAHZL) has been seen among all the experimental rats (Fig. 2).

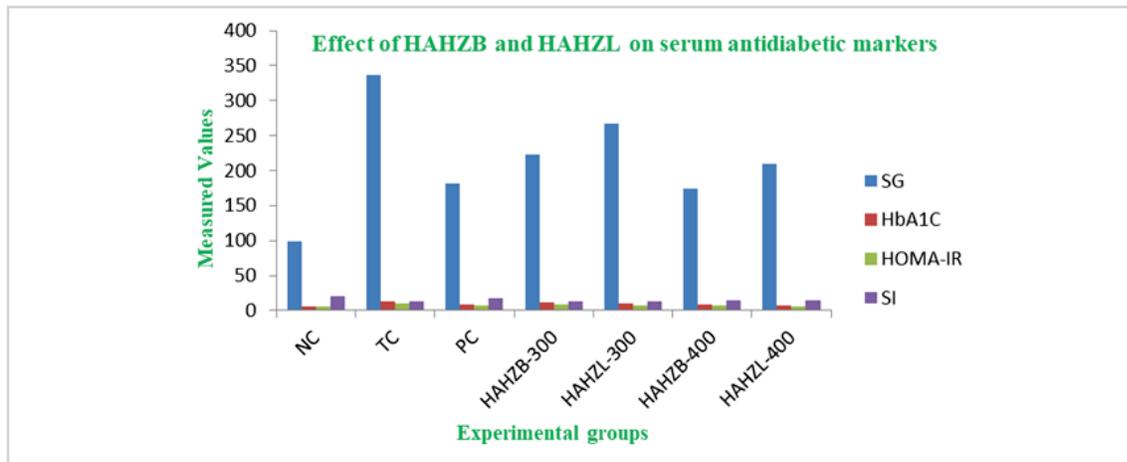


Fig. 2. Effect of HAHZB and HAHZL on serum antidiabetic markers of experimental rats.

Similarly, the lipid profile of toxic control rats showed elevated level of TC, LDL-C, TG and decreased level of HDL-C in comparison to normal rats. However, treatment with HAHZB and HAHZL at 400 mg/kg significantly ($p < 0.001$) restored all the lipoprotein markers to near control levels (TC, 74.66 ± 0.59 mg/dL, HAHZB; 73.53 ± 1.33 mg/dL, HAHZL; LDL-C, 38.06 ± 0.69 mg/dL, HAHZB; 38.43 ± 4.02 mg/dL, HAHZL; TG, 171.92 ± 1.01 mg/dL, HAHZB; 166.41 ± 2.12 mg/dL, HAHZL; HDL-C, 22.08 ± 0.46 mg/dL, HAHZB; 21.87 ± 0.97 mg/dL, HAHZL) (Fig. 3).

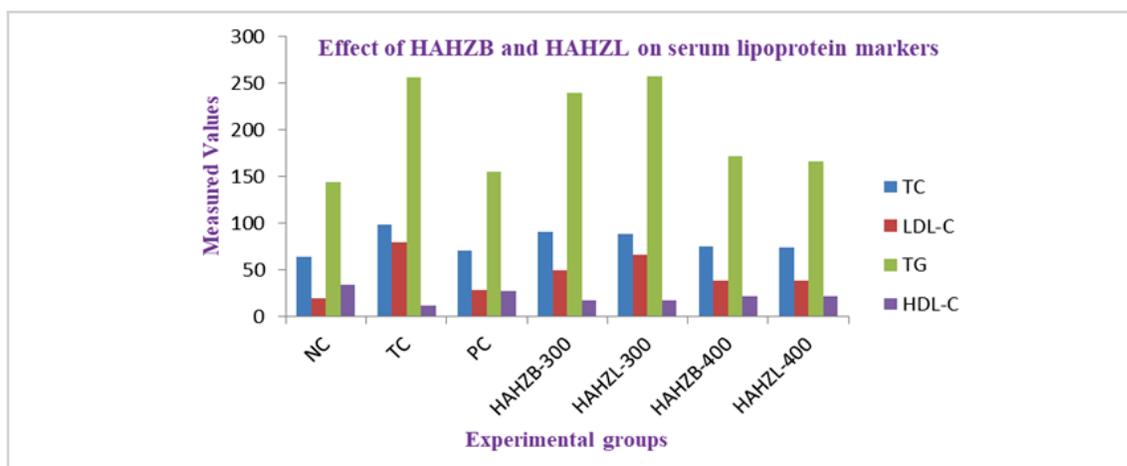


Fig.3. Effect of HAHZB and HAHZL on serum lipoprotein markers of experimental rats.

The renal functional markers such as total protein (TP), blood urea nitrogen (BUN) and creatinine (Cr) have been elevated among toxic control rats. But, pre-treatment with HAHZB and HAHZL at 400 mg/kg remarkably ($p < 0.001$) reduced the level of BUN (23.79 ± 0.14 mg/dL, HAHZB; 23.56 ± 0.22 mg/dL, HAHZL) and Cr (1.34 ± 0.04 mg/dL, HAHZB; 2.23 ± 0.02 mg/dL, HAHZL) and increased the serum TP (6.39 ± 0.10 mg/dL, HAHZB; 4.42 ± 0.04 mg/dL, HAHZL) level to near normalization (Fig. 4).

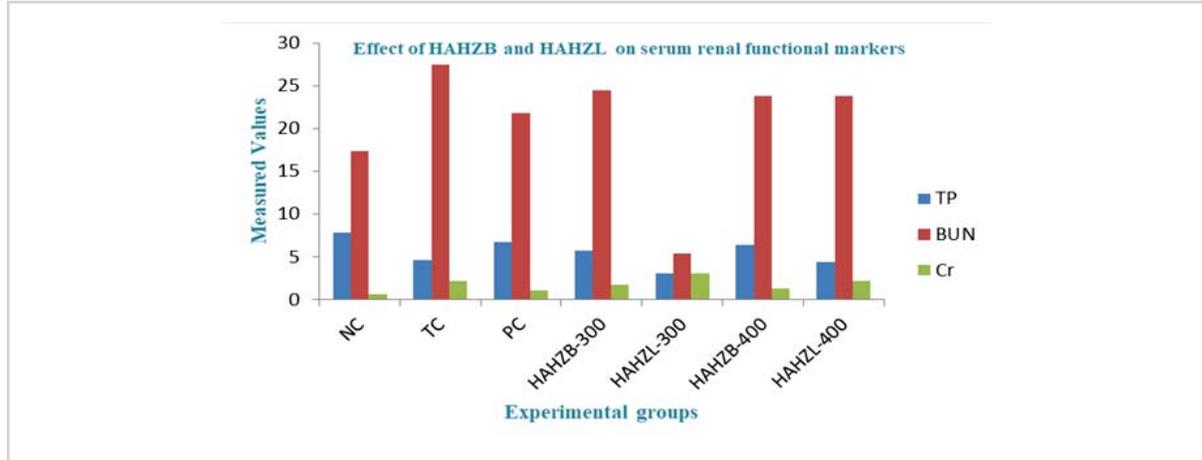


Fig.4. Effect of HAHZB and HAHZL on serum renal functional markers of experimental rats.

The thyroid functional marker such as serum thyroglobulin (Tg) has been found to be elevated among toxic control rats but treatment with HAHZB and HAHZL at 400 mg/kg successfully restored serum Tg level (Tg, 291.26 \pm 0.57 pg/mL, HAHZB; 329.44 \pm 27.81 pg/mL) (Fig. 5).

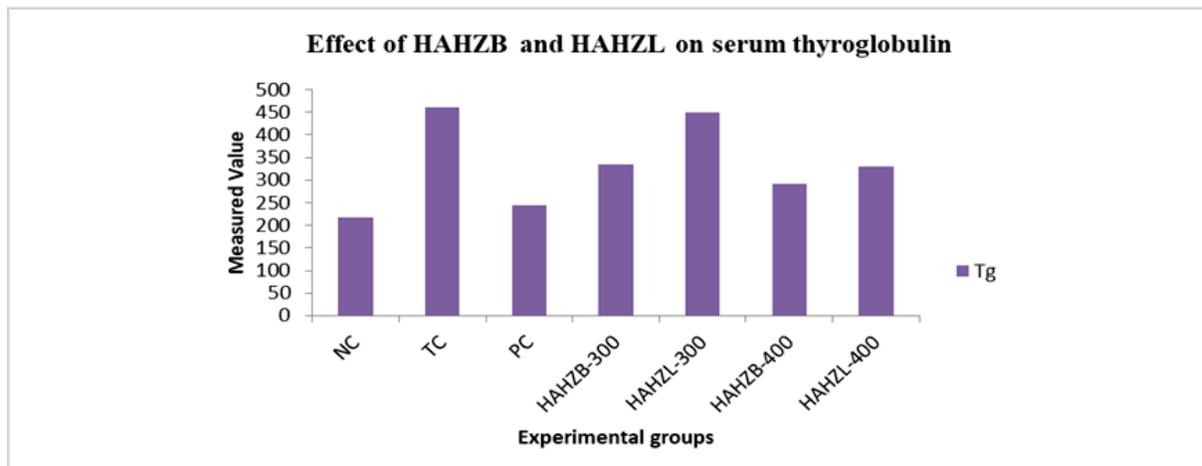


Fig.5. Effect of HAHZB and HAHZL on serum thyroglobulin of experimental rats. vEffect of HAHZB and HAHZL on oxidative stress markers of pancreas of experimental rats

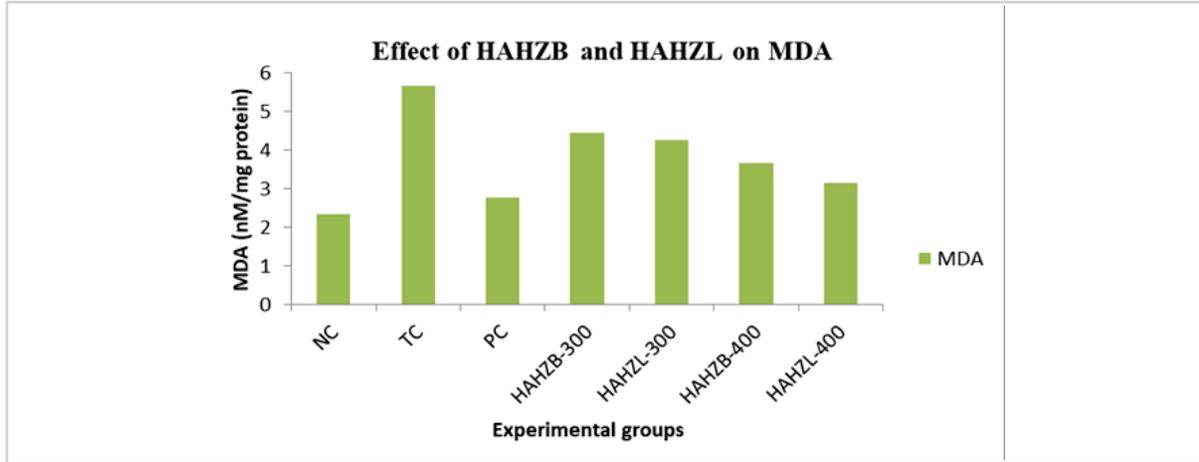


Fig.6A. Effect of HAHZB and HAHZL on pancreatic MDA level of experimental rats.

The current investigation showed significant ($p < 0.001$) increase in pancreatic MDA level in toxic control rats as compared to normal control. Treatment with HAHZB and HAHZL at 400 mg/kg resulted in significant improvement in MDA level among Diabetic treated rats (MDA, 3.65 ± 0.17 nM TBARS /mg protein, HAHZB; 3.15 ± 0.05 nM TBARS /mg protein, HAHZL). Likewise, enzymatic and non-enzymatic antioxidant such as SOD, CAT and GSH level in pancreas were decreased in toxic control rats in comparison to normal control. But, pre-treatment with HAHZB and HAHZL at 400 mg/kg significantly ($p < 0.001$) increased the SOD (3.14 ± 0.28 U/mg protein, HAHZB; 3.36 ± 0.10 U/mg protein, HAHZL), CAT (7.88 ± 0.23 U/mg protein, HAHZB; 7.25 ± 0.21 U/mg protein, HAHZL) and GSH level (12.63 ± 0.28 μ M/g of tissue, HAHZB; 14.30 ± 0.18 μ M/g of tissue, HAHZL) these results were found comparable to positive control (Fig. 6A and B).

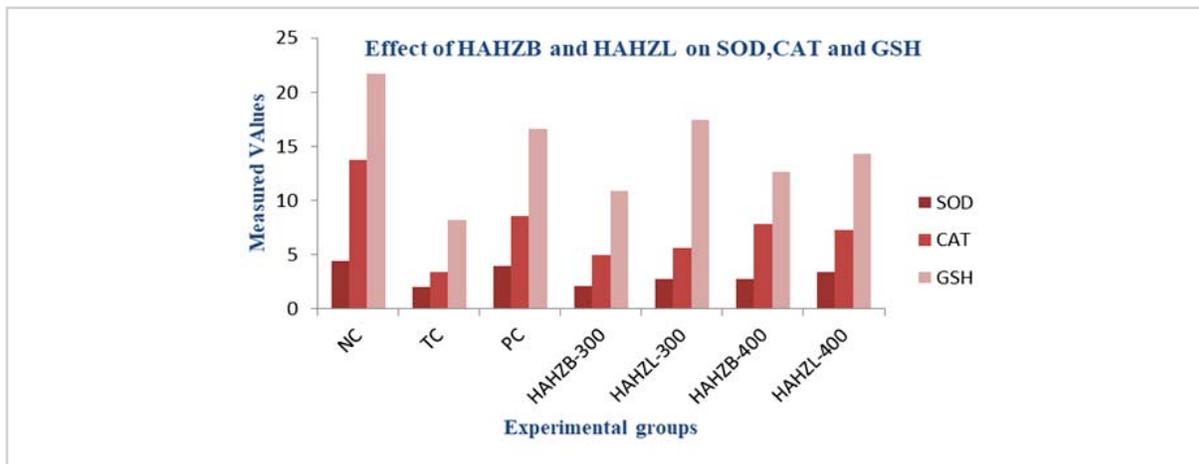


Fig.6B. Effect of HAHZB and HAHZL on pancreatic SOD, CAT and GSH level of experimental rats.

Effect of HAHZB and HAHZL on inflammatory cytokine and oxidative DNA damage markers of pancreas of experimental rats

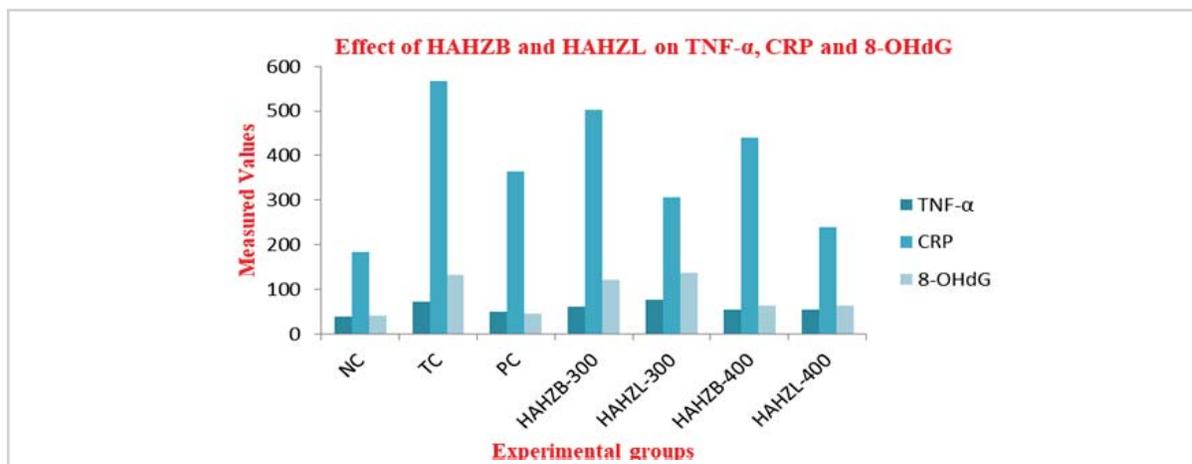


Fig.7. Effect of HAHZB and HAHZL on pancreatic TNF- α , CRP and 8-OHdG level of experimental rats.

This study demonstrates that there were significant ($p < 0.001$) elevation of the level of TNF- α and CRP in toxic control rats as compared to their respective normal control rats. However, HAHZB and HAHZL at 400 mg/kg showed significantly ($p < 0.001$) better activity towards nullifying the risk of inflammation among the diabetic rats by decreasing the level of TNF- α and CRP (TNF- α , 54.48 ± 3.19 pg/mL, HAHZB; 54.80 ± 2.80 pg/mL, HAHZL; CRP, 440.22 ± 7.86 ng/mL, HAHZB; 238.75 ± 1.46 ng/mL, HAHZL). In healthy individuals GSH was efficient at inhibiting 8-OHdG formation. With decrease in GSH, as can be seen among the diabetic rats imparts a great effect on the hydroxylation of guanidine residue forming more of 8-OHdG. The results obtained for the present study indicated an elevated level of 8-OHdG in toxic control rats when compared with the normal control rats. Administration of HAHZB and HAHZL, particularly at a dose of 400 mg/kg significantly ($p < 0.001$) improved the assist in the recovery of damaged DNA (63.65 ± 1.84 ng/mL, HAHZB; 64.30 ± 0.14 ng/mL, HAHZL) (Fig. 7).

Histopathological analysis of pancreas of experimental rats

A study on the histopathological changes of the pancreas in normal control and toxic control rats were done to assess the preservative role of HAHZB and HAHZL on the tissue. Normal control rats showed normal histological architecture with rounded normal proportions of islet of Langerhans and pancreatic acini (Fig. 8A, 9A). Toxic control rats exhibited degeneration of pancreatic islets with necrotic and fatty infiltrate (Fig. 8B, 9B). However, rats taken as positive control showed near normal pancreatic islets and also revealed an increase in the size of islet cells (Fig. 8C, 9C). HAHZL treated diabetic rats showed marked improvement of the cellular injury as (Fig. 8D, E and Fig. 9D,E) evident from the partial restoration of islet cells, reduced β -cell damage, whereas HAHZL at 400 mg/kg exhibited a better protection of pancreatic islet cells from necrosis and β -cell damage. In streptozotocin induced diabetic rats, HAHZL protected the pancreatic β cells by decreasing oxidative stress and preserving pancreatic cellular integrity.

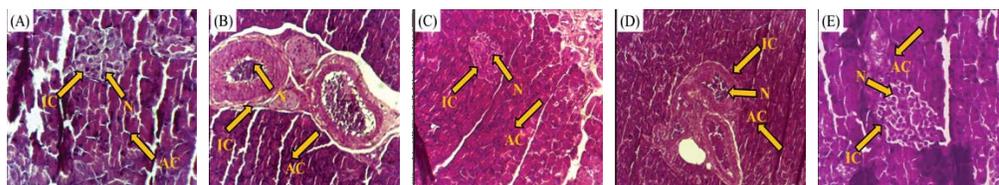


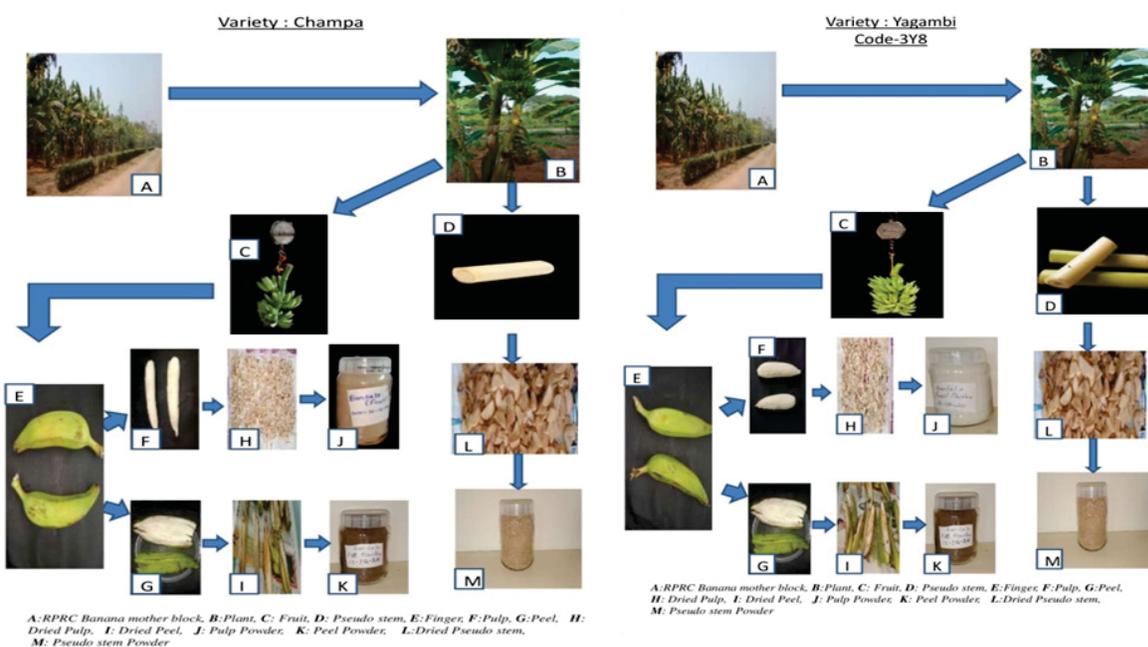
FIGURE 8. Histological tissue section of pancreas of different experimental groups. A. T.S. of normal control rat. The sections showed normal cellular architecture with islets of Langerhans cells (IC) and acinar cells (AC). B. T.S. of toxic control rat. The sections shows severe vacuolation pronounced degeneration of islet cells. C. T.S. of positive control showed normal architecture of islet and acinar cells in comparison to toxic control rats. D. T.S. of HAHZL-300 showed moderate effect in recovering the structure of islet and acinar cells. E. T.S. of HAHZL-400 displaying nearly normal structure of islets of Langerhans. All sections captured at 40X magnification, using optical system H&E, 135X, NIB 100, Lawrence & Mayo, India. HAHZL- Hydroalcohol fraction of *Indigofera zeylanicum*

Fig.9. Histological tissue section of pancreas of HAHZL treated experimental rats

Collection and characterization of germplasm and nutritional analysis of different wild species and cultivated varieties of Musa in Odisha

PI: Dr. (Mrs.) Bandita Deo, Senior Scientist

Nutritional traits which include food or feed value are important for many vegetables, fruits, and forages. Composition and nutrient values of banana fruits are varieties of each cultivar. It is indicated by the increase of carbohydrates level in form of starch when the fruit turn mature. The high nutrient values of bananas are important to fulfil the daily needs for people at any ages.



The Project mainly focused to describe the morphology and analyse nutrient values of mature fruits of different genome groups cultivated in Odisha. In the study, the collected variety of banana was cv. Champa, Gaja Bantala, Patakpara and Yangambi KM5. Different parts of Banana (matured fruit, Fruit peel, Fruit pulp and pseudostem) were dried and processed for nutritional analysis. Higher content of protein and fibre was found in Champa and Yangambi variety comparing to the other varieties of banana. Other parameters like minerals, vitamins, carbohydrates and sugar content are likely to be analysed.

Control of lethal browning with antioxidants during in vitro culture of Banana

PI: Dr. (Mrs.) Bandita Deo, Senior Scientist

Production of quality planting material through in vitro micropropagation is one of the most important advantages and techniques of plants tissue culture. But due to secretion of phenolic compounds during invitro culture of these varieties (Patakpura and Champa), browning of explants resulting which inactivates the growth. Mass micropropagation of these varieties have a serious problem of high mortality due to lethal browning of plantlets.



Fig 1. SwordSucker



Fig2. Removal of SwordSucker

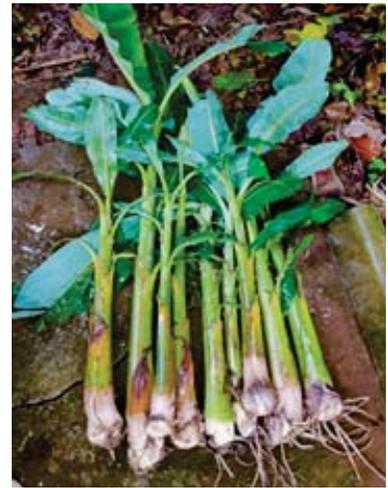


Fig3. Champa Sucker



Fig4. Processing of Explant



Fig5. Initial Culture



An experiment was conducted to study the effect of Ascorbic acid and Citric acid during the initial culture of Champa and Patakpura to minimize the browning effect during initial preparation. Different concentration of ascorbic acid was used in initial media along with plant growth regulator to overcome the Lethal browning (Fig6).

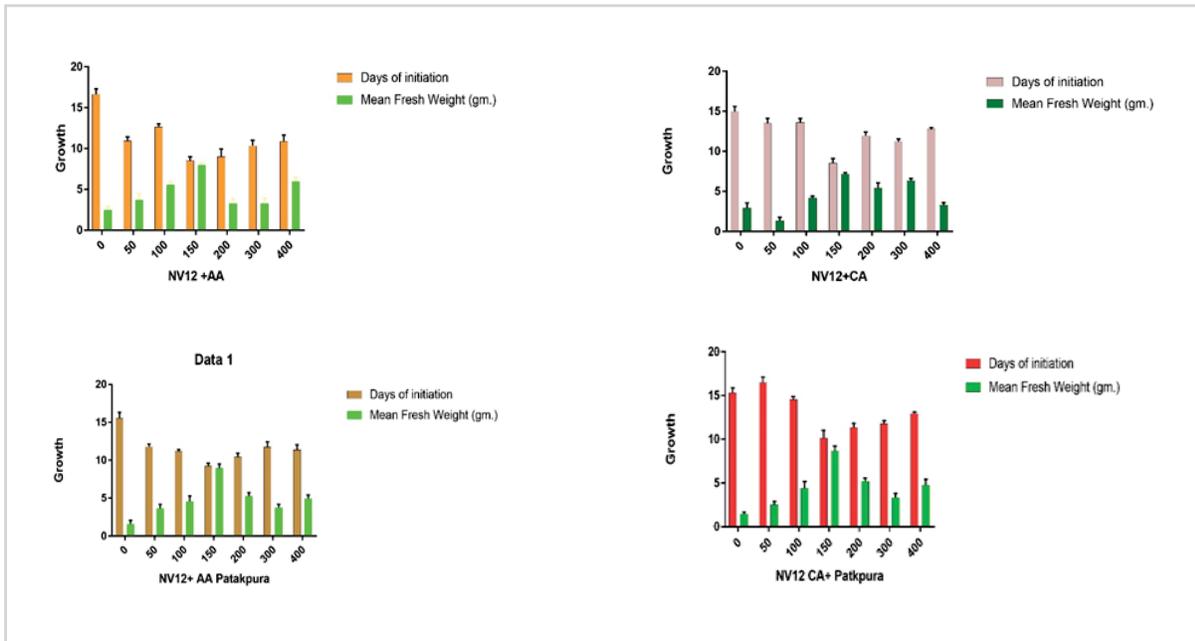


Fig6. Effect of various concentrations of ascorbic acid in controlling the lethal browning



Fig7. Effect of BAP without Ascorbic acid cv. Champa



Fig8. Effect of BAP with Ascorbic acid cv. Champa

From the initial study it was observed that ascorbic acid and citric acid showed better result during initial culture of Champa and Patakpara for controlling lethal browning and can also use as pretreatment during initial culture of Banana Fig7&8.

Mass Propagation of Local *Musa* varieties of Odisha, Commercialization using Tissue Culture Techniques

PI: Dr. (Mrs.) Bandita Deo, Senior Scientist

Banana is typically propagated vegetatively. Tissue culture as a propagation technique provides a robust means to prepare disease-free planting materials that can provide an integrated disease-management program for banana. Tissue-culture techniques established for banana include shoot and meristem culture, callus culture, somatic embryogenesis, cell suspension, and protoplast cultures. However, commercial tissue-cultured banana seedlings are not always conveniently available. Larger-scale banana production farmers, may wish to establish a banana tissue-culture facility in-farm to ensure availability of disease-free seedlings for replanting in conjunction with a practice of destroying diseased banana plants.



Fig1. Establishment of Tissue Cultured Banana Plants at RPRC Mother Block

Four nos. of *Musa* varieties (cv. Patakpura, Bantala, Champa and Yangambi) 40 nos. each which were produced at Banana Tissue Culture Laboratory through tissue culture were Planted at Banana Mother Block of Regional Plant Resource Centre Bhubaneswar Fig1.

Morphological Data (height of plant, no. of leaves, leaf length, shoot diameter and no. of suckers) of tissue cultured variety Gaja Bantala and Patakpura were taken from first month till harvesting. The no of leaves in nine months plant ranges from 16 – 18 nos.in Patakpura where as the no. of leaves in case of Gaja Bantala is 16-20. Plant height in the ninth month is 7.4 ft -9.6 ft in case of Patakpura but in case of Gaja Bantala it is 7.5 ft-9.7ft Fig.2. Maximum no. of suckers in Patakpura was 6-8nosper plant and 8-10 nos per plant in case of Gaja Bantala

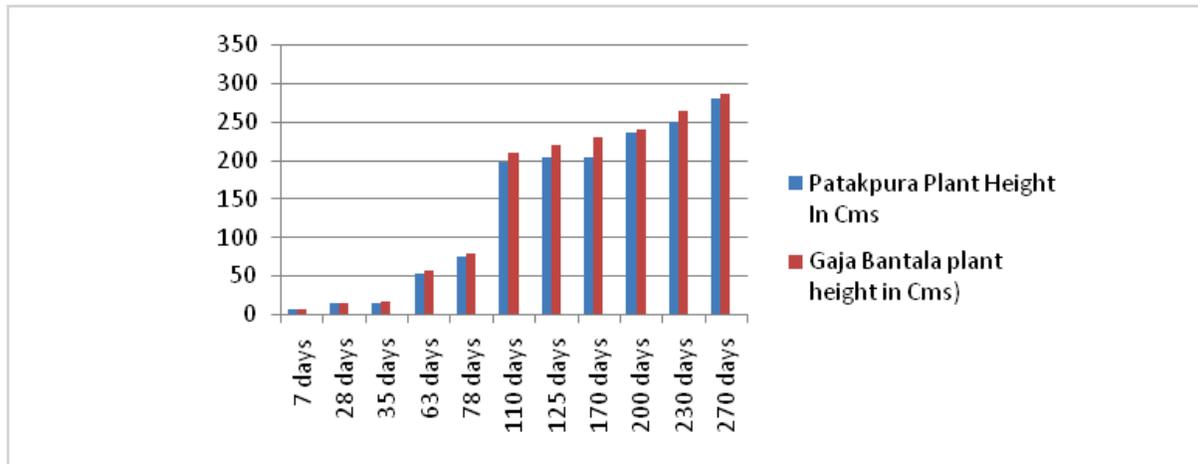


Fig2. Plant height of cv. Patakura and Gaja Bantala at RPRC Mother Block

Different Stages of Mass Propagation of Banana

Suitable sucker of Tissue Cultured Gaja Bantala and Patakura variety were collected from Banana Mother Block inside RPRC premises and were excised it to obtain 4 inches of inner pseudostem, exposing banana meristem. The explants were subjected to surface sterilization through different sterilants like Mercuric chloride, Silver nitrate Sodium hypochlorite and Calcium hypochlorite (Fig3). Murashige and Skoog (1962) media was initially tried for shoot culture in vitro. The medium was supplemented with differing concentrations of various phytohormones to find their effect on shoot induction individually or in combination. The phytohormones used for the shoot culture studies were Benzyl Amino Purine (BAP), Kinetin (kn.), Indole -3-acetic acid (IAA), Indole -3-butiric acid (IBA) and Adenine Sulphate (ADS) in suitable combination were determined. The explants were inoculated in the test-tubes containing induction medium.



Fig3. Surface Sterilization of cv. Gaja Bantala and Patakura



Fig4. Different Stages of Initial Culture (cv. Gaja Bantala)

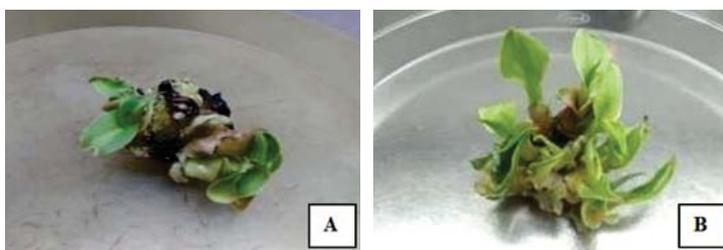


Fig3. Surface Sterilization of cv. Gaja Bantala and Patakura



Fig6. Rooted Plantlets (cv. Gaja Bantala)

This sterilized tissue block was cut longitudinally into two, the half containing the apical dome was retained and used as an explant; the remainder was discarded. The second set of explants were prepared as above but had their apical domes together with subjacent leaf primordia removed so that the effect of apical dome on shoot initiation could be determined. The bottles containing the semi-solid media were kept in culture rack. The culture was maintained at $25 \pm 2^\circ\text{C}$, 16 h photo period of 35-50 $\mu\text{Em}^{-2}\text{s}^{-1}$ intensity provided by cool white fluorescent tubes. Then the explants were undergone initial, multiplication, and rooting culture followed by primary and secondary hardening (Fig.4,5&6).

Genetic fidelity of cv. Gaja Bantala and Patakapura using ISSR markers Isolation of DNA

Fresh leaves from the plants growing in the RPRC Mother Block, Secondary Hardening as well as Tissue Culture raised plants were used for DNA extraction. The DNA was extracted using HipurA plant genomic DNA miniprep Purification kit (HIMEDIA). Quality and quantity of DNA were checked by standard spectrophotometry and also on 0.8 % (w/v) agarose gel for estimating the amount and purity of DNA.

Molecular markers have been used successfully to determine the degree of relatedness among individuals or group of accessions to clarify the genetic structure or variation. One ISSR primer was used (UBC818) to produce reproducibility bands. Bands with same mobility were considered as identical irrespective of their band intensity. Very weak band with negligible intensity and smear bands were both excluded from the final analysis. In Bantala a total of 15 fragments were scored with an average of 5 bands per primer. The size of scored bands ranged from above 1.5 to 0.3 Kbp. In Patakapura a total of 24 fragments were scored with an average of 8 bands per primer and ranged from 1.5 to 0.4Kbp. Genetic similarity among the varieties was estimated using similarity coefficient matrix based on ISSR bands scored.

Analysis of genetic fidelity of in vitro raised plantlets

The quality of in vitro derived regenerates was screened with ten ISSR primers that have showed monomorphic among the plantlets. The banding pattern of PCR amplified product from micro propagated plantlets was found to be monomorphic for most of the primer tested.

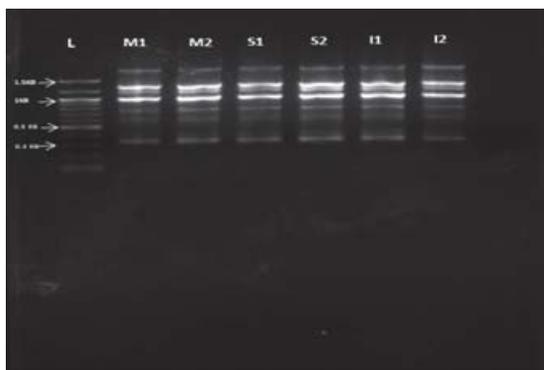


Fig 7. Gel showing the homogenous amplification pattern of Bantala M1 & M2-Mother plant, S1 & S2-secondary hardening and I1 & I2- tissue culture raised plantlets

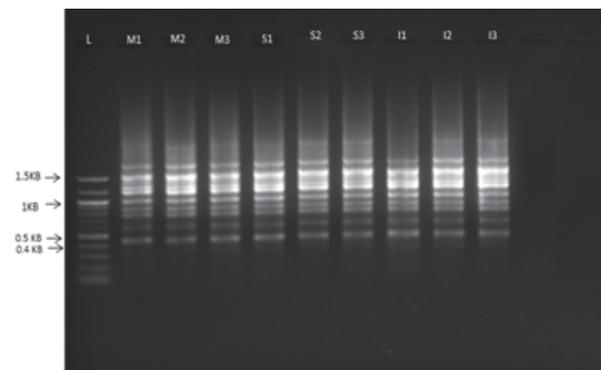


Fig 8. Gel showing the homogenous amplification pattern of Patakapura M1, M2&M3-Mother plant, S1, S2&S3-secondary hardening and I1, I2&I3- tissue culture raised plantlets

In 'Bantala' and 'Patakapura', no polymorphism was detected in the Profile. The identical ISSR banding pattern of in vitro, secondary hardening raised plantlets and their mother control plant have shown in Fig 7 and Fig 8. This shows there is no genetically differences between all the varieties.

Standardization of various macropropagation methods of useful wild edible fruits of Odisha

PI: Dr. Uday Chand Basak, Senior Scientist

Propagation trial has been initiated with 10 no of target species viz. *Antidesma ghaesembilla*, *Carissa carandas*, *Carissa spinarum*, *Ehretia microphylla*, *Cordia dichotoma*, *Eugenia roxburghii*, *Polyalthia suberosa*, *Glycosmis pentaphylla*, *Toddalia asiatica*, *Ziziphus oenoplia*. Out of 10, 9 species were propagated following different methods of vegetative propagation like girdled stem cuttings (black taping), micro stem cutting, juvenile stump cutting, root cuttings and wildling. So far, all together more than 600 saplings have been raised and studied for their hardening and adaptation for suitable domestication.



Saplings of *Glycosmis pentaphylla* and *Eugenia roxburghii*

Evaluation and characterization of pectin in selected wild edible fruits of Odisha.

PI: Dr. Uday Chand Basak, Senior Scientist

Optimization and characterization of pectin in target wild edible fruit species viz. *Ficus auriculata*, *Citrus medica*, *Limonia acidissima*, *Phyllanthus embelica*, *Diospyros malabarica* and *Syzygium cumini* have been carried out. Pectin yield ranged from 5% to 27%. The highest pectin yield was recorded in *Citrus medica* i.e 27% where as lowest pectin yield was recorded in *Limonia acidissima* i.e 5%. Further analysis of pectin components with reference to galacturonic acid and uronic acid are being carried out using advance instrumentation technique i.e. through FTIR spectroscopy.



Pectin from *Citrus medica* (Bada limbu); *Diospyros malabarica* (Kendu); *Ficus auriculata* (Raja dimiri)

Re-introduction of rare and endangered mangrove spp. *Heritiera fomes* and *H. littoralis* in protected areas of Odisha coast.

PI: Dr. Uday Chand Basak, Senior Scientist

To create and enhance awareness amongst stakeholders on mangrove biodiversity conservation, promote social, ecological & economical benefits of the state, very important rare and endangered mangrove species *Heritiera fomes* (sundari) and *Heritiera littoralis* (dhala sundari) are being propagated, hardened for re-introduce in protected coastal areas of Odisha. So far, more than 1500 saplings of *H. fomes* and 100 saplings of *H. littoralis* have been raised and studied (morpho-physico-chemical) for their hardening process against varied salt-stress under captive shade-net house condition. Currently, hardened saplings are being subjected to re-introduce in different suitable sites of Bhitarkanika for their adaptation studies.



Comparative growth analysis of *Heritiera* spp. grown in RPRC and Bhitarkanika before re-introduction



Chief Executive, RPRC monitoring re-introduction work of *Heritiera fomes* undertaken by RPRC in Balijoda Site at Bhitarkanika (February, 2021)

Assessment of antifungal activity of *Indigofera astragalina* and *Stachytarpheta indica* solvent extracts against *Aspergillus flavus*

PI: Dr. Sunita Bhatnagar, Senior Scientist

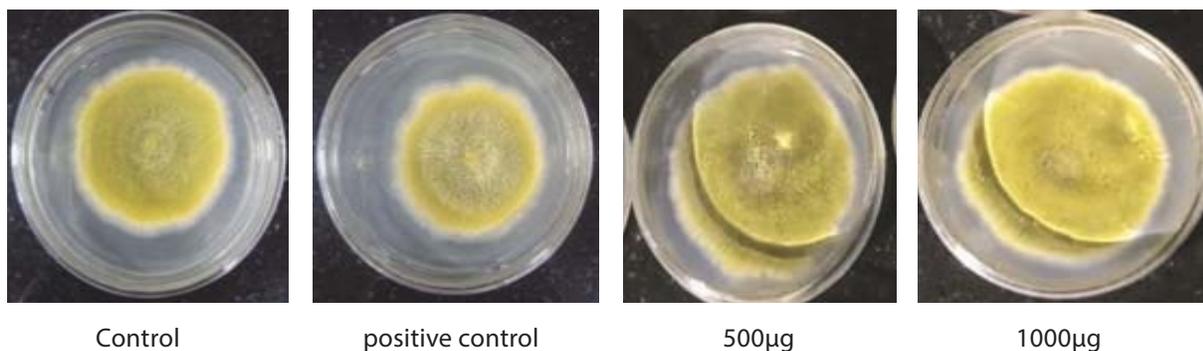
Evaluation of antifungal activity of solvent extracts of *Indigofera astragalina* and *Stachytarpheta indica* leaves against *Aspergillus flavus* has been carried out. Antifungal activity of *Indigofera astragalina* and *Stachytarpheta indica* against *Aspergillus flavus* was tested using three methods, radical growth method, Agar diffusion method and biomass reduction method. Four solvent extracts of leaves were prepared using soxlet extraction method. Same methods were used for all the activities.

Indigofera astragalina

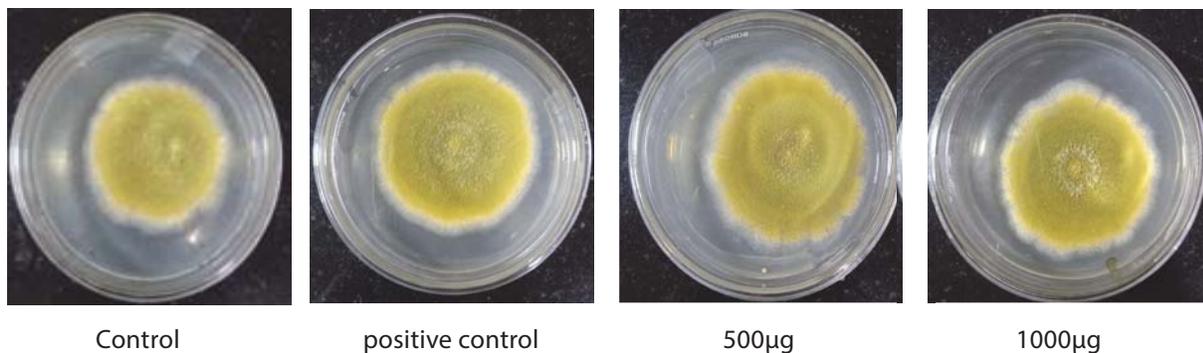
A. Radical growth method

Extracts did not show any inhibitory activity instead they supported the growth of fungus when compared with the controls.

Hexane extract (DAY9)



Chloroform extract (DAY 9)



B. Biomass reduction after radical growth of solvent extracts of *Indigofera astragalina*

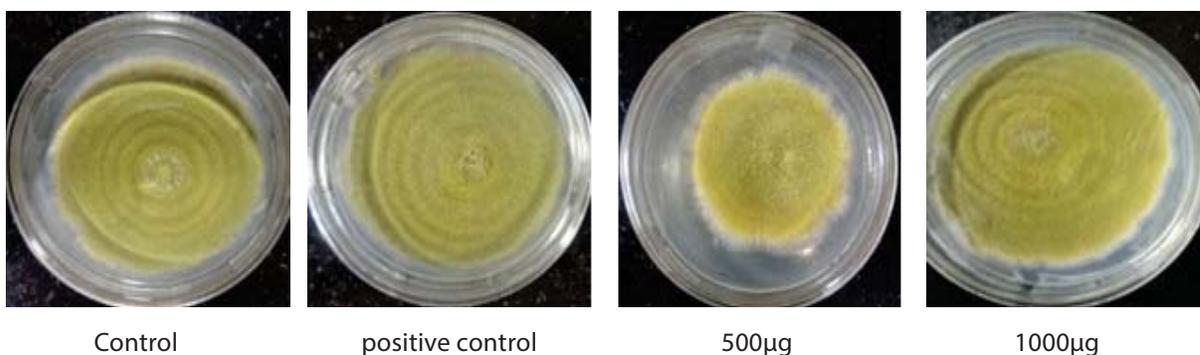
Although there was no inhibition of *Aspergillus flavus* in any of the extracts but except for methanol extract all the extracts exhibited biomass reduction (Table 1).

Sl. No	Dose (microgram/ml)	Hexane	Chloroform	Ethyl acetate	Methanol
1	500	40.6	30.81	43.34	No Inhibition
2	1000	ND	47.615	ND	No Inhibition

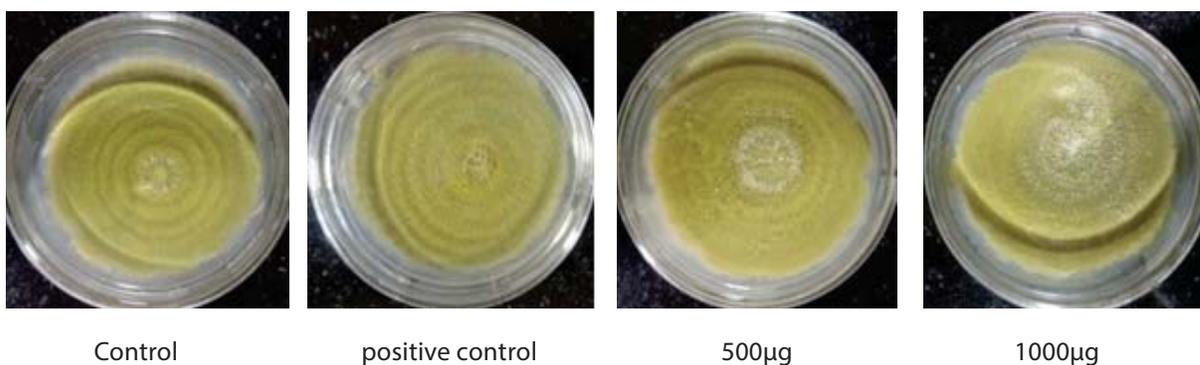
Stachytarpheta indica

Amongst all the extracts only chloroform extract showed mild activity against *Aspergillus flavus*. Whereas methanol extract supported better growth of fungus as compared to control samples. Here as lower doses were fully inactive so a higher dose of 2000 microgram was tested.

Chloroform extract (DAY 9)



Chloroform extract (DAY 9)



B. Biomass reduction after radical growth of solvent extracts of *Stachytarpheta indica*

Although there was no inhibition in the growth of *Aspergillus flavus* in any of the extract but all the extracts showed mild reduction in the biomass when compared with the control samples.

Sl. No	Dose (microgram/ml)	Hexane	Chloroform	Ethyl acetate	Methanol
1	1000	20.32	5.04	12.10	11.23
2	2000	NIL	16.88	NIL	19.59

C. Zone of inhibition. No zone of inhibition was obtained in any of the samples.

Selection of suitable protocols for propagation of vulnerable medicinal plants *Mesua ferrea* and *Paederia foetida* & their biological evaluation.

PI: Dr. Sunita Bhatnagar, Senior Scientist

Vegetative propagation of above mentioned Medicinal plants and their biological evaluation were conducted.

Vegetative propagation

Three methods of vegetative propagation of the medicinal plants was attempted.

A) Air Layering: - In this method a thick branch was selected and a ring of bark was removed just below the node, moist moss was wrapped around with the help of plastic sheet and two ends were tied using thread. This leads to development of roots in the wounded nodal area. After sufficient growth of roots, branch was removed from the main plant and transferred to the poly bags and later to the field.

B) Stem Cuttings: - Stems of all were semi hard, hence after application of rootex (root hormone) stems were placed in plastic trays with a mixture of soil and Farm yard manure (1:1).

C) Root cuttings: - 2-6 Inch root cuttings were taken and were placed directly in polybags consisting of soil and Farm yard manure(1:1) . This was done in shade with the development of new shoots, plants were planted into pots.

Achievements

Mesua ferrea

Conventional method of seed germination is best in *Mesua ferrea*, but the seeds viability is a big problem. Air layering method of propagation was successful but rate of germination is very low.

Around 268 numbers air layerings were done, out of which only 6 numbers successfully gave rise to roots and same were planted first in polythene bags and later after one month were transferred to the medicinal germplasm garden.



Air layering of Mesua ferrea plants.



Transfer to polypacks



Well developed plant from air layering

Propagation and reintroduction of endangered species of Odisha

PI: Dr. C. Kalidass, Scientist

During 2020-21, the present investigation, mass propagation of endangered species of the Eastern Ghats of Odisha, such as *Oroxylum indicum* L., *Cordia macleodii* Hook.f. & Thoms and *Symplocos racemosa* Roxb and are selected for the conservation study. These endangered medicinal plant species have getting been reduced significantly and hence is an urgent need for to evolve conservation and management strategies.

The *Oroxylum indicum* seeds were collected from different regions of Odisha and also was conducted the various methodologies to raise in the aspect of conserve the endangered plant. This seed materials were treated with cold and hot water for different time intervals and also sown the seeds were without treat in the sand medium, in poly house condition. The results showed an increase in the seed germination percentage was high rate, which is compared with the stem cuttings. The stem cutting was treating with different auxins concentrations such as IAA, IBA and NAA. The experiment showed the response after 50 -60 days with new sprouting in the axillary buds, and number of response is very low. The 1st phase experiment on seed germination of *Oroxylum indicum* has been completed and mass propagated around 650 seedlings out of 1000 seeds and the rooted plants were then transferred to poly bags containing soil, sand and composite mixture with 1:1:1 ratio (Fig.1 & 2), which is kept for reintroduction programme in future. In addition the 2nd batch 2000 seeds were sowed in the sand bed for germination of seeds with different physical and chemical treatments and data being regularly recorded for ascertaining percentage of seed germination and the experiment will be continued.

Cordia macleodii Hook.f. & Thoms is an important ethno medicinal plant of India. It is found in the moist and dry deciduous forests of peninsular India of Odisha, Madhya Pradesh, Chhattisgarh, Tamil Nadu. This plant is used as an ethno-medicinal for various treatment like healing wounds, mouth sores, jaundice and also as an aphrodisiac by the indigenous people where it is found. Its pharmacological activities include anti-microbial, wound healing, anti-oxidant and hepatoprotective, anti-venomous, analgesic and anti-inflammatory activity. Many photochemical like quercetin, p-hydroxyphenylacetic acid and phytosterols such as β -sitosterol, stigmasterol, camphesterol and cholest-5-EN-3OL (3β)-carbonyl chlorinated are isolated from this plant. Techniques of propagation of the selected endangered medicinal plant through the conventional vegetative propagation of *Cordia macleodi*, such defoliated stem cutting & air-layering method were taken up using various concentration of plant growth hormone (IBA & NAA) for root induction of the same. The basal ends of the explants were treated with various concentrations (1000 – 3000 ppm) of IAA, IBA, NAA and combinations of IBA & NAA. The treated cuttings were planted in sand beds in mist house. The application of 1000 ppm IAA was found to be the most suitable root promoting substance producing roots in 60% of stem cuttings, this experiment to be continued for desirable level (Fig. 3).

Symplocos racemosa Roxb., commonly known as Lodhra in Sanskrit has been given much importance due to its Pitta and Kapha doshas. It cleans wounds, arrests bleeding and initiates fast healing process of wounds. According to Ayurveda, it has been widely used to treat GI tract disorder, reduces fever, and cures spongy gums or bleeding and used to treat skin diseases as dropsy and liver complaints, anti-diarrheal property, diarrhoea. Pittaja arbuda and medoja arbuda tumors are treated with the bark of Lodhra in combination

with other drugs. *S. racemosa* in combination with *Curcuma domestica*, *Soymida febrifuga* and honey has been used as an external remedy for tumors. It is widely used as an Ayurvedic remedy mainly for Gynecological disorders, useful in abortions and miscarriages. Unani medicine uses it as an emmenagogue and aphrodisiac. It is a potent remedy for inflammation and cleaning of the uterus. This is also used to treat leucorrhoea and menorrhagia. These various healing properties made Lodhra a common name in Ayurveda. For air-layering propagation method, barks of healthy stems were girdled during the month of October-December in the Karanjia forest region i.e. Patabil, Kendumundi and the exposed portion of the stem was treated with different concentrations and combinations of IBA (3000 ppm). The ringed surface was covered with moistened moss and wrapped with transparent polythene strips and tied at both the ends. After 60-75 days the rooted branches were cut from the lower end and transferred to poly bags in nursery (Fig. 4). Defoliated stem cuttings measuring 10-15 cm were collected from healthy trees. The basal ends of the explants were treated with rootex. The treated cuttings were planted in sand beds in mist house. The application was found to be the one of the most suitable root promoting substance producing roots in of stem cuttings. Root stump cutting is one of the most effective methods in propagation of this plant. Root of 6 month to 1 year old *Symplocos racemosa* was collected from Karanjia forest division. The collected root segment was then cut into few segments and treated with organic rooting powder and planted in sand beds in mist house.





Fig. 2. Mass propagation and reintroduction of endangered plant (*Oroxylum indicum* L.) of Odisha.



Fig. 3. A) Stem cuttings of *Cordia macleodii*, B) Stem cuttings treated with Growth regulators (IAA, IBA, NAA), C) Stem cuttings treated with Rootex, D) Callus Formation , E) & F) Leaf proliferation



Fig. 3. Vegetative propagation of planting materials of *Cordia macleodii* Hook.f. & Thomson



Fig. 2. A) Stem cuttings of *S. racemosa*, B) & C) Growth in stem cuttings, D) Prominent leaf growth in stem cutting, E) Root cuttings of *S. racemosa*, f) Sapling growth from root cuttings.

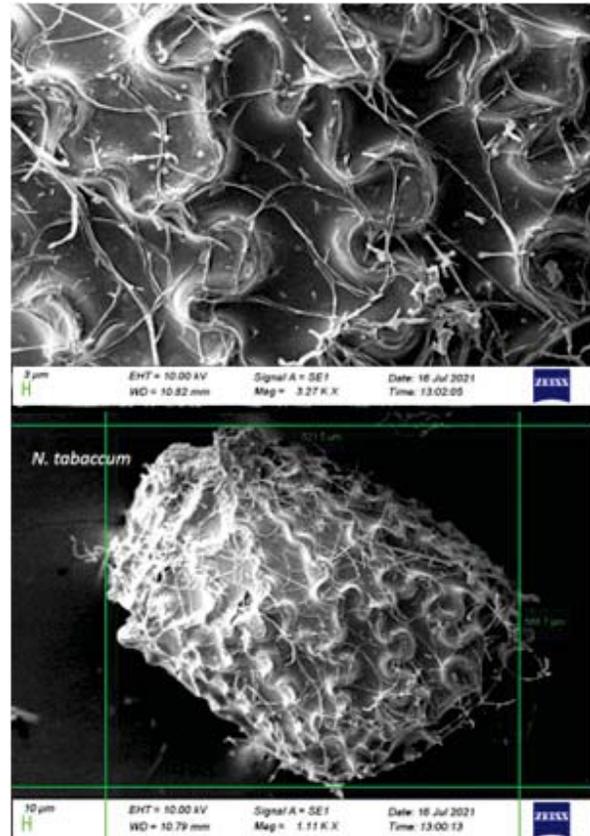
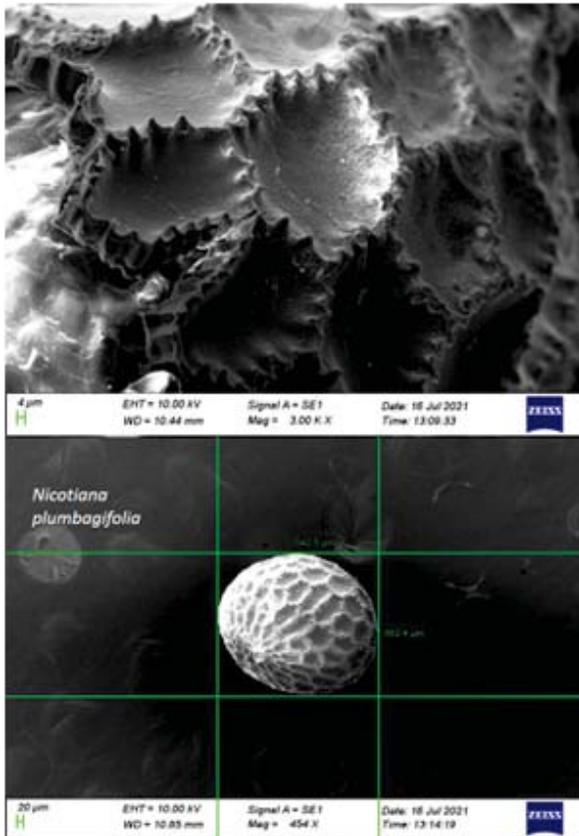
Taxonomic revision of the genus *Physalis*, *Datura*, *Capsicum* and *Nicotiana* (Solanaceae) in Eastern Ghats of India

PI: Dr. C. Kalidass, Scientist

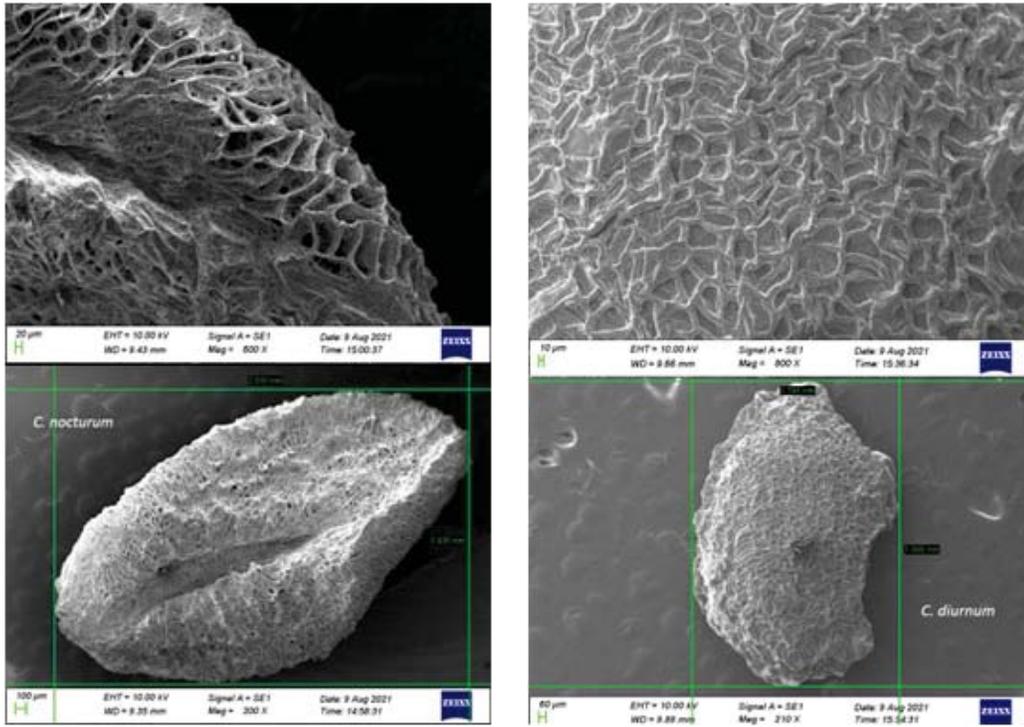
The present study on systematic, morphology, taxonomy and ethnobotany of *Physalis*, *Datura*, *Capsicum*, *Withania*, *Lycianthes*, *Nicotiana*, *Nicandra* and *Cestrum* from the Eastern Ghats were carried out on the basis of fresh samples collected from the field trips and analysis of existing relevant herbarium specimens during 2020-21. The first-hand information of habitat, description, ecology, phenology and medicinal values has been recorded in different field from major hills of Odisha. We have collected seed materials from various species in different localities of Eastern Ghats, India for SEM (Scanning Electron Microscopic) studies. In addition, herbarium consultation tour were conducted to National herbaria of BSI, CNH, Howrah (CAL) and which checked and identity of 464 sheets for *Physalis* species, 279 sheets for *Datura* species, 190 sheets for *Withania somnifera*, 75 sheets for *Lycianthes*, 430 sheets for *Cestrum* species and additionally virtual materials of Royal Botanic Gardens, Kew (K) and NYBG through the online sources also consulted for confirmation. So far, *Lycianthes neesiana*, *Withania coagulance* and *P. angulata* var. *pendula*, newly reported from Odisha as well as Eastern Ghats of India and also described two more new species from the genus *Cestrum*. Finally, a key prepared to the genus of Solanaceae with following the description for different genus of Solanaceae such as *Physalis*, *Datura*, *Capsicum*, *Withania*, *Brugmansia*, *Lycianthes*, *Nicotiana*, *Nicandra* and *Cestrum* including various level of taxonomic treatment with the photo plates for the following species *Physalis angulata*, *P. prinuosa*, *P. peruviana*, *P. angulata* var. *pendula*, *Datura metal*, *D. stramonium*, *D. innoxia*, *Cestrum diurnum*, *Cestrum nocturnum*, *Capsicum annum*, *C. frutescence*, *Withania somnifera*, *Withania coagulance*, have been completed with herbaria were prepared and deposited at RPRC herbarium. In addition, ethno-medicinal values of all species of Solanaceae of Odisha have been completed. The seeds of one genus belonging *Physalis* were studied by Scanning Electron Microscope. Micro and macro-morphological characters including the seed shape, colour, size, seed shape pattern were studied and their data are presented here. The seeds were distinguished on the basis of seed topography, presence of pores and hirsutous nature. Special emphasis was given to the primary sculpture, preclinical walls and secondary sculpture. SEM morphology of seed (Scanning Electron Microscope) and systematics of Solanaceae of Eastern Ghats of India has not been done so far. Moreover we will be completing the Scanning Electron Microscope study of the rest of the genus. Least all other species are to be collected and described in the upcoming time duration. Additional to that the ethno botany of all the species for the genus Solanaceae will be discussed.



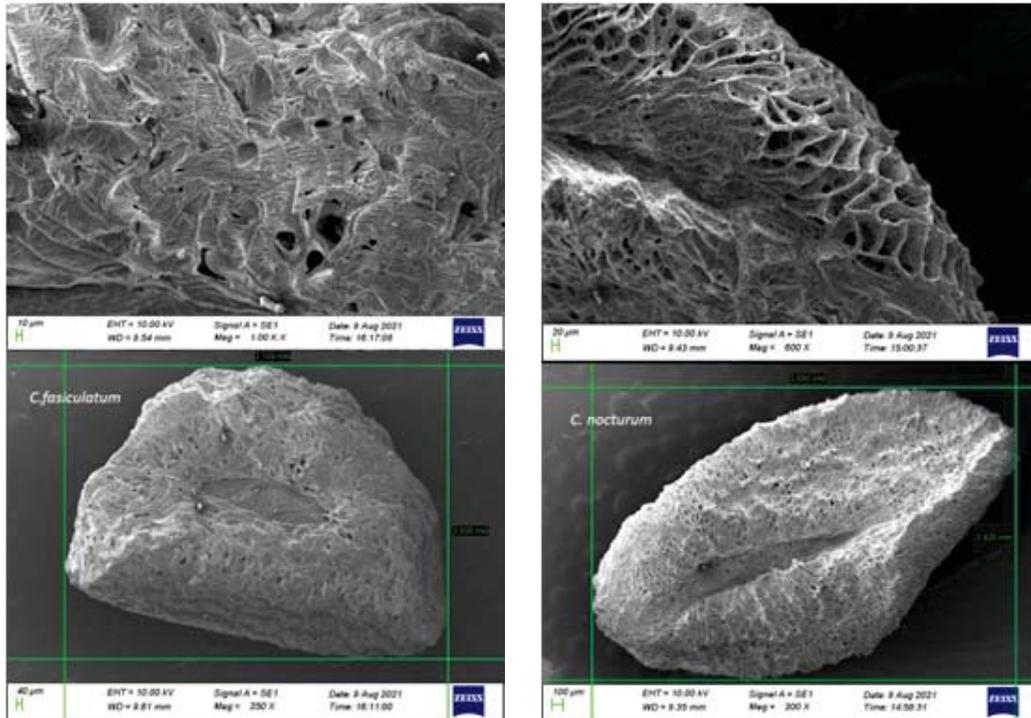
Brugmansia suaveolens (Humb. & Bonpl. Ex Willd.) Bercht. & Presl. & *Nicandra physalodes* (L.) Gaertn.



Nicotiana tabacum - Seeds obovoid or ellipsoid, lateral slight flattish, apex blunt rounded, base narrowed with hilum in one of the prolonged side.; *Nicotiana plumbaginifolia*- Seeds trigonous to ellipsoid or rhomboid. Divided into different feildlets Surface reticulate, tuberculate, hardly lustrous.



Cestrum auranticum-Seeds obovoid to reniform, laterl flattish, apex blunt rounded-off, base narrowed. & *Cestrum diurnum*- Seeds ovoid to rhomboidal or orbicular, flattish, Surface reticulate- foveate, lustourous, brown or greyish brown.



Cestrum fasciculatum- Seeds reniform to orbicular, lateral flattish, dorsal side bluntly rounded off, surface reticulate to foveate. Lustourous , border part pale yellow to yellowish brown. & *Cestrum nocturum*- Seeds reniform or obovate, lateral flattish, base stump, surface reticulate foveate, lustrous, greyish black.

RESEARCH PROJECTS

EXTERNALLY FUNDED PROJECTS

Sl. No	Title	PI	Funding	Period
1	Harnessing the potential of endophytes against root knot nematode <i>Meloidogyne icognita</i> in banana	Dr. N.Gupta Principal Scientist	DBT,MS&T, GOI	2018-2021
2	Establishment of mass propagation and breeding facility for orchids	Dr. N. R. Nayak Senior Scientist	RKVY, GOI	2018-2021
3	Establishment of tissue culture based mass propagation facility of banana and plantains	Dr. N. R. Nayak Senior Scientist	RKVY, GOI	2018-2021
4	Harnessing the potential of traditional knowledge based medicinal plants for identifying SARS CoV2 compounds	Dr. N. R. Nayak Senior Scientist	DBT,MS&T, GOI	2020-2021
5	Omics'- approach to regulate ripening and enhance fruit shelf life in banana: an important fruit crop for food security.	Dr. G.K.Surabhi Senior Scientist	RKVY, GOI	2017-2021
6	Mass Propagation of Local Musa Varieties of Odisha, Commercialization using Tissue Culture Techniques.	Dr. B. Deo Senior Scientist	RKVY, GOI	2017-2021
7	Evaluation of unexplored <i>Ardisia solanacea</i> and <i>Aegiceras corniculatum</i> plants of Myrsinaceae family as embelin and other related compounds producing substitutes for overexploited RET medicinal species <i>Embelia ribes</i> & <i>E. tsjeriam-cottam</i>	Dr. U.C. Basak Senior Scientist	NMPB, GOI	2016-2020
8	Conservation of salt-sensitive back mangroves <i>Heritiera fomes</i> and <i>H. littoralis</i> through re- introduction in protected area: application of vegetative propagation technique	Dr. U.C. Basak Senior Scientist	DBT, MS&T, GOI	2018-2021

STATE PLAN FUNDED PROJECTS (2020-21)

Sl. No	Title	PI	Funding	Period
1.	Development of bioinoculant for growth and development of <i>Dalbergia latifolia</i> and <i>Desmodium oogeinense</i> by using native microflora	Dr. N. Gupta Principal Scientist	F & E Dept. GoO	2020-21
2.	Morphoraxonomic characterization and documentation of fungi of Odisha	Dr. N. Gupta Principal Scientist	F & E Dept. GoO	2020-21
3	Production, purification and evaluation of anticancer properties of extracellular secondary metabolite from <i>Penicillium</i> sp.	Dr. N. Gupta, Principal Scientist	F & E Dept. GoO	2020-21
4	Mass production of selected medicinal plants of Odisha through tissue culture.	Dr. N.R. Nayak Senior Scientist	F & E Dept. GoO	2020-21
5	Developing micropropagation methods for <i>Pterocarpus Santalinus</i> , <i>Anogeissus latifolia</i> <i>Santalum album</i> , and <i>Desmodium oogeinense</i> , some endangered forest trees species of Odisha	Dr. G.K. Surabhi Senior Scientist	F & E Dept. GoO	2020-21

6	Deciphering molecular regulation of fruit ripening in banana through quantitative proteome analysis following advanced (iTRAQ) isobaric tags for relative and absolute quantitation.	Dr. G.K. Surabhi
Senior Scientist	F & E Dept. GoO	2020-21
7	Strategic approach to establish the lead findings from <i>Geophila repens</i> as cholinesterase inhibitor for the treatment of Alzheimer's disease.	Dr. A. K. Sahoo
Senior Scientist	F & E Dept. GoO	2020-21
8	Therapeutic validation of <i>Pterocarpus marsupium</i> and <i>Piper trioicum</i> as a cholinesterase inhibitor for the treatment of Alzheimer's disease	Dr. A. K. Sahoo,
Senior Scientist	F & E Dept. GoO	2020-21
9	Nutritional analysis of different indigenous banana (<i>Musa</i>) varieties grown in Odisha	Dr. B. Deo
Senior Scientist	F & E Dept. GoO	2020-21
10	Control of lethal browning with antioxidants during in vitro culture of banana	Dr. B. Deo
Senior Scientist	F & E Dept. GoO	2020-21
11	Standardization of various micropropagation methods of useful wild edible fruits of Odisha	Dr. U. C. Basak
Senior Scientist	F & E Dept. GoO	2020-21
12	Evaluation and characterization of specific nutritional properties in selected wild edible fruits of Odisha	Dr. U. C. Basak
Senior Scientist	F & E Dept. GoO	2020-21
13	Assessment of antifungal activity of <i>Indigofera astragalina</i> and <i>Stachytarpheta indica</i> solvent extract against <i>Aspergillus flavus</i>	Dr. S. Bhatnagar
Senior Scientist	F & E Dept. GoO	2020-21
14	Selection of suitable protocols for propagation of the vulnerable medicinal plant <i>Mesua ferrea</i> , <i>Paederia foetida</i> and <i>Rauwolfia serpentina</i> and their biological evaluation.	Dr. S. Bhatnagar
Senior Scientist	F & E Dept. GoO	2020-21
15	Taxonomic revision of the genus <i>Physalis</i> , <i>Datura</i> , <i>Capsicum</i> and <i>Nicotiana</i> (<i>Solanaceae</i>) in Eastern Ghats of India	Dr. C. Kalidass
Scientist	F & E Dept. GoO	2020-21
16	Propagation and reintroduction of endangered species of Odisha	Dr. C. Kalidass
Scientist	F & E Dept. GoO	2020-21

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Dash, S., Gupta, N. (2021). Bioinoculation Response on Growth of *Adenanthera Pavonina* Seedlings Under Nursery Conditions. *Tropical Agroecosystem*, 2(1): 44-51.

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BOOK

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Sunita Bhatnagar(2021) Forest canopy and medicinal plants. Published by Regional Plant Resource Centre, Bhubaneswar.

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BOOK CHAPTER

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REPORT

Basak U.C and Mekro P. (2021). Research and Activity Report 2019-20. Published by Regional Plant Resource Centre, Bhubaneswar.

TRAINING & EDUCATION

Regional Plant Resource Centre provides short term training to the students of M.Sc. / B.Tech. / M/Tech. and other courses every year from January to June for a duration of 6 months. Training is imparted on various subjects pertaining to the "Advance Plant Biotechnology". The students have to submit their CV along with the forwarding letter from their Institutes head or the Project head. The applications are to be received by end of November every year. Students enroll themselves by end of December and the training starts from January (6 months). Students are allotted to various Scientists and have to work under them for completing their Thesis. Training/course completion Certificate is issued at the end of the scheduled course period in favour of the student trainee.

Detailed account of the Training provided in the Year 2021

Sl.No.	Name of the candidate/ Univ.	Title of the Dissertation	Guide	Year
1	Ms. Alora Sahoo SOA University, Bhubaneswar	Production of organic acid and antifungal bioactive compound from bacteria	Dr. N. Gupta, Pr. Scientist	2021
2	Mr. Soumya Ranjan Rout SOA University, Bhubaneswar	Development of inter simple sequence repeat (ISSR) molecular marker profiles of <i>Dendrobium regium</i> (Orchidaceae)	Dr. N. R. Nayak Sr. Scientist	2021
3	Ms. Puja Samal SOA University, Bhubaneswar	Development of inter simple sequence repeat (ISSR) molecular marker profiles of <i>Dendrobium 'Sonia'</i> (Orchidaceae)	Dr. N. R. Nayak Sr. Scientist	2021
4	Mr. Siddharth Satapathy SOA University, Bhubaneswar	In vitro micropropagation of banana through tissue culture technique.	Dr. B. Deo, Sr. Scientist	2021
5	Mr. Malay Kumar Murmu SOA University, Bhubaneswar	Influence of plant growth regulators on in vitro multiplication in <i>Musa</i> spp.	Dr. C. Kalidass Sr. Scientist	2021

LIBRARY

The library of the centre has collection of books on the thrust areas of Taxonomy, Biotechnology, Medicinal and Aromatic Plants, Tissue Culture, Microbiology, Physiology and Biochemistry, Forestry and Ecology, Molecular Biology, Horticulture and Floriculture, Ornamental Plants, Orchids and many other areas. Number of periodicals and journals of leading institutions and firms on related areas of importance are subscribed by the library. Several Indian Journals of repute are included as annual subscription.

HERBARIUM

The Centre has a modern Herbarium with a collection of 14,000 accessions belonging to 1600 species. The herbarium specimens have been digitized and made available to researchers as well as scientific communities through a web-based application.

EX-SITU CONSERVATION & GERmplasm COLLECTION

Germplasm collection and ex-situ conservation of various plant groups are major activities of RPRC. It has rich living collections of different plant groups like cacti and other succulents, wild and exotic orchids, species with fragrant flowers, endangered and threatened plants, medicinal plants, mangroves, palms, bamboos, wild edible fruit plants, cultivars of Hibiscus and Roses. These have been introduced to the living collection division and are being studied.

The gist of some notable ex-situ collections established in RPRC are mentioned below.

Wild Edible Fruits Garden

With a view to create awareness among various stake holders including foresters, plant lovers, researchers and general citizen about various wild edible fruits occurring in Odisha, RPRC has created an ex situ conservation garden housing more than 100 species of fruits, nuts & berries. Since plant conservation and research are major objectives of the centre, this germplasm collection is also meant to provide important wild fruit bioresource for undertaking research on propagation, cultivation, analysis and utilization.



RET Corner: Conserved more than 35 RET species like *Lasiococca comberi*, *Hildegardia populifolia*, *Cycas sphaerica*, *Homalium tomentosum*, *Hypericum gaitii*, *Cordia macleodii*, *Gnetum ula*, *Homalium tomentosum* etc.



Jagannath Vatika: A special garden housing 125 species of plants used in different rituals of Lord Jagannath and the plants are grouped under 7 categorized such as -1.Construction of chariots and special carts to transport the holy logs of Neem for making the idols.2.Selection of holy Neem plants for making the idols and special characteristics of the site.3.Making a special floral crown "Tahia" for the deities.4.Preparation of "Dasamula"- an Ayurvedic drug.5.Preparation of fragrant herbal oil called "Phuluri"6. Leaves and flowers used in different attires, rituals and festivals and 7.Fruits and seeds used in daily rituals, attires and special occasions.



Fragrance Flower Garden: "Garden of Species with Fragrant Flowers" of around 70 species like *Magnolia coco*, *Cananga odorata*, *Gardenia jasminoides*, *Murraya paniculata* etc.



Rose Garden: The Centre has collection of around 1000 varieties of roses. The available varieties include Alec's Red, Black Lady, Double delight, French perfume, Nurjahan, Surkhab, Tiara etc,

Palmetum: Representing nearly 60 species of palms including *Archontophoenix alexandrae*, *Calamus* spp, *Corypha umbraculifera*, *Dypsis lutescens*, *Livistonia chinensis*, *Ravenea rivularis* etc.



Bambusetum

Having collection of around 30 species of bamboos like *Arundinaria chino*, *Arundo donax*, *Bambusa balcooa*, *Dinochloa maclellandii*, *Melocanna baccifera*, *Phyllostachys nigra*, *Pseudosasa japonica* etc.



Hibiscus Garden: The garden has a collection of 52 varieties of Hibiscus species



Medicinal Plant Garden:

This germplasm garden houses 250 species of medicinal plants collected from all over the country.

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This germplasm garden houses 250 species of medicinal plants collected from all over the country.



Orchidarium: Having germplasm collection of nearly 100 species of Orchids. Some species/hybrid orchids are also displayed in orchadarium in botanical garden & many hybrid orchids are available for sale.



ESTABLISHMENT OF ORCHID RESEARCH LABORATORY

Research laboratory has been established that will carry out different research activities related to orchids. The laboratory is equipped with modern equipment like Real-Time PCR for the detection of orchid viruses. In addition, this will also assist in analyzing the gene expressions patterns under different conditions. Polymerase Chain Reaction equipment installed to develop the molecular markers required for the production of quality planting materials. Other equipment like Gel-Documentation Unit, Gel Electrophoresis Units, Cold Centrifuges, Spectrophotometer, Incubated Shakers, Microscope, Laminar Air Flows have been installed in the laboratory.



ESTABLISHMENT OF OMICS RESEARCH LABORATORY



ESTABLISHMENT OF ORCHID PROPAGATION FACILITY

As the tissue culture technology is the only viable method for the production of the planting materials of orchids, one facility has been developed for the production of planting materials of Dendrobium, Vanda, Phalaenopsis, Cymbidium and other orchids with a capacity of 20,000 plants per year. The facility have dedicated media preparation room, media sterilization room, inoculation room and plant growth rooms. The facility is equipped with the Autoclaves, Laminar Air Flows, Plant Growth Chambers and other small equipment.



Media Preparation Room



Sterilization Room



Inoculation Room



Growth Room

ESTABLISHMENT OF VISITORS SHED IN BOTANICAL GARDEN



FLOWER SHOW 2021

The Regional Plant Resource Centre (RPRC), in collaboration with Plant Lovers' Association (PLA), Bhubaneswar organised the Annual Flower Show in the premises of the Botanic Garden of RPRC (Ekamrakanan) on 23rd and 24th January, 2021 with the support of Odisha Mining Corporation Ltd. and the Directorate of Horticulture, Odisha.

On this occasion, Dr. Mona Sharma, IAS, Additional Chief Secretary, Forest & Environment Department, Government of Odisha inaugurated the Annual Flower Show, 2021 as Chief Guest and visited various floral displays. She visited the Exhibition Stall and Floral arrangements made by Regional Plant Resource Centre, Bhubaneswar and released the Research and Activity Report 2019-20 and other two books brought out by the Institute. Dr. Sandeep Tripathi, IFS, Principal Chief Conservator of Forests and HoFF, Odisha attended the function as Guest of Honour. Smt. Pusazhule Mekro, IFS, Chief Executive, RPRC, Sri Khirod Pattnaik, President, Plant Lovers' Association and other dignitaries attended the function. The Scientists of RPRC, Administrative Officer and office bearers of RPRC and Plant Lovers Association were also present. An attractive "Floral Gate" was erected at the entry point of the flower show premises, which has become a major attraction of the event.

A total 10 organizations/ firms/ nurseries have participated and exhibited ornamental plants for display. A "Orchid" section has been introduced comprising of Cattleya, Dendrobium, and Vanda where 20 attractive varieties are on display.



Floral Gate of RPRC Annual Flower Show 2021; Incoming of Chief Guests (Dr. Mona Sharma, IAS) accompanied by Chief Executive, RPRC



Flower Arrangement Display by OMC-ODISHA; Visits of Various Flower Display by the Chief Guest

The Plant Lovers' Association also organised "Garden Competition" in the Capital city of Bhubaneswar to encourage the residents for raising Gardens and growing plants to add to the beauty of the City. The winners of Garden are being awarded with prizes and trophies in the valedictory function held on 24th January, 2021. Shri Susanta Nanda, IFS, Director, Environment-cum-Special Secretary, Forest & Environment Department, Odisha and Shri Balwant Singh, IAS, Managing Director, Odisha Mining Corporation Ltd., Odisha were the Guests of the function and gave away prizes to the winners of Garden competitions. Shri Rohit Kumar Lenka, IFS, Director of Horticulture, Department of Agriculture and Farmers Empowerment Department, Odisha was the Guest of Honour.

In the wake of the pandemic Covid-19, this year, entry to the Botanic Garden was restricted to a permissible number for the visitors as allowed by the Municipal Corporation of Bhubaneswar.

The scientists, staff, students and workers of RPRC; the office bearers and members of PLA had put their best efforts to make this Annual Flower Show, 2021 a continuity. The financial assistance from OMC and Directorate of Horticulture, Odisha and help and assistance from different participating institutions and individuals have were gratefully acknowledged.



Inauguration of Visitors' Shed at Botanic Garden; Visits of RPRC Stall by the Chief Guest (right most)



Media Addressing by the Chief Guest during Inauguration of the Flower Show; COVID 19 Floristic Art displayed at the Flower Show



Prize Distribution Ceremony during valedictory session of Flower Show at RPRC

Inauguration of Research Laboratory and Nursery Complex



Inauguration of new Tissue Culture Laboratory (Lab. No.3)



Inauguration of New Nursery of RPRC; Inauguration of Modern Nursery facility



Visit of Special Nursery Facilities ;Chief Guest addressing media Regarding Modern Nursery at RPRC



Visiting of Modern Nursery by Chief Guest Dr. Mona Sharma, IAS

Inauguration of Research Laboratory and Nursery Complex



Release of Annual Research and Activity Report 2019-20 of RPRC by Dr. Mona Sharma, IAS, Chief Guest DURING Flower show 2021; Release of Books published by RPRC & Plant Lovers Association (PLA)



Book Released titled 'Natural Pectin from Edible Wild Fruits of Odisha' published by RPRC during World Forestry Day (21 March, 2021) by Hon'ble Minister, Forest, Environment & Climate Change Department, Govt. of Odisha in presence of Dr. Mona Sharma, IAA, Additional Chief Secretary, Forest, Environment & Climate Change Department, Govt. of Odisha.

OTHER MAJOR ACTIVITIES CARRIED OUT DURING 2020-21

- i. Strengthening of existing Model Nursery & Sale facilities along with provision of Toilet facilities for visitors
- ii. Development and strengthening of high-tech propagation facilities for production of Quality Planting Materials of ornamental plants like rose, hibiscus, foliages etc.
- iii. Strengthening of botanical garden amenities such as Visitor sheds.
- iv. Renovation of Old Cambridge House (Polyhouse)
- v. Establishment of display huts in the nursery
- vi. Strengthening of peripheral compound wall damaged during Cyclone Fani.



New initiatives under taken during 2021 in RPRC

Reintroduction of Mangrove (Sundari) in Devi River Mouth



Reintroduction of Mangrove (Sundari) in Bhitarkanika



Development and strengthening of high-tech propagation facilities, during 2021



Various innovative display during Flower Show 2021



REGIONAL PLANT RESOURCE CENTRE

NAYAPALLI, BHUBANESWAR-751015

BALANCE SHEET AS ON 31.03.2021

LIABILITIES	SCHEDULE	AMOUNT(RS.)	ASSETS	SCHEDULE	AMOUNT(RS.)
General Fund	1	10,76,73,321	Fixed Assets	5	9,49,01,036
Grant for Non-recurring Expenses	2	15,25,75,414	Work-in-Progress	6	3,69,86,813
Advance Received for Contract Work	3	72,20,370	Fund Transfer to opening new scheme account		45,65,034
Current Liabilities	4	81,49,626	TDS receivable		8,96,175
			<u>Current Assets</u>	7	9,81,18,946
			Loans & Advances		3,146
			Cash in Hand		4,01,47,581
			Cash at Bank		27,56,18,730
Total		27,56,18,730	Total		27,56,18,730

UDIN:22301929AAAAAAB7370

Place: Bhubaneswar

DATE:08/11/2021


 Chief Executive
 Regional Plant Resource Centre
 Bhubaneswar



Regional Plant
Resource Centre

www.rprcbbsr.in

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