



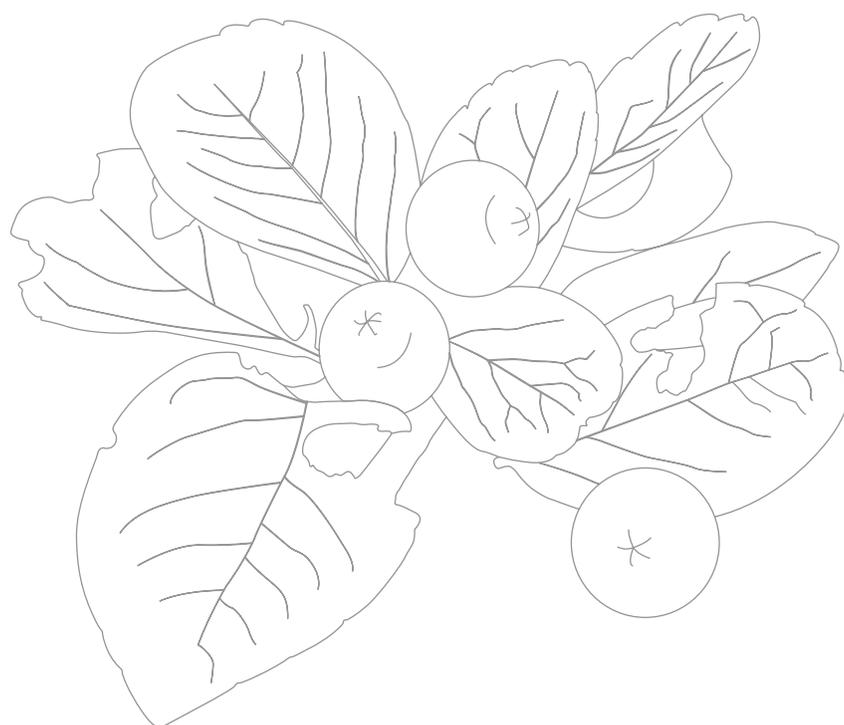
# RESEARCH AND ACTIVITY REPORT 2023-24



Regional Plant  
Resource Centre  
Bhubaneswar



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REGIONAL PLANT RESOURCE CENTRE, BHUBANESWAR

## **Research & Activity Report 2023-24**

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*Published by*

Chief Executive  
Regional Plant Resource Centre  
Nayapalli, Bhubaneswar-751015, Odisha, India

Phone : 0674-2557925  
Email : rprcbbsr@gmail.com  
Website : www.rprcbbsr.in

Printed: 2025

*Compiled & Edited by*

Dr. U. C. Basak  
Shri Suresh Pant

*Design and Print:*

Third Eye Communications  
A brand and unit owned by Ketaki Enterprises Pvt. Ltd.  
Bhubaneswar 751015, Odisha  
Email: info@thirdeyeco.in  
www.thirdeyeco.in

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*Flacourtia indica* (Baincha Koli)



**Shri Ganesh Ram Singhkhuntia**

Minister

Forest, Environment & Climate Change Department  
Government of Odisha



I am pleased to learn that RPRC, a renowned Research & Development Organization, has been engaged in various basic as well as applied research activities in the field of plant genetic resource conservation, biotechnology, biochemistry, microbiology, medicinal and aromatic plants, taxonomy, horticulture and floriculture. In addition to core research on plant biodiversity conservation, microbial applications, wild edible fruits, mangroves, orchids and phyto-chemicals of selected medicinal plants, few other aspects like production of economically important crops including tissue culture of banana, orchids are noteworthy as important thrust and frontier areas of plant sciences. It has consistently maintained and added to its unique 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 conservation. I wish that the outcomes of the work carried out in RPRC would find its way to benefit all stakeholders in general and Odisha state in particular.

I am hopeful that the findings of various research activities highlighted in this Research & Activity Report (2023-24) published by the center would be of immense help to academicians, biodiversity conservationists, students, teachers, farmers and researchers.

Best Wishes.

(Ganesh Ram Singhkhuntia)



*Polyalthia suberosa* (Gua Koli)



**Shri Satyabrata Sahu, 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, Govt. of Odisha which distinguished itself in the field fundamental and applied research in plant sciences. The centre has been implementing research and developmental 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 centre. Recent initiative on development of Breeding & Tissue Culture facilities, re-introduction of RET plants into field for conservation, is highly appreciable. The centre has made significant stride in initiating several innovative research projects for bioprospecting indigenous plants and microflora for wider use. Accreditation for Certification of T.C. Banana plantlets, Orchid breeding & propagation are significant achievements of RPRC. I also hope the Institute would continue to endeavor to find solutions to meet new challenges in conserving the biological diversity of the State.

I express my appreciation for bringing out this Research & Activity Report (2023-24) which would be of source of information and dissemination of the findings of various works being undertaken by the centre.

Best Wishes.

(Satyabrata Sahu)



*Suregada multiflora* (Khakada)



**Shri Suresh Pant, IFS**  
Chief Executive



It is my pleasure to bring out this Research & Activity Report 2023-24 which appraises implementation of various prioritized research programmes 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 for benefiting forest species and micro-propagation of plantation crops and endangered plants.

In order to implement various relevant research activities, Scientists of RPRC are provided with research funds from state Forest, Environment & Climate Change Department under state plan budget after rigorous screening & 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 state as per recommendation of the RAC. Besides, Scientists are implementing research projects funded by external funding agencies like DBT, NMPB, RKVY, Gol etc. All the cumulative research activities & achievements are being reviewed & evaluated by Scientific Advisory Committee (SAC) at regular interval to guide & advice the scientists of RPRC in their scientific endeavours.

The centre has been in the forefront for several years since its inception in encouraging and nurturing academic intellect by guiding Ph.D. and M.Sc. students. A six month Project training programme 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.

The scientific research groups, 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 Secretary, 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.

(Suresh Pant)



*Walsura trifoliata* (Mundica)

## 1 INTRODUCTION

Being an autonomous R&D institute of Forest, Environment & Climate Change Department, Govt. of Odisha, Regional Plant Resource Centre (RPRC), Bhubaneswar, has been implementing various R&D activities primarily through execution 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. Various research programmes have been undertaken with the financial support from Forest, Environment & Climate Change Department, Govt. of Odisha, RKVY, Science & Technology, 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 the state of Odisha.

The centre has prioritized research 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, useful secondary metabolites from fungi, micro-propagation of edible mushroom, forest species, plantation crops and endangered plants.

A total 3 external funded, 20 state plan funded projects have been implemented during the year 2023-24, engaged around 30 research fellows, published 20 research papers, 2 Books, 1 Research & Activity Report, trained 5 M.Sc. Biotech students for their PG degrees.



## 2 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 layout, 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.



**Mangroves**

### 3 EXECUTIVE SUMMARY

Highlights of various research activities implemented during 2023-24 in Regional Plant Resource Centre are summarized below:

#### **Microbiological Applications**

Bioactive lead molecule from fungal endophytes has been extracted, purified and characterized with Endophytic fungi having potential candidate for the production of various secondary metabolites to explore and investigate alternative avenues for searching novel bioactive molecules.

Bioprocess optimization for enhanced recovery of L-glutaminase free L-asparaginase of fungal origin has been undertaken. Determination of effect of different K, P, Mn, Mg, Ca, Fe salts, amino acids, organic acids, vitamins, hormones for improvement in enzyme production is in process. Lyophilised enzyme samples being subjected to analysis for evaluation of anticancer properties. The fungal strain has already been submitted for Molecular Identification.

Morphotaxonomic characterization of fungi of Odisha have been done for documentation of micro fungi in forest soils of Odisha. Till date 87 no. soil samples collected. Total 321 no. fungal isolates obtained. Segregated into 170 no. of fungal isolates & Plate culture of 162 no. of fungi completed. Slide culture of 152 no. of fungi completed

Extraction, purification and characterization of piperine content have been undertaken to harness the potential of endophytes of *Piper longum* as an alternate source for piperine production through optimization of protocol for laboratory production.

#### **Tissue Culture & its application on various important plant spp. (banana, orchids, medicinal & forest spp. & mushrooms)**

In vitro regeneration techniques of red banana has been standardized for establishment in Odisha climate condition. After micropropagation, hardening experimentation has been undertaken. Impact of various hardening media like Soil, Vermicompost; Soil mixture and Vermicompost (1:1), Soil mixture and Vermicompost (2:1) & Soil mixture and Vermicompost (1:2) have been assessed. Impact of varying concentrations of Auxins and the salt content of MS (Murashige and Skoog) media on the in vitro rooting of red banana micro-shoots. Vermicompost effectively decreases the mortality percentage while simultaneously increasing the survival percentage (up to 95%). Growth performances are also being studied.

Developing protocols for spawn production and cultivation of few selected wild edible mushroom species in Odisha: *Termitomyces clypeatus*, *Termitomyces medius*, *Russula cyanoxantha*, *Russula lepida*, *Tuber rufum*, and *Calocybe indica* are subjected to micro propagation to explore ideal explant, media and optimizing temperature, pH and humidity for achieving pure cultures.

Developing efficient micro-propagation methods for some RET listed forest tree species of Odisha has been standardizing. Collection of nodal explant source from tree & culture establishment for *Pterocarpus santalinus*, shoot initiation and growth of nodal segments (explants collected from tree/germinated seedlings), explant source from the seedling & culture establishment, axillary shoot, Multiple shoot initiation and growth (from germinated seedlings) Have been achieved. Besides, shoot initiation and growth of *Lasiococca comberi* nodal segments has also been achieved.

Standardization of propagation methods for *Bulbophyllum*, *Pomatocalpa decipiens* and *Cymbidium bicolor* orchids through tissue culture has been achieved through seeds as well as vegetative parts as explants like young shoot buds.

Generation of Genetic Variants for *Dendrobium*, *Cattleya*, *Cymbidium* and *Spathoglottis* Orchids Through Mutation Breeding towards the development of Novel Flowers has been initiated. Seeds/ protocorms are subjected to chemical mutagen (EMS- Ethyl methanesulphonate). Mutation induction has been started with the protocorms of *Cymbidium aloifolium*, *Dendrobium Sonia*, *Cattleya pink*, *Spathoglottis plicata*.

Using Mass Propagation and Breeding Facility of Orchids, established in RPRC, in vitro propagation of *Dendrobium*, *Cattleya* orchids has been undertaken. Development of molecular markers for the *Dendrobium* orchids has also been worked out.

In order to regulate ripening and enhance fruit shelf-life in banana, an important fruit crop for food security, studies through Omics-approach have been undertaken. In this context RNA isolation and cDNA synthesis started for different developmental and ripening stages and tissues (peel and pulp) of banana along with studies on RTqPCR for the selective genes/proteins concerned.

### **Wild Edible Fruits : Conservation and nutraceutical analysis.**

Sugar Profiling and Antinutrient Analysis of Some Unexplored Wild Edible Fruits of Odisha : Sugar profiling and antinutrients analysis were performed for eight different wild edible fruits to identify fruit species containing less amount of anti-nutrients and sugar content for selective & legitimate consumptions as well as to encourage its conservation. Sugar profiling (Total Sugar, Reducing Sugar and Non-reducing sugar) & Antinutrient (Oxalate, Tanin, Phytate, Phenolic content) were quantitatively analysed through spectrophotometer and further isolation and qualitative analysis using HPLC is going on.

Characterization Of  $\alpha$ -tocopherol & Polyphenols in Some Immune Boosting Wild Edible Fruits Used by Tribal Communities for Therapeutic Value: This research aims to characterize, isolate, and analyze  $\alpha$ -tocopherol and Polyphenols in ten wild edible fruit species to identify potent and immune-boosting fruit species. Fruits were collected from three different agro-climatic zones of Odisha for better comparison and selection of promising one. Isolation and Qualitative study of  $\alpha$ -tocopherol and Polyphenols (Tannin, Flavonoid, Phenolic, Quercetin, Catechin) were planned to carried out using UV-Vis Spectrophotometer followed by HPLC. Screening and quantitative analysis of polyphenols were performed through UV-Vis Spectrophotometer and HPLC analysis for polyphenols are going on.

Conservation of wild edible fruit plants through field introduction in different protected wild areas of Odisha: Field introduction of wild edible fruit species done at Site: 1 Podagada, Nayagarh Forest Range under Nayagarh Forest Division. And Site: 2 Sorisiapada, Kapilash Forest Range under Dhenkanal Forest Division. Field visit to Chandaka WL Division completed for site survey. Each site was introduced with around 900 saplings of *Antidesma ghaesembilla*, *Carmona retusa*, *Eugenia roxburghii*, *Glycosmis pentaphylla*, *Polyalthia suberosa* and *Toddalia asiatica*.

### **Propagation and reintroduction of Mangrove plants**

Conservation of rare mangrove species of *Xylocarpus* through vegetative propagation & re-introduction in protected areas of Odisha: Artificial regeneration of these rare, endangered, and threatened mangroves viz. *Xylocarpus* spp. through vegetative propagation methods has been attempted. Various methods, such as air-layering and Black-taping, were employed for rooting and

artificial regeneration using rooting hormones. Air-layering was effective in *X. granatum* and *X. mekongensis*, but *X. moluccensis* acted as difficult to root species. Further mass propagation and hardening are in progress.

### **Medicinal plant and its application**

A study has been undertaken to explore ameliorative effect of *Aporosa octandra* against carbon tetrachloride-induced oxidative stress and hepatocellular injury in experimental animal model along with the phytochemical investigation of the leaf extracts.

The fruit extract of *Buchanania lanzan* Spreng., a medicinal plant, has been studied for its protective diabetic neuropathy effect in streptozotocin induced type 2 diabetic rats for exploring the therapeutic potential.

Biological evaluation of leaf extracts of *Zingiber zerumbet* and *Hedychium spicatum*. Both of these are rhizomatous plants and rhizomes have been reported to Possess significant medicinal properties, In the project leaf extracts of the same are being explored for their medicinal properties through Phytochemical analysis for the presence of secondary metabolites, antioxidant activity of extracts using qualitative and quantitative assays and Cytotoxic activity of leaf extracts using brine shrimp assay.

An exploration study on cytotoxic potential of methanolic leaf extract of medicinal plant *Crinum defixum* has been undertaken . In order to isolate active principle, large scale preparation of methanolic extract of leaf of the plant has been initiated which is followed by column chromatographic separation various gradients of acetonitrile and methanol for isolation of single molecule.

### **Propagation and reintroduction of endangered species**

Conventional propagation & reintroduction for conservation of a few endemic and endangered plants in Odisha such as *Rademachera xylocarpa*, *Nothopegia racemosa*, *Cryptocarya amygdalina* and *Alphonsea madraspatana* by collecting seeds, air-layering and rooting stem cuttings have been undertaken. Raised *Cryptocarya amygdalina* plantlets (1500 nos) during the first phase.

### **Taxonomical Study**

Taxonomical and ethno botanical significance of the Leguminosae family in Odisha have been studied and remarkable evolutionary diversification in morphology, physiology and ecology has been examined.

### **Other major achievements**

- Up-scaling of QPM production & Sale: Ornamental & Garden plants, Seasonal & Orchids, wild edible fruit plants.
- Establishment of Modern Plant Sale Counter
- Celebration of Van Mahotsav 2023: Introduction of RET Plants in RET Garden,RPRC
- Release of RPRC Publications on occasion of World Environment Day 2023
- Organize Exposure Visits to RPRC by various organizations/institutions
- Inauguration of RPRC Sale Counter 'Blossom Bazaar' (December, 2023)
- Organized Annual Flower Show 2024 (January, 2024)

## 4 RESEARCH ACHIEVEMENT

### 4.1. Microbiological Applications

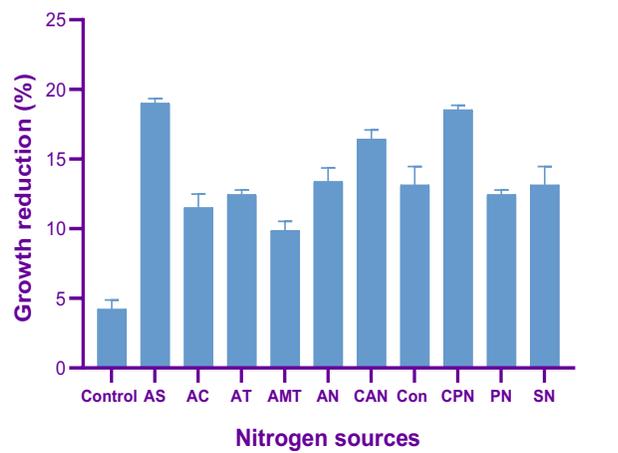
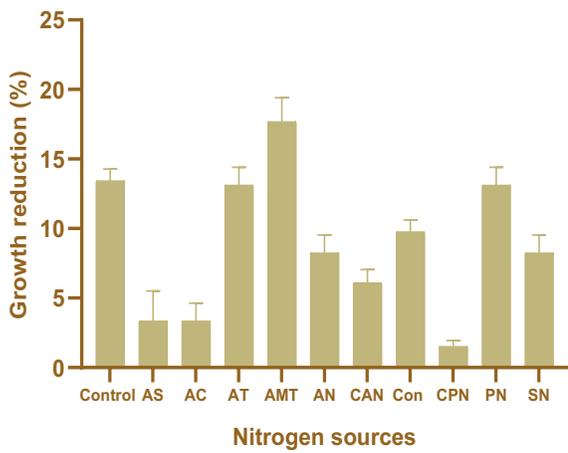
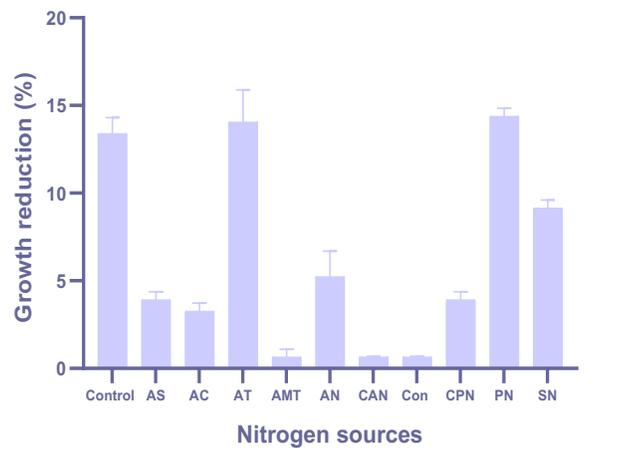
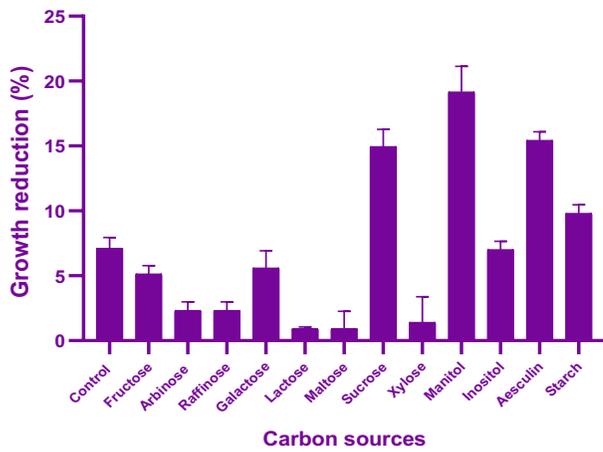
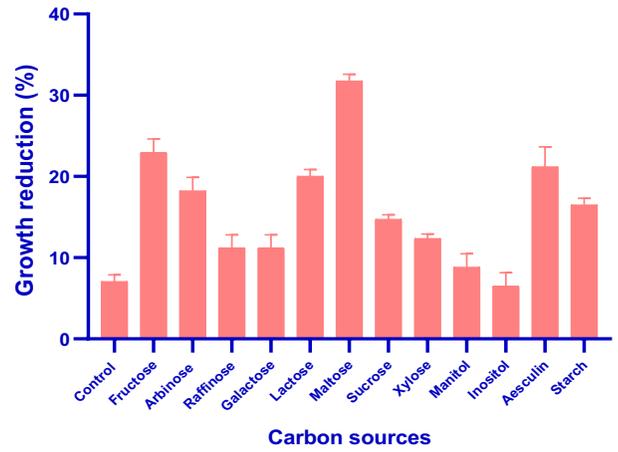
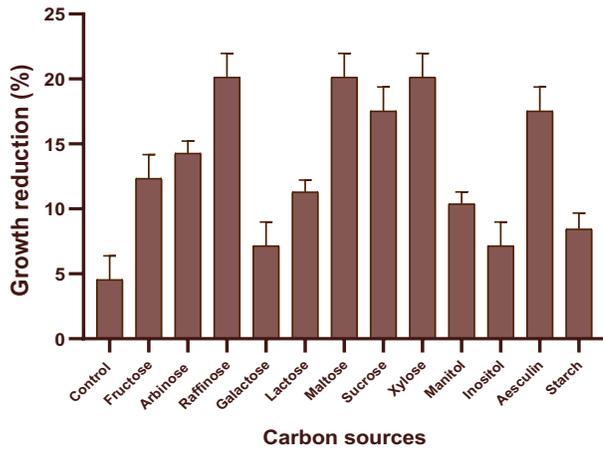
#### 4.1.1. Bioactive Lead Molecules from Fungal Endophytes: Extraction, Purification, and Characterization

**Principal Investigator: Dr. Nibha Gupta, Principal Scientist**

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Microorganisms like fungi are useful for the growth and development of plants in different sectors like forestry, agriculture, horticulture, etc. Fungi are an essential part of the microbial world, they have a mass of branched, tubular filaments enclosed in stiff cell walls and perform a variety of activities, including transporting nutrients in various environments, decomposing, and recycling. By improving and altering the root surface, fungi play a crucial function in enabling plants to better absorb nutrients and quickly mobilize minerals. Many fungi live in the soil or water for free, while others interact with plants and animals in parasitic or symbiotic ways. Endophytic fungi are fungal species that live within plants without causing illness or harm to the host. Fungal endophytes reside in the different organs of the host like leaves, stems, barks, roots, fruits, flowers, and seeds and they form a mutual relationship with the plant in which the plant gives shelter & harm to the endophytes whereas endophytes produce bioactive substitutes which increase the resistance and benefit the plant growth. Several researchers studied endophytic fungi for their sources, taxonomic categorization, biological, & metabolic activity, and their industrial importance. The microscopic creatures known as endophytic fungi live inside the plant without contaminating it or harming it in any way. These may have an impact on the host plants' distribution, ecology, physiology, and biochemistry. Several scientists studied about the endophytic fungi, their sources, categorization, biological properties, industrial importance, & therapeutic value. Endophytic fungi are considered as "gold mine" for bioactive compounds having antimicrobial, anti-diabetic, anticancer, antioxidant, and many more medical, industrial & biotechnological applications. They are well studied for their synthesis of secondary metabolites from different sources, these are not only useful for agriculture instead used in the food industry and many of them have therapeutic potential. Current study aimed to optimize the media using different nutrient factors, mass scale production, solvent extraction, and column chromatography. Furthermore, the extract's biological activities were investigated.

Nutritional changes at the carbon and nitrogen source levels, as well as culture conditions such as specific incubation periods, resulted in the production of a novel and modified medium in which our fungus demonstrated increased levels of various bioactivity. Phytochemical test of selected fungal endophyte showed the presence of various secondary metabolites. Antioxidant properties were shown in the fungal culture though differed as per culture condition and nutritional factors. The highest antifungal activity was seen in the 18-day-old culture and maltose-potassium nitrate is the chosen C and N sources. Achievement of this changed medium composition may be effective in eliciting the synthesis of secondary metabolites, useful for pharmaceutical research and antagonistic principle against plant pathogens.



Effect of nutrient factors on antifungal activity of F1 against three *Fusarium* spp.

Mass scale production with selected carbon source and selected nitrogen source. Inoculation of selected fungi F1 and incubated. Solvent extraction was carried out after 18 days of incubation and evaporated. Silica column was prepared. The evaporated sample was dissolved in Dimethyl sulfoxide and passed through the column. Fractions were collected and solvent extraction from the first column was done. All methanolic extracts of different nutrient factors were rich in alkaloids, phenols, flavonoids, tannins, and saponins. Extracts of F1 displayed DPPH activity at varied percentages, highest percentage of DPPH activity was observed in lactose followed by maltose and aesculin in contrast highest percentage of antioxidant activity was observed in sodium nitrate followed by calcium nitrate.



*Mass scale production of selected fungi*

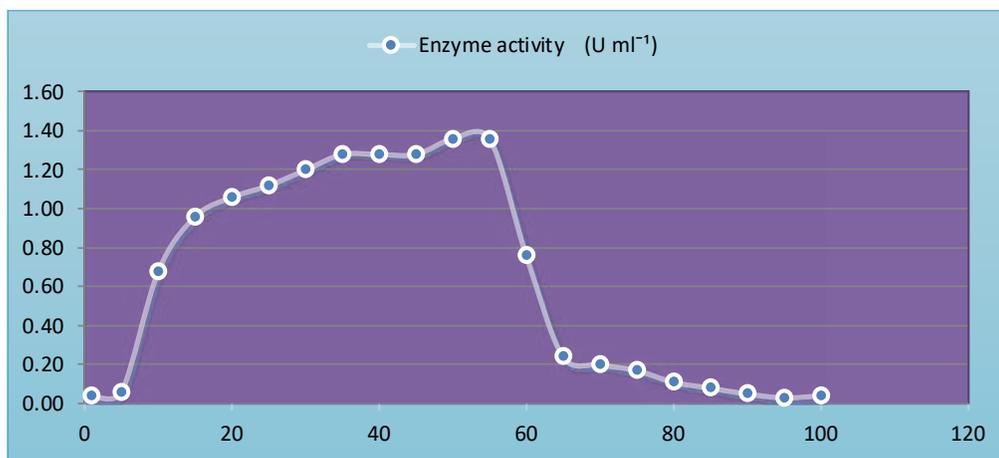
#### **4.1.2. Bioprocess optimization for enhanced recovery of L-glutaminase-free L-asparaginase of fungal origin**

**Principal Investigator: Dr. Nibha Gupta, Principal Scientist**

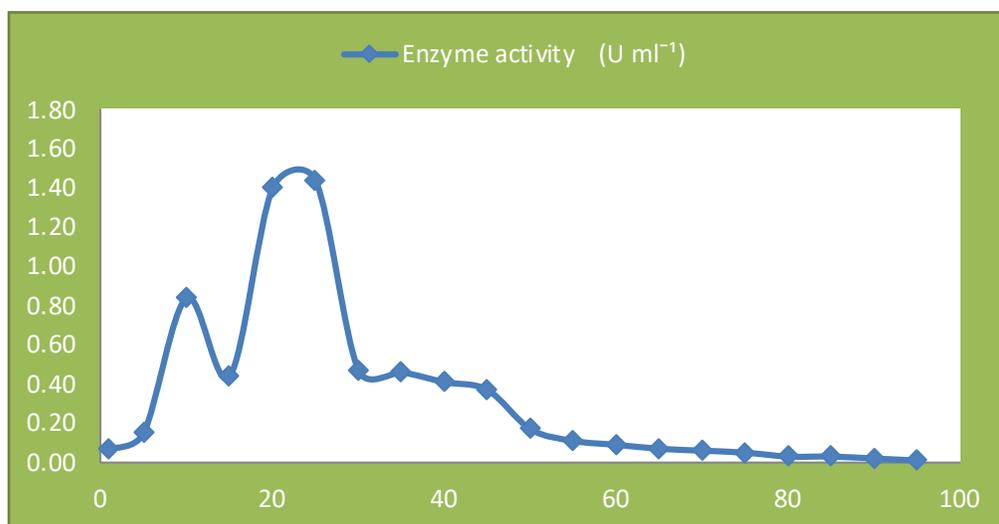
L-asparaginase is an enzyme that mostly helps to break down asparagine into L-aspartic acid and ammonium in water. This enzyme can be found in many living things, like bacteria, plants, and some animals, like the serum of some rodents. Especially for Acute Lymphoblastic Leukaemia (ALL) and Hodgkin's lymphoma, it is an important chemotherapeutic drug for treating lymphoproliferative diseases and lymphomas. When L-asparaginase comes in contact with water, it breaks down more easily. At the moment, biotechnological methods using certain microorganisms are mostly used to make L-asparaginase. Still, industrial manufacturing needs a study that focuses on both increasing production yields and coming up with new ways to do things, like using different microbes to make enzymes useful in more situations. L-asparagine is an amino acid required both by normal cells as well as cancer cells for the production of protein. Normal cells synthesize this amino acid by the catalytic activity of asparagine synthetase from aspartic acid and glutamine. However, leukemic cells cannot produce L-asparagine on their own due to the low levels of L-asparagine synthetase, which is very essential for the growth of tumour cells. So, the tumor cells take L-asparagine from blood circulation or body fluid as it cannot synthesize L-asparagines. The presence of L-asparaginase

enzyme as chemotherapeutic agent may degrade the L-asparagine present in blood circulation and indirectly starve tumor cells and lead to cell death. This idea aims to optimize process parameters for L-asparaginase production, its large-scale production, extraction and purification followed by characterization of the partially purified enzyme using substrate specificity, pH optima, temperature tolerance and incubation period.

L-asparaginase was made by first removing the crude enzyme then precipitating it with ammonium sulphate, filtering it through a Sephadex column, and finally making it even purer through ion exchange chromatography. Different sources and concentrations of carbon, nitrogen and amino acid inducers (nutritional factors) as well as pH and temperature (physical parameters) were optimized to achieve enhanced L-asparaginase production. The findings indicated that the most favourable conditions for enzyme production were a pH of 8.0 at 37°C. The carbon and nitrogen sources that demonstrated the highest efficacy were Fructose and Ammonium sulphate respectively. The yield was also high in vitamin-M, Calcium phosphate, proline, Tryptophan, and sodium Nitrate. The production of L-asparaginase on a large scale can be achieved through continuous fermentation using the medium composition.



*Purification of L-asparaginase using gel filtration column chromatography*



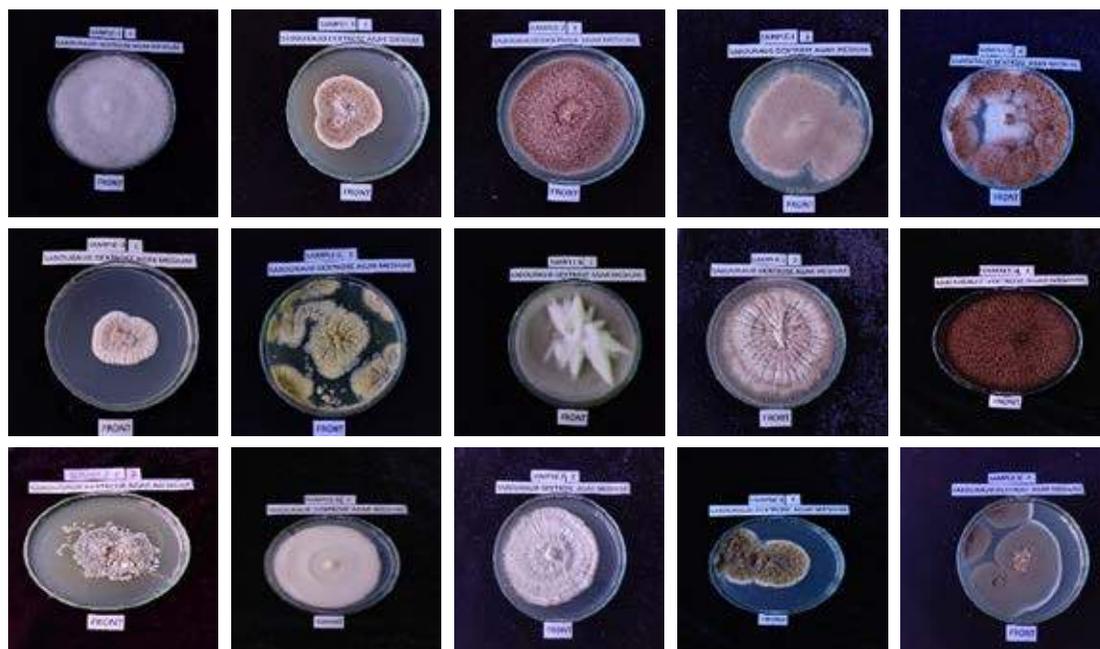
*Purification of L-asparaginase using ion exchange column chromatography*

The examination of substrate specificity revealed that the enzyme functions as a catalyst for L-asparagine, specifically acting on it as its substrate. We also investigated the optimal incubation period for an enzyme and substrate to achieve maximum enzyme activity and the most rapid substrate degradation rate. This was done by incubating the enzyme with the substrate for different amounts of time under the best conditions.

#### 4.1.3. Documentation of Microfungi in forest soils of Odisha

**Principal Investigator: Dr. Nibha Gupta, Principal Scientist**

Natural forests are sites of high biodiversity, where complex relationships among fauna, flora and microflora are maintained due to structural richness of the habitat. Soil microorganisms are essential components of the biotic community in natural forests, responsible for the breakdown of organic materials, mobilization of nutrients, maintenance of soil-plant quality and ecosystem. Microorganisms in the soil are helpful in enhancing soil fertility. Among these microorganisms, fungi are one of the dominant groups present in soil. Soil fungi play an important role as major decomposers in the soil ecosystem. Besides, they also provide mankind with very useful pharmaceutical products. On the other hand, some of them are very harmful causing food spoilage and diseases to plants, animals and humans with significant economic losses. Recent studies suggested that there may be around 1.5 to 5.1 million fungal species and about 1200 new species are reported each year. This indicated that there are many more fungal species to be explored and identified. Present study aimed for isolation and characterization of fungal isolates found in different forest soils of various physico-chemical parameters. Overall 441 no. of fungal isolates obtained by applying different isolation methods. Total 203 morphotypes of fungal isolates recovered and segregated at genus level. The soils have been found with domination of *Aspergillus*, *Fusarium*, *Penicillium*, alongwith scattered occurrence of *Cladosporium*, *Colletotrichum*, *Chaetomium*, *Trichoderma* and few mycelia sterilia.

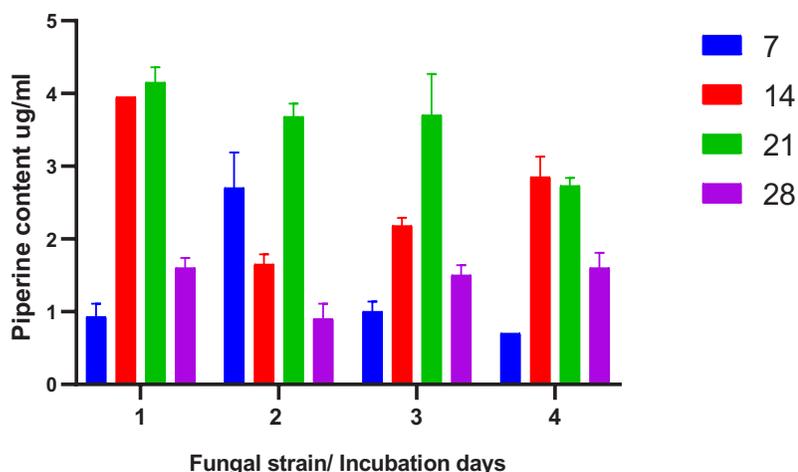


*Fungal isolates from forest soils*

#### 4.1.4. Harnessing the potential of endophytes of *Piper longum* as an alternate source for piperine production: optimization of protocol for laboratory production

Principal Investigator: Dr. Nibha Gupta, Principal Scientist

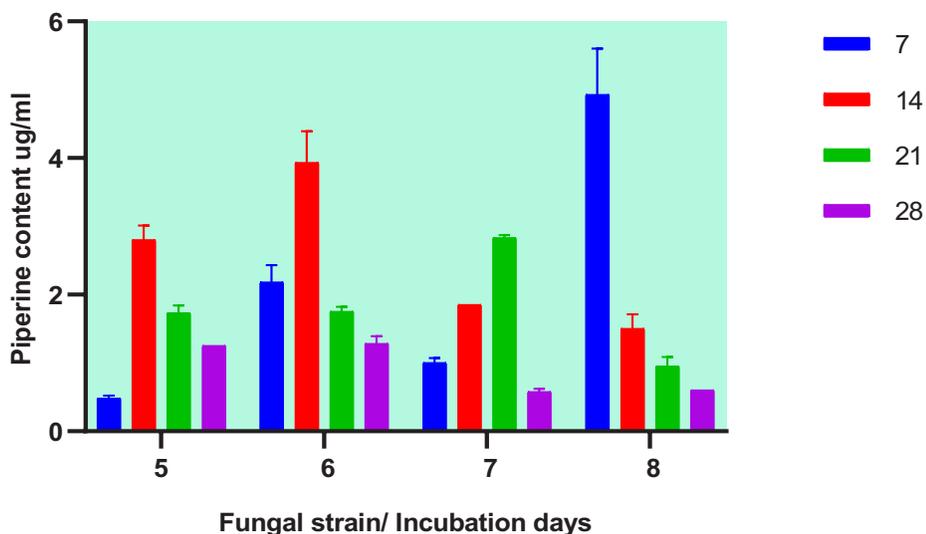
Endophytic fungi are remarkable microorganisms that exhibit extraordinary diversity and are renowned for their capacity to generate a vast spectrum of secondary metabolites. This study of endophytic fungi and their bioactive compounds not only showcases their existing uses but also emphasizes the immense potential for identifying new compounds with substantial advantages. The future prospects in this field are promising, with ongoing research focused on optimizing production processes, understanding the mechanisms of action, and exploring the untapped diversity of endophytic fungi to discover new bioactive compounds. Piperine is an alkaloid compound isolated from several members of the Piperaceae family including *Piper nigrum*, *Piper longum*, *Piper chaba*, *Piper guineense*, *Piper sarmentosum*. Plants of this family are also prominent sources of other bioactive compounds such as flavonoids, phenolics, amides, alkaloids, steroids, lignans, chalcones, terpenes, and various other phytochemicals. This compound has attracted the attention of medicinal chemists and health professionals due to its numerous benefits including antioxidant, antitumor, anti-inflammatory,



antibacterial, immunomodulatory, antifungal, antiapoptotic, antithyroid, antimutagenic, antimetastatic, and anti-spermatogenic. This report is based on the endophytes isolated from *Piper longum*. Research has identified that an endophytic fungus, *Periconia sp.*, from *Piper longum L.*, can produce piperine, though specifics on culture condition. This study aimed to determine the culture conditions and nutritional factors for enhanced production of piperine. Mass scale production and solvent extraction

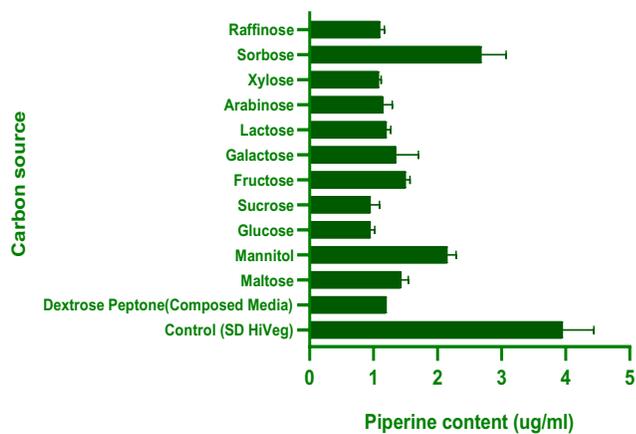
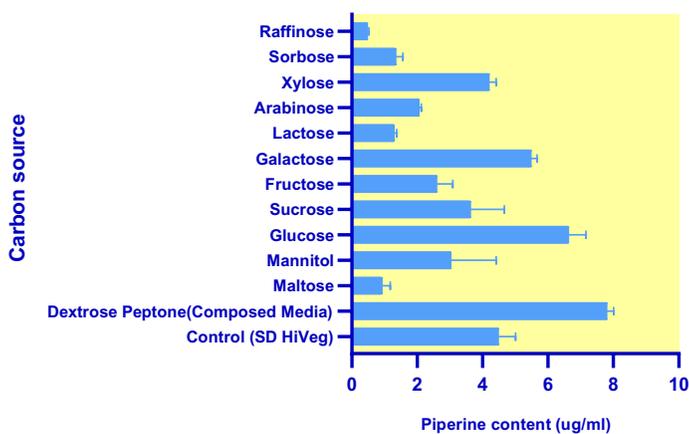
of metabolites through column chromatography in comparison to standard revealed the presence of piperine in the selected fungal strain. Further clarification for the presence of piperine content demands higher advanced instrumentation analysis.

All fungi belong to *Piper longum* were isolated and screened for piperine production by following the solvent extraction and UV spectrophotometric analysis protocols. Subsequently, three fungi F1, F2 and F3 have been selected for further experiments carried out on effect of carbon sources on piperine production in liquid culture conditions. The screening experiment carried out in different incubation period of fungal growth revealed variation in production of piperine at different growth period by different fungal species. Most of them preferred 21 days for better production. The three selected fungi exhibited production of piperine in 7 days of growth period.



*Piperine production by Fungal strains under different incubation period*

Further experiments on effect of various carbon sources on metabolite production expressed the usefulness of dextrose and mannitol for enhanced production in in-vitro condition. Additional clarification for the presence of piperine content demands higher advanced instrumentation analysis. This detailed optimization process maximizes piperine yield and enhances the practicality of large-scale fungal production.



*Piperine production by fungal strains in media supplemented with different carbon sources*

## 4.2. Tissue Culture & its application on various important plant spp. (banana, orchids, medicinal & forest spp. & mushrooms)

### 4.2.1. Standardization of Propagation Methods for *Bulbophyllum* Orchids Through Tissue Culture

**Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist**

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*Bulbophyllum* is the largest genus in the orchid family with more than 2,000 species, and also one of the largest genera of flowering plants. The species are mostly epiphytic and lithophyte orchids. The plants are typically small to medium, in some of the species although some have leaves up to a meter long, creeping epiphytes, having a sympodial growth habit with prominent pseudo bulbs, with one or two fleshy leaves. There is a wide range of fantastic flower shapes and sizes (2 mm to 400 mm). So far 124 species have been reported from India and many of them are located in the northeast region and maximum species are found in Manipur. From the state of Odisha, eight numbers of species have been reported such as *Bulbophyllum guttatum*, *B. carniflorum*, *B. crassipes*, *B. macraei*, *B. panigrahanum*, *B. polyrhizum*, *B. triste*, *B. umbellatum*. Some *Bulbophyllum* species are threatened with extinction and are recognized as such by the World Conservation Union (IUCN). However, with the development of plant tissue culture technology planting materials could be produced on a mass scale to be used for the re-introduction purpose which is the most viable method for the conservation of the orchids. Taking this in view in this study procedures have been developed for the production of planting materials through tissue culture technology.

Seeds of *Bulbophyllum crassipes* were collected from RPRC Orchidarium and inoculated on MS (Murashige and Skoog) medium with numbers of different supplements to examine the effects of different strengths of macro & micro nutrients and plant growth regulators on growth and development of plants. By comparing different strengths of MS medium on seed germination it was observed that seeds initiated the germination process after 30 days of culture, however, no difference was observed. At 60 days and 90 days culture it was observed that highest rate of germination was reported on the full strength of MS medium (Fig. 14).

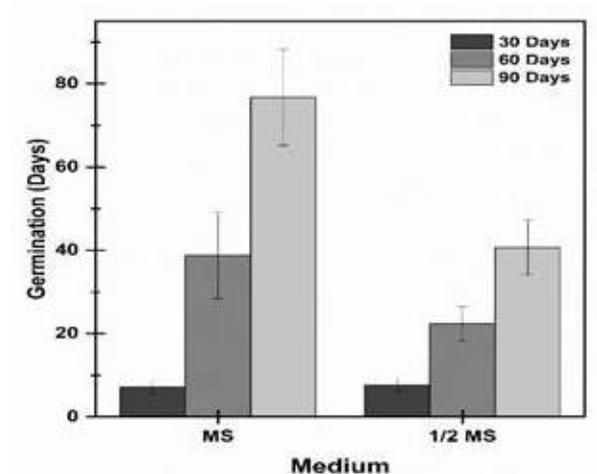


Fig. 14. Effects of two strengths of MS medium on seed germination.

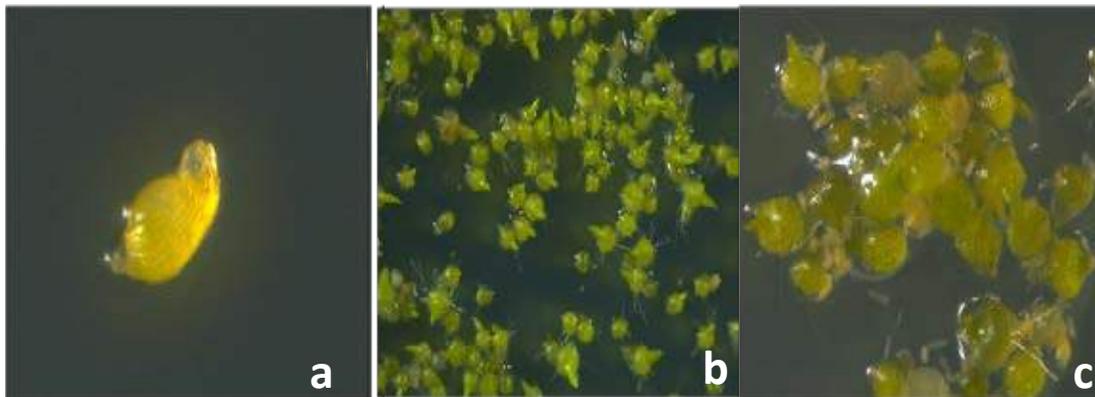


Fig. 15. Seed germination on MS medium: Germinating seed on MS medium; b) Seed germination at 60 days of culture; c) Seed germination at 90 days of culture.

Young shoot buds developed in vitro were cultured on MS medium containing different hormone combinations containing cytokines (MS + BAP 2.5 mg/l, MS + BAP 5.0 mg/l, MS + BAP 2.5 mg/l + KN 2.5 mg/l). Among these, MS containing BAP 2.5 mg/l had produced highest number of shoots (10-15) after 90 days of culture (Fig.16). These new shoots were then transferred to shoot elongation and rooting medium for production of new plantlets for primary hardening.

a) MS + BAP 2.5 mg/l, b) MS + BAP 5.0 mg/l, c) MS + BAP 2.5 mg/l + KN 2.5 mg/l).



Fig.16. Shoot multiplication on MS medium containing different growth regulators.

About 70 numbers healthy plantlets of *Bulbophyllum crassipes* were transferred on a moss bed for necessary moist and humid environment for acclimatization. Nearly after one month these plantlets were transferred to pots with coconut husk, coal and brick pieces for further growth.

#### 4.2.2. Generation of Genetic Variants for *Dendrobium*, *Cattleya*, *Cymbidium* and *Spathoglottis* Orchids Through Mutation Breeding Towards Development of Novel Flowers.

Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist

##### (a) Cultivation of *Dendrobium* orchids

Orchids are highly valued because of their attractive flowers. At Regional Plant Resource Centre facilities have been developed for the cultivation of ornamental orchids being used for cut flower production also for potted flower production. Since last three years cultivation of three important hybrids like *Dendrobium Earsakul*, *Dendrobium Sanan White* & *Dendrobium Singapore White* has been initiated and quality flowers are produced (Fig. 1, Fig. 2 & Fig. 3). Details of the cultivation practices are under development. As these three orchids are perennial in nature for which it is required to understand the growth and development at different times at least for 15 years. Taking this in view 7000 numbers of plants growing under protected cultivation conditions were selected and different parameters were observed as mentioned in the (Table 1). It was observed that each plant had accrued 6 to 7 shoots and attained 32 cm in height. In the three varieties, each stem had produced 18 to 22 leaves with the leaf width of 4 to 6 cm. With regard to the flower features it was observed that the inflorescences were reached to the height of 30 to 43 cm and each had 6 to 8 flowers. In comparing the quality of the flowers among the used varieties it was found that the flowers of the *Dendrobium Earsakul* has a greater number of flowers than that of the other two white varieties

**Table 1. Different features of the three varieties of *Dendrobium* growing under protected cultivation conditions.**

Name of the varieties	No of shoots per plant	Leaf No.	Leaf Width (in cm)	Shoot length (in cm)	Length of inflorescence (in cm.)	No. of flower per inflorescence
<i>Dendrobium Earsakul</i>	7	18	5.03	21.78	42.8	8
<i>Dendrobium. Sanan White</i>	6	22	5.25	32.60	30.5	6
<i>Dendrobium. Singapore White</i>	7	19	4.70	25.80	31.4	5



*Fig. 1. (a) Dendrobium Earsakul under cultivated condition*

*Fig. 1(b) Inflorescence of D. Earsakul*



*Fig. 2 (a) Dendrobium Singapore White under cultivation condition*

*Fig. 2 (b) Inflorescence of D. Singapore White*

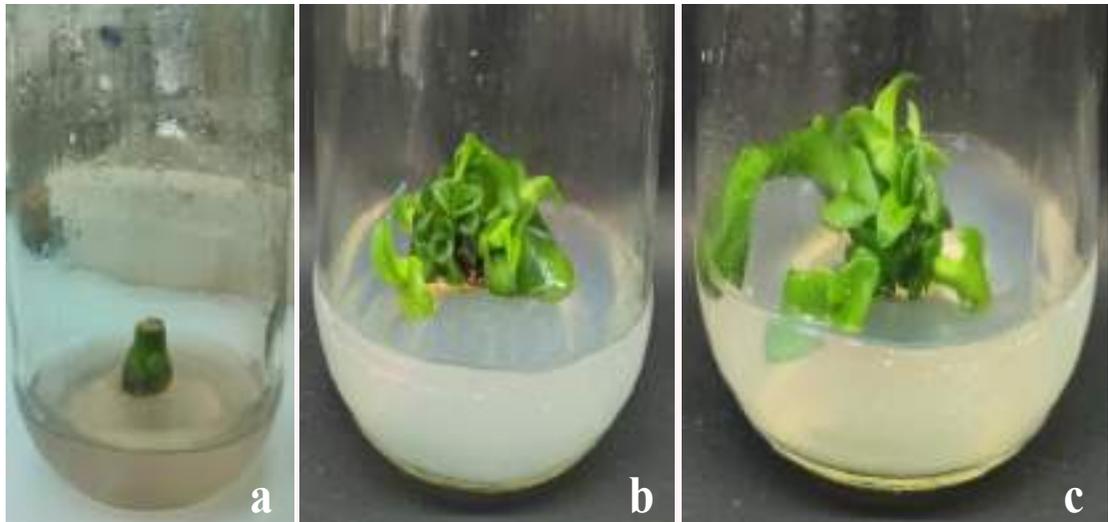


*Fig. 3 (a) Dendrobium Sanon White under cultivation condition*

*Fig. 3 (b) Inflorescence of D. Sanon White*

### **(b) Propagation of Dendrobium Earsakul**

Planting material production of the Dendrobium Earsakul orchid is challenging as these requires the use of the plant tissue culture technology. The orchid growers like to grow genetically uniform and virus free plants for which use of tissue culture methods are highly essential. The plants growing at different polyhouses are considered as the mother plants; from these plants young shoot buds were collected and after sterilization were cultured on the Murashige and Skoog's, 1962 nutrient medium. The medium was supplemented with two plant growth regulators (MS+BAP7.5 + NAA 0.5mg/l) for induction of new shoots. It was observed that new shoots were produced after 180 days of culture. The new shoots were transferred to another multiplication medium for further production of shoots.



*Fig.4. Propagation of Dendrobium Ersakul through tissue culture (a) Shoot bud cultured on nutrient medium; (b) Initiation of new shoots buds; (b)(c) New shoot production on multiplication medium.*

***(c) Generation of Genetic Variants for Dendrobium, Cattleya, Cymbidium and Spathoglottis Orchids Through Mutation Breeding towards the development of Novel Flowers”***

Orchids are grown predominantly as ornamental and are valued as cut flowers because of their exotic beauty; they are the most popular in ornamental trading. Mutation breeding refers to the method of using artificial mutagenesis to obtain new biological cultivars, mainly through chemical or radiation mutagenesis. Chemical mutagenesis refers to the biochemical reaction between chemical agents and genetic material, and the result is mostly point mutations in genes. Comparatively, radiation mutagenesis has the characteristics of more complex genetic mutations and more beneficial mutant phenotypes. In vitro plant breeding and propagation is one approach that can also be used to modify the nature of orchids, from their appearance to their growing capacity. The resulting mutants are expected to increase the variety of orchids as well as their selling price. In this study experiments have been conducted for the generation of the genetic variants of Dendrobium, Cattleya, Cymbidium, and Spathoglottis orchids using chemical and physical radiations.

**Mutation through EMS(Ethyl methanesulphonate)**

The protocorms of Dendrobium, Cattleya, Spathoglottis and Cymbidium first formed using manual pollination followed by tissue culture. The protocorms were treated with EMS with different dosages(0.05%, 0.075%, 0.1%) for different hours. After the treatment the growth and development of the protocorms were observed in the culture room (Fig. 5). It was observed that the growth of Cymbidium aloifolium multiplied more rapidly; however, for other varieties a significant growth has been recorded. It was observed that among the three concentrations of EMS used, the high concentration of 0.10% found to be more toxic to the respective orchid tissue. In Dendrobium ‘Sonia Ersakul’ the toxicity is less as compared to that of the other orchids. The tissue of the S. plicata completely died with the treatment of high concentrations of EMS. Thus for further studies the EMS concentrations of 0.05 % and 0.08 % were used for the generation of the mutants.

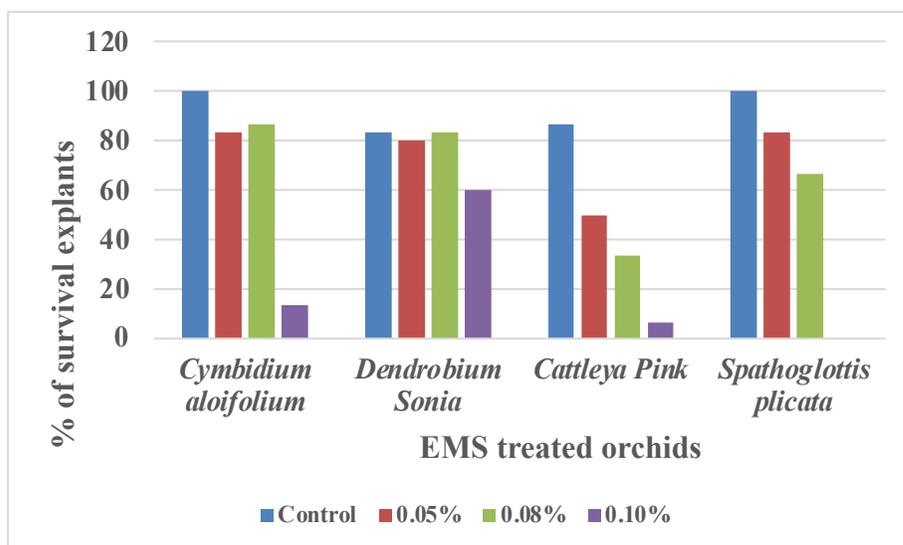


Fig.5:-Survival of the protocorms after exposure to different concentrations of EMS.

The survived protocorms were cultured on MS medium for the development of roots and leaves under in vitro conditions. It was observed that in all the species and varieties, the protocorms had produced healthy shoots and roots in all the orchids except for the *Dendrobium 'Sonia Ersakul'*. In all responding orchids, the protocorms initiated leaves after 60 days of culture and roots at 180 days of culture in *C. aloifolium*.

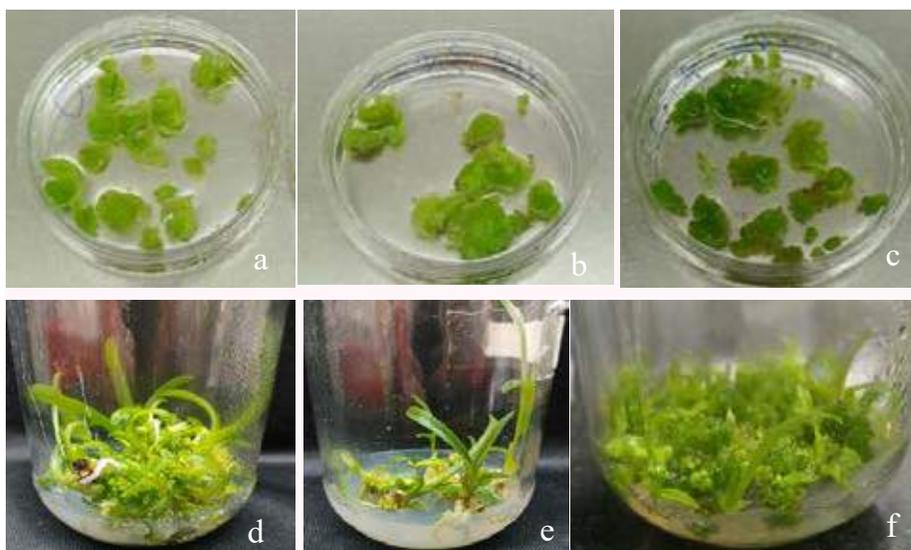


Fig 6:-Plant production from the EMS Treated *Cymbidium aloifolium* protocorms.  
 Pa, b)0.5%, c, d)0.075%, e, f)0.10%

**Mutation through Gamma Radiation:**For Gamma radiation, PLBs of *Dendrobium Sonia*, *Cattleya Pink*, *Cymbidium aloifolium*, *Spathoglottis plicata* were inoculated in 60mm petri plates containing nutrient medium. The PLBs were irradiated by Gamma Chamber 1200 at NBRI, Lucknow. The protocorms were irradiated with different doses (10Gy, 25Gy, 35Gy). After the irradiation, the explants were transferred to multiplication medium and kept under 16h light and 8hrs dark photoperiodic condition for production of new plants (Fig. 7).

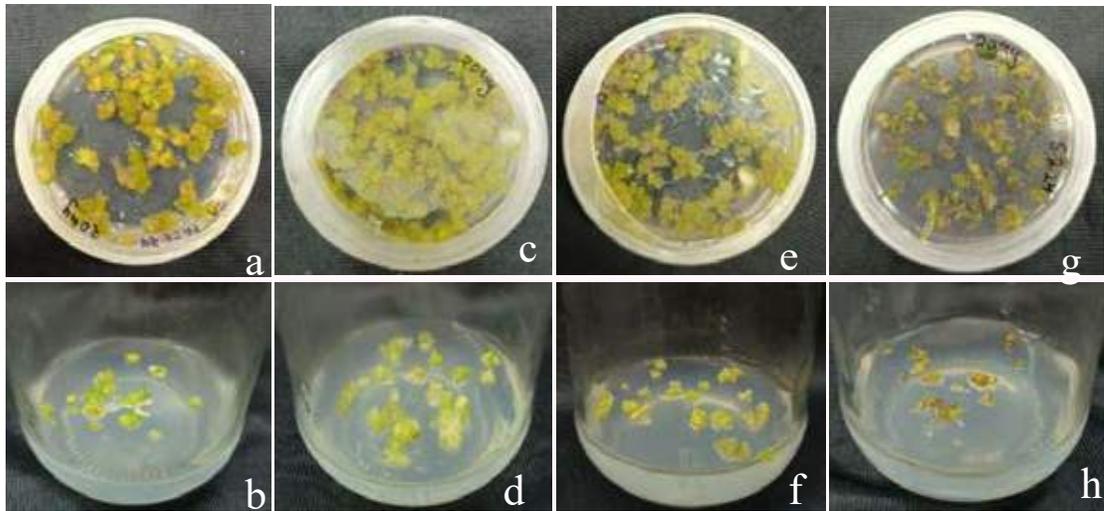


Fig 7:- Gamma irradiated protocorms a,b) *Cymbidium aloifolium*, c,d) *Cattleya Pink*, e,f) *Dendrobium Sonia*, g,h) *Spathoglottis plicata*

The effects of the different gamma radiation are presented in (Fig. 8); it was observed that the survival rates decreased with the increase of the radiation doses. In the orchid *C. aloifolium* the survival rates was extremely low even if the at the lowest dose of 10gy in which only 34% of the protocorms survived. With the use of the 35 gy, none of the protocorms survived. The protocorms of *Cattleya Pink* and *Dendrobium Sonia* survival rates were higher as compared to other orchids used in this study. In *CattleyaPink* all the protocorms survived with the use of the lower doses.

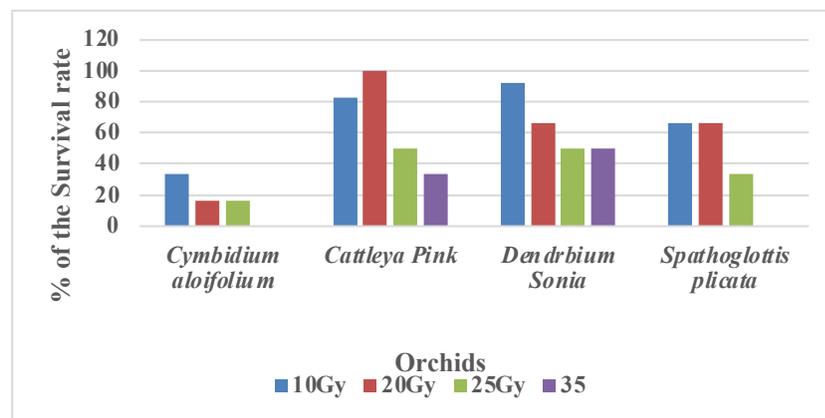


Fig. 8. Gamma irradiation of protocorms of different orchids.

Plants will be produced using tissue culture technology from the treated protocorms and will be evaluated for the generation of any mutants.

### 4.2.3. Standardization of efficient tissue culture based propagation methods for *Pomatocalpa decipiens* (Lindl.) J.J.Sm and *Cymbidium bicolor* (Lindl.): Rare Orchids of Odisha.

**Principal Investigator: Dr. Nihar Ranjan Nayak, Senior Scientist**

Orchids are considered to be the most diversified and evolved in flowering plants. The Orchidaceae comprising of >26000 species considered as one of the largest family in terms of the number of species among the angiosperms; is facing severe threats and found to have a high proportion of threatened members as compared to other families because of their complex life cycle. In India, the family Orchidaceae is represented by 1331 species under 185 genera, of these 309 species is endemic to this subcontinent. *Pomatocalpa decipiens* is one of the extremely rare orchids of India; populations are confined only to the state of Odisha. Within Odisha, again in a few pockets, the major populations are growing at the Barbara Reserve Forest. From the preliminary studies, it has been found that population growth is extremely slow and requires immediate attention. Similarly, *Cymbidium bicolor* also one of the extremely rare orchids of Odisha reported from the Similipal Biosphere Reserve Forest of Mayurbhanja district. Planting materials need to be produced for these orchids to be used for reintroduction purpose. Taking the above facts in view the project has been developed to standardize the propagation methods of the rare orchids.

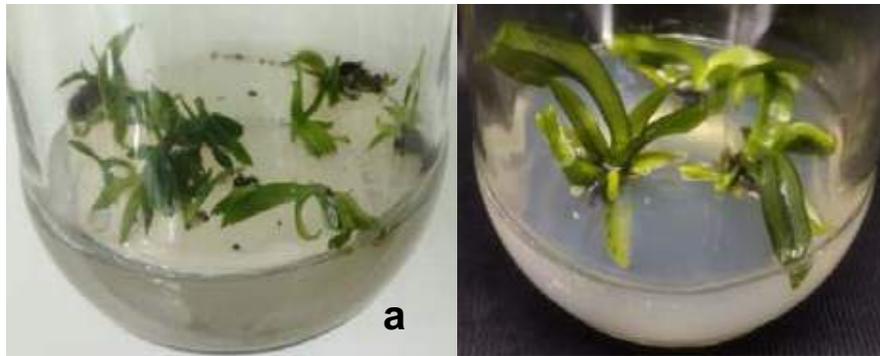
#### **In vitro propagation of *Pomatocalpa decipiens***

Capsules (seeds bearing fruits) were collected from natural habitat and brought to the laboratory, then surface sterilized and inoculated onto the MS medium containing various concentrations of nutrients and growth regulators. By examining under microscope, it was found that seeds took 90 days for the initiation of the germination process. Swelling of the seeds was observed at 60 days of culture, later the embryo grew bigger in size, finally ruptured the seed coat and completed the germination process. The seeds on BAP 0.5 mg/l, 80% of the seeds completed the germination process after 120 days of culture. With the application of the BAP and GA3 together, 90% of the seeds completed the germination process.



**Fig. 9.** Different stages of seed germination of *P. decipiens*  
a) Initiation of seed germination process; b) Completion of the seed germination; c) Initiation of leaf

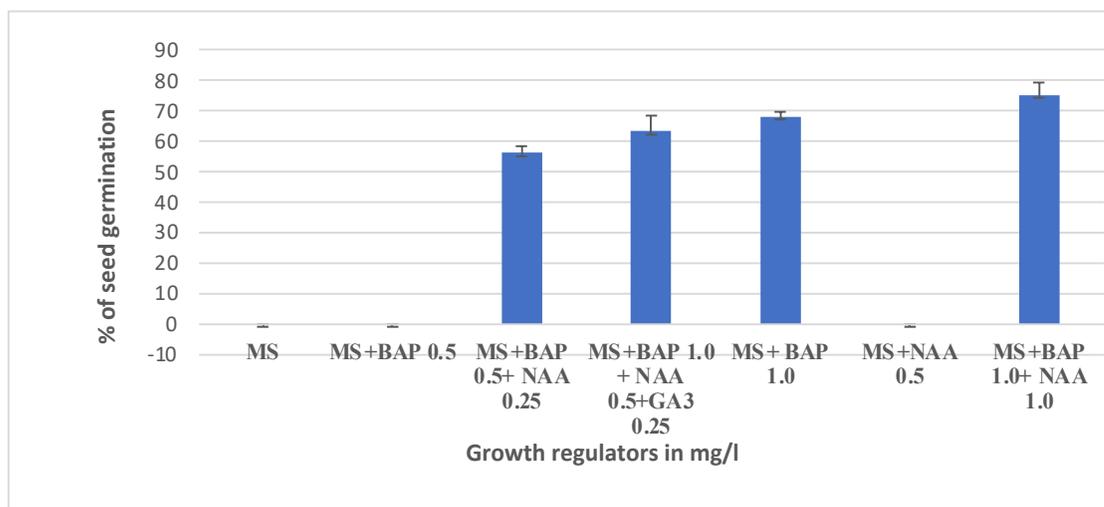
The seed inoculated on the MS medium containing BAP 5.0 mg/l after germination were maintained in the culture room for further growth and development. It was found that after one year of culture, the seedlings attained 1.5 cm of height, each had 2-3 leaves. However roots were not induced (Fig.10). The shoots were then transferred to root induction medium (MS+ IBA 2.0 mg/l) (Fig. 6), enhanced both the root and shoot induction.



**Fig.10.** Leaf and root induction in *P. decipiens*  
 a) Leaf induction on MS medium containing BAP 5.0 mg/l  
 b) Root induction on MS medium containing IBA 2.0 mg/l

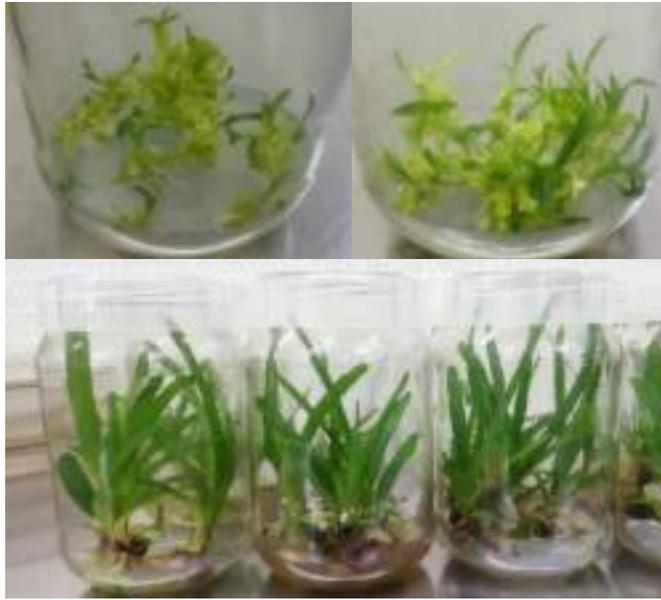
#### **In vitro propagation of *Cymbidium bicolor*-**

Seeds were collected from Similipal Biosphere Reserve during the month November 2023. These were examined under the microscope and found that all of them were matured with well-developed embryos. Seeds did not germinated on MS medium even after four months of culture and it was found that the inclusion of growth regulators was essential for the completion of the seed germination process. Among the three concentrations of BAP tested, the inclusion of lower concentrations of 0.5 mg/l did not initiate the germination process. With the increase in the concentrations of the BAP to 1.0 mg/l, 67% of seeds completed germination. Different combinations of the BAP and NAA were tested, and the best response was observed with the inclusion of BAP 1.0 mg/l and NAA 1.0 mg/l in which 75% of the seeds completed germination (Fig.11).



**Fig.11** Influence of plant growth regulators on the seed germination of *C. bicolor*

Young shoots buds produced through tissue culture were transferred to MS medium containing different combinations of growth regulators. The shoots produced were multiplied on transferring to MS medium containing 0.5 mg/l BAP. At 180 days of culture, each had 12-15 new shoots (Fig. 12 a & b). The new shoots were transferred to medium containing IBA 2.0 mg/l for shoot elongation and roots development. The overall process of shoots elongation and leaf developments were slow. At 120 days of culture on the IBA medium, the shoots reached to a height of 5- 6 cm (Fig. 12 c). These were then successfully acclimated on the coconut husk pieces (Fig. 13).



**Fig.12.** Plant propagation from in vitro culture of shoot buds  
 a) New shoot production on MS+ BAP 0.5 mg/l  
 b) Shoot elongation and root production on MS + IBA 2.0 mg/l  
 c) shoots reached to a height of 5-6 cm at 120 days of culture on the IBA medium



**Fig.13.** Acclimated shoots on the coconut husk pieces.

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

**Principal Investigator: Dr. Giridara Kumar Surabhi, Senior Scientist.**

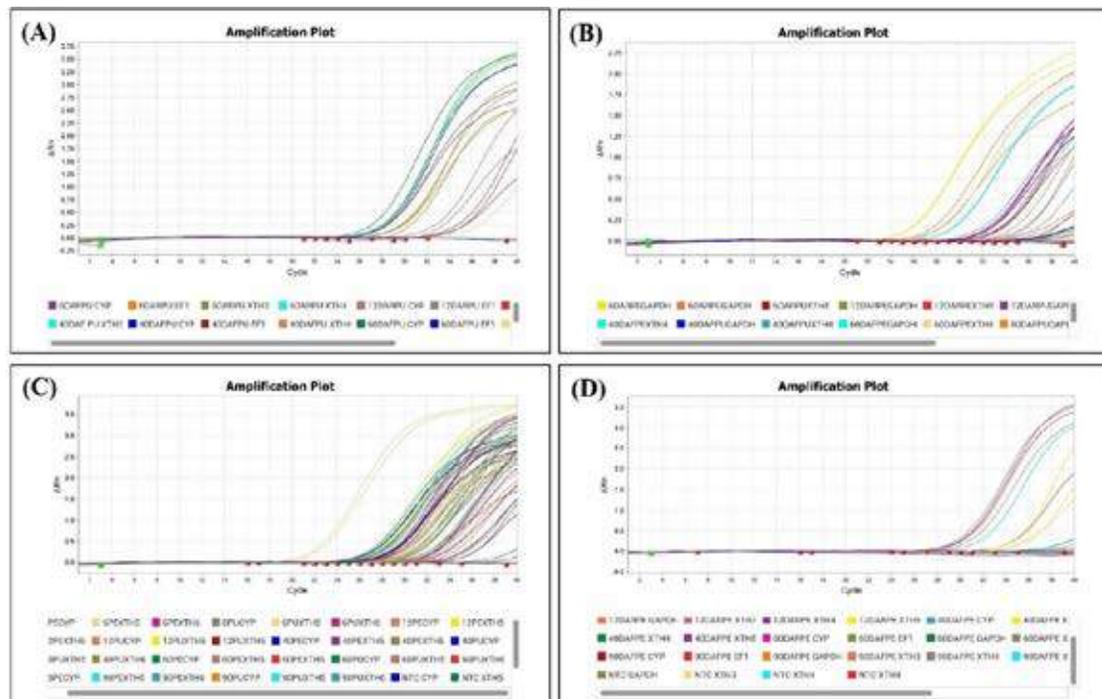
*Realtime Quantitative PCR based gene expression studies of a major cell wall modification genes in banana during fruit development and ripening.*

The process of fruit softening is a significant transformation that takes place during ripening. It involves extensive restructuring of the cell wall at the biochemical level. The plant cell wall is an intricate macromolecular matrix consisting of pectin, hemicelluloses, cellulose and structural proteins that undergoes dynamic alterations in the composition and structure throughout the fruit ripening. Structural changes in cell wall fractions are typically the result of the degradation of cell wall polymers catalyzed by various enzymes, such as cellulase, polygalacturonase (PG),  $\beta$ -galactosidase ( $\beta$ -gal), pectate lyase (PL), and xyloglucan endotransglucosylase/hydrolases (XTH). In total, seven genes from

different metabolic pathways and having significant role in fruit ripening process was selected for the study i.e. XTH-3, 4, 5, 6, 8, pectate lyase (PL) and sucrose synthase (SuSy) by considering cyclophilin (CYP) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as reference genes. The relative expression for each gene was examined during developmental (i.e., 40, 60 and 90-DAF) and ripening stages, (i.e., 6-and 12-DAR) and in different tissues.

Majority of XTH gene expression was not explored previously during fruit ripening, to the best of our knowledge and at least for banana. Enhanced expression of XTH3 and XTH4 was noticed at 6- and 12-DAR in both peel and pulp tissues, compared to fruit developmental stages. XTH-5 exhibited reduced gene expression from developmental to ripening stages in both the tissue type. Whereas, XTH-6 demonstrated elevated expression in pulp and reduced expression in peel tissue during ripening. XTH-8 expression was elevated in peel tissue during ripening, but at later stage of ripening the expression level was low as compared to early ripening stage.

In line with XTH gene expression, another important gene i.e., PL expression was also increased during the ripening stage, confirming its role in degrading the cell wall pectins, thereby, softening of the fruit. Further, SuSy gene expression was decreased from developmental to ripening stages, leaving minimal expression at fruit ripening, it reflects the involvement of SuSy in starch breakdown and the accumulation of simple sugar during fruit ripening. Hence the current study discloses valuable and novel information on XTHs, a major cell wall modifying class of genes. Identified genes can be further validated using either through Genome Editing or RNAi technology to assign individual functional role in fruit ripening and shelf-life.



**Figure 1A-D:** RTqPCR amplification plots with cycle number and relative fluorescence ( $\Delta Rn$ ). CYP,EF1a with XTH3 gene(1A), GAPDH with XTH4 gene(1B), CYP with XTH5 & XTH6 gene (1C),GAPDH with XTH3, XTH4 & XTH8 gene(1D).

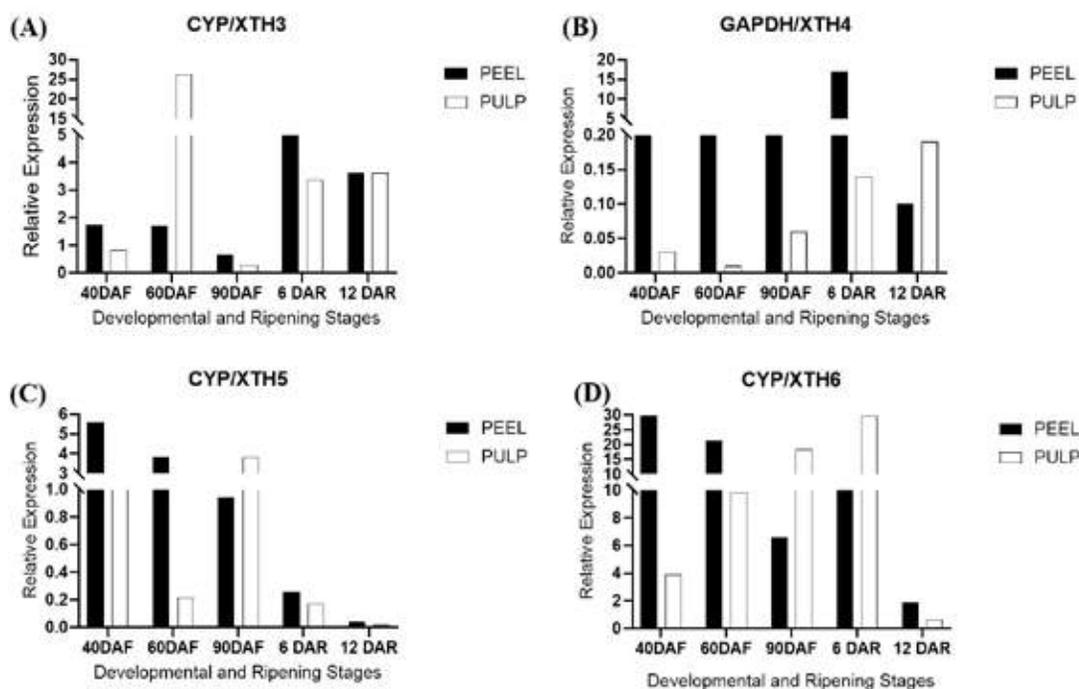


Figure 2A-D: Relative gene expression profiles of XTH3 (2A), XTH4 (2B), XTH5 (2C) and XTH6 (2D) during different developmental and ripening stages, and tissues of banana. The relative transcript abundance was normalized using reference genes CYP and GAPDH. Quantitative real time PCR of the genes was carried out using cDNA which was synthesized from the total RNA isolated from the respective stages and tissues of banana fruit.

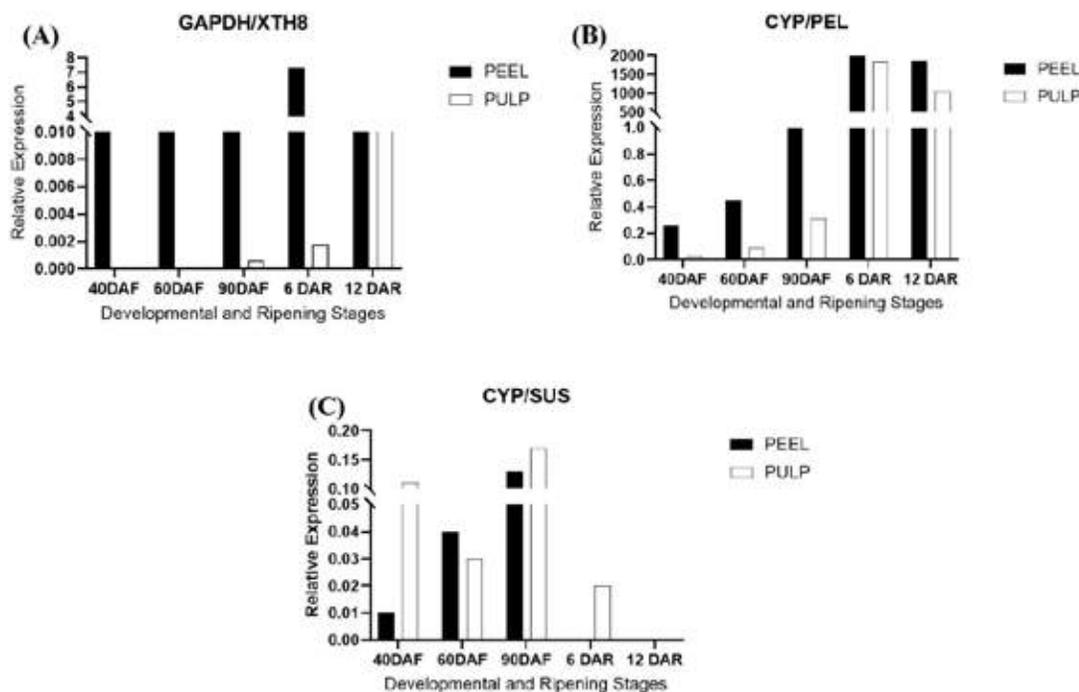


Figure 3A-C: Relative gene expression profiles of XTH8 (3A), PL (3B) and SuSy (3C) during different developmental and ripening stages, and tissues of banana. The relative transcript abundance was normalized using reference genes CYP and GAPDH. Quantitative real time PCR of the genes was carried out using cDNA which was synthesized from the total RNA isolated from the respective stages and tissues of banana fruit.

**Table 1. Selected candidate and reference genes primers sets and amplicon characteristics.**

Sl. No.	Gene	Gene accession No.	Primer Sequence(5'-3')	Amplicon (Tm°C)	Amplicon length (bp)
Housekeeping Genes/ Reference Genes					
1	CYP-F	HQ853241	ATAGCGGGTCCAC-CAAGAAG	59.35	230
2	CYP-R		GGCTCCTGCTGACGATA-ATG	59.35	
3	GAPDH-F	AY821550	GCAAGGATGCCCAATGT	55.97	101
4	GAPDH-R		AGCAAGACAGTTGGTTGT-GCAG	60.25	
Gene of interest/Candidate Genes					
5	XTH3-F	FJ264506	GACAGGATGAGGTGGGTG-CAGAAGA	66.26	222
6	XTH3-R		TGCTAATCCGGTAGACG-CAGAACAGA	64.80	
7	XTH5-F	FJ264508	CACATTCCCGGACTGC-GATTACGTC	66.26	247
8	XTH5-R		CTTCGAACACCAATC-CCCGATGCTC	66.26	
9	XTH6-F	FJ264509	TGCTACGACCAGCATCGA-TATGGCA	64.62	167
10	XTH6-R		GATGGTTGATCGTCGACG-GCACTTG	66.26	
11	XTH4-F	FJ264507	CGACTGATGGCTGCTGGAT	58.82	101
12	XTH4-R		TCCATCTTTTACATA-CAAAACGGAACT	58.89	
13	XTH8-F	FJ264511	TACAATACTGCAACGAC-GCCAAGC	64.62	204
14	XTH8-R		CCTGAGAACATGGTTTGC-GCAGGTT	64.62	
15	PL1-F	EC 4.2. 2.2	TGCT-CATTTCTCTTTTCACG	56.53	153
16	PL1-R		TCCAAGTCAAGTAGTAT-CAACACA	59.70	
27	SuSy-F	chr10:25291305.25295732	AACTACAAGGGCATGTC-GATG	57.87	270
28	SuSy-R		GGTGTGATGCGATACT-CAATAG	61.01	

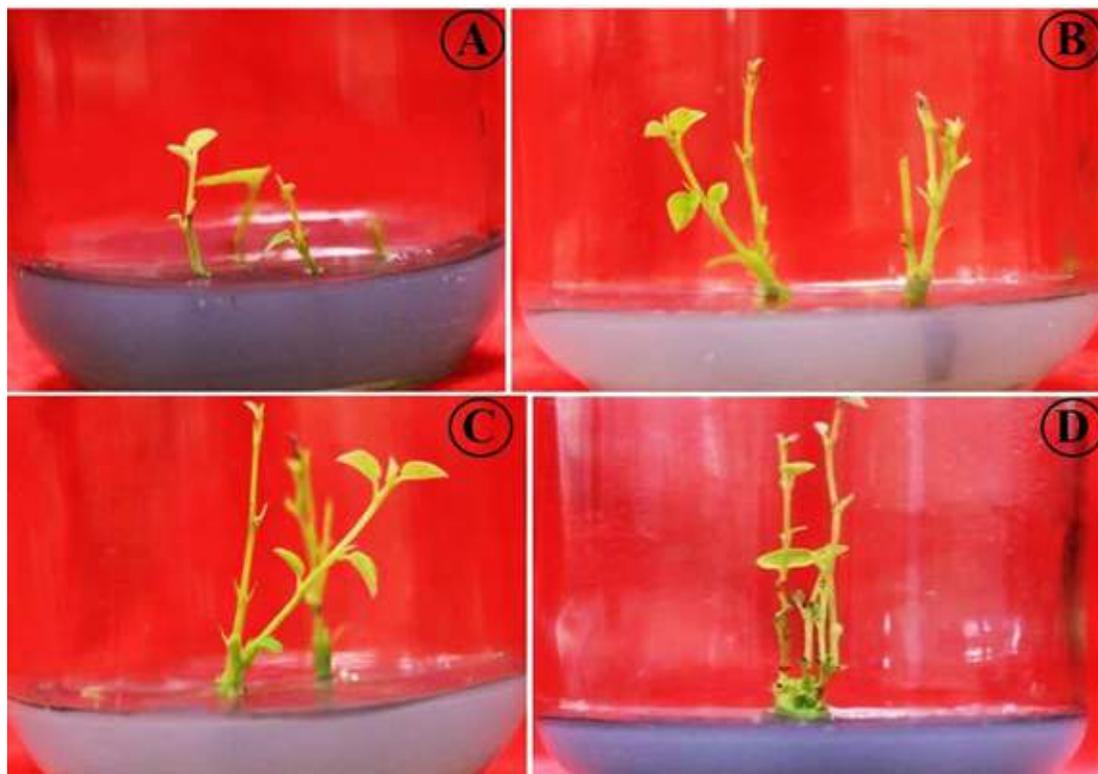
#### 4.2.5. Developing efficient micro-propagation methods for some RET listed forest tree species of Odisha.

Principal Investigator: Dr. Giridara Kumar Surabhi, Senior Scientist.

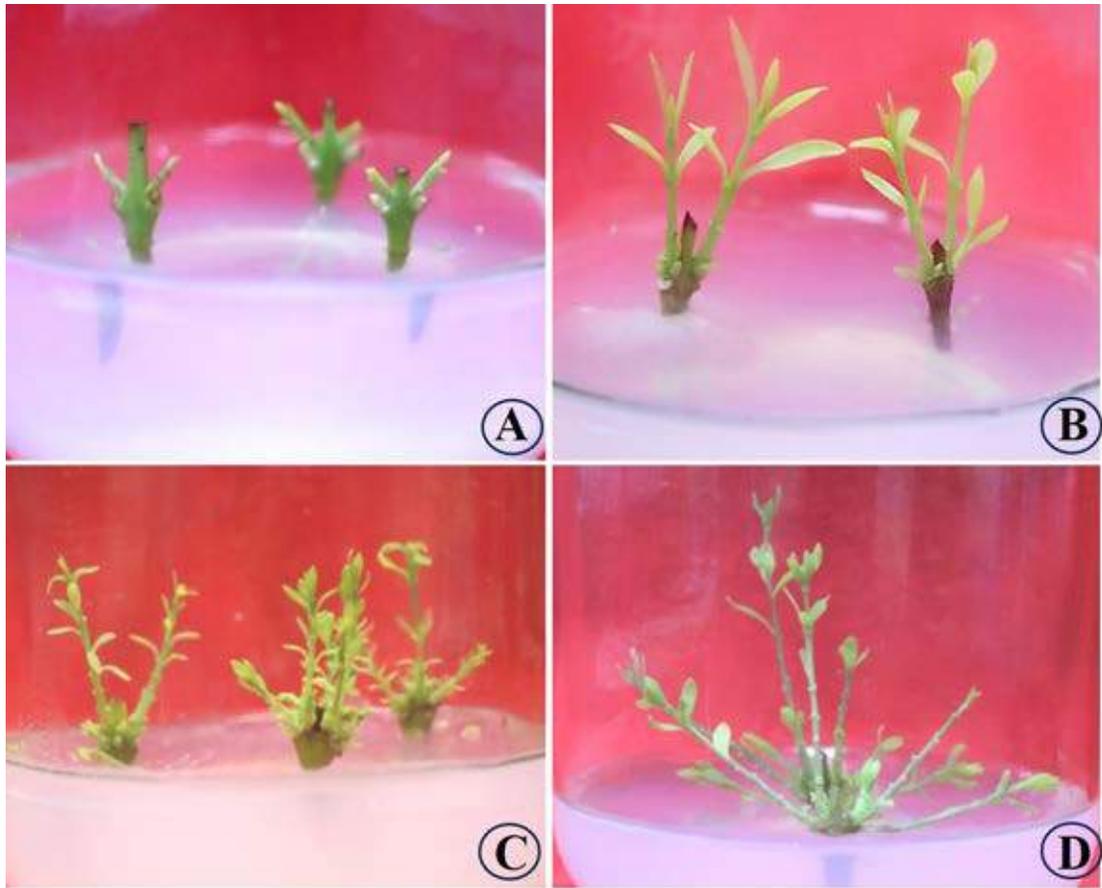
Sandalwood, also known as Chandan (*Santalum album* L.), is a valuable and economically significant member of the Santalaceae family. Its aromatic heartwood contains sandal oil, which is utilized in the agarbathi, cosmetics, perfume, and medicinal sectors. *Santalum album* is recalcitrant to both *in vitro* and *in vivo* multiplication, thus limited success so far. Another uncommon and endangered forest tree *Pterocarpus santalinus* L. belongs to the family Fabaceae, referred as red sanders, is extremely important both ecologically and economically.

Due to a hard seed coat and low viability, seed propagation is frequently quite challenging. In addition, Fabaceae family's members have proven challenging to propagate *in vitro* because of their recalcitrant nature.

*In vitro* propagation of forest tree species involves three crucial steps: (i). induction and growth of shoot, (ii). induction and growth of multiple shoots, and (iii). rooting. In this study, successfully established the protocol for the shoot induction and growth and multiple shoot induction and growth (Fig.1A-D & Fig.2A-D). Further, efforts are on in induction of root for both the tree species.



**Fig.1A-D:** Shoot induction and growth, and multiple shoot induction and growth in *P. santalinus*, (A-C) shoot induction and growth on MS+ BAP1.0mg/L +0.5 mg/L NAA +additives +activated charcoal, (D) multiple shoot initiation and growth on MS + BAP 1.0mg/L + NAA 0.5 mg/L + additives + activated charcoal.



**Fig.2A-D:** Micropropagation stages of *Santalum album*, (A) nodal explants showing shoot induction on MS+1.0mg/L BAP, (B) shoot growth on MS+1.0mg/L BAP, (C) and (D) development of multiple shoots on MS+1.0mg/L BAP + additives.

#### 4.2.6. Standardization of in vitro regeneration techniques in red banana and establishment of red banana in Odisha climate condition

Principal Investigator: **Dr. Kalidass C., Scientist**

##### ***Efficient Micropropagation Protocol for Red Banana:***

This project aimed to develop a standardized and efficient protocol for micro-propagating red bananas using sward suckers on Murashige and Skoog's (MS) medium. We investigated different auxin hormones and surface sterilant to establish a contamination-free aseptic in vitro culture.

##### ***Optimizing Root Formation:***

The experiment identified optimal root formation in treatments containing IBA 1.0 mg/L (T2) or IAA 1.5 mg/L (T1). IBA played a significant role, with the highest average root number observed in T2 (11.81 roots). Conversely, the lowest root numbers were found in the control group (2.32 roots) and the treatment with NAA 0.5 mg/L (3.40 roots). and control (2.32). This ongoing experiment has demonstrated significant progress in the micro-propagation of red bananas.

**Acclimatization and Potting Media:**

Following successful *in vitro* rooting, plantlets were transferred for acclimatization through primary and secondary hardening stages using various potting media: soil mixture, vermicompost, cow dung and garden soil.

**Primary Hardening:** Vermicompost demonstrated the highest survival rate (95.26%) compared to the control group (78.56%) which lacked a balanced media dose. Vermicompost also significantly influenced leaf and root growth parameters, with the highest values observed for leaf length (4.81 cm), root length (22.33 cm), number of leaves (8.66) and leaf width (2.88 cm) compared to the control group. Treatments 3 and 4 showed statically similar results (Fig.1).

**Secondary Hardening:** A potting media mix of cow dung, garden soil and vermicompost (T2) resulted in the highest survival rate. Treatments 2 and 3 were statistically similar, while the control group exhibited the lowest survival rate. This demonstrates that diverse potting media combinations can increase plant survival and reduce mortality (Fig.2).



The figure shows the propagation of the media bed and the transfer to the primary hardening chamber of red banana plants

Fig.1: A view of the primary chamber in different growth media: (a) Soil Mixture and (b) Vermicompost.



Fig.2: A view of the secondary chamber, showing tissue culture banana plantlets ready for sale.

#### 4.2.7. Developing protocols for spawn production and cultivation of few selected wild edible mushroom species in Odisha

Principal Investigator: Dr. Kalidass C., Scientist

Wild mushrooms thrive in natural environments and are primarily consumed by indigenous communities near forests. Rich in protein, vitamins, minerals and antioxidants, they offer a valuable source of nutrition and food security. Cultivating these mushrooms can help conserve species, support local farmers and address food in security.

This project focuses on: Collection and development of pure culture of specific wild edible mushrooms. Optimization of spawn production for the cultivation of selected wild edible mushroom species, using various substrates and techniques

The project includes the cultivation of 10 wild mushroom species from Odisha, including *Calocybe indica*, *Termitomyces clypeatus*, *Termitomyces medius*, *Termitomyces microcarpus*, *Russula cyanoxantha*, *Russula lepida*, *Russula emetica*, *Amanita losii*, *Amanita caesarea* and *Tuber rufum* (Fig.1). Tissue culture techniques are used to develop pure cultures, produce spawn, and cultivate fruiting bodies, which will then be reintroduced into their natural habitat for the restoration. Different culture media, such as potato dextrose agar (PDA) and malt yeast extract agar (MYA) were tested, along with substrates like wheat and sorghum, MYA gave the best results for *Calocybe indica*, and sorghum was most efficient for spawn production. Healthy fruiting bodies were achieved with treated paddy straw beds at temperatures above 25°C and humidity above 70%.



Fig.1:A. *Termitomyces clypeatus* B. *Russula lepida* C. *Russula rosea* D. *Termitomyces heimii* E. *Russula breviceps* F. *Russula cyanoxantha* G. *Termitomyces microcarpus* H. *Ganoderma lucidum* I. *Tuber rufum* J. *Calocybe indica*

### Exploration and Collection of wild mushrooms:

In August, a week-long field visit was conducted in Kandhamal district, focusing on Mandasaru, Raikia, and Baliguda for wild mushroom collection. Mandasaru, a tropical moist deciduous forest, is rich in biodiversity, supporting both edible and inedible mushrooms. The Sal forests of Raikia and Baliguda are ideal habitats for *Russula* species. Local residents and tribal communities were interviewed to gather information on the mushrooms' edibility, health benefits, potential side effects, and distribution. These mushrooms are not only consumed locally but also sold in markets, providing income. The collected mushrooms included *Calocybe indica*, *Termitomyces clypeatus*, *Termitomyces medius*, *Russula cyanoxantha*, *Russula rosea*, and *Tuber rufum*. *Russula* species were mostly found in Sal forests, while *Termitomyces* species grew near termite colonies, and *Calocybe* species thrived on cellulosic substrates. Pure cultures of the mushrooms were started, and some specimens were preserved in formalin for further study (Fig. 2).



Fig 2: A view of Survey, collection and interview with senior indigenous tribe groups and local communities with confirmed

### Milky mushroom pure culture, spawn & fruiting bodies preparation:



Fig.3: a.Pure culture, b.Spawn, c.Fully matured fruiting body

#### Pure Culture Preparation:

Young, healthy mushrooms were carefully cleaned using distilled water, dried with tissue paper and sliced length wise. The tissue at the junction of the stipe was removed, and the slices were then inoculated onto agar media plates. These petri plates were incubated for four weeks at 20+1°C. After a few days, a petri plate filled with mycelial growth produced a pure culture.

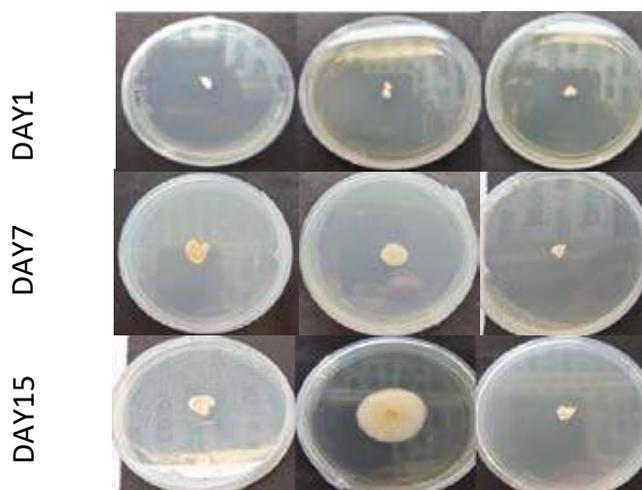


Fig 4 Comparative study of different media for pure culture preparation in which MYA (malt yeast agar) showed better results than PDA (potato dextrose agar) and MEA (malt extract agar).

#### Spawn Substrate Preparation:

Sorghum grains were boiled for one hour, and dried. They were mixed with 30-40g of CaCO<sub>3</sub> per kg of grain, packed into polypropylene bags or bottles and sterilized. Under aseptic conditions, the mother culture was inoculated and inoculated for 2-3 weeks, resulting in fully colonized spawn (Fig3b).

#### Bedding Substrate preparation:

Paddy straw was cut, soaked for 48 hrs, drained and treated with hot water for 45-60 mins. Air-drying 65-70% moisture, chalk powder was added to adjusted the pH. Spawn was inoculated in layers of straw and spawn inside a bedding bag with holes for air exchange. The bags were sealed and incubated at temperatures above 25°C with 70-90% humidity (Fig5).



*Fig5: Views of substrates soaked 48 hrs and 1 hour of hot water treatment then mixed with calcium carbonate for avoiding the contamination and final bed is ready and placed for the experimental conditions*

### **Casing:**

The vermicompost was autoclaved for 45 min, then left to 24 to 72 hrs and before being mixed with chalk powder. A 2 cm layer of this mixture was applied on the top of spawn run bags and the casing layer was kept moist by regular watering (Fig 6).

### **Cropping:**

After introducing fresh air and maintaining a temperature above 25°C, it took 10-15 days for the mycelium to reach the top of the casing layer. Fruiting body initiation occurred within 3-5 days, starting as pin heads, which matured in about a week. Mushrooms, measuring 7-8 cm diam., were harvested by twisting, then cleaned and packed in perforated polythene or polypropylene bags (Fig7).



*Fig6: A view of bed preparation after mycelium developed, then vermicompost casing*



Fig7: Different stages of Development of Milky Mushroom (Starting Pinheads to Fruiting Bodies Which Takes Near About 7-10 days)



Fig.8: A view of flow chart involved the different steps in pure culture, spawn and mushroom development

## Conclusion

Mushrooms are a valuable source of vitamins, minerals and other essential nutrients. However, wild edible mushrooms are declining in forest due to changing rainfall patterns and rising temperatures. To address this, it is important to conserve these species by reintroducing them into forests and promoting cultivation through various methods. This can create self-employment opportunities and combat food insecurity. Research has developed a cultivation protocol for milk mushrooms, focusing on factors like temperature, humidity, vermicomposting, and agar media. Standardizing media and substrates is vital for efficient spawn production and successful cultivation.

## 4.3. Medicinal plant and its application

### 4.3.1. The protective diabetic neuropathy effect of *Buchanania lanzan* Spreng in Streptozotocin- induced type 2 diabetic rats

**Principal Investigator: Dr. Atish Kumar Sahoo, Senior Scientist**

This study aimed to evaluate the antioxidant, antidiabetic, and anticholinesterase activities of *Buchanania lanzan* Spreng seeds. The therapeutic significance of phytochemicals, minerals, and nutrition were quantified. Hydroalcohol-extract (HABL) possessed the highest phenol contents demonstrated superior inhibitory potential against oxidants in DPPH, CAP-e, and imparted total antioxidant capacity in ORAC test. HABL exhibited mixed-competitive inhibition against  $\alpha$ -amylase and  $\alpha$ -glucosidase. HABL exhibited mixed-competitive and competitive inhibition against acetyl-, and butyryl-cholinesterase. Seeds were a rich source of minerals, amino acids, protein, carbohydrates, volatile oil, and energy content. Reported Vit B3 and B6 in seeds were responsible for brain function, and neurotransmitter-synthesis. HPLC quantified phenols (gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin), and GC-MS identified (Catechol, 2-Hydroxy-5-methylbenzaldehyde, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, 8-Octadecenoic acid, methyl ester, 9,12-Octa-decadienoic acid, ethyl ester, (E)-9-Octadecenoic acid ethyl ester, Heptadecanoic acid, 15-methyl-, ethyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxy-methyl)ethyl, and  $\beta$ -Sitosterol) in HABL exhibited antioxidant, antidiabetic, and anticholinesterase activities. *B. lanzan* seeds are given adequate attention for making medicines and dietary supplements in the pharmaceutical and nutraceutical industries.

Diabetes mellitus is a well-known metabolic syndrome that originates from a complete or virtual requirement of insulin and low insulin action, which leads to hyperglycemia and malformation in the regulation of lipids, proteins, and carbohydrates. Among the different types of diabetes, the most prevalent form is type 2 diabetes mellitus (T2DM) which accounts for 90% of cases. The global prevalence of T2DM is rapidly increasing with more than half a billion affected as a result of aging populations and lifestyle changes. Prolonged undiagnosed diabetes or improper management can lead to micro and macrovascular complications such as neuropathies, nephropathies, retinopathies, and cardiomyopathies affecting multiple organs such as the heart, blood vessels, nerves, eyes, and kidneys. Diabetic neuropathy, a chronic microvascular complication of diabetes, resulting from diffuse and focal nervous system damage leads to significant morbidity and mortality.

The study included Material collection, Gradient solvent extractions and fractionation of hydroalcohol extract, Spectrophotometric quantification of secondary metabolites contents of *B. lanzan*, Estimation of Total carbohydrate, energy, and volatile oil content, Estimation of radical scavenging assay and *In vitro* anti-diabetic activities of *B. lanzan*.

### **Plant material collection.**

The fruits of *Buchanania lanzan* Spreng. (Anacardiaceae) were collected in April 2021 from Harishankar, Balangir Forest Division, Balangir, Odisha, India (Latitude: 20° 45.5643'N; Longitude: 82°53.8893'E) and validated by Dr. C. Kalidass, Scientist, Taxonomy and Conservation Division, Regional Plant Resource Centre, Bhubaneswar, India. The voucher specimen of the sample was deposited at the herbarium of the Centre (1990/RPRC).

### **Gradient solvent extractions and fractionation of hydroalcohol extract**

The seeds were dried in the shade for 1 week and then ground into a coarse powder. The powdered sample (500 g) was extracted by hexane, chloroform, ethyl acetate, and methanol sequentially using soxhletor for each extraction of 72 h. Also, the hydroalcohol extraction (70% ethanol and 30% distilled water) and water extraction of each powdered seed sample (100 g) were carried out continuously by hot maceration for 72 h. Then, hexane (HBL, 2.2%), chloroform (CBL, 7.6%), ethyl acetate (EABL, 8.3%), methanol (MBL, 7%), hydroalcohol (HABL, 10.3%), and water (WBL, 9.2%) extract were concentrated under reduced pressure using a rotary vacuum evaporator (R-100, Buchi, Switzerland). All extracts were desiccated at 4°C for further experimentation.

### **Spectrophotometric quantification of secondary metabolites contents of *B. lanzan***

The phytochemical profiles of HBL, CBL, EABL, MBL, HABL, and WBL fractions of *B. lanzan* revealed a significant presence of secondary metabolites belonging to phenols, flavonoids, tannins, steroids, and alkaloids. Spectrophotometric quantification indicated that the HABL fraction had a higher level of total alkaloids (TAC; 140.91 mg ATEqv/g), phenolics (TPC; 207.33 mg GAEqv/g), and flavonoids (TFC; 214 mg QEeqv/g) compared to other fractions. HABL fraction exhibited elevated levels of alkaloids, phenols, and flavonoids, suggesting a greater likelihood of possessing antioxidant, anti-apoptotic contributions to neuro-protection, and anti-diabetic properties with the ability to reduce reactive oxygen species, suppressing proinflammatory signalling, and mitigating oxidative stress.

### **GC/MS analysis of HABL**

GC-MS analysis of HABL allowed for the identification of various compounds by comparing their mass spectra with the standard mass spectra found in the NIST library (Version-11). A total of 15 different compounds were successfully identified with their chemical classes of alcohols, aldehydes, fatty acid esters, fatty acids, and steroids. The identification of each compound was established based on specific parameters like peak number, retention time (RT), molecular formula, molecular weight, peak area, and m/z (mass-to-charge) ratio. Out of 15 compounds, 12 compounds were reported to have possessed antioxidant, antidiabetic, and anticholinesterase activities. Such compounds were Catechol, 2-Hydroxy-5-methylbenzaldehyde, Hexadecanoic acid, methyl ester, n-Hexadecanoic, Hexadecanoic acid, ethyl ester, 8-Octadecenoic acid, methyl ester, 9,12-Octa- decadienoic acid, ethyl

ester, (E)-9-Octadecenoic acid ethyl ester, Heptadecanoic acid, 15- methyl-, ethyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl,  $\beta$ -Sitosterol.

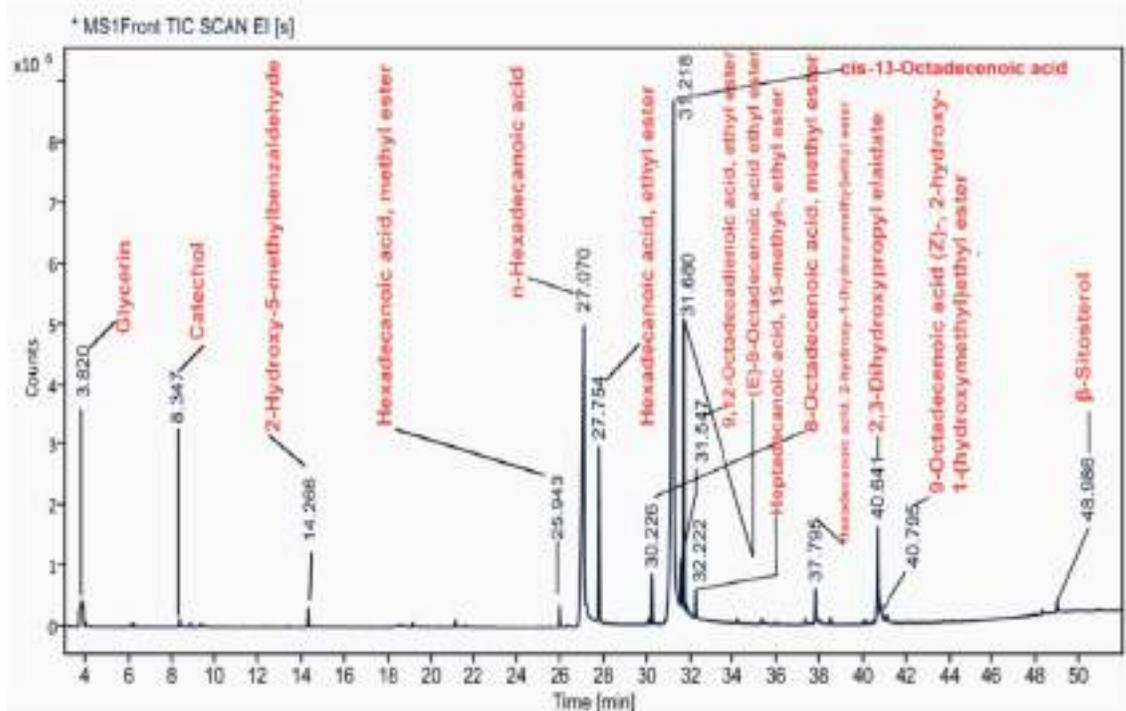


Fig. 1. GC-MS chromatograms and list of identified compounds in the seeds of *B. lanzan*.

### Estimation of Total carbohydrate, energy, and volatile oil content

The carbohydrate content was determined by subtracting the water content, ash content, protein content, and fat content from its 100%. This method relies on a reduction factor to calculate the carbohydrate content, as carbohydrates significantly impact the composition of other nutrients (AOAC, 2012, pp. 237-242). The reason behind this approach is that carbohydrates exert a substantial influence on the overall nutrient profile. The energy content is ascertained using the total calorie calculation method, represented as the below equation. Protein is assigned an energy value of 4 kcal/g. Similarly, the assigned energy value of fat is 9 kcal/g and carbohydrates are 4 kcal/g for energy calculation purposes.

The HPLC analysis was conducted by using a Shimpack column measuring 4.6 mm  $\times$  150 mm with a particle size of 3  $\mu$ m, maintained at a column oven temp of 35°C. Mobile phase A consisted of 25 mM HK<sub>2</sub>PO<sub>4</sub> adjusted to pH 6.5, while mobile phase B was methanol. The HPLC system was configured with an injection volume of 20  $\mu$ L, a flow rate set to 0.45 mL/min, and a sample thermostat temp of 5°C. Data acquisition was performed over 25 min. A gradient elution method was also employed, commencing with B (1%), gradually increasing to 40% over 10 min, maintaining this concentration until 15 min, then decreasing to 1% by 20 min. The analysis concluded at 25 min with the controller stopping. This protocol ensured efficient separation and analysis of the target compounds within the specified parameters.

The mineral content of the samples was assessed through the wet digestion technique, utilizing perchloric acid as the solvent. Subsequently, the digested samples underwent filtration via glass filters and were diluted to a final volume of 100 mL using distilled water. Atomic Absorption Spectrometry (AAS) was employed to determine the mineral concentration of the samples.

The method of Sadasivam and Manickam (1992) was followed to determine the crude fat content in *B. lanzan* seeds. A thimble containing 2 g of dry material was placed in the Soxhlet apparatus. Pre-weighed solvent flasks ('a' g) were placed below the apparatus, and the appropriate amount of petroleum ether was added. The condenser was connected to extract the sample for 16 h at a temp to collect distillate at a rate of 2–3 drops per h. After removal of the thimble Once the thimble was removed, the ether was retained in the apparatus. The excess ether in the solvent flask was evaporated using a hot water bath and then cooled before being weighed.

A. Proximate analysis, B. Presence of amino acids, C. Mineral analysis, and determination of Vitamin B-complex, and D. GC-MS analysis of Composition and Quantification of Phenolics of *B. lanzan* seeds.

Parameters		A. Proximate analysis																
		Protein	Moisture	Fat	Ash	Crude fiber	Carbohydrate	Volatile oil content	Energy									
Concentration (mg/g)		11.09	12.54	10.15	5.20	23.89	73.56	0.86	429.95 Kcal									
B. Presence of amino acids																		
Amino acids	Aspartic acid	Glutamic acid	Serine	Histidine	Glycine	Threonine	Arginine	Alanine	Tyrosine	Cystine	Valine	Methionine	Phenylalanine	Isoleucine	Leucine	Lysine	Proline	
Concentration (mg/g)		1.8922	5.3863	5.0019	2.8369	0.3527	3.4719	1.4879	2.6723	3.5751	0.7692	1.4929	0.6465	1.4319	1.1894	2.4897	1.0660	1.6220
C. Mineral analysis and determination of Vitamin B-complex of <i>B. lanzan</i> seeds.																		
Sl. No.	Mineral analysis (mg/kg)						Vitamin B-complex (Conc. mg/kg)											
1	Sodium as Na						63.87	Vitamin B1			Below Detection Limit (BDL)							
2	Potassium as K						19822.34	Vitamin B2			BDL							
3	Magnesium as Mg						3560.85	Vitamin B3			3.09							
4	Calcium as Ca						5117.94	Vitamin B5			BDL							
5	Manganese as Mn						59.72	Vitamin B6			3.45							
6	Iron as Fe						328.94	Vitamin B9			BDL							
7	Zinc as Zn						21.67	Vitamin B12			BDL							
D. Biochemical estimations and composition of phenolic compounds in <i>B. lanzan</i> seeds.																		
Biochemical estimation		Total Phenol Content (TPC; mg GalEq/g)				Total Flavonoid Content (TFC; mg QE/g)		Total Alkaloid Content (TAC; mg AT/g)										
Quantification		207.33				214		140.91										
GCMS analysis of Composition and Quantification of Phenolics by HPLC (mg/kg)		Gallic acid (26.32), Chlorogenic acid (BDL), Caffeic acid (24.45), Rutin (BDL), Quercetin (3.01)																

### Oxygen radical absorbance capacity assay (ORAC)

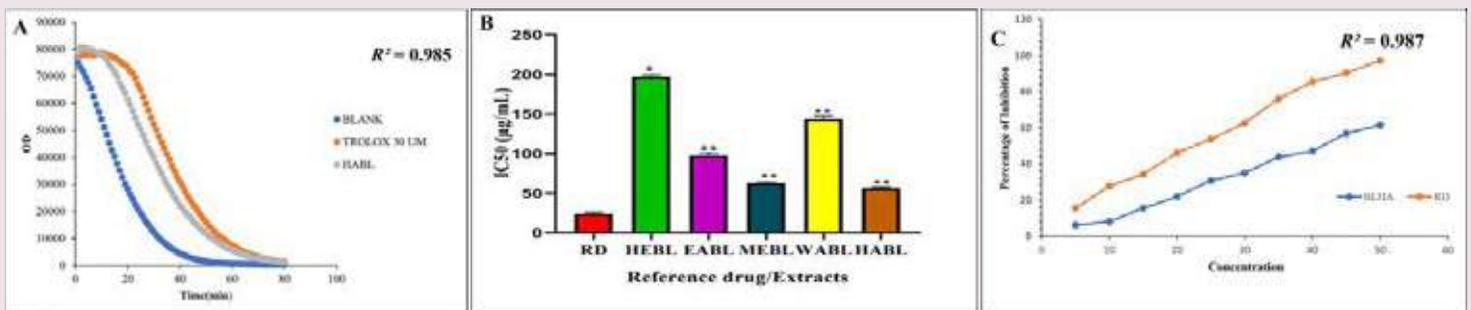
ORAC assay of HABL was performed by measuring the AUC of HABL (14.715) which was found comparable to that of the AUC of standard drug trolox (19.878) (Fig. 2A). The antioxidant capacity of HABL was attributed due to the presence of antioxidant principles e.g., oleic acid, ethyl tri-decanoate, phytol, stigmasterol, and  $\beta$ -sitosterol and the poly phenols like gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin which can quench in situ generated peroxy radicals. As a result, the increase in fluorescent decay was observed with a decrease in the fluorescence intensity of fluorescein.

### DPPH free radical scavenging assay of *B. lanzan*

Radical scavenging activities of various extracts of *B. lanzan* in DPPH assays were performed and it was found that HABL demonstrated a significant ( $p < 0.01$ ) inhibitory potential against free radicals with IC 50  $57.16 \pm 1.09 \mu\text{g/mL}$ , outperforming other extracts which were comparable to the standard drug ascorbic acid ( $25.03 \pm 0.89 \mu\text{g/mL}$ ) (Fig. 2B). The phenol and flavonoid rich free radical scavengers like gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin were responsible for the antioxidant properties of HABL.

### CAP-e assay of *B. lanzan*

CAP-e test was performed to assess the antioxidant potential of HABL in safeguarding live cells from oxidative damage. This evaluation was based on the extent of inhibition observed in DCF-DA fluorescence over time. The protective effect graph depicted in Fig. 2C displayed a dose-dependent reduction in AAPH-induced DCF-DA fluorescence due to HABL, with IC<sub>50</sub> 44.2 ± 0.89 µg/mL. This result was comparable to the reference drug gallic acid (IC<sub>50</sub> 23.44 ± 0.62 µg/mL). Notably, HABL exhibited superior cellular protection against oxidative damage owing to its higher phenol content bearing compounds gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin and the presence of phytochemicals oleic acid, ethyl tri-decanoate, phytol, stigmasterol, and β-sitosterol. These compounds possess significant antioxidant properties, enabling them to effectively penetrate and shield living cells from oxidative harm caused by the free radical generator AAPH.



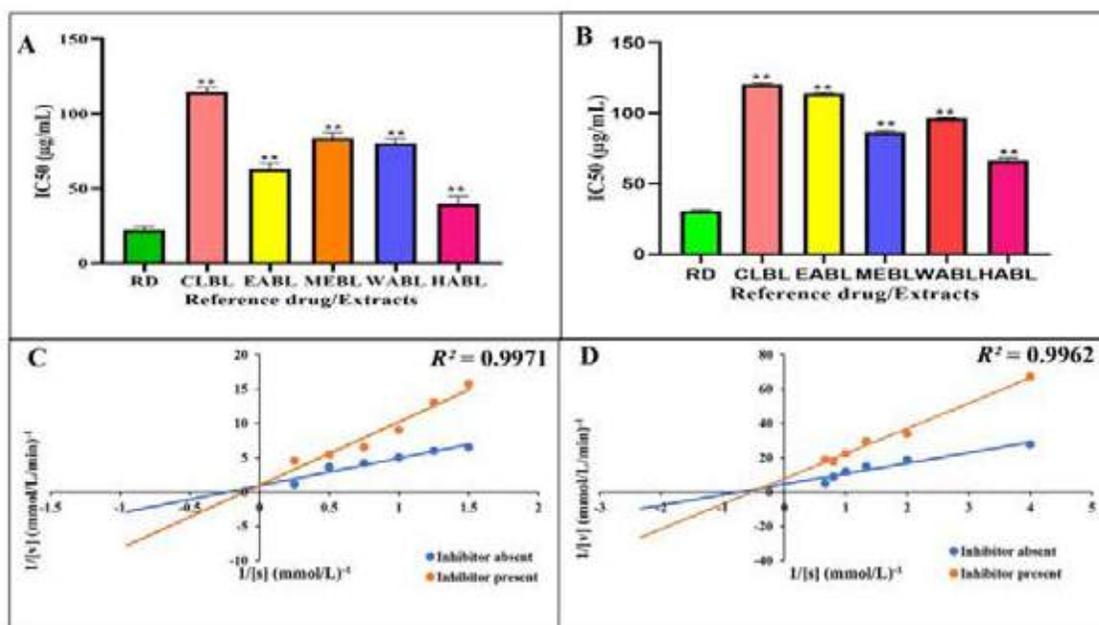
**Fig.2.** ORAC, DPPH, and CAP-e assays of *B. lanzan*. A. Oxygen radical absorbance capacity (ORAC) assay revealed that the HABL achieved an area under the curve (AUC) of 14.715, which was comparable to the reference drug trolox (AUC 19.878). B. DPPH assay of various extracts of *B. lanzan* seeds. Test samples (except HEBL) demonstrated significant (\*\*p < 0.01) activities in scavenging free radicals, but HABL demonstrated impressive capacity in scavenging free radicals with IC<sub>50</sub> 1.09 µg/mL as compared to the reference drug (Ascorbic acid) with IC<sub>50</sub> 57.16 ± 25.03 ± 0.89 µg/mL. C. Cell-based antioxidant protection in erythrocytes (CAP-e) assay, HABL demonstrated impressive capacity in scavenging cellular oxidants with IC<sub>50</sub> ± 0.62 µg/mL. The experiments were conducted in triplicate, IC<sub>50</sub> 44.2 ± 0.89 µg/mL as compared to the reference drug (Gallic acid) with IC<sub>50</sub> 23.44 was determined to access the half maximal inhibitory concentrations and results were expressed as means ± standard deviation by using MS Excel (STDEV, 2010).

### *In vitro* anti-diabetic activities of HABL

#### Inhibition and mode of inhibition of HABL on α-amylase and α-glucosidase

The assessment of HABL's potential to inhibit α-amylase and α-glucosidase activities was conducted as these enzymes play a pivotal role in reducing glucose absorption in the bloodstream. HABL exhibited significant (p < 0.01) inhibition towards α-amylase and α-glucosidase showed IC<sub>50</sub> 46.62 ± 0.51, and 67.46 ± 0.32 µg/mL respectively (Fig. 3A and B). The observed outcomes closely resembled those of acarbose exhibited α-amylase inhibition and α-glucosidase inhibition at IC<sub>50</sub> 30.01 ± 0.49 and 24.48 ± 0.42 µg/mL, α-amylase and α-glucosidase are responsible for breaking down carbohydrates, leading

to elevated postprandial glucose levels in individuals with diabetes. Elevated postprandial plasma glucose level is linked to a range of complications such as hyperglycaemic surges, atherosclerosis, and cardiovascular issues. So, effective management of these levels holds significance in the treatment of diabetes mellitus. Acute hyperglycemia elicits its impacts on the generation of free radicals. Inhibition of these enzymes is regarded as an oral treatment strategy for diabetes, aiming to slow down the absorption of ingested carbohydrates. This approach helps in diminishing the postprandial spikes in glucose and insulin levels, thereby working towards achieving normal blood sugar levels. Hence, HABL is an effective antidiabetic agent due to its phenol-rich compounds like gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin. Also, GCMS reported antioxidant and antidiabetic compounds like Catechol, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, (E)-9-Octadecenoic acid ethyl acetate, 9,12-Octadecadienoic acid, ethyl ester, 8-Octadecenoic acid, methyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester,  $\beta$ -Sitosterol were also responsible for anti-diabetic properties.

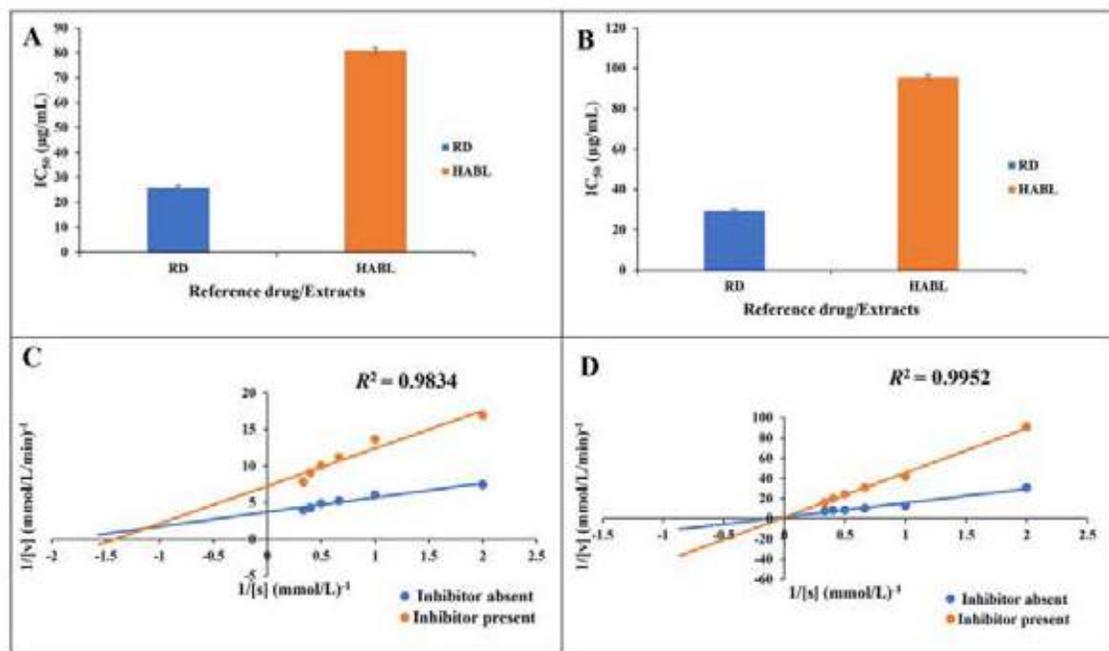


**Fig. 3.** *In vitro* antidiabetic and mode of inhibition studies of *B. lanzan*. **A.** HABL inhibition towards  $\alpha$ -amylase demonstrated IC<sub>50</sub> at  $46.62 \pm 0.51 \mu\text{g/mL}$ . **B.** HABL inhibition towards inhibition towards  $\alpha$ -glucosidase with IC<sub>50</sub>  $67.46 \pm 0.32 \mu\text{g/mL}$ . The IC<sub>50</sub> of acarbose was found to be  $24.48 \pm 0.42$  and  $30.01 \pm 0.49 \mu\text{g/mL}$  for  $\alpha$ -amylase and  $\alpha$ -glucosidase, respectively. Each bar from Fig. A & B. represents mean  $\pm$  SEM with the significance level of  $**p < 0.01$  compared with the reference drug (Acarbose); **C. and D.** Lineweaver-Burk plot of the inhibition modes of  $\alpha$ -amylase ( $V_{\text{max}}$  0.995 approx.), and  $\alpha$ -glucosidase ( $V_{\text{max}}$  0.128 approx.) depicted mixed competitive inhibition towards  $\alpha$ -amylase and  $\alpha$ -glucosidase, respectively and results were expressed as mean  $\pm$  standard deviation by using MS Excel (STDEV, 2010).

### Anti-cholinesterase activities and mode of inhibition of HABL

The current investigation demonstrated acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activities of HABL. HABL showed potent inhibition (IC<sub>50</sub>) towards AChE ( $81.02 \pm 1.09 \mu\text{g/mL}$ ), and BChE ( $95.56 \pm 1.05 \mu\text{g/mL}$ ) as compared to the reference drug galanthamine showed

50% AChE and BChE inhibition at  $25.89 \pm 0.65$  and  $29.32 \pm 0.99$   $\mu\text{g/mL}$ , respectively (Fig. 4 A and B). Acetylcholine and butrylcholine are the neurotransmitters extensively distributed in CNS. In AD, the decrease in the level of these neurotransmitters results in the decline of memory and cognition. Also, it is evident that free radicals cause oxidative damage to brain cells and affect neurotransmitters, which can deteriorate cognitive aging and CNS disorders. It has already been reported that cognitive performance and age-related brain performance can be significantly improved by a diet rich in antioxidants which are the effective cholinesterase inhibitors. Several chemical classes of GC-MS identified compounds (Catechol, 2-Hydroxy-5-methyl benzaldehyde, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, (E)-9-Octadecenoic acid ethyl ester, Heptadecanoic acid, 15-methyl-, ethyl ester, and  $\beta$ -Sitosterol) and phenol quantified antioxidants by HPLC (gallic acid, chlorogenic acid, caffeic acid, rutin, and quercetin) in HABL contributed anticholinesterase activities. The enzyme inhibition kinetics study of HABL against AChE and BChE was conducted using Line weaver-Burk double reciprocal plots, which depicted  $1/[s]$  against  $1/[v]$ , with 's' representing substrate concentration and 'v' representing reaction velocity. Analysis of the inhibition mode through these plots using graphical tools revealed that HABL exhibited a mixed competitive and competitive inhibition for AChE and BChE, respectively. This was evident from the point of intersection on the Y-axis, with  $V_{\text{max}}$  and D).



**Fig. 4.** Anticholinesterase and mode of inhibition of HABL. A&B. The inhibitory activity of HABL against acetylcholinesterase (AChE) with  $IC_{50}$  HABL inhibition activity against butyrylcholinesterase (BChE) with  $IC_{50} 95.56 \pm 1.05$  and  $81.02 \pm 1.09$   $\mu\text{g/mL}$ . In comparison, the reference drug galantamine demonstrated  $IC_{50} 25.89 \pm 0.65$  and  $29.32 \pm 0.99$   $\mu\text{g/mL}$  for AChE and BChE inhibition, respectively. C. Lineweaver-Burk graph presents the inhibition modes of AChE ( $V_{\text{max}}$  0.137) and D. BChE ( $V_{\text{max}}$  0.736) activities across various substrate concentrations ranging from 0.05 to 3 mmol/L. These findings indicated that in the presence of HABL, AChE exhibits mixed competitive inhibition, while BChE exhibits competitive inhibition. HABL, hydroalcohol extract of *B. lanzan*.

## Conclusion

The investigation supports the potential role of *B. lanzan* in the antidiabetic neuropathy condition of type 2 diabetic Miletus. The status of hyperglycemia, oxidative stress, and inflammation associated with T2DM is evidenced by histological architecture of pancreatic islet cells. Investigation of hydroalcoholic fraction of *B. lanzan* has potential antioxidant effect (MDA, SOD, CAT, and GSH) in cellular levels and improving type 2 diabetes-induced neurodegenerative condition by inhibition of  $\alpha$ -amylase,  $\alpha$ -glucosidase and cholinesterase (AChE and BChE) and  $\beta$ -secretase (BACE1 and BACE2) activity in streptozotocin-induced Wister rat model is going on. Future investigations such as QSAR analysis of compounds, various signalling pathways, and mechanism of action of lead compounds in the cortex region of the brain are necessary to further validate its therapeutic use in Alzheimer's disease.

**A study has been undertaken to explore ameliorative effect of *Aporosa octandra*** against carbon tetrachloride-induced oxidative stress and hepatocellular injury in experimental animal model along with the phytochemical investigation of the leaf extracts.

### 4.3.2. Biological evaluation of leaf extracts of *Zingiber zerumbet* and *Hedychium spicatum*.

Principal Investigator: **Dr. Sunita Bhatnagar, Sr. Scientist**

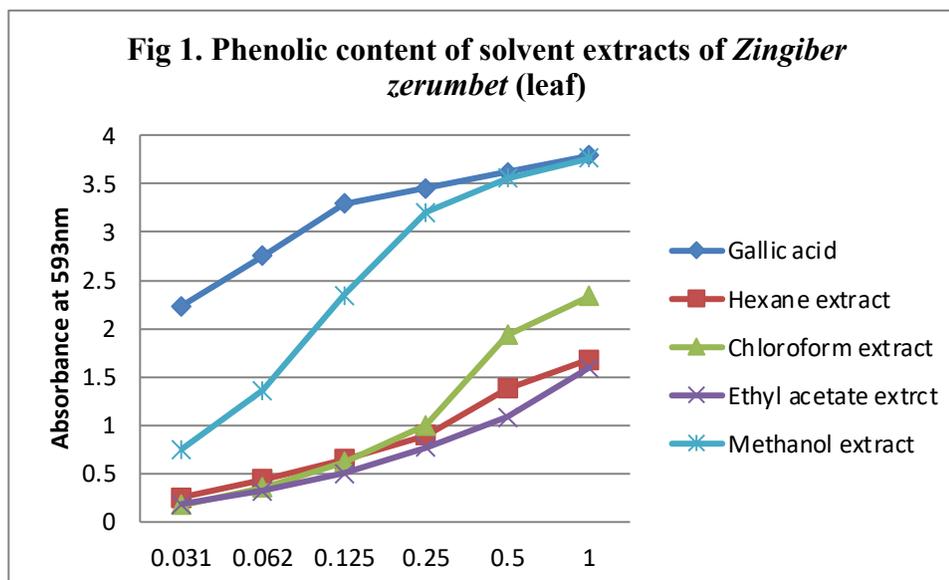
#### *Zingiber zerumbet*

Presence of alkaloid was predominant as can be seen in Table 1. it was found positive in all three tests. Tannin was present in all the extract. Cardiac glycoside was present in all the extracts except chloroform extract. Thus, just like the rhizome of the plant leaf extracts also possessed medicinally important molecules like alkaloids, tannins and cardiac glycosides which are invariably used as anti-inflammatory and antioxidant purpose.

Secondary metabolite	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Alkaloids	+	+	+	+
Mayer's test	+	+	+	+
Wagner's test	+	+	+	+
Dragendroffs test	+	+	+	+
Flavonoids	-	-	-	-
Anthraquinone	-	-	-	-
Saponin	-	-	-	-
Tannin	+	+	+	+
Terpenoids	-	-	-	-
Phlobotanin	-	-	-	-
Cardiac glycoside	+	-	+	+

**Table 1:** Phytochemical analysis of leaf extracts of *Zingiber zerumbet*

As can be seen from Fig 1 at higher dose, absorbance of methanol extract was similar to the standard gallic acid suggesting an amount of Phenolic content equivalent to gallic acid. Whereas other extract possessed less content when compared with the standard molecule.



Antioxidant activity of solvent extracts of *Zingiber zerumbet* was explored using qualitative as well as quantitative assays.

#### Qualitative Antioxidant Activity

Antioxidant activity of solvent extracts of *Zingiber zerumbet* was conducted using TLC based DPPH antioxidant assay. Best separation was obtained in BEA solvent where hexane, chloroform and ethyl acetate extract showed antioxidant bands respectively. Whereas a complete yellow streak was obtained in methanol extract which suggested presence of antioxidant molecules in close proximity. Thus all the extracts of *Zingiber zerumbet* had antioxidant potential.

SAMPLE	SOLVENT	No. of bands	Rf values
HEXANE	BEA	10	0.28, 0.35, 0.38, 0.42, 0.53, 0.56, 0.65, 0.7, 0.79, 0.8
	CEF	Nil	Nil
	EMW	1	0.65
CHLOROFORM	BEA	7	0.27, 0.34, 0.38, 0.41, 0.49, 0.75, 0.8
	CEF	1	0.75
	EMW	Nil	Nil
ETHYL ACETATE	BEA	2	0.75, 0.77
	CEF	Nil	Nil
	EMW	Nil	Nil

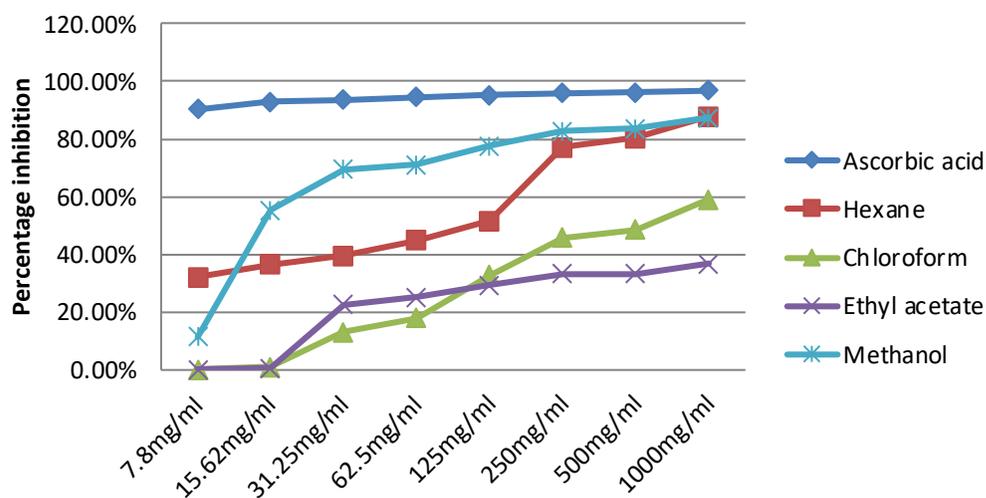
METHANOL	BEA	Complete yellow streak	Infinite number of antioxidant molecules in close proximity.
	CEF	Nil	Nil
	EMW	Nil	Nil

**Table 2:** TLC Based DPPH ASSAY of solvent extracts of *Zingiber zerumbet*

### Quantitative Antioxidant assay

#### DPPH free radical scavenging assay :

The antioxidant activity of different extracts of *Zingiber zerumbet* was analyzed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of free radical scavenger, the absorption decrease and the resulting discoloration related to the number of electrons gained. As can be observed from the (Fig 2), all solvents showed less amount of antioxidant activity as compared to the standard ascorbic acid. However, hexane and methanol extract showed more than 80% inhibition at higher dose of 1mg/mL.



**Fig 2.** DPPH radical scavenging assay of solvent extracts of *Zingiber zerumbet*

#### Ferric reducing antioxidant power assay :

The antioxidant can donate an electron to free radicals, which leads to neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of  $Fe^{3+}$  to  $Fe^{2+}$  in the presence of TPTZ. The product was visualized by forming an intense blue colour complex and then measured at 593nm. As can be seen from the Fig 3, Ethyl acetate extract showed better antioxidant value at higher dose in comparison to the standard molecule.

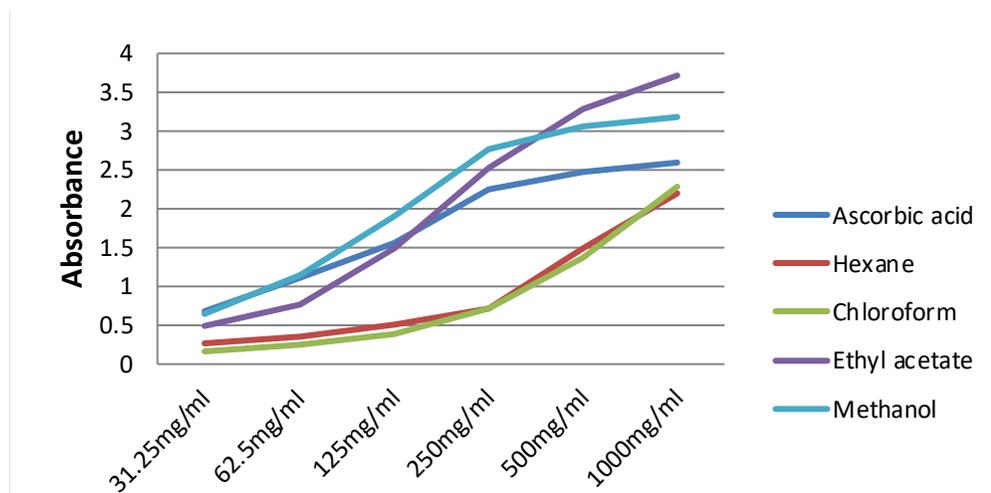


Fig 3. Ferric reducing ability of different extracts of *Zingiber zerumbet*

### Cytotoxic activity using brine shrimp assay

All the extracts were tested in three doses (2000, 1000, 500 microgram/ml). Cytotoxic activity was found highest in ethyl acetate extract (45.16%) at the dose 2000microgram/ml followed by chloroform extract which showed 37.2% activity at the dose 2000microgram/ml. Remaining extracts showed mild activities against brine shrimp assay.

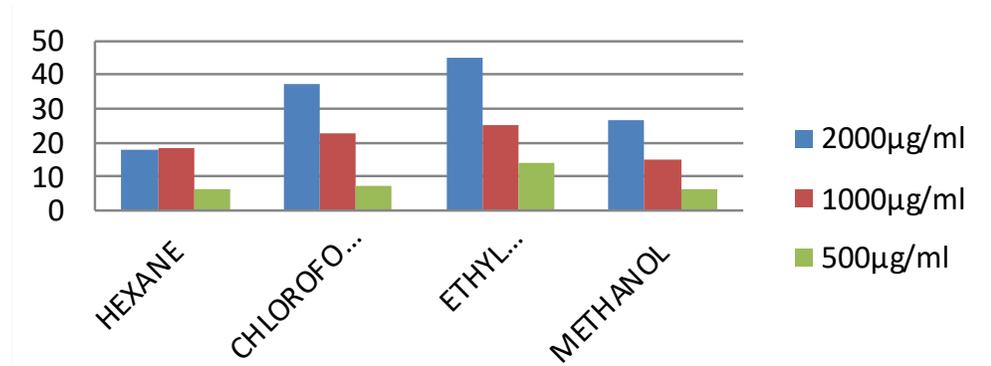


Fig 4. Cytotoxic activity of solvent extracts of *Zingiber zerumbet*

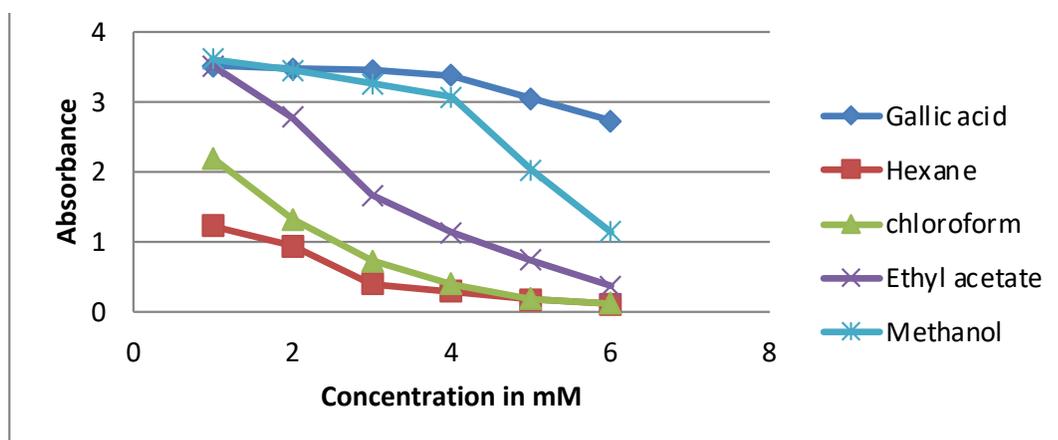
### Hedychium spicatum

Phytochemical screening of the plant material revealed the presence of alkaloids and tannins. The tannins have anti proliferative and apoptotic effects against a variety of compounds and protect the plants from being infected by bacteria or fungi. All the tests for alkaloids were positive in all the solvents. Methanol extract showed the presence of terpenoids and cardiac glycosides as well. Terpenoids are used by plants as primary as well as secondary metabolite, as primary metabolite it provides support in the growth of plants and as secondary metabolites it acts as protective agent for plants. Cardiac glycosides are considered as potent anticancer agents as they target senescent cells in the body. Thus, phytochemical analysis of leaf extracts has provided a lead that leaves of the plant are also rich in medicinal potential.

Secondary metabolite	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract
Alkaloids	+	+	+	+
Mayer's test	+	+	+	+
Wagner's test	+	+	+	+
Dragendroffs test	+	+	+	+
Flavonoids	-	-	-	-
Anthraquinone	-	-	-	-
Saponin	-	-	-	-
Tannin	+	+	+	+
Terpenoids	-	-	-	+
Phlobotanin	-	-	-	-
Cardiac glycoside	-	-	-	+

**Table 1:** Phytochemical screening of leaf extract of *Hedychium spicatum*

Total phenolic content of leaf solvent extracts of *Hedychium spicatum* was determined using Gallic acid as standard. As can be seen from Figure 1, at higher concentration methanol extract exhibited similar phenolics content as the standard molecule. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging.



**Table 1:** Phenolic content of solvent extracts of *Hedychium spicatum*

## Anti-oxidant Activity of *Hedychium spicatum* :

### Qualitative Antioxidant Screening

As per the standard protocol, all the extracts were run in three different solvents i.e. CEF, BEA and EMW. DPPH is reduced and resulting molecule gives a yellow band when chromatograph is sprayed with 0.2% DPPH solution. Best separation was obtained in BEA solvent where bands all the extracts showed maximum number of antioxidant activity with maximum number of 8 bands in the chloroform extract. Number of bands is directly correlated with the extent of antioxidant activity of the extract.

Solvent extract	Solvent system	No. of bands	Rf values
Hexane	BEA	0	0
	CEF	4	0.21, 0.23, 0.25, 0.42
	EMW	0	0
Chloroform	BEA	3	0.21, 0.24, 0.4
	CEF	3	0.7, 0.75, 0.78
	EMW	2	0.13, 0.31
Ethyl acetate	BEA	0	0
	CEF	3	0.42, 0.52, 0.7
	EMW	0	0
Methanol	BEA	0	0
	CEF	2	0.46, 0.55
	EMW	0	0

**Table 2:** TLC based DPPH ASSAY of solvent extracts of *Hedychium spicatum*

### Quantitative Antioxidant Activity

#### TLC based DPPH Assay

The reactivity of different extract of *Hedychium spicatum* was analysed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of free radical scavenger, the absorption decreased and the resulting discoloration was related to the number of electrons gained. The DPPH free radical scavenging activity of different extract (Hexane, Chloroform, Ethyl acetate, Methanol) was determined by spectrosopic assay at 517nm. *Hedychium spicatum* hexane extract exerted an inhibition of 73.26% at 31.25µl/ml, chloroform extract exerted an inhibition 41.75% at 62.5µl/ml, ethyl acetate extract exerted an inhibition of 91.75% at 31.25µl/ml, methanol extract exerted an inhibition of 95.99% at 250µl/ml and that of ascorbic acid was 97.09% at 250µl/ml. Methanol extract's activity was similar to the standard molecule ascorbic acid.

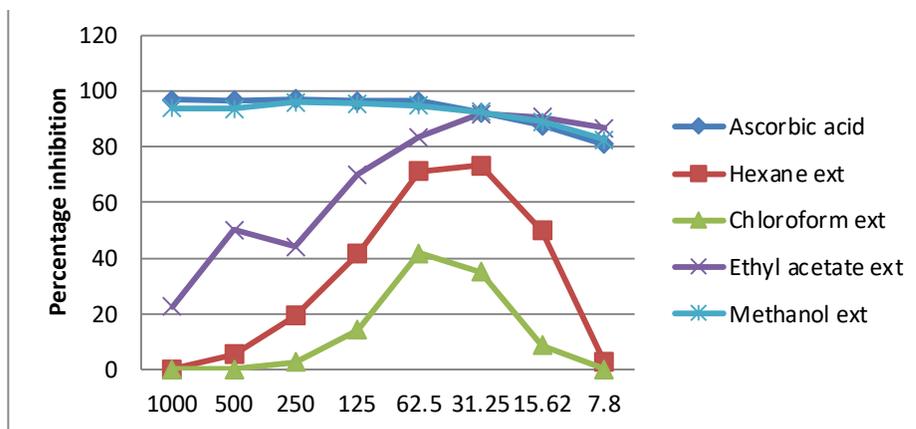


Fig 2: DPPH radical scavenging assay of solvent extracts of *Hedychium spicatum*

**Ferric reducing antioxidant power assay**

The antioxidant can donate an electron to free radicals, which leads to neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> in the presence of TPTZ. The product was visualized by forming an intense blue colour complex and then measured at 593nm. As can be seen in the Fig 3, none of the extracts showed similar activity when compared with the standard molecule ascorbic acid. All the extract showed mild activity.

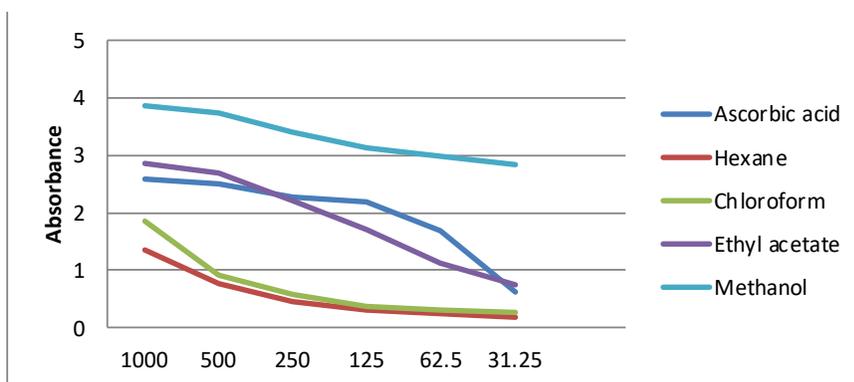


Fig 3. FRAP assay of solvent extracts of *Hedychium spicatum*

**Cytotoxic activity of solvent extracts of Hedychium spicatum**

All the extracts showed mild activity as can be seen from Fig 5.

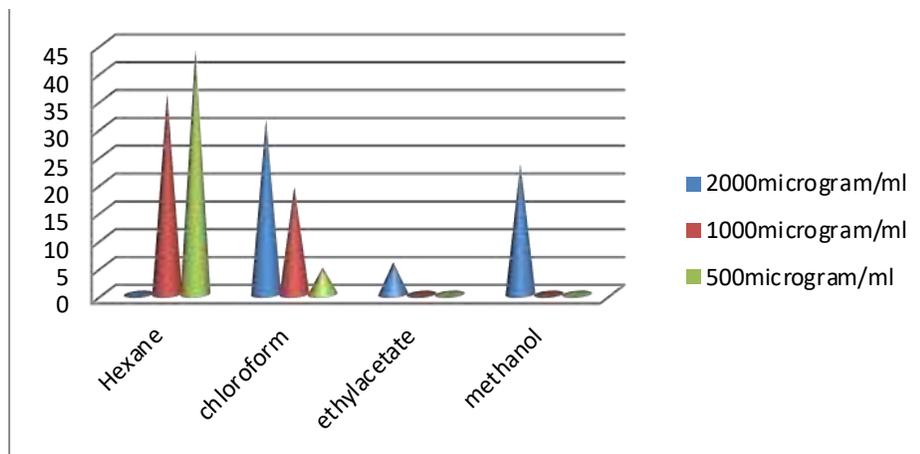


Fig 4: Brine shrimp assay of solvent extracts of *Hedychium spicatum*

Overall it can be concluded that methanol extract of the plant holds promise as a potential antioxidant candidate.

### 4.3.3. Exploration of cytotoxic potential of methanolic leaf extract of medicinal plant *Crinum defixum*.

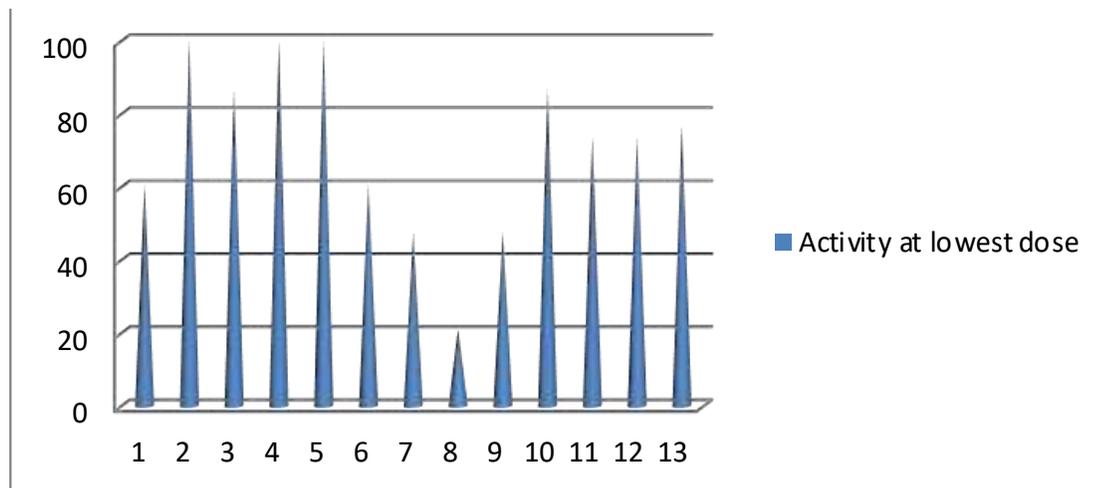
Principal Investigator: **Dr. Sunita Bhatnagar, Sr. Scientist**

Moisture content was quite high for the leaf it was 86.16% and yield of methanol extract was 49.6% and rest was residual mass. On the basis of TLC analysis 70 fractions were reassembled into 13 major fractions. Thin layered analysis of the fractions is shown in Table 1. It can be observed that majority of fractions possess more than one compound, Only fractions 8, 12 and 13 showed single band.

Fraction	Rf Values	No. of bands
1	0.48,0.5	2
2	0.3,0.36,0.38,0.42,0.45,0.47,0.51,0.52	8
3	0.12,0.3,0.38,0.49,0.53	5
4	0.12,0.3,0.37,0.49,0.52,0.54	6
5	0.41,0.45,0.74,0.78	4
6	0.42,0.45,0.79	3
7	0.13,0.32,0.37,0.47,0.5	5
8	0.48	1
9	0.11,0.52,0.54	3
10	0.47,0.54	2
11	0.37,0.4	2
12	0.45	1
13	0.5	1

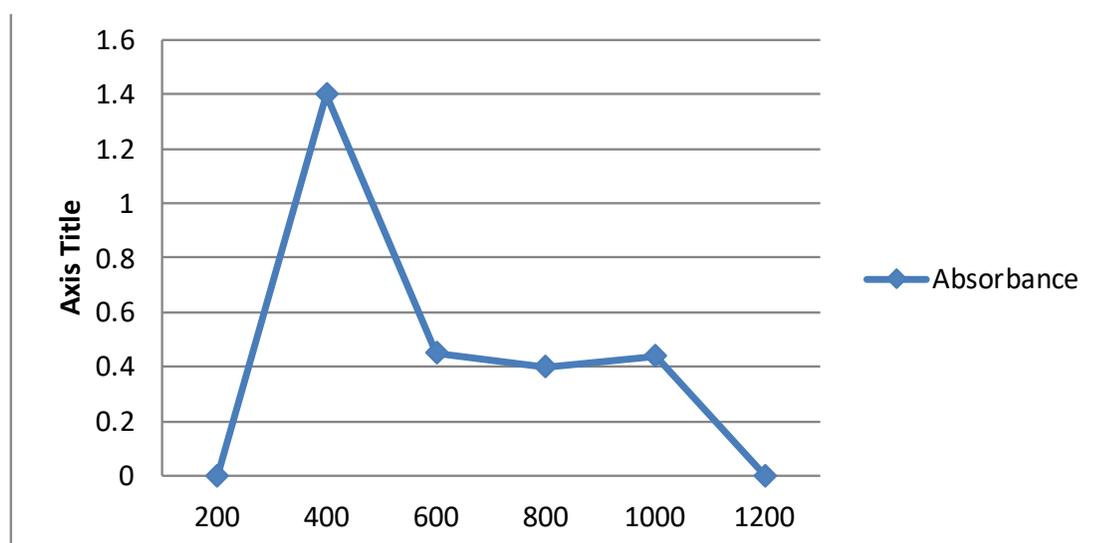
**Table 1:** TLC based analysis of fractions of the methanolic extract of *Crinum defixum*.

All the above fractions were subjected to cytotoxic activity using brine shrimp assay. As can be seen in Fig 1. Fractions 2, 4 and 5 exhibited highest activity suggesting that all the molecules are acting synergistically. However, all the bands were in close proximity hence could not be separated.



**Fig 1:** Cytotoxic activity of fractions of methanol extract of *Crinum defixum*.

Preparative TLC of fraction 2 was conducted and one major band was isolated. One major spectral analysis peak was obtained at 400nm where as minor peaks were obtained at 600, 800 and 1000(Fig2). Thus, fraction is not a single entity but a mixture of molecules. Thus methanolic leaf extract of the plant is a good candidate for the isolation of cytotoxic principle and a number of molecules could be isolated which is beyond the scope of this project.



**Fig 2.** Spectral analysis of isolated fraction by preparative TLC

## 4.4. Wild Edible Fruits : Conservation and nutraceutical analysis

### 4.4.1. Sugar Profiling and Anti-nutrient Analysis of Some Unexplored Wild Edible Fruits of Odisha.

**Principal Investigator: Dr. Uday Chand Basak, Senior Scientist**

Forests play an indispensable role in improving quality of life of many tribal's, rural people and forest dwellers. Wild fruits are the natural resources of the forest acts as an important source of food as well as folk medicine. However, potential of these wild palatable fruits still remains unexploited and their economic importance has not yet been realized.

Sugar, the primary product of photosynthesis of green plants initially occurs as monosaccharides, which are then transformed into disaccharides, trisaccharide, and sugar alcohols. The presence of sugar in the fruits is very essential in determining the quality of fruits as well as its caloric value. It plays crucial role in the production of flavouring compounds as well as secondary metabolites that can be used as an indicator to determine the maturity. The taste of fruits is vitally affected by the concentration of soluble sugar like Glucose, Fructose and organic acids. Fruits do not contain same combination of soluble sugar in them that greatly changes with fruit development and peaking at ripening. Sugar Profiling refers to the test which helps to identify and quantify the monosaccharides and disaccharides present in wide sample range. Thus it is essential to have proper knowledge about the fruits having proper sugar content and suitable for everyone in general and diabetic persons in particular.

Apart from the nutritional benefits these wild palatable fruits may contain one or more anti-nutrient factors. They include oxalic acid, tannin, phytic acid, saponin, etc. can negatively impact health by impeding protein digestion, growth, and the absorption of minerals in particular. Oxalic acid, commonly found in certain fruits, can chelate with calcium, potentially causing oral and pharyngeal irritation. Phytic Acid binds with macronutrients to form insoluble phytate salts that inhibits these crucial elements bioavailability. Tannins have the ability to precipitate specific proteins and can also bind to digestive enzymes, rendering them inactive and unavailable for digestion. Similarly, when injected into the bloodstream, saponin, can be extremely lethal since they rapidly cause the rupture of red blood cells as well as interferes with the absorption of fat-soluble vitamin A and vitamin E. Although WEFs are tasty and nutritious but more consumption of such wild fruits may be hazards to our body. Thus, before consuming one, it must be checked whether it contain proper amount of antinutritional factors or not.

In this context, the following objectives are taken into consideration for the study i.e. Qualitative and Quantitative analysis of anti-nutrient and profiling of sugar in selected wild edible fruits of Odisha, in order to identify potent species having less anti-nutrients and sugar contents to encourage its conservation as well as to avoid over consumption.

For this, the eight selected wild fruits species i.e. *Ardisia solanacea*, *Elaeocarpus serratus*, *Eugenia roxburghii*, *Flacourtia jangomas*, *Ficus racemosa*, *Grewia asiatica*, *Pithecellobium dulce* and *Syzygium jamobs* were collected from forest regions of Mid Central Table Land (MCTL) and East & South Eastern Coastal Plain (ESECP) regions of Odisha and preserved at -20°C until further use.



For sugar profile of fruit species at various stages of maturity the spectrophotometric and HPLC methods was followed. Spectrophotometric quantification of Total sugar, reducing sugar and non-reducing sugar in fruit samples was done following the method of Rangana (1970) and Lam *et al.* (2021). The concentration of sugar was determined by comparing the results obtained to the standard calibration plot. Reverse phase HPLC method with UV detection has been used for identification and quantification of fructose, glucose and sucrose were performed according to a study performed by Sabeetha *et al.*, 2017, with slight modification using HPLC system (Thermo Fisher Scientific, Dionex™ Ultimate 3000) equipped with binary pump and porous silica with 5µm diameter C-18 column controlled by software Chromeleon™7.2.8.10783 version. The isocratic mode of RP-HPLC was conducted using C18 column at temperature (30 °C) with flow rate of 1 mL/min using suitable mobile phase. Separation and detection of fructose, glucose and sucrose was carried out by injecting 20 µL of standard solution and fruit sample solution in triplicate into C18 column coupled with refractive index detector (RID) at 30°C at 200nm. Antinutrients like oxalic acid, tannin, phytic acid, saponin were determined qualitatively and quantitatively using UV-Vis Spectrophotometer using the methods of Nayak and Basak (2017).

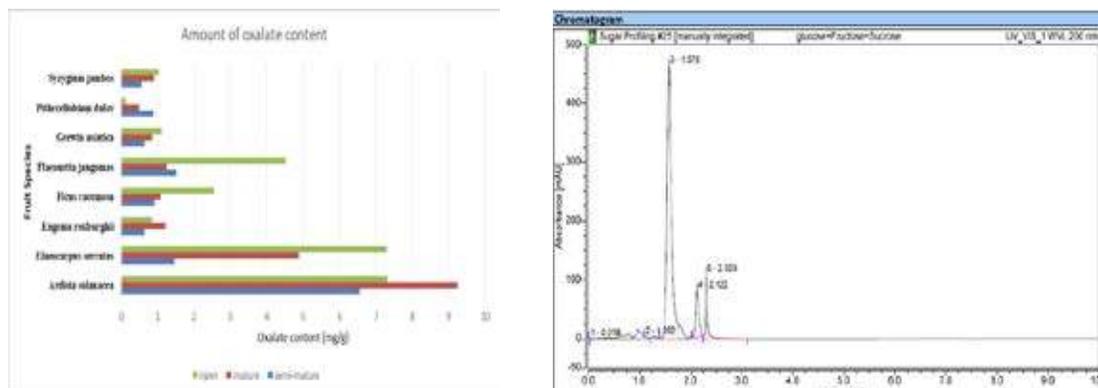


Fig 1: Quantification of Oxalate through Uv-Spectrophotometer Fig 2: Chromatogram for Sugar Profiling through HPLC

The results revealed that Fruits of *Ardisia solanacea* (ESECP), *Eugenia roxburghii* (NCP), *Flacourtia jangomas* (NCP), *Syzygium jambos* (ESECP) and *Pithecellobium dulce* (NCP) have significant amount of Sugar and are lower in anti-nutrient content. Hence, these wild edibles should be popularized for consumption to maintain a healthy life for everyone in general and diabetic person in particular. They should be domesticated for prevention of future food scarcity as well as benefit the stakeholders of the state.

To get a comprehensive view, a systematic and multi-faceted approach to identify fruit species and stages with beneficial profiles, by connecting regional variation, maturity stages, and advanced profiling techniques, the study will enable a nuanced comparison of fruits that considers both sugar and anti-nutrient factors. The findings may support the development of healthier fruit-based products, guide agricultural practices, and inform consumer choices for better health outcomes. Further FT-IR analysis will complement HPLC and spectrophotometry by confirming and refining the analysis, ensuring accurate profiling of sugar and anti-nutrient content. It strengthens the study's ability to assess and validate findings about optimal species, regions, and stages.

#### 4.4.2. Characterization of $\alpha$ -Tocopherol and Polyphenols in some immune boosting wild edible fruits used by Tribal communities for the therapeutic value.

**Principal Investigator: Dr. Uday Chand Basak, Senior Scientist**

Until now different studies have been carried out to provide information about the immune modulatory activity of bioactive compounds in fruits. However, there was an absence of a comprehensive study on wild edible fruits used by tribals' that includes different commonly consumed fruits and the role played by phytochemicals in strengthening specific part of the immune system along with its mechanism. In this piece of research work it is thus, tried to present the immune-boosting effects of various bioactive constituents present in some wild edible fruits and highlight their essential role in protecting us from the invasion of various microorganisms. It might be useful for future researchers while planning strategies like selecting effective bioactive ingredients to prepare functional foods with immune enhancing potential.

Following objectives were targeted to achieve i.e. a) Screening of  $\alpha$ -tocopherol & Polyphenols in selected wild fruits b) Characterization of  $\alpha$ -tocopherol & Polyphenols through FTIR c) Selection of potent species with immune-enhancing potential for conservation.



*Antidesma ghaesembilla*



*Artocarpus lakoocha*



*Carmona retusa*



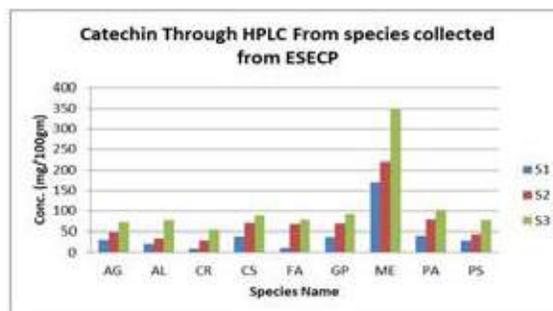
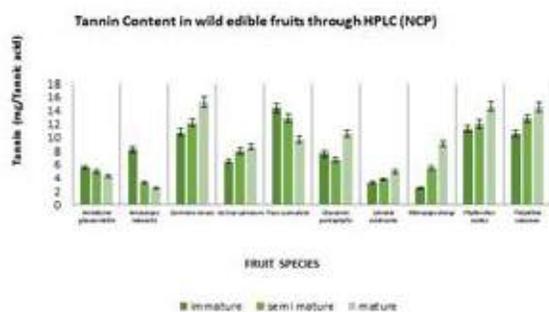
*Carissa spinarum*

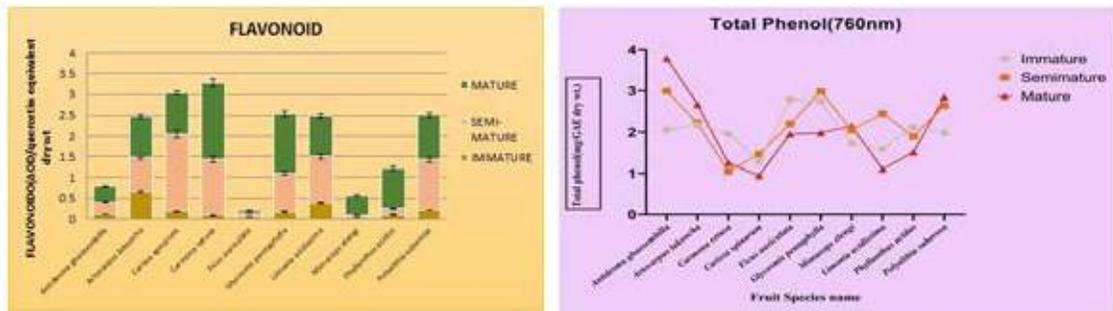


*Ficus auriculata*

*Glycosmis pentaphylla**Mimusops elengi**Limonia acidissima**Phyllanthus acidus**Polyalthia suberosa*

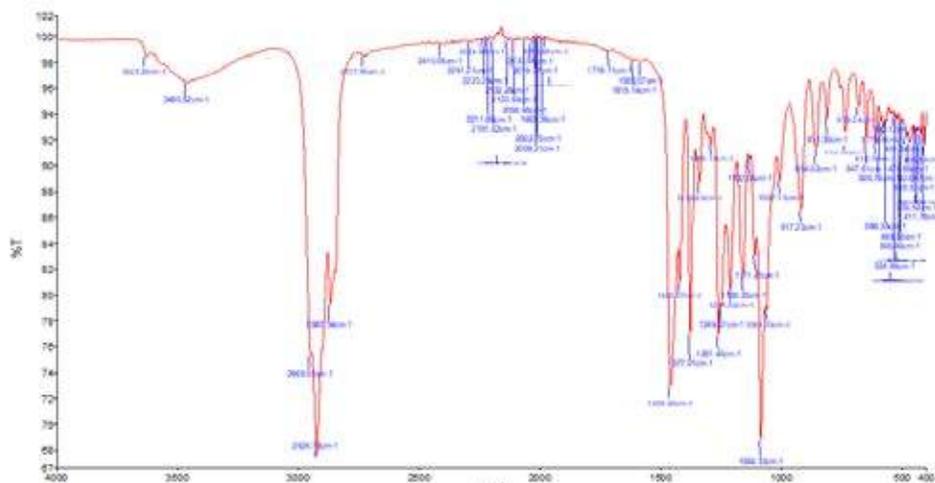
Ten distinct wild edible fruits at various stages of ripeness; semi-mature, mature and ripen were collected in accordance with the objectives of the project. These fruits were gathered from areas that included the Mid Central Table land (MCTL), the East and South Eastern Coastal Plains (ESECP) and North Central Plateau (NCP) zones of Odisha. They were then subjected to analysis in order to determine and measure their Polyphenol levels and  $\alpha$ -Tocopherol. Agro climatic zones exert a profound influence on the growth, development, and nutritional composition of foods, including wild edible fruits traditionally used by tribal communities. The findings of our study reveal intriguing insights into the antioxidant properties of various wild edible fruits, shedding light on their potential health benefits. Among the ten species analyzed, *Polyalthia suberosa* stood out for its remarkable tannin content, particularly in its ripe stage, suggesting its potential as a rich source of this antioxidant compound. Furthermore, the significant flavonoid content observed in *Polyalthia suberosa* underscores its importance as a valuable dietary component for combating oxidative stress. *Carissa spinarum* also exhibited noteworthy flavonoid content, particularly at its mature stage, indicating its potential as a source of these beneficial compounds. Similarly, *Carmona retusa* demonstrated significant flavonoid levels in its mature stage, highlighting its potential as a dietary antioxidant. *Antidesma ghaesembilla* displayed the highest total phenol content at its mature stage, particularly when collected from the East and South Eastern Coastal Plain. This suggests the importance of geographical factors in influencing the antioxidant composition of wild fruits, with environmental conditions potentially impacting the synthesis and accumulation of phenolic compounds. Interestingly, *Mimusops elengi* (NCP) contains highest Catechin, a flavan-3-ol, a type of secondary metabolite providing antioxidant roles in plants. Catechin have numerous health benefits, including anti-inflammatory properties, immune system support, anticancer effects, antioxidant activity, heart health improvement, and aid in weight management.





**Figure 1:** Polyphenol analysis from wild edible fruits

The FTIR spectra of dried fruit pulps are shown in the graph below. From the spectra, it looks difficult to make straight forward assignment of frequencies present in the spectra of the molecule. However, bands between 1250 and 740  $\text{cm}^{-1}$  are characteristic of 7-cis configuration while those at 780  $\text{cm}^{-1}$  are characteristic of 15-cis configuration. Catechin vibrations results from its polymer backbone which includes C=C-C stretching, and C=C-C and C-C-C deformations. Gallic acid isomers exist as stretched (all-trans), terminal bent (7-cis) and central bent (15-cis) configurations. There is considerable coupling between C=C and C-C stretching with C-H in-plane bending and between C-H out-of-plane vibrations with C=C torsions. Peaks at 1720 and 1680 are characteristic of C=C-C stretching. The  $\alpha$ -tocopherol exhibits peaks at 2922  $\text{cm}^{-1}$  (asymmetric stretch) and 2862  $\text{cm}^{-1}$  (symmetric stretch) associated with vibrations of the CH<sub>2</sub> and CH<sub>3</sub> respectively.



**Figure 2:** ATR-FTIR spectrum for fruit samples

Overall, our findings underscore the diversity and significance of antioxidant compounds present in wild edible fruits, emphasizing their potential role in promoting human health and well-being. The relationship between agro climatic zones and the antioxidant and nutritional composition of wild edible fruits highlights the intricate interplay between environmental factors and food quality. By acknowledging and leveraging these agro climatic influences, we can unlock the full potential of indigenous food resources to support immune health, address nutritional deficiencies, and promote

overall well-being within tribal communities and beyond. Moreover, consumers can make informed choices by selecting locally grown produce, which may offer higher nutritional quality and antioxidant content tailored to the specific agro climatic conditions of their region.

Further research into the bioavailability and bioactivity of these antioxidant compounds, as well as their potential synergistic effects, could provide valuable insights into their therapeutic applications and dietary recommendations. Additionally, exploring cultivation and conservation strategies for these antioxidant-rich fruits could contribute to their sustainable utilization and preservation of biodiversity.

#### **4.4.3. Conservation of Wild Edible Fruit plants through field introduction in different protected wild areas of Odisha**

**Principal Investigator: Dr. Uday Chand Basak, Senior Scientist**

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The study focuses on the propagation and conservation of wild edible fruits in Odisha's forests, which are vital sources of nutrition and income for local communities. Approximately 150 species of wild edible fruits exist in the region, offering unique flavors and nutritional benefits. However, these species face threats from deforestation, climate change, and overexploitation.

The research involved propagating several wild edible fruit species including *Antidesma ghaesembilla*, *Carmona retusa*, *Eugenia rothii*, *Glycosmis pentaphylla*, *Polyalthia suberosa*, and *Toddalia asiatica* through macro-propagation methods. The propagated saplings were introduced in two different forest areas - Nayagarh and Dhenkanal Forest Divisions - to study their growth and adaptability.

The field introduction trials were conducted during the monsoon period of 2023. The study monitored various parameters including growth rate, collar perimeter, number of branches, leaf counts, and survivability rate. Photosynthetic efficiency was analyzed using chlorophyll fluorescence parameters (Fv/Fm and Plabs), and stress response was evaluated through antioxidant activity measurements.

Key findings revealed that Nayagarh Forest Division showed higher survivability rates (82.40%) compared to Dhenkanal Forest Division (52.65%). Among the species, *Carmona retusa* demonstrated the highest survivability rate with 90% in Nayagarh and 78% in Dhenkanal. Other species like *Glycosmis pentaphylla*, *Polyalthia suberosa*, and *Eugenia roxburghii* also showed favorable performance.

The research included extensive biochemical analysis of leaf samples to assess environmental stress adaptation. Species like *Antidesma ghaesembilla*, *Carmona retusa*, and *Toddalia asiatica* showed higher antioxidant capacity in both field-introduced and nursery-grown saplings. Enzymatic antioxidant activities (SOD, CAT, and POX) were particularly high in field-introduced saplings of *Antidesma ghaesembilla*, *Carmona retusa*, and *Glycosmis pentaphylla*, indicating their ability to cope with oxidative stress.

The study concluded that while nurseries provide controlled environments for initial growth, introducing plants into natural habitats promotes genetic diversity and enhances their resilience to environmental stresses. The success of field introduction varied between locations, with Nayagarh Forest Division showing better results overall. The research emphasizes the importance of monitoring both morphological growth and biochemical adaptations for successful conservation efforts.

The project's outcomes include the production of quality planting materials, successful field introduction at two sites, assessment of growth and adaptability, and comprehensive analysis of photosynthetic efficiency and stress response. These efforts contribute to the preservation of wild edible fruit species and enhancement of biodiversity in Odisha's forests, while ensuring their availability for future generations.

Field introduction of wild edible fruit species done at Site: 1 Podagada, Nayagarh Forest Range under Nayagarh Forest Division. And Site: 2 Sorisiapada, Kapilash Forest Range under Dhenkanal Forest Division. Field visit to Chandaka WL Division completed for site survey. Each site was introduced with around 900 saplings of *Antidesma ghaesembilla*, *Carmona retusa*, *Eugenia roxburghii*, *Glycosmis pentaphylla*, *Polyalthia suberosa* and *Toddalia asiatica*.



Field introduction of wild edible fruit species at Podagada, Nayagarh Forest Division



Field survey for field introduction of Wild edible Fruit species at Dhenkanal Forest Division

## 4.5. Propagation and Reintroduction of Mangrove Plants

### 4.5.1. Conservation of rare mangrove species of *Xylocarpus* through vegetative propagation and reintroduction in protected areas of Odisha

**Principal Investigator: Dr. Uday Chand Basak, Senior Scientist**

Conservation efforts have been made for rare mangrove species of the genus *Xylocarpus* through vegetative propagation and reintroduction in protected areas of Odisha. This part of research project has been focused on production of suitable saplings through vegetative propagation techniques followed by reintroduction trials, and to evaluate the adaptability analyzing various growth & developmental parameters of three species: *Xylocarpus granatum*, *X. mekongensis* and *X. moluccensis* belonging to the Meliaceae family which are environmentally as well as socio-economically important mangrove plants. Various vegetative propagation methods, including air layering and black-taped layering have been standardized to produce saplings during previous year (2022-23). In continuation, these techniques were further carried out This year (2023-24) at the Bhitarkanika National Park, where vegetative propagation was performed on pre-existing mother plants at specific geo-tagged locations within the park. The hardening experiments, crucial for preparing the saplings for reintroduction, were conducted at the institutional (RPRC) nursery conditions which involved applications of varying levels of salt stress to the rooted saplings to acclimatize them to the conditions they would face in natural mangrove habitats.



*Vegetative propagation in mangrove mother plant *Xylocarpus granatum* at Bhitarkanika to produce planting saplings (22 44°3.63' N 86 52°7.69' E Dangmal & Kanika)*

The project's findings have significant implications for mangrove conservation strategies. The successful standardization of vegetative propagation protocols for *X. granatum* and *X. mekongensis* provides valuable tools for future conservation efforts. These techniques can potentially be adapted for other rare mangrove species, contributing to broader biodiversity conservation in coastal ecosystems. Moreover, the production of hardened saplings ready for reintroduction represents a crucial step in preserving these important components of mangrove ecosystems.

The success of this project extends beyond the immediate conservation of *Xylocarpus* species. By demonstrating effective propagation and hardening techniques, it sets a precedent for similar efforts with other mangrove species. This work contributes to the broader goal of maintaining and restoring mangrove habitats, which are critical for coastal protection, carbon sequestration, and supporting diverse marine and terrestrial ecosystems.

The hardening experiments, which involved subjecting the rooted plants to varying levels of salt stress, proved crucial in preparing the saplings for reintroduction. The biochemical analysis during this phase showed positive stress responses and increased enzymatic activities, indicating that the plants were successfully adapting to the challenging conditions they would face in the wild. This process is essential for improving the survival rates of reintroduced plants and ultimately contributing to the restoration of mangrove populations.

Despite the overall success of the project, several challenges were encountered, particularly in the vegetative propagation of *X. moluccensis*. The lack of success in air layering for this species highlights the need for further research to develop species-specific propagation protocols. The varying rooting and survival rates between species also emphasize the importance of tailored approaches in mangrove conservation efforts.

The study also included a comprehensive approach to assess the health and viability of the propagated plants. This included productivity assessments using chlorophyll fluorescence analysis and extensive biochemical assays. The chlorophyll fluorescence measurements provided insights into the photosynthetic efficiency of the plants, serving as an indicator of their overall health and stress tolerance. These measurements were taken at different stages of growth and compared between mother plants, nursery-grown seedlings, and hardened saplings.

Biochemical analyses played a crucial role in understanding the physiological responses of the plants to propagation and hardening processes. The study examined various biochemical markers, including proline, total phenolic content, flavonoids, catalase activity, peroxidase activity, and superoxide dismutase activity. These parameters were compared between plants grown in Bhitarkanika and those cultivated in the RPRC nursery, as well as at different stages of the hardening process. The results provided valuable insights into the stress responses and adaptability of the propagated plants.

The chlorophyll fluorescence analysis revealed interesting patterns in photosynthetic efficiency. Newly propagated saplings initially showed low chlorophyll activity, which improved over time. This suggests that the plants require an adaptation period after propagation before reaching

optimal photosynthetic performance. Notably, nursery-grown saplings demonstrated good energy absorption, likely due to the controlled conditions and proper management in the nursery setting. These findings highlight the importance of careful nurturing during the early stages of propagation to ensure the long-term viability of the plants.

The biochemical analyses provided a wealth of information about the physiological state of the plants under different conditions. Generally, *X. granatum* showed higher levels of most biochemical markers compared to the other two species. This could indicate a greater stress tolerance or adaptability in *X. granatum*, which aligns with its higher propagation success rate. The study observed differences in biochemical profiles between naturally grown and nursery-propagated plants, suggesting that the growing environment significantly influences the plants' physiological responses.



*Collection of rooted air layers of Xylocarpus granatum from Bhitarkanika (20 44'8. 18" N 86 52'30.79" E Mahisamara & Suajore Riverside)*



*Hardened plants of Xylocarpus granatum at RPRC Nursery ready to reintroduction (20 18'11.4" N 85 48'13.63" E RPRC Nursery)*

During the hardening process, both *X. granatum* and *X. mekongensis* showed increases in phenolic content, flavonoids, saponins, and tannins. These changes are indicative of the plants' responses to the induced salt stress and suggest successful acclimatization to conditions similar to their natural habitat. The increases in these compounds are often associated with enhanced stress tolerance in plants, which bodes well for the saplings' ability to survive when reintroduced to natural mangrove ecosystems.

Looking ahead, several key steps will be crucial for building on this project's success. First, reintroduction trials in natural mangrove habitats should be conducted to assess the long-term viability of the propagated plants in their native environments. This will involve careful site selection, considering factors such as tidal influence, soil composition, and existing vegetation. Long-term monitoring of reintroduced plants will be essential to evaluate their survival rates, growth patterns, and integration into the existing ecosystem.

The reintroduction of *Xylocarpus granatum* at various sites within Bhitarkanika represents a critical step in restoring the ecological balance of these mangrove ecosystems. This initiative focuses on enhancing biodiversity and stabilizing the coastal environment by reintroducing this rare and important species, which plays a key role in maintaining the health of mangrove forests. Through a carefully planned process involving the cultivation of healthy saplings via vegetative propagation, followed by their reintroduction into suitable habitats, the project aims to re-establish *Xylocarpus granatum* populations at selected sites in Bhitarkanika. These efforts not only contribute to the recovery of degraded mangrove areas but also enhance the overall resilience of the ecosystem against climate change impacts and coastal erosion. Monitoring and adaptation of reintroduction strategies will ensure the long-term survival and integration of this species into the broader mangrove forest system, supporting the preservation of Odisha's unique coastal biodiversity.

Further research could explore the genetic diversity of the propagated plants to ensure population viability. This is particularly important for conservation efforts, as maintaining genetic diversity is crucial for the long-term resilience of the species. Additionally, expanding the project to include other rare mangrove species would contribute to a more comprehensive approach to mangrove conservation in the region.



*Transportation of propagated saplings to Bhitarkanika for Re-introduction*

### Re-Introduction at Bhitarkanika



Re-Introduction of vegetatively prepared saplings of *Xylocarpus* species at Bhitarkanika  
(20°43'45.78N 86°52'8.19E Dangamal & Kanika)

The project also highlights the importance of collaboration between research institutions, forest departments, and local communities in conservation efforts. The involvement of multiple stakeholders can ensure the long-term success and sustainability of such initiatives. Public awareness and education programs about the importance of mangrove ecosystems and these conservation efforts could be developed to engage local communities and promote broader support for mangrove preservation.

In conclusion, this project work has made significant progress on conservation of rare *Xylocarpus* mangrove species, particularly through the development of vegetative propagation techniques and conservation via initiation of reintroduction. The vegetatively propagated hardened saplings may be used for reintroduction trial at the suitable habitats especially in Bhitarkanika mangrove ecosystems. By combining propagation methods with detailed physiological assessments, this piece of research work has established a robust framework for future conservation efforts. While challenges remain, especially for species like *Xylocarpus moluccensis*, the overall success including conservation through reintroduction activities at Bhitarkanika, offers renewed hope for the preservation and restoration of these crucial coastal habitats. Continued research, monitoring, and collaboration are essential to build on these achievements and ensure the long-term survival of rare mangrove species in Odisha and beyond. Keeping this fact in mind, further work on re-introduction of these *Xylocarpus* spp. required to be implemented in order to establish the sustainable conservation strategies of these vulnerable mangrove plants.

## 4.6. Propagation and reintroduction of endangered species

### 4.6.1. Propagation and reintroduction of endangered species of Odisha

Principal Investigator: **Dr. Kalidass C., Scientist**

#### *A study on Raderamachera xylocarpa and other species:*

The reintroduction and propagation of endangered plant species is a critical conservation strategy to preserve biodiversity. In Odisha, India, several plant species, including in the present study on the propagation and reintroduction *Raderamachera xylocarpa*, *Nothopegia racemosa*, and *Alphonsea madraspatana*, are at risk of extinction. These species are not only significant from an ecological perspective but also have medicinal and economic value. Our study involved several field surveys in Odisha to collect seeds and seedlings of these endangered species, with the goal of propagating and reintroducing them into their natural habitats (Fig.1).



**Fig.1:** Field Observations of *Raderamachera xylocarpa* for population assessment, seed collection and natality and mortality rates.

#### **Propagation methods for endangered species:**

In this study, we explored different propagation techniques for the listed species, including seed-based methods and vegetative propagation through stem cuttings and air-layering. These methods were applied to each species based on their specific characteristics and requirements.

##### **1. Seed propagation of *Radermachera xylocarpa*:**

We began our experiments with *Radermachera xylocarpa*, a species that is naturally propagated through seeds. The seeds were collected from mature fruits in the Bargarh and Bolangir districts of Odisha. The initial germination tests revealed a low germination rate of less than 30%. To improve seed germination, we applied cold water treatment (100°C) for 24 hours, which significantly improved germination, increasing the rate to 63.7%. This result suggests that cold water treatment can be an effective strategy for enhancing seed germination in this species.

Further experiments involved treating the seeds with different concentration of gibberellic acid (GA3). Seeds were subjected to GA3 treatment of 1000 to 4000 ppm for 24 hours. The highest success

was observed with the 4000 ppm GA3 treatment, which promoted better germination rates. However, treatments with concentration higher than 4000 ppm did not yield satisfactory results, as the seeds did not respond well (Fig.2).



*Fig.2: Mature fruits and seeds for of Radermachera xylocarpa used for seed morphology studies, hormones treatment and germination analysis.*

## **2. Vegetative propagation of Radermachera xylocarpa:**

Given the low seed viability and germination rates, we also explored vegetative propagation techniques, such as stem cuttings and air-layering, to propagate Radermachera xylocarpa.

**2.a. Stem cuttings:** for vegetative propagation through stem cuttings, we collected 1-2 cm thick cuttings bearing two nodes from RET garden plants. These cuttings were treated with different concentrations of root-promoting hormones, including IAA, IBA and NAA. Hormones treatments were applied at different intervals and the cuttings were planted in a polyhouse. Despite these efforts, the stem cutting showed slow growth and the propagation process is still under observation.

**2.b. Air-layering:** Air-layering was another method tested for propagating Radermachera xylocarpa. For this technique, we treated the branches with hormones concentrations of 1000 ppm IAA, 2000 ppm IBA and 3000 ppm NAA. Similar to stem cuttings, air layering also showed slow growth and the results are still being monitored (Fig.3) .



*Fig.3: Air-Layering of Radermachera xylocarpa used in propagation studies and rooting of stem cuttings.*

### **3. Challenges in Natural propagation of *Radermachera xylocarpa*:**

The natural propagation of *Radermachera xylocarpa* faces significant challenges. One of the main obstacles is poor seed set and low seed viability due to seed abortion. The species is pollinated by bats and is self-incompatible, which limits its ability to reproduce successfully without external pollination. Furthermore, over-exploitation of the species for medicinal purposes and habitat destruction has contributed to its endangered status. These factors highlight the need for alternative propagation methods, such as the ones explored in our study.

### **4. Air-layering and other methods of vegetative propagation:**

Air-layering is a well-known method of vegetative propagation that is particularly effective for species that are difficult to root from cuttings. This method has been successfully used for various mangrove species and other endangered plants. It involves removing a ring of bark from a stem and treating the exposed area with applied for rooting development hormones, followed by wrapping it in a moist environment to encourage root development. The success of air-layering largely depends on the specific hormone concentration, environment conditions and the time take for root formation.

In our experiments, air-layering was conducted on branches with diameters ranging from 6 to 15 mm. we used different hormone treatments, including IBA, IAA and NAA at concentrations of 1000 to 5000 ppm. The branches were covered with moist sphagnum moss and wrapped in polyethylene sheets to maintain a humid, warm environment. Rooting was evaluated after 5-12 months and the results showed and their length and the most successful treatments were those using IBA.

### **5. In-Situ Conservation and the Importance of Protected Areas:**

In-situ conservation refers to the protection of endangered species in their natural habitats. The approach is considered the most effective strategy for preserving biodiversity. For *Radermachera xylocarpa* and other endangered species, in situ conservation is crucial. Efforts should focus on protecting the remaining natural habitats of these species, preventing habitat destruction and implementing sustainable land-use practices. Moreover, raising awareness among local communities about the importance of these plant species can help mitigate over-exploitation and encourage their conservation.

### **6. Conclusion:**

The propagation of endangered plant species in Odisha, particularly *Radermachera xylocarpa*, presents several challenges but also offers valuable insights into effective conservation techniques. Our study highlights the potential of seed treatments, stem cuttings and air-layering as viable propagation methods. However, the slow growth observed in some of the propagation techniques suggest that further research is needed to refine these methods and improve success rates.

Future studies should explore the optimal environmental conditions for propagation, including temperature, humidity and light levels. Additionally, the genetic diversity of the species should be considered to ensure that propagated plants maintain the resilience and adaptability of wild populations. Long-term monitoring of the reintroduced plants will also be essential to assess their survival and establish strategies for their continued conservation.

The successful propagation and reintroduction of endangered species like *Radermachera xylocarpa* are critical steps in preserving the biodiversity of Odisha and ensuring the survival of these valuable plants for future generations. Through continued research and conservation efforts, we can improve the methods of propagation and enhance the chances of these species' survival in the wild.

## 4.7. Taxonomical Study

### 4.7.1. Taxonomical and ethno botanical significance of the Leguminosae family in Odisha

Principal Investigator: **Dr. Kalidass, C. Scientist.**

The Leguminosae family is the third-largest angiosperm family, following Asteraceae and Orchidaceae in terms of species diversity. This family holds significant economic and ecological value. Leguminosae species are widely distributed and play a crucial ecological role across nearly all biomes, thriving even in the most arid environments. Its members vary greatly, from large tropical trees to small annual herbs, climbing annuals or perennials with tendrils, lianas, shrubs, but exclude aquatic plants. The flower within the family exhibits a wide range of symmetry, from radially symmetric to bilaterally symmetric and even asymmetric.

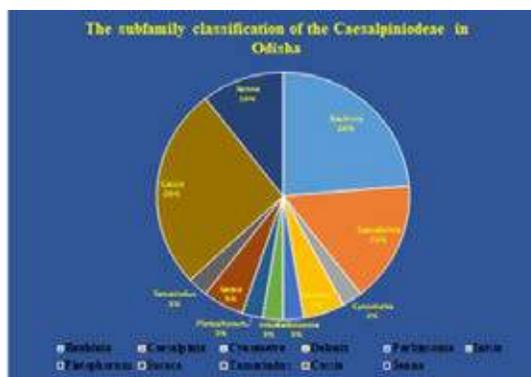
The family's remarkable evolutionary diversification is evident in its vast differences in morphology, physiology and ecology. The subfamily Caesalpiinoideae (Caesalpiaceae), for example, is distinguished by its characteristic raceme inflorescence (e.g., *Cassia*), hypogynous

and zygomorphic flowers, and distinct aestivation patterns. The calyx follows a descending imbricate arrangement (with the odd sepal anterior), while the corolla exhibits an ascending imbricate pattern. The androecium contains 10 polyandrous stamens in a diplostemonous arrangement, with dehiscence through pores or longitudinal slits. In genera like *Tamarindus*, the stamens are monodelphous, while in *Sindora*, they are diadelphous. Staminodes are present in *Cassia*. The gynoecium is monocarpellary, with a superior, often stalked ovary that is unilocular, featuring marginal placentation. The fruit produced is typically a lomentum. Prominent plants in this subfamily include *Caesalpinia pulcherrima*, *Cassia fistula*, *Bhaunia variegata*, *Delonix regia*, *Saraca indica*, *Tamarindus indica*, *Heematoxylon campechianum* and *Parkinsonia aculeata*.

In our ongoing work, we propose new categorizations for the subfamilies of the Leguminosae genus, based on current systematic patterns that have successfully addressed previous nomenclature issues. Over the past year, we have made substantial progress, achieving 75% completion of the project. Our



efforts include re-describing species, confirming valid names and synonyms, and verifying the protologue and nomenclature of each species with supporting images. Additionally, field studies have been conducted on selected Caesalpiinoideae, including surveys, ethnobotanical documentation, herbarium preparation, and depositing the specimens in the RPRC herbarium, further strengthen our research.



Initially, species of Cassia and Senna were classified under a single genus. However, due to significant morphological differences, they were later separated into two distinct genera. For example, in Cassia, out of the 10 stamens, 3 are staminodes, while in Senna all 10 stamens are functional. Another key difference is in the petals: in Cassia, all the petals are of equal size, whereas in Senna, the petals are unequal in size. These and other differences led to the reclassification of these species into separate groups.



A. *Senna alata*, B. *S. hirsuta* C. *S. sophera* D. *S. occidentalis* E. *S. saimea* F. *S. surratensis*

**Ethnobotany of the Caesalpiinoideae subfamily:** preliminary phytochemical screenings of species in the Caesalpiinoideae subfamily have revealed a rich composition of bioactive compounds, including flavonoids, alkaloids, tannins, proteins, reducing sugars, carbohydrates, phytosterols, coumarins, saponins, and triterpenoids. In traditional medicine, various parts of these plants, especially the seeds, are commonly used to treat a wide range of ailments. Other plant parts, such as the leaves, flowers, roots, fruit, bark, and seed oil, are also utilized for medicinal purposes.

Species of Cassia, for example, have traditionally been used for their antipyretic, analgesic, antifilarial, antidiabetic, anti-inflammatory, hypolipidemic, antimalarial, anthelmintic, antiulcer, and antioxidant properties. Similarly, various parts of Bauhinia species, including the leaves, seeds, and seed kernels, are employed in the treatment of different health conditions. The seeds of Saraca

species are particularly notable for their use in therapeutic ointments. When mixed with castor oil, these seeds form a remedy that is applied to the skin to treat hydrocele and orchitis.

In addition, the leaves of *Tamarindus* species are crushed and mixed with fermented shrimp paste to create a condiment. *Caesalpinia* and *Peltophorum* species are also widely used for ornamental purposes in gardens around the world.

## 5. RESEARCH PROJECTS

### STATE PLAN FUNDED PROJECTS

Sl.No.	Title	P.I	Funding	Period
1	Documentation of Micro-Fungi in forest soil of Odisha	Dr. Nibha Gupta Principal Scientist	State Plan	2023-24
2	Bioactive lead molecule from fungal endophytes: Extraction, purification, characterization	Dr. Nibha Gupta Principal Scientist	State Plan	2023-24
3	Bioprocess optimization for enhanced recovery of Glutaminase free Lasparaginase of Fungal Origin	Dr. Nibha Gupta Principal Scientist	State Plan	2023-24
4	Standardization of Propagation Methods for <i>Bulbophyllum</i> Orchids Through Tissue Culture	Dr.N.R. Nayak Senior Scientist	State Plan	2023-24
5	Generation of Genetic Variants for <i>Dendrobium</i> , <i>Cattleya</i> , <i>Cymbidium</i> and <i>Spathoglottis</i> Orchids Through Mutation Breeding Towards Development of Novel Flowers.	Dr.N.R. Nayak Senior Scientist	State Plan	2023-24
6	Standardization of efficient tissue culture based propagation methods for <i>Pamatoalpadeciens</i> (Lindl.)J.J.Sm and <i>Cymbidium bicolour</i> (Lindl.): Rare Orchids of Odisha.	Dr.N.R. Nayak Senior Scientist	State Plan	2023-24
7	Developing efficient micro-propagation methods for some important RET listed forest tree species of Odisha	Dr.G.K. Surabhi Senior Scientist	State Plan	2023-24
8	The protective diabetic neuropathy effect of <i>BuchanialanzanSpreng</i> in Streptozotocin- induced type 2 diabetic rats	Dr. A.K. Sahoo Senior Scientist	State Plan	2023-24
9	Ameliorative effect of <i>Aporusaoctandra</i> against carbon tetrachloride induced oxidative stress and hepatocellular injury in experimental rats	Dr. A.K. Sahoo Senior Scientist	State Plan	2023-24

10	Anticholinesterase effect of Carnosic acid on Sodium azide-induced memory impaired in rat model	Dr. A.K. Sahoo Senior Scientist	State Plan	2023-24
11	Sugar profiling and anti-nutrient analysis of some unexplored wild Edible Fruits of Odisha.	Dr. U.C. Basak Senior Scientist	State Plan	2023-24
12	Characterization of a-tocopherol & Polyphenols some immune boosting wild edible fruits used by Tribal Communities for therapeutic value.	Dr. U.C. Basak Senior Scientist	State Plan	2023-24
13	Conservation of rare mangrove species of Xylocarpus through vegetative propagation & re-introduction in protected areas of Odisha	Dr. U.C. Basak Senior Scientist	State Plan	2023-24
14	Conservation of rare mangrove species of Xylocarpus through vegetative propagation & re-introduction in protected areas of Odisha	Dr. U.C. Basak Senior Scientist	State Plan	2023-24
15	Conservation of wild edible fruit plants through field-introduction in different protected wild areas of Odisha.	Dr. Sunita Bhatnagar Senior Scientist	State Plan	2023-24
16	Exploration of cytotoxic potential of methanolic leaf extract of medicinal plant Crinum defixum.	Dr. Sunita Bhatnagar Senior Scientist	State Plan	2023-24
17	Biological evaluation of leaf extracts of Zingiberzerumbet and Hedychimum spicatum	Dr. Kalidass C Scientist	State Plan	2023-24
18	Standardization of in vitro regeneration techniques in red banana and establishment of red banana in Odisha climate condition.	Dr. Kalidass C Scientist	State Plan	2023-24
19	Taxonomic and ethnobotanical significance of the Leguminosae family in Odisha.	Dr. Kalidass C Scientist	State Plan	2023-24
20	Conservation of a few endemic and endangered plants in Odisha.	Dr. Kalidass C Scientist	State Plan	2023-24

## EXTERNAL FUNDED PROJECTS

Sl.No.	Title	P.I	Funding	Period
1	Harnessing the potential of endophytes of <i>Piper longum</i> as an alternate source for piperine production: optimization of protocol for laboratory production and exploration of its anticancer properties	Dr. Nibha Gupta Principal Scientist	NMPB	2022-25
2	Establishment of mass propagation and breeding facility for orchids	Dr.N.R. Nayak Senior Scientist	RKVY	2022-23
3	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	2022-23

## 6. PUBLICATIONS

### Research Paper

#### Year 2024

Acharya Rupa , Birendra Bindhani and Nibha Gupta (2024). Microbial L-asparaginases: Therapeutic and Industrial applications. *Defence Journal of Life Sciences* VOL 9 (3) :233-241

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Dash Umesh Chandra, Sandeep Kumar Swain, Atala Bihari Jena, Jagnehswar Dandapat, Atish Kumar Sahoo (2024).The ameliorative effect of *Piper trioicum* in attenuating cognitive deficit in scopolamine-induced neurotoxicity in experimental rats. *Journal of Ethnopharmacology*. 318, Part A, 116911.

Gupta N, Niharika Mohanty and Atmaja Elina Mishra (2024). Impact of nutrient media on growth and antagonistic behavior of some fungi. *International journal of current advanced research*, vol.13 (3):2916-2919

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Mishra Atmaja Elina, Biswasana Bhuyan and Nibha Gupta (2024). Bioinoculation strategies for enhanced growth and development of economically important *Dalbergia latifolia* Roxb *Journal of Biotechnology Research* 10, Issue. 4, pp: 41-48

Mishra Atmaja Elina, Nibha Gupta, Uday Chand Basak and Birendra Kumar Bindhani (2024). Piperine production by fungal endophytes isolated from *Piper longum* L.:Effect of carbon sources *Bioscene* vol 21 (3): 323-335

Mishra S. & Sunita Bhatnagar (2024) Comparative analysis of methanol extract (leaf) of three species of *Sansevieria* using chemical and biological parameters. *Wjpls*. 10(2): 116-118

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**Year 2023**

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#### **Abstract of Research Paper Published in Conferences**

Anjeela A., Basak U. C., Bindhani. B.K. (2023) Characterization of L-Ascorbic Acid (Vitamin-C) in *Antidesma ghaesmbilla*, a wild edible fruit used by Tribal communities of Odisha; International conference on Recent Trends in Biotechnology on 18th – 19th July 2023, Centurion University, Bhubaneswar.

Anjeela A., Basak U. C., Bindhani. B.K. (2024) Assessing Antioxidant capacities for fruits of *Polyalthia suberosa* and *Glycosmis pentaphylla*: Insights from two agroclimatic zones of Odisha; International conference on Materials for sustainable Environment on 27th – 28th March, Centurion University, Bhubaneswar.

Mahatab S., Basak U.C., (2024) In Vivo Analysis of Photosynthetic Efficiency of Artificially Grown *Heritiera* Spp: An Adaptation in Bhitarkanika Mangrove Ecosystems of Odisha.; International Conference on Multidisciplinary Research In Global Warming, Climate Change & Economic Development. By International Society of Teachers, Administrators and Researchers Bangkok, Thailand.

Meher Neelam and Atish Kumar Sahoo. (2024) In vitro evaluation of antioxidant, antidiabetic, and anticholinesterase activities of secondary metabolites rich extracts from *Buchanania lanzan* Spreng. International conference on materials for a sustainable environment. Centurion University. 27th-28th March 2024.

Mohanty S, Pradhan D, Surabhi GK, (2024) 'Deciphering molecular regulation of fruit ripening in banana: a gel-free proteome approach', poster presented at National Seminar on Recent Advancement in Plant Science for Sustainable Development, organized by P.G Department of Botany, Utkal University, Bhubaneswar on dt.30th March, 2024.

Pradhan D, Gupta M, Surabhi GK, (2024) 'Transcriptome analysis of banana reveals a major role of cell wall modifying genes in fruit softening', poster presented at National Seminar on Recent Advancement in Plant Science for Sustainable Development, organized by P.G Department of Botany, Utkal University, Bhubaneswar on dt.30th March, 2024.

## Book

Gupta N., C. Manoharachary, S. K.Singh, Resmita Behera, P. Mekro (2023),. Fungi of Odisha : Part II. Published by Regional Plant Resource Centre, Bhubaneswar pp. 1-253. (ISBN: 978-93-6013-296-5)

Basak U. C. , Kalidass C. , Nayak N R. , Mekro P. (2023). Initiatives on Re-introduction of some RET and Special Group of Plants. Published by Regional Plant Resource Centre, Bhubaneswar pp. 1-154. (ISBN: 978-93-6013-115-9)

## Research Report

Basak UC,P Mekro , K Murugesan and S Pant. 2024. Research and Activity Report, 2022-23.Regional Plant Resource Centre, Forest, Env& CC. Dept,Govt. of Odisha,Nayapalli,BBSR-15,Odisha,India.104pp..

Basak U.C and Mekro P. (2023). Research and Activity Report 2021-22. Published by Regional Plant Resource Centre, Forest, Env& CC. Dept,Govt. of Odisha,Nayapalli,BBSR-15,Odisha,India.104pp..

## 7. 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 infavour of the student trainee.

### Detailed account of the Training provided

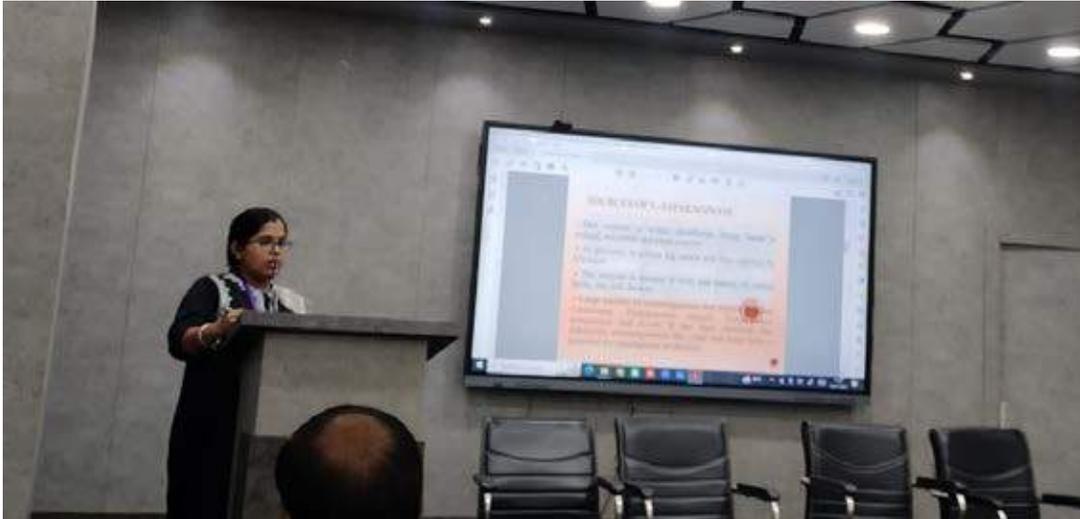
Sl.no.	Name of the Student Trainee	Topic	Supervisor/ Guide
1.	Sanjana Singh SOA Univ, BBSR	Evaluation of different plant growth regulators on germination of <i>Spathoglottis plicata</i> (Bl.) and <i>Cattleya 'Pink'</i> (Orchidaceae) seeds under in vitro conditions.	Dr. N.R. Nayak, Sr. Scientist
2.	Megha Gupta SOA Univ, BBSR	Real Time Quantitative PCR based gene expression studies of a major cell wall modifying genes in banana during fruit development and ripening	Dr. G.K. Surabhi, Sr. Scientist

**Ph.D. Pursuing/Submitted/Awarded**

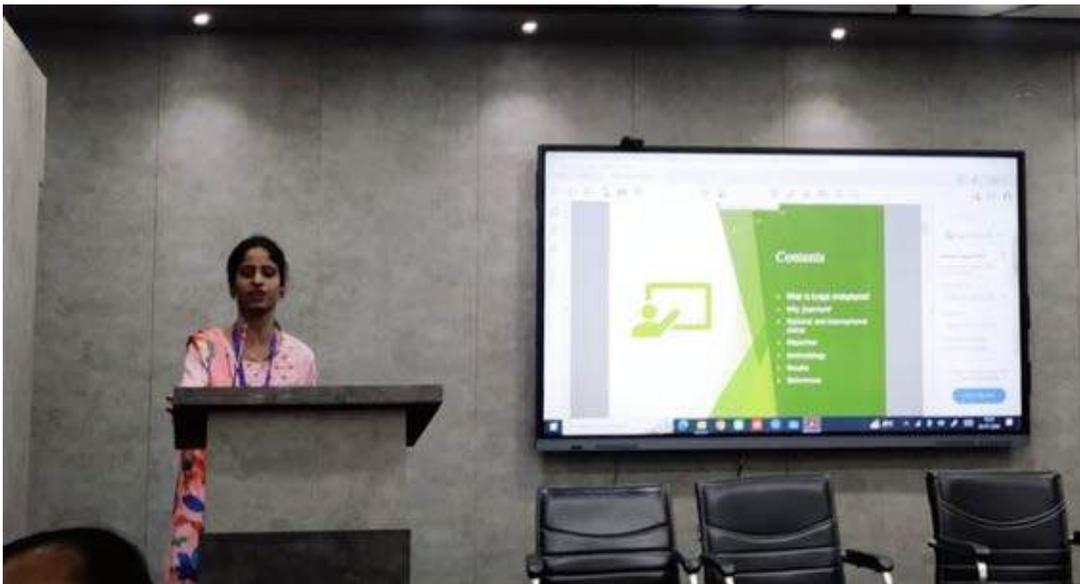
Sl. No.	Name of the Supervisor/ candidate	Title of the Doctoral program	University registered/ Year	Status
1	<b>Dr. Nibha Gupta, Pr. Scientist</b>			
	Mrs. Smita Behera	Optimization of cultural and nutritional conditions for enhanced production of exopolysaccharide by some fungi.	Utkal University, Bhubaneswar (Reg. No.07 –Life Science 2015-16)	Awarded
	Ms. Atmaja Elina Mishra	Process optimization for enhanced recovery of bioactive secondary metabolite from endophytic fungi	KIIT University, BBSR in Biotechnology, Registration no. – 21467381857	Pursuing
	Mrs. Rupa Acharya	Optimization, production, purification and characterization of L-asparaginase enzyme form some fungi.	KIIT University, BBSR in Biotechnology Registration no. – 20667579426	Pursuing
	Ms. Debjani Samantray	Bioprospecting endophytic fungi from Terminalia species for antifungal properties”	Utkal University, Bhubaneswar Registration no. 001-Life Science, 2020-2021	Pursuing
2	<b>Dr. Nihar Ranjan Nayak, Sr. Scientist</b>			
	Mrs. Johnnita Tirkey	Development of molecular tools for dendrobium species and their hybrids (Orchidaceae) for application in horticulture industry.	Dept. of Botany Utkal University. (Reg. No 06-Biotechnology, 2017-18)	Pursuing
	Mr. Debabrata Dash	Optimization of Various Parameters for the Production of Second Generation Bioethanol from the Efficient Biomass Producing Plants of Odisha.	Utkal University, Bhubaneswar. (Reg. No 07-Biotechnology, 2017-18)	Pursuing
3	<b>Dr. Giridara Kumar Surabhi, Sr. Scientist</b>			
	Mr. Subhankar Mohanty	A proteome approach to investigate fruit ripening and identification of key ripening proteins/genes in banana.	Utkal University, Bhubaneswar. 06-Biotechnology, 2017-18	Submitted
	Mr. Dinesh Pradhan	Transcription profiling and molecular characterization of candidate fruit ripening associated genes in banana.	Utkal University, Bhubaneswar. 03-Biotechnology, 2018 -19	Pursuing

4				
Dr. A.K. Sahoo, Sr. Scientist				
	Mr. Satish Kanhar	Phytochemical and Biological Evaluation of three Indian Homalium species with special reference to Hepatoprotective activity in CCl <sub>4</sub> -Induced oxidative stress in Wistar rats.	Utkal University, Bhubaneswar. (Regd. No. 02-Pharmacy-2016-17).	Submitted
	Mr. Umesh Chandra Dash	Pharmacological profiling of Geophilarepens and Bacopa floribunda and evaluation of their therapeutic potential against Alzheimer disease.	Utkal University, Bhubaneswar. (Regd. No. 10-Biotechnology-2016-17).	Awarded
	Mr. Sandeep Kumar Swain	Ethnopharmacological significance and therapeutic evaluations of Hydroleazeylanica in experimentally induced type 2 diabetes in rats.	Utkal University, Bhubaneswar. (Regn.no. 01-Biotechnology-2017-18)	Awarded
	Mrs. Deepmayee Rout	Ameliorative effects of Homaliumzeylanicum on diabetes induced oxidative stress and inflammation in Wistar rats.	Utkal University, Bhubaneswar. (Regd. No. 11-Biotech-2016-2017).	Awarded
	Mrs. Neelam Meher	Pharmacological validation of Buchanania lanzan fruits and its protective effects on high-fat-diet and streptozotocin induced type-2 diabetic neuropathy in Wistar rats (Rattus norvegicus)	Utkal University Regd. No. 03-BIOTECH-2021-22; Dt. 06-02-2021	Pursuing
	Mr. Bikash Kisan	Phytochemical and Biological Evaluation of Aporosa octandra with specific reference to Hepatoprotective activity in CCl <sub>4</sub> -induced oxidative stress in Wistar rats	Utkal University	Pursuing
5				
Dr. U. C. Basak, Sr. Scientist				
	Mrs. Swadha Baral	Essential amino acid profiling of some wild edible fruits of Odisha. (Reg. No. 04-Biotechnology-2017-18)	Utkal University, Bhubaneswar. (Reg. No. 04-Biotechnology-2017-18)	Awarded
	Ms. Abhipsa Anjeela	Identification and Characterization of some immune-enhancer bioactive compounds in wild edible fruits for therapeutic value.	KIIT University, Bhubaneswar, in Biotechnology (Regd. No. 21467481858, 2021-2022)	Pursuing
6				
Dr. Kalidass C., Scientist				
	Mrs. Madhusmita Mallia	Study of diversity, distribution and systematics of the family Solanaceae in Eastern Ghats of India	Utkal University (Reg. No. 09-Botany-2018-19)	Pursuing
	Mrs. Sasmita Pati	Study of the ethnobotany and traditional conservation practices of the Saora tribes of Odisha	Utkal University (Reg. No. 09-Botany-2020-21)	Pursuing

## 8. SEMINAR/CONFERENCES



*Rupa Acharya attended the conference on "Screening and process optimization of L-asparaginase production by fungi" at International conference on Recent Trends in Biotechnology ICRTB 2023 organized by Department of Biotechnology, Centurion University on 19th July 2023.*



*Debjani Samantaray attended the conference on "Analysis of bioactive potential of fungal endophytes against Fusarium sp." at International conference on Recent Trends in Biotechnology ICRTB 2023 organized by Department of Biotechnology, Centurion University on 18th July 2023 and received best oral presentation award.*



Oral Presentation by Abhipsa Anjeela on topic entitled "Characterization of L-Ascorbic Acid (Vitamin-C) in *Antidesma ghaesmbilla*, a wild edible fruit used by Tribal communities of Odisha" at International conference on Recent Trends in Biotechnology on 18th – 19th July 2023 organized by Department of Biotechnology, School of Engineering and technology, Centurion University, Bhubaneswar.



Poster presentation by Abhipsa Anjeela on topic entitled "Assessing Antioxidant capacities for fruits of *Polyalthiasuberosa* and *Glycosmis pentaphylla*: Insights from two agroclimatic zones of Odisha" at International conference on Materials for sustainable Environment 27th – 28th March 2024 organized by Centurion University, Bhubaneswar.



*Dinesh Pradhan, Ph.D. Scholar has attended and poster presented on Transcriptome analysis of banana reveals a major role of cell wall modifying genes in fruit softening at National Seminar on Recent Advancement in Plant Science for Sustainable Development, organized by P.G Department of Botany, Utkal University, Bhubaneswar on dt.30th March, 2024.*



*Subhankar Mohanty, Ph.D. Scholar has attended and poster presented on Deciphering molecular regulation of fruit ripening in banana: a gel-free proteome approach at National Seminar on Recent Advancement in Plant Science for Sustainable Development, organized by P.G Department of Botany, Utkal University, Bhubaneswar on dt.30th March, 2024.*



*Subhankar Mohanty, Ph.D. Scholar has secured best poster presentation for his poster entitled Deciphering molecular regulation of fruit ripening in banana: a gel-free proteome approach at National Seminar on Recent Advancement in Plant Science for Sustainable Development, organized by P.G Department of Botany, Utkal University, Bhubaneswar on dt.30th March, 2024.*

## 9. ORIENTATION PROGRAMME

Regional Plant Resource Centre, Bhubaneswar has organized an Orientation Programme of Project Fellow/Project Associate/Field-cum-Lab Assistants joined in 20 State Plan Projects and 1 External Project being implemented in RPRC for the year 2023-24 on 08.05.2023. The programme was chaired by PCCF-cum-Chief Executive Smt. Pusazhule Mekro, IFS in presence of all faculty Scientists , Administrative Officer, ACF. A total 31 researchers were present in this orientation programme and joined under various projects on Microbiological Applications, Tissue Culture & its application on various important plant spp. (banana, orchids, medicinal & forest spp. mashrooms), Medicinal plant and its application, Wild Edible Fruits : propagation and nutraceutical analysis, Propagation and reintroduction of RET & Mangrove plants.



*Orientation Programme of Project Fellow/Project Associate/Field-cum-Lab Assistants Chaired by Chief Executive Smt. Pusazhule Mekro, IFS in presence of all faculty Scientists Administrative Officer, ACF. In RPRC on 08.05.2023.*

## 10. 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.

## 11. 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.

## 12. EX-SITU CONSERVATION & GERMLASM COLLECTION

RPRC has its 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.

Wild Edible Fruits Garden : In order 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 110 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*, *Heritiera fomes*, *Heritiera littoralis*, *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: The iconic Botanic Garden (Ekamrakanan) of RPRC offers visitors a living museum of native and exotic plant collections of fragrant flowers, both wild and cultivated, in its themed section called 'Fragrant Flower Garden'. With a collection of more than 70 species of plants with scented flowers, the fragrance garden established in a unique landscape over about 3.5 acres of land and thus a must-visit site for everyone. The centrally placed giant Ashoka Chakra is surrounded by several landscapes represented by majority of Magnolias, Jasmines, Gardenias, Tabernaemontanas and many more scented flower groups inter-connected with network of visitors path and shed to enjoy the beauty and fragrance of the garden.



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.

Cacti and other Succulents: The Centre houses more than 1000 Varieties and cultivars of cacti & succulents, both for sale and display for visitors.



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.



## 13. FLOWER SHOW 2024

The Regional Plant Resource Centre (RPRC), Ekamra Kanan, Bhubaneswar has organised the Annual Flower Show 2024 in the premises of its Botanic Garden ) on 13th -14th January, 2024 in association with Plant Lovers' Association (PLA), Bhubaneswar with the support of Odisha Mining Corporation Ltd., Directorate of Horticulture, Odisha and Odisha Forest Development Corporation Ltd., NALCO, MGM Minerals, (P) Ltd. & SOA University, Bhubaneswar.

Inaugural Function:

Shri Debidutta Biswal, IFS, PCCF & HoFF, Odisha has inaugurated the Annual Flower Show, 2024. Shri Suresh Pant, IFS, PCCF & Chief Executive, RPRC accompanied Shri Biswal during the Inaugural Function and briefed the whole events of the Flower Show. All Scientists of RPRC, Senior Officers and office staff of RPRC and Plant Lovers Association were also present during the Inaugural Function in the morning session.

Shri Biswal has released 'Research and Activity Report 2022-23' of RPRC & Sabujima , souvenir of Plant Lovers' Association (PLA).

Prize Distribution Ceremony

In the afternoon session (at 3.30 PM on 13th Jan, 2024), Smt. Pusazhule Mekro, IFS, Former PCCF & Chief Executive of RPRC had graced the Prize Distribution Ceremony as Chief Guest in presence of Shri Rohit Kumar Lenka, IFS, Director, Directorate of Horticulture, as Guest of Honour. Prizes were given to the winners for Painting, Poster & Garden competitions.

An attractive "Floral Gate" has been erected at the entry point of the Flower Show premises, which has become a major attraction of the event along with other photo point sites.

A total 36 organizations/ firm, 19 nurseries (open space), 44 stalls have participated and exhibited ornamental plants for display and sale through Plant Bazaar. Apart from Cut Flower display, a "World of Special Group of Plants with landscape" were on display.

Valedictory Function (held at 3 PM on 14th January, 2024)

As a major part of the Flower Show, Floral Display Competition has been organized to encourage the plant lovers for growing flowering plants (seasonal/perennial/annuals) to add the beauty to the society. The winners of Floral Display Competition are being awarded with prizes and trophies in the Valedictory Function held on 14th January, 2024.

Shri Satyabrata Sahu , IAS, Additional Chief Secretary, Forest, Environment & Climate Change Dept., Odisha has graced the Valedictory Function as Chief Guest. Shri Balwant Singh, IAS, Managing Director, Odisha Mining Corporation Ltd., Shri K. Murugesan, IFS, Director, Environment-cum-Spl. Secretary to Govt., Odisha had graced the occasion as the Guest of Honour.

Under the able guidance of the Chief Executive, all scientists, Officers, staff, students and workers of RPRC had put their best efforts to make this Annual Flower Show, 2024 a grand success. The financial assistance obtained from OMC, Directorate of Horticulture, Odisha, OFDC Ltd., NALCO, MGM Minerals, (P) Ltd., SOA and help & assistance received from different participating institutions and individuals are gratefully acknowledged.

# Annual Flower Show 2024

on 13<sup>th</sup> to 14<sup>th</sup> January, 2024

In the premises of  
Botanic Garden (Ekamra Kanan)  
Bhubaneswar



**PROGRAMME**

**13<sup>th</sup> January, 2024 (Saturday)**  
07.00 AM - 09.00 AM: Registration of Exhibits  
09.00 AM - 10.00 AM: Judging of Exhibits  
10.00 AM - 10.45 AM: Inaugural Function  
Inauguration of Annual Flower Show 2024.  
Release of publication of RPRC and PLA.  
3.30pm - Prize distribution ceremony.

**14<sup>th</sup> January, 2024 (Sunday)**  
03.00 PM: Valedictory Function  
Prize Distribution Ceremony



Annual Flower Show 2024 of RPRC inaugurated by PCCF & HoFF, Odisha in presence of PCCF & Chief Executive, RPRC on 13th January, 2024



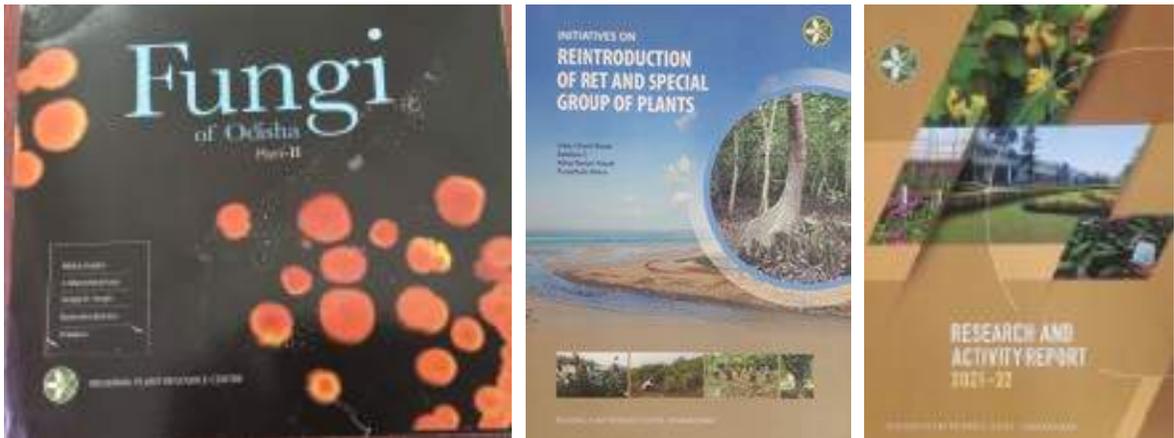




## 14. RELEASE OF BOOKS AND OTHER PUBLICATIONS



*RPRC Publications (2023 )Released on occasion of World Environment Day (June 5th , 2023): Book: i.Fungi of Odisha, Part-II; ii. Initiations of Reintroduction of RET and other Speial Group of Plants.*



### Book

- i. Fungi of Odisha, Part-II
- ii. Initiations of Reintroduction of RET and other Speial Group of Plants
- iii. Annual Research and Activity Report 2021-22

## 15. EXPOSURE VISIT OF VARIOUS INSTITUTIONS/ ORGANIZATIONS TO RPRC



*Exposure visit of Forest Personnel's from G.Udaigiri (2023)*



*Visit of Forest Trainees & Visit of Director, BSI, Kolkata*



*Exposure visit of Students from different educational institutions (2023)*



*Exposure visit of Students from OUAT, Bhubaneswar (2023)*



*Exposure visit of Students from different educational institutions (2023)*



## 16. CELEBRATION OF VAN MAHOTSAV 2023 IN RPRC: INTRODUCTION OF RET PLANTS IN RET GARDEN, RPRC



*Introduction of RET plants in RET Garden, RPRC during Van Mahotsav 2023*

## 17. INAUGURATION OF RPRC SALE COUNTER: BLOSSOM BAZAAR



*Inauguration of RPRC Sale Counter Blossom Bazaar by Additional Chief Secretary, FE&CC Dept, Odisha in presence of PCCF & HoFF & PCCF & Chief Executive, RPRC on 22nd December, 2023*

## 18. BALANCE SHEET

### REGIONAL PLANT RESOURCE CENTRE

NAYAPALLI, BHUBANESWAR-751015

BALANCE SHEET AS ON 31.03.2024

LIABILITIES	SCHEDULE	AMOUNT(RS.)	ASSETS	SCHEDULE	AMOUNT(RS.)
General Fund	1	15,73,89,113	Fixed Assets	5	7,75,85,415
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	1,17,93,162	Current Assets		
			Loans & Advances	7	8,39,23,690
			Cash in Hand		2,077
			Cash at Bank		12,59,15,030
<b>Total</b>		<b>32,89,78,058</b>	<b>Total</b>		<b>32,89,78,058</b>

For PARTHA S MISHRA & CO.  
Chartered Accountants

*Sanjaya kumar Patra*

CA S.K. Patra (FCA, DISA)  
Partner, M. No- 301929



UDIN: 24301929BKAKMJ6402  
Place: Bhubaneswar  
DATE: 27.09.2024

*[Signature]*  
Administrative-cum-Finance Officer  
Regional Plant Resource Centre  
Bhubaneswar



**Regional Plant Resource Centre**

Forest, Environment and Climate Change Department, Govt. of Odisha  
Nayapalli, Bhubaneswar 751015, Odisha, India,  
Phone: (0674) 2557925, E-mail: rprcbbsr@gmail.com

[www.rprcbbsr.in](http://www.rprcbbsr.in)