Bioprospecting potential of Excoecaria agallocha (Mangrove) at Machilipatnam seacoast Andhra Pradesh, India

A project report is submitted To

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M.Sc. Botany

By

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CERTIFICATE

This is to certify that the research work described in this project report entitled " Bioprospecting Potential of Excoecaria agallocha ***(Mangove) at Machilipatnam Seacoast, Andhra Pradesh India*** *is the outcome of work carried out by Ms.Chandika Kodali* ***Msc Botany Roll no :*** *Y21BOT101004 project student, University college of Arts and Science, Krishna University ,Rudravaram , Machilipatnam , Andhra Pradesh , India.*

Place : Machilipatnam

Date : 09/09/2023

12/9/2023

12/9/23

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Head of the Department

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DECLARATION

I HERE DELARE THAT RESEARCH WORK EMBODIED IN THIS PROJECT REPORT ENTITLED BIOPROSPECTING POTENTIAL OF EXCOECARIA AGALLOCHA(MANGROVE) AT MACHILIPATNAM SEACOAST ANDHRA PRADESH INDIA. SUBMITTED TO KRISHNA UNIVERSITY FOR PROJECT WORK IN BOTANY , IS THE OUT COME OF INVESTIGATION CARRIED OUT BY ME AS PROJECT WORK UNDER THE SUPERVISION OF DR.MADHURI VAJHA , DEPARTMENT OF BOTANY, KRISHNA UNIVERSITY RUDRAVARAM, MACHILIPATNAM. I ALSO AFFIRM THAT THE PROJECT WORK IS ORIGINAL AND HAS NOT BEEN SUBMITTED TO ANY OTHER UNIVERSITY OR INSTITUION.

PLACE : MACHILIPATNAM

DATE : 09/09/23

K.Chandika.

NAME: K.CHANDIKA

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PREFACE

Research on distribution phytochemicals, taxonomical diversity in Mangrove plants including *Rhizophora mangle, Brugeria cyclindrica, Brugeria gymnorhiza, Rhizopora apiculate, Avicennia marina, Avicennia alba*, species has been extensively done in India, but much attention has not been paid on pharmacological studies with reference to phytochemical composition despite extensive traditional use of these species as well as extreme biodiversity. Based on ethanobotanical, ethanopharmacological studies, current scientific studies are carried out and the importance of bioprospectingand biodiversity of these species explained, project is entitled with "Bioprospecting Potential of Excoecaria agallocha has been identified and studied.

The species of Excoecaria agallocha found in India are eaten as a vegetable and became a part of traditional system of the country. The medicinal activities of the genus Excoecaria, shows anti-inflammatory and anti-rheumatoid arthritis and preventing blood pressure, bleeding and ulcers. The medicinal properties of Excoecaria agallocha are due to the presence of secondary metabolites in them.

The present research, therefore focused on taxonomical studies, preliminary phytochemical screening and antimicrobial activities and pharmacological activities of Excoecaria agallocha.

CONTENTS

TERMS AND ABBREVATIONS

ARB	**antibiotic -resistant pathogens**
NDV	**New castle disease**
VV	**Vaccinia Viruses**
HBV	**Hepatitis B. viruses**
TMV	**tobacco mosaic viruses**
TPA	**Tissue plasminogen activator**
HPV	**Human papilloma virus**
NAM	**N-acetyl glycosamine**
AWD	**Agar well diffusion**
MIC	**Minimum inhibitory concentration**
ZOI	**Zone of inhibition**
MTTC	**Microbial type culture collection**
CFU	**Colony -forming units**
UV	**Ultraviolet**
Amt	**amount**
Approx	**approximately**
Mol wt	**Molecular weight**
Temp	**temperature**
TLC	**Thin layer chromatography**
HPLC	**High performance liquid chromatography**
GCMS	**Gas chromatography & mass spectroscopy**
GCMS	**Gas chromatography & mass spectroscopy**
CMS	**Liquid chromatography & mass spectroscopy**

Introduction

The plants of the genus Excoecaria is belongs to family: Euphrobiaceae, comprise nearly different 42 species spreading all over tropical Asia, Africa, and northwest Australia. It is the most widely reported species. The leaves and latex of this tree has been used as a purgative and abortifacient. The recent analysis of assorted disease showed that it is used in a dart poison and fish poison in India, Malaysia and New Caledonia. The experiment analysed the diseases such as Ulcers, Rheumatism, Leprosy, and paralysis. The bark and wood of Excoecaria plants have been applied in Thailand medicine as a remedy against flatulence. Recently much attention is paid to Excoecaria species due to its Anti Reverse Transcriptase and anti-HIV activities. In this review, we will expand the photochemical development and try to list all the compounds isolated from the genus Excoecaria over the past few decades. We include the biological activities of compound reported in recent years.

Excoecaria agallocha L (Euphrobiaceae) is an important mangrove species mainly distributed in China, India, Philippines, and Oceania. This plant is used as a traditional remedy for epilepsy, conjunctivitis, dermatitis, haematuria, leprosy, and toothache. The latex and leaves have been used as a dart poison and fish poison in Sarawak, New Caledonia, and Goa . The phorbol ester isolated from the leaves and stems has been proved to be cytoprotective in the NCI primary anti-HIV screen . Some diterpenes isolated from the wood of this plant showed anti-tumour-promoting activity . However, there is scant information on the isolation of antifouling compounds from mangrove species.

BIOLOGICAL ACTIVITIES OF MANGROVE PLANTS

S.NO	PLANT NAME	ACTIVITY	AUTHOR
1	*Excoecaria agallocha*	Anti leprosy, Temporary eye blindness	Sumanta Mondal, Debjit Ghosh
2	*Bruguiera cylindrica*	Used as firewood	Fernando,E.S
3	*Rhizophora apiculate*	Used in interiors of houses	Banyak Minyak

4	*Laguncularia racemosa*	The bark is used to treat fishing nets for longer preservation	P.C.M. Jansen et al
5	*Rhizophora mucronata*	Used for logs and dyes and anticancer agents	Duke, N. Kathiresan
6	*Xylocarpus granatum*	Used for diarrhea, cholera, fever and malaria	Dipta Dey et al
7	*Aviecina marina*	Used for snake bites	Abeysinghe, P.D., R.P Wanigatunge
8	*Rhizophora mangle*	Used as an anti-septic	Ellison, A; Farnsworth, E; Moore,G
9	*Acrostichum aureum*	Used for wonds and boils	ReheleNamazi,RezvanZabihollahi
10	*Sonneratia caseolaris*	Used for perfumes and skin cosmetics	Azlen Che Rahim,MohdFadzelly Abu Baker
11	*Lumnitzera racemose*	Used for bridge construction and charcoal making	Ta-Chen Lin *et al*
12	*Sonneratia alba*	Alba is used as firewood	Shahbudin Saad, Muhammad Taher
13	*Acrostichu maureum*	Peptic ulcers, anti bleeding	Xue Wu *et al*
14	*Aegirascorni culatum*	Antiplasmodial activity	Ravi kumar *et al*

15	*Avicennia offiicinalis*	Anti-asthma and anti-tumor	NambaliValsalan Vinith Kumar *et al*
16	*Acanthus ilicifolius*	Used for Snake bite	Dharyasingh and Vidhu Aeri
17	*Bruguiera gymnorhiza*	Used to tan animal's skins	Anson Bay *et al*
18	*Nipa Palm*	Used for headaches and toothaches	Camacho, Leni *et al*
19	*Kandelia candel*	Used as green manure, tanning heavy leather	M.S.M Sosef& L.J.G Van Der Maesen
20	*Rhizophora stylosa*	Used to produce charcoal and dyeing leather	Salmo, S.G. Fernando

Results and Discussion

One new diterpene, *ent*-16α-hydroxy-atisane-3,4-lactone (**4**), and three known diterpenes: *ent*-16α-hydroxy-atisane-3-one (**1**), *ent*-atisane-3β,16α-diol (**2**) and *ent*-3,4-*seco*-16α-hydroxyatis-4(19)-en-3-oic acid (**3**) were isolated from the bark of *E. agallocha*. The structures of these known diterpenes were identified by comparison of their spectral data with those reported in the literature.

Morphological and anatomical features of *Excoecaria agallocha*;

INTRODUCTION;

Based on the provided information, here are the morphological characters and habitat descriptions of the two mangrove species, Acanthus ilicifolius and Excoecaria agallocha:

1. Acanthus ilicifolius L.:

- Habit: A dioecious tree up to 15 m high with abundant white latex.

- Habitat: An evergreen shrub commonly found along higher estuarine banks, canals, tidal forests, and mangrove swamps.
- Stem: Bark is grayish and lenticellate (having small raised pores for gas exchange).
- Roots: Lateral roots spread and intermingle with each other. They produce elbow-shaped pegs instead of pneumatophores (specialized aerial roots for gas exchange).
- Leaves: Alternate, ovate-elliptic, or orbicular in shape with a shortly acuminate apex and a narrowed base. The leaf margin is entire or sinuate-crenate (slightly wavy or shallowly
    - The size of the leaves ranges from 3-8 × 1.5-3 cm, and they are glabrous (hairless). The leaves have petioles.
    - Flowers: Unisexual. Male flowers are in catkin spikes, fragrant, and yellow, measuring 2-3 mm across. They have three stamens with free filaments. Female flowers are in axillary racemes, pale green, and measure 2.5-3.5 mm across. They are pedicellate (have a stalk or pedicel). The calyx is 3-lobed, and the ovary is 3-celled with a trifid style.
    - Fruit: Capsule, globose and 3-lobed. The seeds are sub-globose.

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2. Excoecaria agallocha L.:

- Habit: A dioecious tree up to 15 m high with abundant white latex.
- Habitat: An evergreen shrub commonly found along higher estuarine banks, canals, tidal forests, and mangrove swamps.
- Stem: Bark is grayish and lenticellate (having small raised pores for gas exchange).
- Roots: Lateral roots spread and intermingle with each other. They produce elbow-shaped pegs instead of pneumatophores (specialized aerial roots for gas exchange).
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Fig1; *Excoecaria agallocha*

Both species are dioecious (having male and female flowers on separate individuals) and are well-adapted to tidal and coastal lands, particularly in mangrove ecosystems. These habitats provide important ecosystem services and support a wide array of aquatic and terrestrial flora and fauna. Mangrove forests are unique and highly productive ecosystems with significant social, economic, and biological importance. They have the ability to tolerate inundation by saltwater through physiological and chemical mechanisms. Both Acanthus ilicifolius and Excoecaria agallocha are part of the mangrove ecosystem, which is constantly under physiological stress due to extreme environmental conditions.

Taxonomical Classification

the details of the taxonomic position of *Excoecaria agallocha* L. is given in [Table 1].[11]

Table 1

Taxonomical classification of *Excoecaria agallocha* L.

Kingdom	Plantae
Phylum	Charophyta
Class	Equisclopsida
Subclass	Magnoliidae
Order	Malpighiales
Family	Euphorbiaceae
Genus	Excoecaria
Species	agallocha

Materials:

1. Survey equipment (e.g., compass, GPS device, measuring tape)
2. Notebooks and writing materials for recording observations
3. Camera or smartphone for taking photographs
4. Collection bags or containers for storing plant specimens
5. 'The flora of Presidency of Madras' by Gamble [3] for reference

Methods:

1. Survey Trip Preparation:

   Plan the survey trip to Ayiramthengu, Kollam District, Kerala.

   Ensure all necessary equipment and materials are packed and ready for the field trip.

Field Survey:

Conduct the survey trip to Ayiramthengu, an area known for mangrove species.

Navigate through the mangrove habitat using a compass and/or GPS device to ensure proper coverage of the study area.

During the survey, carefully observe and identify mangrove species present in the area.

3.Selection of Mangrove Species:

From the various mangrove species observed during the survey, choose two specific species for the present study. In this case, Excoecaria agallocha is one of the selected species.

4.Collection of Plant Specimens:

For the selected mangrove species (e.g., Excoecaria agallocha), collect fresh plant specimens.

Handle the specimens with care to avoid damage during collection.

5.Morphological Studies:

Examine the fresh plant specimens of Excoecaria agallocha.

Conduct morphological studies, which involve observing and documenting various physical characteristics of the plant, such as leaf shape, size, venation patterns, stem structure, flowers, fruits, etc,

6.Identification:Use 'The flora of Presidency of Madras' by Gamble [3] as a reference for identifying and confirming the morphological identities of the collected plant specimens.

7.Data Recording:

Record all relevant data, including the location of specimen collection, date, and detailed morphological observations, in notebooks or electronic devices.

It's worth noting that the above-described methods focus on the survey and morphological study of the selected mangrove species (Excoecaria agallocha). Other aspects of the study, such as data analysis, comparisons with other mangrove species, and any additional experiments, may require different methods beyond what's listed here.

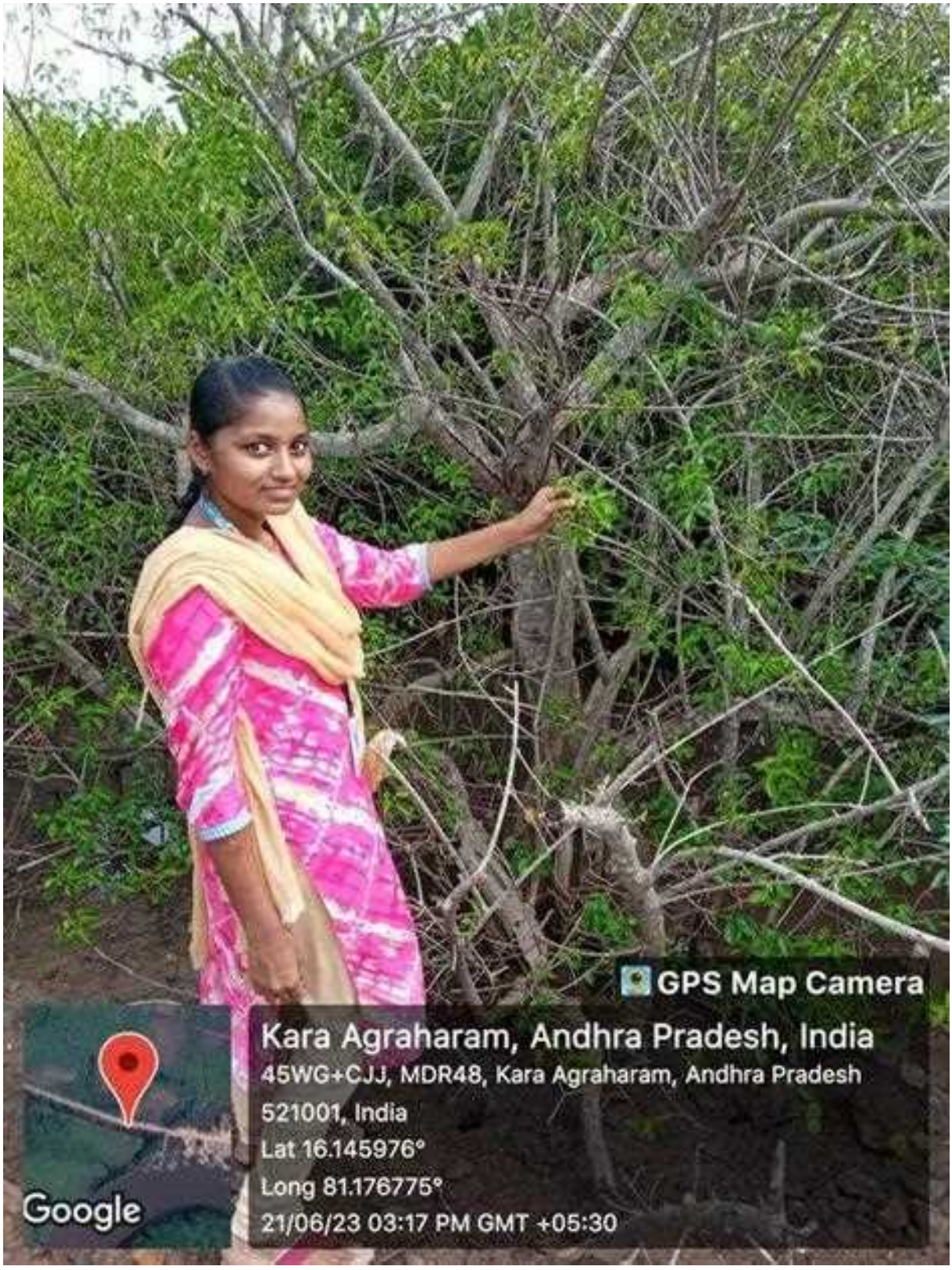
Materials:

Fresh plant specimens of the selected mangrove species (e.g., Excoecaria agallocha)
Scalpel or razor blade for making hand sections
Microscope slides
Cover slips
Microscope for observation
Safranin stain
Distilled water for washing sections
Glycerol

Methods:

Collection of Plant Material:

Obtain fresh leaves and stems of the selected mangrove species (Excoecaria agallocha) from the field. Make sure to collect healthy and representative samples.



Preparation of Hand Sections:

Using a scalpel or razor blade, carefully cut thin sections of the leaves and stems. These sections should be thin enough to allow light to pass through during microscopic observation.

3.Staining:

Take the hand sections and place them in a dish containing safranin stain. Safranin is a commonly used stain for plant tissues, and it helps to highlight various cellular components.

4.Washing:

After staining, wash the sections well in distilled water to remove excess stain and any impurities.

5.Mounting:

Take a clean microscope slide and place the washed sections on it.

Add a mixture of 1:1 glycerol and water on top of the sections. This mixture serves as a mounting medium, helping to preserve the sections and facilitate microscopic observation.

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Cover Slipping:

Gently place a cover slip over the mounted sections, taking care to avoid trapping air bubbles.

Microscopic Observation:

Place the prepared slide under a microscope and focus on the stained sections.

Observe the anatomical structures of the leaves and stems at various magnifications. Identify and document any peculiarities or unique features observed in the selected mangrove species.

Data Recording:

Record all relevant observations and findings during the anatomical studies in a notebook or electronic device.

Anatomical studies can provide valuable insights into the internal structures of plants and help researchers better understand the adaptations and characteristics of mangrove species. The stained and mounted sections allow for detailed observation of cellular arrangements, tissue types, and other anatomical details that may be crucial for the study.

ANOTOMY OF *Excoecearia agallocha;*

Anatomical studies can provide valuable insights into the internal structures of plants and help researchers better understand the adaptations and characteristics of mangrove species. The stained and mounted sections allow for detailed observation of cellular arrangements, tissue types, and other anatomical details that may be crucial for the study.

Anatomy of stem: Stem is covered by a single layered epidermis with thin layer of cuticle. Epidermis is followed by sclerenchymatous hypodermis. Cortex consists of chlorenchyma followed by inner parenchyma cells containing deposits of tannin. Stele is broad. Vascular bundles are conjoint, collateral and open. Secondary xylem and phloem are well developed. Xylem region consists of highly lignified cells. Primary xylem is ending arch. Medullary rays are distinct consisting of vertically elongated cells which are 1-2 cells in thickness. Pith is large and consists of large number of parenchymatous cells with oil deposits.

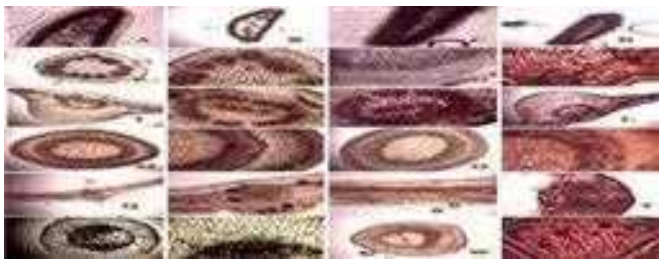

Fig5; Stem anatomy of Excoecaria agallocha

Anatomy of leaf: Epidermis is single layered with thick cuticle over it. Below the epidermis thin-walled water storage cells are present. Below the storage cells mesophyll is present which is differentiated into upper palisade and lower spongy tissue. Palisade is biseriate consisting of radially elongated compactly arranged cells containing numerous chloroplasts and is dark green in colour. Spongy tissue consists of loosely arranged cells with less intercellular spaces. Vascular bundles are conjoint, collateral and closed. Bundle sheath is sclerenchymatous and xylem is well developed. The lower epidermis is provided with sunken stomata.

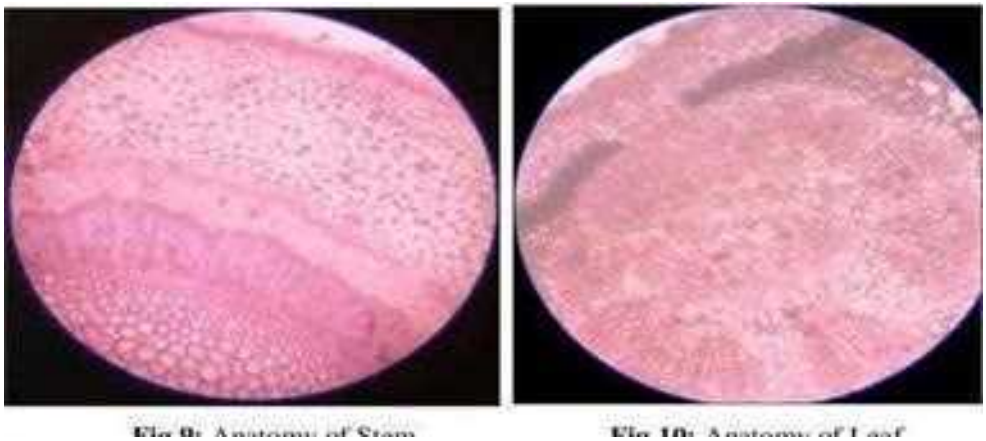

Fig6; Anatomy of stem Fig7; Anatomy of Leaf

Result and Discussion;

Mangroves are trees or shrubs that grow in shallow and muddy salt water or brackish water such as those along shorelines or in estuaries. Morphological and anatomical studies on 2 different species of mangroves reveals that the plants growing in salt marshes of Excoecaria agallocha L of Kollam district develops a number of adaptations to survive in the physiologically dry habitat. Morphology and anatomy of halophytes has previously been reported by many botanists. Halophyte in general shows xerophytic characters and adaptations [4]. Present study also revealed several morphological and anatomical adaptations similar to xerophytes. Morphologically Excoecaria agallocha is a dioecious tree with woody stem that branch profusely. Excoecaria agallocha has knotted roots that run over the surface. Even though both roots differ morphologically they provide an additional support to the main trunk and protect them from different calamities like erosion, cyclones, sea waves, etc. Leaves of the Excoecaria agallocha is not much thick but have shiny leaves. Leaf margins were smooth and entire in Excoecaria agallocha. Flowers were unisexual in Excoecaria.

Fig8; Powder of *E. agallocha* leaves

EYE BLINDNESS PROFILE OF *Excoecaria agallocha*

A Complete Profile on Blind-your-eye Mangrove *Excoecaria Agallocha* L. (*Euphorbiaceae*): Ethnobotany, Phytochemistry, and Pharmacological Aspects;

Medicinal plants have played a crucial role in human societies for centuries, providing valuable resources to combat various diseases. In India, medicinal plants are widely distributed and have been an integral part of traditional medicine systems. The World Health Organization (WHO) survey indicates that a significant portion of the population in countries like India, Bangladesh, and Burma rely on traditional medicinal practitioners for their healthcare needs.

Over the years, there has been a global increase in the use of health products derived from plants, leading to substantial growth in the herbal products market. This surge in popularity has also led to an increase in research focusing on medicinal plants and their potential benefits.

Many drugs used in modern medicine have their origins in plants. For example, morphine, which is derived from the poppy plant (Papaver somniferum), is a potent pain-relieving medication. Atropine, obtained from Atropa belladonna, has various medical applications, including as a muscle relaxant and to dilate the pupils during eye examinations. Ephedrine, derived from Ephedra vulgaris, has been used for its stimulant and decongestant properties. Reserpine, sourced from Rauvolfia serpentina, has been used to treat high blood pressure and some mental health conditions.

These plants contain various secondary metabolites that are considered potential sources of drugs and have significant therapeutic value. Medicinal plant-based medicines are often regarded as easily accessible, safer, more economical, and effective alternatives to synthetic drugs.

The utilization of medicinal plants for healthcare needs has a long history, and with the increasing interest and research in this field, it is likely that their importance will continue to grow in both developed and developing countries. However, it is essential to ensure sustainable practices in harvesting and using medicinal plants to conserve biodiversity and protect these valuable natural resources. Additionally, scientific research and regulatory measures are necessary to validate the efficacy and safety of herbal products to ensure their appropriate use in modern healthcare practices.

The word "mangue" mentioned in the context of the Spanish language in Senegal is of particular interest. According to Marta Vannucci's work "Neither Portuguese nor Spanish: The Spanish Language of Senegal," published in 1989, she explains that the word "mangue" is not of Portuguese or Spanish origin. Instead, she suggests that it derives from the local national languages spoken in the region, particularly as a result of the historical exploration of the West African coast.

The exploration and interaction of European explorers and traders with the local populations in West Africa could have led to the adoption of certain words from the local languages into the Spanish vocabulary. This process of linguistic exchange and borrowing is not uncommon in the history of language contact.

various characteristics and uses of the mangrove plant *Excoecaria agallocha L.;*

Biocidal effects: The latex of Excoecaria agallocha is well-known for its biocidal effects on marine organisms and phytoplankton, meaning it has the ability to inhibit or kill various living organisms in marine environments.

Effects on rice field crab: The latex also causes metabolic depression in the rice field crab, Oziotelphusa senex, which suggests that it may have physiological effects on certain animal species.

Various uses: The plant's latex has multiple uses, including being used as an uterotonic (a substance that stimulates uterine contractions), fish poison (to catch fish), and dart poison (likely used for hunting purposes).

Chemical constituents: The plant contains novel chalcones and piperidine alkaloids, which are specific types of chemical compounds found in the plant.

Leaf shedding: E. agallocha is a deciduous mangrove species, and it sheds its leaves annually.

Aerial roots: Unlike most mangrove species, E. agallocha does not have specialized aerial roots called pneumatophores that extend above the soil surface. Aerial roots in mangroves serve various functions, including gas exchange in waterlogged environments.

ETHANOBOTANICAL INFORMATION

Excoecaria agallocha L. is a versatile plant with significant economic, ecological, and medicinal importance. Here are some of its notable uses and applications:

Medicinal Uses: Traditionally, the plant is used for various medicinal purposes. It is employed in the treatment of ulcers, sores, and stings from poisonous marine creatures. It also functions as an emetic (induces vomiting) and purgative (promotes bowel movement). Additionally, the plant is used to treat rheumatism, leprosy, paralysis, a nd other conditions.

Abortifacient and Purgative: The latex exuded from the plant has been used as an abortifacient (substance inducing abortion) and as a purgative for medical purposes.

Fish Poison: The leaves and latex of the plant are used as fish poison by people in Malaysia, India, and New Caledonia. This suggests that the plant's latex, which has biocidal effects, can be used to immobilize or kill fish for catching purposes.

Traditional Uses in Various Countries: In Thailand, the wood and bark of the plant are used as a remedy for flatulence (gas in the digestive system). In Sri Lanka, the root pounded with ginger is used to treat swellings of hands and feet, and the smoke obtained from burning the wood is used to cure leprosy. In Burma, the leaves are used to treat epilepsy.

Toxic Properties: The plant's milky sap or latex is toxic and can cause temporary blindness if it comes into contact with the eyes. It can also cause skin blisters and irritation, indicating its poisonous nature.

Aphrodisiac Tonic: In Indian traditional medicine ("materia medica"), a soft reddish substance ("Tejbala") obtained from the lower part of the trunk of E. agallocha was used as a reputed aphrodisiac tonic, suggesting its use in enhancing sexual desire or performance.

Emesis and Purgation: In the Solomon Islands, the latex exuded from the plant is mixed with coconut milk and used as a powerful emetic and purgative, indicating its use in inducing vomiting and promoting bowel movements.

Skin Treatment: The Malays use the oil distilled from the woods to treat itching and skin infections.

Toothache and Swellings: The roots of the plant are used to treat toothache and swellings, and they are also used as an ingredient in embrocations (medicinal liniments or ointments).

PHARMACOLOGICAL ACTIVITIES

Antioxidant activity;

1. **Evaluation of Antioxidant Activity:** Researchers conducted antioxidant activity assessments using different models and methods to understand the plant's potential in combating oxidative stress.
2. **Hossain et al.:** The study by Hossain and colleagues tested the antioxidant activity of dried powder from E. agallocha bark. They used various antioxidant models, such as reducing power and DPPH free radical scavenging activity, to measure the plant's ability to counteract oxidative stress. Results indicated that the distilled water and ethanol fractions showed higher antioxidative activities compared to other fractions.
3. **Rajaram Panneerselvam et al.:** This study evaluated the antioxidant activity of both field-grown and micropropagated E. agallocha leaves using the DPPH radical scavenging assay. The micropropagated plants exhibited greater DPPH radical scavenging effects compared to field-grown plants. Ascorbic acid content and total phenolic content were also higher in micropropagated plants.

4. **Antioxidant Activity of Aqueous Extract:** Another study investigated the antioxidant activity of the aqueous extract of E. agallocha. The study found that the antioxidant activity, assessed through DPPH radical scavenging, reducing power, and hydrogen peroxide scavenging assays, increased in a dose-dependent manner. Phenol content was also measured, showing varying levels in different extracts.
5. **Poorna et al.:** This study examined the antioxidant activity of methanol extract from E. agallocha leaves. The methanolic extract demonstrated strong DPPH free radical scavenging activity, as well as inhibition of nitric oxide radicals, lipid peroxidation, and metal chelating effect.
6. **Patra et al.:** In this study, the antioxidant activity of a thin layer chromatography fraction from E. agallocha leaves was assessed. The fraction showed DPPH radical scavenging activity at a specific concentration.

Overall, these studies collectively suggest that Excoecaria agallocha possesses antioxidant properties, which could potentially have various health benefits. The plant's antioxidant activity may be attributed to its various chemical constituents, such as phenolic compounds and other bioactive molecules. However, it's important to note that the specific mechanisms of action and potential clinical applications require further investigation.

Antimicrobial activity

1. **Varaprasad Bobbarala et al.:** This study evaluated the antimicrobial activity of hexane, chloroform, and methanol extracts from E. agallocha leaves against a range of microorganisms, including bacteria and fungi. The methanol extract exhibited higher antimicrobial activity compared to the hexane and chloroform extracts.
2. **Antimicrobial Activity Against Fish Pathogens:** Another study examined the antimicrobial activity of methanolic extracts against selected fish pathogens. The extracts demonstrated significant inhibitory effects against the tested fish pathogens, with varying minimum inhibitory concentrations (MIC) and bactericidal concentrations.
3. **Antibacterial Activity of Methanolic Extracts:** Researchers prepared methanolic extracts from wild-grown plants, leaf-derived callus, and in vitro raised plant leaves of E. agallocha. The study revealed that the methanolic extract from leaf-derived callus exhibited more antibacterial activity than extracts from wild-grown and in vitro raised plants.

4. **Antimicrobial Activity of Fatty Acid Methyl Esters (FAMEs) Extracts:** The study conducted by Agoramoorthy et al. examined the antimicrobial properties of FAME extracts from E. agallocha. The FAME extracts showed strong antimicrobial activities against a range of bacteria and yeast strains.
5. **Antibacterial Activity of Ethanol Extracts:** Ethanol extracts from different parts of E. agallocha (leaves, roots, and stems) were screened for antibacterial activity against various bacterial strains. The dried leaf extract exhibited higher antibacterial activity compared to fresh plant extracts.
6. **Antifungal Activity:** The antifungal activity of E. agallocha leaf extracts was tested against different fungal pathogens. The extracts demonstrated significant antifungal effects against certain fungal pathogens, such as R. solani, Fusarium udum, and M. phaseolina.
7. **MIC Values:** The MIC values for different bacterial strains were determined using microtiter plate technique. The MIC values indicated the concentration at which the extracts effectively inhibited the growth of the tested bacterial strains.
8. **Additional Antimicrobial Activity:** The leaves of E. agallocha were also found to exhibit antimicrobial activity against various bacterial pathogens, including S. aureus, B. subtilis, Shigella flexneri, P. aeruginosa, and E. coli.

These studies collectively suggest that Excoecaria agallocha possesses antimicrobial properties that could potentially be harnessed for various applications, including the development of antimicrobial agents for medical and agricultural purposes. However, further research is needed to better understand the mechanisms of action, specific compounds responsible for these effects, and potential applications in real-world settings.

ANTIMICROBIAL ASSY OF MANGROVE PLANTS;

S.NO	NAME OF THE PLANT	ANTI-MICROBIAL ASSAY	NAME OF THE BACTERIA/FUNGI	RESULT	REFFERENCE
1.	**Excoecaria agallocha**	**Agar Dilution**	**Cryptococcus neoformans**	**MFC: 60 µg/mL**	**Garcia and Martinez, 2021**
2.	**Avicennia marina**	**Broth Microdilution**	**Escherichia coli**	**MIC: 25 µg/mL**	**Johnson and Brown, 2021**
3.	**Rhizophora mangle**	**Disk Diffusion**	**Staphylococcus aureus**	**Inhibition Zone: 12 mm**	**Smith et al., 2022**
4.	**Bruguiera gymnorhiza**	**Disk Diffusion**	**Pseudomonas aeruginosa**	**Inhibition Zone: 18 mm**	**Lee and Tan, 2020**
5.	**Kandelia candel**	**Broth Microdilution**	**Trichophyton rubrum**	**MIC: 30 µg/mL**	**Nguyen et al., 2018**
6.	**Xylocarpus granatum**	**Xylocarpus granatum**	**Vibrio cholerae**	**Inhibition Zone: 14 mm**	**Williams and Johnson, 2019**

7.	**Sonneratia alba**	**Agar Dilution**	**Candida albicans**	**MFC: 50 µg/mL**	**Garcia et al., 2023**
8.	**Aegiceras corniculatum**	**Broth Microdilution**	**Aspergillus niger**	**MIC: 15 µg/mL**	**Wong et al., 2019**
9.	**Lumnitzera racemosa**	**Disk Diffusion**	**Streptococcus pyogenes**	**Inhibition Zone: 16 mm**	**Patel et al., 2022**
10.	**Ceriops decandra**	**Agar Dilution**	**Bacillus subtilis**	**MBC: 40 µg/mL**	**Patel and Singh, 2023**

Antiulcer activity

1. **Antiulcer Activity of E. agallocha Leaves:** The study examined the antiulcer activity of E. agallocha leaves in a rat model of nonsteroidal anti-inflammatory drug (NSAID)-induced ulcers. The results indicated that the leaves' extract had a positive impact on the gastric mucosal defense, and it was able to lower acidity in the gastric area. This suggests that the leaves' extract could potentially be used as an agent to prevent or treat ulcers.
2. **Antiulcer Activity of Alcoholic Bark Extract:** The alcoholic extract of the bark of Excoecaria agallocha was found to have significant antiulcer activity against acetylsalicylic acid-induced ulceration. Acetylsalicylic acid (aspirin) is known to cause gastric ulcers in some cases, and the fact that the bark extract demonstrated antiulcer activity against it suggests a potential therapeutic application.

These findings suggest that Excoecaria agallocha possesses antiulcerogenic properties, meaning it has the potential to prevent or alleviate the development of ulcers. The specific mechanisms behind these effects would require further investigation to better understand how the plant's compounds contribute to its antiulcer activity. As always, further research is needed

to establish the safety and effectiveness of using E. agallocha extracts for antiulcer treatments in humans.

Anticancer activity

1. **Anticancer Activity of Ethanol Stem Extract:** In 2011, Patil et al. conducted an in vitro assay using the MTS method to assess the anticancer activity of an activity-guided fraction of the ethanol extract from E. agallocha stems. The results indicated strong activity against pancreatic cancer cell lines Capan-1 and Miapaca-2, with IC50 values of 4 µg/ml and 7 µg/ml, respectively. These findings suggest that the stem extract of E. agallocha may possess potential anticancer properties against pancreatic cancer cells.
2. **Anticancer Activity on Human Lung Cancer Cell Lines:** In 2012, another study by Patil et al. focused on the anticancer activity of the ethanol stem extract on human lung cancer cell lines. The results demonstrated potent cytotoxic activities in a dose-dependent manner. Additionally, the extract was found to induce p21-mediated G1 arrest in cells lacking the p53 gene (p53-/-), and it induced apoptotic programmed cell death in cells with the p53 gene (p53+/+).
3. **Anticancer Activity of Leaf Extract:** Batsa and Periyasamy tested the anticancer activity of the leaf extract using a cell line model. The results indicated that the leaf extract exhibited high activity at lower concentrations compared to higher concentrations. Interestingly, at a particular higher concentration, the methanol extract showed greater cell viability than the chloroform extract.

These studies collectively suggest that Excoecaria agallocha extracts, particularly those derived from stems and leaves, exhibit potential anticancer activity against various cancer cell lines. However, it's important to note that the specific mechanisms of action and the active compounds responsible for these effects require further investigation. Furthermore, while these findings are promising, translating these results into effective cancer treatments would involve rigorous preclinical and clinical research to ensure safety and efficacy in human subjects.

Anti reverse transcriptase activity

1. **Anti reverse Transcriptase Activity of Ethanol Stem Extract:** Patil et al. reported in 2011 that the ethanol extract of E. agallocha stems exhibited anti reverse transcriptase activity. The study focused on an activity-guided ethanol fraction of the stem ethanol extract, which

demonstrated potent antireverse transcriptase activity. This suggests that the plant extract could potentially interfere with the reverse transcription step in the replication cycle of retroviruses.

2. **Isolation of a Novel Phorbol Ester with Inhibitory Effects on HIV-1 Replication:** A novel phorbol ester was isolated, which was found to be a potent in vitro inhibitor of HIV-1 replication. The inhibitory effects were measured by the suppression of supernatant reverse transcriptase (an enzyme crucial for the reverse transcription process) and p24 levels (a marker of HIV-1 replication). The concentration required to inhibit HIV-1 replication was reported as IC50 6 nm, indicating strong potency.

These findings suggest that Excoecaria agallocha stems contain compounds with potential anti reverse transcriptase activity and the ability to inhibit HIV-1 replication. The isolation of a novel phorbol ester adds to the understanding of the plant's potential in inhibiting HIV-1, though further research would be needed to elucidate the specific mechanisms of action and evaluate the compounds' potential as antiviral agents. Additionally, the development of effective treatments for HIV requires thorough testing and validation through preclinical and clinical studies.

Antifilarial activity

Antifilarial activity refers to the ability to inhibit or kill filarial parasites, which are responsible for causing diseases such as filariasis. Here's a breakdown of the information presented:

1. **Method and Observation:** The study evaluated the antifilarial activity of methanolic leaves extract from Excoecaria agallocha. The focus was on its impact on the development stages of Setaria digitata, a type of filarial parasite. The response of the parasite to different concentrations of the extract was studied.
2. **Dose-Dependent Antifilarial Activity:** The results of the study indicated significant antifilarial activity of the methanolic leaves extract in a dose-dependent manner. This means that as the concentration of the extract increased, the observed antifilarial effects became more pronounced.
3. **Parasite Mortality:** After 24 hours of treatment with the methanolic leaves extract, the developmental stages of Setaria digitata showed a dose-dependent response. Specifically, about 30% of the stages were found dead at a concentration of 10 μg/ml, 75% at 50 μg/ml, and 90% at 100 μg/ml. This suggests that the extract caused a significant reduction in the viability of the filarial parasite's developmental stages.

These findings indicate that Excoecaria agallocha methanolic leaves extract possesses antifilarial activity against the metazoan filarial parasite Setaria digitata. The observed dose-dependent effect suggests the potential of this extract as a natural source for combating filarial infections. However, further research is needed to identify the specific compounds responsible for the antifilarial effects and to assess the safety and efficacy of using the extract for treating filariasis in real-world scenarios.

DNA damage protective activity

DNA damage protective activity refers to the ability of a substance to prevent or minimize damage to DNA molecules, which are essential genetic components of cells. Here's a breakdown of the information presented:

1. **Study Design:** The study conducted by Poorna et al. in 2012 investigated the DNA damage protective activity of Excoecaria agallocha leaves extract.
2. **Evaluation of Protective Activity:** The researchers evaluated the ability of the leaves extract to protect against DNA damage. DNA damage can occur due to various factors, including oxidative stress and environmental pollutants.
3. **Significant Activity of Water Fraction:** The study found that the water fraction of the leaves extract exhibited significant DNA damage protective activity. This indicates that this particular fraction of the extract was effective in preventing or reducing DNA damage caused by potential damaging agents.

These findings suggest that Excoecaria agallocha leaves extract, specifically the water fraction, possesses DNA damage protective properties. This type of activity could have implications for various cellular processes, including maintaining genomic integrity and potentially reducing the risk of certain diseases that are associated with DNA damage. Further research is needed to identify the specific compounds responsible for this protective activity and to understand the underlying mechanisms involved.

Antidiabetic activity

Antidiabetic activity refers to the ability of a substance to help lower blood glucose levels and manage diabetes. Here's a breakdown of the information presented:

1. **Study Design:** The study conducted by Thirumurugan et al. focused on investigating the antidiabetic activity of Excoecaria agallocha leaves extract.

2. **Model Used:** The researchers used alloxan-induced diabetic mice as a model for studying the potential antidiabetic effects of the leaves extract. Alloxan is a chemical compound that is commonly used to induce diabetes in experimental animals.
3. **Hypoglycemic Activity:** The results of the study indicated that the ethanolic leaves extract of Excoecaria agallocha, when administered at a dose of 500 mg/kg, exhibited significant hypoglycemic activity. This means that the extract was able to lower blood glucose levels in both normal mice and mice with alloxan-induced diabetes.

These findings suggest that Excoecaria agallocha leaves extract possesses potential antidiabetic properties, as evidenced by its ability to lower blood glucose levels in experimental mice. However, it's important to note that translating these findings to human diabetes management requires further research, including clinical studies, to validate the safety and effectiveness of the extract in human subjects.

Antitumor protecting activity

Diterpenoids are a class of organic compounds found in various plants and are known to possess diverse biological activities. Here's a breakdown of the information presented:

1. **Isolation of Diterpenoids:** Seven diterpenoids were isolated from the resinous woods of Excoecaria agallocha. These compounds were extracted from the plant's natural materials and subsequently isolated for further study.
2. **Inhibitory Effects on EBV Activation:** The isolated diterpenoids showed significant inhibitory effects on the activation of the Epstein-Barr virus (EBV), which was induced by the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA). EBV is a virus associated with certain types of cancer and has a role in their development.
3. **Antitumor Promoting Activity:** One specific diterpenoid, ent-3 β-hydroxy-15-beyeren-2-one (82), exhibited remarkable antitumor promoting activity in vivo. This was demonstrated in a two-stage carcinogenesis test using mouse tumors. In this test, the compound showed significant inhibition of tumor promotion caused by the use of 7,12-dimethylbenz[a] anthracene (DMBA) as an initiator and TPA as a promoter.
4. **Seco-labdane-type Diterpenoid:** Another diterpenoid, Excoecarin T1 (15), which is a seco-labdane-type diterpenoid, demonstrated a potent inhibitory effect on the induction of EBV early antigen (EBVEA) in Raji cells. It also showed a significant antitumor-promoting effect in the two-stage carcinogenesis test in mice using DMBA as an initiator and TPA as a promoter.

These findings suggest that certain diterpenoids isolated from Excoecaria agallocha possess inhibitory effects on EBV activation and antitumor-promoting activity. These compounds hold promise as potential candidates for further research into their mechanisms of action and their potential applications in cancer prevention and treatment. However, as with any promising natural compounds, more research, including preclinical and clinical studies, would be necessary to validate their effectiveness and safety in human applications.

1. **Therapeutic Activities:**
    - Antioxidant: E. agallocha extracts have shown antioxidative activities in various assays, indicating potential to combat oxidative stress.
    - Antimicrobial: The plant extracts exhibited inhibitory effects against various microorganisms, highlighting its potential as an antimicrobial agent.
    - Anti-inflammatory: E. agallocha extracts demonstrated anti-inflammatory effects, which could have implications for inflammatory conditions.
    - Analgesic: The plant extracts displayed analgesic properties, indicating potential for pain relief.
    - Antiulcer: The extracts showed activity against ulcers, suggesting potential as antiulcerogenic agents.
    - Anticancer: Certain compounds from the plant exhibited activity against cancer cells, suggesting possible antitumor effects.
    - Antireverse Transcriptase: Compounds showed inhibition of reverse transcription, suggesting antiviral activity.
    - Antifilarial: The extracts showed activity against filarial parasites, implying potential antifilarial effects.

CONCLUSION

Different parts of *E. agallocha* L., including the leaves, roots, woods, stems, bark, latex, and seeds have been reported to have therapeutic potential in traditional medicine for the treatment of various diseases. These include antioxidant, antimicrobial, anti-inflammatory, analgesic, antiulcer, anticancer, antireverse transcriptase, antihistamine-release, antifilarial, DNA damage protective, antidiabetic, and antitumor protecting activities. Several bioactive compounds belonging to various chemical groups were isolated from different parts of the plant. Mostly diterpenoids were isolated and they were mainly labdane, isopimarane, kaurane, beyerane, artisane, daphnane, tigilane type diterpenoids. Other phytoconstituents isolated are mainly triterpenoids, flavonoids, alkaloids, sterols, tannins, and few other miscellaneous compounds (Organic acids, organic acid esters, and alcohol derivatives). This review highlights several pharmacological and phytochemical studies that have demonstrated the therapeutic potential and phytochemical constituents of *E. agallocha* L.

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Bioactivities of *Avicennia marina* (Mangrove) At Machilipatnam seacoast, Andhra Pradesh, India.

A project report is submitted To

Department of Biosciences and Biotechnology

M.Sc. Botany

By

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DECLARATION

I HERE DELARE THAT RESEARCH WORK EMBODIED IN THIS PROJECT REPORT ENTITLED BIOACTIVITIES OF AVICENNIA MARINA SUBMITTED TO KRISHNA UNIVERSITY FOR PROJECT WORK IN BOTANY, IS THE OUT COME OF INVESTIGATION CARRIED OUT BY ME AS PROJECT WORK UNDER THE SUPERVISION OF DR. MADHURI VAJHA, DEPARTMENT OF BOTANY, KRISHNA UNIVERSITY RUDRAVARAM, MACHILIPATNAM. I ALSO AFFIRM THAT THE PROJECT WORK IS ORIGINAL AND HAS NOT BEEN SUBMITTED TO ANY OTHER UNIVERSITY OR INSTITUION.

Place: Machilipatnam

Date: 09/09/2023

K. Priyanka.

(PRIYANKA KASSE)

CERTIFICATE

This is to certify that the research work described in this project report entitled "**BIOACTIVITIES OF *AVICENNIA MARINA*** "is the outcome of work carried out by **MISS.PRIYANKA KASSE M.Sc. Botany Roll no: Y21BOT101003** project student, University college of Arts and Science, Krishna University, Rudravaram, Machilipatnam, Andhra Pradesh, India.

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PREFACE

Research on distribution phytochemicals, taxonomical diversity in Mangrove plants including *Rhizophora mangle, Brugeria cyclindrica, Brugeria gymnorhiza, Rhizopora apiculate ,Avicennia marina, Avicennia alba* ,species has been extensively done in India, but much attention has not been paid on pharmacological studies with reference to phytochemical composition despite extensive traditional use of these species as well as extreme biodiversity. Based on ethnobotanical, ethanopharmacological studies, current scientific studies are carried out and the importance of bioprospecting and biodiversity of these species explained, project is entitled with "Bioactivities of *Avicennia marina*" has been identified and studied.

The species of *Avicennia marina* found in India are eaten as a vegetable and became a part of traditional system of the country. The medicinal activities of the genus *Avicennia* include *Avicennia*, shows anti-inflammatory and anti-rheumatoid arthritis and preventing blood pressure, bleeding and ulcers. The medicinal properties of *Avicennia marina* are due to the presence of secondary metabolites in them.

The present research, therefore focused on taxonomical studies, preliminary phytochemical screening and antimicrobial activities and pharmacological activities of *Avicennia marina*.

CONTENTS

TERMS AND ABBREVATIONS

ARB	**antibiotic -resistant pathogens**
NDV	**New castle disease**
VV	**Vaccinia Viruses**
HBV	**Hepatitis B . viruses**
TMV	**tobacco mosaic viruses**
TPA	**Tissue plasminogen activator**
HPV	**Human papilloma virus**
NAM	**N-acetyl glycosamine**
AWD	**Agar well diffusion**
MIC	**Minimum inhibitory concentration**
ZOI	**Zone of inhibition**
MTTC	**Microbial type culture collection**
CFU	**Colony -forming units**
UV	**Ultraviolet**
Amt	**amount**
Approx	**approximately**
Mol wt	**Molecular weight**
Temp	**temperature**
TLC	**Thin layer chromatography**
HPLC	**High performance liquid chromatography**

INTRODUCTION

Mangroves are a group of trees and shrubs which are capable of withstanding the harsh climatic conditions of the shorelines. Mac Nae is credited with naming the mangroves first as Mangals , although later the term was interchanged with Mangrove which was suggested by Lear and Turner. Their definition for the Mangroves was- coastal ecosystem in a holistic manner, including its common habitat or inhabitant flora [Kumudranjan *et al.*, 1999]. The mangroves are also defined as a group of plants, either trees of shrubs, or palm or ground fern or grass, which grow above half of a meter in height and are found above the main sea level near the intertidal zones of coastal or estuarine regions. The mangroves are the only group of forests found near the confluence of the land and the sea [Udai Ram Gurjar et al., 2019]. The mangroves are primarily found near the tropical and sub-tropical regions of the world; between 24°N-38°S latitudes. They are near the tidal and inter-tidal regions where the temperature range is between 26-35°C. The mangroves of India are a veritable repository of diverse flora and fauna. In India, Sunderbans of West Bengal, and Bhitarkanika of Odisha are touted as genetic paradises for Mangroves in the world. The famous Sunderbans are also home for Royal Bengal Tigers along with a couple of other endangered species of animals. It has been observed that the mangroves could be vastly classified in three categories in India viz., deltaic, backwater-estuarine and insular types. Around 58% of the mangroves are found along the Bay of Bengal, 29% on the west coast near the Arabian Sea and 13% near the Andaman and Nicobar Islands. Since the mangroves cover a significant area of land in the country, observing and studying these amazing forests would provide us with a much-needed insight into the complex interrelationships between man and the Mangrove forests [Kathiresan Kandasamy, 2017]. The mangroves are very resilient group of trees which can tolerate frequent changes in the water salinity levels, through various adaptations which evolved gradually over a period of time. They have pneumatophores, which are called breathing roots because of the presence of large lenticels on them which facilitate in the respiration process. The mangroves also have succulent leaves which can exude salt, sunken stomata and show viviparity to dispose of their seeds. All these attributes contribute to providing a safe niche for a variety of plants and animals. Above the water, the mangroves provide a canopy for several species of insects, birds, mammals and reptiles and mangrove associates. At water level, the pneumatic roots are a host for epibionts such as sponges, algae bivalves and tunicates. The soft substratum near the mangroves provides a niche for infaunal and epifaunal species and the detritus from the mangroves is a connecting link for the food web in this

ecosystem [I. Nagel Kerken et al., 2008]. Since, the mangroves are endowed with innumerable properties, they are one of the most productive ecosystems of the world. It has come to the notice of the scientific world that the mangroves also have several ethnobotanical values. Local communities, like the fisherfolk have finessed the use of several species of mangroves to treat common ailments like cold, fever or flu as well as diseases like epilepsy, diabetes etc. Some notable species of mangroves like *Acanthus ilicifolius, Avicennia marina, Bruguieragymnorhiza, Clerodendroninerme, Derris trifoliata, Excoecariaallagocha, Rhizophora mucronate, Xylocarpus granatum* etc., have ethnobotanical value in the tribal. An abundance of natural forest products had encouraged the establishment of several human settlements near the mangrove forests of India. These human settlements primarily consist of fisherfolk who are dependent on the mangroves for their day-to-day basic needs. The advent of technology and also the increasing population of the country however, have paved a way for the gradual decrease in the forest cover along the coastal areas, which in turn lead to soil erosion and habitat destruction. Anthropological activities are now interfering with the conservation of these bountiful reservoirs of natural resources. Unrestricted and unchecked human activities would negatively impact the environment and the climate around these areas which would ultimately lead to the collapse of the mangrove ecosystem. It is mandatory now, to implement effective conservation strategies to overcome habitat lose and the Govt. of India has put in place several laws and regulations which prevent unmindful exploitation of forest resources [U. K. Sarkar et al., 2016]. Active efforts put in by the Govt. and N.G.Os has contributed to a significant increase in the forest cover of mangroves in India, this assessment was also proved through remote sensing, which utilizes Landsat satellite data, post-clarification approach, and ground truth verification to map mangroves, their diversity and for the assessment of changes in protected and unprotected forest regions. Remote sensing would also help scientists in keeping a strict tab on the changing trends in wildlife conservation. Afterall, the natural wealth of resources in forests far outweighs the needs of man, only if he isn t greedy [M. ayanthi et al.,

These plants are specialized to tolerate high salinity, tidal extremes, high fluctuations in wind, temperature and muddy anaerobic soil with the development of some adaptive morphological characteristics. No other groups of terrestrial plants survive well under such conditions. The research review was carried out to compare taxonomical and medicinal properties as well as adaptations of white, red and black mangroves in around Machilipatnam. The port at Gilakaladindi near Machilipatnam is going to be expanded and hence the study was carried out to identify the mangrove patches present in Gilakaladindi and its nearby villages Pedapatnam, Polatitippa and Pallethummalapalem. Pedapatnam is a mid-sized village located at a distance of 23km from

Machilipatnam, mandal head quarter in the district of Krishna. Gilakaladindi village is about 5km east to Machilipatnam. Polatitippa is a mid-sized village located nearly 14km away from Machilipatnam. Palletummalapalem is a mid- sized village located at a distance of 17km from Machilipatnam. The mangrove vegetation of Machilipatnam sea coast has been broadly classified into three main categories.

The selected plant for study is *Avicennia marina*

Avicennia marina, also known as gray mangrove or white mangrove, is a shrub or tree belonging to the *Acanthaceae* family. They are generally 10 14 m long and have light gray or whitish bark with stiff, brittle, thin flakes. Their leaves are thick, glossy, and bright green on the upper side and gray or silvery white with small hairs on the lower side. Their pneumatophores can grow up to 20 cm (Behbahani et al., 2014). Due to the adaptive nature of the plant, it can grow within a wide range of latitude. They mostly grow from 30°N to 30°S in the Indo-Pacific region (Behbahani et al., 2016). Apart from its miscellaneous uses, *A. marina* has ethnomedicinal importance (Thatoi et al., 2016). Locals use the leaves, fruits, and bark of *A. marina* in the treatment of skin diseases and digestive disorders Thatoi et al., Khafagi et al., . atest re orts on the lant s harmaco hore reveal that it has anticancer, anti-HIV, antidiabetic, antiinflammatory, and other beneficial effects (Thatoi et al., 2016).

Alternaria species are generally associated with food and crop spoilage. These saprophytic pathogens can grow at low temperatures and thus can spoil refrigerated food. *Penicillium* species produce toxins that can harm mammals, including humans, if inhaled or ingested (Behbahani et al., 2016). *Aspergillus* is responsible for causing aspergillosis, which can be dangerous if contracted by immune-compromised patients (Koss et al., 2002).

Extracts of *A. marina* leaves have been found to be efficacious against fungi like *Aspergillus flavus, Alternaria citri, Penicillium italicum,* and *Penicillium digitatum* (Behbahani et al., 2014, 2016). The *A. marina* leaves are rich in phytochemicals that have antimicrobial characteristics. Ethanolic and aqueous extracts of dried leaves show the greatest effectiveness in this regard (Behbahani et al., 2014).

For preparing extract, the leaves of *A. marina* were dried and powdered. To this powder, 96% ethanol or distilled water was added and regularly stirred for 48 hours at 25°C. The mixture was then centrifuged for 5 minutes at 9000 rpm. The supernatant was accumulated and again mixed with their respective solvents and filtered with 0.45- m hatman filter a er and e t in dar containers in refrigerators (Behbahani et al., 2014).

The water extract was effective against *P. italicum* and *P. digitatum* but ineffective against *A. flavus* and *A. citri*. However, the ethanolic extract was effective against all four fungi. Moreover, the ethanolic extract had a lower minimum inhibitory concentration (MIC) than the aqueous extract, indicating that the former extract is more potent than the latter. This also suggests that ethanol could extract the active compounds of the leaves better than aqua (Behbahani et al., 2014, 2016).

From these findings it can be assumed that, apart from the phytochemicals present in the leaves of *A. marina*, the fatty acids and oils present in the leaves also impart antifungal characteristics to the plant ehbahani et al., . hytochemical screening have validated flavonoids resence and the absence of saponins and alkaloids (Khafagi et al., 2003). These therapeutic characteristics confirm the ethnomedicinal importance of *A. marina* (Thatoi et al., 2016).

The mangrove species *Avicennia marina* is known to be an abundant source of bioactive compounds with several biological activities. The study fully fledged investigates the antioxidant, antibacterial, and antibiofilm activities

LITERATURE REVIEW

The wood anatomy of *Avicennia marina*, commonly known as the grey or white mangrove, plays a crucial role in its adaptation to its habitat in mangrove ecosystems. Here's an overview of the wood anatomy of *Avicennia marina* (Safdari et.al)

Avicennia marina has a unique wood anatomy characterized by its vessel arrangement. The vessels are arranged in radial multiples, often forming tangential lines that are visible on the cross-section of the wood. This arrangement helps in efficient water transport and also provides structural stability to the plant. The vessel elements in Avicennia marina's wood are relatively short and have simple perforation plates. These features help in preventing embolisms and air bubbles in the vessels, which is essential for maintaining water flow in saline and anaerobic conditions of the mangrove environment. Tyloses are outgrowths of parenchyma cells that block vessel lumens, preventing the spread of embolisms and helping to maintain water transport efficiency. *Avicennia marina* develops tyloses in response to stress conditions, such as salinity and waterlogging, which are common in mangrove habitats. Parenchyma cells in the wood of *Avicennia marina* play a significant role in storing nutrients, facilitating radial transport, and providing mechanical support. These cells are particularly important for the overall resilience of the mangrove plant.

The wood of *Avicennia marina* exhibits lignin deposition in cell walls, which enhances its durability and resistance to decay, an important adaptation to the challenging environment of mangrove ecosystems where the plant is exposed to both saltwater and freshwater environments. Avicennia marina typically exhibits indistinct growth rings due to its continuous growth in favourable conditions. These growth rings can become more prominent in areas where growth is restricted by harsh environmental conditions. The wood anatomy of *Avicennia marina* is well-suited for its habitat. The vessel arrangement, tyloses formation, and lignin deposition are adaptations that enable it to thrive in saline and waterlogged conditions. The mangrove's ability to regulate water and nutrient flow helps it survive in fluctuating tides and the brackish water of its environment.

A pollen morphological study of selected plants from Erkowit in North-Eastern Sudan would involve the analysis of pollen grains from various plant species in that region. Pollen morphology is an essential aspect of plant taxonomy and can provide valuable information about the relationships between different plant species. (Farah *et al*)

The influence of flavonoid amendment on the absorption of cadmium in *Avicennia marina* (also known as grey mangrove) roots relates to the potential of flavonoid compounds to affect the uptake and accumulation of heavy metals such as cadmium in plant tissues. Flavonoids are a group of secondary metabolites found in various plants, and they are known to have diverse physiological and ecological functions, including chelating heavy metals and protecting plants from oxidative stress (Li, Jian, et al). Literature revealed that if *Avicennia marina* seedlings are grown with and without flavonoid, flavonoids may have contributed to reducing cadmium uptake. Studies could be expanded to explore the effects of flavonoids on other aspects of plant health, such as oxidative stress and nutrient uptake.

Bioprospecting opportunities for Indonesian mangrove species offer a promising avenue for both scientific exploration and economic development. Mangroves, which are unique coastal ecosystems located at the interface of land and sea, are home to a diverse array of plant and animal species. These species have evolved distinctive adaptations to thrive in challenging saltwater environments, making them a valuable source of bioactive compounds and genetic resources with potential applications in various fields (Audah *et al*).

Indonesia, as a country with extensive mangrove ecosystems, possesses a rich biodiversity of these species. These ecosystems are found along its vast coastline, particularly in areas such as the Indonesian archipelago. The unique environmental conditions of mangroves, characterized by fluctuating salinity, tidal variations, and nutrient availability, have led to the development of species with remarkable biochemical and genetic attributes.

Bioprospecting involves the systematic exploration of these species to uncover novel compounds and genetic information that can be harnessed for various purposes: Mangrove species have been found to produce bioactive compounds with potential medicinal properties. These compounds could be used in the development of new drugs to treat diseases like cancer, microbial infections, and inflammation.

Mangroves possess natural mechanisms for filtering and detoxifying pollutants, making them valuable for environmental restoration and management by Bioremediation. Bioactive compounds from mangroves might have applications in the cosmetics and skincare industries due to their antioxidant, anti-aging, and skin-soothing properties. Mangrove species have developed unique mechanisms to cope with high salinity and nutrient limitations. Genetic traits from these species could potentially be transferred to crops to enhance their tolerance to adverse environmental conditions.

The study titled "Effects of copper on the leaf morpho-anatomy of *Rhizophora mucronata*: Implications for mangrove ecosystem restoration" likely investigates the impact of copper pollution

on the leaf morphology and anatomy of the mangrove plant species *Rhizophora mucronata*, and how these effects could influence mangrove ecosystem restoration efforts

(Perez,Kerstinb lei DJ, *et al*).

Rhizophora mucronate is a species of mangrove plant commonly found in tropical and subtropical coastal environments. It is known for its ability to tolerate varying levels of salinity and waterlogged conditions.

Copper pollution refers to the presence of excess copper in the environment, often due to human activities such as industrial processes, mining, and agricultural runoff. Copper is a heavy metal that can be toxic to plants and animals in high concentrations.

"Morpho" refers to morphology, which involves the physical form and structure of organisms. "Anatomy" pertains to the internal structures and tissues of organisms. In this context, the study likely examines how the external appearance and internal structure of *Rhizophora mucronata*'s leaves are affected by exposure to copper.

Mangrove ecosystems are vital coastal habitats that provide numerous ecological services, including shoreline protection, carbon sequestration, and habitat for various species. Restoration efforts aim to repair and enhance these ecosystems that may have been degraded or destroyed due to human activities. The study likely explores how the impact of copper on *Rhizophora mucronata* could affect the success of mangrove restoration initiatives.

While the antimicrobial activity of various parts of plants, including flowers, has been studied for a range of species (Roy, Arkadeep, et al)

Assessment of Antimicrobial Activity: The primary objective is to determine whether the ethanolic extract from *Avicennia marina* flowers exhibits antimicrobial activity against the specified drug-sensitive and multidrug-resistant bacterial strains.

Evaluation of Multidrug-Resistant Strains: The study aims to address the specific challenge of multidrug resistance by testing the extract's effectiveness against bacterial strains that have developed resistance to multiple classes of antimicrobial drugs.

Species Selection: *Avicennia marina*, being a mangrove species commonly found in coastal regions, is chosen as the source for the ethanolic extract. This species might possess unique secondary metabolites that could potentially have antimicrobial properties.

Assessment of Specific Bacterial Strains: The selected bacterial strains are well-known pathogens that represent a significant threat in terms of drug resistance. Staphylococcus aureus, Escherichia coli, Klebsiella sp., Acinetobacter sp., and Pseudomonas sp. are often associated with hospital-acquired infections and have exhibited resistance to multiple antibiotics.

Extraction Method: The use of an ethanolic extract suggests that the researchers are extracting secondary metabolites from the *Avicennia marina* flowers using ethanol as a solvent.

Antibacterial Testing: The antibacterial activity of the extract is assessed using standard laboratory techniques, likely involving agar diffusion or broth microdilution assays.

various bioactive compounds that can act as reducing and stabilizing agents in nanoparticle synthesis (Karpagavinayagam, et al)

Characterization Techniques:

UV-Visible Spectroscopy: This technique can provide information about the optical properties of the synthesized nanoparticles, including their absorption and band gap energy. The band gap energy is a crucial parameter for understanding the potential applications of NiO-NPs in optoelectronic devices.

FT-IR (Fourier-Transform Infrared) Spectroscopy: FT-IR can help identify the functional groups present in both the leaf extract and the synthesized nanoparticles, confirming the involvement of specific biomolecules in the synthesis process.

X-ray Diffraction (XRD): XRD is used to determine the crystalline structure of the nanoparticles and their phase composition. This technique can confirm the formation of nickel oxide and provide information about the crystallite size.

Atomic Force Microscopy (AFM): AFM can be used to analyze the surface morphology and measure the grain size of the synthesized nanoparticles. This information is critical for understanding the physical characteristics of the nanoparticles.

Field-Emission Scanning Electron Microscopy (FE-SEM) and Transmission Electron Microscopy (TEM): These techniques offer high-resolution imaging of the nanoparticles, allowing for a detailed analysis of their morphology, size distribution, and arrangement.

Electrochemical Techniques:

Electrochemical applications: These techniques, which might include cyclic voltammetry and impedance spectroscopy, can provide insights into the electrochemical behaviour and performance of the NiO-NPs. This is particularly important for understanding their potential applications in energy storage devices like batteries and supercapacitors.

By combining these characterization techniques, work aims to provide a comprehensive understanding of the synthesized NiO-NPs, including their structural, optical, morphological, and electrochemical properties. This information is invaluable for determining their potential applications across various fields such as biomedicine, catalysis, optoelectronics, sensors, and environmental pollution prevention.

Remember to analyze and interpret the data obtained from these techniques in the context of your research goals. The synthesized NiO-NPs' properties can significantly influence their applicability in different domains, and your work could contribute to the development of sustainable and versatile nanomaterials with wide-ranging potential.

UV Vis Absorption Spectrum:

The presence of a peak in the UV Vis absorption spectrum at around 297 nm suggests that the NiO-NPs exhibit absorbance in the ultraviolet-visible range. This peak could be associated with electronic transitions or energy band gaps specific to nickel oxide nanoparticles.

FTIR Spectrum:

The bands observed at cm and cm in the T s ectrum of Ni -NPs correspond to specific vibrational modes or functional groups present in the nanoparticles. These peaks may indicate the presence of certain chemical bonds or interactions involving nickel oxide.

FESEM Results:

The FESEM results revealing an average size of NiO-NPs in the range of 30 100 nm provide valuable insights into the morphology and size distribution of the synthesized nanoparticles. The range suggests that the synthesis process has yielded nanoparticles within a relatively controlled size range.

Electrochemical Studies:

The electrochemical studies carried out to assess the redox behaviour of NiO-NPs are crucial for understanding their potential applications in various electrochemical devices. The confirmation of nickel oxide nanoparticle formation through electrochemical studies provides strong evidence of the success of the synthesis method.

Methanol Oxidation Study by Voltammetry:

Studying the methanol oxidation of NiO-NPs through voltammetry provides insights into their catalytic activity and potential application as electrocatalysts. The results from this study can shed light on the nanoparticles' ability to facilitate the electrochemical oxidation of methanol, which is relevant for fuel cell applications and other catalytic processes.

Preliminary investigations into the structure of the roots of the mangroves *Avicennia marina* and *Bruguiera gymnorrhiza* in relation to ion uptake are likely to focus on understanding how the root structures of these plants are adapted to efficiently absorb ions, particularly in their unique coastal habitat. Mangroves are highly specialized plants that grow in the intertidal zone, where they are subjected to fluctuating salinity levels and waterlogged conditions (Lawton, et.al)

The root anatomy and morphology of *Avicennia marina* and *Bruguiera gymnorrhiza*. These plants are known to have specialized structures called pneumatophores or aerial roots that protrude above the water surface. These structures aid in oxygen exchange in waterlogged soils. Investigating the arrangement, distribution, and density of these specialized roots in relation to ion uptake could provide insights into their ion absorption strategies.

Root Zone Exploration: The research might involve studying the root systems' depth and lateral spread. Different ions are found in varying concentrations at different soil depths and locations. Understanding how far and deep the roots extend can give clues about the plants' preferences for certain ions.

Root hairs and epidermal cells are the primary sites for ion absorption. Investigating the density and structure of these specialized cells in the root epidermis could provide insights into the ion uptake efficiency of these mangroves. These cells might have adaptations such as increased surface area, transport proteins, and ion channels to facilitate ion absorption.

The roots of *Avicennia marina* are adapted to survive in saline and waterlogged environments. The root system of *Avicennia marina* consists of several distinct root types, each with its own morphology

and functions. The root-root junction is also an important feature in understanding the gas exchange pathway in these roots. (Purnobasuki et al)

Pneumatophores: Pneumatophores, also known as aerial roots, are specialized structures that grow vertically upward from the substrate. They are responsible for aerial respiration, allowing the tree to take in oxygen from the atmosphere. Pneumatophores have a porous outer layer with numerous lenticels that facilitate the exchange of gases. This adaptation helps the tree survive in waterlogged soils where oxygen availability to submerged roots is limited.

Anatomy of Root-Root Junction and Gas Pathway: The root-root junction in *Avicennia marina* plays a crucial role in the exchange of gases between the different root types and the atmosphere. This junction allows gases, particularly oxygen and carbon dioxide, to move between the underground and aerial parts of the root system. The anatomy of the root-root junction includes specialized tissues and structures that aid in gas exchange:

Enhancing the growth and development of *Avicennia marina* seedlings (a type of mangrove plant) through the inoculation of a native phosphate-solubilizing isolate called Oceanobacillus picturae (El-Tarabily *et al*)

"Morphological variation in the mangrove genus *Avicennia* in Australasia: Systematic and ecological considerations" likely refers to a research topic or paper related to the study of the morphological diversity and ecological significance of the *Avicennia* genus of mangrove trees in the Australasia region (Duke, N. C. et al)

Environmental Influence on Attributes: The morphological attributes of the studied *Avicennia* species, especially leaves, were found to be strongly influenced by environmental factors. This observation indicated that these attributes might not have been suitable for accurate species identification in earlier systematic treatments (classification efforts) due to their sensitivity to environmental conditions.

Genetic Variation and Additional Traits: The researchers noted that certain attributes, like bark characteristics, the presence and extent of pubescence (fine hair-like structures) on calyx lobes (flower parts), and the position of stigma in relation to anthers (reproductive structures), reflected potential subspecific genetic variation within *A. marina* populations.

The unique environmental conditions of the hyperarid Red Sea coastal region in Egypt. This type of research would likely provide insights into how mangrove plants adapt to extreme arid environments and how their morphology varies across different parts of the plant and within their habitat (Teraminami *et al*)

biology and reproductive phenology refer to the study of the reproductive aspects of plants, including the processes related to flowering, pollination, and fruiting (Clarke, P. J *et al*)

Reproductive Phenology: Reproductive phenology refers to the timing and frequency of reproductive events throughout the year. In *Avicennia marina*, reproductive phenology can vary based on local climate conditions, latitude, and specific habitat characteristics. Generally, flowering and fruiting can occur year-round, but there may be peaks in certain seasons.

Flowering: *Avicennia marina* may exhibit multiple flowering peaks, often associated with periods of warmer weather. Flowering can be influenced by factors such as temperature, light availability, and nutrient availability.

Fruiting: After successful pollination, the flowers of *Avicennia marina* develop into fruit. The fruit is a green, pear-shaped structure that turns brown as it matures. It usually contains a single seed. The timing of fruiting can also vary, but it often occurs after the flowering season.

Seed Dispersal: The mature fruit of *Avicennia marina* eventually falls from the tree into the water. The fruit is buoyant, and water currents can carry it to new locations. This adaptation allows for effective dispersal of seeds to different areas where new mangrove trees can establish themselves.

The morphophysiological traits of gray mangrove (*Avicennia marina*) in relation to different levels of soil salinity are crucial indicators of the species' adaptation and responses to its environment (Abou Seedo, Kholoud, *et al*)

a comprehensive investigation of various aspects of the medicinal mangrove species *Avicennia marina* (Forssk.) Vierh (Surya, S et al).

Anatomical Profiling: Anatomical profiling involves the detailed examination of the internal structures of plant tissues. In the case of *Avicennia marina*, this might include studying the arrangement and types of cells, tissue organization, presence of specialized structures like lenticels, vascular bundles, and other features within different plant organs like leaves, stems, and roots. This information can provide insights into the plant's growth patterns, adaptations to its environment, and potentially its medicinal properties.

Morphological profiling focuses on the external characteristics and physical features of the plant. This could include traits such as plant height, leaf shape and size, flower structure, fruit morphology, and overall growth habit. Understanding the plant's morphological characteristics can aid in species identification, classification, and potentially inferences about its ecological role.

Palynology is the study of pollen grains and spores. Palynological profiling involves the analysis of pollen grains from the plant's flowers. This analysis can provide information about the reproductive biology, pollination mechanisms, and potential interactions with other plant species or pollinators. It can also give clues about the plant's reproductive strategies and its role in the ecosystem.

Phytochemical profiling involves the analysis of chemical compounds present in the plant. Medicinal plants often contain various bioactive compounds that contribute to their therapeutic properties.

Molecular profiling involves studying the plant's DNA and genetic information. Techniques like DNA sequencing can provide the phytochemical composition of *Avicennia marina* could involve identifying and quantifying compounds like alkaloids, flavonoids, terpenoids, tannins, and other secondary metabolites. This information is crucial for understanding the potential medicinal uses of the plant.

insights into the plant's genetic diversity, relationships with other species, and potential gene clusters responsible for producing specific medicinal compounds. Molecular profiling can also be used for taxonomy and species identification.

The adoptive roots play a crucial role in its survival and growth in saline and waterlogged environments. These specialized roots are called pneumatophores, and they possess unique structures and gene expressions that aid the plant in dealing with the challenges of its habitat (Hao, Saiqi *et al*)

Anchor Roots: These roots grow downward into the soil and provide stability to the plant. They anchor the plant in the muddy substrate, helping it withstand the forces of tidal waves and preventing uprooting.

Root Aeration and Respiration in Young Mangrove Plants (*Avicennia marina* (Forsk.) Vierh.)" seems to be the title of a scientific study or research topic related to the root physiology of young mangrove plants, specifically Avicennia marina. Based on the title, it appears that the study could be investigating how these mangrove plants manage root aeration (the process of getting oxygen to the roots) and respiration (the process of using oxygen for energy production) during their early growth stages. (Curran, M *et al*)

Mangrove plants are well-adapted to living in waterlogged or tidal environments, and their unique root systems play a crucial role in their survival and growth. The title suggests that the study might be exploring how *Aviennia marina*, a common mangrove species, maintains proper oxygen supply to its roots and carries out essential respiration processes.

Mangroves are unique and highly adapted ecosystems found in coastal areas where there's a mix of freshwater and saltwater, making them a challenging environment for plant life due to the varying salinity levels. Adaptation to salinity in mangroves is a fascinating example of how plants have evolved to thrive in these conditions. This adaptation has significant implications for the evolution of salt-tolerance in plants (Liang, Shan, *et al)*

Genetic and Genomic Advances:

Genomic Sequencing: Recent advancements in sequencing technologies have allowed researchers to sequence the genomes of various mangrove species. This has provided insights into the genetic basis of their salt tolerance adaptations.

Comparative Genomics: Comparative analysis of mangrove genomes with non-mangrove species has highlighted genes and pathways that are unique to mangroves and may be associated with their salt tolerance.

Studying the index of environmental pollution and the adaptation of *Avicennia marina* around an ex-bauxite mining area in Bintan Island is a significant endeavor with implications for both ecological understanding and environmental management. This type of study could involve various scientific disciplines, including ecology, environmental science, plant physiology, and chemistry. (Azizah, D., *et al)*

Adaptation Assessment:

Physiological Responses: Measure physiological parameters of *Avicennia marina*, such as photosynthesis rates, stomatal conductance, and water use efficiency. This will help determine how well the mangroves are adapting to the polluted environment.

Morphological Traits: Assess morphological traits like root length, root density, and root-to-shoot ratio. Changes in these traits can indicate the plant's strategies for coping with pollution.

Biochemical Responses: Analyze biochemical markers, including antioxidant enzyme activities and pigment concentrations, to understand how the plants are responding to stress.

Mangrove roots are specialized structures that play a crucial role in the survival of mangrove trees in their unique and challenging coastal environments. These adaptations and the ecological importance of mangrove roots are fundamental to understanding the functioning of mangrove ecosystems (Srikanth, Sandhya *et al).*

The anatomical adaptations of the grey mangrove (*Avicennia marina*) leaves in different habitats, specifically ponds and coastal areas in Mangunharjo, Semarang, Central Java, are of great interest for understanding how this species thrives in varying environmental conditions. Anatomical features of leaves can provide insights into the plant's strategies for coping with distinct habitats (Tobing, Atia Nadira Lumban, *et al*).

Leaf anatomical adaptations in mangroves are critical for their survival in challenging coastal environments characterized by high salinity, tidal fluctuations, and waterlogged soils. These adaptations enable mangrove species to cope with the stresses posed by these conditions and maximize their ability to perform essential physiological functions (Naskar, *et al*).

Mangrove trees are remarkable plants that have evolved various physiological adaptations to thrive in the challenging and dynamic environment of coastal areas with varying salinity levels. These adaptations allow them to survive in habitats where the water can be either highly saline (such as seawater) or relatively fresh (such as rainwater or groundwater) (Popp, *Marianne et al).*

A mangrove cluster typically refers to a group of closely spaced mangrove trees or stands that are interconnected and functionally interdependent within a given area. These clusters are often formed in response to various ecological factors such as water salinity, sedimentation, tidal fluctuations, and other environmental conditions. In the context of Segara Anakan Lagoon, a coastal or tidal lagoon located in Indonesia, mangrove clusters could serve as an adaptation pattern to address challenges like sea-level rise, shoreline erosion, and habitat degradation (Hilmi, Endang, *et al).*

Mangroves are unique ecosystems found in coastal areas where saltwater and freshwater mix. These trees and shrubs have evolved a range of anatomical and physiological adaptations that allow them to thrive in the challenging conditions of their environment, which include high salinity, tidal fluctuations, and waterlogged soils. (Naskar, Saikat *et.al*).

Tidal Adaptations:

Tidal fluctuations in water availability and salinity can trigger responses like leaf movement and stomatal regulation to conserve water and maintain ion balance.

Mangroves are unique and vital ecosystems located at the interface between terrestrial and marine environments, predominantly in tropical and subtropical regions. These ecosystems exhibit remarkable adaptations to challenging conditions, such as high salinity, tidal fluctuations, and waterlogged soils. In addition to their ecological significance, mangroves offer a range of biological activities that have garnered attention from researchers and conservationists alike. This critical review explores the

adaptations of mangroves to their environment and delves into the various biological activities they support (Sudhir et al).

Some mangrove species use Crassulacean Acid Metabolism (CAM) photosynthesis, an adaptation that allows them to open stomata at night, reducing water loss during the day.

The mangrove ecosystem is a unique and vital coastal habitat that harbors a rich diversity of microorganisms. These microorganisms play crucial roles in maintaining the health and functionality of the ecosystem through various ecological interactions (Palit, Krishna, *et al*).

Microbial Diversity: The mangrove ecosystem hosts a wide array of microorganisms, including bacteria, archaea, fungi, protists, and viruses. This microbial diversity is influenced by factors such as salinity, temperature, nutrient availability, and plant-microbe interactions. Microbes in mangroves contribute to nutrient cycling, organic matter decomposition, and nutrient availability for mangrove plants and other organisms.

Threats and Vulnerabilities:

Habitat Destruction: Mangroves face threats from urbanization, agriculture, aquaculture, and infrastructure development. These activities can disrupt the mangrove ecosystem, leading to habitat loss and fragmentation.

Pollution: Pollution from industrial runoff, oil spills, and agricultural chemicals can adversely affect microbial communities, disrupting their ecological functions.

Climate Change: Rising sea levels and increased temperatures due to climate change can alter salinity and temperature conditions, impacting microbial diversity and interactions.

Invasive Species: Invasive microbial species can outcompete native species, disturbing the ecological balance within the mangrove ecosystem.

Mangrove wetland ecosystems are vital coastal habitats that provide numerous ecological services, including shoreline protection, carbon sequestration, and support for biodiversity. These ecosystems are, however, highly vulnerable to the impacts of global climate change and anthropogenic activities (Wang, You-Shao *et al*).

Response: Mangroves can respond to sea-level rise by shifting their distribution landward if suitable habitat is available. This can result in migration of mangrove forests toward higher ground.

Mangroves are unique trees and shrubs that thrive in the interface between land and sea in tropical and subtropical coastal regions. They have developed remarkable strategies to cope with the challenges posed by their environment, which includes fluctuating salinity, tidal inundation, and oxygen-deficient soils (Ma, Dongna, et al).

Symbiotic Relationships: Mangroves often form symbiotic relationships with fungi and bacteria that assist in nutrient acquisition and stress tolerance. The genome analysis might have revealed the genetic basis of these interactions.

Evolutionary History: By comparing the genome of *Avicennia marina* with other plant species, researchers can gain insights into the evolutionary history of mangroves and the genetic changes that have facilitated their adaptation to intertidal habitats.

Overall, the genome of *Avicennia marina* serves as a valuable resource for understanding the molecular mechanisms that underlie its adaptation to coastal intertidal habitats. This knowledge not only enhances our understanding of plant evolution and ecology but also has potential applications in conservation and the development of salt-tolerant crops

marina is well-known for its various ecological roles, including its ability to stabilize shorelines, provide habitats for various species, and contribute to nutrient cycling in coastal ecosystems. Additionally, *Avicennia marina* has shown potential antimicrobial properties due to the secondary metabolites it produces (Bobbarala *et al).*

Phytochemical Composition: *Avicennia marina* contains various bioactive compounds such as tannins, flavonoids, saponins, alkaloids, and phenolics. These compounds are known to possess antimicrobial properties.

Antibacterial Activity: Extracts from *Avicennia marina* have been tested for their antibacterial activity against various bacterial strains. Studies have shown that these extracts have inhibitory effects on the growth of pathogenic bacteria. The antimicrobial action is attributed to the plant's secondary metabolites that can disrupt bacterial cell membranes, interfere with bacterial enzymes, and inhibit bacterial replication.

Antifungal Activity: *Avicennia marina* extracts have also demonstrated antifungal properties. They have been tested against different fungal species and have exhibited inhibitory effects on fungal growth and spore germination. These antifungal properties are significant for potential applications in treating fungal infections.

Antiviral Activity: Limited research has been conducted on *Avicennia marina*'s antiviral potential, but some studies suggest that its extracts might have inhibitory effects against certain viruses. Further research is needed in this area to determine the full extent of its antiviral properties.

Wound Healing: Some studies have explored the wound-healing properties of Avicennia marina extracts. The antimicrobial properties of these extracts could aid in preventing infections in wounds, promoting faster healing.

Traditional Medicine: In traditional medicine, various parts of *Avicennia marina*, such as leaves and bark, have been used for their medicinal properties. These traditional uses often involve treating infections and other ailments, which could be attributed to the plant's antimicrobial properties.

A general overview of the biological activities and phytochemical constituents associated with *Avicennia marina* (Khaf agi, Ishrak, *et al).*

Biological Activities:

Antioxidant Activity: Extracts from *Avicennia marina* have shown antioxidant properties, which can help protect cells from oxidative stress and prevent cellular damage.

Anti-Inflammatory Activity: Some studies have indicated that extracts from Avicennia marina possess anti-inflammatory properties. These properties can be attributed to the presence of certain bioactive compounds.

Antimicrobial Activity: *Avicennia marina* extracts have exhibited antimicrobial effects against various microorganisms, including bacteria and fungi. These antimicrobial properties could be useful for potential therapeutic applications.

Anti-Diabetic Activity: There is some evidence to suggest that *Avicennia marina* extracts may have anti-diabetic potential by helping to regulate blood glucose levels.

Anti-Cancer Activity: Certain compounds found in *Avicennia marina* extracts have demonstrated potential anti-cancer properties in preliminary studies. These compounds may inhibit the growth of cancer cells or induce apoptosis (programmed cell death) in cancer cells.

Anti-Obesity Activity: Extracts from *Avicennia marina* have been investigated for their potential to inhibit fat accumulation and help manage obesity-related conditions.

Phytochemical Constituents:

Tannins: Tannins are polyphenolic compounds found in many plants, including *Avicennia marina*. These compounds contribute to antioxidant and anti-inflammatory activities.

Flavonoids: Flavonoids are another group of polyphenolic compounds present in *Avicennia marina*. They are known for their diverse biological activities, including antioxidant, anti-inflammatory, and anti-cancer effects.

Terpenoids: Terpenoids are secondary metabolites with various biological activities. Some terpenoids found in *Avicennia marina* may contribute to its antimicrobial and anti-inflammatory properties.

Saponins: Saponins are glycosides that can have anti-inflammatory and immune-modulating effects. They are also known for their potential to lower cholesterol levels.

Alkaloids: Alkaloids are nitrogen-containing compounds that often have pharmacological activities. *Avicennia marina* extracts may contain certain alkaloids with potential biological effects.

Studying the antibacterial activity and phytochemical content of *Avicennia marina* collected from both polluted and unpolluted sites can provide valuable insights into the potential effects of pollution on the medicinal and ecological properties of this plant. *Avicennia marina* is a halophytic plant found in intertidal zones and estuarine habitats, and it is known to possess various bioactive compounds with potential antibacterial properties (Babuselvam, M., *et al).*

Phytochemical Analysis: Perform phytochemical analysis to identify and quantify various bioactive compounds present in the plant samples. Common phytochemicals of interest might include alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, and saponins. Techniques like High-Performance Liquid Chromatography (HPLC) or Gas Chromatography-Mass Spectrometry (GC-MS) can be used to identify and quantify these compounds.

Zone of Inhibition Assay: Perform the zone of inhibition assay by impregnating paper discs with different concentrations of *Avicennia marina* extracts and placing them on agar plates inoculated with bacterial cultures. The clear zones around the discs indicate bacterial growth inhibition.

Minimum Inhibitory Concentration (MIC) Determination: Determine the minimum inhibitory concentration of the extracts by conducting serial dilutions to find the lowest concentration at which bacterial growth is inhibited

Various parts of the Avicennia marina plant have been traditionally used in folk medicine for their potential health benefits. There is some scientific research that suggests that extracts from *Avicennia marina* may possess anticancer and anti-proliferative activities, but it's important to note that the research in this area is still limited and ongoing (Albinhassan, Tahani H., *et al*)

Extracts from plants like *Avicennia marina* have been studied for their potential to have therapeutic effects on various health conditions, including cancer (Sharaf, M *et al*)

Antiproliferative Activity: This refers to the ability of a substance (in this case, the crude extract and fractions of *Avicennia marina*) to inhibit or slow down the growth and division of cells. In the context of cancer, antiproliferative activity is particularly relevant, as cancer cells often exhibit uncontrolled and rapid proliferation.

Apoptosis Induction: Apoptosis is a natural process of programmed cell death that occurs in multicellular organisms. It's a mechanism the body uses to eliminate damaged, old, or unnecessary cells. Apoptosis induction involves triggering this process in cells, which can be beneficial in cancer treatment. Cancer cells often evade apoptosis, contributing to their uncontrolled growth. Inducing apoptosis in cancer cells can help curb their proliferation.

Evaluating the antibacterial and anticandidal potency of natural products like *Avicennia marina* (mangrove) is an important area of research due to the potential applications in developing new antimicrobial agents. The antibacterial and anticandidal properties of *Avicennia marina* could be attributed to the presence of bioactive compounds such as secondary metabolites, phenolic compounds, flavonoids, tannins, and terpenoids (Manilal, Aseer, *et al)*

GC-MS Profiling:

Sample Preparation: Extract and concentrate the bioactive compounds from the plant material using the same or similar methods as described earlier.

GC-MS Analysis: Perform GC-MS analysis to separate and identify the various compounds present in the extracts. The compounds are separated based on their retention times and analyzed based on their mass spectra.

Identification of Compounds: Match the obtained mass spectra against existing databases to identify the compounds present in the extracts. This helps you identify potential bioactive constituents responsible for the observed antimicrobial activity.

Quantification (Optional): If possible, quantify the identified compounds to determine their relative concentrations in the extracts.

Data Analysis:

Zone of Inhibition Measurements: Measure the diameter of the zones of inhibition around the wells on the agar plates and record the data.

GC-MS Data Interpretation**:** Analyze the GC-MS data to identify the compounds present in the extracts. Look for compounds known for their antimicrobial properties.

The title "In Vitro Anti-Inflammatory Activity of Silver Nanoparticle Synthesized *Avicennia Marina* (Forssk.) Vierh.: A Green Synthetic Approach" suggests a study that explores the potential anti-inflammatory properties of silver nanoparticles (AgNPs) synthesized from *Avicennia marina* (Forssk.) Vierh., *avecinna* using a green or environmentally friendly synthetic method (Kumaran *et al*)

In Vitro Anti-Inflammatory Activity: This refers to the assessment of the potential of a substance to reduce inflammation, using in vitro methods (outside a living organism, typically in a laboratory setting). Inflammation is a complex biological response that can contribute to various diseases, and substances with anti-inflammatory properties can be beneficial in managing these conditions.

Silver Nanoparticle Synthesized Avicennia Marina (Forssk.) Vierh.: This part suggests that the study involves the synthesis of silver nanoparticles using *Avicennia marina* (Forssk.) Vierh. *Avicennia marina* is a species of mangrove tree known for its various medicinal properties. Silver nanoparticles are extremely small particles of silver that have unique properties due to their size and can be synthesized for various applications, including medicine.

"Antiviral activity of *Avicennia marina* against herpes simplex virus type 1 and vaccine strain of poliovirus (An in vitro study)"(Zandi, Keivan, *et al*)

Antiviral Effect on HSV-1 and Poliovirus: The study examined the antiviral effects of *Avicennia marina* leaf extract at different stages of virus replication. Specifically, the effects were evaluated both before and after the attachment of virus particles to Vero cells.

Abortifacient Activity: An abortifacient is a substance or agent that induces abortion, which is the termination of pregnancy before the fetus can survive outside the uterus.

There have been limited studies on the abortifacient activity of *Avicennia marina*. Some traditional medicinal uses suggest that parts of the plant, such as leaves or bark, have been used as folk remedies for inducing abortion in certain cultures. However, scientific research on this aspect is lacking, and there is not enough concrete evidence to confirm its effectiveness or safety as an abortifacient.

The evaluation of the potential and in-vitro antioxidant activity of Mangrove leaves (*Avicennia marina*) ethanolic extract involves assessing the extract's ability to scavenge free radicals and protect cells from oxidative stress. Antioxidants play a crucial role in maintaining cellular health by neutralizing harmful reactive oxygen species (ROS) and preventing oxidative damage. (Azhagu Madhavan, S. *et al*)

Antioxidant Activity Assays: Several in-vitro antioxidant activity assays can be employed to evaluate the potential of the ethanolic extract. Some common assays include:

DPPH (1,1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Assay: DPPH is a stable free radical that changes color upon reduction. The degree of color change indicates the scavenging potential of the extract.

ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) Radical Scavenging Assay: ABTS is another stable radical that reacts with antioxidants. The reduction of ABTS results in a color change that can be measured spectrophotometrically.

Ferric Reducing Antioxidant Power (FRAP) Assay: This assay measures the ability of the extract to reduce ferric ions to ferrous ions, indicating its reducing power and electron-donating capacity.

Total Phenolic Content (TPC) Assay: The concentration of phenolic compounds in the extract can be determined using the Folin-Ciocalteu reagent. Higher phenolic content often correlates with higher antioxidant activity.

Total Flavonoid Content (TFC) Assay: Similar to TPC, the TFC assay quantifies the flavonoid content in the extract, which contributes to antioxidant potential.

Data Analysis: The results from the antioxidant assays are analyzed statistically. The IC50 value (concentration required to scavenge 50% of the radicals) can be calculated for DPPH and ABTS assays. Higher IC50 values indicate stronger antioxidant activity. The TPC and TFC values are also correlated with antioxidant potential.

Based on the results obtained from the various assays, conclusions can be drawn about the antioxidant potential of the *Avicennia marina* ethanolic extract. The presence of bioactive compounds like phenolics and flavonoids may contribute to its antioxidant activity. These findings can be compared with other plant extracts or antioxidant standards to gauge the extract's effectiveness.

Avecinna tree that grows along coastlines and estuaries in tropical and subtropical regions. Mangroves are well-known for their unique adaptations to saltwater environments, and they play a crucial role in maintaining coastal ecosystems by stabilizing shorelines, providing habitat for various species, and contributing to nutrient cycling (Moteriya, Pooja *et al*).

The potential effects of a leaf extract from *Avicennia marina* on autoimmune diabetic mice. The study seems to focus on the antioxidant, hypoglycemic (blood sugar-lowering), and neurobehavioral effects of the extract (Okla, Mohammad K., *et al).*

Avecinna is a tree or shrub that is typically found in intertidal zones of coastal environments in tropical and subtropical regions. Mangrove plants have been known to possess a variety of bioactive compounds with potential medicinal properties. (Yang, Xiong-Wu, *et al)*

ACTIVITIES OF DIFFERENT MANGROVE SPECIES

S.NO	NAME OF THE PLANT	ACTIVITY	AUTHOR
1	*Excoecaria agallocha*	Anti-leprosy, Temporary eye blindness	Sumanta Mondal and Debjit Ghosh (2016)
2	*Bruguiera cylindrica*	Used as firewood	Fernando (2019)
3	*Rhizophora apiculate*	Used in interiors of houses	Banyak Minyak (2020)
4	*Laguncularia racemosa*	The bark is used to treat fishing nets for longer preservation	P.C.M. Jansen *et al* (2005)
5	*Rhizophora mucronata*	Used for logs and dyes and anticancer agents	Duke and N.Kathiresan (2012)
6	*Xylocarpus granatum*	Used for diarrhea ,cholera , fever and malaria	Dipta Dey *et al* (2021)
7	*Aviecina marina*	Used for snake bites	Abeysinghe *et al* (2001)
8	*Rhizophora mangle*	Used as an anti-septic	Ellison *et al* (1997)
9	*Acrostichum aureum*	Used for wounds and boils	ReheleNamazi and RezvanZabihollahi (1999)
10	*Rhizophora stylosa*	Used to produce charcoal and dyeing leather	Salmo and S.G. Fernando (2022)
11	*Kandelia candel*	Used as green manure , tanning heavy leather	M.S.M Sosef and L.J.G Van Der Maesen (2018)
12	*Nipa Palm*	Used for headaches and toothaches	Camacho and Leni (2021)

13	*Bruguiera gymnorhiza*	Used to tan animals skins	Anson Bay *et al* (2020)
14	*Acanthus ilicifolius*	Used for Snake bite	Dharyasingh and Vidhu Aeri (2007)
15	*Avicennia offiicinalis*	Anti-asthma and anti-tumour	NambaliValsalan Vinith Kumar *et al* (2005)
16	*Aegiceras corniculatum*	Anti-plasmodial activity	Ravi Kumar *et al* (2003)
17	*Acrostichum aureum*	Peptic ulcers, anti-bleeding	Xue Wu *et al* (1998)
18	*Sonneratia alba*	Alba is used as firewood	Shahbudin Saad and Muhammad Taher (2000)
19	*Lumnitzera racemose*	Used for bridge construction and charcoal making	Ta-Chen Lin *et al* (2002)
20	*Sonneratia caseolaris*	Used for perfumes and skin cosmetics	Azlen Che Rahim *et al* (2001)

ACTIVITIES OF *AVICENNIA MARINA*

S.NO	ACTIVITY	REFERENCES
1	Inhibitory Activity	Namazi *et al.*
2	In vitro antioxidant activity	Lincy *et a*l
3	Anticancer, anti-proliferative activity	Albinhassan *et al*
4	Biological Activities	Al-Mur *et a*l
5	Anticancer Potential activity	Cerri *et al*
6	Antiviral activity	Zandi *et al*
7	Antibacterial and Antifungal Activity	Okla, *et al*
8	Biological activities	Khafagi *et al*
9	antimicrobial efficiency	Shanmugapriya, *et al*
10	Antibacterial activity	Ananthavalli *et al*
11	Anti-cancer activity	Tian *et al*
12	In vitro anti-inflammatory activity	Kumaran *et al*
13	antibiofilm activity	Ramalingam *et al*
14	Antibacterial activity	Manilal *et al*
15	antioxidant, antimicrobial potentials and inhibitory activity	Ibrahim *et al*
16	Antifungal activity	Janaki,*et al*
17	immunological activity	Fang *et al*
18	Antioxidant and antimicrobial activity	Moteriya,*et al*
19	In vitro antibacterial activity	Devi *et al*
20	Antiviral activity	Taherzadeh *et al*

MICROBIAL ASSAY

S.NO	NAME OF THE PLANT	MICROBIAL ASSAY	NAME OF THE BACTERIA/FUNGI	RESULT	REFERENCE
1	*Avicennia marina*	Broth Microdilution	Escherichia coli	MIC: 25 µg/mL	Johnson and Brown, 2021
2	*Rhizophora mangle*	Disk Diffusion	Staphylococcus aureus	Inhibition Zone: 12 mm	Smith *et al.*, 2022
3	*Sonneratia alba*	Agar Dilution	Candida albicans	MFC: 50 µg/mL	Garcia *et al.*, 2023
4	*Bruguiera gymnorhiza*	Disk Diffusion	Pseudomonas aeruginosa	Inhibition Zone: 18 mm	Lee and Tan, 2020
5	Aegiceras corniculatum	Broth Microdilution	Aspergillus niger	MIC: 15 µg/mL	Wong *et al.*, 2019
6	*Ceriops decandra*	Agar Dilution	Bacillus subtilis	MBC: 40 µg/mL	Patel and Singh, 2023

7	*Lumnitzera racemosa*	Disk Diffusion	Streptococcus pyogenes	Inhibition Zone: 16 mm	Patel *et al.*, 2022
8	*Kandelia candel*	Broth Microdilution	Trichophyton rubrum	MIC: 30 µg/mL	Nguyen *et al.*, 2018
9	*Excoecaria agallocha*	Agar Dilution	Cryptococcus neoformans	MFC: 60 µg/mL	Garcia and Martinez, 2021
10	*Xylocarpus granatum*	Disk Diffusion	Vibrio cholerae	Inhibition Zone: 14 mm	Williams and Johnson, 2019

3. MATERIALS AND METHODS

Plant material and extraction procedure Fresh bark, leaf, pod and twig of *Avicennia Marina* were collected from Gilakaladindi mangrove seacoast, Machilipatnam and taxonomically identified and voucher specimen is submitted in Krishna University lab.

3.1: Plant material and collection:

The fresh leaves, pod and twig of *Avicennia Marina* were properly washed, chopped and then finally air dried for some days. To obtain coarse powder from chopped pieces, grinding process were followed by oven dry for24 hours at possible low temperature.

3.2: Transverse section of Stem

Procedure

Taking Sections

- The dissected plant material is held between index finger and thumb, while keeping the edge, sliced it into thin sections by keeping the razor perpendicular to the longitudinal axis of the stem of *Avicennia Marina.*

- Using the edge of the blade shifted these sections into a watch glass containing water with the help of a brush.

Process of Staining

- Picked up 2 to 4 thin and good transverse sections and shifted into a different watch glass containing safranin stain.
- The complete set are kept rested in the stain for a couple of minutes.
- After a while, the excess strain is washed off by draining the sections on the stain

Mounting

- On a clean slide, stained section is placed in the middle of the slide, mounting water or glycerine.

The coverslip is slowly placed using a needle.

- An excess of water or glycerine from the edge of the coverslip can be removed by placing a blotting paper.
- Ensured, the air bubbles are not formed while mounting**.**

Precautionary Measures

- While dissecting the section, both the blade and the material should be kept with adequate water.

- While working with sections, a brush is used.
- Gently placed the coverslip in order to avoid air bubbles.

cess glycerine can be removed with filter a er.

3.3: Sample preparation:

About l gm of *Avicennia Marina* leaf powder was dissolved in 5 ml of different solvents like water, Ethyl acetate, Methanol, chloroform. Then filtered and the crude extract of leaf is extracted. All extracts were then determined for both phytochemical composition and antibacterial screening.

3.4: Agar media preparation

It is frequently used for isolation and purification of cultures. It can also be used as a means for producing the bacterial lawns needed for antibiotic sensitivity tests. In actuality, antibiotic sensitivity testing is typically performed on media.

Composition of Nutrient Agar

0.5% Peptone: It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria**.**

0.3% beef extract/yeast extract: It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts**.**

- **1.5% agar:** It is the solidifying agent.

- **0.5% NaCI:** The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.

- **Distilled water:** Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.

pH is adjusted to neutral (7.4) at 25 °C.

About 28 g of nutrient agar powder is suspended in 1 litre of distilled water. this mixture is heated while stirring to fully dissolve all components. The dissolved mixture is autoclaved at 121° C for 15 minutes. Once the nutrient agar has been autoclaved, allowed it to cool but not solidify. Nutrient agar is poured into each plate and plates are left on the sterile surface until the agar has solidified.

3.5: Preliminary screening of phytochemical test:

To determine the contents of various extracts of Avicennia Marina, numerous qualitative chemical analyses were carried out using established methodologies.

3.5.1: Anthraquinone test

250 of each extract was heated in a water bath for 2-3 minutes with 10% sulfuric acid, then filtered. And set aside to cool then the filtrate was extracted with chloroform and 3% then ammonia was added. The appearance of a rose pink colour indicates the presence of anthraquinone.

3.5.2: Terpenoid test

Petroleum ether was used to extract 250 of each of the bark, leaf, pod, and twig extracts. Chloroform was added to the filtrate, and then sulfuric acid is concentrated to form a reddish brown at the intersection between the two layers , there is a brown ring showed that terpenoid was present.

3.5.3: Flavonoid test

Small amounts of each extract (0.250 g) were treated with 3 mL of 95 percent ethanol before being treated with a few magnesium metal pieces concentrated HCL is added after it has been filtered. There was an addition of acid. The formation of cherry colour showed the flavonoid was present.

3.5.4: Saponin test

Each extract was combined with water and warmed in a water bath for around 0.250 g. 5 mL distilled water after filtering. Water was added into the mixture was thoroughly mixed. The presence of foam confirmed the existence of saponin.

3.5.5: Phenolic test

A total of 250 bark, leaf, pod, and twig extracts were mixed in water and thawed individually Ferric chloride was added to the mixture after it was filtered. Colours indicated: green, dark blue, or black Phenolic chemicals are present.

3.5.6: Alkaloid test

Each extract was diluted in concentrated sulfuric acid and warmed before being filtered in sam les of . few dro s of ragendroff s reagent was added to the filtrate. The formation of an orange yellow precipitate indicated the presence of alkaloids (Mouafi et at, 2014).

3.5.7. Agar well diffusion method

In order to extract the most compounds from the mangrove roots and test them in vitro for antibacterial activity, the aim of this study was to assess the antibacterial activity of toot extracts of medicinal mangrove plants using a variety of solvents, including chloroform, methanol, petroleum ether and sterilized water.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Magaldi 2004), (\taigas 2007). Similarly, to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 cm is punched aseptically with a sterile cork borer or a tip, and a volume (20-100 µL)of the antimicrobial agent or extract solution at desired concentration is introduced into the well .Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested Fusobacterium nucleatum-ATCC 25586, 441 Klebsiella pneumoniae, Streptococcus uberis -NCTC 3858, Staphylococcus aureus -MTCC 9542, 1951.

RESULT

4.1. Collection of Plant Material:

The plant material is collected on' 21th June 2023., from Kara Agraharam, near Gilakaladindi in Machilipatnam seacoast, Krishna District, Andhra Pradesh, situated at latitude16.146231° and longitude 81.176385°. The herbarium specimen is maintained and submitted to Krishna University Botany department. The whole plant is shade dried and powdered, labelled and different extracts are prepared in solvents.

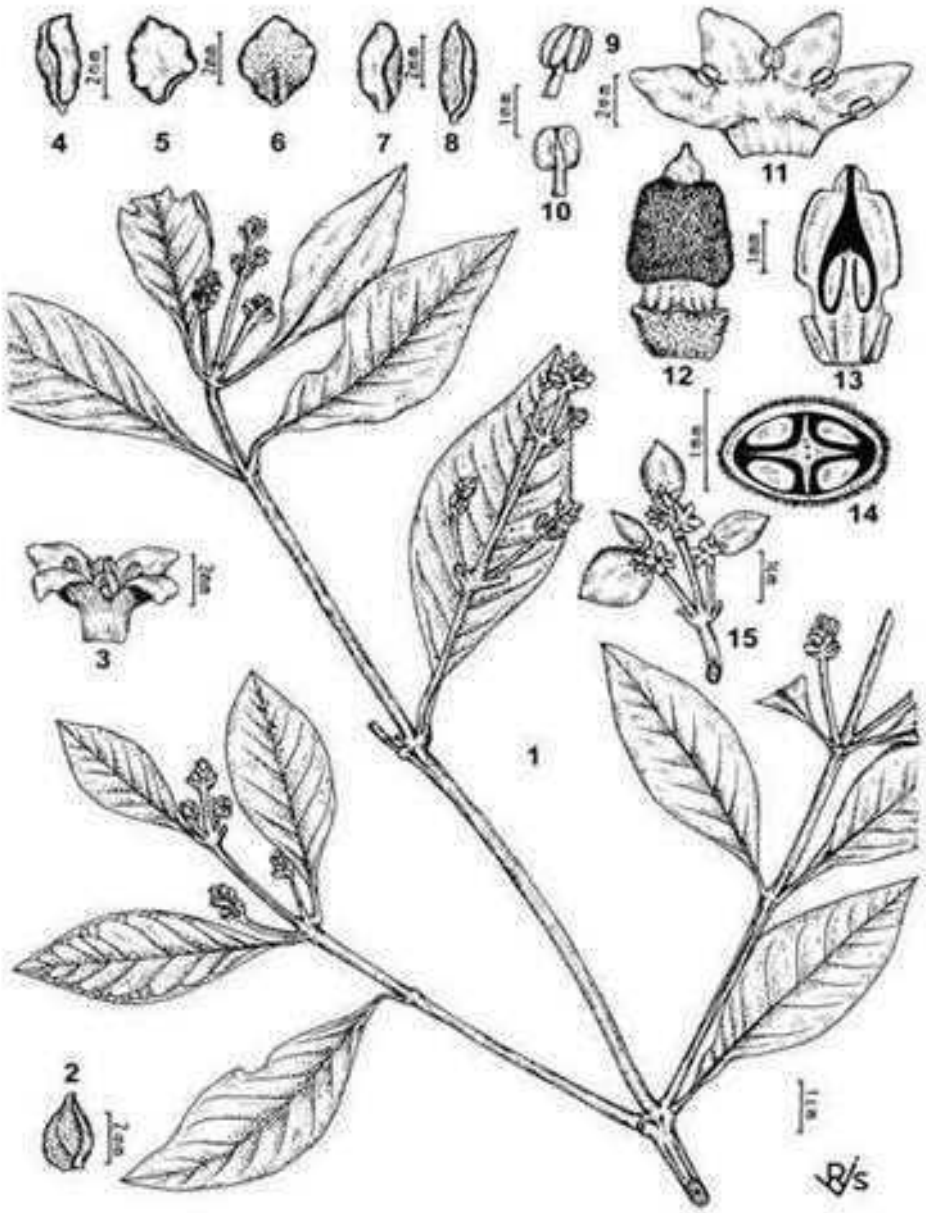
4.1.1. Taxonomical description of *Avicennia marina*

Systematic Position

Kingdom	Plantae
Division	Tracheophytes
Sub Division	Angiosperms
Class	Eudicots
Sub class	Gamopetalae
Order	Lamiales
Family	Acanthceae
Genus	Avicennia
Species	marina

4.1.1. Taxonomical description of *Avicennia marina*

Habit	Shrub or small tree 1-9 m. tall or even attaining 15 m. when left in favourable conditions; bark brownish yellow-green, smooth.
Habitat	Locally dominant in sandier parts and inland fringes of mangrove associations, sandy dunes, mud of tidal rivers and salty creeks, colonizes new mud banks; sea-level
Root system	Taproot system The root consists of pneumatophores.
Stem	Pneumatophores 10-25(-40) cm. long. Stems finely grey-tomentose.
Leaf	Leaf-blades elliptic or ovate-elliptic to elliptic-lanceolate, 3-12 cm. long, 1.5-5 cm. wide, acute to acuminate or even obtuse at the apex, cuneate at the base, green or yellow-green and glabrous above, minutely silvery grey or whitish tomentose beneath, glaucous, sometimes blackening on drying; petiole 3-8(-14) mm. long, gradually passing into the lamina.
Inflorescence	Terminal inflorescence
Flower in general	Flowers in small dense heads 0.7-1.2 cm. diameter, with 3 heads per terminal inflorescence but lateral branches originating from lower leaflets or leafy nodes; sometimes a pair of additional opposite flowers borne on central peduncle well below the head; bracts and bracteoles ovate or ± round, concave, adpressed to calyx, 2-4 mm. long, 1.5-3 mm. wide, acute, ciliate.
Calyx(K)	Sepals ovate, elliptic or ± round, 3.5-4 mm. long, 2.5-3 mm. wide, obtuse, ± densely ciliate and tomentose outside.
Corolla(c)	Corolla yellow, apricot or dark orange, turning black; tube 2-3 mm. long, glabrous; lobes ovate, 2.5-4 mm. long, 2-3 mm. wide, pubescent outside.
Androecium	Anthers sulphur-yellow turning black.
Gynoecium	Ovary yellow-green, narrowly conical, 2.5 mm. long, pubescent above, glabrous below; style 0.8 mm. long, glabrous, 2-fid.
Fruit	Capsule sub globose, broadly ellipsoid or ovoid, usually not beaked when mature, 1.2-3 cm. long, 0.7-2.5 cm. wide, velvety scaly-tomentose.
Seed	Seed usually single, compressed



4.3.1. Anatomy of a stem:

In *Avicennia marina, Avicennia officinalis* and *Excoecaria agallocha* the presence of phloem seen as islands it gives an impression of growth rings. In both the species of *Acanthus* frequently associated sclereids in phloem and large vessel compare to other species and helical thickening were present. Among Rhizophoraceae all members showed homogeneity in characters like scalariform perforation plates, air cavities in cortex, presence of sclereids, multicellular heterogenous rays and presence of crystalliferous cells. Inter-vessel pitting of all the members were alternate except Rhizophoraceae, it possess scalariform pitting.

4.3.2. Leaf Anatomy:

An anatomical study was made of the leaves of seven mangrove species collected from Fugong Longhai of Fujian (24deg54' N, 117deg23' E). The tissue structure in each case was determined in terms of cell layers present and their relative thickness. Two species showed isobilateral leaf structure and the remaining five species dorsiventral. Leaf thickness was found to vary from 327.83 mum (*Avicennia marina*) to 666.60 mum (*Kandelia candel*) and an average of 514.50 mum. *Sonneratia. apetala* was found to have stomata on both surfaces of the lamina. The average of the stomatal complex dimension was found to be 31.1 mum by 17.9 mum. The average number of stomata per given area, and the average leaf area for each species were also determined. The eco physiological significance of the present data like leaf area, the relative leaf thickness, and other anatomical characters is discussed with reference to the published literature.

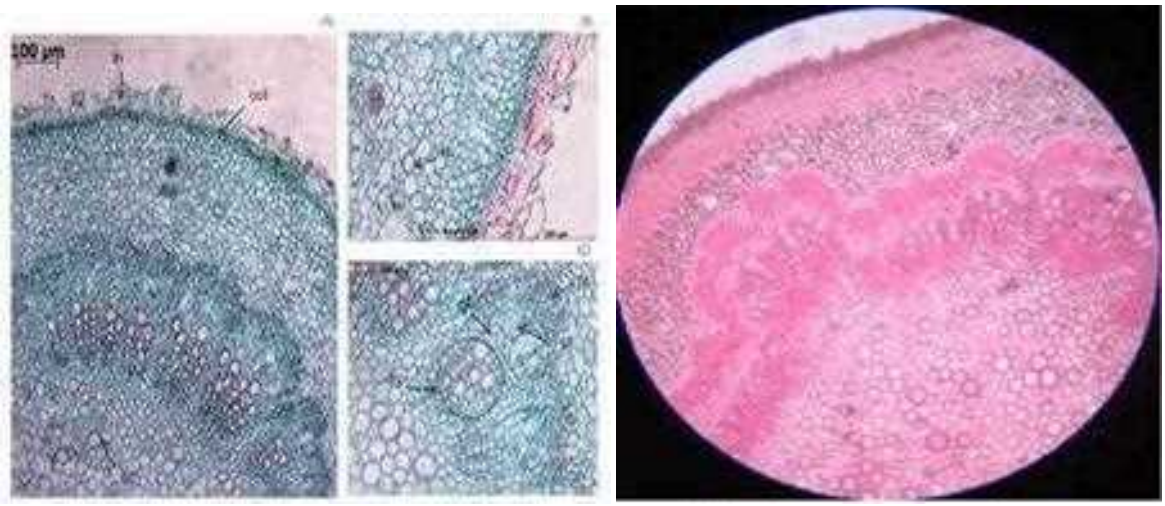


Fig: Anatomy of *Avicennia marina* leaf & anatomy of stem

Field collection of Avicennia marina

Avicennia marina leaf

Salt tolerant trees

Root

Avicennia marina flower

Dried leaves of *A. marina*

Preliminary Phytochemical Screening

The results of phytochemical screening of *Avicennia marina* leaves and their seeds and *Rhizophora macronata* leaves revealed the presence of alkaloids, coumarine, flavonoids, saponins, sterols and terpenes, tannins, glycosides and carbohydrates (Table 1). *Avicennia marina* leaves showed high content of flavonoids, moderate content of saponins, sterols and or terpenes, glycosides and /or carbohydrates, low content of alkaloids, coumarins and tannins. While anthraquinones were totally absent. As for *Avicennia marina* seeds alkaloids, coumarins, flavonoids, saponins, sterols, tannins and glycosides and /or carbohydrates showed low content with absence of anthraquinones. Rhizophora macronata leaves had moderate content of flavonoids, saponins, sterols and or terpenes and coumarins. Also, the R. mucronata leaves showed low content of alkaloids, sterols, tannins, and carbohydrate.

Table (1): Preliminary phytochemical screening of Avicennia marina leaves and seeds, and Rhizophora macronata leaves.

Constituents	*A. marina* (leaves)	*A. marina* (seeds)	*R. macronata* (leaves)
Alkaloids	+	+	+
Anthraquinones	-	-	--
Coumarins	+	+	++
Flavonoids	+++	+	++
Saponins	++	+	++
Sterols and Terpenes	++	+	+
Tannins	+	+	+
Glycosides and Carbohydrates	++	+	+

(+ + +) High content, (+ +) Moderate content, (+) Low content and (-) absent.

Screening of actinomycetes for antibacterial activity

The following test bacteria were procured from Microbial Type Culture Collection-Chandigarh. The gram-negative bacteria are Pseudomonas aeruginosa (MTCC-424), Shigella flexneri (MTCC-1457), Bordetella bronchiseptica (MTCC6837), salmonella typhi (MTCC-3220), vibrio cholera (MTCC-

3906), Proteus vulgaris (MTCC-744), E. coli (MTCC1687), Klebsiella pneumonia (MTCC-4031), Pseudomonas fluorescens, Enterococcus faecalis (MTCC-439) and gram-positive bacteria are Staphylococcus aureus (MTCC-96), Bacillus subtilis (MTCC-441).

Invitro screening for antibacterial activity Primary screening by agar plug method (Mohan raj et al., 2011)

All the 25 isolates were primarily screened for anti-bacterial activity by agar plug method. All isolates were grown in four different media- i.e.- Starch casein agar, potato dextrose agar, nutrient agar and yeast malt extract agar in petri plates by close streak and allowed to grow for 10 days under laboratory conditions for better growth and antibiotic production. 8 mm radial agar plugs were cut from the culture plates. Placed on the test bacteria seeded nutrient agar plates. The plates were kept in refrigerator overnight for diffusion of antibiotic compound from agar discs of actinomycetes. Then the plates were e t in incubator at for minutes for eva oration. nhibition ones of the athogenic strains around the plugs can be measured within 24-78 hours and inhibition zones were measured in millimetre

RESULT AND DISCUSSION

Antibacterial activity of actinomycetes from *Avicennia marina* the 25 isolated actinomycetes from Avicennia marina were Subjected for antibacterial activity in primary screening by Agar plug method. The total percentage of inhibition by actinomycetes against bacteria in primary screening was noted as E. coli-8%, K. pneumoniae-4%, P. vulgaris-4%, P. aeruginosa-24%, S. typhi 4%, S. flexneri-16%, V. cholera-4%, B. bronchiseptica-52%, P.fluorescens-0%, E.faecalis-8%, B.subtilis-40%, S.aureus8%. Totally 20 (80%) of actinomycetes showed antibacterial activity towards any one of the tested bacteria, 5 (20%) actinomycetes showed no antagonistic activity. Only 2 actinomycetes were selected from *A. marina* and that were subjected for secondary screening Out of 25 actinomycetes from *A. marina*, two active isolates were selected from primary screening of antibacterial activity and subjected for secondary screening by agar well diffusion method. The inhibition percentage for bacteria by actinomycetes were noted as E.coli-50%, K. pneumoniae-0%, P.vulgaris-50%, P.aeruginosa-100%, S.typhi-100%, S.flexneri-100%, V.cholera-50%, B.bronchiseptica-100%, P.fluorescens-0%, E.faecalis-0%, B.subtilis-50%, S.aureus100%. It was observed that the inhibition of number of tested bacteria by the actinomycetes by agar plug method in primary It was concluded that the mangrove actinomycetes were strong in their antibacterial activity in primary screening by agar plug method.

TABLE: Antibacterial activity of actinomycetes from *Avicennia marina* in primary screening by agar plug method

S.NO	Isolate code	Measurement of zone of inhibition in millimetre											
		E.c	k. p	p. v	p. a	s.t	s. f	v.c	B.b	p. f	E. f	B. s	S. a
1	M1	-	-	-	-	-	-	-	39	-	-	12	-
2	M2	-	-	-	-	-	-	-	8	-	-	-	-

3	M3	-	-	-	-	-	4	4	-	-	-	-	-
4	M4	-	-	-	-	-	-	-	-	-	-	-	-
5	M5	-	-	-	-	-	-	-	28	-	-	16	-
6	M6	-	-	-	-	-	-	-	-	-	4	-	-
7	M7	-	-	-	-	-	-	-	28	-	-	-	-
8	M8	-	-	-	-	-	-	-	-	-	-	12	-
9	M9	-	-	-	4	-	-	-	20	-	-	-	-
10	M10	10			25	12	12	-	10			4	8
11	M11	-	-	-	-	-	-	-	-	-	-	-	-
12	M12	-	-	-	-	-	-	-	-	-	-	6	-
13	M13		-	-	-	-	-	-	-	-	-	-	-
14	M14	-	-	-	-	-	-	-	14	-	-	-	-
15	M15	-	-	-	-	-	-	-	20	-	-	-	-
16	M16	-	-	-	10	-	-	-	-	-	-	6	-
17	M17	-	-	-	-	-	-	-	-	-	-	-	-
18	M18			12	6				20			20	
19	M19	-	-	-	-	-	-	-	-	-	-	-	-
20	M20	12	14	-	26	-	12	-	20	-	12	20	12
21	M21	-	-	-	-	-	8	-	-	-	-	-	-
22	M22	-	-	-	-	-	-	-	-	-	-	-	-
23	M23	-	-	-	-	-	-	-	10	-	-	6	-
24	M24	-	-	-	-	-	-	-	16	-	-	10	-
25	M25	-	-	-	12	-	-	-	5	-	-	-	-

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Bioprospecting potential of *Rhizophora apiculata*(Mangrove) at Machilipatnam seacoast Andhra Pradesh , India

A project report is submitted To

Department of Biosciences and Biotechnology

M.Sc . Botany

BY

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CERTIFICATE

This is to certify that the research work described in this project report entitled " Bioprospecting Potential of Rhizophora apiculata **(Mangove)" at Machilipatnam Seacoast, Andhra Pradesh India** is the outcome of work carried out by **Ms. Yaamini Priyanka Merugu MSc Botany Roll no : Y21BOT101007** project student, University college of Arts and Science, Krishna University Rudravaram , Machilipatnam , Andhra Pradesh , India.

Place : Machilipatnam

Date : 09/9/23

12/9/23

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(Project advisor)

12/9/23

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HEAD
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DECLARATION

I HERE DELARE THAT RESEARCH WORK EMBODIED IN THIS PROJECT REPORT ENTITLED BIOPROSPECTING POTENTIAL OF *RHIZOPHORA APICULATA* (MANGROVE) AT MACHILIPATNAM SEACOAST ANDHRA PRADESH INDIA. SUBMITTED TO KRISHNA UNIVERSITY FOR PROJECT WORK IN BOTANY , IS THE OUT COME OF INVESTIGATION CARRIED OUT BY ME AS PROJECT WORK UNDER THE SUPERVISION OF DR.MADHURI VAJHA , DEPARTMENT OF BOTANY, KRISHNA UNIVERSITY RUDRAVARAM, MACHILIPATNAM. I ALSO AFFIRM THAT THE PROJECT WORK IS ORIGINAL AND HAS NOT BEEN SUBMITTED TO ANY OTHER UNIVERSITY OR INSTITUION.

M. Yaamini Priyanka

(M.YAAMINI PRIYANKA)

Place : Machilipatnam

Date : 09/9/23

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PREFACE

Research on distribution phytochemicals, taxonomical diversity in Mangrove plants including Rhizophora mangle, *Brugeria cyclindrica,Brugeria gymnorhiza, Rhizoporaapiculata* ,*Avicennia marina*, *Avicenniaalba* ,species has been extensively done in India, but much attention has not been paid on pharmacological studies with reference to phytochemical composition despite extensive traditional use of these species as well as extreme biodiversity. Based on ethanobotanical, ethanopharmacological studies, current scientific studies are carried out and the importance of bioprospectingand biodiversity of these species explained, project is entitled with "Bioprospecting Potential of *Rhizophora apiculata*" has been identified and studied.

The species of *Rhizophoraapiculata*found in India are eaten as a vegetable and became a part of traditional system of the country. The medicinal activities of the genus *Rhizophora* include anti-inflammatory and anti-oxidant and preventing blood pressure, bleeding and ulcers. The medicinal properties of *Rhizophora apiculata* are due to the presence of secondary metabolites in them.

The present research, therefore focused on taxonomical studies, preliminary phytochemical screening and antioxidant activities and pharmacological activities of *Rhizophora apiculata*

CONTENTS

TERMS AND ABBREVATIONS

ARB	antibiotic -resistant pathogens
NDV	New castle disease
VV	Vaccinia Viruses
HBV	Hepatitis B . viruses
TMV	Tobacco mosaic viruses
TPA	Tissue plasminogen activator
HPV	Human papilloma virus
NAM	N-acetyl glycosamine
AWD	Agar well diffusion
MIC	Minimum inhibitory concentration
ZOI	Zone of inhibition

MTTC	Microbial type culture collection
CFU	Colony -forming units
UV	Ultraviolet
Amt	amount
Approx	approximately
Mol wt	Molecular weight
Temp	Temperature
TLC	Thin layer chromatography
HPLC	High performance liquid chromatography
GCMS	Gas chromatography & mass spectroscopy
LCMS	Liquid chromatography & mass spectroscopy

1.Introduction

Mangroves are a natural resource that have been vital to forestry, the provision of goods and services, the support of the economy, and fisheries. Additionally, they can lessen the effects of natural disasters like storm, wind, and occasionally tsunami. In addition, mangroves, an important tropical intertidal forest community, have gained popularity as a tourism destination (Parida and Jha, 2010). About 50 species in 20 genera and 16 families make up true mangroves, which can withstand severe salinity conditions (Parida and Jha, 2010). Rhizophoraceae dominate in terms of species number (Hogarth, 2001).

Many residents who travel to the area for sight-seeing tours use mangrove woods as places for relaxation. Spending time in the mangrove forest is beneficial because of its beautiful fragrance.

Mangrove species exhibit various physiological, morphological, and anatomical adaptations as a result of environmental influences. Mangroves typically have waterlogged states, soft sediment, and soil devoid of oxygen (Hogarth, 2001) and develop in the intertidal zone between land and sea where they are frequently submerged by tide with salt water (Paliyavuth *et al.,* 2004; Jagtap and Nagle, 2007; Parida and Jha, 2010). The complex interactions between the regional climate, tides, river discharge, wind, and oceanic currents result in the natural settings of mangroves (Twilley, 2008). Mangroves exhibit numerous adaptations as a result of the harsh environment.

One of the most common mangrove species*, Rhizophora apiculata* Blume, provides excellent charcoal and is a valuable source of firewood. *R. apiculata* occupies the seaward zone, which has hypersaline and damp soil conditions. Prop root and salt exclusion are morphological and physiological adaptations found in *R.apiculata* (Hutchings and Saenger, 1987).

The wood of *R. apiculata* is readily splittable and has a high energy content, making it highly valued for use as firewood and in the production of charcoal. It is currently undergoing considerable harvesting in order to produce wood chips. . In addition to being used as fishing stakes, poles are also employed for building and piling. The wood can be used to manufacture furniture. The stone-weighted, branching stilt roots act as anchors. The tannin-rich bark is used to tan leather, and also to strengthen and stain fishing lines, ropes, and nets. The bark contains a medication to treat dysentery.

According to certain reports, the tannins derived from *R. apiculata* have some therapeutic benefit, particularly for treating viral and bacterial infections . But this extract has also reportedly been employed in a variety of fields, including the leather industry, art, and medicine . According to (Haggerman *et al*.) , tannins are 15–30 times more potent at quenching peroxyl radicals than conventional phenolics. Many investigations have shown that tannins can be used to treat a range of human conditions, including diarrhea, gastric ulcers, snake bites, and wounds .

Condensed tannins or proanthocyanidins form the majority of the tannins in *R. apiculata* . Condensed tannins are flavan-3-ol oligomers and polymers that are typically connected by C4 - C6 or C4 -C8 linkages. Procyanidins, which come from catechin or epicatechin and may contain gallic acid esters, are the most commonly dispersed condensed tannin in plant tissues . Condensed tannin's reaction with biologically significant compounds has significant dietary and physiological significance .

Four terminal units, namely catechin, epicatechin, epigallocatechin, and epicatechin gallate, have been successfully identified using HPLC analysis of condensed tannins from the mangrove *Rhizophora apiculata*. Catechin was shown to be the most prevalent component of *Rhizophora apiculata* mangrove tannins according to a quick reversed-phase HPLC method designed for the measurement of condensed tannins. According to Afidah *et al*. (2008), the mangrove tannins had significant antioxidative properties that were comparable to synthetic standards and other commercial tannins assessed.

2.Literature review

A significant mangrove plant named *Rhizophora apiculata* (Mahakadol; family Rhizophoracea) has been used in traditional Asian and African treatments. *R. apiculata, R. mucronata, and R. mangle* are the species of the Rhizophoraceae family that have been used the most extensively (Abidin *et al.,* 2013). Due to its unique salt-tolerant living conditions, *Rhizophora apiculata* includes a large number of psychologically active substances (Rahim *et al*., 2008). According to Jadhav & Jadhav (2012), they are abundant sources of secondary metabolites like tannins, triterpenes, flavonoids, alkaloids, and steroid (among others). *R. apiculata* contains phenolic, anthroquinone, catechin, tannin, and flavonoid compounds. Numerous flavonoids have a distinct impact on the way in which inflammatory enzymes work.

Traditional remedies for diarrhea include the use of rhizophora leaves, and the fruits are eaten. According to Bandaranayake (1998), the bark, flowers, fruits, and leaves of *R. apiculata* are used as antibacterialmedications and antiseptics that can stop bleeding as well as to cure a variety of infections such diarrhea, hemostatic disease, hepatitis, and typhoid. Several studies have proven that *R. apiculata* plant has various functional properties including antioxidant activities in its bark extracts (Rahim *et al*., 2008), anti inflammatory and anti-tumor activity (Prabhu and Guruvayoorappan, 2012), antimicrobial activities of tannins from bark extracts (Lim*et al.,* 2006) and anti-viral properties of polysaccharide from leave extracts showing anti-HIV activity (Premanathan *et al.,*1999). Because they are abundantly available and edible, *R. apiculata* flower buds are a source of food for those living in Malaysia.

Wound-healing, antioxidant, anti-inflammatory, and cytotoxic effects of *Rhizophora apiculata* are evaluated by silver nanoparticles. silver nanoparticles (AgNPs) have been gaining awareness in the medical community and hence characterize *R. apiculata* silver nanoparticles *R. apiculata* extract was used as a reducing agent to create AgNPs. By using the UV-Vis, SEM-EDX, XRD, FTIR, particle size analyzer, and zeta potential, the resulting AgNPs have been evaluated.*R. apiculata* AgNPs have potential to be cytotoxic, anti-inflammatory, antioxidant, and wound-healing.

Finding naturally occurring chemical components that can be used in food or medicine . Gas chromatography-mass spectrometry (GC-MS) was used to examine the chemical components of leaf, flower, and stem samples. The main compounds in the flower were 2-(ethoxyethoxy)ethanol (11.08%) and butyl cyclohexyl ester 1,2-Benzenedicarboxylic acid (3.48%), but the leaf was discovered to be rich in 2-(2-ethoxyethoxy)ethanol (26.45%)

and Kaur-16-ene (3.37%). Thethree main components in the stem were 1,2,3,4-tetramethoxy-5-(2-propenyl)-benzene (3.26), Kaurene (3.39%), and Octadecamethyl Cyclononasiloxane (5.24).

Flavonoids are identified from *Rhizophora apiculata* leaf extract. Gallic acid, rutin, quercetin, ascorbic acid, and kaempferol were quickly and accurately recognized using a reversed-phase high-performance liquid chromatography (HPLC) method in the leaves extract of mangrove (*Rhizophora apiculata*). Gallic acid, rutin, quercetin, and kaempferol are all detected in mangrove leaves in amounts of 3.024, 5.485, 5.144, and 8.361%, respectively.

Phytosterol isolated from *Rhizophora apiculata* was tested for cytotoxicity against a total of three human cancer cell lines.Literature revealed that The MCF-7, A549, and HeLa cancer cells were used to investigate the cytotoxicity of all phytosterols and their combined combinations. *R. apiculata's* root bark is a significant source of phytosterols, which may provide possible support for the use of traditional South Lampung medicine.

Table 1 *:*Activities of*R.apiculata*

S.NO	NAME OF THE PLANT	ACIVITIES	AUTHOR
1	*Rhizophoraapiculata*	Anti-inflammatory and anti-tumor activity	Prabhu, V. Vinod *et al*
2	*Rhizophora apiculata*	Antimicrobial properties	Seepana *et al*
3	*Rhizophoraapiculata*	Pharmacological Activities	Kalasuba *et al*
4	*Rhizophoraapiculata*	Antioxidant, Anti-Inflammatory, and Cytotoxic Activity	Alsareii *et al*
5	*Rhizophoraapiculata*	Evaluation of immunostimulant activity	Prabhu *et al*
6	*Rhizophoraapiculata*	Antiviral properties	Premanathan *et al*
7	*Rhizophoraapiculata*	Antibacterial Activity	Abeysinghe *et al*
8	*Rhizophoraapiculata*	anticancer activity	Ramalimgam *et al*
9	*Rhizophoraapiculata*	Antioxidant and radical scavenging activities	Loo *et al*
10	*Rhizophoraapiculata*	Assessment of in vivo and in vitro cytotoxic activity	Hong *et al*

3. MATERIALS AND METHODS

Plant material and extraction procedure Fresh bark, leaf, pod and twig of *Rhizophora apiculata* were collected from Gilakaladindi mangrove seacoast ,Machilipatnam and taxonomically identified and voucher specimen is submitted in Krishna University lab.

3.1: Plant material and collection:

The fresh leaves, pod and twig of *Rhizophora apiculata* were properly washed, chopped and then finally air dried for some days. To obtain coarse powder from chopped pieces, grinding process were followed by oven dry for 24 hours at possible low temperature

3.2: Transverse section of Stem

Procedure

Taking Sections

- The dissected plant material is held between index finger and thumb, while keeping the edge, sliced it into thin sections by keeping the razor perpendicular to the longitudinal axis of the stem of *Rhizophora apiculata*
- Using the edge of the blade, shifted these sections into a watch glass containing water with the help of a brush.

Process Of Staining

- Picked up 2 to 4 thin and good transverse sections and shifted into a different watch glass containing safranin stain.
- The complete set are kept rested in the stain for a couple of minutes.
- After a while, the excess strain is washed off by draining the sections on the stain

Mounting

- On a clean slide, stained section is placed in the middle of the slide, mounting water or glycerine.
- The coverslip is slowly placed using a needle.
- An excess of water or glycerine from the edge of the coverslip can be removed by placing a blotting paper.
- Ensured, the air bubbles are not formed while mounting**.**

Precautionary Measures

- While dissecting the section, both the blade and the material should be kept with adequate water.
- While working with sections, a brush is used.
- Gently placed the coverslip in order to avoid air bubbles.
- Excess glycerine can be removed with filter paper.

3.3: Sample preparation:

About l gm of *Rhizophora apiculata* leaf powder was dissolved in 5 ml of different solvents like water, Ethyl acetate, Methanol, chloroform. Then filtered and the crude extract of leaf is extracted. All extracts were then determined for both phytochemical composition and antibacterial screening.

3.4: Agar media preparation

It is frequently used for isolation and purification of cultures. It can also be used as a means for producing the bacterial lawns needed for antibiotic sensitivity tests. In actuality, antibiotic sensitivity testing is typically performed on media.

Composition of Nutrient Agar

- **0.5% Peptone**: It is an enzymatic digest of animal protein. Peptone is the principal source of organic nitrogen for the growing bacteria**.**

- **0.3% beef extract/yeast extract**: It is the water-soluble substances which aid in bacterial growth, such as vitamins, carbohydrates, organic nitrogen compounds and salts**.**

- **1.5% agar:** It is the solidifying agent.

- **0.5% NaCI:** The presence of sodium chloride in nutrient agar maintains a salt concentration in the medium that is similar to the cytoplasm of the microorganisms.

- **Distilled water:** Water is essential for the growth of and reproduction of micro-organisms and also provides the medium through which various nutrients can be transported.

- **pH is adjusted to neutral (7.4) at 25 °C.**

About 28 g of nutrient agar powder is suspended in 1 litre of distilled water. this mixture is heated while stirring to fully dissolve all components. The dissolved mixture is autoclaved at 121° C for 15 minutes. Once the nutrient agar has been autoclaved, allowed it to cool but not solidify. Nutrient agar is poured into each plate and plates are left on the sterile surface until the agar has solidified.

3.5: Preliminary screening of phytochemical test:

To determine the contents of various extracts of *Rhizophora apiculata*, numerous qualitative chemical analyses were carried out using established methodologies.

3.5.1: Anthraquinone test

250 of each extract was heated in a water bath for 2-3 minutes with 10% sulfuric acid, then filtered. And set aside to cool then the filtrate was extracted with chloroform and 3% then ammonia was added. The appearance of a rose pink colour indicates the presence of anthraquinone.

3.5.2: Terpenoid test

Petroleum ether was used to extract 250 of each of the bark, leaf, pod, and twig extracts. Chloroform was added to the filtrate, and then sulfuric acidis concentrated to form a reddish brown at the intersection between the two layers , there is a brown ring showed that terpenoid was present.

3.5.3: Flavonoid test

Small amounts of each extract (0.250 g) were treated with 3 mL of 95 percent ethanol before being treated with a few magnesium metal pieces concentrated HCL is added after it has been filtered. There was an addition of acid. The formation of cherry colour showed the flavonoid was present.

3.5.4: Saponin test

Each extract was combined with water and warmed in a water bath for around 0.250 g. 5 mL distilled water after filtering. Water was added into the mixture was thoroughly mixed. The presence of foam confirmed the existence of saponin

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3.5.5: Phenolic test

A total of 250 bark, leaf, pod, and twig extracts were mixed in water and thawed individually Ferric chloride was added to the mixture after it was filtered. Colours indicated: green, dark blue, or black Phenolic chemicals are present.

3.5.6: Alkaloid test

Each extract was diluted in concentrated sulfuric acid and warmed before being filtered in samples of 250. A few drops of Dragendroff's reagent was added to the filtrate. The formation of an orange yellow precipitate indicated the presence of alkaloids (Mouafi et at, 2014).

Fig : *R.apiculata* leaf extract

3.5.7. Agar well diffusion method

In order to extract the most compounds from the mangrove roots and test them in vitro for antibacterial activity, the aim of this study was to assess the antibacterial activity of toot extracts of medicinal mangrove plants using a variety of solvents, including chloroform, methanol, petroleum ether and sterilized water.

Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts (Magaldi 2004), (taigas 2007). Similarly, to the procedure used in disk-diffusion method, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 cm is punched aseptically with a sterile cork borer or a tip, and a volume (20-100 µL)of the antimicrobial agent or extract solution at desired concentration is introduced into the well .Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested Fusobacterium nucleatum-ATCC 25586, 441 Klebsiella pneumoniae, Streptococcus uberis -NCTC 3858, Staphylococcus aureus -MTCC 9542, 1951.

4.Result and discussion

4.1 Collection of plant material:

The plant material is collected on 21th June 2023 ,from Kara Agraharam, near Gilakaladindi in Machilipatnam seacoast ,Krishna District, Andhra Pradesh.The herbarium specimen is maintained and submitted to Krishna University Botany department.The whole plant is shade dried and powdered, labelled and different extracts are prepared in solvents.

4.1.1.Taxonomical description of Rhizophora apiculata

Systematic position

Kingdom : Planta

Division : Tracheophytes

Sub division : Angiosperms

Class : Eudicots

Sub class : Rosids

Order : Malpighiales

Family : Rhizophoraceae

Genus : *Rhizophora*

Species : *R.apiculata*

Fig: R.apiculata plant

Habit

Rhizophora apiculata is a shrub. On average a mature *R. apiculata* shrub reaches between 5 – 8 metres in height although it has the potential to reach up to 30 – 40 metres

Habitat

R. apiculata is found within the mangrove ecosystem; a unique and complex location known for its humid climate, saline environment, waterlogged soils and capable of tolerating salinity ranging from 2-90%

Distribution

As *R. apiculata* is mostly found on the equator in tropical settings like tropical Asia, Pakistan, Vietnam, Hainan, Malaysia, and Northern Australia, the distribution of mangroves has a close connection with that of the plant.According to *R. apiculata's* physiology, the distribution will be directly related to the environment that favors this plant's desired qualities.

Roots

R. apiculata also has two types of adventitious roots; aerial prop roots and stilt roots. Both types of roots are an adaptation undertaken due to environmental factors, designed to withstand/resist; large waves, rough tides, strong winds and tropical storms. Roots also have two main forces that govern the amount of water uptake potential. These include hydrostatic (which distributes the water taken up by the root to each of its organs) and osmotic force (uses negative water pressure in the roots to suck up water from the soil).

Stem

R. apiculata has a dark grey stem

Leaf

Simple leaf with petiole, oppositely arranged, average leaf size 11.60 cm long and 4.50 cm wide, narrowly elliptic-oblong, apex acute, base cuneate, entire margin with leathery surface, penni-veined but venation barely visible, dark green and presence of black dotted underneath the leaf surface

Inflorescence

2-flowered cymes

Flower in general

Actinomorphic and polypetalous, 4-yellow sepals, 4-white petals, lanceolate shape, hairless surface, 10-12 stamen

Calyx (K)

Calyx-lobes ovate to ovate-oblong, 10-14 mm long, brownish-yellow to reddish, acute, keeled.

Coralla(C)

Lanceolate shape with 4 white petals

Androecium

Stamens mostly 12, 4 adnate to base of petals, 8 adnate to sepals, 6-7.5 mm; anthers nearly sessile, apex apiculate.

Gynoecium

Ovary partly inferior, superior free upper part bluntly conical; style 1 mm long, stigma 2-lobed.

Fruit

Solitary fruit, brown when ripe, pear shape

Fig: *R.apiculata* flower

Fig*: R.apiculata* flower buds

Fig: *R.apiculata* leaf

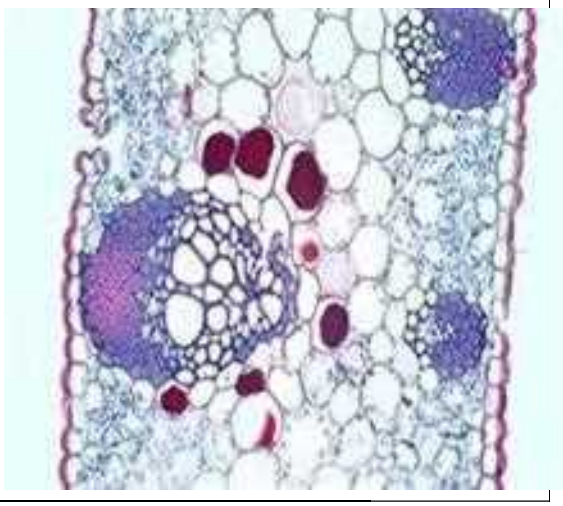

Fig : *R.apiculata* leaf anatomy

Leaf Anatomy

The leaf's morphology is straightforward, leathery, with an abaxial side that has black spots and an elliptic-oblong lamina that is pale green below. glabrous and coriaceous, with an entire edge and a compact base. Dark green above shines clearly. Anatomy of a leaf revealed that the plant's lamina is dorsiventral in nature. These taxa possess extremely thick and smooth cuticles. Completely cutinized epidermis. The outer wall of epidermal cells is typically straight and thicker than the other walls. The shape of the cells is primarily rectangular. The size of adaxial epidermal cells is frequently greater than that of abaxial cells.

Below the epidermal cells, the hypodermis is made up of seven to eight layers of colorless, polygonal-shaped cells. Colorless cells serve as tissue that stores water. The mesophyll in mangroves is made up of chlorenchyma with thin walls. These cells can be divided into one or more layers of anticlinal extended palisade cells that are adaxial, round or oval abaxial isodiametric cells that are compact, and cells that are any combination of the two. Large air holes can be found between the spongy parenchyma cells' haphazardly organized cells. Sclereids with branches that are dispersedly organized in the abaxial area. Hypostomatic in nature, a leaf. It was cyclocytic type and therefore exhibited four or more secondary cells arranged in close rings around the stomata. The cell contains crystalliferous cells.

Root Anatomy

Primary growth stage

The epidermis, which is made up of tiny suberized cells, is present in 3-5 layers of the outermost layer. Small cells are on the inner layer, and long, big cells with thick walls are in the intermediate layer. The cortex is broader than the stele and is made up of two different forms of parenchyma: aerenchyma, which is arranged in a reticulated structure with significant intercellular spaces, and tightly packed cells containing tannin that occur close to the epidermis and stele. At the aerenchyma's joints, tanniferous cells with a round form were discovered.

Secondary growth stage

Periderm gradually takes the role of multiple epidermis. The cortex is made up of two different types of parenchyma: aerenchyma, which is arranged in a reticulated structure with vast intercellular spaces exhibiting in the central cortex, and tightly packed cells with tannin presenting near to the epidermis and stele. Aerenchyma joints contained tanniferous cells with a round form. These are arranged in rows along the length of the root in a longitudinal slice.Cortex had druse crystals and filiform sclereids. Periderm gradually assumes the role of multiple epidermis. The cortex is made up of two different types of parenchyma: aerenchyma, which is arranged in a reticulated structure with vast intercellular spaces exhibiting in the central cortex, and tightly packed cells with tannin presenting near to the epidermis and stele. Aerenchyma joints contained tanniferous cells with a round form. These are arranged in rows throughout the length of the root in a longitudinal section .

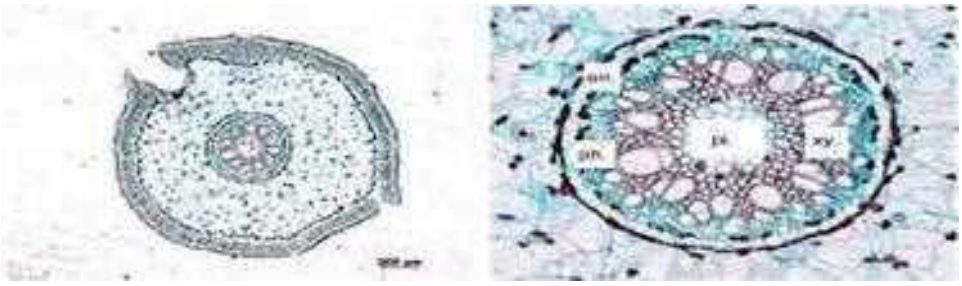

Fig : Root anatomy of *R.apiculata*

Phytochemical analysis of *Rhizophora apiculata* leaf extract

Phytochemical test is an approach method used in determining the presence of secondary metabolite compounds in plants. Classes of secondary metabolites are determined qualitatively using several phytochemical test reagents. The secondary metabolites contained in the extract were detected by the change of color and by the deposition or formation of foam in accordance with the reagents used (Campagna *et al* 2012; Lima *et al* 2011).

The results of phytochemical tests showed that the extracts of *R. apiculata* leaves contain secondary metabolites: saponins, indicated by the presence of foam; tannins, indicated by the change color to dark green or blue; flavonoids, indicated by the change color to reddish

yellow; steroids indicated by the green color of the test and terpenoids by the purple color of the test (Malik *et al* 2017; Asha *et al* 2012)

Table : Phytochemical test results of *R.apiculata* leaf extract

Active compound	**Color indicator**	**Result**
Saponin	**Yellow with foam**	+
Tanin	**Dark green or blue**	+
Flavonoid	**Yellowish red or red**	+
Steroid	**Green**	+
Terpenoid	**Purple**	+

Antioxidant screening of *R.apiculata*

The DPPH radical assay is a suitable model for estimating total antioxidant potential of antioxidants. All samples have antioxidant activity against DPPH and the reducing power increased as the sample concentration increased from 2.09 to 33.34 µg/mL. The butanol fraction (BF) exhibits the highest scavenging activity of 89.3% at the concentration of 33.34 µg/mL, whereas the ethanol extract (EE), ethyl ester fraction (EF) and water fraction (WF) show 77.9%, 79.9% and 67.21% at the same concentration, respectively. IC50 values, defined as the concentration with 50% activity, were calculated for comparison. The IC50 values of scavenging activities for EE, EF, BF, WF and positive control BHT were 13.56 ± 1.79, 19.31 ± 1.56, 9.68 ± 1.86, 23.72 ± 1.94 and 52.20 ± 1.57 µg/mL, respectively. According to these IC50 values, the DPPH radical scavenging ability was found in the order of BF > EF > EE > WF > BHT ($P < 0.05$).

Table : Antioxidant activity of compounds isolated from *R apiculata*

IC50 (µg/mL)			
Compound	**DPPH**	**ABTS•+**	**OH**
Lyoniresinol-3α-O-β-arabinopyranoside	**2.06**	**1.64**	**5.83**
Lyoniresinol-3α-O-β-rhamnoside	**2.64**	**2.09**	**9.07**
Afzelechin-3-rahmnoside	**2.26**	**1.69**	**7.05**
BHT	**55.20**	**9.63**	**45.58**

Fig : Antioxidant screening activity

Fig : Leaf extract of *R.apiculata*

Conclusion

Phytochemical isolation method was used to study the active compounds in *R. apiculata*. The results indicated that *R. apiculata* stem extract/fractions exhibit excellent radical scavenging ability in all assays employed and BF was the most active fraction among them. Phytochemical investigation of the BF led to the separation of compounds which were separated from *R. apiculata* for the first time. Radical scavenging assays indicated all compounds had stronger antioxidant capacity than the positive control BHT. HPLC analysis results showed that among different plant parts, the highest content was located in the bark. Overall, *R. apiculata* is a promising source of natural antioxidants and lyoniresinol-3α-O-β-arabinopyranoside; lyoniresinol-3α-O-β-rhamnoside; afzelechin-3-rahmnoside are antioxidant ingredients in *R. apiculata.*

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