Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

"In Vitro Bioactivity and Phytochemical Profiling of Leaf Extracts from the Mangrove Plant *Excoecaria agallocha*"

Dr Shilaja Yougender Nayini[1], Deepthimahanthi Divya[2], Dr. Govindarao Kuna[3], Dr S.Narsaiah[4]

[1] Associate Professor, Department of Zoology, Sarojini Naidu Vanita Maha Vidyalaya, Hyderabad, Telangana, India

[2]Associate Professor, Department of Zoology, St.Ann's College for Women, Hyderabad, Telangana, India

[3]District Program Manager, PMMSY, Kakinada District,Andhra Pradesh,India

[4]Associate Professor, Department of Zoology, Govt Degree college Hayathnagar, Rangareddy(Dist),Telangana, India

*** Shilajayougendernayini@gmail.com**

Cite this paper as: Dr Shilaja Yougender Nayini, Deepthimahanthi Divya, Dr. Govindarao Kuna, Dr S.Narsaiah (2024) "In Vitro Bioactivity and Phytochemical Profiling of Leaf Extracts from the Mangrove Plant Excoecaria agallocha"". *Frontiers in Health Informatics,* (8), 5677-5692

Abstract

The most prevailing microbial diseases and antibiotic resistance exhibited by many pathogens has prompted to discover new antimicrobials of plant origin owing to their minimal side effects. Recent widespread interest in mangrove ecosystem, reflects its validity regarding the potential medicinal application in various diseases. Considering the biomedical importance of mangroves, the present study evaluates the phytochemical screening, antimicrobial, antioxidant activities and characterization using TLC and LC-MS of leaf fractionated extracts of *Excoecaria agallocha (Euphorbiaceae),* a small milky mangrove extensively studied for its chemical composition and many bioactivities. Initial crude extract preparation of leaves was carried out using four dissimilar solvents viz., acetone, diethyl ether, petroleum ether and water to study the phytochemical constituents which revealed the bearing of saponins, flavonoids, glycosides, steroids, phytosterols and reducing sugars particularly with relatively high abundance in petroleum ether extract. The antimicrobial activity was evaluated by means of zone of inhibition using agar well diffusion assay. Among the tested bacterial and fungal species, the maximum zone of inhibition was recorded in fungal species ranging from 5 to 12 mm compared to bacterial species ranging from 0.2 to 0.7mm, indicating compounds to be better antifungal agents. Also the performed antioxidant activity of petroleum ether extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) recorded total free radical scavenging capacity with IC_{50} value of 247.92 µg/ml and with ferric reducing antioxidant potential (FRAP) method showed higher activity of 47.66 ug/ml. Further partial characterization of the petroleum ether extracts was carried out by thin layer chromatography and LC-MS analysis indicated the presence of bioactive compounds in the extracts of the *Excoecaria agallocha* species.

Keywords: *Excoecaria agallocha* ,Phytochemicals,Bioactive compounds,Antibacterial activity

Introduction

Fish are an integral component in the human diet. They are a staple in various ethnic groups and regions around the world, have unique nutritional properties, and have higher production efficiency compared to other animal production systems; therefore, they deserve more attention in food policies than it currently receives (FAO, 2016). Fish are susceptible to several infections, that aid in the development of an active fish disease which is directly associated to the effect of different pathogenic microorganism mainly when reared in high densities conditions. These disease outbreaks are responsible for elevated mortality rates and decrease of the productivity efficiency, causing high economic losses to the fish farmers. In aquaculture, up to 50% of production loss is caused by diseases (Assefa & Abunna 2018). A significant increase in the use of antimicrobial drugs for the control and treatment of disease-related issues in fish farming improves the emergence and occurrence of the resistance phenomenon. As an alternative to conventional antimicrobial drugs, medicinal plants have been widely used in veterinary and human medicine. Nowadays, medicinal plants have a significant role in aquaculture as prophylactic and therapeutic agents against fish pathogens presenting with antiviral, antibacterial, antifungal, and anti-parasitic properties(Reverter, M.,et al,2017). Although the efficacy of plant-based medicines against fish pathogenic microbes has been investigated and are currently playing a major role in the treatment of disease because of their efficacy, minimally observed side effects and cost effectiveness. Several studies showed that Boesenbergia pandurata, Zingiber zerumbet and Solanum ferox have the ability to suppress the Aeromonas hydrophila and Pseudomonas sp. bacteria growth (Hardi et al 2016a; Hardi et al 2016b). Also a study indicates that enriched fish feed with Sonneratia alba leaf extracts against Aphanomyces invadans, a oomycete fungus causing epizootic ulcerative syndrome (EUS) enhanced the non-specific immunity and survival rate of the goldfish, suggesting that the extract may serve as a potential prophylactic treatment against this disease (Afzali, S.F. and Wong, W.L., 2019). The plant *Rheum palmatum* derived products rhein and aloe-emodin, are studied for the prevention and treatment efficacy on *Saprolegnia* infection of grass carp and found that rhein has promising anti-*Saprolegnia* activity and may be an option in preventing and controlling *Saprolegnia* infection *(*Yao, J.-Y,2017). Thus, there is an increasing interest in the scientific and modern medicine world and a simultaneous intensifying effort towards the evaluation of valuable medicinal plants.

Among the various medicinal plants that have been exploited in various herbal folklores, mangrove species gained importance and are used as an alternative base in finding the novel compounds to treat various diseases around the world. These plants have been used because of their therapeutic traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant. The secondary metabolites produced by these plants present an alternative route to address the ever increasing need for new drugs, because of their low production costs, novelty and structural diversity(Vinoth R et al,2109; Nabeelah Bibi, Sadeer, et al.2019). Many secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangrove plants which have toxicological, pharmacological and ecological importance. Extracts from mangroves and mangrove-associated species have proven to possess therapeutic activities against humans, animals, and plant pathogens. One mangrove species, *Excoecaria agallocha* (Euphorbiaceae) has been widely reported as a

traditional remedy for several diseases. *Excoecaria agallocha*, an angiosperm, commonly known as milky mangrove or blind your eyes mangrove, grows as small trees and widely distributed throughout Asia, Africa and northwest Australia (Kaliamurthi and Selvaraj, 2016; Mondal S et al,2016). It also known as a back mangrove, is found at higher elevations back away from the ocean where salinity is lower(Durgadevi. S.etal,2020). The milky latex discharged from Excoecaria agallocha bark is poisonous and may cause temporary blindness and blistering of the skin (Chan et al.2019). The latex is also well known for its biocidal effects on marine organisms and phytoplankton, causes metabolic depression of the rice field crab, Oziotelphusa senex and is used as an uterotonic, fish poison, dart poison, and contains novel chalcones and piperidine alkaloids (Mondal S et al,2016).The bark oil has been found effective against rheumatism, leprosy and paralysis. This plant also has been traditionally used to treat sores and stings from marine creatures, and ulcers, as a purgative and an emetic. Recently, studies on different parts of this mangrove reported various biological activities like anti-oxidant (Sofia S et.al,2016; Laith AA etal,2016), anticancer (RajeswaryHarietal.2019; Bhuvaneswari et al. 2017), antimicrobial (Laith AA etal,2016), anti-convulsant(M.K. Shelar et al.2018) anti-larvicidal activity ((Mendhulkar et al., 2017) and numerous other studies of applicability of its extracts or phytocompounds.

With this background and considering the biomedical importance of mangroves, the present study was conducted with an objective to carry out a phytochemical analysis of the various solvents acetone, diethyl ether, petroleum ether and water extracts of *Excoecaria agallocha* dried leaf and the petroleum ether leaf extracts are used to determine the antimicrobial and antioxidant activity. Further, the TLC and LCMS profile of the petroleulm ether fractions are also analysed.

Materials and methods

Plant material

Excoecaria agallocha plant leaves were collected from the Gilakaladindi mangrove fields located in Machilipatnam (AP, India). The plant was identified and authenticated at Botanical Survey of India. The leaves of the plant were cleaned and dried for 15 days and then pulverized into fine powder using pestle and mortar. The powdered plant material was stored in polythene air tight containers at room temperature for further use.

Extraction procedure

The fine powdered leaves of *Excoecaria agallocha* was added to a soxhlet apparatus for sample extraction along with solvent. Solvent fractionation in organic solvents are on the basis of increasing order of polarity. The aqueous extraction and extraction with organic solvents acetone, diethyl ether and petroleum ether was carried out for a period of 24 hours at 60ºC. At the end of the extraction, the extracts were filtered using Whatman No. 1 filter paper and are concentrated at 40°C under reduced pressure using a rotary evaporator and the resultant green color residue considered as the crude extracts were stored in refrigerator for use in subsequent experiments.

Phytochemical Characterization

Phytochemical screening was carried out for all the four leaf extracts of *Excoecaria agallocha* for alkaloids, saponins, glycosides, fats, phenols, tannins, flavonoids, terpenoids, steroids, phytosterols, anthraquinones, cardiac glycosides reducing sugars and proteins according to standard methods.In general, test for the presence or absence of phytochemical compounds using standard methods involves the addition of an appropriate chemical agent to all the extracts in a test tube and shaken. The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition.

Test for Tannins: A small portion of the extract was diluted with 20 ml of distilled water and boiled in a boiling tube. Then few drops of 0.1% ferric chloride was added. The appearance of brownish green or blue-black colour indicates the presence of tannins.

Test for Saponins: One mL of the extract was diluted with 20 ml of distilled water and shaken vigorously. The formation of stable foam indicates the presence of saponins.

Test for Flavonoids: About 1 ml of the extract was mixed with few fragments of magnesium ribbon and concentrated hydrochloric acid. The appearance of pink or magenta-red colour indicates the presence of flavonoids.

Test for Phenols: A small portion of the extract was mixed with 2 ml of ferric chloride solution. The appearance of green or blue colour indicates the presence of tannins.

Test for Alkaloids: Two ml of the extract was mixed with 0.2 ml of 1% HCl. Then 1 ml of Mayers' reagent was added. Any precipitate or turbidity indicates the presence of alkaloids.

Test for Steroids: A small portion of the extract 2 ml of sulphuric acid was added by the sides of the test tube. The appearance of bluish-green or violet colour indicates the presence of steroids.

Test for Terpenoids: A small portion of the extract wasmixed with 2 ml of chloroform. Then 3 mL of sulphuric acid was carefully added. The appearance of reddish brown or pinkish brown ring/colour indicates the presence of terpenoids.

Test for Glycosides: A small portion of the extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of ferric chloride solution. The mixture was then poured into another test tube containing 2 ml of concentrated sulphuric acid. The appearance of brown ring indicates the presence of glycosides.

Test for anthraquinones: About 5 ml of the extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. This extract was further extracted with strong ammonia. A pink or deep red coloration of the aqueous layer indicates the presence of anthroquinones.

Reducing sugar-fehling's test: Few drops of Fehling's solution A and B in equal volume were added in dilute extracts and heated for 30 min and observed for the formation of brick red colored precipitate.

Bioactivity Evaluation

Microbial Strains:

The four bacterial and two fungal microorganisms are used in the study. The bacterial pathogens are *Klebsiella pneumonia* (MTCC 3384), *Pseudomonas aureginosa*, (MTCC 1688) *Staphylococcus*

aureus (MTCC737) and *Bacillus thuringiensis* (MTCC 1953). The fungal pathogens are *Rhizopus, Mucor, Penecillium* and *Puccine.* The bacteria were maintained on Nutrient agar medium and fungi were maintained on Potato Dextrose agar (PDA) medium. A loopful of bacterial (12 hrs grown) and fungal (36 h grown) culture were sub cultured on Muller Hinton Agar (MHA) and PDA respectively. Colonies of the pure organism were cultured in 10ml broth medium and incubated at 37°C overnight.

In Vitro Antibacterial Activity

The Antibacterail activity of petroleum ether extract of *E.agallocha* is carried out using the agar well diffusion method. For antibacterial susceptibility tests, MHA media is used for bacterial species growth. The medium was prepared separately by pouring 20 ml of molten media into sterile Petri plates. The plates were allowed to solidify and 100µl of an overnight broth culture of test bacterial strains are swabbed uniformly on the medium and allowed to dry for 5 min. For agar well diffusion method, the 6mm diameter equidistant wells were cut from the agar with the help of a cork-borer. 40 µl of petroleum ether extracts containing 4 mg concentration was loaded on wells. The standard antibiotic disc Amikacin was used as a positive reference to determine the sensitivity of one strain/isolate in each microbial species tested. The plates were kept for incubation for 24 hrs at 37°C to find their antibacterial efficacy. Antibacterial activity was evaluated by measuring the diameter of zone of inhibition around the well containing samples and standard.

Invitro antifungal activity

The antifungal activity of the petroleum ether extract of *E.agallocha* is carried out using the agar well diffusion method. For antibacterial susceptibility tests, PDA media is used for fungal species growth. PDA were prepared. With sterile cotton swabs 36h culture of test fungal strains were seeded individually over the surface of PDA plates. Well of 6mm diameter was made in the centre of the agar plate with a sterile cork borer. 40 µl of petroleum ether extracts containing 4 mg concentration was loaded on wells. The standard antibiotic disc streptomycin was used as a positive reference to determine the sensitivity of one strain/isolate in each fungal species tested. Plates were then incubated for 72 h at 28°C. Antifungal activity of the extracts was determined by measuring the diameter of the inhibition zone around the well.

In Vitro Antioxidant Activity

The antioxidant activity of the crude extracts of E. agallocha was evaluated using following assays such as Scavenging of DPPH radical and Evaluation of the reducing power FRAP assay

DPPH Radical Scavenging Activity

The percentage antioxidant activity (AA%) of the petroleum ether leaf extract was obtained using the DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical absorbance assay. The DPPH is a stable free radical and widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H. This transformation results in a colour change from purple to yellow, which is measured spectrophotometrically at 517 nm. The reaction mixture was made to 5ml containing DPPH solution at different concentrations of extact (31.25, 62.5, 125, 250, 500 μg/mL). The reaction mixture are incubated for 30 min in the dark, and then the absorbance was measured at 517 nm against ascorbic acid as standard, and Methanol as a blank using UV-Vis

spectrophotometer. The DPPH radical scavenging activity was calculated by the following equation;
% DPPH radical scavenging activity= (Ao-A1)/Aox100%
Where Ao is the absorbance of the control reaction and A1 is the absorbance of the sample of the tested extracts.
Each experiment was carried out twice and the results are expressed as mean % antiradical activity. The inhibitory concentration IC_{50} was calculated by interpolation from linear regression analysis. Percentage of free radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC50 value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay solution. IC50 values are inversely proportional to the antioxidant potential.

Ferric reducing antioxidant power (FRAP) assay:
FRAP was used to measure the direct electron donating ability of extract. FRAP assay was performed according to the method of Benzie and Strain (1999). FRAP reagent was prepared in acetate buffer (300mM) by adding 10mM 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) solution in 40mM HCl and 20mM FeCl3 solution in proportion of 10:1:1 (v/v), respectively. The mixture was incubated for 15 min at 37 °C before use. A standard curve was prepared by using curve was obtained by using its concentrations ranging from15.62ul/ml to 500ul/ml in methanol. The sample at different concentrations 15.62, 31.25, 62.5, 125, 250 and 500ul/ml or standard ascorbic acid are added to FRAP reagent. The mixture was incubated for 30 mins in the dark, and its absorbance was measured at 593 nm. The results were recorded as µg of ascorbic acid equivalents (AAE) per ml.

Chromatographic characterization
Thin Layer Chromatography(TLC):
TLC is the method mainly uses to investigate the presence of chemical constituent qualitatively in the plant extract. It is used to investigate alkaloids, glycosides, triterpenoids, steroids, saponins, phenolic compounds etc. It is an easy, versatile and reliable method to establish authenticity, identity and purity. The petroleum ether extract of *Excoecaria agallocha* was subjected to TLC studies, to find the presence of number of compounds which support by the chemical test. TLC of *Excoecaria agallocha* was performed in the solvent solvent system comprising Butanol: Acetone: Water(12:6:3). The TLC plates (precoated TLC plates Silica Gel G) were trimmed to strips and the position of the origin marked by a straight line. The plant petroleum ether extract was spotted on the origin with 100 µl syringe on pre coated silica gel TLC plates (10 × 10 cm) with band length of 8 mm and track separation of 12 mm using Linomat V applicating device. and put in a lidded tank containing a solvent system. The procedure was followed until good resolution was noticed. The level of solvent in the tank was about 1 cm beneath the origin. The solvent travelled up the plate by capillary action till it reached the solvent front (also marked by a straight line across). The lid was lifted off and the strip dried before it was viewed by spraying with silver nitrate and iodine vapour and visualized under UV light. The retention factor (Rf) values of all the spots were determined by the following formula:
Retention factor = Distance traveled by the plant extract/distance traveled by the solvent system.

Preparative TLC

For preparative thin layer chromatography, slurry of 30 g of silica gel in 60 ml of distilled water was applied to a hundred glass plates totally (20.3 cm square), with a thin- layer spreader (Research Specialties Co.,) producing a gel layer of 250 - micron thickness. The plates were allowed to stand for 10 minutes at room temperature for 1 hour at 105°C in hot air oven and then in a desiccator for 2 hours. Later petroleum ether extract of leaves was spotted on the plates (10 µl each spot). Formation of the bands was observed under the UV light.

Results and discussion

Phytochemical characterization

The powdered material of *Excoecaria agallocha* was extracted sequentially with acetone, diethyl ether,petroleum ether and water. All the four extracts of *Excoecaria agallocha* leaves were examined for the presence of phytoconstituents. Results from this study indicated a variation in phytochemicals compounds in the four extracts of leaves of *Excoecaria agallocha.* The results are tabulated in Table 1. The results revealed that flavonoids, steroids, phenols, cardiac glycosides reducing sugars presence in the acetone extracted fractions of leaves, only steroids in diethylether extracts, saponins, flavonoids, glycosides, steroids, phytosterols and reducing sugars in petroleum ether extracts and saponins, glycosides, phenols, phytosterols and reducing sugars in aqueous extracts. There were high amounts of phytochemicals present in the petroleum ether extract of *Excoecaria agallocha* and found to be the most effective solvent for extracting compounds in the present study as compared to all other extracts, with diethyl ether showing very poor extractability of these compounds and fairly by acetone and water.

A study by Mondal et al. 2016, revealed a wide array of phytoconstituents isolated from *E. agallocha* which include flavonoids, glycosides and Alkaloids. In previous studies it was reported that the aqueous leaf extract of C. erectus L. contained several bioactive leads such as coumarins, tannins, saponins, triterpenes, alkaloids and flavonoids (Nascimento et al. 2016).

The methanol leaf extracts from Suaeda maritima have phytochemicals namely Saponins, terpenoids, tannins, alkaloids, steroids and are reported for antimicrobial and scavenging activity (Santhi and Sengottuvel, 2016). The mangrove species: Avicennia schaueriana, Laguncularia racemosa and Rhizophora mangle leaf extracts shown the presence of alkaloids, coumarins, saponins, steroids, tannins and triterpenoids (Mendes et al. 2018). The mangrove species Sonneratia alba leaf extract has shown the presence of several bioactive leads such as flavonoids,
steroids, triterpenoids, alkaloids, saponin and tannins (Syafitri et al. 2017).

Table 1: Phytochemical Screening and analysis of *Excoecaria agallocha* leaves extracts

Name of test	ACETONE	DIETHYLETHER	PETROLEUM ETHER	WATER
TANNINS	Negative	Negative	negative	negative
SAPONINS		Negative	**positive**	**positive**
FLAVONOIDS	**Positive**	Negative	**positive**	negative
GLYCOSIDES	Negative	Negative	**positive**	**positive**
TERPENOIDS	Negative	negative	negative	negative
STEROIDS	**Positive**	**positive**	**positive**	negative
PHENOLS	**Positive**	negative	negative	**positive**
PROTEINS	Negative	negative	negative	negative
PHYTOSTEROLS	Negative	negative	**positive**	**positive**
ANTHRAQUINONES	Negative	negative	negative	negative
CARDIAC GLYCOSIDES	**Positive**	negative	negative	negative
REDUCING SUGARS	Positive	negative	positive	positive

Invitro Antibacterial activity

The antibacterial activity of the petroleum ether leaf extracts of *Excoecaria agallocha* was assayed *in vitro* by agar well diffusion method against four bacterial (*Staphylococus aureus, Bacillus thuringiensis, Klebsiella pneumonia, Pseudomonas aeruginosa* which exhibited a low activity. Table 2 shows the results on antibacterial activity of petroleum ether extract of E.agallocha. The petroleum ether extract of E. agallocha exhibited in vitro antibacterial activity against the tested strains with the zones of inhibition ranging from 0.2 to 0.7 mm (table 2). Among the tested bacterial strains, the highest bacterial inhibition zone was observed against *Bacillus thuringenesis* (0.7mm), followed by *Staphylococcus aureus* (0.4 mm), *Klebsiella pneumoniae* (0.3 mm) and least inhibition zone was noted *Pseudomonas aeruginosa* (0.2 mm).

A study on antimicrobial analysis against Micrococcus luteus, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsella pneumonia and Bacillus subtilis of selected mangrove species, Suaeda nudiflora, Lumnitzera racemosa, Ipomoea tuba and Avicennia alba extracts with different solvents was performed by G. Eswaraiah et al.,2020. The methanolic leaf extract from the selected mangroves showed highest antimicrobial activity in comparison with other solvent extracts and the highest the zone of inhibition found on Staphylococcus aureus from the leaf extract of Suaeda nudiflora. The order of antimicrobial activity expressed as inhibitory zones of methanol > acetone > hexane observed for pathogenic strains.

Alizadeh Behbahani et al. (2018) , evaluated the antimicrobial effects against Klebsiella pneumoniae, Streptococcus pneumoniae and Enterococcus faecium of different combinations of four solvents (water, ethanol, methanol and glycerin) extracted using response surface method with mixture optimal design. Their results showed that Enterococcus faecium had the highest
antimicrobial effect and highest resistance against mangrove leaf extract in Klebsiella pneumoniae, respectively. They assigned the difference of resistance may have been because of the structure of the cell wall in the gram positive and negative bacteria.

The antibacterial effect of various extracts of root, stem and leaves from E. agallocha extracted in different organic solvents (petroleum ether, chloroform, ethyl acetate and n-butanol, in the increasing order of polarity) was analysed against six bacterial strains and it was found that all the extracts showed antibacterial effect in varying degrees. The differences observed in the inhibition of test organisms could be due to the presence of various compounds present in the extract and also the morphology of the test organisms (Deepa and Padmaja, 2017).

In previous studies reported that leaf extract of Avicennia officinalis showed higher degree of inhibitory zones against other bacterial strains (Thatoi et al., 2016).

The crude extract of Suaeda nudiflora with different solvents like hexane, ethyle acetate, methanol and chloroform showed higher degree of inhibitory zones against selected human pathogenic bacterial strains (Nayak et al., 2018).

The methanol leaf extract of marine mangrove plant Avicennia marina showed the highest antimicrobial activity against pseudomonas aeruginosa (Thamizharasan and Anbusaravanan, 2016).

Table 2: Antibacterial activity of petroleum ether extract of E.agallocha

Type of plant extract	**organism**	**of inhibition (mm)**
Petroleum extract of E.agallocha	*lus thuringenesis*	0.7mm
	ylococcus aureus	0.4mm
	iella pneumoniae	0.3mm
	lomonas aeruginosa	0.2mm

Invitro antifungal activity

The antifungal activity of the petroleum ether leaf extracts of *Excoecaria agallocha* was assayed *in vitro* by agar well diffusion method against the four fungal strains (Rhizopus, Mucor, Penecillium and Puccinea) which showed significant results than the anti bacterial activity. The results on antifungal activity of are shown in Table 3. The in vitro antifungal activity against the tested fungal strains exhibited the zones of inhibition ranging from 5 to 12 mm. Among the tested fungal strains, the highest fungal inhibition zone was observed against *Penecillium* (12 mm), followed by *Rhizopus* (8 mm), *mucor* (7 mm) and least inhibition zone was noted *Puccinea* (5 mm).

Results obtained from the present study evidently substantiated that, the petroleum ether extracts

had significantly inhibited the growth of fungal pathogens with varying level of inhibitory zones. A study on antifungal activity of E.agallocha of Ethyl acetate, Hexane and Methanol extacts of which highest zone of inhibition noted in Ethyl acetate extracts against Aspergillus niger (2.1 ± 0.12 at 50µl concentration) and least also recorded from methanol extract against Aspergillus niger (0.8 ± 0.04 at 50µl concentration), Aspergillus flavus in methanol. Hexane extracts were resistant and methanol of Aspergillus fumigatus also resistant because there were no zones of inhibition observed(Ella. Sai Kumar,2020).

Alizadeh Behbahani B et al,2016, studied the antifungal activity of ethanolic and aqueous extracts of A. marina on Penicillium digitatum and Alternaria citri. In this study, ethanolic extract compared to the aqueous extract was more effective and has a greater inhibition effect in 2 mg/mL, on the growth of P. digitatum and A. citri.

A study on Anti-fungal activities of extracts of some species of Mangrove plants towards some selected strains of which methanol extract of E. agallocha is effective towards the strains of C.albicans, C.neoformans and C.glabrata (Karnati Rajeswari et al,2016).

Antifungal potential of the leaf, root and bark extracts of mangrove plant *Bruguiera gymnorrhiza* with combination of organic solvents against Candida albicans, Aspergillus niger and A. fumigatus was studied by S. Acharya, et al.. (2020). Results of antifungal activity revealed the
highest toxicity to Candida albicans, having the lowest MICs (9.04–10.87 µg/ml) and Aspergillus fumigatus was the next susceptible strain having MIC 11.77–33.43 µg/ml, while Aspergillus niger was found to be the most resistant one to the extracts of B. gymnorrhiza showing MIC in the range of 306.1–3292 µg/ml.
A study by Somayeh Rastegar et al . (2017) aimed at evaluating the antifungal properties of ethanol and water extracts of leaves of Rhizophora Mucronata and Avicennia Marina mangrove plant species against five postharvest pathogenic fungal. Results showed that the ethanol extracts
of both species had antifungal activities and none of the water extracts showed antimicrobial activity on the studied fungi.

Table 3: Antifungal activity of petroleum extract of E.agallocha

Type of plant extract	**Test organism**	**Zone of inhibition (mm)**
Petroleum extract of E.agallocha	*Rhizopus*	8mm
	Mucor	7mm
	Penecillium	12mm
	Puccinea	5mm

Invitro Antioxidant activity

Antioxidant activity of petroleum ether extract of E.agallocha was analyzed using two different methods viz., DPPH radical scavenging activity and FRAP assay. The DPPH radical scavenging activity was determined on the basis of concentration providing 50% inhibition. The results obtained for antioxidant activity using DPPH are show in Table 4. The results revealed significant free radical scavenging activity of petroleum ether extract of E.agallocha on DPPH with IC50 value of 247.92 µg/ml. The positive control ascorbic acid showed the IC50 values of 6 µg/ml. The scavenging activity increased with increase in concentration. Figure 1. shows result of the petroleum ether extract exhibited the highest radical scavenging activity with 76.038 % at 500 µg/ml. According to these results, it was concluded that plant extracts from E.agallocha have potent antioxidant activity.

In previous studies it was reported the ethanol leaf extract of Rhizophora mucronata has shown antioxidant activity . The free radical scavenging activity of 127.5 µg/mL was reported by DPPH assay method (Ray et al. 2016).

In a previous study, the antioxidant ability of Methanolic extracts of selected mangrove plans i.e., Aegiceras corniculatum, Excoecaria agallocha and Lumnitzera racemose using DPPH are evaluated. Among the three mangrove species and standard, tested for in vitro antioxidant activity, the crude Methanolic extracts of Aegiceras corniculatum, Excoecaria agallocha and Lumnitzera racemosa with inhibition percentage of 75.13 ± 0.52, 41.43 ± 0.34 and 79.1 ± 0.62 (at 1 mg concentration) respectively. The Lumnitzera racemosa extract showed highest antioxidant activity and Excoecaria agallocha extract showed lowest antioxidant activity(Reddy ARK, Grace JR (2016).

A study Sofia and Teresa, 2016 determined DPPH activity in in leaf, stem and root samples of *E. agallocha*, L. Leaf showed maximum (IC_{50}-141.56µg/ml) antioxidant property in methanolic extract and stem showed minimum (IC_{50}-931.3µg/ml). The range of DPPH radical scavenging activity in the solvent is Methanol > Ethyl acetate > Ethanol > Chloroform.

In previous studies, antioxidant activities for different plant parts of A. laurina as determined by DPPH assay using the different extraction solvents are studied. The results revealed that ethanol extracts of A. laurina have the highest levels of DPPH from leaves as 76333.5 µmol TE/100 g, followed by stem bark extract with 65262.3 µmol TE/100 g. These results suggest that ethanol was found to be the most efficient solvent to extract DPPH from different parts of A. laurina (Gbago Onivogui et.al,2017)

The methanol leaf extract of Avicennia marina having phenolic compound namely gallic acid was reported for high antioxidant activity using DPPH assay
(Molaee et al. 2017)

Table 4: Results of the DPPH radical scavenging activity of the petroleum ether extracts of E.agallocha leaves.

Concentration (ul)	Abs Reading 1	Abs Reading 2	Mean Abs	Mean Abs (Sample-Blank)	% Inhibition
Blank	0.05	1.567	0.065.5		
Control	0.08	1.572	1.5695	1.5045	0
Ascorbic acid	0.816	0.189	0.8175	0.7525	49.9833
31.25	1.551	1.548	1.5495	1.4845	1.329
62.5	1.105	1.109	1.107	1.042	30.7411
125	0.934	0.938	0.936	0.871	42.107
250	0.606	0.603	0.6045	0.5395	64.1409
500	0.422	0.429	0.4255	0.3605	76.0385
IC50	**247.92(µg/mL)**				

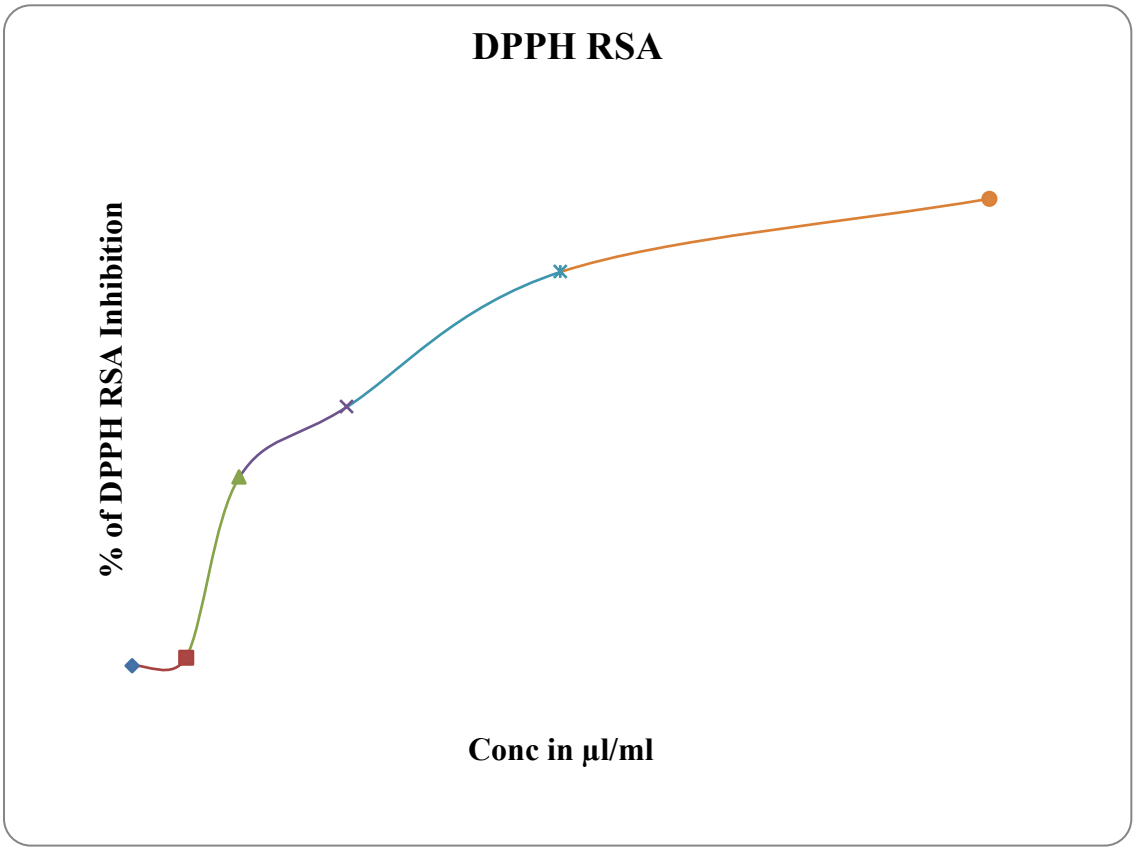

Figure. 1: DPPH free radical scavenging activity of crude petroleum ether extracts of E.agallocha

The ability of antioxidants to reduce Fe3+ to Fe2+ in the presence of TPTZ, was determined in FRAP assay. Reducing potential of crude petroleum ether extracts of E.agallocha was expressed in AAE µg/ml. The results obtained for antioxidant activity using FRAP assay are show in Table 5 . The

reducing power gradually increased with increase in concentration. Strongest antioxidant activity was observed at the the highest test concentration (250 µg/mL) with an AAE value of 47.66 µg/mL as shown in figure 2. From the results, the petroleum ether extract of E.agallocha exhibited moderate reducing activity.

In a study by Reddy ARK, Grace JR (2016), it was found that the reducing power of all studied mangrove plants(Aegiceras corniculatum, Excoecaria agallocha and Lumnitzera racemose) increased with increase in their concentrations. Among these three plant extracts, the reducing power was found to be highest in Lumnitzera racemosa which was significantly followed by Excoecaria agallocha and Aegiceras corniculatum extracts.

In a study by G. Onivoguiet.al 2017, the antioxidant activity of leaves and stem bark of A. laurina are examined and found that the highest levels of antioxidant activities evaluated by the FRAP assay were observed in ethanol extract of stem bark (36060.9 µmol TE/100 g). The results of FRAP showed good variation and strong antioxidant activity in different extracts, and the ranges were from 19146.6 to 32375.2 µmol TE/100 g in leaves and 10146.6 to 36060.9 µmol TE/100 g in stem bark.
The antioxidant activity plant extracts of Brugeiera Gymnorrhiza and Aegialitis Rotundifolia were also measured by FRAP assay in a study.

Table 5: results of the FRAP assay of petroleum ether extracts of E.agallocha.

Concentration (ul)	**Abs Reading 1**	**Abs Reading 2**	**Mean Abs**	**Mean Abs (Sample-Blank)**	**AAE (ug/ml)**
Blank	0.042	0.048	0.045		
Control	0.115	0.12	0.1175	0.0725	0
15.62	0.136	0.139	0.1375	0.0925	2.833333333
31.25	0.159	0.163	0.161	0.116	10.66666667
62.5	0.224	0.228	0.226	0.181	32.33333333
125	0.256	0.247	0.2515	0.2065	40.83333333
250	0.275	0.269	0.272	0.227	47.66666667

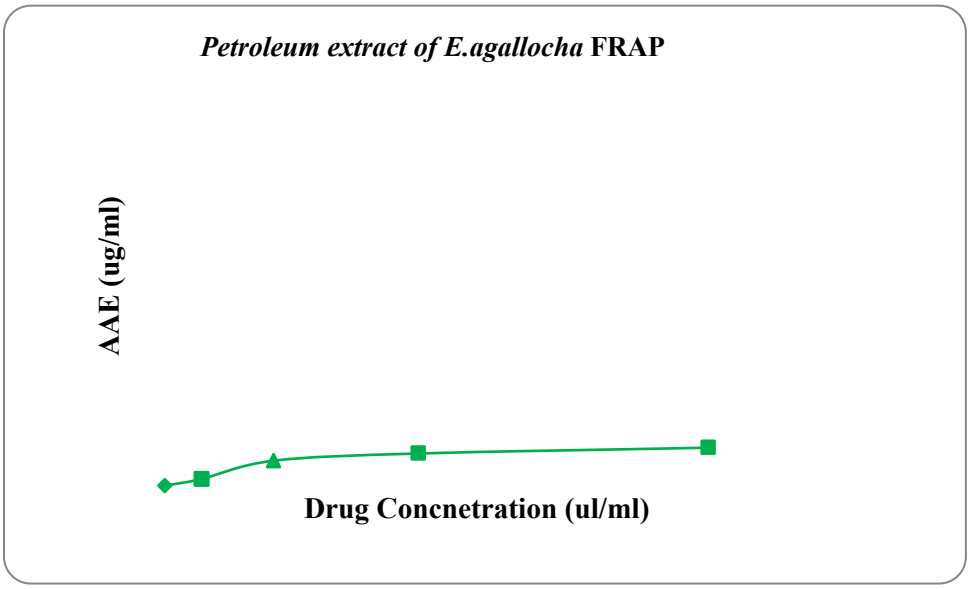

Figure 2: Reducing power activities of the crude petroleum ether extracts of E.agallocha.

Chromatographic characterization

Thin-layer chromatography is particularly valuable for the qualitative determination of small amounts of constituents. Thin Layer Chromatography was used to detect spots from different solvent extracts. TLC studies of petroleum ether extract of *Excoecaria agallocha* was performed in Butanol: Acetone: Water solvent system. The sample spots of the extract showed colored spot on the solvent system. The Rf value was found to be 0.70.

Figure 3: TLC of petroleum ether extracts of leaf of E. agallocha.

A study on thin layer chromatography (TLC) analysis revealed a great potential for E. agallocha that appeared in the form of the many bands on the TLC-plates. Combinations of ethyl acetate and methanol

solvents were used to carry out the TLC. All pure solvents separated into seven bands and TLC chromatograms of plant extracts showed the presence of phenolic compounds, such as tannins and flavonoids(Laith A. A and Mazlan A. G, 2016)

G.Eswaraiah,(2019) performed TLC studies for alkaloids, tannins, phenols, emodins, terpenoids etc. The colour spots represent presence of bioactive compounds and pale yellow spots on TCL plates was indicative for antioxidants from mangrove species. The report of TLC indicates presence of Alkaloids and flavonoids.

References:

Afzali, S. F., & Wong, W. L. (2019). Effects of dietary supplementation of *Sonneratia alba* extract on immune protection and disease resistance in goldfish against *Aphanomyces invadans*. *Tropical Biomedicine*, 36(1), 274–288.

Assefa, A., & Abunna, F. (2018). Maintenance of fish health in aquaculture: Review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary Medicine International*, 2018. https://doi.org/10.1155/2018/5432497

Durgadevi, S., Vinodhini, K., Santhanakrishnan, T., Elamparithi, D., Moorthy, V., & Nagaraja Suryadevara. (2020). Isolation and characterization of biopotential antimicrobial compounds from mangrove plant species (*Excoecaria agallocha*). *International Journal of Engineering Research & Technology (IJERT)*, 9(3).

Food and Agriculture Organization. (2016). *State of world fisheries and aquaculture 2016 (French)*. Food & Agriculture Organization.

Hardi, E. H., Kusuma, I. W., Suwinarti, W., Agustina, A., Abbas, I., & Nugroho, R. A. (2016a). Antibacterial activities of some Borneo plant extracts against pathogenic bacteria of *Aeromonas hydrophila* and *Pseudomonas* sp. *AACL Bioflux*, 9(3), 638–646.

Hardi, E. H., Kusuma, I. W., Suwinarti, W., Agustina, A., & Nugroho, R. A. (2016b). Antibacterial activity of *Boesenbergia pandurata*, *Zingiber zerumbet* and *Solanum ferox* extracts against *Aeromonas hydrophila* and *Pseudomonas* sp. *Nusantara Bioscience*, 8(1), 18–21.

Kaliamurthi, S., & Selvaraj, G. (2016). Insight on *Excoecaria agallocha*: An overview. *Natural Products Chemistry & Research*, 4, 203. https://doi.org/10.4172/2329-6836.1000203

Laith, A. A., Mazlan, A. G., Effendy, A. W., Ambak, M. A., Jabar, A., Najiah, M., et al. (2016). Phytochemical composition and in vitro antimicrobial, antioxidant activities of methanolic leaf extracts from *Excoecaria agallocha*. *Biosciences Biotechnology Research Asia*, 13(1), 599–608.

Mendhulkar, V. D., Yeragi, L. A., & Kumar, H. (2017). Bioassay of vector larvae with latex of blind eye mangrove plant *Excoecaria agallocha* Linn. *International Journal of Mosquito Research*, 4, 33–36.

Mondal, S., Ghosh, D., & Ramakrishna, K. (2016). A complete profile on blind-your-eye mangrove *Excoecaria agallocha* L. (Euphorbiaceae): Ethnobotany, phytochemistry, and pharmacological aspects. *Pharmacognosy Reviews*, 10(20), 123–138.

Nabeelah Bibi, S., Fawzi, M. M., Gokhan, Z., et al. (2019). Ethnopharmacology, phytochemistry, and

global distribution of mangroves—A comprehensive review. *Marine Drugs*, 17(4), Article 231. https://doi.org/10.3390/md17040231

Palagati Rohithkumar Reddy, Priyadurairaj, & RajeswaryHari. (2019). Antioxidant and antiproliferative activity of methanolic and ethanolic extract of *Excoecaria agallocha* leaves on human breast cancer cell lines MCF-7. *International Journal of Research in Pharmaceutical Sciences*, 10(4), 3805–3810.

Reverter, M., Tapissier-Bontemps, N., Sasal, P., & Saulnier, D. (2017). Use of medicinal plants in aquaculture. In B. Austin & A. Newaj-Fyzul (Eds.), *Diagnosis and control of diseases of fish and shellfish* (pp. [chapter pages]). Wiley-Blackwell. https://doi.org/10.1002/9781119152125.ch9

Shelar, M. K., Patil, M. J., Bhujbal, S. S., & Chaudhari, R. B. (2018). Evaluation of anticonvulsant activity of the ethanolic extracts from leaves of *Excoecaria agallocha*. *Future Journal of Pharmaceutical Sciences*, 4(2), 215–219.

Sofia, S., & Teresa, M. V. M. (2016). Investigation of bioactive compounds and antioxidant activity of *Excoecaria agallocha* L. *International Journal of Pharmaceutical Sciences and Research*, 7, 5062–5066.

Vinoth, R., Kumaravel, S., & Ranganathan, R. (2019). Therapeutic and traditional uses of mangrove plants. *Journal of Drug Delivery and Therapeutics*, 9(4-s), 849–854.

Yao, J.-Y., Lin, L.-Y., Yuan, X.-M., Ying, W.-L., Xu, Y., Pan, X.-Y., Hao, G.-J., Shen, J.-Y., Wu, J.-C., Ye, T., & Ge, P.-H. (2017). Antifungal activity of rhein and aloe-emodin from *Rheum palmatum* on fish pathogenic *Saprolegnia* sp. *Journal of the World Aquaculture Society*, 48(1), 137–144.