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Research Article

Antibacterial Activity of Two Medicinal Mangroves plants against Selected Human Pathogens

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Abstract

Mangrove ecosystems are rich reservoirs of structurally diverse secondary metabolites with potential antimicrobial applications. This study evaluated the *in vitro* antibacterial efficacy of leaf and bark extracts of two true mangrove species, *Excoecaria agallocha* L. (Euphorbiaceae) and *Rhizophora mucronata* Lam. (Rhizophoraceae), collected from Valapattanam, Kannur, Kerala. Air-dried plant tissues were extracted sequentially using solvents of increasing polarity: chloroform, petroleum ether, ethyl acetate, and methanol. Antibacterial activity was assessed against four human pathogens (*Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) using the agar well diffusion method. Across all tested organisms, polar solvents (methanol and ethyl acetate) yielded significantly larger inhibition zones compared to non-polar solvents (chloroform and petroleum ether), which exhibited baseline activity (8.0 to 9.8mm). Peak antibacterial potency against Gram-positive *S. aureus* was recorded for the ethyl acetate bark extract of *E. agallocha* ($30.3±0.57 mm), followed by the methanol bark extract of *R. mucronata* (27.3± 0.57 mm). Moreover, the methanol leaf extract of *E. agallocha* displayed broad-spectrum inhibition across all four bacterial strains. These results demonstrate that mid-to-high polarity constituents in *E. agallocha* and *R. mucronata* hold potential as natural therapeutic leads. Further investigation into bio-guided fractionation, compound isolation, and minimum inhibitory concentrations (MIC) is recommended.

Keywords: Excoecaria agallocha, Rhizophora mucronata, Antibacterial activity, Mangroves, Solvent polarity, Agar well diffusion.

1. Introduction

Indian systems of medicine have a deep root in our culture heritage and also cater to combat the diseases since times immemorial to a large section of our population. Natural products and related drugs are used to treat 87% of all categorized human disease including bacterial infection, cancer and immunological disorders (1, 2). About 25% of prescribed drugs in the world originated from plants and over 3000 species plants have been reported to have anticancer properties (3, 4). About 80% of the populations in developing countries rely on traditional plant-based medicines for their primary health care needs.

India has a rich and prestigious heritage of mangrove forests. Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, tannins However, the majority of the plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their sources as bioactive compounds (5, 6). The work on traditional records and ecological diversity indicate that Indian plants represent an exciting resource for possible lead structures in drug design. There are ample number of compounds that have the ability to combat disease, antimicrobial, antiviral and antifungal activities (7,

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8). Evaluation of plant species for anti-microbial activity in the discovery of new sources of economically valuable materials and metabolites with new therapeutic agents is an important task. As a preliminary study, it has found that aqueous and ethanol extracts of some mangrove species have antimicrobial activities (9). Until now, more than 200 bioactive metabolites have been isolated from true mangroves of tropical and subtropical populations (10). According to their chemical structures, most of the isolated compounds belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics which having a wide range of therapeutic possibilities (11, 12).

The use of bioassay offers various scientific strategies like screening of extracts, fractions and compounds obtained from plants, which are often used in phytochemical research. The evolution of *in vitro* methods for natural products, the anticancer activity has changed in the past few years; one of the recent developments is comet assay, which gives a ratio between the viable cells in the cell culture to total cells in the culture (13). These techniques are considered rapid and economical for the evolution of anticancer compounds (14). Therefore, it is possible to control infectious agents using natural products responsible for the inhibitory effect on pathogenic microorganisms using mangrove plant extracts. Expanding the same research (instead of using aqueous and ethanol extracts), the aim of this study was to evaluate the antibacterial activity of medicinal mangrove plants *Exoecaria agallocha* L. (Family Euphorbiaceae) and *Rhizophora mucronate* Lam. (Family Rhizophoraceae using different solvents viz. chloroform, petroleum ether, ethyl acetate, and methenol in order to get maximum compounds from the different plant materials and screening them *in vitro* for antibacterial activity against selected human pathogens such as *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeuriginosa* and *Klebsiella pneumoniae*.

2. Materials and Methods

The plant materials used for the present study are leaves and bark of *Exoecaria agallocha* L. (Family Euphorbiaceae), *Rhizophora mucronate* Lam. (Family Rhizophoraceae), both the species belongs to mangrove habitat. The plants were collected from Kannoor (Valapattanam).

The bark and leaves separated from the collected plants were washed in tap water then with distilled water, dried in shade and powdered in mechanical grinder and stored in airtight bottle for future use. Samples weighing about 30gm were extracted sequentially into 300 ml of Chloroform, Petroleum ether, Ethyl acetate and Methanol individually and were stored in 4^0 C. These stored plant extracts were tested for antibacterial activity against four bacterial strains viz. *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeuriginosa* and *Klebsiella pneumoniae* by agar diffusion technique (11,12).

2.1 Culture media preparation

A nutrient medium prepared for growing micro-organisms in a laboratory may vary according to the type of bacteria. The preparation of nutrient medium used for growing bacterial culture in the present study was Fuerst (15) which contain Peptone (10g), Meat extract (10 g), Sodium chloride (5g), Agar (10 g) in a 1000ml distilled water. The Nutrient agar medium was sterilized in steam under pressure in an autoclave at 121^0 C temperature and a pressure of 15 psi for 15 minutes. Time and exposure to this temperature and pressure may be allowed to exceed 15 minutes, if large volume of nutrient is being sterilized (16). After autoclaving, the medium was allowed to remain as such for 1-15 minutes.

2.2 Testing of antibacterial activity for plant extracts

The sterilized medium was transferred into pre-sterile Petri dishes and allowed to solidify. 1.0 ml from the overnight broth cultures of each bacterial strain was added to the petri plate. Wells were made using cork borer on the solidified medium. Prepared wells are filled with 0.5 ml of original crude extraction of each extract obtained by Soxhlet extraction method. All tests were performed in triplicates. For control,

Chloroform, Petroleum ether, Ethyl acetate and Methanol were used devoid of plant extracts. The bacterial cultures used are *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The petri plates were inverted and incubated at 37^{O} C for 24 hours. The diameters of the inhibition zones were measured in millimetres and their means were calculated (5) all experiments were conducted in triplicate.

3. Results

The table 1 shows antimicrobial activity against Gram-positive *S. aureus*. Maximum inhibition is observed in the ethyl acetate bark extract of *Exoecaria agallocha* (30.3±0.57 mm) and the methanol bark extract of *Rhizophora mucronata* (27.3±0.57 mm). No inhibition zone is reported in the control Chloroform, Petroleum ether, Ethyl acetate and Methanol without plant extracts and considered as zero. The results showed lower zone of inhibition with chloroform and petroleum ether extracts of leaf and bark *Exoecaria agallocha* and *Rhizophora mucronate* at the varying range from 8.5 mm to 9.8 mm (Table 1).

Table 1. Antimicrobial activities of chloroform, petroleum ether, ethyl acetate and methanol extract of leaf and bark of *Exoecaria agallocha*, *Rhizophora mucronata*, against *Staphylococcus aureus* on nutrient agar medium at 37^{0}C. Averaga inhibition zone diameter ±sd.

Extract Solvent	*Exoecaria agallocha* (Leaf)	*Exoecaria agallocha* (Bark)	*Rhizophora mucronata* (Leaf)	*Rhizophora mucronata* (Bark)
Chloroform	8.5±0.5	8.4±0.6	8.5±0.6	9.8±0.8
Petroleum Ether	8.6±0.7	9.7±0.8	8.4±0.5	8.5±0.6
Ethyl Acetate	23.0±2.0	30.3±0.57	12.6±0.57	23.6±0.57
Methanol	27.0±1.00	17.3±0.57	15.6±0.57	27.3±0.57

Antibacterial activity of chloroform, petroleum ether, ethyl acetate and methanol extract of leaf and bark of *Exoecaria agallocha*, *Rhizophora mucronata*, against *Proteus mirabilis* is presented in table 2. Organic extracts prepared using methanol exhibited the highest zone of inhibition by *R. mucronata* bark (27.5±1.9 mm) and *E. agallocha* leaf (26.6±0.57 mm). This was followed by ethyl acetate and methanol extract of *Exoecaria agallocha* and *Rhizophora mucronate* 23.3±0.57 and 23±1.0 respectively. Here also the chloroform, petroleum ether extracts of leaf and bark reported an inhibition in between 8.3 -9.6 mm (Table 2).

Table 2. Antimicrobial activities of chloroform, petroleum ether, ethyl acetate and methanol extract of leaf and bark of *Exoecaria agallocha*, *Rhizophora mucronata*, against *Proteus mirabilis* on nutrient agar medium at 37^{0}C. Averaga inhibition zone diameter ±sd.

Extract Solvent	*Exoecaria agallocha* (Leaf)	*Exoecaria agallocha* (Bark)	*Rhizophora mucronata* (Leaf)	*Rhizophora mucronata* (Bark)
Chloroform	9.6±0.5	8.5±0.5	8.6±0.5	8.4±0.6
Petroleum Ether	8.3±0.5	8.3±0.5	8.7±0.6	8.8±0.9
Ethyl Acetate	23.3±0.57	19.0±1.0	20.0±0.8	13.5±1.2
Methanol	26.6±0.57	18.6±0.57	23.0±1.0	27.5±1.9

E. agallocha leaf methanol extract displayed the strongest inhibitory effect (25.3±0.57 mm), followed by *E. agallocha* bark ethyl acetate extract (22.0±1.0 mm) for *P. aeruginosa* (Table 3). This was followed by methanol extract of leaf of *R. mucronata* and bark of *E. agallocha* 20±1.0 mm and 19.6±0.57mm respectively. Just like the previous cases the chloroform and petroleum ether extracts of leaf and bark of *E. agallocha* and *R. mucronata* recorded an inhibition zone in between 8.0 mm and 9.6 mm.

Table 3. Antimicrobial activities of chloroform, petroleum ether, ethyl acetate and methanol extract of leaf and bark of *Exoecaria agallocha*, *Rhizophora mucronata*, against *Pseudomonas aeruginosa* on nutrient agar medium at 37^{0}C. Averaga inhibition zone diameter ±sd and significance at ≤.0.01% P based on one-way ANNOVA.

Extract Solvent	*Exoecaria agallocha* (Leaf)	*Exoecaria agallocha* (Bark)	*Rhizophora mucronata* (Leaf)	*Rhizophora mucronata* (Bark)
Chloroform	8.0±0.5	9.5±0.5	8.6±0.5	9.5±0.5
Petroleum Ether	8.6±0.4	9.6±0.57	8.6±0.57	9.4±0.5
Ethyl Acetate	19.0±0	22.0±1.0	11.6±0.9	10.0±0.5
Methanol	25.3±0.57	19.6±0.57	20.0±1.0	14.6±0.9

Table 4 gives the size of the inhibition zone against *K. pneumoniae*, with chloroform, petroleum ether, ethyl acetate and methanol extract of leaf and bark of *E. agallocha* and *R. mucronata*. Against *K. pneumoniae*, polar solvents consistently outperformed non-polar solvents, with *E. agallocha* leaf methanol extract giving the largest inhibition zone (25.3±0.57 mm) followed by leaf ethyl acetate extract (21.6±1.2) and methanol bark extract (21.2±1.3). As that of other three cases the chloroform, petroleum ether leaf and bark extracts of *E. agallocha*, *R. mucronata*. recorded inhibition zone in between 8.5mm and 9.5 mm.

Table 4. Antimicrobial activities of chloroform, petroleum ether, ethyl acetate and methanol extract of leaf and bark of *Exoecaria agallocha*, *Rhizophora mucronata*, against *Klebsiella pneumonia* on nutrient agar medium at 37^{0}C. Averaga inhibition zone diameter ±sd.

Extract Solvent	*Exoecaria agallocha* (Leaf)	*Exoecaria agallocha* (Bark)	*Rhizophora mucronata* (Leaf)	*Rhizophora mucronata* (Bark)
Chloroform	8.5±0.5	8.6±0.6	9.5±0.5	8.5±0.5
Petroleum Ether	8.6±0.4	9.5±0.5	8.7±0.5	8.6±0.4
Ethyl Acetate	21.6±1.2	18.0±2.0	11.3±0.9	17.3±1.1
Methanol	25.3±0.57	21.2±1.3	15.6±0.57	16.5±1.4

4. Discussion

Extracts from different parts of mangrove plants are reported to possess diverse medicinal properties, which include novel agrochemical products, compounds of medicinal value, and biologically active compounds (12, 17,18). Extracts from different mangrove plants and mangrove associates are reported to have active against human and plant pathogens (19). According to preliminary study reports it has been recorded that, a number of mangrove plant extracts of methanol, ethanol and aqueous showed antibacterial activity against pathogenic isolates as well as antibiotic resistant bacteria (19, 20). Ethanol extracts showed higher inhibition than aqueous extracts. Water extract may contain a low concentration of antibacterial compounds or may not extract antibacterial compounds.

In the present work across all test organisms, polar solvents (Methanol and Ethyl acetate) yielded significantly higher inhibition zones compared to non-polar/weakly polar solvents (Chloroform and Petroleum Ether). This observation aligns with fundamental natural product chemistry principles regarding solvent polarity and phytochemical extraction. Polar solvents like methanol and ethyl acetate effectively dissolve polyphenols, flavonoids, tannins, and saponins, etc. which have strong antimicrobial properties. Non-polar solvents (chloroform and petroleum ether) predominantly extract non-polar substances such as fats, waxes, fixed oils, and sterols, which typically exhibit weaker broad-spectrum antimicrobial activity in agar diffusion assays.

The organic extracts of leaf and bark were effective against all the human pathogenic bacteria selected for the study. The maximum activity shown by ethyl acetate extracts of *Exoecaria agallocha* bark against *Staphylococcus aureus*. *Exoecaria agallocha* leaf and *Rhizophora mucronata* bark effective against *Proteus mirabilis*. Methanol extracts of *Exoecaria agallocha* leaf show significant activity against *Pseudomonas aeruginosa*. Methanolic extract of *Exoecaria agallocha* leaf showed significant activity against *Klebsiella pneumonia*. The minimum zone of inhibition shown by petroleum ether and chloroform extracts. Maximum activity was shown by methanol than ethyl acetate. The ethyl acetate bark extract's remarkable activity (30.3±0.57mm) against *Staphylococcus aureus* suggests that mid-to-high polarity bark constituents - likely specific diterpenoids, tannins, or phenolic compounds - possess potent antibacterial mechanisms against Gram-positive cell walls supports the observation of Raghavanpillai Sabu et al. (21).

The methanol leaf extract's consistently large zones of inhibition (25.3 to 27mm) across all tested strains indicate that polar leaf metabolites offer broad-spectrum antimicrobial activity, effectively targeting both Gram-positive and Gram-negative bacterial structures. Methanol extractions solubilize cell-wall-permeating polyphenolic structures that disrupt outer membrane stability in Gram-negative bacteria while simultaneously coagulating cytoplasmic components in Gram-positive cells (21). The contrast between the

bark extract's targeted peak potency and the leaf extract's broad consistency illustrates how bioactive secondary metabolites are differentially synthesized and localized across different plant tissues.

The antibacterial study also clearly pictures' the antibacterial property of the mangrove community. The study reveals the particular plant community has significant and antibacterial properties. The data strongly reveals the medicinal property of the mangrove species but further studies are recommended for the separation of secondary metabolites and chemical constituents present in the plants make it highly medicinal one.

In the present study dried plant material were extracted sequentially in chloroform, petroleum ether, ethyl acetate and methanol using Soxhlet apparatus. These plant extracts were tested against four types of human pathogens viz. *Staphylococcus aureus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. From this study it is found that the leaves and bark of mangroves plants such as *Exoecaria agallocha* and *Rhizophora mucronata*, possess antibacterial activity. The higher rate of inhibition was observed in *Staphylococcus aureus* and *Proteus mirabilis*. These results demonstrate that mid-to-high polarity constituents in *E. agallocha* and *R. mucronata* hold potential as natural therapeutic leads. Further investigation into bio-guided fractionation, compound isolation, and minimum inhibitory concentrations (MIC) is recommended.

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