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Research Article

A PRELIMINARY OBSERVATION ON AN EXPLICIT ANTIMICROBIAL ACTION OF MANGROVE PLANTS ON *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

Objectives: Due to emerging drug-resistant microorganisms throughout the world, newer antimicrobial agents should be looked for. Plants are enriched with different bioactive chemicals. In this study, we searched antibacterial activities of some mangrove plant extracts against *Pseudomonas aeruginosa*, *Staphylococcus aureus,* and *Escherichia coli.*

Methods: In the present study, the antimicrobial activity of the leaves of *Bruguiera gymnorhiza, Excoecaria agallocha, Avicennia alba,* and *Aegialitis rotundifolia* was evaluated against a few reference pathogenic bacterial strains, namely, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *S. aureus* ATCC 29213, and multidrug-resistant bacterial strains *E. coli* extended-spectrum beta-lactamases strain. Aqueous, ethanolic, methanolic, and dimethyl sulfoxide (DMSO) extracts were studied. The antimicrobial activities of the organic solvent extracts on the various test microorganisms were investigated using agar well diffusion technique followed by determination of minimum inhibitory concentration values by serial dilution in a microtiter plate.

Results: Ethanol and DMSO extracts of *B. gymnorhiza* exhibited promising antimicrobial activity followed by extracts of *A. alba* and *E. agallocha*. Among all microorganisms studied, *P. aeruginosa* ATCC 27853 showed significant growth inhibition with ethanol and DMSO extracts.

Conclusion: Extracts of some mangrove plants, particularly, *B. gymnorhiza* showed very good antimicrobial activities against common microbial agents causing human infections and in general mangrove plants appear to act better on *P. aeruginosa.*

Keywords: *Bruguiera gymnorhiza, Excoecaria agallocha, Avicennia alba, Aegialitis rotundifolia, Pseudomonas aeruginosa*, Antimicrobial activity.

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INTRODUCTION

In the recent years, over-exploitation of antibiotics has caused the emergence of multidrug-resistant (MDR) microbes, and therefore treatment of such MDR microbial diseases demand a search for novel alternative and effective therapeutic compounds [1]. The major search focus has, thus, been shifted toward exploring the natural flora with known antimicrobial and antioxidant properties.

Historically, plants have been proved to be a source of inspiration for novel drug compounds as plant-derived medicines have made large contributions to human health and well-being. Their role is two-fold in the development of new drugs: First, they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or second: A phytomedicine to be used for the treatment of various diseases. It is estimated that today, plant materials are present in or have provided the models for 50% Western drugs [2]. The primary advantage of using plant-derived medicines is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits, and more affordable treatment.

Several research works to date have suggested the potential of mangrove floral community in traditional medicines [3-5]. For centuries, the tribal population employed mangrove plant extracts as their traditional folk medicine for healing several health disorders [6-8]. However, unlike various herbs, seaweeds and higher medicinal plants, the use of mangroves as alternative medicine has been comparatively less explored [9-14].

The Indian Sundarbans, one of the most taxonomically diverse and physicochemically dynamic ecosystems of the Indian subcontinent, sustains some 34 species of true mangroves among which members of the Avicenniaceae family in which our present study plants present, rank second in terms of prevalence [15,16]. In comparison to the normal terrestrial flora, this halophytic mangrove community gets exposed to high and low tides twice in every 24 h [17], and therefore, has developed a unique mode of adaptation, which could have enriched their phytochemical repertoire of medicinal importance. Wu *et al.* showed that *Avicennia marina*, a true mangrove floral species (commonly known as gray mangrove), contains few quinone derivatives with therapeutic and antimicrobial properties [18].

Mangroves are widespread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coastlines and contain biologically active antimicrobial compounds. Previous studies on mangrove plant parts and its chief chemical classes exhibited various levels of biological activities such as antibacterial, antifungal, cytotoxic, hepatoprotective, and free radical scavenging activities [19-25,]. Mangrove plant parts have been used for centuries as popular medication for various health disorders. Numerous studies have been conducted on various natural products screening their antimicrobial property [20,26]. The present study made an attempt to investigate the antibacterial properties of four mangrove plants, namely, *Bruguiera gymnorhiza, Excoecaria agallocha, Avicennia alba,* and *Aegialitis rotundifolia* against pathogenic and antibiotic-resistant bacterial strains.

METHODS

Collection of plant samples

Fresh leaf samples of *B. gymnorhiza, E. agallocha, A. alba,* and *A. rotundifolia* were collected from Bali Island of the Indian Sundarbans during the month of June 2018. The samples were stored at 4°C after collection and utilized within 7 days.

Collection and maintenance of microorganisms

All ATCC strains were procured from HiMedia, India and maintained at Peerless Hospital and B. K. Roy Research Centre, Kolkata, India, and the *Escherichia coli* extended-spectrum beta-lactamases (ESBL) strain was isolated and identified in the VITEK-2 automated system in the hospital. The ESBL enzymes present in the strain are mutants of temoneira and sulfhydryl variables. enzymes and they can hydrolyse third and fourth generation cephalosporins and monobactams. Infections with *E. coli* (ESBL) (ESBL-Ec) results in severe morbidity and mortality in humans, with significant associated health costs and disease burdens. Recently, the WHO launches a global survey of ESBL-Ec strains and thus one such strain has been included in this study. Fresh subcultures were made in the preceding day of the experiment from the stock cultures maintained in the laboratory.

Powder preparation and pharmacognostical study

The leaf samples were oven dried at 60°C till crisp and ground to a fine powder using mortar and pestle. The fine powder obtained was used in the microscopic and physicochemical analysis. Different physicochemical parameters such as pH, acid soluble ash, water-soluble ash, water-soluble extractive values, and ethanol soluble extractive values were measured for the different plant extracts.

Extract preparation

About 1 g of each of the finely powdered plant leaf material was soaked in 10 ml of solvents (ethanol, methanol, and dimethyl sulfoxide [DMSO]) for a period of 1 week at room temperature. Then, the extracts were filtered and concentrated by rotary vacuum evaporator (RotaVap). The final concentration was adjusted to 1 mg/mL for screening the antimicrobial activity.

Antibacterial screening assay

The minimum inhibitory concentration (MIC) assay was done by serial dilution method, using 96 well plates, and plate reader (Erba Lisa Scan II Transasia Mannheim, Germany). 100 µL of Mueller-Hinton broth (HiMedia, India) was dispensed in all the wells of the plate. 100 µL of stock concentration of the extract was added to the first well of each column. Serial double dilution was done till the eighth well. Finally, 10 µL of 0.5 McFarland opacity culture suspension was added to each well of the plate. The plate was then gently shaken to mix the contents properly and immediately a baseline absorbance reading at 620 nm was taken. Then, the plates were kept for incubation for 16–18 h at 37°C and another absorbance reading at 620 nm was recorded.

RESULTS

Microscopical observations of the powders of the plants (Table 1 and Fig. 1) showed variegated but specific findings which were confirmed by repeated observations. The physicochemical analysis of the powder extract (Table 2) showed a characteristic pattern of each extract. Loss on drying varied between 2.8% and 9.7%; total ash ranged between 3.43% and 7.63%; and water-soluble ash was between 3.07% and 5.82%. Water-soluble extraction value and ethanol soluble extraction value were both markedly higher in *A. rotundifolia* powder extract with values of 23.4% and 21.43%, respectively, than others. Detailed physicochemical characters of all the extracts are delineated in Table 2.

B. gymnorhiza extract in ethanol and DMSO was the most effective extract (Fig. 2) studied so far in this experiment in relation to their antimicrobial activities. Ethanolic extract of *B. gymnorhiza* has been inferred from our results to be the most effective extract which has promising antibacterial activity against *Pseudomonas aeruginosa* ATCC 27853 and *E. coli* ESBL strain with an MIC value of around 8 µg/mL and 32 µg/mL, respectively. DMSO extract of *A. alba* is also effective in inhibiting the growth of *E. coli* ATCC 25922 and *P. aeruginosa* ATCC

Table 1: Microscopic observations of the powder

Plant sample	Observations
Bruguiera gymnorhiza	Crystals Stomatal fragments
Excoecaria agallocha	Epidermal cell fragments Crystals (very few) Globular cells
Avicennia alba	Astro-sclereids Branched sclereids Oval and rectangular crystals
Aegialitis rotundifolia	Branched sclereids Oil globules Xylem vessel fibers

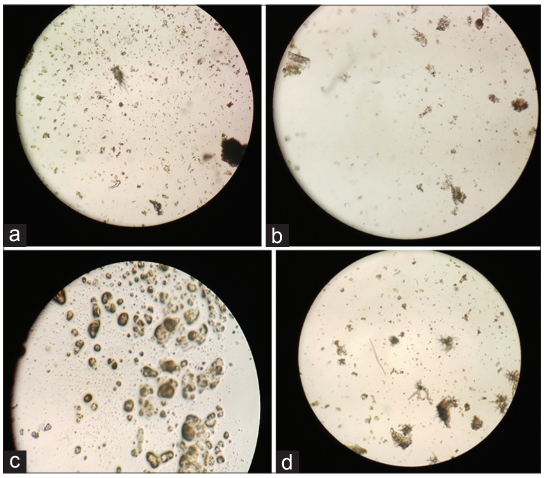

Fig. 1: Microscopic observations of powder study, (a) *Excoecaria agallocha*, (b) *Bruguiera gymnorhiza*, (c) *Avicennia alba*, (d) *Aegialitis rotundifolia*

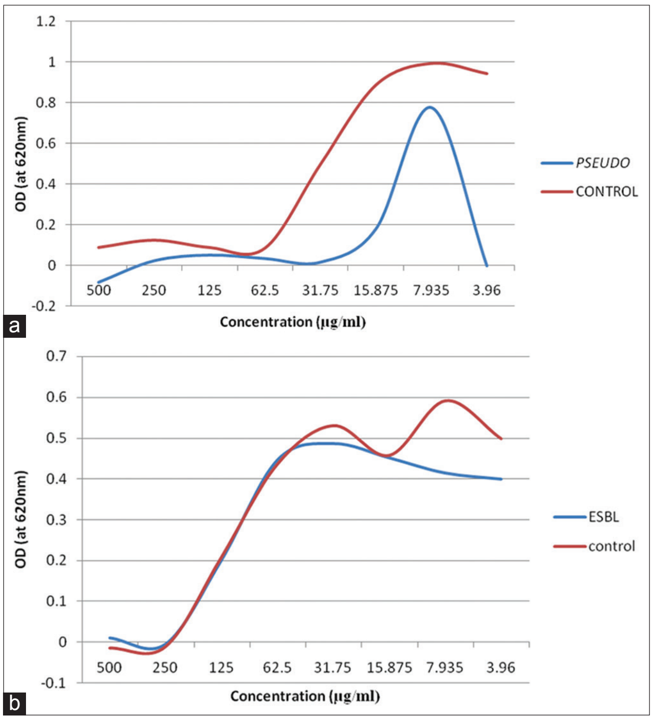

Fig. 2: (a and b) Antibacterial activity of the ethanolic and dimethyl sulfoxide extracts of *Bruguiera gymnorhiza* against *Pseudomonas aeruginosa* (Pseudo) and *Escherichia coli* extended-spectrum beta-lactamases strains, respectively

Table 2: Physicochemical analysis of powder extracts

Plant sample	Parameters	Value (%)
Bruguiera gymnorhiza	Foreign matter	0
	Loss on drying	2.8
	Total ash	7.63
	Acid‑insoluble ash	4.31
	Water‑soluble ash	3.07
	pH	7.1
	Water‑soluble extractive value	10.43
	Ethanol soluble extractive value	9.37
Excoecaria agallocha	Foreign matter	0
	Loss on drying	3.7
	Total ash	3.43
	Acid‑insoluble ash	3.783
	Water‑soluble ash	5.82
	pH	7.6
	Water‑soluble extractive value	8.12
	Ethanol soluble extractive value	10.15
Avicennia alba	Foreign matter	0
	Loss on drying	4.3
	Total ash	7.43
	Acid‑insoluble ash	2.561
	Water‑soluble ash	3.11
	pH	7.4
	Water‑soluble extractive value	7.64
	Ethanol soluble extractive value	11.23
Aegialitis rotundifolia	Foreign matter	0
	Loss on drying	9.7
	Total ash	5.7
	Acid‑insoluble ash	1.233
	Water‑soluble ash	3.742
	pH	7.3
	Water‑soluble extractive value	23.4
	Ethanol soluble extractive value	21.43

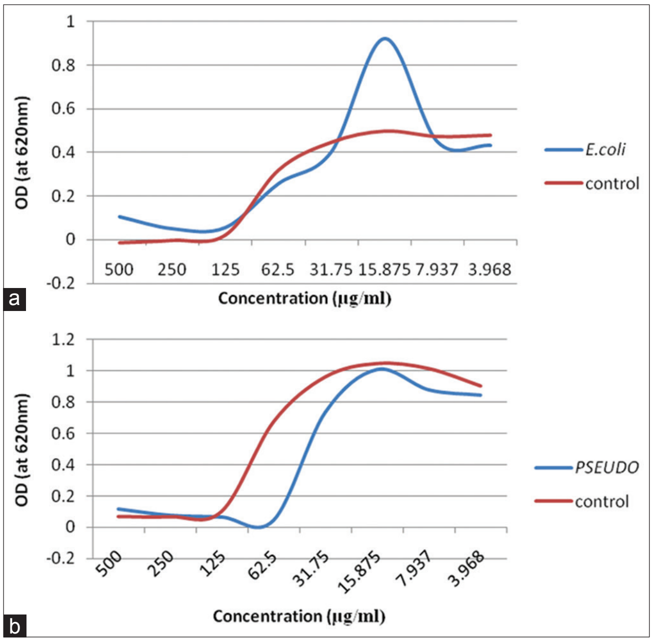

Fig. 3: (a and b) Antimicrobial activity of dimethyl sulfoxide extract of *Avicennia alba* against *Pseudomonas aeruginosa* (Pseudo) and *Escherichia coli* ATCC strains

27853 with an MIC value of 15 μg/mL in both the cases (Fig. 3). Other plant extracts showed antimicrobial activities only against *P. aeruginosa* (Figs. 4 and 5). Thus, in general, almost all the studied plant extracts showed antimicrobial activities against *P. aeruginosa*. However, it is important to note that the methanolic extract of *B. gymnorhiza*, and both ethanolic/methanolic extracts of *A. alba*, did not show any antimicrobial activity against *P. aeruginosa* which was found unique. Another very important finding in this study is the antimicrobial activity of DMSO extract of *B. gymnorhiza* against *E. coli* ESBL strain which was also an important observation. None of the extracts are effective in ceasing or controlling the growth of *Staphylococcus aureus.*

DISCUSSION

Pharmacognostical study of the powder extracts reveals the presence of different kinds of crystals, oil globules, and astro-sclereids that can be used as anatomical markers and these pharmacobotanical properties can also be used for establishing pharmocognostical standards for the scientifically unexplored mangrove plants. The study of physicochemical properties is essential for correct identification and assessment of the crude drug properties.

Plant extracts showed promising results in relation to their antimicrobial actions [27,28]. In this study, it was found that all our plant extracts showed excellent antimicrobial activities against *P. aeruginosa*. Although our findings corroborate with most of the studies done so far [29], and some also claiming as best action on it like us [30], in a few studies they did not find any inhibitory action on *Pseudomonas* [31]. In a few mangrove areas, *P. aeruginosa* remains naturally [32] on some plants, probably those plants failed to show any antimicrobial action on them. However, it may be due to the use of different solvents. It is very difficult to explain the exact cause of anti-*Pseudomonas* action of mangrove plants in this preliminary study but future studies on this line may through new insights of mangrove plants in this area. In this study, we found no action of these plant extracts on *S. aureus*. In most studies, on mangrove plants again no action have been reported against *S. aureus* [30,33] or the MIC values of the extracts are very high >100 μg/mL even >1 mg/ml [34] and inhibition is usually less than that on other microorganisms [35]. As we know that *S. aureus* is a salt tolerant bacteria and also remains in the sea [36], thus some genetic or epigenetic relationship with these plants may participate in this background.

Chemical composition of *B. gymnorhiza* and *E. agallocha* was studied by many workers. *B. gymnorhiza* contains indole-3-carbaldehyde; anthocyanins, tannins, diterpenes, flavans and flavan polymers, phenolic compounds, procyanidins, steroids, and triterpenes [37-40]. Most of them may play a good antimicrobial role. Due to this, *B. gymnorhiza* plant extract possibly showed preeminent results in this study.

E. agallocha contains alkaloids, chalcones, cyclitol, diterpenes, excoecaria toxins, fluratoxin, polyphenols, saponins, steroids, tannins, triterpenes, Phomopsin A, B, C, and Cytosporone B and C [34,41,42]. Many of such chemicals are familiar antimicrobial agents and thus they can convey their function in such biological behavior of this plant extract. There are only few explicit studies on *A. alba* [43] particularly regarding its naphthoquinones and their analogs, or in relation to triterpenoids such as lupeol, taraxerol, betulinic acid, and betulin [44]; however, these groups of plants also have alcohols, steroids, and tannins as observed in *A. marina*. In *A. rotundifolia* saponin, alkaloids are mainly present. These chemicals may hinder growths of *Pseudomonas*. Effectivity of the extracts in particular solvents and specific inhibition against *P. aeruginosa* may be due to enzyme inactivation, target site modification or membrane disruption, which may be studied in details further. It is well known that microbial communities are heterogeneously distributed in mangroves and the aerobic/anaerobic interface is the main determining factor for specific microbial community structure [32] which may be responsible for the selective pattern of antimicrobial action of these plant extracts.

CONCLUSION

Thus, the extracts of these mangrove plant leaves have no effective antimicrobial action against *S. aureus*, variable antimicrobial activities against *E. coli,* and good antimicrobial activities against *P. aeruginosa.*

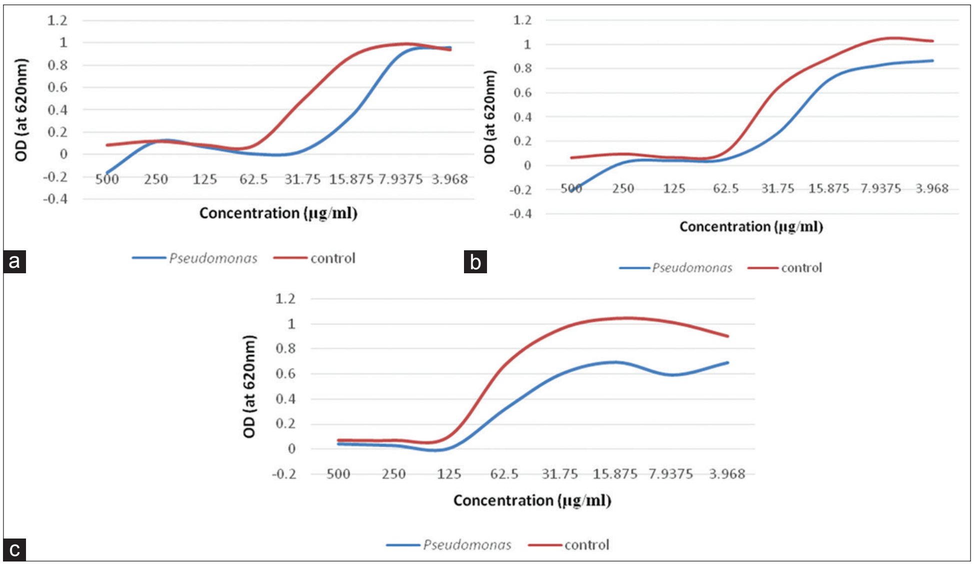

Fig. 4: (a-c) Antimicrobial activities of ethanolic, methanolic, and dimethyl sulfoxide extracts of *Excoecaria agallocha* against *Pseudomonas aeruginosa*

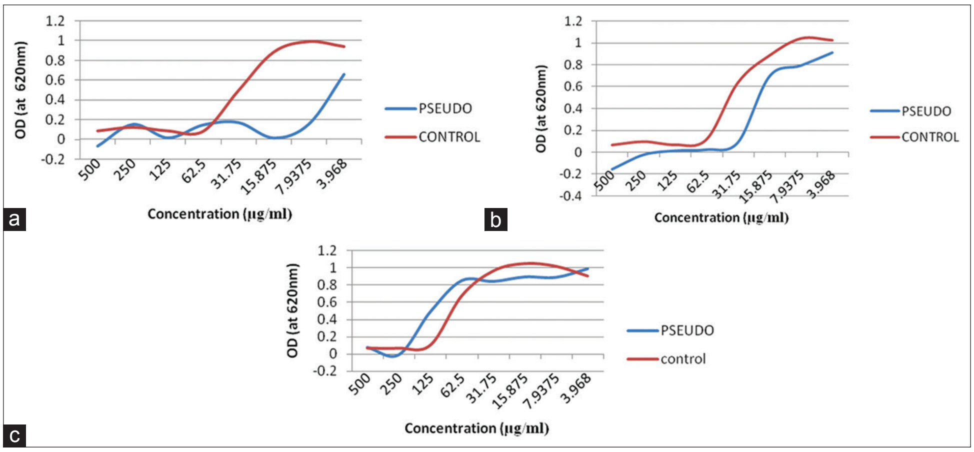

Fig. 5: (a-c) Antimicrobial activities of ethanolic, methanolic, and dimethyl sulfoxide extracts of *Aegialitis rotundifolia* against *Pseudomonas aeruginosa*

CONTRIBUTION DETAILS OF THE AUTHORS

Tamanna Sultana – Literature search, experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation; Arup Kumar Mitra and Satadal Das – Concept, design, definition of intellectual content, manuscript editing and manuscript review.

CONFLICTS OF INTEREST

There are no conflicts of interest of any author.

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