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Research Article

STUDY OF ANTIMICROBIAL POTENTIALITY OF EIGHT DIFFERENT MANGROVE SPECIES AND DEVELOPMENT OF THEIR INTRA-RELATIONSHIP THROUGH CLUSTER ANALYSIS

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ABSTRACT

Objective: The present study investigates the antimicrobial activities of eight different mangrove plants and also the development of their intra-relationship through cluster analysis.

Methods: The dried and powdered leaves of different mangrove species were extracted by cold maceration process with water, methanol, and chloroform. The antimicrobial activity was done using the agar well diffusion method. The cluster analysis of the mangrove plants was analyzed by MINITAB Release 13.1.

Results: The order of extraction yield for each mangrove species was methanol > chloroform > water. The methanol extract of *Suaeda maritima, Avicennia marina, Avicennia officinalis,* and chloroform extract of *Sonneratia apetala* gave the highest inhibition zones of 19 mm, 19 mm, 19.33 mm, and 19.33 mm, respectively, against *Bacillus subtilis.* The methanol extract of *Ceriops decandra, Xylocarpus granatum,* and *Bruguiera gymnorrhiza* found the highest inhibition zones of 21.67 mm, 22 mm, and 20.3 mm, respectively, against *Escherichia coli, Shigella flexneri,* and *Staphylococcus aureus,* respectively. The endangered and endemic species *Heritiera fomes* gave the highest result (18 mm) against both for *Micrococcus luteus* and *B. subtilis* in methanolic extract. The maximum zone of inhibition of fungal strains was found against *Botrytis cinerea* (15 mm), *Fusarium oxysporum* (16.33 mm), and *Rhizopus oryzae* (13.33 mm) with the methanol extract of *X. granatum*, *S. apetala,* and *C. decandra,* respectively. Cluster analysis of 8 mangrove species based on different bacterial pathogens was also shown where it demonstrated their intra-relationship against same bacterial pathogens.

Conclusion: From this study, it may be concluded that mangrove plants can be used to discover bioactive natural products.

Keywords: Antibacterial, Antifungal, Dendrogram, Extraction yield, Mangrove, Phytochemical screening, Sundarbans.

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INTRODUCTION

Infectious diseases have caused of death and disability, accounting for more than about 22% of the global disease burden [1]. In this context, antibiotics are most effective for the control of these diseases. However, the indiscriminate use and abuse of antibiotics have led to the development of antibiotic resistance strains at an alarming frequency [2,3]. In addition, antibiotics sometimes create adverse side effects on the host [4]. For this reason, the isolation of antimicrobial agents less susceptible to regular antibiotics is increasing throughout the world [5,6]. The potential for developing antimicrobials from higher plants plays a central role in the healthcare systems in many populations of the world [7]. According to the World Health Organization, up to 80% of the population in rural areas depends on traditional herbal medicine [8,9]. In comparison with modern medicine, herbal medicines are cheap, safe from undesirable side effects and are more effective to treat chronic diseases [10,11].

The extreme environments such as regular changes in pH of soil and water, salinity, humidity, high temperature, UV irradiance, and tidal cycles may be possible reasons for mangrove plants to synthesize a large number of different bioactive compounds [12]. For these reasons, they show several unique features along with some special biochemical characteristics to prevent microbial growth and can be used as antimicrobial agents [13,14]. As a result, various parts of these plants have been used for a long period of time by the native people for the treatment of many syndromes [15]. Hence, these plants containing various bioactive compounds which are needed to study well to create proper documentation and utilization for many useful purposes.

The aim of the present study was to investigate the antimicrobial activities of eight different mangrove plants of the Indian Sundarbans of West Bengal. These mangrove plants are *Ceriops decandra* (Griff.) Ding Hou, *Xylocarpus granatum* J. König, *Bruguiera gymnorrhiza* (L.) Lam., *Heritiera fomes* Buch.-Ham., *Suaeda maritima* (L.) Dumort, *Avicennia marina* (Forsk.) Vierh., *Avicennia officinalis* L., and *Sonneratia apetala* Buch.-Ham and also the development of their intra-relationship through cluster analysis based on the same bacterial pathogens.

MATERIALS AND METHODS

Plant samples

The leaves of *C. decandra* and *B. gymnorrhiza* were collected from Bakkhali region, West Bengal (latitude 21° 35' N, longitude 88° 15' E) of Indian Sundarbans. On the other hand, leaves of *H. fomes*, *X. granatum, S. maritima, A, marina*, *A. officinalis,* and *S. apetala* were collected from Gosaba region, West Bengal (latitude 22° 15′ 45" N, longitude 88° 39′ 46" E) of Indian Sundarbans. The fresh leaves were transported to Kalyani in airtight containers. The taxonomic identification was confirmed by Prof. S. K Mukherjee, Taxonomy and Plant systematic Unit, Department of Botany, University of Kalyani. The voucher specimens (KU/B/AK/11, KU/B/AK/12, KU/B/AK/14, KU/B/AK/15, KU/B/AK/16, KU/B/AK/22, KU/B/AK/29, and KU/B/AK/30 for *C. decandra, B. gymnorrhiza*, *H. fomes*, *X. granatum*, *S. maritima*, *A. marina*, *A. officinalis*, and *S. apetala,* respectively) were deposited and conserved at the Department of Botany, University of Kalyani, Kalyani, West Bengal, India, for reference. Then, the leaves were air-dried for 12 weeks to obtain constant weight. The dried sample was ground into fine particles (2 mm diameter) with a grinder. The powdered sample was bagged in black plastic bags and stored in an airtight container for further work.

Microorganisms
The test bacterial culture used for the antimicrobial assay were Gram-negative bacteria such as *Escherichia coli* (MTCC 443), *Shigella dysentery* (Clinical isolates), *Shigella flexneri* (MTCC 1457), *Vibrio cholera* (MTCC 3904), and *Pseudomonas aeruginosa* (MTCC 2581) and Gram-positive bacterium cultures were *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 441), and *Micrococcus luteus* (MTCC 1538) which were procured from Institute of Microbial Technology, Chandigarh, India (Microbial Type Culture Collection) except for *S. dysentery* (Clinical isolate). All the bacterial strains were grown in the nutrient broth and maintained on nutrient agar slants at 4°C. The 18 h old bacterial culture was standardized using McFarland standard (10^6 cfu/ml of 0.5 McFarland standard) for this study. The reference strains used in the antifungal assays were *Fusarium oxysporum*, *Botrytis cinerea and Rhizopus oryzae.* All the three fungal strains were procured from the plant Biochemistry Laboratory, Department of Botany, University of Kalyani, India. All the test fungal strains were grown on potato dextrose broth and maintained on potato dextrose agar slants at 4°C.

Extraction of crude material by different solvent
The dried and powdered leaves (10 g) were extracted by cold maceration process with three solvents such as water, methanol, and chloroform (1:10 w/v) for 72 h. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated in vacuum at 40°C using a rotary evaporator. Thereafter the resulting crude extract was lyophilized and kept in the dark in a refrigerator at 4°C until tested. The extraction was done at least 3 times for each plant and respective solvents to calculate the mean values of extractive yields.

Phytochemical screening
The analysis of phytochemicals of selected mangrove plants was carried out using different qualitative tests for alkaloids, flavonoids, carbohydrates, saponins, glycosides, steroids, tannins, and steroids, according to the process described by Sinha *et al*. [16].

Measurement of extraction yield
The yield of evaporated dried extracts (based on a dry weight basis) was calculated from the equation followed –

$$\text{Yield}(\%) = \frac{W1 \times 100}{w2}$$

, Where, W1 = Weight of the extract after evaporation of solvent and

W2 = Weight of the plant sample.

Studies on antibacterial and antifungal activity
The antibacterial and antifungal tests were performed using the agar well diffusion method [17]. By means of a 5 mm cork borer, three cups were bored, well separated, and equidistant from each other in the agar. To each well, 1.5 mg/ml concentration of leaf extracts was added for each solvent extract to about three-quarters full. A control experiment with the respective solvent was done on a different agar plate for each bacterial strain. For antibacterial activities, the plates were incubated aerobically at 37°C and examined for any zone of inhibition after 24 h. For antifungal activities, plates were allowed to incubate at 30°C for 72 h. The experiment was repeated thrice and the average values of inhibition zone of diameter were recorded for antibacterial and antifungal activities.

Data analysis
The results obtained in this study were expressed as mean inhibition zone (mm) ± standard error of three replicates and the Student's *t*-test was applied to evaluate the significance of differences at $p \leq 0.05$ [18]. Cluster analysis of the mangrove plants according to their response against different bacteria was analyzed using MINITAB Release 13.1.

RESULTS AND DISCUSSION

Phytochemical screening
The preliminary phytochemical screening results of leaves of different mangrove plant species showed the presence of various bioactive secondary metabolites constituents (Table 1). The phytochemical screening of *C. decandra* and *B. gymnorrhiza* showed the absence of steroids and terpenoids and the presence of all chemical constituents. Among them, glycosides were present in higher amounts in both the species. The *X. granatum* showed absence of saponins and the presence of alkaloids, flavonoids, and tannins in higher amounts. The leaf of *S. maritima* indicated the presence of glycosides, flavonoids, alkaloids, and steroids only based on this study. The *A. marina* and *A. officinalis* showed similar types of findings and absence of saponins in both species. The *S. apetala* indicated the absence of glycosides but the presence of saponin in much quantity.

Shanmugapriya *et al*. [19] showed that the saponin content is present in leaf of *A. marina* and absent in leaf of *A. officinalis* which was collected from Parangipettai, Tamil Nadu, India. Unlike the result, this study found that the saponin content in leaves of *A. marina* is totally absent, which is supported by another study [20]. However, this study supports the absence of saponin in leaf of *A. officinalis*.

It is well known that the plant secondary metabolites are widely distributed in the plant kingdom and they are vary from plant to plant in presence and quantity [21]. Among the species, it is also varied according to the environmental influences [22]. In the present investigation, *C. decandra* and *B. gymnorrhiza* gave similar types of result; it may be because they are mainly from the same family, that is, Rhizophoraceae. This reason is also applicable for two other mangroves, namely, *A. marina* and *A. officinalis*, which are also from the same family and genus.

Extraction yield
The extraction yields of various solvents, that is, methanol, chloroform, and water are presented in Table 2. In this study, the extraction yields of various mangrove leave obtained from the different solvents ranked in the following order: methanol > chloroform > water. The extraction yield was found maximum in the methanol extract of *X. granatum* (24%) and the minimum for aqueous extract of *S. maritima* (2.3%).

Antibacterial activity
The results showed that the control experiments of pure distilled solvent alone induced no zone of inhibition. The antibacterial activities of the eight various mangrove species with different solvent extracts and their yields has been summarized in Table 2. The observation based on this study found that few bacterial pathogens showed no results in aqueous and chloroform extract in many cases. However, the results of this study showed that the methanol extract of selected mangrove species was found to possess much antibacterial activity than aqueous or chloroform extract except for *S. apetala* extract which showed much antibacterial activity on aqueous extract rather than methanol or chloroform extract. The methanol extract of *C. decandra* showed strong antibacterial activity (21.67 mm) against *E. coli*. The highest (22 mm) and lowest (12 mm) zone of inhibition were found against

Table 1: Qualitative phytochemical screening of different mangrove species

Chemical components	CD	XG	BG	HF	SM	AM	AO	SA
Alkaloids	+	++	+	+	+	+	+	+
Glycosides	++	+	++	+	+	+	+	–
Flavonoids	+	++	+	–	+	+	+	+
Steroids	–	+	–	+	+	+	+	+
Terpenoids	–	+	–	+	–	+	+	+
Saponin	+	–	+	+	–	–	–	++
Tannins	+	++	+	++	–	+	+	+

+ indicates presence or positive reaction, ++ indicates presence in higher amounts, and – indicates absence or negative reaction. CD: *Ceriops decandra*, XG: *Xylocarpus granatum*, BG: *Bruguiera gymnorrhiza*, HF: *Heritiera fomes*, SM: *Suaeda maritima*, AM: *Avicennia marina*, AO: *Avicennia officinalis*, SA: *Sonneratia apetala*

Table 2: Evaluation of antimicrobial activity (mm) and extractive yield (%) of different solvent extracts of eight different mangrove species

Plant species	Solvent extract	Yield (%)	Bacterial name and diameter of zone of inhibition (mm)							
			EC	SA	SD	SF	VC	PA	BS	ML
CD	CH_3OH	22	21.67±0.33	16±1.7	11.33±0.33	19.67±0.33	19±0.58	17.33±0.88	16.33±0.88	20.33±0.67
	H_2O	8.6	15.33±1.8	ND	ND	12.67±0.88	15±0.58	13.33±0.33	13.67±0.88	16±1.16
	$CHCl_3$	16	17.33±0.88	15±0.58	14.33±0.67	16±1.16	13.33±0.33	12.33±0.33	14.33±0.67	18.67±1.20
XG	CH_3OH	24	16.33±0.88	16.33±1.20	21.67±0.67	22±0.58	19±1.00	19±0.58	15±0.58	20.33±0.88
	H_2O	10	13.67±0.33	16±0.58	14±1.0	12±1.16	16±1.16	13.67±0.67	15±0.58	17±1.0
	$CHCl_3$	17	13.67±0.67	15.67±0.33	17±0.58	13.33±0.88	12.33±0.33	14.33±0.67	13.67±0.67	15.6±0.88
BG	CH_3OH	21	18±1.16	20.33±0.88	18.67±1.20	17.67±0.88	19±1.16	18.33±1.12	19±0.58	14.67±0.88
	H_2O	9.6	19±1	15.33±0.67	13.67±0.67	15.67±0.68	17.67±0.33	14.33±0.88	14.67±0.33	13±0
	$CHCl_3$	14	ND	14.33±0.67	12.33±0.33	ND	18.67±0.88	ND	16.33±0.88	12.33±0.33
HF	CH_3OH	18	17.67±0.33	15±0.58	14.33±0.67	16.67±0.88	12.33±0.33	15.67±0.33	18±1.16	18±1.16
	H_2O	7.1	13.33±0.33	15±0	ND	ND	ND	14.67±0.33	ND	17.33±0.67
	$CHCl_3$	11	ND	13.67±0.67	17.67±0.33	14.33±0.88	ND	16.33±0.88	15.67±0.88	ND
SM	CH_3OH	14	15.33±1.8	17±0.58	13.67±0.67	ND	17±0.58	15.6±0.88	19±1.16	14.33±0.67
	H_2O	2.3	ND	16±0.58	ND	ND	15.67±0.33	ND	ND	ND
	$CHCl_3$	7.3	13.67±0.67	ND	17.67±0.88	11.33±0.33	ND	17±0.58	16.33±0.88	ND
AM	CH_3OH	22	15±0	17±0.58	17±0.58	14.33±0.67	16.67±0.33	15.33±1.8	19±0.58	15.6±0.88
	H_2O	8.9	15±0	15.67±0.88	ND	ND	ND	ND	16.67±0.88	ND
	$CHCl_3$	15	14±0.58	ND	15.67±0.33	16.67±0.88	13.67±0.67	17.67±0.33	ND	14±0.58
AO	CH_3OH	23	16.33±0.88	15.33±1.8	16.33±0.88	18±1.16	18.67±0.67	17.67±0.88	19.33±0.88	13±0.58
	H_2O	11	16±0.58	ND	ND	ND	13.67±0.67	ND	13.33±0.33	ND
	$CHCl_3$	17	ND	16±0.58	17.67±0.68	15.6±0.88	ND	15.33±1.8	ND	11.33±0.33
SA	CH_3OH	18	16.33±0.88	17±1.16	15±0	14±0.58	15.67±0.88	17±0	16±0.58	15.6±0.88
	H_2O	6.6	17.67±0.68	13.33±0.88	17.67±0.33	13±0.58	ND	18.67±0.88	ND	ND
	$CHCl_3$	11	18±1.16	14.33±0.67	11.33±0.33	ND	ND	13.67±0.67	19.33±0.88	ND

Here CD: *Ceriops decandra,* XG: *Xylocarpus granatum,* BG: *Bruguiera gymnorrhiza,* HF: *Heritiera fomes,* SM: *Suaeda maritima,* AM: *Avicennia marina,* AO: *Avicennia officinalis,* SA: *Sonneratia apetala.* EC: *Escherichia coli*, SA: *Staphylococcus aureus*, SD: *Shigella dysenteriae*, SF: *Shigella flexneri*, VC: *Vibrio cholerae*, PA: *Pseudomonas aeruginosa*, BS: *Bacillus subtilis*, ML: *Micrococcus luteus.* ND: Not detected

Shigella flexneri with the methanol and aqueous extract, respectively, in the case of *X. granatum*. *B. gymnorrhiza* exhibited maximum zone of inhibition (20.3 mm) against *S. aureus*. The methanol extract of *H. fomes* gave maximum result (18 mm) against both for M. *luteus* and *B. subtilis*. The methanol extract of *S. maritima* (19 mm), *A. marina* (19 mm), *A. officinalis* (19.33 mm), and the chloroform extract of *S. apetala* (19.33 mm) jointly gave the highest zone of inhibition against *B. subtilis.*

Different bacterial strain-based cluster of various mangrove plant species used in this study is shown in Fig. 1 with proper bacterial strain leveling. The *E. coli* based cluster found that *B. gymnorrhiza*, *A. marina,* and *A. officinalis* showed 100% similarity among them. From this analysis, it seems that they may contain the same or different type of compounds which possess the same mechanism to inhibit the growth of *E. coli*. Similarly, the cluster of *S. aureus* showed that there is 100% similarity between *C. decandra* and *A. officinalis* and, also for *H. fomes*, *S. maritima,* and *A. marina*. This cluster seems that *C. decandra* and *A. officinalis* may follow the same mechanism for inhibition of the growth of *S. aureus*. It also found from this cluster that unlike the mechanism of *C. decandra* and *A. officinalis*, the *H. fomes*, *S. maritima,* and *A. marina* possess the same mechanism for inhibition the growth of the respective bacterium. The cluster of *S. dysenteriae* found the 100% similarity between *C. decandra, H. fomes,* and *S. maritima* and indicates having the same mechanism for all these species against the respective microbe. This cluster also showed 100% similarity between *A. marina* and *A. officinalis* which indicates having the same type mechanism for these two species against the *S. dysenteriae* other than the mechanism of *C. decandra, H. fomes,* and *S. maritima*.

The cluster of *S. flexneri* showed 100% similarity between *H. fomes* and *A. officinalis* and also for *B. gymnorrhiza* and *S. apetala*. Based on this cluster, these two different groups of mangrove species may follow two different types of mechanism against the respective bacterium. The cluster of *V. cholerae* demonstrated the 100% similarity between *H. fomes* and *S. apetala* and also for *B. gymnorrhiza* and *A. marina*. In this case, the first group of mangroves has the same mode of action (s) against the growth of *V. cholerae* and the second group of mangroves possesses a similar mechanism rather than the first group of mangroves to inhibit the growth of the respective bacterium. In the present investigation, the *P. aeruginosa* was the only bacterium which showed a unique cluster for all mangrove species. This cluster does not show 100% similarity between any mangrove species. However, there is less than 100 % similarity between *B. gymnorrhiza* and *S. maritima*. This type of similarity indicated that there might be one or more compounds that react with *P. aeruginosa* slightly same or different way. The cluster of *B. subtilis* found that there might be total three different types of pathways to inhibit the growth of respective microbe and each group shared a unique mechanism of pathway among the three ways. The cluster of *M. luteus* also showed three groups of mangroves that found 100% similarity among them, that is, *A. marina* and *A. officinalis* in one group, the other between *X. granatum* and *B. gymnorrhiza* and the last group in between *S. maritima* and *S. apetala*. This result was similar to the cluster of *B. subtilis*. Here also might be three different pathways exit that works to inhibit the growth of *M. luteus* and each group shared a unique technique to inhibit the growth of respective bacterium among the three ways.

Antifungal activity

The antifungal activity of methanol extracts of mangrove plants was presented in Table 3. The chloroform and aqueous extracts of all mangrove species were not able to give antifungal activity against selected fungal pathogens. It was also noticed that the methanol extract of three mangrove species, that is, *S. maritima*, *A. officinalis,* and *A. marina* showed no inhibition zone for any fungal species and, *C. decandra, B. gymnorrhiza,* and *S. apetala* showed antifungal activity against selected fungal pathogens. The maximum zone of inhibition was found against *Botrytis cinerea* (15 mm), *F. oxysporum* (16.33 mm), and *R. oryzae* (13.33 mm) with the extract of *X. granatum, S. apetala,* and *C. decandra,* respectively.

Vundru *et al.* [23] found that the chloroform yielded much phytochemicals from leaves than methanol in the case of *A. marina, B. gymnorrhiza,* and *C. decandra* which was collected from Andhra Pradesh, India and also showed that the chloroform extract of said mangrove species exhibited higher antibacterial and antifungal activities against few strains of total

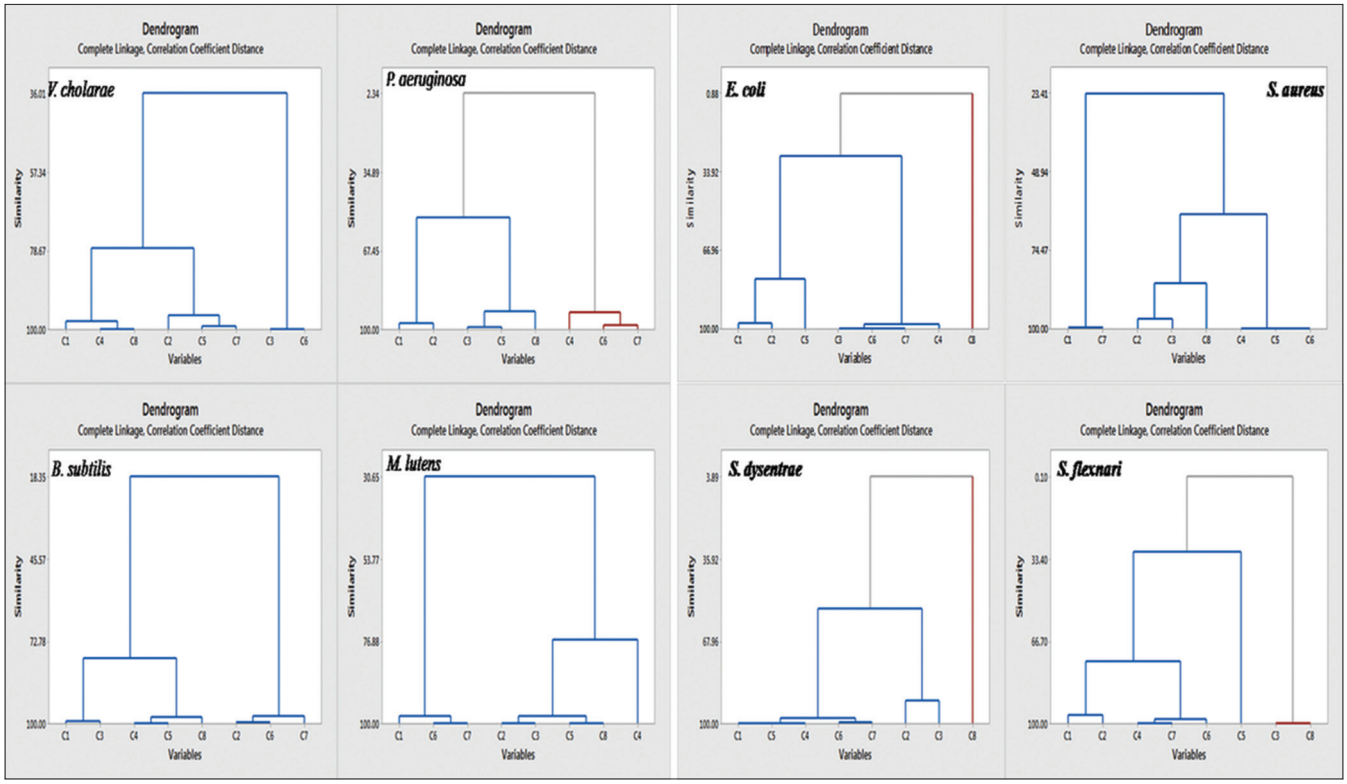

Fig. 1: Dendrogram of the cluster analysis of eight different mangrove plants according to their response against different bacteria (according to similarity index value). C1: *Ceriops decandra*, C2: *Xylocarpus granatum*, C3: *Bruguiera gymnorrhiza*, C4: *Heritiera fomes,* C5: *Suaeda maritima,* C6: *Avicennia marina*, C7: *Avicennia officinalis*, C8: *Sonneratia apetala*

Table 3: Evaluation of antifungal activity (mm) of methanolic extracts of eight different mangrove species

Plant species	*Botrytis cinerea*	*Fusarium oxysporum*	*Rhizopus oryzae*
Ceriops decandra	ND	11±0.58	13.33±0.88
Xylocarpus granatum	15±1.16	15±0.58	13±1.16
Bruguiera gymnorrhiza	13±0.58	ND	11±0.58
Heritiera fomes	14.33±0.88	12.67±0.67	12±0.58
Suaeda maritima	ND	ND	ND
Avicennia marina	ND	ND	ND
Avicennia officinalis	ND	ND	ND
Sonneratia apetala	14.67±0.88	16.33±0.88	ND

ND: Not detected

tested pathogens. However, this study showed that the methanol yielded much phytochemicals than water and chloroform. This study also found that the methanol gave maximum antibacterial activities than other solvents and only methanolic extract exhibited the antifungal activities. This finding may correlate with other findings which indicated that methanol showed a superior degree of inhibitory action as compared to other solvents [24,25].

Methanol is known to be a highly polar solvent and it is used to extract a wide range of molecules [26]. The amount of the biologically active components that can be extracted from a plant material is mainly affected by the efficiency of the extracting solvent to dissolve endogenous compounds might be very important which may probably vary from sample to sample and also from plant species to species [27].

The results of the present study clearly indicated that mangrove plant extracts found antibacterial activity against tested pathogenic bacterial strains, including antibiotic-resistant strains. However, some plant extracts were unable to exhibit antimicrobial activity against tested fungal strains which is a common phenomena during the determination of the antifungal activities [28]. These pathogens may have some kind of resistance mechanisms, for example, inactivation of enzymes, modification of target sites, and the decrease intracellular drug accumulation [29] or the concentration of the extracts applied may not be efficient.

CONCLUSIONS

The present research clearly showed that plant extracts of mangroves have great potential as antimicrobial compounds, particularly for the treatment of infectious diseases caused by resistant microorganisms. From this study, it might be concluded that mangrove plants could be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals. Further phytochemical studies are required to determine the type of compounds responsible for the antimicrobial effects of these medicinal mangrove plants.

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AUTHORS' CONTRIBUTIONS

Abdul Kader carried out the whole research work and wrote the manuscript. Sankar Narayan Sinha provided the bacterial strains, laboratory facilities, and supervised the whole study. Parthadeb Ghosh analyzed the results. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

REFERENCES

1. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global burden of disease study. Lancet 1997;349:1269-76.
2. Nair R, Kalariya T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turk J Biol 2005;29:41-7.
3. Neogi U, Saumya R, Mishra RK, Raju KC. Lipid content and *in vitro* antimicrobial activity of oil of some Indian medicinal plants. Curr Res Bacteriol 2008;1:1-6.
4. Al-Jabri AA. Honey, milk and antibiotics. Afr J Biotechnol 2005;4:1580-7.
5. Cohen ML. Changing patterns of infectious disease. Nature 2002;406:762-7.
6. Hancock EW. Mechanism of action of newer antibiotics for Gram positive pathogens. Lancet Infect Dis 2005;59:209-18.
7. Evans CE, Banso A, Samuel OA. Efficacy of some nupe medicinal plants against *Salmonella* Typhi: An *in vitro* study. J Ethnopharmacol 2002;80:21-4.
8. WHO issues guidelines for herbal medicines. Bull World Health Organ 2004;82:238.
9. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complement Altern Med 2005;5:6.
10. Jagessar RC, Mohamed A, Gomes G. An evaluation of the antibacterial and antifungal activity of leaf extracts of *Momordica charantia* against *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli*. Nat Sci 2008;6:1-14.
11. Rout SP, Choudary KA, Kar DM, Das L, Jain A. Plants in traditional medicinal system-future source of new drugs. Int J Pharm Pharm Sci 2009;1:1-23.
12. Kader A, Sinha SN. Heavy metal contamination in the sediment and plants of the Sundarbans, India. Chem Ecol 2018;34:506-18.
13. Chaudhuri P, Guha S. Potentiality of mangrove plant extracts for biocontrol of a pathogenic fungi, *Fusarium oxysporum*. Sci Cult 2010;76:271-4.
14. Kader A, Sinha SN, Ghosh P. Evaluation of genetic diversity of *Avicenniaceae* family in Indian sundarban by using RAPD and ISSR markers. Iran J Genet Plant Breed 2012;1:22-7.
15. Bandaranayke WM. Traditional and medicinal uses of mangroves. Mangroves Salt Marshes 1998;2:133-48.
16. Sinha SN, Paul D, Halder N, Sengupta D, Patra SK. Green synthesis of silver nanoparticles using fresh water green alga *Pithophora oedogonia* (Mont.) Wittrock and evaluation of their antibacterial activity. Appl Nanosci 2015;5:703-9.
17. Sinha SN, Paul D. Phytosynthesis of silver nanoparticles using *Andrographis paniculata* leaf extract and evaluation of their antibacterial activities. Spectrosc Lett 2015;48:600-4.
18. Bailey NT. Statistical Methods in Biology. Cambridge, UK: Cambridge University Press; 1995. p. 276.
19. Shanmugapriya R, Ramanathan T, Renugadevi G. Phytochemical characterization and antimicrobial efficiency of mangrove plants *Avicennia marina* and *Avicennia officinalis*. Int J Pharm Biol Arch 2012;3:348-51.
20. Dawane V, Fulekar MH. High-performance thin layer chromatography (HPTLC) fingerprinting pattern of mangrove *Avicennia marina*. Int J Pharm Pharm Sci 2017;9:65-72.
21. Harborne JB. Phytochemical methods. In: Harborne JB, editor. A Guide to Modern Techniques of Plant Analysis. London: Chapman and Hall; 1973.
22. Selvam K, Rajinikanth R, Govarthanan M, Paul A, Selvankumar T, Sengottaiyan A. Antioxidant potential and secondary metabolites in *Ocimum sanctum* L. at various habitats. J Med Plants Res 2013;7:706-12.
23. Vundru AK, Kandru A, Busi A. *In vitro* antimicrobial activity of leaf extracts of certain mangrove plants collected from Godavari estuarine of Konaseema delta, India. Int J Med Aromat Plants 2011;1:132-6.
24. Sharief N, Srinivasulu A, Veni PS, Rao VU. Quantification of tannins, antioxidant capacity and antibacterial activities of stem extracts of *Avicennia marina* L. Int J Biol Med Res 2014;5:3869-73.
25. Sharief N, Srinivasulu A, Chittibabu B, Rao VU. Isolation and purification of antibacterial principle from *Avicennia marina* L in methanol. Int J Pharm Pharm Sci 2015;7:38-41.
26. Negi PS, Chauhan AS, Sadia GA, Rohinishree YS, Ramteke RS. Antioxidant and antibacterial activities of various seabuckthorn (*Hippophae rhamnoides* L.) seed extracts. Food Chem 2005;92:119-24.
27. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of *Azadirachta indica, Terminalia arjuna, Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chem 2007;104:1106-14.
28. Pooja A, Arun N, Manider K. Screening of plant essential oils for antifungal activity against *Malassezia furfur*. Int J Pharm Pharm Sci 2013;5:37-9.
29. Schwarz S, Noble WC. Aspects of bacterial resistance to antimicrobials used in veterinary dermatological practice. Vet Dermatol 1999;10:1.