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

ABDUL KADER, PARTHADEB GHOSH, SANKAR NARAYAN SINHA*

Department of Botany, University of Kalyani, West Bengal, India. Email: sinhasn62@yahoo.co.in

Received: 30 April 2020, Revised and Accepted: 14 September 2020

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 [1,3,14]. 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 Bakaldali 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° 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/AA/11, KU/B/AA/12, KU/B/AA/14, KU/B/AA/15, KU/B/AA/16, KU/B/AA/22, KU/B/AA/29, and KU/B/AA/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.

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 2.167 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. 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.

© 2020 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i11.19526
Microorganisms

The test bacterial culture used for the antimicrobial assay were Gram-negative bacteria such as Escherichia coli (MTCC 443), Shigella dysenteriae (Clinical isolates), Shigella flexneri (MTCC 1457), Vibrio cholera (MTCC 3904), and Pseudomonas aeruginosa (MTCC 2581) and Gram-positive bacteria 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. dysenteriae (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 (10⁵ 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, glycossides, 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 –

\[
\text{Yield (\%)} = \frac{W_1 \times 100}{W_2},
\]

Where, \( W_1 = \text{Weight of the extract after evaporation of solvent} \)
\( W_2 = \text{Weight of the plant sample.} \)

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<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, glycossides 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 glycossides, 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 glycossides but the presence of saponin in much quantity.

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

| Plant species | Solvent extract | Bacterial name and diameter of zone of inhibition (mm) |
|---------------|----------------|------------------------------------------------------|
|               |                | EC | SA | SD | VF | VC | PA | BS | ML |
| CD            | CH,OH          | 22 | 16.7±0.33 | 1.7±0.11 | 11.3±0.33 | 19.6±0.33 | 19±0.58 | 17±0.58 | 16.3±0.88 | 20.3±0.67 |
| H$_2$O        | 8.6            | ND | ND | ND | ND | ND | ND | ND | ND |
| XG            | CH,OH          | 24 | 16.3±0.88 | 16.3±0.12 | 21.6±0.67 | 22±0.58 | 19±0.10 | 15±0.58 | 20.3±0.88 |
| BG            | CH,OH          | 21 | 18±1.16 | 20.3±0.88 | 18.6±1.20 | 17±1.16 | 16.3±0.67 | 15±0.58 | 17±1.0 |
| SM            | CH,OH          | 14 | 15.3±0.87 | 15±0.15 | 12.5±0.33 | 16±0.67 | 15±0.33 | 15±0.67 | 15±0.88 |
| H$_2$O        | 7.3            | 14 | 15.3±0.87 | 15±0.15 | 12.5±0.33 | 16±0.67 | 15±0.33 | 15±0.67 | 15±0.88 |
| SM            | CH,OH          | 14 | 15.3±0.87 | 15±0.15 | 12.5±0.33 | 16±0.67 | 15±0.33 | 15±0.67 | 15±0.88 |
| H$_2$O        | 7.3            | 14 | 15.3±0.87 | 15±0.15 | 12.5±0.33 | 16±0.67 | 15±0.33 | 15±0.67 | 15±0.88 |

The cluster of two different types of mechanism against the respective bacterium. This cluster also showed 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. apetala and S. maritima. This result was similar to the cluster of B. subtilis. Here also might be three different pathways exist 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 methanolic 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.

Vundrue et al. [23] found that the chloroform yield 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
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.

### ACKNOWLEDGMENTS

The authors are grateful to the University Grants Commission, New Delhi, for providing financial assistance for the present work. The authors extended their thanks to the Head of the Department of Botany, University of Kalyani, for providing DST-FIST central equipment facility.

### 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.

---

**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            | 13.3±0.88       |
| Xylocarpus granatum    | 15±1.16          | 15±0.58            | 13±1.16         |
| Bruguiera gymnorrhiza  | 13±0.58          | ND                 | 11±0.58         |
| Heritiera fomes        | 14.3±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       | 16.3±0.88          | ND              |

ND: Not detected

---

**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**
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. Rair N, Kalariair V, 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. Evans CE, Banso A, Samuel OA. Efficacy of some Nupe medicinal plants against Salmonella Typhi: An in vitro study. J Ethnopharmacol 2008;1:1-6.

7. WHO issues guidelines for herbal medicines. Bull World Health Organ 2004;82:238.

8. Kader A, Sinha SN. Heavy metal contamination in the sediment and plants of the Sundarbans, India. Chem Ecol 2018;34:506-18.

9. Chaudhuri P, Guha S. Potentiality of mangrove plant extracts for biocontrol of a pathogenic fungi, Fusarium oxysporum. Sci Cult 2010;76:271-4.

10. Bandaranayake WM. Traditional and medicinal uses of mangroves. Mangroves Salt Marshes 1998;2:133-48.

11. Sinha SN, Paul D. Phyto synthesis of silver nanoparticles using Andrographis paniculata leaf extract and evaluation of their antibacterial activities. Spectrosc Lett 2015;48:600-4.