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Evaluation of antimicrobial properties from the mangrove *Rhizophora apiculata* and *Bruguiera gymnorrhiza* of Burmanallah coast, South Andaman, India

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ABSTRACT

Objective: To evaluate the antimicrobial potential of partially purified leaf, bark and root extracts obtained from two mangrove species *Rhizophora apiculata* and *Bruguiera gymnorrhiza* from South Andaman against clinical bacterial and fungal pathogens.
Methods: Roots, bark and leaves were dried in the shade and subjected to organic solvent extraction. Antibacterial and antifungal activities were performed by agar well diffusion technique. Column purified extracts were analyzed by high performance liquid chromatography for compound identification.
Results: Results of the partially purified extracts were analyzed by column chromatography. Fractions collected by high performance liquid chromatography exhibited a wide range of antimicrobial activities against several bacterial and fungal pathogens. Fungal pathogen *Aspergillus niger* (25 mm) was found to be more sensitive against the mangrove extracts as compared with *Klebsiella pneumoniae* (23 mm), *Escherichia coli*, *Shigella flexneri*, *Salmonella typhi* (22 mm). Active fractions were identified as tannin compounds based on the peaks obtained by high performance liquid chromatography.
Conclusions: Present findings reveal that mangrove bark, roots, and leaves contain valuable metabolites, which have significant importance in the pharmacological industries. Hence, this study suggests that these two mangrove plants *Rhizophora apiculata* and *Bruguiera gymnorrhiza* are potential candidates for discovering antimicrobial compounds against clinical pathogens.

1. Introduction

Mangroves are known to be a rich source of various secondary metabolites and these higher plants are widely used for traditional medicine practices. *Rhizophora apiculata* (*R. apiculata*) and *Bruguiera gymnorrhiza* (*B. gymnorrhiza*) belong to the family Rhizophoraceae. They are mainly distributed around seashores and mangrove swamps in coastal proximal and middle zones and these species have been used as traditional medicines for healing various diseases[1]. These genera are known to be used in the treatment of angina, haemorrhages, hematuria and the older leaves and roots are used for childbirth[2,3]. *Rhizophora* contains various phytochemical substances such as diterpenoids, triterpenoids, sesquiterpene, daucosterol, atranorin, palmitone, polyphenols, polymeric tannins and hydrolysable tannins which have significant medicinal values[4]. Bark, roots and leaves of *B. gymnorrhiza* have also been used in treatment of diarrhoea, malaria and burns whereas its fruits have been used in the treatment of shingles and eye diseases[5]. Besides these medicinal importance, *Rhizophora* are rich in tannins, saponins and other volatile oils[6]. Apart from these importance, they are also used for natural anti-insecticidal and antifeedant agents, mosquito repellents and anti-larvicidal properties[7,8]. Mangroves present in Andaman Islands are found to be rich, which is available and important in the medical field. The present investigation was undertaken to extract the antimicrobial compounds from *Rhizophora* and *Bruguiera* species collected from South Andaman.

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2. Materials and methods

2.1. Identification and collection of mangroves

Leaves, bark and roots of the two mangroves *R. apiculata* and *B. gymnorrhiza* were collected from Burmanallah (11°39'281" N, 92°95'452" E), Port Blair, South Andaman. The collected samples were brought to the laboratory and identified by using proper identification keys[3].

2.2. Preparation of crude extracts

Mangroves samples were washed with sterile distilled water to remove epiphytes and other foreign particles and dried under shade for 20 days, and pulverized into fine powder by using mechanical mixer grinder. A total of 25 g of these powders from the bark, roots and leaves were soaked in 100 mL of organic solvent having different polarities like methanol, ethanol, hexane and aqueous solvents at a ratio of 1:4. The containers were sealed and stored for a period of 5 days. The crude extract mixtures were filtered by using Whatmann No. 1 filter paper and the filtrate obtained was concentrated by using a rotary evaporator (Buchi). This crude extract was transferred into air tight bottles and stored at 4 ˚C till further use.

2.3. Partial purification

Crude extracts obtained from different solvents were applied for partial purification by column chromatography. Different fractions collected from different solvents were fractioned at a ratio of 3:2:1 (methanol: acetone: ethyl acetate). The antimicrobial activities of the fractions were analyzed by Shimadzu high performance liquid chromatography (HPLC), column C18 (250 mm × 4.6 mm). The mobile phase [methanol: acetonitrile: water (25:35:40)] was used, and peaks obtained from each extract were compared with the standards.

2.4. Microbial cultures

Five pathogenic bacteria *Staphylococcus aureus* MTCC96 (*S. aureus*), *Escherichia coli* MTCC 443 (*E. coli*), *Salmonella typhi* MTCC 733 (*S. typhi*), *Klebsiella pneumoniae* MTCC 109 (*K. pneumoniae*) and *Shigella flexneri* MTCC 1457 (*S. flexneri*) and four fungal strains *Aspergillus niger* (*A. niger*), *Aspergillus flavus* (*A. flavus*), *Trichoderma* sp and *Rhizopus* sp were used in this study.

2.5. Inoculum preparation for bacteria and fungi

Standard microbial techniques were used for media preparation and other work. Nutrient broth, Mueller-Hinton agar and potato dextrose agar (Himedia, Mumbai) were prepared according to the manufacturer's instructions and then sterilized in an autoclave at 121 ˚C and 15 pounds pressure for 15 min. The bacterial strains were inoculated in sterilized nutrient broth and were incubated at 37 ˚C for 24 h. Fungal inoculums were prepared by diluting fungal strains in sterile distilled water.

2.6. Well diffusion method

The antibacterial and antifungal activities were determined by the following techniques[9]. Suspensions of each bacterial and fungal strain were carefully mixed in the test tube and the respective strains were cotton swabbed on Petri dishes containing Muller-Hinton agar and potato dextrose agar (Himedia, Mumbai). Wells (8 mm) were prepared in these plates using cork borer by maintaining sterile conditions. Then, these concentrated methanol, ethanol, hexane and aqueous extracts were prepared to a final concentration of 1 g/mL. For the minimal inhibitory concentration, 1 mg/mL was used for the activity by loading 50 µL of samples to the wells separately. Methanol, ethanol, hexane and water were used as negative controls and gentamicin as a positive control. All the plates were incubated at 37 ˚C for 24 h. The growth inhibition zones produced by the methanol, ethanol, hexane and water extractions of mangroves were examined and the results were recorded in diameter (mm).

3. Results

In the present study, partial purified extracts obtained from leaves, bark and roots of *R. apiculata* and *B. gymnorrhiza* exhibited antimicrobial activities against a wide range of bacterial and fungal pathogens. Column purified methanolic extracts were found to be tannin from *R. apiculata* (Figure 1). Tannin and some other compounds obtained from bark of *B. gymnorrhiza* (Figure 2) showed zones of inhibition against fungal pathogens *A. niger* (25 mm) and *R. apiculata* roots showed a maximum zone against fungal pathogens *A. niger* (24 mm). Methanolic extracts of *B. gymnorrhiza* leaves also exhibited antifungal activities against *A. niger* (22 mm) whereas bark and root extracts showed moderate activities against *Rhizopus* sp (Figure 3).

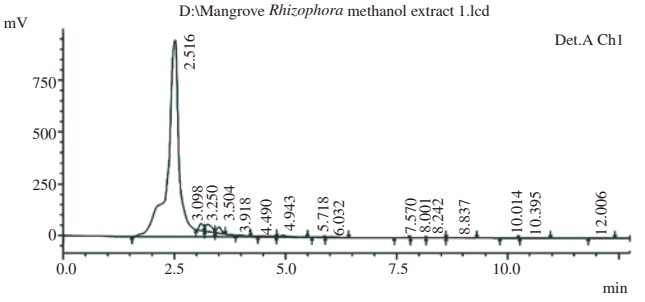

Figure 1. HPLC fractions of *R. apiculata.*

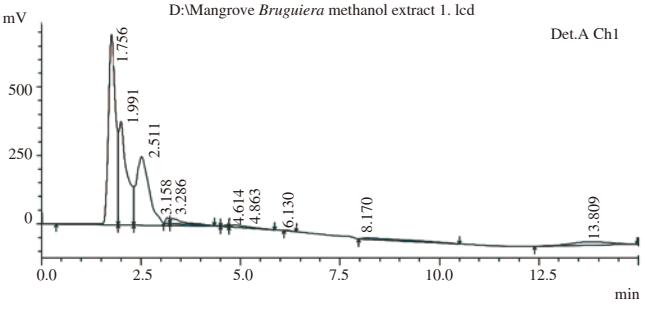

Figure 2. HPLC fractions of *B. gymnorrhiza.*

Hexane root extracts of *R. apiculata* showed moderate activities against *A. niger* (16 mm). The hexane extracts of the leaves, bark, roots of *B. gymnorrhiza* and *R. apiculata* did not show any

inhibition against fungal strains. While ethanol extracts obtained from leaves, bark and roots of *R. apiculata* showed significant activities against *A. niger* (15 mm, 16 mm and 10 mm), respectively, and the leaf extracts of *B. gymnorrhiza* showed moderate activites against *A. niger* (14 mm) (Figure 3).

Aqueous extracts of *R. apiculata* and *B. gymnorrhiza* exhibited inhibition zones against *A. niger* and these extracts did not show any zone of inhibition against other pathogens. Methanol, hexane, ethanol and aqueous extracts showed appreciable activities against fungal pathogens *A. niger* and *Rhizopus* sp. However, it did not inhibit the growth of *A. flavus* and *Trichoderma* sp and they showed their resistance against all the solvent extracts (Figure 3).

Methanolic leaves extract of *R. apiculata* and *B. gymnorrhiza* showed a broad spectrum of activity against *K. pneumoniae* (23 mm) and *E. coli* (22 mm), respectively. Moderate antibacterial activity has been observed in leaves, bark and roots extracts of *R. apiculata* and *B. gymnorrhiza* against the rest of other pathogens, *S. typhi*, *S. flexneri* and *S. aureus* (Figure 4).

Hexane bark and leaves extracts of *B. gymnorrhiza* showed the highest activity against *K. pneumoniae* (23 mm), *S. typhi* (22 mm), *S. aureus* (19 mm) and *S. flexneri* (22 mm), respectively. Hexane extracts showed noticeable activity against all bacterial pathogens (Figure 4).

Ethanol extracts from leaves and bark of *B. gymnorrhiza* exposed the highest activity against *S. typhi* (18 mm and 20 mm), respectively. Root and bark extracts of *R. apiculata* showed inhibition zones against *K. pneumoniae* (18 mm and 14 mm). Ethanolic extracts of leaves, bark and roots of two species have not shown any inhibition zones against *S. flexneri* and *E. coli* (Figure 4).

Leaf and bark aqueous extracts of *R. apiculata* showed noticeable activity against *S. aureus* (14 mm and 17 mm), respectively. *R. apiculata* bark and root aqueous extracts showed moderate activity against *S. typhi* (13 mm and 12 mm), respectively. While *B. gymnorrhiza* leaves and bark aqueous extracts showed moderate activity against *S. typhi* (11 mm and 15 mm). Aqueous extract of *R. apiculata* and *B. gymnorrhiza* did not show any activity against *E. coli*, *S. flexneri* and *K. pneumoniae* (Figure 4).

4. Discussion

Over the past two decades, bacterial pathogens are becoming resistant to the existing antibiotics, hence researchers are targeting compounds from marine plants, animals and bacteria for the discovery of new drugs to control the pathogens. In the present study, the antimicrobial activity was observed from the powder of leaves, bark and roots of two mangroves *R. apiculata* and *B.*

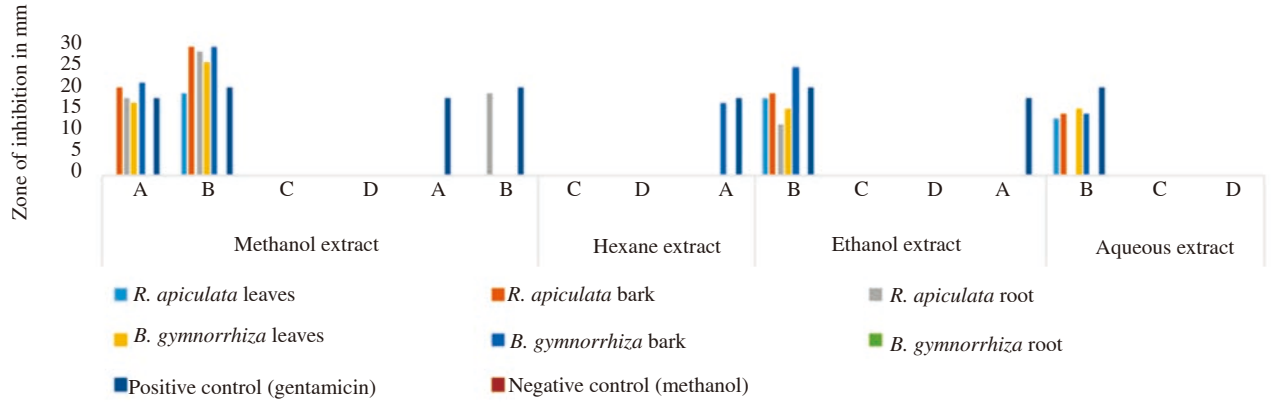

Figure 3. Antifungal activities of *R. apiculata* and *B. gymnorrhiza* at 50 µL concentration.
A: *Rhizopus* spp; B: *A. niger*; C: *A. flavus*; D: *Trichoderma* spp.

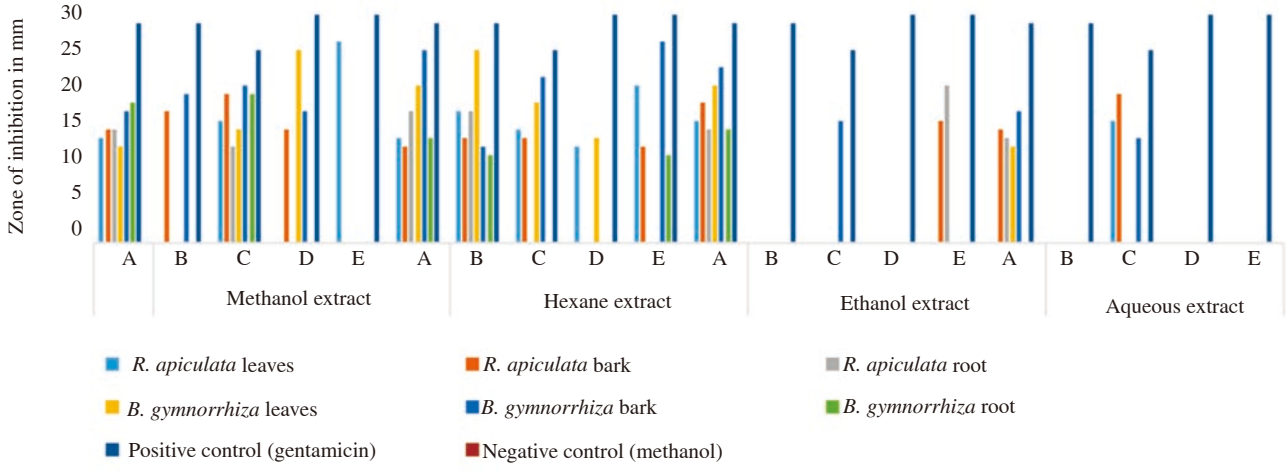

Figure 4. Antibacterial activities of *R. apiculata* and *B. gymnorrhiza* at 50 µL concentration.
A: *S. typhi*; B: *S. flexneri*; C: *S. aureus*; D: *E. coli*; E: *K. pneumoniae*.

gymnorrhiza. Earlier antifungal and antibacterial properties were reported from *R. apiculata*[10]. A similar kind of activity was obtained from leaves extracts, which was found to inhibit multi-drug resistant pathogen (*S. aureus*)[11]. *Rhizophora mucronata* (*R. mucronata*) contains various compounds which are very active against *A. niger* and *A. flavus*[12]. *R. mucronata* extract is also found remarkable activities against methicillin resistant *S. aureus*[13]. Present attempts also revealed that methanol extracts of *R. apiculata* obtained from its bark showed higher zone of inhibition against *A. niger* (25 mm) but showed their resistance against *A. flavus*. *B. gymnorrhiza* showed moderate activities against the human pathogens *E. coli* and *S. typhi* and exhibited activities against *S. aureus*, *E. coli* and *A. niger*[14,15]. The present investigation found similar results obtained from the earlier studies of *B. gymnorrhiza* plant extracts and also found significant antibacterial and antifungal activities against *E. coli*, *S. typhi*, *S. aureus* and *A. niger*. Saponins, glycosides, tannins, flavonoids, phenols and volatile oils are the main components which are found in the extracts obtained from the leaves of *R. mucronata* which are responsible for the antibacterial activity[6]. Similar kind of compound tannins were commonly found in the barks of Brazilian mangrove *Rhizophora mangle*, which showed significant activity against yeast[16]. The present study also agreed with the earlier findings that methanol extracts of *R. apiculata* and *B. gymnorrhiza* showed significant activities against *S. aureus* (17 mm and 18 mm), respectively. Tannin-like compounds were observed in the partially purified extracts. Cold ethanol extract of *R. apiculata* exhibited moderate activity against *S. typhi* and significant activity towards other tested pathogens[17]. The present investigation also found that the ethanol extracts of *R. apiculata* leaves showed similar activity. Methanol and ethanol crude extracts of *B. gymnorrhiza* leaves and bark exhibited considerable zones of inhibitory activity against *E. coli* and *S. aureus*[5]. The present study was also in agreement with the earlier findings that methanolic extracts of *B. gymnorrhiza* leaves (22 mm) and bark (15 mm) showed maximum activity against *E. coli* and ethanol extracts of leaves (13 mm) and bark (18 mm) showed appreciable activity against *S. aureus*. Laith *et al.* found that the inhibition zone was very less (9 mm) with *R. apiculata* leaves extract obtained from methanol against *K. pneumoniae*[18]. In this study, zone of inhibition (23 mm) was observed in leaves extract of *R. apiculata* obtained from high polar solvent methanol at lower concentration against *K. pneumoniae*. Highlights of this study show that roots, leaves and bark of *R. apiculata* and *B. gymnorrhiza* were found to be potential sources for the characterization of potential antimicrobial compounds. Tannins are found to have potent antimicrobial activity. Further screening is being undertaken to find out the characterization of compound from these active extracts.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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