

Evaluation of Antibacterial Activity and Characterization of Phytochemical Compounds from Selected Mangrove Plants

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Abstract

The aim of this study was to investigate the antibacterial activity of n-butanol, petroleum ether, ethyl acetate, ethanol, methanol, and chloroform extracts of the sponges and leaves of different mangrove species (*Bruguiera cylindrica*, *Suaeda maritima*, *Ceriops decandra*, *Avicennia officinalis*, *Rhizophora apiculata*, *Suaeda monoica*, *Avicennia marina*, and *Rhizophora mucronata*) against *Proteus mirabilis* ATCC 7002.

Methods and Results: Agar well diffusion method was used to evaluate the antimicrobial activity of selected mangrove extracts. The active compounds from mangrove sponges and leaves with various solvents were examined under Fourier transform infrared (FTIR) spectroscopy. The ethyl acetate extracts, methanol extracts, chloroform extracts, n-butanol extracts, and petroleum ether extracts of selected mangrove leaves (*B. cylindrical*, *S. maritime*, *A. officinalis*, *S. monoica*, *A. marina*, *R. mucronata*) showed good antibacterial activity against *P. mirabilis* ATCC 7002. FTIR analysis of crude methanol extract of *S. monoica* showed the presence of amide and alkane groups in phytochemicals. For crude methanol extract of *R. mucronata*, the presence of phenol, nitro, amide, and alkane groups was revealed.

Conclusion: the results obtained revealed industrially important mangrove extracts and the functional groups of plant compounds responsible for great antibacterial activity. (International Journal of Biomedicine. 2022;12(4):640-643.).

Keywords: mangrove species • solvents • antibacterial activity

For citation: Duraipandian M, Abirami H, Musthafa KS, Karuthapandian S. Evaluation of Antibacterial Activity and Characterization of Phytochemical Compounds from Selected Mangrove Plants. International Journal of Biomedicine. 2022;12(4):640-643. doi:10.21103/Article12(4)_RA22

Introduction

Among billions of microorganisms, bacteria are the dominant cause of many diseases in higher eukaryotes. Bacteria are present everywhere and have various sizes and shapes. Beneficial bacteria protect the bodily functions of organisms and also have the ability to preserve the environment. But some bacteria cause deadly diseases to people. An exponential increase in bacteria that are resistant to multiple drugs against antibiotics is a critical issue.⁽¹⁻³⁾ Currently, it has been shown that bacteria are highly interactive and exhibit several social behaviors, such as swarming motility, bioluminescence, conjugal plasmid transfer, antibiotic resistance, biofilm maturation, and virulence.⁽⁴⁻⁶⁾ The dramatically rapid and

continuous emergence of antibiotic resistance in the clinical context necessitates the urgent identification of novel strategies for treating bacterial infections, including research for screening of plants for their antimicrobial activity.

Mangroves, the littoral forests of tropics and subtropics, are well known for their ecological importance. The mangrove ecosystem is characterized by highly changeable environmental conditions, such as salinity, temperature, nutrients, and tidal currents, making it one of the most productive ecosystems.⁽⁷⁻⁹⁾ Mangroves are a rich source of several bioactive compounds (secondary metabolites) that have therapeutic significance, such as steroids, triterpenes, saponins, flavonoids, phenolic compounds, alkaloids, and tannins.⁽⁸⁾ Recently, many studies have been directed toward investigating the biological activity of mangroves for the treatment of various diseases.⁽¹⁰⁾ Due to the presence of the varied levels of bioactive compounds, research interest in mangrove plants for their therapeutic activities, including antimicrobial effects, is increasing continuously.⁽¹¹⁻¹⁴⁾

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The present study conducted in vitro experimental evaluation of the antibacterial activity of extracts of plant parts of *Bruguiera cylindrica* (B. *cylindrica*) [M1], *Suaeda maritima* (S. *maritima*) [M2], *Ceriops decandra* (C. *decandra*) [M3], *Avicennia officinalis* (A. *officinalis*) [M4], *Rhizophora apiculata* (R. *apiculata*) [M5], *Suaeda monoica* (S. *monoica*) [M6], *Avicennia marina* (A. *marina*) [M7], and *Rhizophora mucronata* (R. *mucronata*) [M8] in different solvent systems (methyl acetate, methanol, ethanol, n-butanol, chloroform and petroleum ether) against *Proteus mirabilis* ATCC 7002.

Proteus mirabilis (P. *mirabilis*), a kind of motile gram-negative bacteria in the Enterobacteriaceae family, is widely distributed in the environment, mainly in water, soil, and human and animal gastrointestinal tracts.⁽¹⁵⁾ It is an opportunistic pathogen.⁽¹⁶⁾ Among the gram-negative bacteria, P. *mirabilis* is the dominant biofilm producer in urinary tract infections, next to E. *coli*. Urease production and robust swarming motility are the 2 hallmarks of this organism. Clinically, P. *mirabilis* is most frequently a pathogen of the urinary tract, particularly in patients undergoing long-term catheterization.⁽¹⁷⁾

The aim of this study was to investigate the antibacterial activity of n-butanol, petroleum ether, ethyl acetate, ethanol, methanol, and chloroform extracts of the sponges and leaves of different mangrove species (M1-M8) against P. *mirabilis* ATCC 7002.

Materials and Methods

Plant collection

B. *cylindrical*, S. *maritime*, C. *decandra*, A. *officinalis*, R. *apiculata*, S. *monoica*, A. *marina*, R. *mucronata* were collected from the Thondi region of Rameswaram District, Tamil Nadu. The taxonomical identification of this plant was made by mangrove plants of Tamil Nadu Publication from M.S. Swaminathan Research Foundation.

Plant sample preparation and extraction methods

The fresh plant was washed under running tap water and dried in a warm place for 3 to 5 days. The samples were ground into a fine powder and the extract was prepared with different solvents, such as methanol, ethyl acetate, n-butanol, ethanol, petroleum ether, and chloroform. The extract was prepared by adding 0.5 g of sample powder in 10 ml solvents and keeping it in a shaker; then the collected solvent layer was dried in a water bath. Finally, the dried samples were kept in 2 ml Eppendorf tubes, to store for future use.

Bacterial strain

The target pathogen used in this study was P. *mirabilis* ATCC 7002, which was streaked and maintained in Luria-Bertani agar plates. For the antibacterial assay, the strain was cultivated in 2 ml of the sterile nutrient broth overnight, and 1% inoculum was subcultured for 3 hours in 2ml of sterile nutrient broth.

Antibacterial activity by the agar well diffusion method

The agar well diffusion assay was performed in nutrient agar plates. The nutrient agar medium was sterilized at 121°C for 20 minutes and poured into sterile Petri plates. The plates were allowed to solidify. Then the subcultured test pathogens

were swabbed on the nutrient agar plates and kept for a few minutes for drying. Wells with 5 mm diameter were made on each plate and 100 µl of crude extract obtained from sponges and leaves of mangroves were loaded on the well. The plates were incubated for 16 hours at 30°C, and the zone of inhibition against each pathogen was measured.

FTIR analysis of crude of mangrove leaves

The active compounds from mangrove sponges and leaves with various solvents were examined under Fourier transform infrared (FTIR) spectroscopy. FTIR spectrum of crude extract mixed with potassium bromide pellet was recorded using an FTIR spectrophotometer.

Results

The shade-dried samples of sponges and leaves of mangroves were extracted with various solvents, and the weight of each crude extract was weighed in a pre-weighed Eppendorf tube.

Antibacterial activity of different extracts of mangroves against P. mirabilis

A total of 8 different solvent extracts of mangrove plants were tested for their antibacterial activity against P. *mirabilis*. Optimum inhibitory activity was shown by the following extracts: The crude ethyl acetate extracts of M2, M4, M6, and M8 showed inhibitory zones of 16 mm, 17 mm, 18 mm, and 17 mm, respectively (Figures 1-2, Plates 1-2).

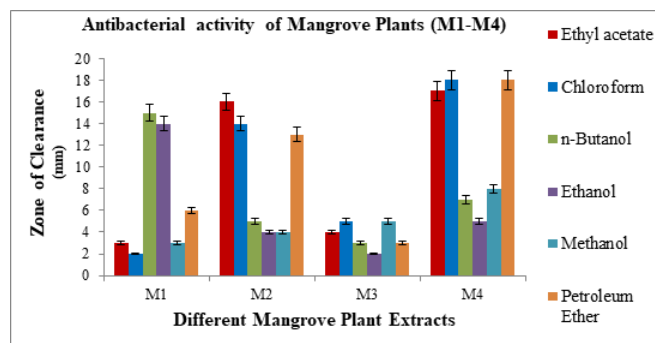


Fig. 1. Antibacterial activity in terms of zone of inhibition of different mangrove extracts (M1 to M4) in different solvent systems

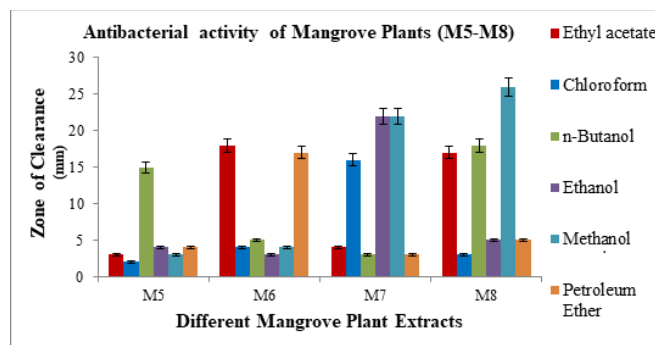


Fig. 2. Antibacterial activity in terms of zone of inhibition of different mangrove extracts (M5 to M8) in different solvent systems



(1) (2) (3)

Plates 1-3. The maximum antibacterial activity in terms of zone of inhibition of different mangrove extracts of M5 to M8 in ethyl acetate (2) and M6, M7 & M8 in methanol (3).

The crude methanol extracts of M7 and M8 showed inhibitory zones of 24 mm and 26 mm, respectively (Figure 2, Plate 3) The crude n-butanol extracts of M5 and M8 showed inhibitory zones of 15 mm and 18 mm, respectively (Figure 2). The crude petroleum ether extracts of M2, M4, and M6 showed inhibitory zones of 14 mm, 18 mm, and 17 mm, respectively (Figures 1-2). Out of chloroform extracts, the crude extracts of M4 and M7 showed inhibitory zones of 18mm and 16mm, respectively (Figures 1-2). Out of ethanol extracts, the crude extracts of M1 and M7 showed inhibitory zones of 14mm and 16mm, respectively (Figures 1-2).

The FTIR spectroscopy was done to identify the functional group of the antibacterial compound present in the crude methanol extracts of M6 and M8. The FTIR peaks of methanol crude extract of M6 showed the presence of amide and alkane groups (Figure 3), whereas FTIR peaks of crude methanol extract of M8 showed the presence of phenol, amide, nitro, and alkane groups (Figure 4).

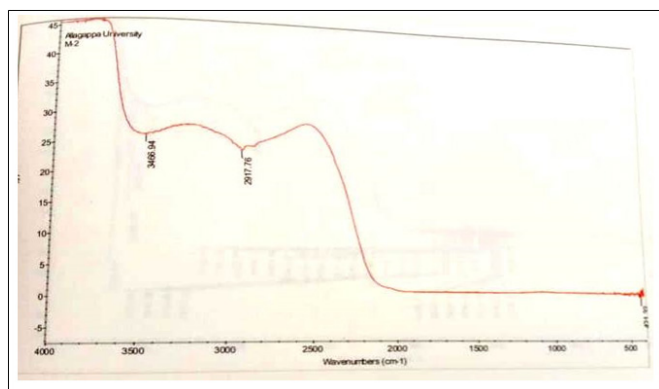


Fig. 3. FTIR spectrum of methanol extracts of mangrove leaves of M6

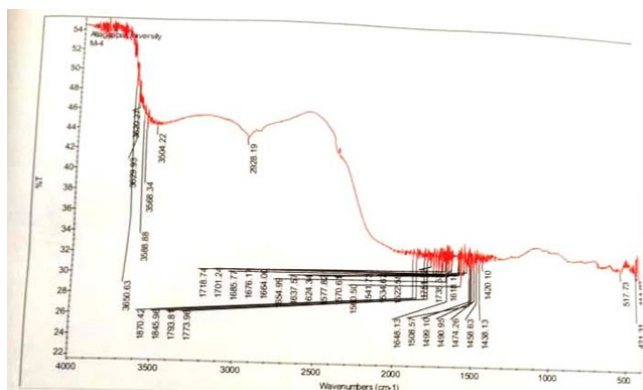


Fig. 4. FTIR spectrum of methanol extracts of mangrove leaves of M8

Discussion

The mangrove plants are the sources of potent drugs.⁽¹⁸⁾ These drugs have many potential properties, such as antiviral, antibacterial, anthelmintic, antifungal, or antimalarial.⁽¹⁹⁻²⁰⁾ However, the extraction of novel natural chemical compounds from mangroves, in addition to those already known to the pharmacological compound, is still far from complete. Abeyasinghe et al.⁽²¹⁾ reported the promising antibacterial activity of *A. marina*. Moderate antibacterial activity is shown by *S. maritima* (Chenopodiaceae family) due to the presence of phenolic compounds like tannins.⁽²²⁾ The presence of glycosides, saponins, tannins, flavonoids, alkaloids, and terpenoids may influence the antibacterial activity of *A. officinalis*.⁽²³⁾ Das et al.⁽²⁴⁾ reported promising antibacterial activity due to the presence of terpenoids, phenolics, and alkaloids. Thus, pharmacological screening of natural products can be a source of innumerable therapeutic agents.⁽²⁵⁾

In our study, different crude extracts of the selected mangrove leaves made with different solvents showed a clear zone of inhibition. The ethyl acetate extracts, methanol extracts, chloroform extracts, n-butanol extracts, and petroleum ether extracts of selected mangrove leaves (*B. cylindrical*, *S. maritima*, *A. officinalis*, *S. monoica*, *A. marina*, *R. mucronata*) showed good antibacterial activity against *P. mirabilis* ATCC 7002. FTIR analysis of crude methanol extract of *S. monoica* showed the presence of amide and alkane groups in phytochemicals. For crude methanol extract of *R. mucronata*, the presence of phenol, nitro, amide, and alkane groups was revealed.

In conclusion, the results obtained revealed industrially important mangrove extracts and the functional groups of plant compounds responsible for great antibacterial activity.

Competing Interests

The authors declare that they have no competing interests.

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