OPEN ACCESS
Saudi Journal of Biomedical Research
Abbreviated Key Title: Saudi J Biomed Res
ISSN 2518-3214 (Print) |ISSN 2518-3222 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Antibacterial and Antifungal Activities of *Avicennia marina* Extract Against Various Multiple Drug Resistant Microorganisms

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DOI: 10.36348/sjbr.2023.v08i05.002 | **Received:** 08.04.2023 | **Accepted:** 11.05.2023 | **Published:** 16.05.2023

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Abstract

Avicennia marina is a commonly found mangrove species in coastal regions, and is known for its medicinal properties. In this study, the antibacterial and antifungal activities of ethanolic extract of *Avicennia marina* leaves were evaluated against MDR microorganisms. The antimicrobial activity of the extract was assessed against a range of bacterial strains, including *Salmonella* sp. (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), *Acinetobacter* sp. (MDR), *Staphylococcus aureus as well as the fungal strain Candida albicans* (ATCC 10231*), Candida parapsilosis* (ATCC 22019). The results showed that the leaf extracts of *Avicennia marina* exhibited significant antimicrobial activity against all tested MDR microorganisms. The phytochemical content of the plant includes many bioactive compounds - flavonoids, tannins, and alkaloids, which may be responsible for the observed antimicrobial activities. These findings suggest that *Avicennia marina* may be a potential source of natural antimicrobial agents, which could be used to develop new drugs for the treatment of resistant bacterial and fungal infections. Further studies are needed to identify and isolate the active compounds responsible for the observed activity and to evaluate their efficacy and safety *in vivo*.

Keywords: *Avicennia marina;* Antibacterial activity; Antifungal activity;*Salmonella* sp.*; Klebsiella* sp.*;Pseudomonas* sp.*; Acinetobacter* sp.*;Staphylococcus aureus; Candida albicans; Candida parapsilosis*.

INTRODUCTION

For a long period of time in history, plants play a crucial role as a source of natural products used for the health of human beings and they are capable for producing various types of drugs. Now mangrove plants are mostly used for producing various antibacterial and antifungal agents. The reason behind having this unique property is that they live in wetlands of subtropical coastal regions where they can survive against the high salinity and high temperature. The mangrove plants are also used for the production of bioactive compounds in traditional medicines. The effectivity of mangrove extracts from plant leaves has been detected against many pathogens (Fiori et al, 2000) Now in the healthcare industry, multi-drug resistance (MDR) bacteria is exponentially increased against many antibiotics, and for that reason failure of various treatments is happening. On the other hand, fungal resistance is also rising against various antibiotics due to the formation of biofilms by fungal strains (Candida parapsilosis, *Candida albicans*, etc). Microorganisms attach to the surface and form a very complicated microbial consortium called biofilms. Hence, it is time to develop such antibacterial and antifungal agents (Neu, 1992). In this study, *Avicennia marina*, a mangrove plant belonging to the Acanthaceae family, and its antimicrobial activity have been investigated. This tree can be survived against various environmental stresses such as high levels of salinity and temperature because of the presence of a large number of phytochemicals. *Avicennia marina* is used for the treatment of various health elements. Ulcers and rheumatism are treated by using leaves of this plant and fever and food poisoning are treated by using leaf decoction. Roots, stems, leaves, fruits, and seeds are the primary extract of *Avicennia marina* which are made in ethanol and evaluated the antibacterial activity against many bacterial species (Febriani et al, 2020; Mouafi et al, 2014). The effectiveness of these plant extracts depends on both the plant part and the solvent used for the extraction. And both the bacterial species and fungal species were grown in Mueller Hinton Broth and for preparing the bacterial suspension three bacterial colonies from each plate were emulsified in sterile 0.9%

NaCl to obtain 10^8 CFU per mL (0.5 McFarland scale) as inoculums. McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. Leaf extract's minimal inhibitory concentration (MIC) is determined against *Salmonella* sp. (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), *Acinetobacter* sp. (MDR), *Staphylococcus aureus as well as the fungal strain Candida albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 22019). Minimal inhibitory concentration is the lowest concentration of an antibiotic that inhibits the growth of a given strain of bacteria. It is used to determine the susceptibilities of these bacteria against drugs and helps to evaluate the activity of new antimicrobial agents (Sasidharan et al, 2011; Prihanto et al, 2011).

MATERIALS AND METHODS

Plant materials and Extraction

A.marina is commonly known as gray mangrove and its vernacular name is Tella belongs to the family Aviceniaceae, grows as a shrub or tree to a height of three to ten meters, or up to 14 meters in tropical regions, growing in the saline intertidal zones of sheltered coastlines. It has been reported to tolerate extreme weather conditions and high winds. The material was taxonomically identified and the voucher specimen is stored. The aerial plants are collected from Jharkhali (22.0306^{O}N, 88.7013^{O}E) of the Sundarbans, West Bengal, India- the largest Mangrove forest of the world. The leaves of the plant (Fig. 1) were collected in the Month of February, 2023, and were separately washed thoroughly with seawater to remove epiphytes, shells, and various extraneous matters. The cleaned plant parts were packed separately in polyethylene bags and brought to the laboratory. The leaves were cut into small pieces by scissors. Then Ethanol extracts of the samples were obtained by the following procedure. For extraction 1 g of the leaves was kept in 5 ml of ethanol for 72 h at room temperature. After that the extract was separated by centrifugation (Fig.1). The collected samples were centrifuged at 3000 rpm for 10 minutes (Goyal, 1989).

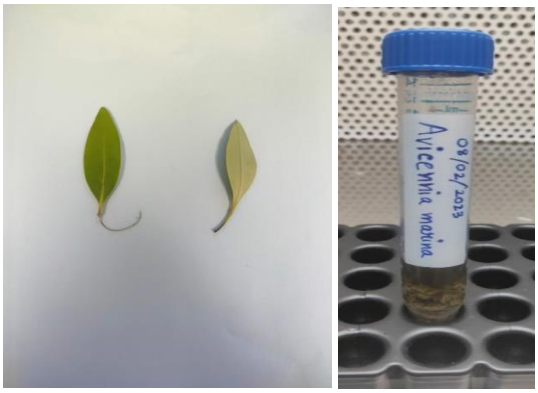

Figure 1: The leaf of *Avicennia marina* and ethanolic extract

Test Microorganisms and Culture Media

Five bacterial strains such as *Salmonella* sp. (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), *Acinetobacter* sp. (MDR), *Staphylococcus aureus* and two fungal strains of *Candida* sp. were selected for this study. Among these bacteria, *Salmonella* sp. (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), *Acinetobacter* sp. (MDR) are gram-negative in nature and *Staphylococcus aureus* is Gram positive in nature. The fungal strains were- *Candida albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 22019). The bacterial species and fungal species both were grown in Mueller–Hinton broth (Hi-media).

Inoculum Preparation

From the stock cultures, each strain of bacteria and fungi were streaked on the agar plate. The plate was then incubated for 24 h at 37 °C. Three bacterial and fungal colonies from each plate were emulsified in sterile 0.9% NaCl (w/v) to obtain 10^8 CFU per mL (0.5 McFarland scale) as inoculums for MIC value determination (Onawunmi, 1989).

Minimum inhibitory concentration (MIC) assays

The antibacterial and antifungal activity was determined followed by Minimum Inhibitory Concentration (MIC). In each microtitre well 100µl of plant extract was poured, then 100 µl of the extract was mixed with it in the first well. Then 100µl of this was added to the next well with 100µl of Mueller Hinton broth, this procedure was continued up to the eighth well. Thus concentration of the extract became half in the first well and serially double diluted till the eighth well. Then 10µl of the different bacterial suspension was added to each well in separate rows. On the other hand, there was a control row chosen for each bacterial strain where the plant extracts were not added; only MH broth, bacterial suspension, and ethanol were present serially diluted. Then the optical density was measured at 620 nm to be used as a baseline absorbance values. After completion of Optical density measuring, then the plate was incubated at 37°C for 24 hours. After 24 hours again the optical density was measured at 620 nm. After this the optical density values of the wells in initial readings were subtracted from the final readings, The MIC value was determined by finding out the lowest concentration of the extract which produced lower optical density value than the control well optical density.

RESULTS

The analysis of the results showed *A. marina* extract was effective against all microbial strains tested in this study – both ATCC strains and MDR strains. *Salmonella* sp. MDR strain showed a MIC value of 6.12 mg/ml; *Pseudomonas aeruginosa* sp. MDR, *Acinetobacter baumannii* sp. MDR, *Staphylococcus aureus*, *Candida parapsilosis* ATCC 22019 sp. All showed MIC value of 3.6 mg/ml; *Klebsiella*

pneumoniae sp. MDR showed MIC value of 1.8 mg/ml. All results are shown in Figure 2-8.

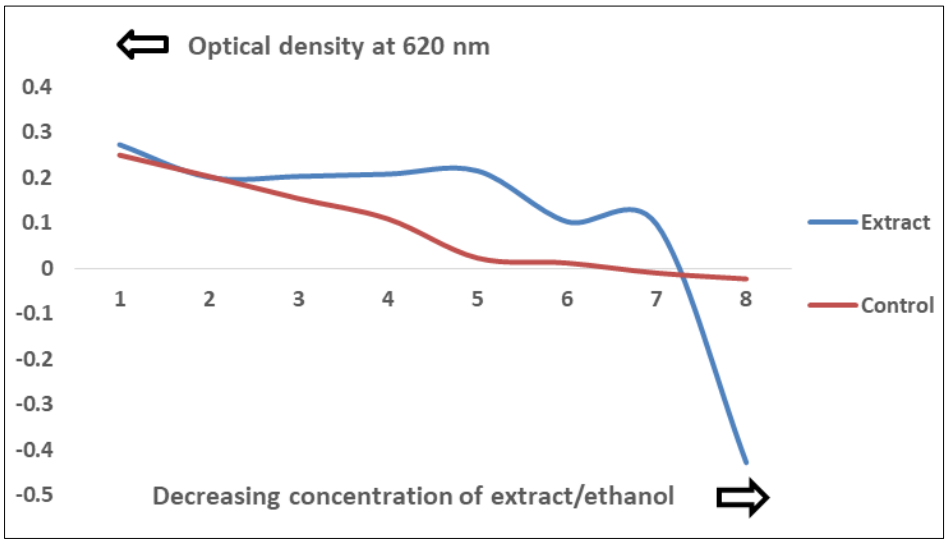

Figure 2: Effect of *A. marina* extract on *Salmonella sp.* (MDR) showing a MIC value of 6.12 mg/ml. Ethanol could act when it became 70% concentration

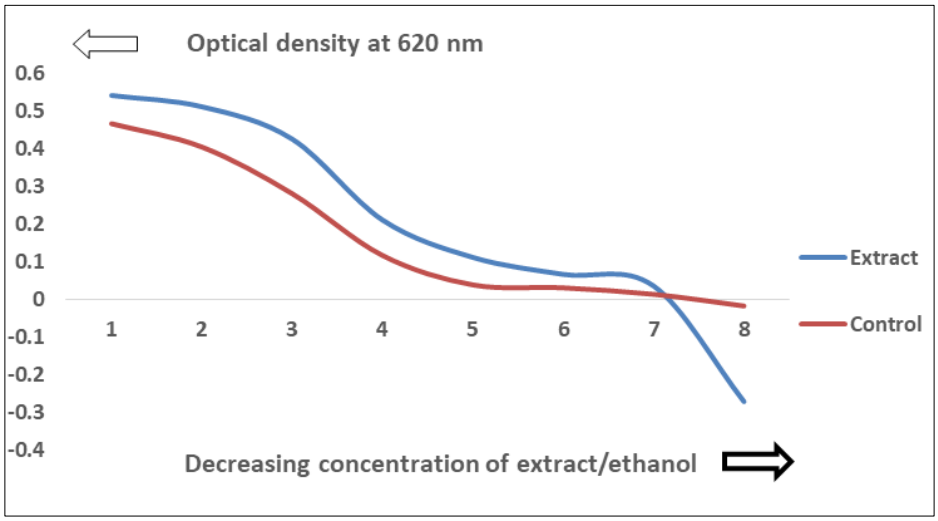

Figure 3: Effect of A. *marina* extract on *Klebsiella sp.* (MDR) showing a MIC value of 1.8 mg/ml. Ethanol could act when it became 70% concentration

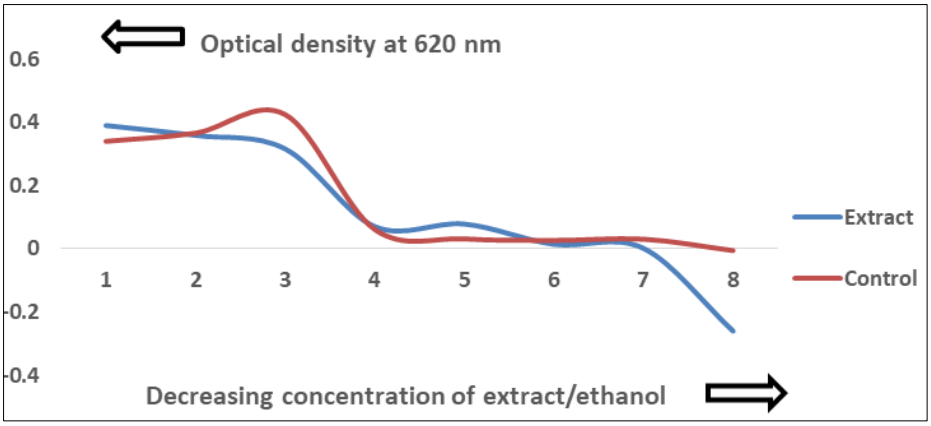

Figure 4: Effect of A. *marina* extract on *Pseudomonas sp.* (MDR) showing a MIC value of 3.6 mg/ml. Ethanol could act when it became 70% concentration

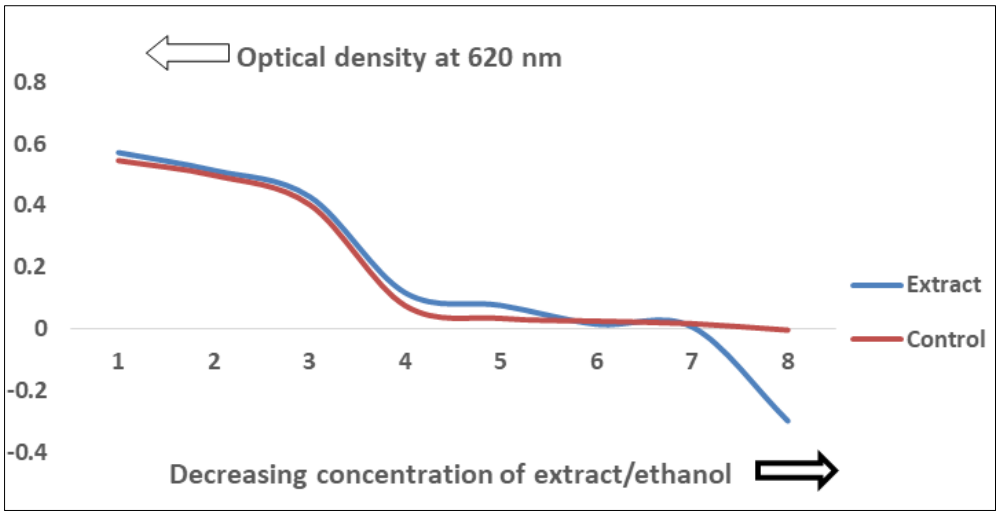

Figure 5: Effect of A. *marina* extract on *Acinetobacter sp.* (MDR) showing a MIC value of 3.6 mg/ml. Ethanol could act when it became 70% concentration

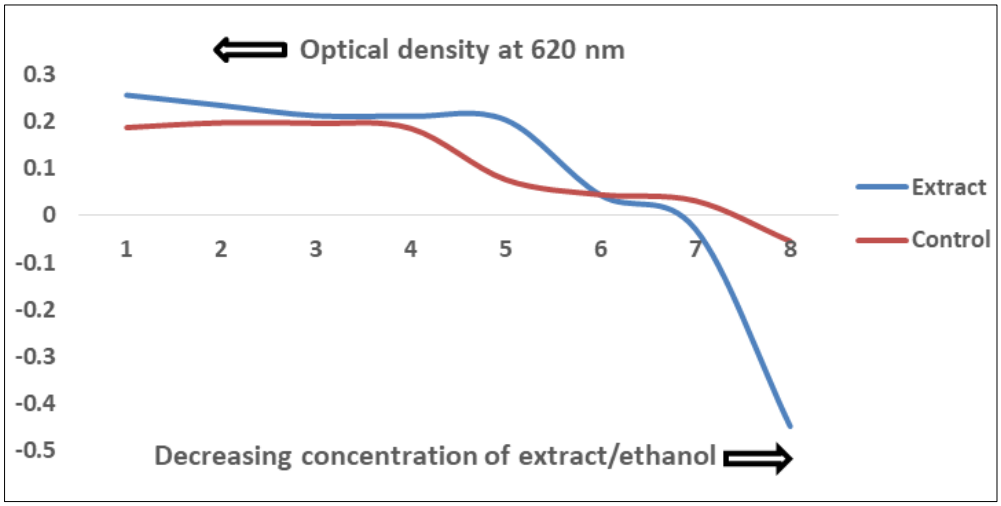

Figure 6: Effect of A. *marina* extract on *Staphylococcus aureus* showing a MIC value of 3.6 mg/ml. Ethanol could act when it became 70% concentration

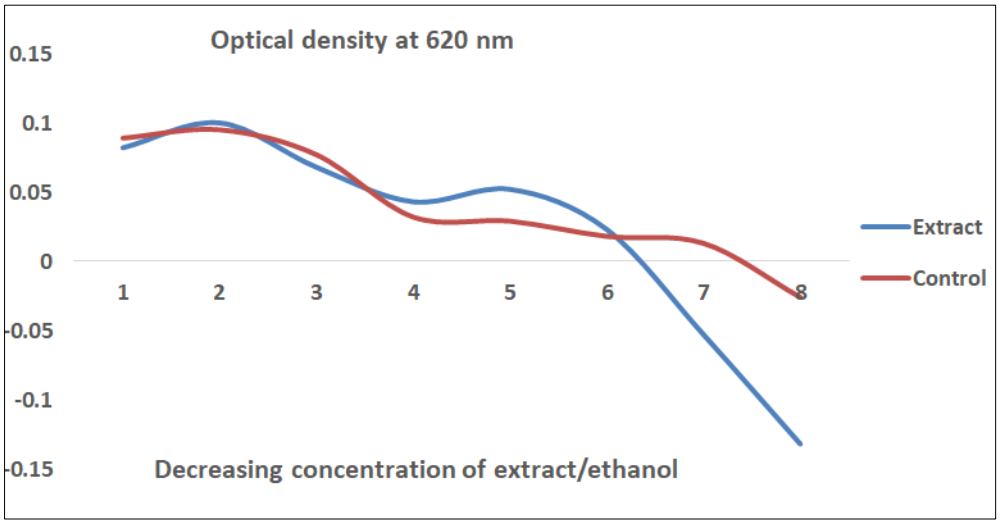

Figure 7: Effect of A. *marina* extract on *Candida parapsilosis*(ATCC 22019) showing a MIC value of 3.6 mg/ml. Ethanol could act when it became 70% concentration

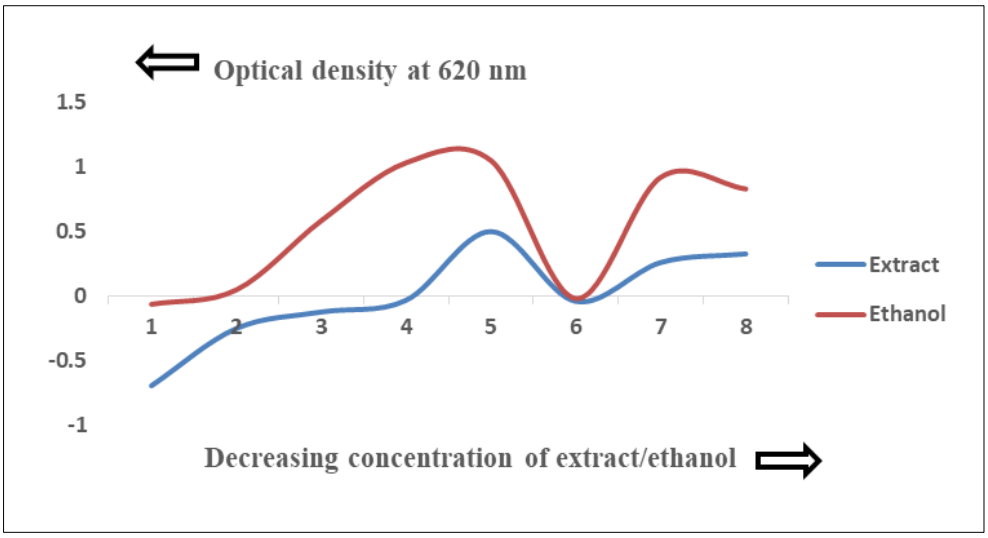

Figure 8: Effect of A. *marina* extract on *Candida albicans* (ATCC 10231) showing a MIC value of 3.6mg/ml. Ethanol could act when it became 70% concentration

DISCUSSION

Throughout ancient times, people have used plants to treat a variety of contagious ailments. Scientific studies are being conducted to demonstrate the therapeutic effectiveness of numerous medicinal plants. In many nations today, medicinal plants are utilized to treat a variety of infectious disorders. Because of the rise of drug-resistant bacteria and the emergence of novel dangerous bacterial strains, there is currently a global interest in medicinal plants as therapeutic agents. It has been observed that extracts and pure components of many medicinal plants are particularly efficient against bacterial strains following *in vitro* testing of a large number of plants against various bacterial strains. The findings of this investigation made it quite evident that mangrove tree *Avicennia marina* extracts demonstrated antibacterial activity against tested pathogenic strains, including those that are resistant to antibiotics.

The effectiveness of the active compounds present in plant extracts causes the inhibition of the growth of these tested pathogenic strains in the microtiter well. Some bacterial strains may have some resistance mechanisms, such as decreased intracellular drug accumulation, target site alteration, and enzyme inactivation. No inhibition was observed with controls, demonstrating that solvents could not behave as antibacterial agents. Nearly all the tests showed that crude ethanolic extracts showed better inhibition against all tested bacterial strains, indicating that active ingredients in plant materials could be extracted into ethanol. To successfully separate, purify, and characterized physiologically active compounds requires further research using chromatographic methods and spectroscopic techniques. The various components that are present in plant extracts of *A. marina* are being separated using column chromatography in further research (Janaki et al, 2016; Mahady et al, 2008).

CONCLUSION

In conclusion, the leaf extract of *Avicennia marina* has demonstrated potent antibacterial and antifungal activity in various studies. The presence of secondary metabolites such as tannins, flavonoids, alkaloids, and phenols in the extract is believed to be responsible for its antimicrobial activity. The extract has shown effectiveness against a range of pathogenic bacteria and fungi, including multi-drug resistant strains.

The findings of these studies suggest that *Avicennia marina* could be a promising source of natural antibacterial and antifungal agents for use in the development of new drugs and therapeutic agents. However, further research is needed to identify the specific compounds responsible for the extract's activity and to evaluate their safety and efficacy in clinical settings.

Conflict of Interest

The author declares no conflict of interest.

Author's Contribution

Dr. Satadal Das conceived and designed the study and collected the plant sample. Mr. Sany Sarkar prepared the plant extract and carried out the experiment under Mr. Arup Kumar Dawn with the help of his proper guidance. Mr. Sany Sarkar analysed the data and wrote the manuscript. Dr. Satadal Das reviewed and edited the manuscript.

Funding Source

This study was not supported by any funding.

Acknowledgement

We hereby acknowledge Managing Director, Peerless Hospital & B K Roy Research Centre, Kolkata for providing me an opportunity to pursue this work in this esteemed institute.

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