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Original Research Article

Antimicrobial Activity of *Avicennia marina* Flowers

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Abstract: Antimicrobial drug resistance is gradually increasing throughout the globe, and there is practically no newer antimicrobial agent coming soon. Plant secondary metabolites are well known for their various biological activities, and there are few studies targeting mangrove plants. Thus, in this study, we aim to assess the effectiveness of the ethanolic extract of the flowers of *Avicennia marina*, a commonly found mangrove species in coastal regions, against multi-drug resistant (MDR) microorganisms. The study focused on evaluating the extract's antibacterial activity against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR), *Pseudomonas* sp. (MDR). The results of the study demonstrated that the flower extracts of *Avicennia marina* exhibited noteworthy antimicrobial properties against all the tested MDR microorganisms. The plant contains several bioactive compounds, such as flavonoids, tannins, and alkaloids, which are believed to contribute to the observed antimicrobial effects. These findings indicate that *Avicennia marina* flowers could serve as a valuable natural source for developing antimicrobial agents that could combat drug-resistant bacterial infections. However, future studies should focus on evaluating the efficacy and safety of this extract in live organisms (*in vivo*) to determine its potential as a new drug for treating resistant infections.

Keywords: *Avicennia marina;* Antibacterial activity; *Staphylococcus aureus, Escherichia coli, Escherichia coli, Klebsiella sp., Acinetobacter sp., Pseudomonas sp.*

INTRODUCTION

Throughout the ages, plants have consistently demonstrated their potential as valuable resources for the well-being of humanity. They have been utilized continually for aspects such as food and a variety of drugs. Taking this into account, mangrove plants are known to exhibit a degree of antimicrobial and antifungal properties. These are to survive in marshy habitats containing high salinity and temperatures. Some mangrove species are an abundant source of bioactive compounds. Studies have been done researching the antioxidant, antimicrobial, and antibiofilm properties of these plants. Mangroves are a distinctive plant community that possesses the morphological, anatomical, physiological, and molecular capacity to respond to a variety of environmental challenges (Dasgupta *et al*., 2010).

In this study, *Avicennia marina* also termed gray mangrove is researched for its antimicrobial properties. It is a shrub belonging to the Acanthaceae family and is characterized by its leaves being thick, glossy, and bright green on the upper side and possessing silvery hair on the underside. *Avicennia marina* also seems to have ethnomedicinal importance (Thatoi *et al*., 2016), locals employ the leaves, fruits, and bark of *A. marina* in the treatment of skin diseases and digestive disorders (Thatoi *et al*., 2016; Khafagi *et al*., 2003). A primary extract from the roots, leaves, stems, seeds, and fruits of *Avicennia marina* was made in ethanol to test for its antibacterial properties (Febriani *et al*., 2020; Mouafi *et al*., 2014). The plant ingredients and the solvent used for extraction directly affect the effectiveness of the process. In this study, the bacterial species were grown in Mueller Hinton Broth and for the preparation of 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. To standardize microbial testing, McFarland standards were used as a

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guide to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a specific range. The flower extract's minimal inhibitory concentration (MIC) was determined against *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR), *Pseudomonas* sp. (MDR). The smallest concentration of an antibiotic required to prevent the growth of a certain strain of bacteria is known as the minimal inhibitory concentration. It aids in assessing the effectiveness of novel antimicrobial treatments and is used to ascertain how susceptible certain bacteria are to medications (Sasidharan *et al*., 2011; Prihanto *et al*, 2011).

MATERIALS AND METHODS

Plant materials and Extraction

Avicennia marina also termed grey mangrove belongs to the Aviceniaceae family and grows as a shrub or tree to a height of 3 to 10 meters or even 14 meters under certain conditions. It is mainly seen in tropical regions, growing in the saline intertidal zones of sheltered coastlines, and is reported to tolerate extreme weather conditions and high winds. The material was classified taxonomically, and the voucher specimen was stored. The aerial plants were collected from Gosaba (22.1652° N, 88.8079° E) of the Sundarbans, West Bengal, India- the largest mangrove forest in the world. The flowers of the plant (Fig 1) were collected in the Month of June 2023 and were thoroughly washed with seawater to remove epiphytes, shells, and other substances. The cleaned plant components were brought to the lab in individual polyethylene bags. With scissors, the flowers were divided into little fragments. The samples were then used to create the ethanol extract. For the extraction process, 1 g of the flowerswas submerged in 5 ml of ethanol at room temperature for 72 hours. The extract was then separated using centrifugation (Fig 1). The samples were centrifuged for 10 minutes at 3000 rpm (Goyal, 1989).

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

Test Microorganisms and Culture Media

Five bacterial strains such as *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Acinetobacter* sp. (MDR), *Pseudomonas* sp. (MDR) were used in this study.Among these bacteria,*Escherichia coli* (ATCC 25922), *Escherichia coli* (MDR), *Klebsiella* sp. (MDR), *Pseudomonas* sp. (MDR), *Acinetobacter* sp. (MDR) are gram-negative in nature and *Staphylococcus aureus*(ATCC 25923) is Gram-positive in nature. The bacterial species were grown in Mueller–Hinton broth (Himedia).

Inoculum Preparation

From the stock cultures, each strain of bacteria was streaked on the agar plate. The plate was then incubated for 24 h at 37 °C. Three bacterial colonies from each plate were suspended 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 of the plant extract was evaluated by determining the

Minimum Inhibitory Concentration (MIC). The experimental procedure involved using a microtitre plate. Initially, 100 µl of Mueller Hinton broth was addedto each well. Then 100 µl of the extract was added to the first well, followed by mixing it. 100 µlof this mixture was then transferred to the second well which already contains 100 µl of Mueller Hinton broth. This serial dilution process was repeated up to the eighth well, resulting in a halving concentration of the extract in each subsequent well.

Next, 10 µl of different bacterial suspensions were added to each well in separate rows. Control rows were also prepared for each bacterial strain, where only Mueller Hinton broth, bacterial suspension, and ethanol were present and serially diluted. The optical density of the plate was measured at a wavelength of 620 nm to establish a baseline absorbance value.

Following the optical density measurement, the plate was incubated at 37°C for 24 hours. After incubation, the optical density was measured again at 620 nm. By subtracting the initial optical density readings from the final readings, the change in optical density for each well was obtained. The MIC value was determined by identifying the lowest concentration of the plant extract which resulted in a lower optical density value than the control well optical density.

RESULTS

The extract from *A,marina* was tested against both ATCC and MDR strains and the findings indicated that the extract exhibited efficacy against some of the microbial strains assessed in the study (Fig.2-Fig.8). *Staphylococcus aureus* (ATCC 29213) showed a MIC value of 6.25 mg/ml.There was no antimicrobial action against *Escherichia coli* (ATCC 25922). However, peculiarly *Escherichia coli* (MDR) showed a MIC value of 3.125 mg/ml.There was no antimicrobial action of the extract against *Klebsiella* sp. (MDR). MIC value of the extract against *Acinetobacter* sp. (MDR) showed a MIC value of 25 mg/ml. MIC value of the extract against *Pseudomonas* sp. (MDR) showed a MIC value of 3.125mg/ml.

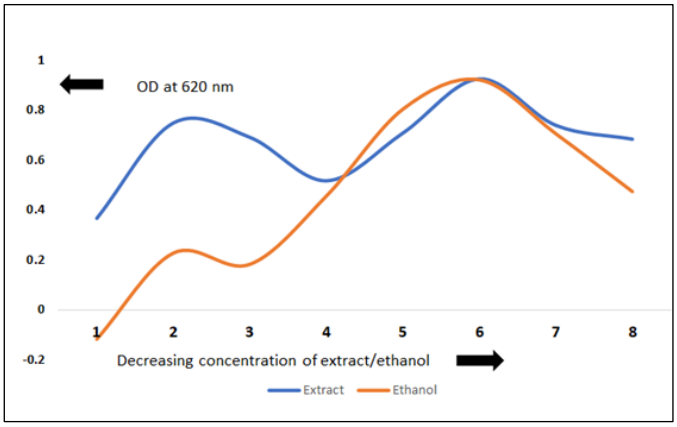

Figure 2: Showing MIC value of the extract against *Staphylococcus aureus* (ATCC 29213). MIC value is 6.25 mg/ml. The concentration of the extract: 1-100mg/ml, 2-50mg/ml, 3-25mg/ml, 4-12.5mg/ml, 5-6.25mg/ml, 6-3.125mg/ml, 7-1.56mg/ml, 8-0.78mg/ml. Ethanol could start its action at 70% concentration

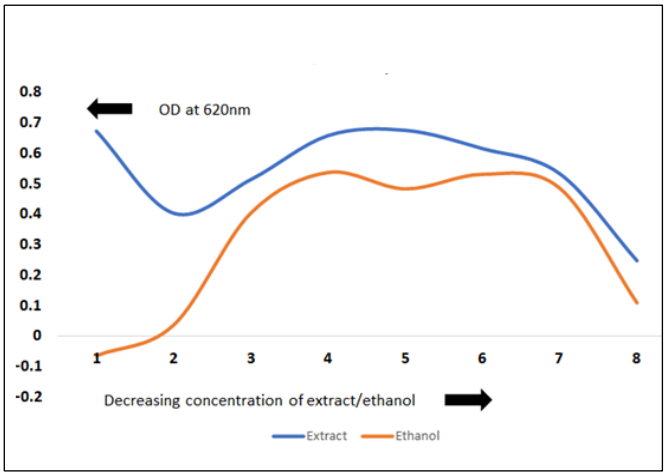

Figure 3: Showing action of the extract against *Escherichia coli* (ATCC 25922). MIC value could not be determined and there was no antimicrobial action of the extract. The concentration of the extract: 1-100mg/ml, 2-50mg/ml, 3-25mg/ml, 4-12.5mg/ml, 5-6.25mg/ml, 6-3.125mg/ml, 7-1.56mg/ml, 8-0.78mg/ml. Ethanol could start its action at 70% concentration

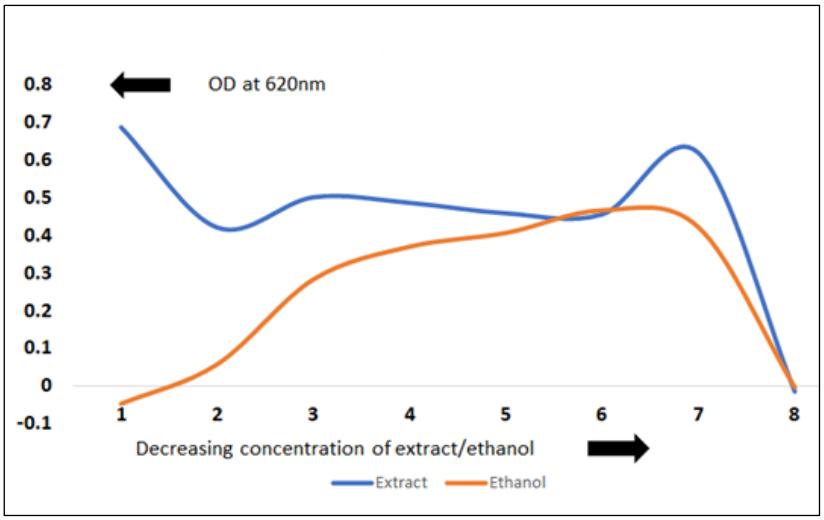

Figure 4: Showing MIC value of the extract against *Escherichia coli* (MDR), MIC value is 3.125 mg/ml. The concentration of the extract: 1-100mg/ml, 2-50mg/ml, 3-25mg/ml, 4-12.5mg/ml, 5-6.25mg/ml, 6-3.125mg/ml, 7-1.56mg/ml, 8-0.78mg/ml. Ethanol could start its action at 70% concentration

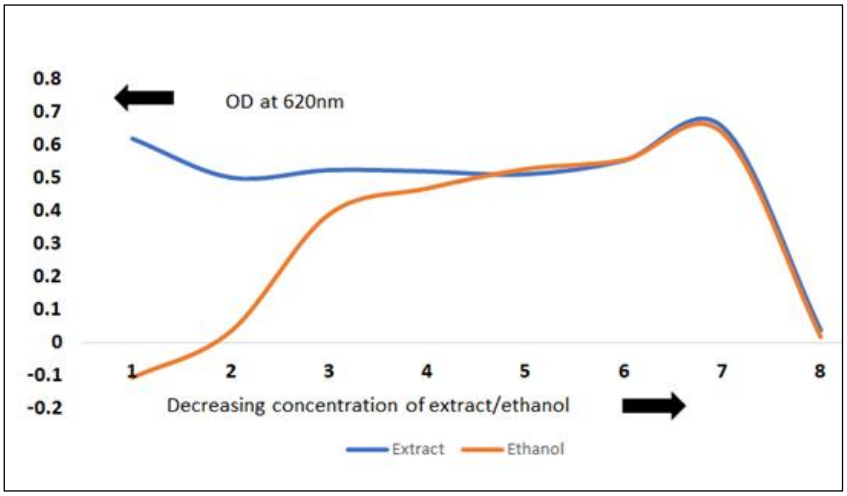

Figure 6: Showing action of the extract against *Klebsiella* sp. (MDR). There is no antimicrobial action of the extract. The concentration of the extract: 1-100mg/ml, 2-50mg/ml, 3-25mg/ml, 4-12.5mg/ml, 5-6.25mg/ml, 6-3.125mg/ml, 7-1.56mg/ml, 8-0.78mg/ml. Ethanol could start its action at 70% concentration

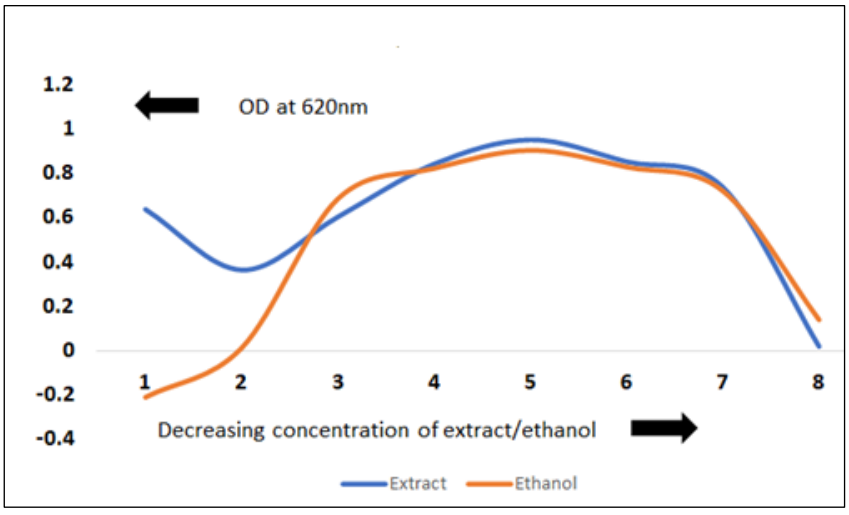

Figure 7: Showing MIC value of the extract against *Acinetobacter* sp. (MDR). MIC value is 25mg/ml. The concentration of the extract: 1-100mg/ml, 2-50mg/ml, 3-25mg/ml, 4-12.5mg/ml, 5-6.25mg/ml, 6-3.125mg/ml, 7-1.56mg/ml, 8-0.78mg/ml. Ethanol could start its action at 70% concentration

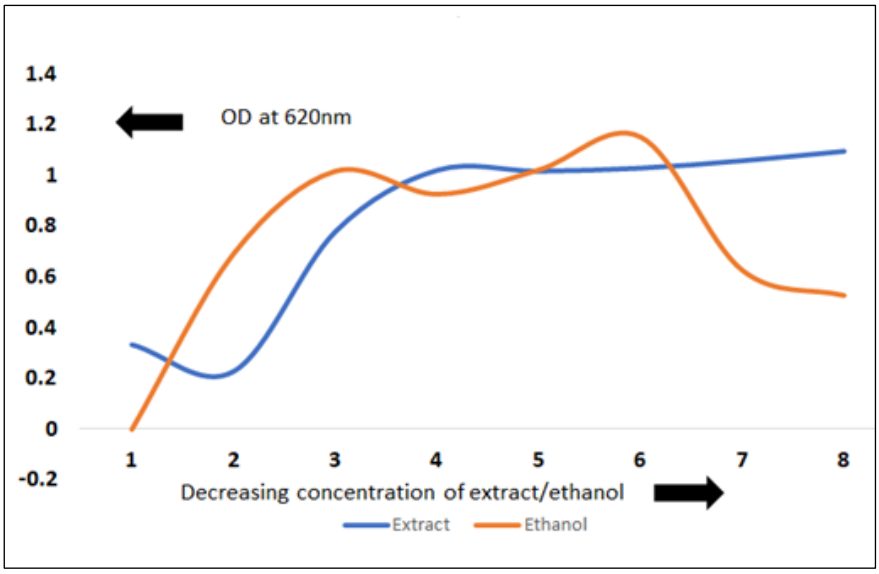

Figure 8: Showing MIC value of the extract against *Pseudomonas* sp. (MDR). MIC value is 3.125mg/ml. The concentration of the extract: 1-100mg/ml, 2-50mg/ml, 3-25mg/ml, 4-12.5mg/ml, 5-6.25mg/ml, 6-3.125mg/ml, 7-1.56mg/ml, 8-0.78mg/ml. Ethanol could start its action at 70% concentration

DISCUSSION

Throughout ancient history, medicinal plants have been utilized by people to treat various contagious ailments. In modern times, scientific studies are being conducted to provide evidence for the therapeutic effectiveness of numerous medicinal plants. Many countries today rely on medicinal plants to address different infectious diseases. The global interest in medicinal plants as therapeutic agents has grown due to the increase in drug-resistant bacteria and the emergence of new and dangerous bacterial strains.

Extensive *in vitro* testing of numerous plants against various bacterial strains has shown that extracts and pure components derived from medicinal plants are highly effective against these strains. Remarkably, the antibacterial activity of flowers of *Avicennia marina*, a mangrove tree, has been observed against pathogenic strains, including those resistant to antibiotics.

The active compounds present in plant extracts inhibit the growth of the tested pathogenic strains in the microtiter well. Some bacterial strains may possess resistance mechanisms such as reduced drug accumulation within the cells, alteration of target sites, or enzyme inactivation. Control experiments confirm that the observed inhibition is not due to the solvents used.

Crude ethanolic extracts have shown superior inhibition against most tested bacterial strains compared to other extracts, indicating that the active ingredients in the plant material can be extracted effectively using ethanol. Further research employing chromatographic methods and spectroscopic techniques is necessary to isolate, purify, and characterize the physiologically active compounds present in the plant extracts of *A. marina*. Ongoing studies involve the separation of various components from the plant extracts using column chromatography (Janaki *et al*., 2016; Mahady *et al*., 2008).

CONCLUSION

Several studies have provided evidence of the strong antibacterial and antifungal properties exhibited by the leaf extract of *Avicennia marina*. The antimicrobial activity of the extract is attributed to the presence of secondary metabolites such as tannins, flavonoids, alkaloids, and phenols. This broad spectrum of activity has been observed against various pathogenic bacteria and fungi, including strains that are resistant to multiple drugs.

These findings highlight the potential of *Avicennia marina* flowers as a valuable natural source for the development of novel antibacterial and antifungal agents. The extract holds promise for the creation of new drugs and treatments. However, additional research is necessary to identify the specific compounds responsible for the extract's activity. Furthermore, evaluating the safety and efficacy of these compounds in clinical settings is crucial for their potential application in the medical field.

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. Arkadeep Roy prepared the plant extract and experimented under Mr. Arup Kumar Dawn with the help of his proper guidance. Mr. Arkadeep Roy analyzed the data and

wrote the manuscript. Dr. Satadal Das and others reviewed and edited the manuscript.

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