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Anti bacterial activity of *Avicennia marina* leaf extract

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Abstract
Grey mangrove (Avicennia marina), is a mangrove plant species widely distributed in the intertidal zones of tropical and subtropical coasts. This genus of the plant has both ecological and economic benefits. The plant has been used in traditional medicine for the treatment of various diseases such as cancer, diabetes, malaria, rheumatism, asthma, small pox and ulcer since centuries and is known to possess various pharmacological properties, including antimicrobial activity. This plant contains some unique metabolites of varied chemical classes which are the reason of its antimicrobial, antifungal, antibacterial, anti-inflammatory activities. In this study, we observed the antibacterial activity of Avicennia marina leaf extract against the MDR bacterial strains along with control ATCC strains. The antibacterial activities were determined by disc diffusion method, well diffusion and MIC method. Our study indicated this extract is sensitive to both MDR and ATCC strains with MIC values varied between 6.25-12.5 mg/ml. In recent years, there has been a growing interest in exploring the potential of Avicennia marina as a source of natural antimicrobial agents. This study indicates a possible role of this mangrove plant extract in MDR bacterial infections.

Keywords: *Avicennia marina,Grey mangrove, Tropical and subtropical region, Antibacterial activity, Pharmacological property.*

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I. Introduction

As a mangrove *Avicennia* is a taxonomically diverse group of halophytic plant communities which need warm conditions for development and survival, they are found only in tropical climates. These plants are highly specialized, flourishing under inhospitable environmental conditions of extreme tides, high salinity, high temperature, strong winds and anaerobic soil which are the key physiological features of their survival in this adverse condition[1]. The genus *Avicennia* (L.) came from a Persian word Avicenna or Abdallah Ibn Sina (980-1037 AD), named by a Persian physician [2]. There are near about 84 mangrove plant species belong to 24 genera and 16 families are present in the whole world out of which only 70 species of them are reported as real mangroves[3]. *Avicennia* plant family is a member of true mangrove which has one genus, 11 species and several sub species[4]. There are eight species found based on the database prepared by the collaboration between The Royal Botanic Gardens, Kew and Missouri Botanical Garden and others states(http://www.the plantlist.org/). Those species are *Avicennia balanophora* Stapf & Moldenke., *Avicennia bicolor* Standl., *Avicennia germinans* (L.) L., *Avicennia integra* N.C. Duke., *Avicennia marina* (Forssk.) Vierh., *Avicennia officinalis* L., *Avicennia schaueriana* Stapf & Leechm. ex Moldenke and *Avicenniatonduzii* Moldenke[5].

Figure 1: *Avicennia marina* leaves collected from island of Sundarbans mangrove forest.

The antimicrobial activity of *Avicennia marina* has been extensively studied using various extracts, such as leaves, stems, roots, and bark. These studies have consistently shown that the plant has significant antimicrobial activity against a wide range of microorganisms, including bacteria, fungi, and viruses. The characterization of thirty bioactive compounds has detected by using the Gas Chromatography- Mass Spectrometry, which are responsible for the antimicrobial activity of *Avicennia marina* have been identified as terpenoids, flavonoids, tannins, and phenolic acids[6]. These compounds have been found to exhibit antibacterial, antifungal, and antiviral properties, providing a scientific basis for the use of *Avicennia marina* as an antimicrobial agent.

The ethanolic solution of *Avicennia marina* root shows antibacterial activity against *Pseudomonas aeruginosa* (MIC= 10.8 +/- 0.78 mg/mL), *Bacillus subtilis* (MIC= 6.1 +/- 0.27 mg/mL), *Staphylococcus aureus* (MIC= 2.3 +/- 0.08 mg/mL) and *Escherichia coli* (MIC= 6.3 +/- 0.28 mg/mL)[7]. The leaf of *Avicennia maria* extracted into ethyl acetate also active against some bacteria -*E. coli* and *S. aureus* [8]. In vitro studies have confirmed, the antibacterial activity of *Avicennia marina* extract has been attributed to the presence of terpenoids and flavonoids, which have been shown to inhibit the growth of bacterial cells by disrupting the cell membrane[9]. A study proved that different solvent of *Avicennia marina* extract shows toxicity toward larva of three major mosquito vector. The highest larvicidal mortality rate was found against *Culex quinquefasciatus* (LC_{50} = 0.197 mg/mL; LC_{90} = 1.5011 mg/mL), *Anopheles stephensi* (LC_{50}= 0.176 mg/mL; LC_{90} = 3.6290 mg/mL) and *Aedes aegypti* (LC_{50} = 0.164 mg/ mL; LC_{90} = 4.3554 mg/mL) using the acetone with the *Avicennia marina* leaf extract[10]. Hence, it is considered to act against various kind of mosquito vector-borne diseases like malaria, dengue, chikungunya, yellow fever, Zika virus, Japanese encephalitis, lymphatic filariasis etc.[10]. According to the current study, silver nanoparticles synthesized from the aqueous *Avicennia marina* seed extract has an immense antibacterial potentiality[11]. The main purpose of this study is to find the antibacterial property of leaf ethanolic extract of *A.marina*.

II. Materials and Methods

- **Collection of plant extract**

The leaves of *Avicennia marina* (Fig. 1) were collected from an island of Jharkhali, Sundarban Mangrove Forest, West Bengal. Leaves were cleaned with tap water thoroughly. Leaves were dried and cut into very small pieces with the help of scalpel. Then 1gm leaf was dissolved in 5ml ethanol and left it for 24hrs in optimum temperature(37°C) to get a proper plant extract (Fig.2).

Figure 2: Extract of Grey mangrove (*Avicennia marina*)

- **Media preparation**

Mueller Hinton broth was used to perform a MIC (Minimal inhibitory concentration) test of *A.marina* extract. To prepare MH broth 4.2gm Muller Hinton broth powder was added in 200ml distilled water. Then mixed well and autoclaved.

- **Inoculums preparation**

Two ATCC strains *Staphylococcus aureus*(ATCC 25923), *Escherichia coli*(ATCC 25922) and two MDR strains *E.coli* and *Klebsiella sp.*were used in this test. The McFarland standard matched suspension of the bacterial samples were obtained by the following procedure. Wire loop was sterilized by flaming it, and then it was allowed to cool. The slant/petri plate was opened, a colony or a little amount growth was picked up from solid medium. A tube containing little amount of sterile distilled water was taken. The growth picked up with the help of sterile loop was transferred to the distilled water. The wire loop was shaken to dislodge the culture from the wire loop. Then the wire loop was withdrawn and the suspension tube was closed. The wire loop was flame and placed aside.

- **Antibacterial assay**

The antibacterial activity of the *A. marina* extract was evaluated using the Minimal inhibitory concentration (MIC).

MIC method

A microtiter plate was divided and marked according to the strains used. 100µl Mueller Hinton broth was pipetted out into each well of the microtiter plate. 100µl *A. marina* extract was added in every first well of all test rows and serial dilution (double dilution in each step)was performed until the last well of the row. Same process was done for the control rows as well. 10µl of bacterial suspension was added into each well of every row which were specifically marked according to the strain. Then 0hr reading was taken and lastly a reading was taken after 24 hours.

III. Results

The result of antibacterial activity of *A.marina* plant leaf extract against *Staphylococcus aureus* shown in the Figure 3 graph. It showed a MIC value of 6.25 mg/ml in 70% ethanol concentration.The result of antibacterial activity of grey mangrove leaf extract against *Escherichia coli* is shown in the Figure 4 graph. It showed a value of approx. 3.125 mg/ml in 70% ethanol concentration. The result of antibacterial activity of the extract against the MDR1 strain is shown in the Figure 5 graph. It shows a MIC value of 6.25mg/ml against the MDR1 strain. The result of antibacterial activity of the extract against the *Klebsiella sp.* strain is shown in the Figure 6 graph. MIC value of 0.781mg/ml.

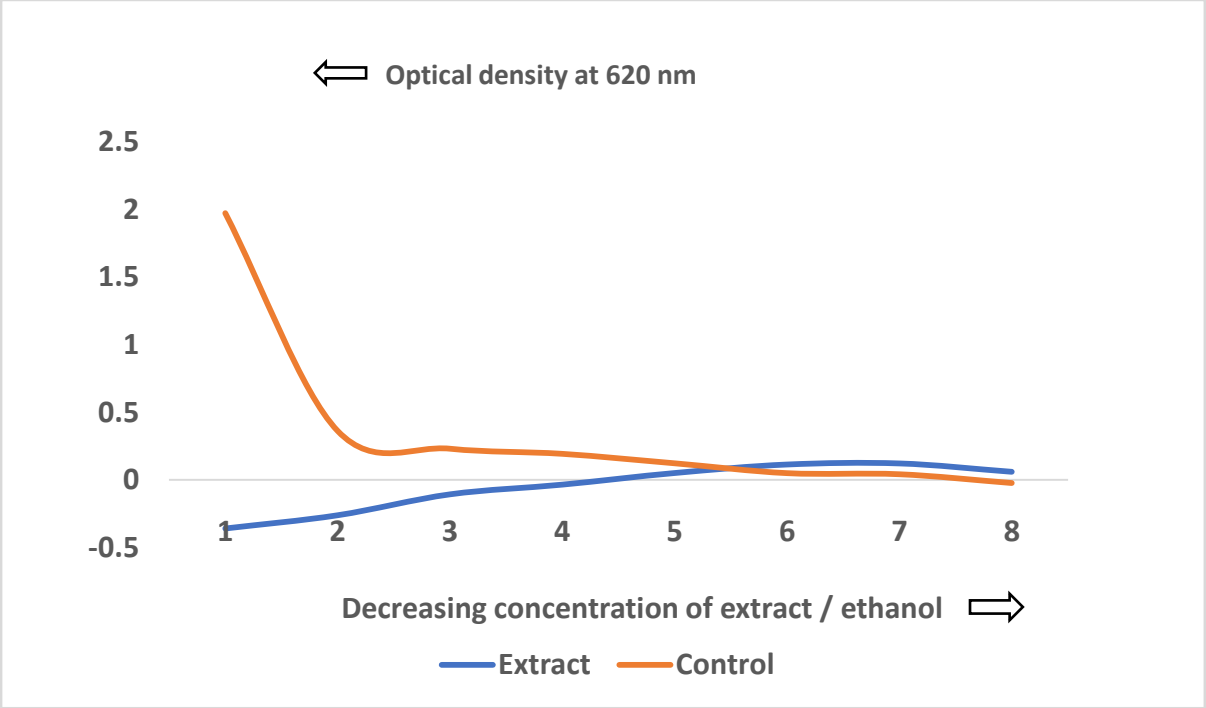

Fig. 3: Effect of *A. marina* extract on *Staphylococcus aureus* (ATCC 25923) showing a MIC value of 6.25 mg/ml. Ethanol could act when it became 70% concentration. Concentration of the extract: 1(100mg/ml), 2(50mg/ml), 3(25mg/ml), 4(12.5mg/ml), 5(6.25mg/ml), 6 (3.125mg/ml), 7(1.562mg/ml), 8 (0.781mg/ml).

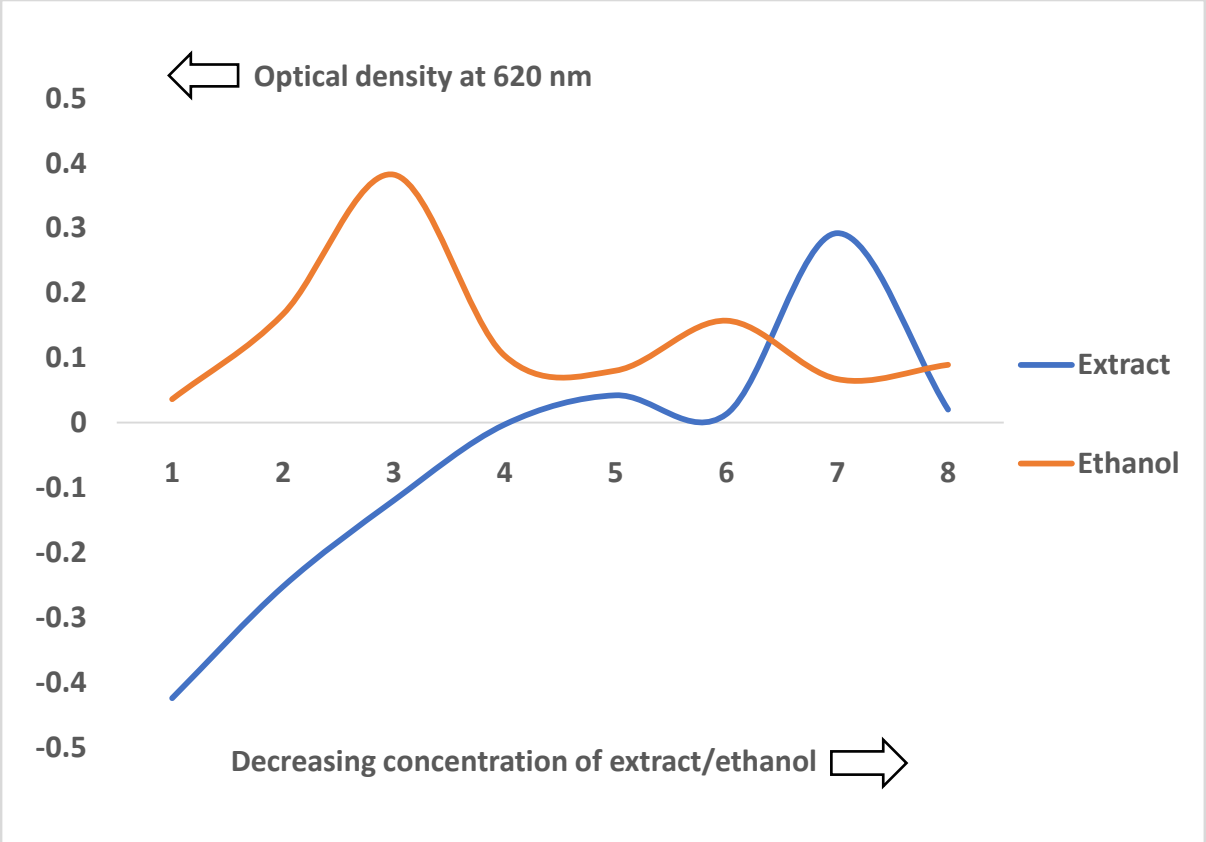

Fig. 4: Effect of *A. marina* extract on *Escherichia coli* (ATCC 25922) showing a MIC value of approximate 3.125mg/ml. Ethanol could act when it became 70% concentration. Concentration of the extract: 1(100mg/ml), 2(50mg/ml), 3(25mg/ml), 4(12.5mg/ml), 5(6.25mg/ml), 6 (3.125mg/ml), 7(1.562mg/ml), 8 (0.781mg/ml).

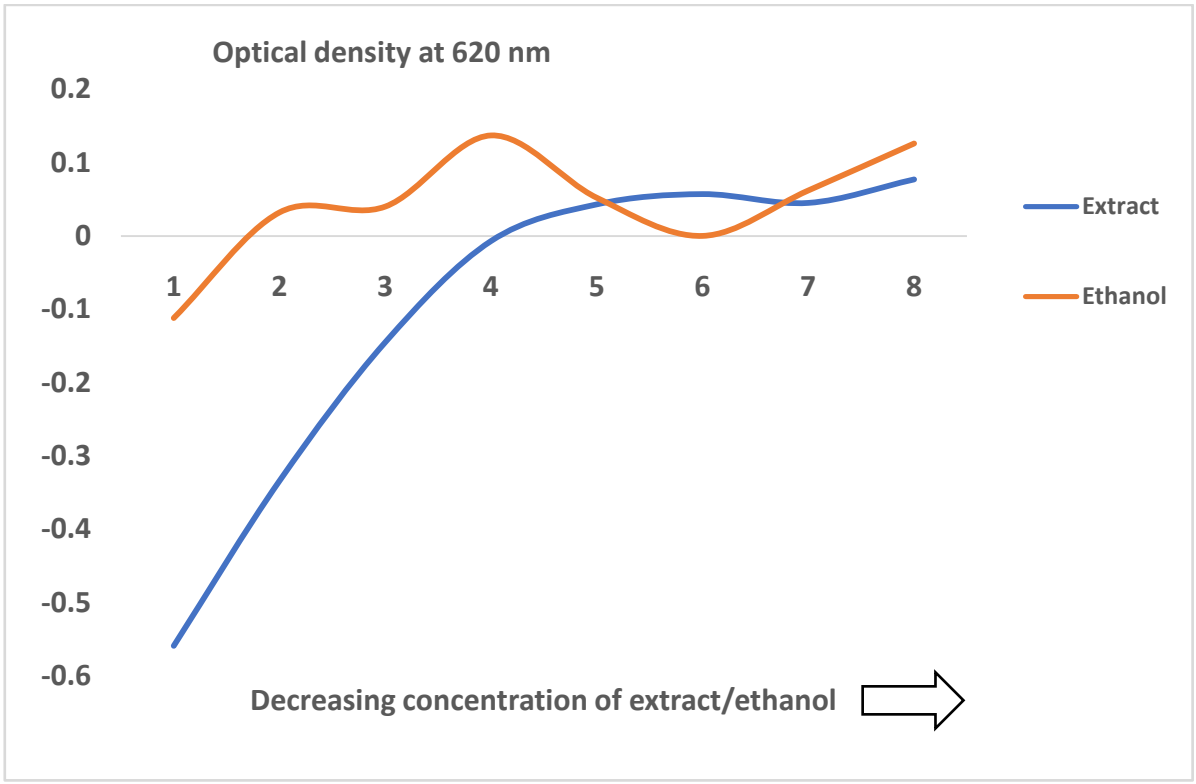

Fig. 5: Effect of A. *marina* extract on *E.coli* (MDR) 1 showing a MIC value of 6.25 mg/ml. Ethanol could act when it became 70% concentration. Concentration of the extract: 1(100mg/ml), 2(50mg/ml), 3(25mg/ml), 4(12.5mg/ml), 5(6.25mg/ml), 6 (3.125mg/ml), 7(1.562mg/ml), 8 (0.781mg/ml).

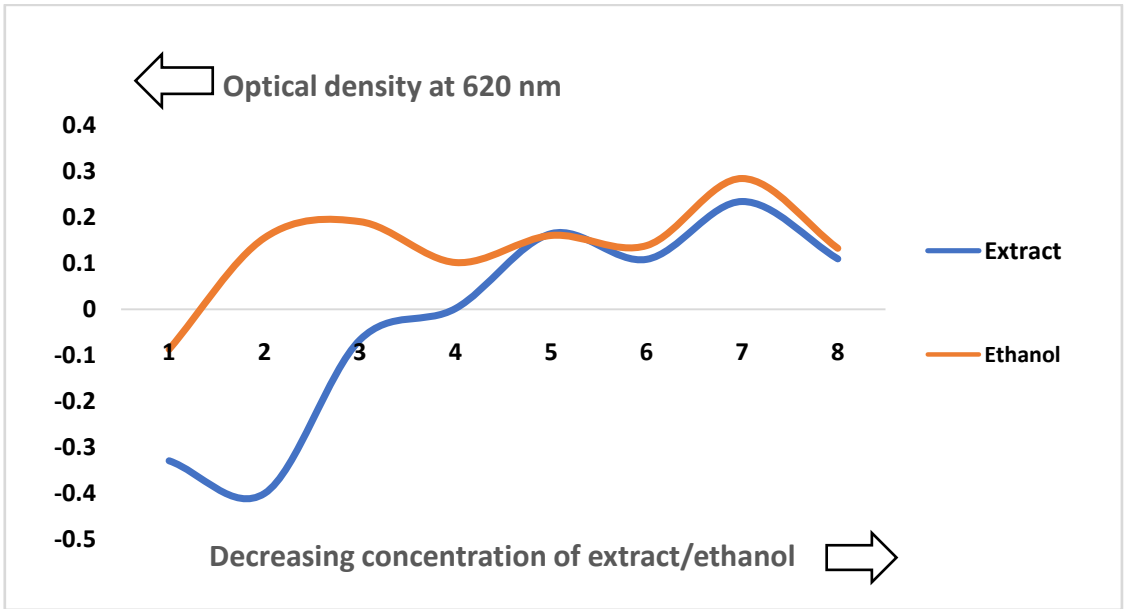

Fig. 6: Effect of A. *marina* extract on *Klebsiella sp.*(MDR) 2 showing a MIC value of 0.781 mg/ml. Ethanol could act when it became 70% concentration. Concentration of the extract: 1(100mg/ml), 2(50mg/ml), 3(25mg/ml), 4(12.5mg/ml), 5(6.25mg/ml), 6 (3.125mg/ml), 7(1.562mg/ml), 8 (0.781mg/ml).

IV. Discussion

Since the ancient time, mangrove plants have been a vital source of drug. Mangrove plant grows in very stressful environment. Due to this reason this type of plant possesses chemical mechanisms to protect themselves from the predators[12]. This type of mangrove plant has a broad spectrum of therapeutic medicinal value due to its phytochemical properties such as flavonoids, tannins, steroids, alkaloids, and sugar in the extract of its green vegetation [13].The present study showed that the antibacterial activity of *Avicennia marina* leaf

extract against MDR bacteria. The *A. marina* extract is highly effective on *Staphylococcus aureus* as well ason *E.coli*. On the other hand, it has been observed that the extract of *A. marina* leaf has a higher effect on *Klebsiella* sp. bacterial strain but it is less effective on MDR of *E. coli* bacterial strain. So, *Avicennia marina* leaf has antibacterial property in its leaf extract. Its extract can be used in the medicine which may work well against the diseases caused by both *Staphylococcus aureus, E. coli* and *Klebsiella sp.*.

V. Conclusion

The present study demonstrated that *Avicennia marina* leaf extract has potential as a natural source of antibacterial agent. Further studies are required to identify the active compounds responsible for the observed antibacterial effect and to evaluate their safety and efficacy.

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. Ms. Tanushka das prepared the plant extract and carried out the experiment under Mr. Arup Kumar Dawn provided the technical help of this work. Ms. Tanushka Das analysed the data and wrote the manuscript. Dr.Bhaskar Narayan Choudhuri, Dr.ParthaGuchhait and Dr. Satadal Das finally checked the analysis and the whole manuscript.

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