

## RESEARCH ARTICLE

# *Avicennia Marina*: A Novel Convivial Phyto Medicine for Antibiotic Resistant Pathogenic Bacteria

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## Abstract

This study investigated two different concentration of methanol extracts of the leaves of *Avicennia marina* against five human pathogenic bacteria, to determine their efficacy against multidrug resistant microbes. Powdered leaves of the tree were treated with two different concentration of methanol (10% w/v and 20% w/v) using hot extraction method. Crude methanol extracts of the leaves of *Avicennia marina* was investigated for their antibacterial activity against a wide range of bacteria (both gram-positive and gram-negative) by disc diffusion method. Multidrug resistant (MDR) strains of *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853) were used in the study. Ciprofloxacin was used as standard. The antimicrobial activities of the crude extracts were increased with increasing the concentration. The methanolic leaves extracts of *A. marina* showed a remarkable inhibition of the microorganisms. The potency shown by these extracts recommends their use against multidrug resistant microorganisms. It is clear that n-hexane extract was the most effective extract. Additionally, Multidrug resistant (MDR) strains of *E. coli*, *Bacillus subtilis* and *Staphylococcus aureus* was strongly inhibited by both concentration of methanol extracts of *A. marina* leaves while MDR strains of *Salmonella enterica*, *Pseudomonas aeruginosa* also inhibited by methanolic extracts of leaves of *A. marina* but comparatively less.

The present study suggests that the methanol extract of the leaves of *A. marina* exhibited a potential antibacterial activity against the tested microorganisms and could be a potential source of new antimicrobial agents.

**Keywords:** *Avicennia marina*; Antibacterial; Ciprofloxacin; Leaves; Microorganisms

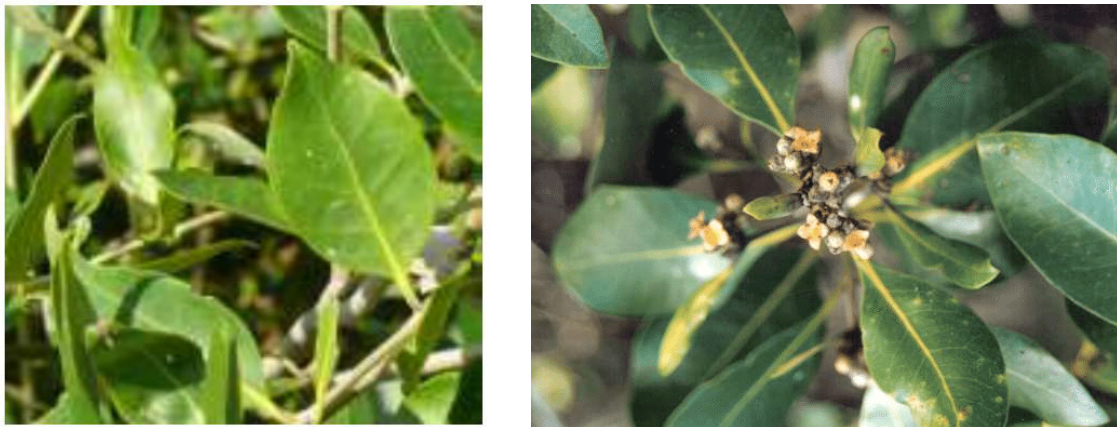
**List of Abbreviations:** *A. marina*: *Avicennia marina*; SD: standard deviation; MDR: Multidrug resistant; ATCC: American Type Culture Collection; E1: Extract 1; E2: Extract 2; h: hours; C: ciprofloxacin

## Introduction

Infectious disease account for the highest proportion of health problem especially in developing countries and amongst them, bacterial infections are a major threat [1]. It is the leading cause of death in world-wide. The only solution to this problem is use of antibiotics or chemicals. The emerging trends of multidrug resistance among several groups of microorganisms against different classes of antibiotics led different researchers to develop efficient drugs from plant sources to counter multidrug resistant strains. Nowadays, medicinal plants play a major role in treatment of infectious diseases and they are easily available and more affordable as compared to synthetic compounds [2]. Scientists have put great attention to the plant extracts and the isolated compounds because of their less side effects and the strong resistance towards various microorganisms. Antimicrobial from plant sources represent a vast untapped source for medicines and further exploration of plant antimicrobials is needed as antimicrobials of plant origin have enormous therapeutic potential [3].

## Botany and Uses

*Avicennia marina* (Forssk.) Vierh. of the family Avicenniaceae is a mangrove tree that can grow more than 10 m in height [4,5,6] that is extraordinarily adaptable with a wide latitudinal range closely associated with its flexible growth pattern. In traditional medicine, the leaves, fruits and bark of *A. marina* are used to treat skin diseases [7] while the stems are used to treat rheumatism, small pox and ulcers [8]. Seeds and seedlings are cooked and eaten as vegetable [6,9,10]. The species produces numerous erect pencil-like pneumatophores with lenticels. The bark is greenish-grey, mottled and peeling in patches. Leaves are elliptic-oblong, pale green on the lower surface with acute to round apex (Figure 1).



**Figure 1:** Leaves (left), and propagules and flower (right) of *Avicennia marina*.

Although the chemical constituents of most mangrove plants still have not been studied extensively, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents. In general, mangroves are trees and shrubs, which grow in saline coastal habitats in the tropics and subtropics. The mangrove dwellers get food and wide variety of traditional products and artifacts from mangroves. Extracts and chemicals from mangroves are used mainly in folkloric medicine (*e.g.* bush medicine), as insecticides and pesticides and these practices continue to this day [11].

Although the chemical constituents of most mangrove plants still have not been studied, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents [12]. Mangroves have been used in fisher folk medicine to treat diseases. In our previous study we investigated that ghaf is a potential desert nutraceutical and antimicrobial agent [2,12,13]. Furthermore, to continue our research to detect the potency of mangrove leaves as source of new antimicrobial agent and also to meet the increasing demand of antimicrobial agent, alternative strategies, this study have been considered recently. With this view, the present investigation was initiated to study the antimicrobial activity of methanolic extract of leaves of mangrove plant against Multi Drug Resistance (MDR) bacterial pathogens. This research was carried out as an awareness of medicinal value of mangrove plant in pharmaceutical.

## Material and Methods

### Plant material collection

The leaves of *A. marina* (Three samples) were collected from Khuzam Road, Ras Al Khaimah, UAE in the month of March 2021. The leaves were sun dried for 5-7 days or more and then oven dried for better grinding. The dried leaves were then ground to a coarse powder using high capacity of grinding machine and then stored in airtight bottles.

### Preparation of the extracts

About 5 g of the coarse powder was extracted with 25.0 ml of methanol followed by continuous hot extraction method. Stirred well and kept for incubation in closed container. Then we centrifuged the tubes at 4000 rpm for 30 min. After that we transferred the supernatant extract for drying for 10 min and finally got residue of leaves sample. 0.1 gm of leaves residue were weighed accurately and transferred in two different test tubes and then we added 0.5 mL of methanol in one test tube [20 % (w/v) solution (E1)] and 1.0 mL of methanol [10 % (w/v) solution (E2)] in other test tube. These were two different final concentration of extracts for experiment. All the extracts were then stored in refrigerator at -20°C till use [2].

### Chemicals

The chemicals used in the present investigation were of analytical grade and of high purity from Merck. Standard used for analysis were purchased from Germany and USA.

### Test organisms

In the present study, the bacterial strains we used were *Bacillus subtilis* (ATCC 6633), *E. coli* (ATCC 8739), *Salmonella enterica* (ATCC 14028), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853) obtained from the American Type Culture Collection (ATCC) to determine the antibacterial activity of *A. marina*. The bacterial strains were procured from LTA srl Italia. Pure culture of bacteria was maintained at 4 °C on nutrient agar slants.

### Methodology for detection of antibacterial activity

#### Inoculums preparation

The bacterial isolates were first grown in 5 ml of nutrient broth in to sterile test tubes for 18 h before use.

#### Agar well diffusion assay

The antibacterial activity of methanolic extracts of *A. marina* leaves was tested against bacterial isolates by agar-well diffusion method. An aliquot of 100 µl inoculum for each bacterial isolate was evenly spread by a sterile glass spreader onto Muller Hinton Agar using sterilized cotton swab and was allowed at room temperature. With the use of Cork borer, we punched 6 mm diameter well in agar plates to cut uniform wells. Two wells were bored in agar plates for two different concentrations. The concentrations of the extract were 20% (w/v) and 10% (w/v), prepared using methanol as solvent. Subsequently, we poured 30 µl extracts of leaves into the wells. Ciprofloxacin 30 µg was used as positive control. Methanol was used as a negative control. Then the plates were kept at 37 °C for 24 h. The diameter of zone of inhibition was measured to the nearest millimeter [14,15]. The formation of clear inhibition zone of ≥7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the extract (Okwori 2007). The effect was compared to those of antibiotic discs. The tests were performed in triplicates and the mean was taken. The whole experiments were performed under strict aseptic conditions.

## Statistical analysis

The tests were performed in triplicates. Data are expressed as mean. Pair wise comparisons were performed. Experimental error was determined for triplicate and expressed as standard deviation (SD).

## Results

According to the present research findings, we used the methanolic extracts of the leaves of *A. marina* (0.1 gm of leaves residue in two different test tubes and added 0.5 mL of methanol in one test tube [20 % (w/v) solution (E1)] and 1.0 mL of methanol [10 % (w/v) solution (E2)] in other test tube) which exhibited antibacterial activity with all the tested strains of microorganisms on comparison with the standard 30 mcg ciprofloxacin by producing zone of Inhibition (Kirby Bauer's zone of inhibition method which describes that - Place the metric ruler across the zone of inhibition, at the widest diameter, and measure from one edge of the zone to the other edge. Use millimetre measurements). *Pseudomonas aeruginosa*, *E. coli*, *B. subtilis*, *S. enterica* are resistant to chloramphenicol. Antibacterial activity of leaves extracts using agar well diffusion. The extract of both concentrations showed antibacterial activity as indicated by the zone of growth inhibition ranged from  $7 \pm .000 - 25 \pm .000$  mm (Figure 2). Similar work was reported by Sahoo et al 2012 [16]. According to present research finding *S. aureus* strongly inhibited by methanol extract of leaves of mangrove plant showed significant difference with the positive control ciprofloxacin in both the concentrations and showed zone of inhibition 25mm and 20 mm at 20% and 10% (w/v) and *S. enterica* strain in a concentration dependent fashion and had the zone of inhibition ( $15.00 \pm 0.05$  mm) at concentration of 20% (w/v) and ( $12.00 \pm 0.05$  mm) at concentration of 10% (w/v) respectively followed by *P. aeruginosa* showed less activity as compared to other bacterial isolates and showed 9 mm of zone of inhibition at 20% (w/v) and 7 mm at 10% (w/v) and *E.coli* showed 22 mm and 18 mm at a concentration of 20% (w/v) and 10% (w/v) respectively while *B. subtilis* showed the zone of inhibition ( $18 \pm .000$  mm) at a concentration of 20% (w/v) and 15 mm at 10% (w/v). (Table 1). Similar results were reported by [17,18].

S. No	Microorganisms	Zone of Inhibition (mm)	
		E1	E2
1.	<i>Bacillus subtilis</i> (ATCC 6633)	15± .000	18± .000
2.	<i>E.coli</i> (ATCC 8739)	18± .000	22± .000
3.	<i>Salmonella enterica</i> (ATCC 14028)	12± 0.05	15± 0.05
4.	<i>Staphylococcus aureus</i> (ATCC 6538)	20± .000	25± .000
5.	<i>Pseudomonas aeruginosa</i> (ATCC 27853)	7± .000	9± .000

**Table 1:** Diameters of the inhibition zone to leaves extracts in comparison with standard reference antibiotic Ciprofloxacin

0.1 gm of leaves residue in two different test tubes and added 0.5 mL of methanol in one test tube [20 % (w/v) solution (E1)] and 1.0 mL of methanol [10 % (w/v) solution (E2)] in other test tube



*Bacillus subtilis* (ATCC 6633)

*Staphylococcus aureus* (ATCC 6538)

*E.coli* (ATCC 8739)



*Salmonella enterica* (ATCC 14028)

*Staphylococcus aureus* (ATCC 6538)

*Pseudomonas aeruginosa* (ATCC 27853)

**Figure 2:** Extracts (E1 and E2) of leaves extract of *A. marina* showed antibacterial activity as indicated by the zone of inhibition against different microorganism’s strain

## Discussion

Since ancient times, plants have been a veritable source of drugs. However, modern societies tend to ignore the importance of herbal medicine. Recently, much attention has been directed towards extracts and biologically active compounds of plants. Therefore, basic factors rationalize the study of antimicrobial properties in mangrove is that these plants can exist in a very stressful condition such as violent environment, high concentration of moisture, high and low tides of water and abundant living microorganism and insect. Due to that, they may develop some mechanisms to overcome these obstacles. Plants that are growing in harsh environments usually possess chemical mechanisms to protect themselves from the predators (Rojas et al 2003). This reason coupled with the extensive using of mangrove plants in folklore medicine are the main reason of choosing mangrove plant.

Mangroves and mangrove associated plants are remarkable source of therapeutic agents having broad medicinal value (Bandaranayake, 2002) [10]. This rich property of mangrove plant extracts is demonstrated to be due to the presence of phytochemicals such as alkaloids, steroids, tannins, flavonoids and sugars in the extracts of this green vegetation [19]. For instance, the antibacterial activity of flavonoids is attributed to the phenolic structure possessing one carbonyl group that complexes with extracellular and soluble protein and, moreover, with bacterial cell wall [20]. These complexes assist in exhibiting the antibacterial activity. Interestingly, the organic extracts from mangrove plant exhibited a varying degree of bactericidal activity as revealed by different size of zone of growth inhibition.

Multiple drug resistance has developed due to indiscriminate use of commercial antimicrobial drugs that are commonly used in the treatment of infectious diseases, making it a global growing problem. There is an urgent need to develop new antimicrobial drugs for the treatment of infectious diseases from medicinal plants, which may be less toxic to humans and possibly with a novel mechanism of action [21-35].

## **Conflict of Interest**

We declare that we have no conflict of interest. All procedures followed were in accordance with the ethical standards (institutional and national).

## **Availability of data and materials**

The relevant data and materials are available in the present study.

## **Acknowledgements**

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## **Author's contributions**

VB supervised the entire project. Supervision of the laboratory work was performed by VB. VB analysed the data and wrote the manuscript. VB did all experiment work.

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