

## Antimicrobial activity of *Avicennia marina* (Forsk) Vierh from Back water area of Puducherry, India

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**Abstract:** Mangroves are the ecologically important coastal wetland. In the tropics, they are especially rich in flora and fauna. They are one of the most productive ecosystems of great ecological and economical significance. Mangrove plant extracts have been used for centuries as popular method for treating several health disorders. Plant derived substances have recently become of great interest owing to their versatile applications. Mangroves are biochemically unique, producing a wide array of novel natural products with antiviral, antibacterial and antifungal activity. The present work deals with antimicrobial activity of the mangrove plant *Avicennia marina* (*Aviceniaceae*) against selected bacteria and fungi. Fresh and healthy leaves of *Avicennia marina* (Forsk) Vierh were collected from Ariyankuppam Back Water area, air dried, pulverized and extracted (5g/25ml solvent) overnight in solvents like ethyl acetate, ethyl alcohol, chloroform and ethyl methyl ketone and screened for antimicrobial activity. The test organism used were Gram negative bacteria; *Pseudomonas aeruginosa* and *Proteus vulgaris*, Gram positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus*, unicellular fungus *Candida albicans* and phytopathogenic fungi viz., *Rhizoctonia solani*, *Colletotrichum gloeosporioides*, *Curvularia lunata* and *Fusarium oxysporum*. Bioactivity was determined by disc diffusion method and well diffusion method. Difference in activity was noticed between the two methods employed. More activity was noticed in well-diffusion method than in disc diffusion method. The test bacteria varied in their sensitivity to the various solvent extracts. Similar trend was noticed for the fungi also. The inhibitory effect observed may be attributed to the inhibitory compounds present in the leaf extract of *A. marina*

**Key words:** *Avicennia marina*, Antimicrobial activity, Pathogenic bacteria.

### Introduction

As mangroves need warm conditions for development and survival, they are found only in tropical climates. Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment; inhibiting bacterial or fungal growth<sup>1</sup>. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world<sup>2</sup>. Mangrove plant extracts have been used for centuries as a popular method for treating several health disorders. Plant derived substances have recently become of great interest owing to their versatile applications. Mangroves are biochemically unique, producing a wide array of novel natural products. Medicinal compounds in the mangroves have long been used in folk medicine to treat diseases<sup>3</sup>. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds<sup>4</sup>. The effects of Mangrove extracts on some microorganisms including *Shigella* sp., *Staphylococcus* sp., *Pseudomonas* sp. has been reported in some studies in the area of pharmacology<sup>5</sup>. Mangrove plants are a rich source of steroids, triterpenes, saponins, flavanoids, alkaloids and tannins<sup>3</sup>. Many researchers reported that the presence of compounds like tannins, alkaloids, steroids and poly phenols in mangroves play important role in the suppression of pathogenic microbes. The present study was carried out to screen the antimicrobial activities of *Avicennia marina* and search for new compounds from mangrove plants.

## Materials and methods

### Plant material

The leaves of *Avicennia marina* were collected from the Ariyankuppam Back water area. Branches and leaves of the plants were cleaned with tap water. Leaves were dried and made into powder form. *Avicennia marina*, commonly known as grey mangrove, belongs to the family Avicenniaceae. It 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 inter tidal zones of sheltered coast lines. It has been reported to tolerate extreme weather conditions and high winds<sup>6</sup>.

### Extract preparation

Four solvents-Ethyl acetate, Ethyl alcohol, Chloroform and Ethyl methyl ketone were taken for extraction of bio compounds from leaf powder. Each 5 gram of *Avicennia marina* leaf powder was added to each 15ml of the four solvents and the leaf powder in four solvents are soaked overnight for two times. The collecting supernatant was centrifuged by 3000 rpm for 10 min. The supernatant were removed and then the samples packed in dark containers and stored at refrigerator temperature after filtered by 0.45  $\mu$  Whatman filter paper<sup>7</sup>.

### Inoculums Preparation

The test fungi *Fusarium oxysporum*, *R. solani*, *Colletotrichum gleosporioides*, *Curvularia lunata*, were maintained in the Potato Dextrose Agar, pH-5.6 were plated in PDA medium. *Candida albicans* were maintained in Nutrient Agar Broth. The test bacteria *P. aeruginosa*, *B. subtilis*, *Staphylococcus aureus*, *Proteus vulgaris* were maintained in Nutrient Agar medium in pH-7.

### Antimicrobial activity

The antimicrobial assay was performed by two methods viz, paper disc method and disc well diffusion method.

#### Paper disc method

In paper disc method, filter paper discs were cut in the diameter of 6 mm and sterilized. These paper discs were soaked in their respective 0.5 ml of solvent extracts. There were 10 discs soaked in each solvent extract. The discs in solvent extracts were dried and used for antimicrobial study. The 350ml of potato dextrose agar-pH 5.6 was used for antifungal activity. After preparing the PDA, 20ml of medium was poured in each plate, so, 16 plates were poured. Triplicates were used for determining the antifungal and antibacterial activity. 300ml of Nutrient Agar was prepared, poured in 15 Petri plates. The plates were allowed to solidify. The discs which had leaf extract impregnation were placed in the 4 corners leaving the Centre for fungal disc inoculation. The results observed from 3-5 days for fungi. The discs were placed on the bacterial lawn in Petri plates for bacteria. The results were observed within 24-48 hrs for bacteria.

#### Well diffusion method<sup>8</sup>

The 350ml of PDA with pH 5.6 and 300 ml NA with pH-7 were poured 16 plates and 15 plates respectively. Triplicates were maintained. The media was allowed to solidify later on the agar plates were holed using a cork borer (6 mm) and 50 $\mu$ l of leaf solvent extract was poured in each well. Test fungi were inoculated in the centre of the plate. For bacteria, bacterial lawn was made followed by removal of disc by cork borer. 5 $\mu$ l of plant leaf extract in 4 different solvents was transferred using a micropipette. Inoculated Petri plates were allowed for diffusion. The antibacterial activity was observed from 24 to 48hrs and for fungi from 3-5 days.

## Results

Bioassay results shown by the Ethyl acetate, Ethyl alcohol, Chloroform and Ethyl Methyl Ketone solvent extracts in well diffusion method are listed in Table 1.

**Table1: Antimicrobial activities of leaf extract of *Avicennia marina***

Solvents	R.so	C.gl	C.lu	F.ox	C.alb	P.aer	B.sub	P.vul	S.aur
E Ac(1)	10	8	18	18	-	-	-	-	-
E A(2)	12	6	6	16	4	18	6	-	-
C F(3)	8	8	12	22	-	-	-	-	-
E M K(4)	6	4	-	20	-	-	-	-	-

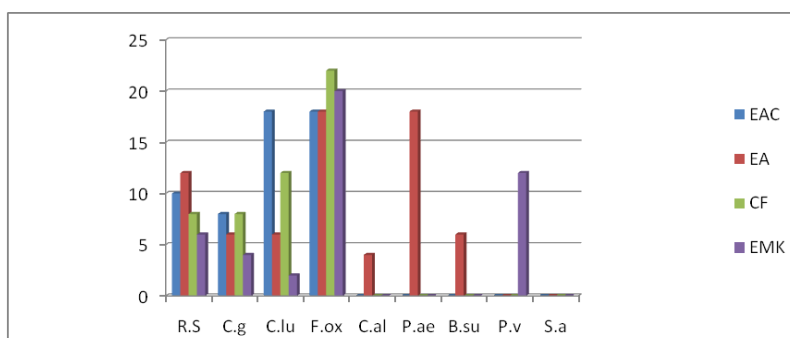
(1) Ethyl acetate (2) Ethyl alcohol (3) Chloroform (4) Ethyl Methyl Ketone

Among these extracts, Ethyl alcohol extract (50µl) showed wide inhibition against all the fungi used for the susceptibility test and showed inhibition against *P. aeruginosa* (18mm) and *Bacillus subtilis* (6mm). *Staphylococcus aureus* found to be resistant against all the 4 solvent extracts used in the study. Ethyl Methyl Ketone showed inhibition against *Proteus vulgaris* (12 mm). The Ethyl acetate and Chloroform extracts showed no inhibition against the test bacteria. All the solvent extracts showed inhibition against the test fungi; *R. solani*, *C. gleosporioides*, *C. lunata*, *F. oxysporum* except *C. albicans*. The growth of *C. albicans* was inhibited in Ethyl alcohol extract. Paper disc method showed less inhibitory effect than the well diffusion method. The variation in inhibition in the 4 solvents exhibited that there were presence of antimicrobial substances which varies from fraction to fraction of crude extract. From the antimicrobial activities, it is concluded that the leaf extract of *Avicennia marina* has both antifungal and antibacterial activities.



a. *C. gleosporioides*      b. *R. solani*      c. *C. lunata*

**Fig 1: Antifungal activity of the leaf extracts of *Avicennia marina* by well diffusion method.**



**Fig 2: Histogram of the antimicrobial activity of leaf extract of *Avicennia marina*.**

Numbers in Y axis shows inhibition of growth of microbes against leaf solvent extracts. Letters in X axis shows microbes used for this study. R.s- *Rhizoctoniasolani*, C.g- *Colletotrichum gleosporioides*, C.lu- *Curvularia lunata*, F.ox- *Fuzarium oxysporum*, C.al- *Candida albicans*, P.ae- *P. aeruginosa*, B.su- *Bacillus subtilis*, P.v- *Proteus vulgaris*, S.a- *Staphylococcus aureus*.

**Discussion**

The plants used in the work are important source for traditional medications.<sup>9</sup> It is essential to study about the results scientifically that mangroves are used as traditional medicines to determine the source of antimicrobial compounds<sup>10</sup>. The use of plant extract in the treatment of diseases caused by various bacteria and

fungi were reported. Anti-fungal properties of plant extracts are were recognized<sup>11</sup>. The results showed that ethanolic leaf extract of *Avicennia marina* had good inhibitory activity for both fungi and bacteria used in this study compared to EMK, EAC. Chloroform extracts and EAC leaf extract had more inhibitory activity towards fungi used in this study for the wide antifungal activity, which may be corroborated with the presence of Xanthone in the mangrove plants. These compounds have toxicological characteristic towards fungi reported by earlier workers<sup>1</sup>. Presence of fatty acids in the mangrove plants play vital role in inhibitory activity of fungi & bacteria<sup>12, 13</sup>. The results of our study indicated that the four solvent leaf extracts showed antifungal activities. This shows that there are many secondary metabolites produced by the mangrove *Avicennia marina* to resist the phytopathogens and human pathogens as reported by many researchers in mangroves. Ethanolic leaf extract of *Avicennia marina* showed both antifungal and antibacterial activity as reported<sup>14</sup>.

## Conclusion

It is an important period to know about the values of medicinal properties in mangroves to resist the pathogenic bacteria, fungi and viruses which cause the infectious diseases among the human beings, animals and plants. These medicinal properties can be of great significance for therapeutic treatments. Results of our findings revealed that the Ethyl alcohol extract exhibited growth inhibitory activity against bacteria and fungi followed by other solvents used for the study. Since the leaf extract of *A. marina* controls both phytopathogens and human pathogens, the problems which arise from both sides can be controlled by using the leaf extract of *Avicennia marina* in the field of Pharmacology.

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