

# ANTIMICROBIAL ACTIVITY OF A TRUE MANGROVE- AVICENNIAOFFICINALIS LEAF EXTRACT AGAINST SOME PATHOGENS

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**Abstract:** Methanolic crude leaf extracts of a true mangrove commonly called as Indian mangrove *Avicenniaofficinalis* L. has been evaluated for its antimicrobial activity. The results has shown that the crude extracts exhibits the inhibition of growth of three species of bacteria and two species of fungus which are *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* in addition to fungus *Aspergillusniger* and *Aspergillusflavus*. The methanolic Crude extracts of leaf had significant effect on the bacterial pathogens with inhibition zones ranging from 14 to 26 mm and 13 to 16 mm respectively whereas it showed no antimicrobial activity with aqueous solution.

**Key words:** Indian mangrove, Antimicrobial activity, Crude leaf extracts, MDIZ (Mean Diameter of inhibition zone).

**1.Introduction:** Mangroves are salt tolerant evergreen woody plants that have special morphological adaptations naturally growing in the tropical and subtropical intertidal regions of the world , largely confined to the region between 30<sup>o</sup> north and south of the equator. They include approximately 12 families and more than 50 species.*Avicenniaofficinalis* is a species of mangrove also known as Indian mangrove . The species is found in the intermediate estuarine zone in the lower intertidal region.It is shade intolerant with a maximum porewater salinity of 63 ppt( Robertson and Alongi, 1992). This species grows on soft recently consolidated mudbanks. This species is a tree or shrubs that grows to 25 m but is more often seen at 5-10 m. This species is a fast growing species . It has a high tolerance of hypersaline conditions. As antibiotics are increasingly used and misused day by day , the bacterial strains become resistant to antibiotics , the plant derived substances have recently become of great interest owing to their versatile applications. As the rise of antibiotic resistant organisms is one of the severe problem in health care system , hence there is a need for searching infection fighting strategies to control microbes. Mangroves are highly source of several bioactive compounds and secondary metabolites like alkaloids, phenolic compounds, steroids, tannins, flavonoids, triterpenoids etc. There is a continuous and urgent need to discover several new antimicrobial agents with chemical structure and novel mechanism of action from mangrove trees and other terrestrial plants [4].

In India, this species is used for fodder for domestic livestock (goats, buffaloes, cattles). It is also harvested for timber and used for firewood and also as toothache pain reliever [14].In our present study, an attempt has been made to evaluate the antimicrobial activities of *Avicenniaofficinalis* leaf extracts.

## Taxonomic position of the plant:

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Lamiales

Family: Avicenniaceae (APG: Acanthaceae)

Genus: *Avicennia*

Species: *Officinalis*

## 2. Materials and Methods:

2.1. Collection of *Avicenniaofficinalis* plant material.

Plant material (fresh leaf) were collected from sundarbans ecosystem (21<sup>o</sup>13'N to 22<sup>o</sup>40'N and 88<sup>o</sup>03'E to 89<sup>o</sup>07'E) at the apex of Bay of Bengal is a mangrove dominated delta in the lower Gangetic region in West Bengal, India. The specimen had been identified at Dept. of marine science , university of Calcutta, W.B, India.

## 2.2. Preparation of plant (leaf) extract :

In the present study, *A. officinalis* fresh leaf had cleaned with tap water and shade dried for 72 hrs under appropriate conditions. The shade dried samples were pulverized into small pieces with the help of sterilized pestle and mortar. 20 g of crushed material had taken separately into 500 ml of methanol and sterilized water that had kept on a rotary shaker at 120 rpm for 24 hrs.

After shaking, it is filtered through eight layers of muslin cloth and centrifused at 5000 rpm for 15 minutes. Resultant extracts were evaporated and concentrated to dryness.

## 2.3. Determination of antimicrobial activities:

Antimicrobial activities of *A. officinalis* leaf extracts were assessed against five pathogens . Three bacterial strains – *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus aureus* and two fungal strains- *Aspergillusniger* and *Aspergillusflavus*. Bacterial and fungal strains were collected from the Dept. of Microbiology, University of Calcutta, W. B., India. The organisms were maintained on nutrient agar slopes at 4<sup>0</sup> c.

Extracts of *A. officinalis* had been tested for antifungal and antibacterial activity by agar diffusion method. 48 hrs old broth fungal culture grown on potato dextrose agar were used for inoculation of plates while 24 hrs old broth bacterial cultures grown on nutrient agar plates were used. An aliquot (0.05 ml) of inoculums was introduced to the molten agar medium poured into a petri-dish by pour plate technique.

In agar, 0.1 ml of each extract were introduced serially. Incubation period of 24-48 hrs at 28<sup>0</sup>c for fungal growth and 30<sup>0</sup>c for bacterial growth were maintained for observation of antimicrobial activity. Amoxycillin( 15µg/disc) was used as standard. Minimal inhibitory concentration ( MIC) had been determined by two fold micro dilution method[8,9]. Minimal inhibitory concentration (MIC) and Minimal bacterial concentration( MBC) had been seen in those strains which showed zone of inhibition (IZ) against the leaf extracts. The following formula had been used for comparison of antimicrobial activity of the sample with that of the standard.

Antimicrobial index = Inhibition zone of sample /Inhibition zone of the standard\*100

## 3. Results and Discussion:

Antimicrobial activity of the two extracts (methanol and water) of *A. officinalis* were carried in vitro by agar well diffusion method against three bacterial strains and two fungal strains. The methanolic extract of leaf were more effective against bacteria and fungus whereas it showed negative activity against aqueous extract. The significant antibacterial activity of the active leaf extracts had been compared with standard antibiotic( 100 mg/ ml at 15 µl/disc). The zone of inhibitions ranging from 14 to 26 mm were recorded for methanol extracts against bacteria. Among the tested microbial strains , fungal pathogens exhibited less inhibition effect than bacteria ranging from 13 to 16 mm. In contrast to other hand, *A. officinalis* leaf extracts had the highest effect on *Staphylococcus aureus* bacterial strain. According to relative resistance to the leaf extract *A. niger* and *A. flavus* showed the most resistant strains. Aqueous solution had not been considered for the antimicrobial activity. It could be argued , that the methanolic extract of *A. officinalis* suppressed the growth of tested strains in varying degree, indicating presence of broad spectrum inhibitory activities[13]. However, difference in inhibitory effect may be due to the variation of cell wall structure of Gram-positive and Gram- negative or thickness of bacterial cell wall. On the other hand, fungal cell wall contain mainly chitin which causes rigidity of cell wall and may suppress efficiency of inhibitory attack[11].

Table 1: Methanolic extract of *A. officinalis* showed antimicrobial activity against tested microorganisms:

tested organisms	mdiz( mean diameter of inhibition zone) in mm	
	methanol	water
Bacteria: 1. <i>Bacillus subtilis</i>	15	-
2. <i>Staphylococcus aureus</i>	24	-
3. <i>Escherichia coli</i>	14	-
Fungus : 1. <i>Aspergillusniger</i>	13	-
2. <i>Aspergillusflavus</i>	12	-

Table 2: Methanol extract of *A. officinalis* exhibited Minimal Inhibitory Concentration ( MIC) against standard microorganisms:

microorganisms	concentration in mg/ ml			
	diameter of inhibition zone ( mm)			
	100	50	25	12.5
1. <i>Bacillus subtilis</i>	16	14	13	12
2. <i>Escherichia coli</i>	16	14	13	12

3. <i>Staphylococcus aureus</i>	25	22	19	18
4. <i>Aspergillus niger</i>	14	13	12	12
5. <i>Aspergillus flavus</i>	12	10	8	8

#### 4. Concluding Remarks:

Our attempt of this work is to identify the varying degree of antimicrobial efficiency of a true mangrove species *Avicennia officinalis* leaf extracts using methanol solvent showed the greatest result of inhibition which ranged from 14 to 26 mm whereas there was no effect in case of aqueous solvent. The best result had been found towards *Staphylococcus aureus* bacteria and the lowest against the fungus *Aspergillus flavus*. The viable antimicrobial compounds derived from methanolic extracts are ecologically safe.

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