

**ANTIBACTERIAL ACTIVITY OF THE LEAVES OF MANGROVE
PLANT *AVICENNIA* SPECIES COLLECTED FROM TWO DIFFERENT
LOCATIONS IN MAHARASHTRA**

V. N. Magare* and Charuu P. Kulkarnii

*Principal, Dept. of Zoology, Kirti M. Doongursee College of Arts, Science and Commerce,
Dadar (W), Mumbai.

Dept. of Chemistry, Kirti M. Doongursee College of Arts, Science and Commerce, Dadar
(W), Mumbai.

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***Corresponding Author**

Dr. V. N. Magare

Principal, Dept. of Zoology,
Kirti M. Doongursee
College of Arts, Science
and Commerce, Dadar (W),
Mumbai.

ABSTRACT

Mangroves have wide applications in folklore medicine since ages due to the presence of several bioactive compounds. There is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and reemerging infectious diseases. The present study was carried out to evaluate the antibacterial potential of the leaf extracts of mangrove plant *Avicennia*

officinalis collected from two separate locations of the Maharashtra state. Extracts prepared using methanol as the solvent showed promising activity against the test organisms. Results also highlighted that the habitat has a role in the antibacterial activity exhibited by the plant.

KEYWORDS: Antimicrobial, Mangroves, *Avicennia*, Chemical characterisation.

INTRODUCTION

Mangroves are perennial plants that grow in coastal regions of tropical regions. They live in hostile environmental conditions such as high salinity, hypoxic (oxygen deficient), water logged soil strata, tidal pressures, strong winds and sea waves. To cope up with such a hostile environment, mangroves exhibit highly evolved morphological and physiological adaptations to extreme conditions.^[1]

Mangrove plants and their products have been used in folklore medicines for centuries in the treatment of several health disorders. It is well known that the mangroves are a rich source of bioactive compounds.^[2,3] Earlier studies on mangrove plant parts and its major chemical classes displayed various level of biological activities such as antibacterial, antifungal, antiplasmodial, cytotoxic, antifouling, hepatoprotective, ichthyotoxic, and free radical scavenging activities.^[4-7]

Species belonging to genus *Avicennia* are important members of Indian mangroves. India's traditional healers used *A. officinalis* to treat smallpox infection in the past. Attempt has been made to evaluate the antimicrobial potentialities of *A. marina*.^[8] However, recent reports on *A. officinalis* from Maharashtra are hardly available especially reporting a comparative study on samples collected from two different locations. With this background, the present study was undertaken with an aim of evaluating the antibacterial activity of the methanol extracts prepared from the leaves of *A. officinalis*.

MATERIALS AND METHODS

Sample collection

The fresh mangrove plants leaves were collected from the mangrove forests of Thane and Ratnagiri districts of Maharashtra state (West coast of India). Each collection site was approximately 100 Km away from each other. The collected mangrove leaves were then washed thoroughly under the running tap water in the laboratory order to remove dirt, germs and other contaminants from the sample. After collection, the leaves were shed dried for three days and used further for solvent extraction.

Preparation of extract

The dried leaves were powdered and 100 grams of the powder was taken in a Soxhlet apparatus for extraction using methanol as the solvent. After completion of soxhlet extract, filtration was carried out and further the organic solvent layer was evaporated (under vacuum) by using rotary evaporator.

Test microorganisms and media

Both gram positive and gram negative bacterial strains were taken for the test. A total of five standard human pathogenic bacteria were procured from Government hospital. The organisms were maintained on nutrient agar slants and stored at 4°C with periodic sub-culturing.

Antibacterial assay

Antibacterial activity was tested in triplicate using the standard paper disk diffusion method. Stock solutions of extracts were prepared by dissolving in 50 mg/ml of methanol. The extracts (1 mg per disk) were applied to sterile paper disks (6 mm in diameter). The solvent was evaporated before they were placed onto agar plates that had been seeded with reference bacterial strains. The diameters of the inhibition zones (diameter of inhibition zone minus diameter of disc) were measured in millimeters after incubation at 30°C for 24 hours. Solvent control discs without extracts prepared in the same manner were never observed to inhibit bacterial growth.

UV-VISIBLE spectroscopy

UV-Visible spectroscopy was carried out by using SHIMADZU UV spectrometer. The crude extracts were weighed, dissolved in suitable organic solvents and scanned.

FTIR spectroscopy

5 mg of dried methanol extracts of mangrove leaves collected from two different locations were taken and crushed to a fine powder using mortar and pestle. Kalium bromide was added and evenly mixed with the sample to obtain a homogenized fine powder. The sample was then placed in the moulds and pressed using mechanical strength for 20-30 seconds using a clean alcohol sterilized spatula. Analysis was done using Shimadzu FTIR.

RESULTS**Mangrove leaf extracts**

From 100 grams dried powder of mangrove leaves, approximately 2.8 grams of extracts were obtained from both the collection sites.

Antibacterial activity

Antibacterial activity of methanolic extracts of mangrove leaves were examined and found to exhibit considerable amount of antibacterial activity against most of the gram positive and gram negative organisms as depicted (Table 1).

Table 1: In vitro antibacterial screening of crude methanolic fractions from mangrove leaves, collected from two different locations.

Collection site	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Vibrio cholera</i>	<i>Salmonella typhi</i>
Thane	14±1	-	13±1	-	8±1
Ratnagiri	11±1	-	9±1	-	5±1
Ampicillin	13±	15±	19±	20±	17±1
methanol	-	-	-	-	-

UV spectroscopy

In absence of any additional chromophores, carboxylic acid (RCOOH) or ester (RCOOR) shows typical UV absorption at 195-210 (λ_{max}), thus carboxylic acid functionality may be present in the sample 1 (Thane) and 2 (Ratnagiri). Also carbonyl groups of saturated ketone or aldehyde give a weak absorption in UV region between 270-300 nm. The UV absorption by aromatic systems shows one band of moderate intensity occur near 205 nm and another less intense band appears in the region of 250-275 nm range indicating presence of aromatic skeleton (Fig.1).

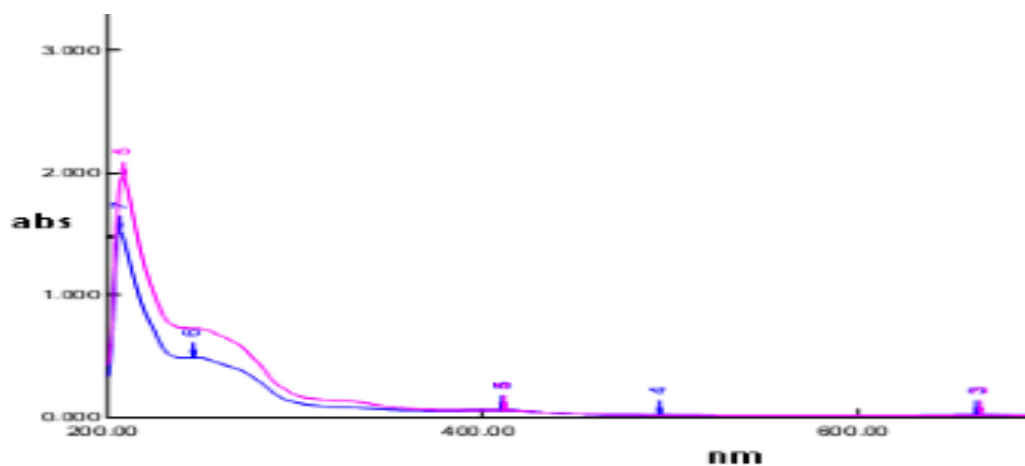


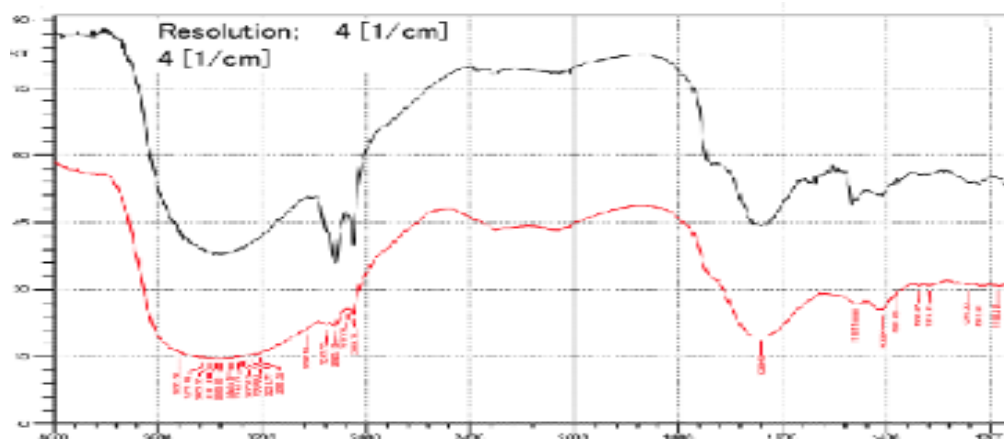
Figure 1 UV spectra of mangrove leaf extracts, collected from two different locations.

FTIR spectra

The FTIR spectrum was used to identify the functional group of the active components present in extract based on the peaks values in the region of infrared radiation. The results of FTIR peak values and functional groups and the FTIR spectrum profile is illustrated in the Fig. 2 & Table 2.

Table 2 FTIR spectra of mangrove leaf extracts, collected from two different locations.

Sr. No.	Peak value (cm-1)	Functional group
1	3209-3523	Hydroxyl (alcohol, carboxylic acid, phenol, <i>etc</i>)
2	2850-2922	Alkane
3	1600-1750	Carbonyl (ketone, aldehyde, ester, amide <i>etc</i>)
4	1460	Alkene, alkane

**Figure 2 FTIR spectra of mangrove leaves extracts, collected from two different locations.**

DISCUSSION

In the present study, extracts were prepared using methanol as the solvent and these extracts showed promising antimicrobial activity highlighting that active ingredients in mangrove plants can be successfully extracted into methanol. This observation is in the agreement with the Arivuselvan *et al.*, who investigated the antibacterial activity of leaves and bark extracts of mangroves *Ceriops tagal* and *Pemphis acidula* using acetone, methanol, ethanol and water extract against human pathogens and reported that methanolic crude extracts exhibited better inhibition.^[9]

Overall results clearly indicate the antibacterial potential of the leaf extracts of *Avicennia* sp. Bakshi and Chaudhuri highlighted that the *Avicennia marina* extracts were active in lowest concentrations and thus need to be further explored.^[10] Extracts of *A. alba* has also reported to have strong activity against certain plant and human oral pathogens.^[11] Significant antibacterial activity was observed against *S. aureus* by the leaf extract of *Avicennia officinalis* in the present study. Abeysinghe 2010 also reported that leaf and bark extracts of *A. marina* showed promising antibacterial activity including that against an antibiotic resistant *S. aureus* strain.^[12] So overall results suggest the importance of genus *Avicennia* to the field of drug discovery.

Investigation and identification of the antimicrobial potential of common mangrove plants of the Konkan region could lead towards a new dimension to clinical medicine as well as the betterment of the lives of under-privileged local population of this region. This study also highlights the use of mangroves for biological control of pathogens and substitute the chemical agents applied to control those pathogens.

CONCLUSION

The extract prepared from mangrove leaves collected from the mangrove *Avicennia officinalis* from Thane region showed stronger antibacterial activity as compared to the samples from Ratnagiri. This indicates that habitat influences the antibacterial activity of mangroves. However chemical composition of both the collection was found to be similar. To conclude, mangroves are a treasure house of therapeutic compounds.

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