Evaluation of antibacterial activity of *Avicennia marina* leaf extract against *Staphylococcus aureus*

Running Title - Antibacterial activity of *Avicennia marina* leaf extract

ABSTRACT

Introduction: Mangrove are widespread in the tropical and subtropical regions of Indo-West-Pacific area. *Avicennia marina* possesses vast medicinal values. The pharmacological activity of the plant is attributed to the presence of several phytochemicals.

Aim: To evaluate the antibacterial activity of *Avicennia marina* leaf extract against *Staphylococcus aureus.*

Methods and Materials: Plant extract prepared by adding 20g of dried powdered mangrove leaf sample to 100ml of methanol. MDRSA, MRSA & VRSA were cultured in Muller –Hinton Broth for 24 hr at room temperature. From this prepared bacterial suspension, 1ml of was spread over Muller Hinton agar plate and incubated for 24hrs at ambient temperature. Antibacterial activity carried out through the disc diffusion method. Whatman filter paper discs (5mm) were impregnated with various concentrations (50, 100, 150, 200, 250 & 300 µg/ml) of leaf extract. After incubating the plates for 24hr at room temperature the zone of inhibition was measured. Minimum Inhibitory Concentration was determined in 5 concentrations (50 -300 µg/ml) with blank (extract in Muller Hinton broth). The inoculated bacteria in test tubes are incubated for 24hr in ambient temperature. The results are noted as well growth (+) and inhibited (-). Tetracycline (1µg/ml) was used as standard and DMSO as negative control.

Results: The zone of inhibition was greater at 300µg/ml of the extract for MDRSA (13±1.2) , MRSA ((13±1.4))& VRSA (9±1.2). MIC for MDRSA, MRSA and VRSA was 250µg/ml, 200µg/ml 150µg/ml respectively.

Conclusion : The present study showed effective antibacterial activity of *Avicennia marina* against MDRSA, MRSA, VRSA. Hence, this extract may be used for infections against resistant *Staphylococcus aureus.*

Keywords: *Staphylococcus aureus, Avicennia marina*, antibacterial activity, mangroves,natural source

INTRODUCTION

Plants are recently explored for their pharmacological activities in different health conditions such as cancer, diabetes, inflammatory as well as oxidative stress conditions (1–10).Mangroves are a group of trees and shrubs that live in the coastal intertidal zone. Mangrove trees can grow under adverse environmental conditions like low-oxygen soil, high temperature, high salinity, etc. The distribution of mangrove forests is mainly at the tropical and subtropical latitudes (11).

Mangrove plants are a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids, tannins (12,13). Extracts from different mangrove plants are reported to possess diverse medicinal properties such as antibacterial, anthelmintic (14). Marine organisms including coastal plants and mangrove trees are known for their ability to produce secondary metabolites that differ from the terrestrial plants due to their specific adaptation such as aerial roots, viviparous seedlings, high tolerance to extreme temperature, weather and salinity (15)-(16). Mangrove ecosystems serve as a natural filter of land-derived waste materials thus supporting the health of marine ecosystems (17)- (18,19).

The *Avicennia marina* (Forssk.) Vierh., a mangrove tree belonging to the Acanthaceae family, is mostly found in the subtropical and tropical regions of the Indo-West-Pacific area. It is considered a representative example of mangroves that have been widely investigated for their medicinal importance (20). The presence of the various categories of phytochemicals makes this plant an excellent candidate for the treatment of various health ailments. *Avicennia marina* has been used in the traditional medicine system for many centuries(21) (22) (23) (24). For example, leaves are used for the treatment of ulcers, abscesses, and rheumatism. The leaf decoction is also used for the treatment of malarial fever and food poisoning (25).Our team has extensive knowledge and research experience that has translated into high quality publications (26–30) (31). The aim of this study was to evaluate the antibacterial activity of *Avicennia marina* leaf extract against *Staphylococcus aureus*.

METHODS AND MATERIALS

Study setting: Marine Biomedical and Environmental Health Research Lab - Blue Lab,Saveetha dental College. The study was ethically approved by the Scientific Review Board, Saveetha Dental College, Chennai.

Collection of plant material and preparation

The fresh leaves of *Avicennia marina* were collected from Tuticorin, Tamilnadu. The leaves were washed thoroughly with tap water then shade dried on table tissue paper for 2-3weeks and turned into a fine powder.

Preparation of extraction: 20g of dried powdered mangrove leaf samples were mixed with 100ml of methanol/Ethanol and allowed to place for 24 hours at ambient temperature. Then the mixture was passing through whatman filter paper (No.4) then the filtrate was centrifuged at 3000rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4ºC for further analysis.

Bacterial Suspension: Multi drug resistant *Staphylococcus aureus* (MDRSA), Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Staphylococcus aureus*(VRSA) was collected from Department of Microbiology, Saveetha medical college and hospital, Tamilnadu. The bacterial pathogens were cultured in Muller–Hinton Broth for 24 hr at room temperature. From this bacterial suspension was prepared with saline and the optical density was measured at 600 nm. The concentration of microbial suspension was fixed as 10^8 CFU/ml. 1ml of suspension was spread over on Muller Hinton agar plate and incubated for 24hrs at ambient temperature.

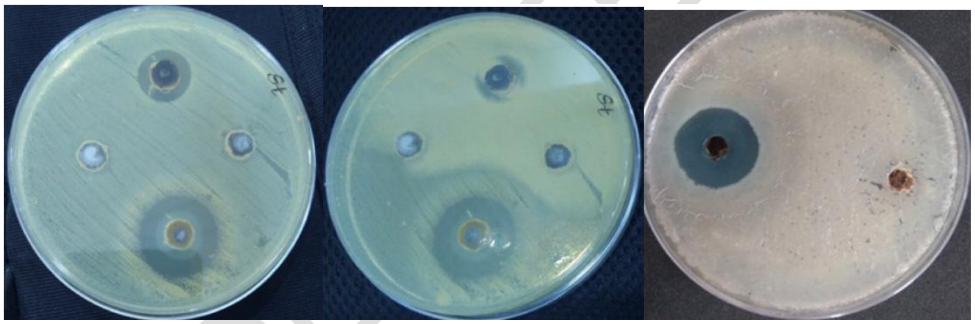
Antibacterial activity: The antibacterial activity of mangrove leaf extract was performed with disc diffusion method. Whatman filter paper discs (5mm) were impregnated with various concentrations (50, 100, 150, 200, 250 & 300 µg/ml) of leaf extract. The inoculated plates were incubated for 24 hr at room temperature and the inhibition zones around the discs were measured. All the results were expressed with mean ± standard error.

Minimum Inhibitory Concentrations:

Minimal Inhibition Concentration of mangrove leaf extract was determined in 5 concentrations (50 -300 µg/ml) with blank (extract in Muller Hinton broth). The inoculated bacteria in test tubes are incubated for 24hr in ambient temperature. The results are noted as well growth (+) and inhibited (-). Tetracycline (1 mg/ml) was used as standard and DMSO was used as negative control.

Figure 1: Collection of *Avicennia marina* plant collection and preparation

RESULTS



A **B** **C**

Figure 2: Images showing antibacterial activity of *Avicennia marina* extract against (A) MRSA, (B) VRSA, (C) MDRSA

Table 1: Table showing zone of inhibition produced by *Avicennia marina* on MDRSA, MRSA and VRSA . The values are given in triplicate with mean ± SE.

Concentration of *Avicennia marina* (µg/ml)	**MDRSA**	**MRSA**	**VRSA**
0	0	0	0
50	2±0.86	0	0
100	5±1.3	2±0.57	2±0.8
150	8±1.07	5±1.2	4±1.07
200	11±1.2	6±1.4	5±1.2
250	12±1.4	9±1.2	8±1.4
300	13±1.2	13±1.4	9±1.2

Table 2: Table showing MIC for MDRSA, MRSA and VRSA

µg/ml	**50**	**100**	**150**	**200**	**250**	**300**	MIC (µg/ml)
MDRSA	**+**	**+**	**+**	**+**	**-**	**-**	**250**
Tetracycline	**+**	**+**	**-**	**-**	**-**	**-**	**150**
MRSA	**+**	**+**	**+**	**-**	**-**	**-**	**200**
Tetracycline	**+**	**+**	**-**	**-**	**-**	**-**	**150**
VRSA	**+**	**+**	**+**	**+**	**-**	**-**	**250**

Tetracycline	**+**	**+**	**-**	**-**	**-**	**-**	**150**

On measuring the zone of inhibition for each pathogen at different concentrations ranging from 50µg/ml to 300µg/ml. The zone of inhibition for MDRSA was minimum i.e. 2mm at 50µg/ml of concentration and maximum i.e. 13mm at 30µg/ml of concentration. The zone of inhibition for MRSA as well as VRSA was minimum i.e. 2mm at 100µg/ml of concentration and maximum i.e. 13mm and 9mm respectively at 300µg/ml of concentration (Table 1). The zone of inhibition at 300µg/ml of concentration for MDRSA and MRSA showed higher effect (13mm) when compared to VRSA (9mm). The minimum inhibitory concentration for MDRSA was 250µg/ml of concentration, MRSA was 200µg/ml of concentration and VRSA was µg/ml of 150µg/ml of concentration (Table 2).

DISCUSSION

The heartwood of Red sanders is known to have antipyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, and diaphoretic activities. It has also been used as a cooling agent. Ethanol extract of stem bark was reported to possess anti-hyperglycaemic activity. The wood in combination with other drugs is also prescribed for snake bites and scorpion stings. Phytochemical investigations of aqueous and ethanol extracts of stem bark has revealed the presence of alkaloids, phenols, saponins, glycosides, flavonoides, triterpenoids, sterols, and tannins (3),(32)-(33).

The present interest in medicinal plants as therapeutic agents has progressed in different parts of the world because of the increasing incidence of bacterial resistance to chemical drugs and the emergence of new pathogenic bacterial strains. On carrying out in vitro testing of a large number of plants against various bacterial strains, it has been demonstrated that extracts and pure compounds of numerous medicinal plants are very effective against bacterial strains. (34)

In India, the epidemiology of MRSA has been changing over the past few decades. The resistance of MRSA to B-lactam like penicillin and amoxicillin is 100%. (35) Previous studies have revealed that plant extracts exhibit antibacterial activity against pathogenic bacteria stains such as, *Staph. aureus*, *E.coli*, Pseudomonas and antibiotic resistant strains. In a similar study conducted by Dhayanithi et al., 2012 reported that A. marina extract showed inhibitory activity against multi-drug resistant *S. aureus*. *Staphylococcus aureus* strains showed resistance to ciprofloxacin, methicillin and vancomycin based on the antibiotic susceptibility test. (36)

Human oral cavity is normally sterile at birth but soon it possesses a predominant streptococcal microbiota. The species often related to "viridans streptococci" comprises *Streptococcus salivarius, Streptococcus sanguis, Streptococcus mitis, and Streptococcus mutans*. (37) Dental caries, a microbial disease is caused by α-hemolytic streptococci usually referred to as *S. viridans*, a major component of dental plaque and primarily responsible for pediatric dental health related problems. (38)

In a study conducted by Mahasneh 2002 using *Avicennia marina* leaves extracts in methanol showed antibacterial activity against *B. subtilis, S. aureus, and Streptococcus pyogenes* with approximately 20 mm zone of inhibition. Butanol crude extract of the plant exhibited a potent antibacterial activity against both Gram-positive and Gram-negative bacteria including *E. coli, B. cereus, S. aureus, P. aeruginosa, A. flavus, C. albicans* with zone of inhibition measuring 15 mm, 14 mm, 10 mm, 14 mm, 13 mm, and 14 mm, respectively (39)

A study conducted by Okla et a., 2021 has shown that root and leaf extracts of *A. marina* exhibited antibacterial activity. Ethanol root extract proved to be effective against bacterial strains, *P. aeruginosa, B. subtilis, S. aureus,* and *E. coli* as well as fungal strains *Aspergillus fumigatus* and *Candida albicans.* Leaf extract in ethyl acetate displayed significant antibacterial activity against *S. aureus* and *E. coli*. (40)

CONCLUSION

The present study showed effective antibacterial activity of *Avicennia marina* against MDRSA, MRSA, VRSA(41)-(42) . Hence, this extract may be used for infections against resistant *Staphylococcus aureus.*

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