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ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF MANGROVE PLANT AVICENNIA OFFICINALIS L. FROM MAHARASHTRA COAST

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Abstract

The objective of present study is to investigate the antibacterial activities of n-hexane, ethyl acetate and methanolextracts of the leaves of AvicenniaofficinalisL.(A. officinalis) against six human pathogenic microbes.The antibacterialactivity was evaluated using disc diffusion and microdilutionmethods. The antibacterialactivities of the crude extracts were increased withincreasing the concentration. It is clear that n-hexane extract was the most effective extract.Additionally, Gram positive Bacillus cereus (B. cereus)appear to be the most sensitive strainwhile Pseudomonas aeruginosa (P. aeruginosa). The inhibition of bacterial growth atconcentration as low as 0.04 mg/mL indicated the potent antibacterial activity of A. officinalisextracts. This concludes that the obtained results are considered sufficient for further study to isolatethe compounds responsible for the antibacterialactivity and suggesting the possibility of finding potentantibacterial agents from A. officinalisextracts.

Keywords: *Mangoves, Avicenniaofficinalis L, antibacterial activity*

1. Introduction

Infectious diseases represent a serious public healthproblem and they remain the leading cause of deaththroughout the world[1-3]. Currently, the problems ofmicrobial drug resistance, an increase of opportunisticinfections and the toxicity effect of continued use of severalantimicrobial drugs[4] have necessitated a search for newantimicrobial drugs from other sources including naturalsources like plants which are the good sources of novelantimicrobial chemotherapeutic agents. Furthermore, plantshave been a major source for drug development[5-7]. Plantextracts and products are used in the treatment of infectiousdisease[8-10].*Avicenniaofficinalis*(*A. officinalis*)(locally known as Tagal) is large mangrove tree (up to 10 – 12 m tall) belongs tothe Avicenniaceae family. It is distributed throughout theIndian coast [11]. However, *A. officinalis*has been known traditionally as an important remedy forsprue[11]. Up to date, there are no study has been conductedon the evaluation of the antibacterial activity of this plant.Therefore, this study aims to investigate

the antimicrobialactivities of *A. officinalis*extracts against four human pathogenicbacteria including two Gram-positive (*Staphylococcusaureus*(*S. aureus*) ATCC25923, *Bacillus cereus*(*B. cereus*)ATCC11778) and two Gram-negative (*Pseudomonas aeruginosa*(*P. aeruginosa*) ATCC27853, *Escherichia coli*(*E. coli*)ATCC35218). The efficacy of n-hexane, ethylacetate and methanol extracts from the leaves of *A. officinalis*were also investigated and described.

2. Materials and methods

2.1. Plant collection

*A. officinalis*was collected from Maharashtra coast at Raigad district in August 2012. The Voucher of the specimenwas deposited in the Department of Botany, DoshiVakil Arts and G.N.S.B. Science and Commerce College,Goregaon-Raigad. The taxonomic identification of this plant was doneby using Flora of Maharashtra.

2.2. Plant preparation and extraction

The fresh plant was washed under running tap water anddried in a warm room for 3 to 5 d. The samples were grindedinto fine powder and extracted by Soxhlet with n-hexane,ethyl acetate and methanol successively to get n-hexane,ethyl acetate and methanol extracts. Then, all the crude waskept at -20 ℃ until further use.

2.3. Samples preparation

A sample of 100 mg from each extract was dissolved in1 mL DMSO. The extract was then sterilized by filtrationthrough sterile syringe filter (0.2 µm pore). Finally thefiltered extract was stored as aliquots until it was used.

2.4. Microbial strains

Four reference strains of human pathogenic bacteria were used in thisstudy including two Gram-positive (*S. aureus*ATCC25923,*B. cereus*ATCC11778) and two Gram-negative (*P. aeruginosa*ATCC27853, *E. coli*ATCC35218).

2.5. Antimicrobial assay

2.5.1. Disc diffusion method

The agar disc diffusion method was employed for thedetermination of antibacterial activities of the extractsaccording to Qaralleh*et al.*[12] with some modification.Briefly, inoculum containing 107 CFU/mL was spread onMueller-Hinton agar plates for four bacteria.Using sterile forceps, the sterile filter papers (6 mmdiameter) containing the crude extracts (1 or 1.5 mg),standard antibiotics (30 µg of chloramphenicol) or negative control (DMSO) were laiddown on the surface of inoculated agar plate. The plateswere incubated at

37 ℃ for 24 hours. Eachsample was tested in duplicate and the zone of inhibitionwas measured as millimetre diameter.

2.5.2. Microdilution method

Minimum inhibitory concentration (MIC) was measuredby determining the smallest amount of extract or standardantibiotic needed to inhibit the visible growth of a test bacterium. This was done using 96-well plates. Theassay plates were filled with Mueller-Hinton broth medium(MHB) containing different concentrations of extracts, tetracycline or solvent control and the test bacterium(107 CFU/mL). Each sample was tested in triplicate andthe observation was recorded by naked eyes after 24 h incubation periods at 37 ℃.Minimal bactericidal concentration (MBC) was determinedby transferring and spreading the treated culture broth ofthe wells containing the concentrations equal to and higherthan the MIC on agar plates. The lowest concentration of the extract or the standard antibiotic required to completelydestroy test bacteria after incubation at 37 ℃ for 24 h was reported as MBC.

3. Results

The antibacterial activity of *A. officinalis*extracts areshown in Table 1. Generally, the results showed thatthe antibacterialactivities of the crude extracts were increased with increasing the concentration. Although theantibacterialactivity of the extracts tested is variable, twoGram-positive bacteria (*S. aureus*and *B. cereus*) and onlygram negative (*E. coli*) were inhibited by the extracts. Quantitative analyses on the antibacterial properties wereobtained through the determination of bacteriostatic andbactericidal concentrations of *A. officinalis*extracts. Table2 shows the MIC and MBC of the extracts that produceinhibition zone more than 12 mm. The results of inhibitionzone were reflected in lower MIC values. The MIC and MBCvalues for bacterial strains, which sensitive to the extracts,were in the range of 0.04-1.11 mg/mL and 0.04-10 mg/mL,respectively. Furthermore, in most cases, the MBC valueswere higher than the MIC values, except for n-hexaneextract against *B. cereus*(MIC = MBC). According to thedisc diffusion results, MIC and MBC values, it is clear thatn-hexane extract was the most effective extract (Table 1 and2). Additionally, Gram positive *B. cereus*appears to be themost sensitive strain with inhibition zone of 19 mm (1.5 mg/disc) and the MIC value is 0.04 mg/mL). The inhibition of microbial growth at concentration as low as 0.04 mg/mL indicatedthe potent antibacterialactivity of *A. officinalis*extracts.

Table 1: Antimicrobial activity of *A. officinalis*extracts

Microorganisms	Zone of Inhibition							
	n-hexane		Ethyl acetate		Methanol		Positive control	Negative control
	1 mg	1.5 mg	1 mg	1.5 mg	1 mg	1.5 mg		
S. aureus	14.5	14.5	11	16	13	17	21	00
B. cereus	16.00	19	09	09	9	9.5	27	00
E. coli	11.5	13.5	13.5	16.5	9.5	11	30.5	00
P. aeuroginosa	00	00	00	00	00	00	30.5	00

Positive control: tetracycline (100 µg); Negative control: DMSO.

Table 2: MIC and MBC of *A. officinalis*extracts and standard antibiotic

Extracts	*S. aureus*		*B. cereus*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC
n-hexane	1.11	>10	0.04	0.04	0.04	0.37
Ethyl acetate	0.37	>10	-	-	0.37	>10.00
Methanol	1.11	10	-	-	-	-
Tetracycline	30.0	-	2.00	-	30.0	-

All data were expressed as (mg/mL) except for tetracycline (µg/mL); -: not determined.

4. Discussion

Traditionally, plants were known as the main sources fordrugs. Interest in this area continues and many new potentdrugs have been isolated. Tropical and sub-tropical areasof the world are rich with many plant species which haveeffective properties, such as antimicrobial, antiviral andantifungal. Many medicine plant extracts have been knownto possess antibacterial effects. Mangroves possess novelbiologically active compounds. The extracts from differentmangrove plants and mangrove associates have beenreported to possess inhibition action against human andplant pathogens[13-22].In this report, three different polarity extracts havebeen tested for antimicrobial activity. With respect to theinhibition panel and the MIC and MBC concentrations,n-hexane extract of *A. officinalis*was the most effective extract.The methanol and dichloromethane extracts of *A. officinalis*also demonstrated antibacterial effect, although they were lower than the antibacterial effects of the n-hexane extract.The presence of the activity in n-hexane, dichloromethaneand methanol extracts might be represented by existenceof more than one active compound. Chemical analysisofthe species belongs to the genus *Avicennia*have shown the presence of various bioactive ingredients including alkaloids, steroids, triterpenoids and flavonoids[14,23]. On theother hand, it is interesting to note that the plant extractsshowed bacteriostatic and bactericidal actions against *S.aureus*, *B. cereus*and *E. coli*. This suggests that they may possess remarkable therapeutic action in the treatment ofinfectious disease caused by these species.The obtained results suggest the possibility of findingpotent antibacterial agents from

*A. officinalis*extracts andconsidered sufficient to isolate the compounds responsible for the activity.

Conflict of interest statement: I declare that I have no conflict of interest.

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