



REGULAR ARTICLE

ANTIBACTERIAL ACTIVITY OF *SALICORNIA BRACHIATA*, A HALOPHYTE

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SUMMARY

Antibacterial activity of *Salicornia brachiata* was studied by the agar diffusion and dilution methods. The methanolic extract of leaves of this species was more active than the aqueous extracts against *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus* and *Staphylococcus aureus*.

Keywords: Antibacterial activity, methanolic extract, shoots, *Salicornia brachiata*.

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1. Introduction

A large proportion of the human population depends on traditional medicine. Medicinal plants have become the focus of intense study recently in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. With the increasing acceptance of traditional medicine as an alternative form of health care, the screening or medicinal plant for active compound is very important. The situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immune compromised, AIDS and cancer patients [4]. It is likely that plant extract showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogen. However, very little information is available on such activity of

medicinal plants [7]. In the present study, we have selected *Salicornia brachiata*, a halophyte, is screened against multi drug resistant bacteria like *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*.

2. Materials and Methods

Plant material: The leaves of *Salicornia brachiata* were collected from the salt marsh area of Pichavaram on the north-east coast of Tamilnadu; about 10 km east of Annamalai University campus contains about 1,100 ha of mangrove vegetation.

Preparation of extracts: Dried shoot material of *Salicornia brachiata* (25g) was finely ground and extracted with 50 ml of water and 80 % methanol for 30 min in an ultrasound bath (Branson 2210, 47 kHz). The plant extracts were macerated overnight, filtered and the clear filtrates evaporated to dryness under

reduced pressure at a temperature of 40 °C. The residues were resuspended in water or 80% methanol to give 100 mg residue/ml.

Antibacterial activity assay

The disc-diffusion assay [10] was used to determine the growth inhibition of bacteria by the plant extracts. The following bacteria were used: *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Esherichia coli*. These were maintained at 4°C on nutrient agar plates.

Petri plates were prepared by pouring 10 ml of Mueller Hinton Agar and allowed to solidify. 0.1 ml of standardized inoculums suspension was poured and uniformly spread. The excess inoculum was drained and the inoculum was allowed to dry for 5 minutes. The discs were then applied and plates were incubated in BOD incubators at 37°C for 24 h. The inhibition zone was measured from the edge of the disc to the inner margin of bacterial colony. DMSO used as a negative control and Ciprofloxacin used as a positive control was also run simultaneously. The experiment was done in triplicate.

Minimum inhibitory concentration (MIC) of the plant extract was tested in Mueller Hinton Broth by the two-fold serial dilution

method. The test extract was dissolved in 5 per cent DMSO to obtain 400 mg/ml stock solutions. Stock solution (0.5 ml) was incorporated into 0.5 ml Mueller Hinton Broth to get a concentration of 100 mg/ml and this was serially diluted by double technique so as to get 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml concentrations. Fifty µl of standardized suspension of the test organism as transferred on to each tube with a control set which contained only organisms and not the plant extract. The culture tubes were incubated in BOD incubators at 37 °C for 24 h. The lowest concentration, which did not show any growth of tested organism after microscopic evaluation was determined as minimum, inhibitory concentration.

Minimum bactericidal concentrations (MBC), which did not show any growth of the bacteria after the incubation period, were first diluted (1:4) in subculture on to the surface of the freshly prepared Mueller Hinton Agar plates and incubated in BOD incubators at 37 °C for 24 h. The maximum bactericidal concentrations were recorded as the lowest concentration of the extract that did not permit any visible bacterial colony growth on the agar plate after the period of incubation.

3. Results and Discussion

Table 1. Antibacterial activity of *Salicornia brachiata*

Bacteria	Mean zone of inhibition (mm) ^a								Ciprofloxacin 5 µg/disc	Methanol (mg/ml)		Aqueous (mg/ml)		Ciprofloxacin (mg/ml)	
	Methanol (mg/ml)				Aqueous (mg/ml)					MIC	MBC	MIC	MBC	MIC	MBC
	100	50	25	12.5	100	50	25	12.5							
<i>B. subtilis</i>	11	8	6	5	5	4	2	1	20	1.7	2	7.2	15.5	0.07	0.14
<i>B. pumilus</i>	10	9	7	6	6	5	3	2	32	1.7	3	14.3	30.5	0.05	0.10
<i>M. luteus</i>	14	12	11	9	10	9	6	4	20	1.7	4	4.5	7.5	0.07	0.14
<i>S. aureus</i>	12	10	9	7	9	8	5	3	16	1.7	4	7.2	16.7	0.13	0.26
<i>P. aeruginosa</i>	6	3	3	4	5	3	2	2	22	30.5	61	6.1	13.5	0.08	0.16
<i>K. pneumoniae</i>	8	6	5	5	5	4	3	2	22	7.2	15.4	6.1	13.5	0.07	0.14
<i>E. coli</i>	9	7	6	5	5	5	3	2	20	7.2	15.4	6.1	13.5	0.13	0.26

^a – Mean of three assays, MIC – Maximum Inhibitory concentration, MBC – Maximum bactericidal concentration

The present study reveals that the shoot extracts of *S. brachiata* were very effective against gram positive bacteria such as *Bacillus subtilis*, *Bacillus pumilus*, *Micrococcus luteus* and *Staphylococcus aureus* than the other species tested. The methanolic extracts of the shoots were more antagonistic than the respective aqueous extracts. This may be due to the different solvent extract relationship resulting into involvement of different constituents having antibacterial activity (Table 1). The antibacterial activity may be due to the presence of alkaloids, flavonoids, tannin, polyphenolic and oil as reported by [6] and [2]. No antibacterial activity could be obtained against the Gram-negative bacteria. This is not unusual as, in general, these bacteria are more resistant than Gram-Positive ones [8]. The volatile oil of *Artemisia afra* has greater activity as an antimycotic than as an antibacterial agent [5].

The MIC values for some of methanolic extracts are shown in Table 1. These extracts do not have good potency level based on their high MIC values, implying the active compounds would probably not be pharmaceutically useful [11]. Reasons for the relatively high MIC values could be that the extracts tested are still in an impure form, or that the active compounds are present in very low concentrations. The values obtained in the MBC studies were generally greater than the MIC values. [1] had also recorded similar observations. Interestingly the antibacterial activities are both strain and dose dependent [9]. Nevertheless some of the plant extracts warrant further investigation using bio assay-guided fractionation to characterize the active constituents. The results of this study is encouraging to the certain degree for the traditional medicinal uses of this plant and this effort can reinforce the concept of ethno botanical approaches to drug discover [3] through screening of plants as potential source of bioactive substances

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