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IN-VITRO ANTIMICROBIAL ACTIVITY OF *SALICORNIA BRACHIATA* (ROXB.) AGAINST SELECTED PATHOGENS

Prasanna Lakshmi K., Lakshmi Narayana V. and G. M. Narasimha Rao*

Department of Botany, Andhra University, Visakhapatnam.

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***Correspondence for Author**
G. M. Narasimha Rao
Department of Botany,
Andhra University,
Visakhapatnam.

ABSTRACT

Salicornia brachiata (Roxb.) is halophyte occurs in the estuarine and mangrove habitats of East & West coasts of India. In the recent years *Salicornia* plants are widely used in several applications such as vegetable, paper making and extraction of edible oil. This communication deals with the antimicrobial activity of *Salicornia brachiata* against some plant and human pathogens. Plant parts of *S. brachiata*, were collected from mangrove habitats of Godavari estuary, Kakinada, dried and extracted successfully with hexane, chloroform, methanol and water, using Soxhlet extraction apparatus. The antimicrobial activity of the plant extracts on the various test organisms, including multiple antibiotic resistant bacteria were investigated. *In vitro* screening of *S. brachiata* plant extracts showed greater activity in inhibiting the growth of bacteria and fungi.

KEYWORDS: Antimicrobial Activity, *In vitro* Screening, Multiple Antibiotic Resistant Bacteria, *Salicornia brachiata*, Well Diffusion Method.

INTRODUCTION

A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources (Farnsworth 1984, Srinivastava *et al.*, 1996). Traditionally important mangroves and halophytic plant species have recently attracted the attention of the pharmaceutical and scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor *et al.*, 2001). Mangroves are biochemically unique, producing wide array of natural products with unique bioactivity. They possess active metabolites with some novel chemical structures which belong to diverse chemical classes

such as alkaloids, phenols, steroids, terpenoids, tannins, etc. (Patra and Thatoi, 2011). Mangroves have been reported to contain compounds like tannins, alkaloids and polyphenols (Combs and Anderson, 1949), which have antimicrobial activity (Nishiyama *et al.,* 1978); (Ross *et al.,* 1980). Halophytes are rich in proteins, oils and fats that are suitable for human consumption (Stanley, 2008). Knowledge of the biological activities and chemical constituents of plants is desirable, not only for the discovery of new therapeutic agents but because such information may be have values in disclosing new sources of already known biologically active compounds.

Salicornia brachiata (popularly described as 'Sea asparagus') is a succulent, erect annual herb belongs to family Acanthaceae. *Salicornia* is rich in vitamins A and C, calcium, iron, iodine, magnesium, sodium and amino acids (Stanley, 2008). Studies on distribution in relation to physiochemical features of *Salicornia brachiata* in Godavari estuary was studied by Narasimha Rao and Murty (2015). Narasimha Rao *et al*., (2015) studied the extraction of edible oil from *Salicornia brachiata* seeds. The present study assessed the antimicrobial activity of *Salicornia brachiata* against some fungal and bacterial pathogens.

MATERIALS AND METHODS

Plant Material

Plant materials of *S. brachiata* were collected from mangrove habitats of Godavari estuary, Kakinada, Andhra Pradesh. Plant materials were identified with the help of authentic specimens available in the Department of Botany, Andhra University, Visakhapatnam. Plant parts such as roots and stems were cleaned properly and shade dried, cut into small pieces and powdered in a mixer grinder, the residues (crude extracts) obtained were finally dried under vacuum.

Test Microorganisms

The microorganisms (including fungi and bacteria) selected were *Bacillus subtilis* [B 2274], *Bacillus megaterium* [B 2444], *Lactobacillus acidophilus* [B 5463], *Escherichia coli* [B 9637], *Enterobacter aerogenes* [B 2822], *Enterobacter cloace* [B 7982], *Klebsiella pneumonia* [B 2405], *Candida albicans* [0227], *Mucar recemosus* [7382], *Rhizoctonia solani* [5642], *Rhizopus stolonifer* [2591] and *Saccharomyces cerevisiae* [0174]. These microorganisms were obtained from Microbial Type Culture (MMTC) from Institute of Microbial Technology, Chandigarh, India. The strains are maintained and tested on Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi.

Preparation of Plant Extracts

The collected plant materials were chopped into small pieces and coarsely powdered in Whilly mill. The coarsely powdered material weighed and extracted with hexane, chloroform, methanol and water in sequential order of polarity using a Soxhlet extractor for five to six hours at temperature not exceeding the boiling point of the solvent. For each gram of dry material 2 ml of solvent was used. The extracted solvents were filtered though Whatman no-1 filter paper and subsequently concentrated under reduced pressure (in vacuum at 40^{o}C) using a rotary evaporator. The residue obtained were designated as crude extracts and stored in a freezer at -20^{o}C until bio assayed.

The dried plant extract residues obtained were redissolved in 0.1% Dimethyl Sulfoxide (DMSO) to get different concentrations (100mg/ml, 300mg/ml and 500mg/ml) of crude extracts and filtration through a 0.45µm membrane filter and stored in sterile brown bottles in a freezer at 20^{o}C until bio assayed. The prepared hexane, chloroform, methanol and water extract samples were tested for antibacterial and antifungal activity against the test organism's (plant and human pathogens) using the agar cup plate/well diffusion method.

***In vitro* Antibacterial Activity Assays**

The antimicrobial activity of the hexane, chloroform, methanol and water extracts of each sample was evaluated by using Well Diffusion Method of Murray *et al*., (1995) modified by Olurinola, (1996). 20ml of nutrient agar was dispensed into sterile universal bottles these were then inoculated with 0.2 ml of cultures mixed gently and poured into sterile petri dishes. After setting a number 3-cup borer (6mm) diameter was properly sterilized by flaming and used to make three to four uniform wells in each Petri dish. The wells were filled with 50µl of the extract concentration of 100mg/ml, 300mg/ml and 500mg/ml and allow diffusion for 45 minutes. The solvents used for reconstituting the extracts were similarly analyzed. The plates were incubated at 37^{o}C for 24 hours for bacteria. The above procedure is allowed for fungal assays but except the media Potato Dextrose Agar instead of nutrient agar and incubates at 25^{o}C for 48 hours. The zones inhibition was measured with antibiotic Zone Scale in mm and the experiment was carried out in duplicates.

RESULTS AND DISCUSSION

Hexane Extracts of 500mg/ml

The data pertaining to the antimicrobial potential of the plant extract (concentrations in 500 mg/ml) of *Salicornia brachiata* was presented in table-1. In the present study hexane,

chloroform, methanol and water extracts of *Salicornia brachiata* exhibited the different degree of growth inhibition against tested bacterial and fungal strains. Highest zone of inhibition were recorded in the concentration of 500mg/ml. As the disc dosage level increased, the inhibitory effect also increased. Hence only 500 mg/ml dosage level results were analysed. Moderate level of antimicrobial activity was found with the hexane extracts of *S. brachiata* whole plant, against fungal strains such as *S. cerevisiae* (15.7 mm) followed by *R. stolonifer* (14 mm). Absence of antibacterial activity observed against all bacterial strains whereas fungal strains such as *C. albicans, M. racemosus* and *R. stolonifer* (Table-1).

Chloroform Extracts of 500mg/ml

Highest level of antimicrobial activity was recorded for the chloroform extracts of *S. brachiata* whole plant, against fungal strain such as *S. cerevisiae* (21.3 mm) whereas moderate activity was reported against bacterial strains such as *B. subtilis* (15.7), *L. acidophilus* (15.7 mm) and fungal strains such as *R. solani* (16.3 mm) and *M. racemosus* (15.3 mm). Less activity was observed against *B. megaterium* (13 mm) while the remaining bacterial strains are resistant to this extract (Table-1).

Methanol Extracts of 500mg/ml

Highest level of antimicrobial activity was found with the methanol extracts of *S. brachiata* whole plant, against fungal strain such as *S. cerevisiae* (26 mm). Moderate level of antimicrobial activity was found against bacterial strains such as *L. acidophilus* (19 mm) whereas fungal strains such as *M. racemosus* (18.3 mm) followed by *R. solani* (17.7 mm) (Table-1).

Water Extracts of 500mg/ml

Highest level of antimicrobial activity was recorded for the water extracts of *S. brachiata* whole plant, against bacterial strains such as *B. megaterium* (21.3 mm) followed by *B. subtilis* (19.7 mm) and *K. pneumonia* (19.7 mm) and fungal strains such as *S. cerevisiae* (23 mm) followed by *R. stolonifer* (22.7 mm) *C. albicans* (21.3 mm). Moderate level of antimicrobial activity was found against bacterial strains such as *L. acidophilus* (19 mm) and fungal strains such as *M. racemosus* (19 mm) and *R. solani* (18.3 mm). *E. coli* is resistant to this extract (Table-1).

Table-1: Antimicrobial activity of hexane, chloroform, methanol and water extracts of *Salicornia brachiata* (whole plant).

Name of the Microorganisms	500 mg/ml				Standard
	Hexane	Chloroform	Methanol	Water	
Bacillus subtilis	-	16.3±0.6	16.7±1.2	19.7±0.6	32
Bacillus megaterium	-	13.0±1.0	14.6±0.6	21.3±1.5	28
Lactobacillus acidophilus	-	15.7±0.6	19.0±1.0	19.0±1.0	30
Escherichia coli	-	-	15.3±1.2	-	34
Enterobacter aerogenes	-	-	15.0±1.0	18.3±0.6	31
Enterobacter cloacae	-	-	15.3±1.2	18.0±1.0	32
Klebsiella pneumonia	-	-	-	19.7±0.6	31
Candida albicans	-	14.6±0.6	15.7±0.6	21.3±1.5	35
Mucar recemosus	-	15.3±1.2	18.3±0.6	19.0±1.0	32
Rhizoctonia solani	14.6±0.6	16.3±0.6	17.7±1.2	18.3±0.6	34
Rhizopus stolonifer	-	14.3±1.0	15.7±0.6	22.7±1.2	30
Saccharomyces cerevisiae	15.7±0.6	21.3±1.5	26.0±1.0	23.0±1.0	29

Volume per well: 50µl; Borer size used: 6mm; (-) indicates No inhibition. Diameter of zone of inhibition (mm) including disc diameter of 6mm: mean of three assays ± standard deviation.

Hexane extracts of *S. brachiata* (whole plant) appears to have less antifungal activity and no activity was observed against all bacterial strains. These exudates are associated with a complex defence mechanism that includes the prevention of phyto pathogenic organisms (Reina *et al.*, 1997).

Chloroform extracts of *S. brachiata* inhibited most bacterial and fungal growth, but their effectiveness varied. Moderate activity observed against all bacterial strains and all fungal strains appears to have less activity. *S. brachiata* whole plant extracts showed less antibacterial activity and moderate antifungal activity. A number of studies have been reported, investigating the anti-microbial activities of some *Suaeda* species such as *Suaeda villosa* and *S. vermiculata* possess antifungal activity against *Candida albicans* and *Fusarium oxysporum* (Mahasneh *et al.*, 1996).

Taha *et al.*, (2000) reported the highest ethanol extract activity was exhibited by *Cressa cretica* against *Penicillium citrinum* (32.2 mm) followed by *Candida albicans* (25.7 mm). The methanolic extract of leaves of *Salicornia brachiata* was more active against *Bacillus subtilis, Bacillus pumilus, Micrococcus luteus* and *Staphylococcus aureus* (Manikandan *et al.*, 2009). In the present investigation, methanol extracts of *S. brachiata* whole plant showed

moderate and highest activity against all pathogens tested. Chandrasekaran *et al.*, (2005) reported higher amount of saturated fatty acids in *Arthrocnemum indicum, Salicornia brachiata, Suaeda maritima.* The highest mean zone of inhibition (20.5 mm), values were obtained for the extract of *S. brachiata* against *B. subtilis.* The extract showed good antimicrobial activity against *S. aureus, C. parapsilosis,C. albicans, C. krusei* and *M. luteus*.

Water extracts showed the highest activities than the hexane, chloroform and methanol. Water extracts of *S. brachiata* whole plant showed most active and significant activity against all pathogens tested. The above results concluded that these plant extracts have greater potential compounds against microorganisms and that they can be used as novel antimicrobial agents (Table-1). Lauric acid, palmitic acid and linolenic acid was reported from *Salicornia brachiata, Suaeda maritima* and they found to have antimicrobial activity against *Bacillus subtilis, Staphylococcus aureus, Candida parapsilosis, C. albicans, C. krusei* and *Micrococcus luteus* (Chandrasekaran *et al.*, 2008). Similarly in the present study *S. brachiata* was effectively inhibiting the bacterial and fungal growth; but the highest values were obtained with the water extract against all pathogens tested. The ashes of *Salicornia brachiata* are used for manage and for the itch. They are considered as emmenagogue and abortive (Kirtikar and Basu, 1991). Kabara, (1978) reported that fatty acids such as oleic, palmitic, stearic, myristic, linoleic and linolenic acids show activity against *Clostridium perfringens* and *Staphylococcus pyogens.* Some *in vitro* studies have indicated that the fatty acid composition could either directly or indirectly affect the aflatoxin contamination (Burrow *et al.*, 1997). The report of Ouattara *et al.*, (1997) which showed that lauric acid possesses antibacterial activity. Similarly, long-chain unsaturated fatty acids, including linoleic acid, are well known to inhibit bacteria like *E. coli* (Sun *et al.*, 2003). Linolenic, linoleic and palmitic acids isolated from *Schotia brachypetala, Pelargonium* sp. and *Pentanisia prunelloides*, respectively, were found to have antibacterial activity (Yff *et al.*, 2002). Other compounds like saponins also have antifungal properties. Many plants release phenolic compounds that are toxic to microbial pathogens (Aboaba *et al.*, 2006). Oleic and arachidonic acids were known to possess antimicrobial activity against human pathogens (Kabara *et al.*, 1972).

Overall, the present study provides enough data to show the antimicrobial activity of halophytic plant such as *S. brachiata.* These findings suggest that the halophytic plant species may be a new source for pharmaceutical industries. Based on the above results it can be

concluded that the above plant extracts have greater potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of infectious diseases caused by resistant pathogenic microorganisms.

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REFERENCES

1. Aboaba OO, Smith SI, Olude FO. Antimicrobial Effect of Edible Plant Extract on Escherichia coli. Pak. J. Nutri, 2006; 5(4): 325-327.
2. Burrow GB, Nesbitt TC, Dunlap J, Keller NP. Seed Lipoxygenase Products Modulate Aspergillus Mycotoxin Biosynthesis. Mol. Plant Microb. Interact, 1997; 10: 380-387.
3. Chandrasekaran M, Venkatesalu V, Anantharaj M. Sivasankari S. Fatty Acid Composition and Antibacterial Activity of Ipomoea pes-caprae L. Indian Drugs, 2005; 42: 275-281.
4. Chandrasekaran M, Kannathasan K, Venkatesalu V. Antimicrobial Activity of Fatty Acid Methyl Esters of Some Members of Chenopodiaceae. Z. Naturforsch, 2008; 63(C): 331-336.
5. Combs CA, Anderson H. Use of mangrove bark. Australian Leather Trade Rev, 1949; 43: 270-274.
6. Farnsworth NR. The role of medicinal plants in drug development, Natural products and drug development, Alfred Benzon Symposium 20, Eds P. Krogsgaardlarson, S. Brogger Christensen, H. Munksgaard, Copenhagen, 1984.
7. Kabara JJ. Fatty Acids and Derivatives as Antimicrobial Agents. In: The Pharmacological Effect of Lipids (Kabara J. J., ed.). The American Oil Chemists Society, St. Louis, MO, 1978; 1-14.
8. Kabara JJ, Swieczkowski DM, Conley AJ, Jruant JP. Fatty Acids and Derivatives as Antimicrobial Agents. Antimicrob. Agents Chemother., 1972; 2: 23-28.
9. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol. IDIV. Lalit Mohan Basu Publishers, Allahabad, India, 1991; 1-2793.
10. Mahasneh AM, Abbas JA Oqlah EI, Ahmad A. Antimicrobial activity of extract of herbal plants used in the traditional medicine of Bahrain. Phytother. Res., 1996; 10(3): 251-253.

11. Manikandan T, Neelakandan T, Usha Rani G. Antibacterial Activity of Salicornia brachiata, a Halophyte, J. Phytol., 2009; 1(6): 441-443.
12. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken HR. Manual of Clinical Microbiol., 6th Edition, ASM Press, Washington DC, 1995; 15-18.
13. Nishiyama Y, Ryuzo Sanchez PC, Kozaki M. Inhibitory functions of mangrove bark towards cell growth of microorganisms. Hakko, Kogaku, Kaishi, 1978; 56: 712-717.
14. Narasimha Rao GM, PrayagaMurty P, Murali Krishna Kumar M. Seeds of Salicornia brachiata as a source of edible oil. Indian Journal of Applied Research, 2015; 5(8): 532-533.
15. Narasimha Rao GM, PrayagaMurty P. Distribution of Salicornia brachiata in Relation to Physico-Chemical and Soil Characteristics in Godavari Estuary, AP, India. IOSR Journal of Pharmacy and Biological Sciences, 2015; 10(4): 13-16.
16. Olurinola PF. A Laboratory Manual of Pharmaceutical Microbiology. Idu Abuja, Nigeria, 1996; 69-105.
17. Ouattara B, Simard RE, Holley RA, Piette GJP, Begin A. Antibacterial Activity of Selected Fatty Acids and Essential Oils against Six Meat Spoilage Organisms. Int. J. Food Microbiol., 1997; 37: 155-162.
18. Patra JK, Thatoi HN. Metabolic diversity and bio acativity screening of mangrove plants: a review. Acta Physiol. Plant, 2011; 33: 1051-1061.
19. Reina M, Gonzalez-Coloma A, Gutierrez C, Cabrera R, Henriquez J. Bioactive Saturated Pyrrololizidine Alkoloids from Heliotropium floridum. Phytochem., 1997; 46(5): 845-853.
20. Ross SA, Megalla SE, Bisby DW, Awad AH. Studies for determining some antibiotic substance in some Egyptian plants, Screening of some antimicrobial activity Fitoterpia., 1980; 51: 303-308.
21. Srinivastava J, Lambert J, Vietmeyer N. Medicinal Plants: An Expanding Role in Development. The World Bank, 1996; 18.
22. Stanley OD. Bio Prospecting marine halophyte Salicornia brachiata for Medical Importance and Salt Encrusted Land Development. J. Cosatal Development, 2008; 11(2): 62-69.
23. Sun CQ, O'Connor CJ, Roberton AM. Antibacterial Actions of Fatty Acids and Monoglycerides against Helicobacter pylori. FEMS Immunol. Med. Microbiol., 2003; 36: 9-17.

24. Taha A, Alsayed H. Brine Shrimp Bio Assay of Ethanol Extracts of Sesuvium verrucosum. Salsola baryosma and Zygophyllum quatiarense Medicinal Plants from Bahrain. Physiotherapy Res., 2000; 14(1): 48-50.
25. Taylor JLS, Rabe T, McGaw LJ, Jager AK, Van Staden J. Towards the scientific validation of traditional medicinal plants. Plant Growth Regul., 2001; 34(1): 23–37.
26. Yff BTS, Lindsey KL, Taylor MB, Erasmus DG, Jager AK. The Pharmacological Screening of Pentanisia prunelloides and the Isolation of the Antibacterial Compound Palmitic Acid. J. Ethnopharmacol. 2002; 79: 101-107.