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Research article

AN ANTIMICROBIAL ACTIVITY OF THE BROWN SEAWEED *PADINA TETRASTROMATICA* EXTRACT IN DIFFERENT CONCENTRATION AGAINST HUMAN PATHOGENIC BACTERIA

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ABSTRACT: The brown seaweed *Padina tetrastromatica* which can be collected from Gulf Of Mannar Sea shore Thoothukudi, India. In this present study was focused on the antimicrobial activity of the selected seaweed against six human pathogenic bacteria (Gram +ve: *Staphylococcus aureus*, *Bacillus subtilis*, *Lactobacillus acidophilus* and Gram -ve: *Pseudomonas aeruginosa*, *Escherichia coli and Proteus mirabilis*) by the agar well diffusion method. Here, different concentrations of the extract from *Padina tetrastromatica* were tested for probable antimicrobial activity and the extracts were prepared by five different solvents such as Acetone, Chloroform, Ethanol, Ethyl acetate and methanol. The experimental results shows that the highest antimicrobial activity 15mm was shown by Ethyl acetate extract against *S. aureus* and the lowest activity observed in methanol extract 1mm against *E.coli*. The experiment concludes that the extract of Ethyl acetate forms a good activity against all the six organisms.

Key words: Seaweed, *Padina tetrastromatia*, Antimicrobial activity.

INTRODUCTION

Seaweeds are rich and varied source of bioactive natural products and many chemically unique compounds of marine origin with various biological activities have been isolated and some of them are under investigations which are considered as lead molecules to develop new pharmaceuticals (Subba Rangaiah *et al*., 2010). Seaweeds serve as an important source of bioactive natural substances. They have some of the valuable medicinal value compounds such as antibiotics, antioxidant (Lekameera *et al*., 2008). In recent years, there are numerous reports of macro algae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumor, cytotoxic and antimitotic activities (Bhosale *et al*., 2002 and Okai *et al*., 1997).

Southwest coast of India is a unique marine habitat with a great variety of seaweed species, spread between the intertidal zone and the deep water regions of the Indian coast (Oza and Zaidi, 2000). It is already documented that seaweeds from southeast coast of India belonging to Chlorophyceae, Rhodophyceae and Phaeo-phyceae has antibacterial property (Kandhasamy and Arunachalam, 2008). Recently, infections have become the leading cause of death worldwide which has led to an increase in antibacterial resistance, making it a global growing problem. Thus, there is an urgent need to discover new antimicrobial compounds from plants with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases. The new therapeutic agents should be effective and have a novel mode of action that renders them impervious to existing resistance mechanisms (Westh *et al*., 2004). The revolutionized therapy of infectious diseases by the use of antimicrobial drugs has certain limitations due to changing patterns of resistance in pathogens and side effects they produced.

These limitations demand for improved pharmacokinetic properties which necessitate the continued research for new antimicrobial compounds for the development of drugs (Al- Haj *et al*., 2009). The present study was undertaken to investigate the antibacterial activities of *Padina tetrastromatica* collected from Thoothukudi seashore against six human pathogenic bacteria.

MATERIALS AND METHODS

Sample collection

The seaweed *Padina tetrastromatica* was collected from collected from Thoothukudi, Gulf of Mannar Marine Biosphere Reserve, South east Coast of India. The collected seaweed samples were washed with seawater and then in fresh water and extraneous matters were removed. After that they were brought into the laboratory in sterile plastic bags. The samples were rinsed with sterile distilled water, shade dried, cut into small pieces and powdered in a lab mixer grinder. The powdered samples were then stored in freezer for further study.

Preparation of seaweed extract

The powdered sample (5g) was extracted in soxhlet apparatus using acetone, chloroform, ethanol, ethyl acetate and methanol (200 ml) as solvents for 8h at a temperature maintain not more than the boiling point of the solvent. The extracts were filtered using Whatman No.1 filter paper and kept it under Hot air oven (40^0C) for the solvent evaporation. The residues obtained were stored in a freezer at -20^0C.

Test organisms

Extracts were tested against six bacterial stains (Gram +ve: *Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus* and Gram -ve: *Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis*). The test pathogens were obtained from the Research Department of Microbiology, VHNSN College, Virudhunagar, Tamil Nadu, India.

Antimicrobial activity

The antimicrobial activity was carried out by using agar well diffusion method. The solvents like acetone, chloroform, ethanol, ethyl acetate and methanol were used to collect the seaweed extract and were tested against the human pathogens at four dose levels like 40µg/ml, 60µg/ml, 80µg/ml and 100µg/ml. The bacterial culture was transferred to sterile Petri plate with Mueller Hinton agar medium (Hi Media Laboratories Limited, Mumbai, India) and was spread with sterile spreader to create a lawn. About 5 wells of 6mm diameter were made in each plate with the help of a sterile cork borer. Among the five, four wells were placed with the different concentration of the extracts using sterile pipettes and remaining one well was mentioned as control had with solvent alone. The Petri dishes were prepared and incubated for 18-24hrs at 37°C and the zone of inhibition around the well was measured in nearest millimeter. Each experimental result was determined by the average of triplicates.

RESULT AND DISCUSSION

The antimicrobial properties of the selected seaweed *Padina tetrastromatica* was extracted with five different solvents like acetone, chloroform, ethanol, ethyl acetate and methanol and determined the activity against six pathogenic organisms both Gram positive *(Staphylococcus aureus, Bacillus subtilis, Lactobacillus acidophilus* and Gram negative *Pseudomonas aeruginosa, Escherichia coli and Proteus mirabilis)* bacteria by agar well diffusion method were tabulated in the Table 1. The highest activity were based on the 100µg/ml from the extract of *Padina tetrastromatica* in ethyl acetate solvent the maximum 15mm against *S.* a*ureus* and the lowest activity in 6mm showed when we used acetone as a solvent against *P. aeruginosa* and *E. coli* and ethanol as a solvent against *S. aureus and B. subtilis* respectively. The maximum activity (13mm) at 80µg/ml concentration level when ethyl acetate used as a solvent against *S.* a*ureus* and the lowest activity (6mm) by chloroform solvent against *L. acidophilus*. We have obtained very limited results from 60µg/ml concentration. Ethyl acetate extracts shows the maximum activity of 11mm against *S.* a*ureus* and the lowest activity of 4mm against *E. coli* when methanol used as a solvent. In the 40µg/ml concentration, all the test organisms were resistant to the extracts of *Padina tetrastromatia* in different solvents, but the highest activity was seen in ethyl acetate extracts (9mm) against *S.* a*ureus* and the lowest activity in methanol extracts (1mm) against *E.coli*. However, the present study showed that the ethyl acetate extracts of *Padina tetrastromatica* have a good activity against the entire six organisms at four different concentrations and the lowest activity were shown in chloroform extracts. There is no activity was recorded in gram negative pathogens when we are using chloroform as a solvent. In our investigation, antibacterial activity varied according to the solvent extract and test organism. In the present study, it was observed that ethyl acetate was the best organic solvent for extracting the effective antibacterial material from the *Padina* species used in this experiment.

Table: 1 Antibacterial activity of *Padina tetrastromatica* extracts against tested pathogens

Test samples	Concentration (µg/ml)	Zone of inhibition in mm					
		S.a	***B.s***	***L.a***	***P.a***	***E.c***	***P.m***
Acetone extract	40	–	–	–	–	–	–
	60	–	–	–	–	–	–
	80	–	–	7	–	–	–
	100	7	8	8	6	6	9
Chloroform extract	40	–	–	–	–	–	–
	60	–	–	–	–	–	–
	80	–	–	6	–	–	–
	100	6	6	7	–	–	–
Ethanol extract	40	–	–	–	–	6	–
	60	6	–	6	–	8	–
	80	9	–	7	–	10	10
	100	10	9	9	8	11	12
Ethyl acetate extract	40	9	6	–	–	–	–
	60	11	9	7	–	–	6
	80	13	11	10	9	7	7
	100	15	14	12	12	8	10
Methanol extract	40	–	–	–	–	1	–
	60	–	–	–	–	4	–
	80	–	–	7	–	8	–
	100	–	7	9	10	11	–

Gram +ve: *S.a.*: *Staphylococcus aureus;* ***B.s.***: *Bacillus subtilis;* ***L.a.***: *Lactobacillus acidophilus;*
Gram -ve: *P.a.*: *Pseudomonas aeruginosa;* ***E.c.***: *Escherichia coli;* ***P.m***.: *Proteus mirabilis*

CONCLUSION

From this study it can be we concluded that the extract of Ethyl acetate forms a good activity against all the six organisms and there is no activity was recorded in gram negative pathogens when we are using chloroform as a solvent.

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