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ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACT OFSYRINGODIUM ISOETIFOLIUM COLLECTED FROM KANYAKUMARI COAST,TAMILNADU AGAINST HUMAN PATHOGENS

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Abstract
The present investigation carried out the antibacterial activity of the seagrass Syringodium isoetifolium and revealed the efficiency of various solvent extracts (methanol, chloroform and acetone)against gram positive (E.faecalis,S.pneumoniae, S.mutans,S.aureus) and gram negative (S.typhi,K.pneumoniae,P.aeruginosa,E.coli,P.vulgaris)human pathogens. Fresh seagrass was collected from Kanyakumari coast and crude extract were prepared by using the three above mentioned solvents. The antibacterial activity was estimated by Muller Hinton Agar plates (MHA) method. In addition to that ,the minimum inhibitory concentration(MIC) and minimum bactericidal concentration (MBC) were determined for Syringodium isoetifolium derived from three solvent with each pathogenic species and it was compared with an appropriate positive control. Among the three solvent the methanol extract exhibited a maximum zone of inhibition (13 ± 0.84 mm) against E.faecalis among the gram positive pathogens and a maximum zone of inhibition (14±0.92) was observed in the methanol extract against P.aeuroginosa among the gram negative pathogens.

Keywords: Syringodium isoetifolium,Antibacteial activity,MIC,MBC,gram positive,gram negative,methanol,chloroform and acetone.

1.1 INTRODUCTION

Since early human history plants have served as the most important source of medicinal natural products. Even in this synthetic age the majority of lead compounds for pharmaceutical development remain of plant origin. The availability of flora and fauna in ocean is much more when compared to that in land. In the course of evolution, marine organisms have adapted excellently to the marine environment such as high salt concentration, low temperature, high pressure and low nutrient availability. These extreme conditions require unique adaption strategies leading to the development of new natural products, which differ from known structures of terrestrial organisms *(Fenical et al., 1997).* Although research on marine natural products started only about 50 years ago, marine organisms have been used in traditional system of medicine much before that. While marine environment is known for its coral reefs, beautiful ornamental fishes, dolphins, sharks and other kind of fishes the importance of sea grasses has never been highlighted. Sea grasses, the gentle yet very important inhabitants of the coastal waters are the lesser known wealth of the ocean that is rated as one of the most valuable ecosystem globally. They are the only flowering plants that have colonized the ocean floor since the time of the dinosaurs. In Asian maritime areas, sea grass extracts were used as antibiotics, antihelmintic, antipyretic, antitumour and anti-diarrhoeal agents, for wound healing and treatment of various maladies like cough, gallstone and goiter *(Uma Maheswari et. al 2009).*

The main objective of this present study is to explore the antibacterial activity of one such sea grass – *Syringodium isoetifolium*.

1.2 MATERIALS AND METHODS

1.2.1 Sample Collection & Drying

The sea grass *S.isoetifolium* was collected in the coastal area of Kanyakumari (8°4’33” N, 77°32’53”E) Tamilnadu, India during the month of July 2014. Live and healthy sea grass samples were collected during the low tide period. Then they were immediately brought to the laboratory in plastic bags containing seawater to prevent evaporation. The sea grasses were washed thoroughly with tap water to remove all sand particles and epiphytes, shade dried at a room temperature (35±2°c) until a constant weight was obtained . The grasses were grinned with electric grinder till having very fine granules and kept at room temperature in a dark bottle until used.

1.2.2 Extraction

The grounded sea grasses powder of 100 gm dry weight was taken and subjected to percolation separately in 300ml of three different air tight glass jars and required quantity of methanol, acetone and chloroform solvents were added and kept for one week at room temperature under dark conditions. After 7 days, the contents were stirred well and then filtered by using Whatman No.1 filter paper. Each filtrate was concentrated under reduced pressure using a rotary evaporator. The dry aqueous extracts were lyophilized and stored in a refrigerator until further analysis.

1.2.3 Strain collection

9 bacterial strains - 4 gram positive and 5 gram negative strains were obtained from Central institute of Brackish water Aquaculture, Chennai. All the bacterial strains were grown on nutrient agar and maintained at 4°c.

1.2.4 Antibacterial activity

The bioassay was carried out using the agar disc diffusion method with paper discs of 6mm diameter prepared from Whatman No.1 filter papers. The antibacterial assay using gram positive and gram negative bacteria were carried out using the agar plate method. The standard disc diffusion method described by Bauer, Kirby, *Sherris and Tvrok (1966)* was followed. For this, Muller Hinton Agar plates (MHA) were prepared separately and overnight culture of test bacterial pathogens were seeded individually over the surface of MHA plates using sterile cotton swabs. Thereafter wells of 6 mm diameter were made over MHA plates using sterile cork borer. The wells were then loaded with 100 µl of sample containing 250µg of crude extracts which was prepared in dimethyl sulphoxide. Streptomycin was used as positive control; while DMSO was used as negative control. The plates were then incubated at 37°C for 24h and growth inhibitory activity in terms of zone of inhibition (mm) formed around each well was measured and recorded. Reference standard discs were prepared with tetracycline to compare the antibacterial activity results of sea grass extracts. After drying, discs with sea grass extracts and tetracycline were placed on Muller Hinton Agar plates where the bacterial culture was swabbed on the surface of the agar and incubated for 24h at 37°c.After the incubation, plates were examined for a clear zone. Diameter more than 2 mm was taken as antibacterial activity and the results were expressed in mm.

1.2.4 Determination of MIC and MBC

The minimum inhibitory concentration(MIC) was determined by using the method of micro-dilution assay following a method described by Kuete, Ngadjui, and *Penlap (2007).* It (MIC) was carried out with the extracts from chosen bacteria. 0.5ml of various concentration (25, 50, 75, 100, 125µg ml-1) of extracts was prepared with Dimethyl sulphoxide (DMSO) and mixed with 50Il of 24 hr old bacterial inoculum and allowed to grow overnight at 37o C for 48 hr. To calculate the MIC, turbidity due to bacterial growth was observed in each concentration. To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds, the minimum bactericidal concentration (MBC) was determined by sub culturing the MIC dilutions on to the sterile agar plates. The lowest concentration of the extracts which inhibits the growth of tested bacteria are observed and tabulated.

1.3 RESULTS

The methanol, chloroform and acetone extracts of *Syringodium isoetifolium* were tested for their antibacterial sensitivity against gram positive bacterial pathogens *E.faecalis*, *S.pneumonia*, *S.mutans* and *S. aureus* and gram negative bacterial pathogens *S.typhi*, *K.pneumoniae*, *P.aeruginosa*, *E.coli* and *P.vulgaris* and the results are tabulated (Table 1).

1.3.1 Antibacterial activity against Gram positive strains

The results reveal that among the three extracts, the methanol extract exhibited appreciable antibacterial activity against the gram positive strains. The methanol extract exhibited a maximum zone of inhibition (13 ± 0.84 mm) against *E.faecalis.*

1.3.2 Antibacterial activity against Gram Negative Strains

Predominant antibacterial activity was observed in the methanol extract against almost all gram negative strains. A maximum zone of inhibition (14±0.92) was observed in the methanol extract against *P.aeuroginosa.*

1.3.3 MIC and MBC

Table showed the MIC of sea grass extracts .MIC of the tested sea grass extract was ranged between 10-100 mg/ml. Lowest MIC value was recorded with crude methanol extract for *P.aeruginosa (1.0µg)* and the highest MIC was observed with Chloroform against *P.aeruginosa (100µg)* .Simultaneously MBC results showed the lowest values in Chloroform extract whereas highest values in Acetone against *S.typhi (100µg), K.pneumoniae (100µg),, P.aeruginosa (100µg), E.coli (100µg), and P.vulgaris (100µg),.*

1.4 DISCUSSION

There were a number of reports demonstrating the antibacterial activity of marine plants and their metabolites. The variance in the efficiency of antibacterial activities of these metabolites might be due to the method of extraction, type of solvent used for extraction and the season at which samples were collected. The present work was paying attention to evaluate the effectiveness of antibacterial ability of sea grass by using three types of solvent extraction. In this investigation sea grass collected from the Gulf of Mannar, South coast of India, Kanyakumari were screened for their antibacterial activities using methanol, chloroform and acetone. The results showed that the highest antibacterial activity was obtained with methanol

extract against all the gram positive and gram negative bacterial strains compared to other solvents tested. Similar studies with methanol extracts of seven different seaweeds showed broad spectrum antibacterial activity against human pathogenic bacteria. Similar results have also been reported with *E.serrulata (Mayavu et al., 2009), Posidonica oceanica (Ayad et. al., 2014) and Halophila johnsonii (Manilal et. al., 2009).*The results of the present study indicate that the susceptibility of gram positive bacteria was more than those of gram negative bacteria. This susceptibility of gram positive bacteria may be due to the differences in their cell wall structure and their com¯ position *(Taskinet al., 2007).* In gram negative bacteria, the outer membrane acts as a barrier to many environmental substances *(Tortora G J et al., 2001).* It is interesting to notice that, the concentration of the extract is minimum, when compared to the previous reports. For instance, the minimum inhibitory concentration of M. jodocodo against E.coli was 2.75 mg ml-1 while that of T. robustus against M. bourtardi was 15.75 mg ml-1 *(Jonathan Gbolagade et al., 2007).* Likewise, Liasu and Ayandele (2008) reported that, the minimum inhibitory concentration of the ethanolic plant extract ranged from 0.01 mg ml- 1 to 100 mg ml-1 against pathogenic bacteria and fungi. *Gandhimathi et al. (2008)* reported that the endosymbiotic marine actinomycetes from sponges exhibited potent antimicrobial activity against the growth of human pathogens.

1.5 CONCLUSION

The promising results for the antibacterial activity of the methanol extract of *S. isoetifolium* obtained in the present study is an indication that the sea grass *S. isoetifolium* could be used to synthesize novel antibiotics. However further research is necessary for the successful separation and purification of bioactive compounds in this sea grass.

Table 1. Antibacterial activity of crude extracts of *S. isoetifolium* and antibiotic (Tetracycline) against bacterial pathogens (Zone of inhibition – mm)

Bacterial pathogens	Crude extracts			Tetracycline (Positive control)	DMSO (Negative control)
	Methanol	Chloroform	Acetone		
Gram positive bacterial strains					
E. faecalis	13 ± 0.84	9 ± 0.44	9 ± 0.5	17 ± 1.08	0 ± 0.00
S. pneumoniae	13 ± 0.76	11 ± 0.67	11 ± 0.72	19 ± 1.22	0 ± 0.00
S. mutans	10 ± 0.56	4 ± 0.20	9 ± 0.45	13 ± 0.87	0 ± 0.00
S. aureus	11 ± 0.65	0 ± 0.00	11 ± 0.86	16 ± 1.10	0 ± 0.00
Gram negative bacterial strains					
S. typhi	10 ± 0.6	0 ± 0.00	6 ± 0.38	12 ± 0.46	0 ± 0.00
K. pneumoniae	10 ± 0.52	7 ± 0.35	4 ± 0.12	6 ± 0.33	0 ± 0.00
P. aeruginosa	14 ± 0.92	0 ± 0.00	11 ± 0.78	12 ± 0.75	0 ± 0.00
E. coli	7 ± 0.22	4 ± 0.18	7 ± 0.28	12 ± 0.82	0 ± 0.00
P. vulgaris	9 ± 0.35	6 ± 0.10	4 ± 0.12	11 ± 0.68	0 ± 0.00

Each value is the Mean ± SD of three replicate

Table 2 (A). One-way ANOVA for the data on antibacterial activity of crude chloroform extract of *S. isoetifolium* as a function of variation due to different bacterial pathogens

Source of variations	Sum of Squares	df	Mean Square	F	P-value
Variation due to bacterial pathogens	396.6667	8	49.6	526.61	P < 0.0001*
Error variance	1.6948	18	0.09	-	-
Total variance	398.3615	26	-	-	-

*Statistically more significant

Table 3(A). One-way ANOVA for the data on antibacterial activity of crude acetone extract of *S. isoetifolium* as a function of variation due to different bacterial pathogens

Source of variations	Sum of Squares	Degrees of freedom	Mean Square	F	P-value
Variation due to bacterial pathogens	198	8	24.75	86.65629	P < 0.0001*
Error variance	5.141	18	0.286	-	-
Total variance	203.14	26	-	-	-

*Statistically more significant

Table 4A. One-way ANOVA for the data on antibacterial activity of antibiotic tetracycline as a function of variation due to different bacterial pathogens

Source of variations	Sum of Squares	Degrees of freedom	Mean Square	F	P-value
Variation due to bacterial pathogens	350.6667	8	43.83333	59.41712	P < 0.0001*

Error variance	13.279	18	0.737722	-	-
Total variance	363.9457	26	-	-	-

*Statistically more significant

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude extracts of *S. isoetifolium* and antibiotic tetracycline against bacterial pathogens

Bacterial pathogens	Crude extracts						Antibiotic	
	Methanol		Chloroform		Acetone		Tetracycline	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis	10	25	10	25	25	50	1.0	10
S. pneumoniae	10	**25**	25	100	10	25	0.1	1.0
S. mutans	10	50	10	50	25	50	1.0	10
S. aureus	25	100	50	NB	10	50	0.1	1.0
S. typhi	25	50	50	NB	50	100	0.1	1.0
K. pneumoniae	25	50	50	100	50	100	10	25
P. aeruginosa	1.0	10	100	NB	25	100	10	25
E. coli	50	100	50	100	50	100	1.0	10
P. vulgaris	50	100	50	100	50	100	10	25

Each value is the mean of six replicates; NB – No bactericidal activity observed.

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