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ASSESSMENT AND SCREENING OF PHYTO-CHEMICAL COMPONENTS OF THE SEA GRASSES *CYMODOCEA ROTUNDATA AND SYRINGODIUM ISOETIFOLIUM* AND ITS ANTIBIOTIC POTENTIAL

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ABSTRACT

Sea grasses have become a recognized potential natural product in pharmaceutical industries. The *Cymodocea rotundata and Syringodium isoetifolium* contains large amount of valuable phytochemicals like saponins, flavonoids and alkaloids and so on which are known for its medicinal uses. The extracts of sea grasses are prepared with methanol solvent and tested against gram positive bacteria like *Staphylococcus aureus, Streptococcus faecalis, Salmonella enteric, Bacillus subtilis.* Gram negative bacteria like *Escherichia coli, Shigella boydii, Pseudomonas aeruginosa,Vibrio cholera* and fungi like *Aspergillus niger, Aspergillus fumigates, Candida albicans and Fusarium.* The result revealed that both the extract showed a remarkable action on the pathogens studied. Among the two extracts studied, the extract of *Cymodocea rotundata* showed a more promising action on the organism studied than *Syringodium isoetifolium.*

INTRODUCTION

Sea grasses are the submerged marine angiosperms growing well in tidal and sub-tidal areas of all seas except the polar regions. They are not true grasses of the family, Poaceae which has only terrestrial grown grasses but belong to two related families of monocotsvis. *Hydrocharitaceae* and *Potamogetonaceae* successfully adapted to saline environment[1]. Sea grasses are an essential part of the marine environment and are central to a web of life. Sea grasses grow fast, producing a great amount of organic material. They supply as the basic energy source for more or less complicated food web. Natural products have been an important resource for the maintenance of life for ages. Marine species are known to produce a large number of structurally diverse secondary metabolites[2]. Microbiologist andPharmocologists are having increased attention during the recent years towards sea grasses and marine algae which constitute the potential bioactive substance[3]. It has been realised that many of these metabolites being biologically active are of biomedical importance and could be used as a potential drugs. Bioactive marine natural products play an important role in chemotherapy. A variety of medicines and chemicals are also prepared from sea grasses and their associates. As antibiotic resistance has developed, medical researchers have fought back with alternative antibiotics and combination therapy but constant over antibiotics in human and live-stocks means that the strains of bacteria resistant to nearly all antibiotics are now known. The resistance problem demand that renewed effort be made to seek antibacterial agents against pathogenic bacteria. The present study is planned to investigate the phyto-constitutents of the sea grasses *Cymodocea rotundata and Syringodium isoetifolium* with its antimicrobial activity against some selective pathogens.

MATERIALS AND METHODS

Sea grasses collection:

Fresh *Cymodocea rotundata and Syringodium isoetifolium* were collected during the low tide period from Tuticorin, Gulf of Mannar southeast coast of India. Then the samples were washed in sea water to remove epiphytes and extraneous matter and brought to the laboratory in plastic bags containing water to prevent evaporation. Samples were then shade dried. The dried leaves were chopped with sterilized knife and powdered using mixer and were stored in the refrigerator for further use.

Extract preparation:

The extract preparation was done by using the method proposed by Padmini Sreenivasa Rao [4]. About 10 grams of the powdered sea grasses*Cymodocea rotundata and Syringodium*

isoetifolium were subjected to extraction with 100 ml of methanol. The extract was filtered through Whatman No. 1 filter paper. The filterate (stock) was evaporated and dried and kept in the refrigerator till use.

Phytochemical analysis:

Preliminary phytochemical analysis for the presence of various compounds by standard methods like Steroids [5], Phytosterols [6], Benedict's test for reducing sugar [7],Hagers test for alkaloid [8], tannins[9], saponins[10], Terpenoids by Salkowski test [11] and compounds like Phenols, Flavonids, Glycosides by Khandelwal[12] were conducted.

Microorganisms used for screening :

The microorganism used for the study were four Gram positive, Gram negative, fungi pathogens *Staphylococcus aureus*,(MTCC 96), *Streptococcus faecalis* (MTCC5383), *Bacillus subtilis* (MTCC 441), *Salmonella enteric* (MTCC 3858), *Escherichia coli* (MTCC1687), *Shigella boydii* (MTCC11947),*Pseudomonas aeruginosa* (MTCC1688),*Vibrio cholera*,*Aspergillus niger*(MTCC1344), *Aspergillus fumigates*(MTCC 3341), *Candida albicans*(MTCC854) and *Fusarium oxysporum* (MTCC 3379)respectively. All the strains were obtained from the Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India.

Antimicrobial activity:

The antimicrobial activity was determined by well diffusion method [13-15]. Muller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) with lawn culture using desire test organism. The inoculated plates were kept aside for few minutes, using well cutter,four wells were made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. The extract for this study was prepared by dissolving 1gm of stock powder in 1ml of DMSO. Using sterilized micropipettes 20ul extract of *Cymodocea rotundata, Syringodium isoetifolium,* dimethylsulfoxide (DMSO), Streptomycin (10 mcg) were added separately in each well of the bacterial culture plates (MHA). Like-wise, 20ul extract of *Cymodocea rotundata, Syringodium isoetifolium,* dimethylsulfoxide (DMSO) and Ketoconazole (10mcg) were added separately in each well of the fungal culture plates (PDA). The Streptomycin and Ketoconazole were considered as positive control and DMSO as negative control in the study. Each test was executed in triplicates. The plates were incubated at 37^{o} C and 30^{o} C for 24 and 48 hours respectively. After incubation the inhibition of growth was analyzed and results were recorded.Antimicrobial activities were evaluated by measuring the zone of inhibition in millimeters by using graduated scale. The following formula was

used for comparison of the antimicrobial activity of the sample with that of the standard (antimicrobial index) [16-19].

$$\text{Antimicrobial index} = \frac{\text{Inhibition zone of sample X 100}}{\text{Inhibition zone of the standard}}$$

RESULTS AND DISCUSSION

The Phytochemical screening of the sea grasses (Table.1) studied showed positive sign for Tannin compounds and negative for Phytosterols. Tannins are secondary metabolites responsible for antimicrobial properties in various plants [20]. In both, *Cymodocea rotundata* and *Syringodium isoetifolium*the compounds Tanins and Terpenoids are present which are attributed for analgesic and anti-inflammatory activities. Apart from this, Tannins also contribute the property of astringency that is faster healing of wounds and inflamed mucous membrane [21]. The component Saponins was present in both the sea grasses in a good level which is commonly used in several industries. In addition to industrial application as foaming and surface active agents Saponins have been extensively used as a detergent, pesticides, mollusicides and beneficial health effects [21]. In *Cymodocea rotundata* the phenolic component observed in a positive level and in*Syringodium isoetifolium* it was observed in a moderately positive level. The phenolic compounds are one of the largest and most ubiquitous group of plant metabolites [22] and they possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammatory, anti-atherosclerosis and some cardiovascular properties. The phytocomponent steroids and flavonoids are well represented in*Cymodocea rotundata* while it was absent in *Syringodium isoetifolium*. Flavonoids are hydoxylated phenolic substance known in response to microbial infection and they have been found to be antimicrobial substance act against a wide array of microorganisms[23]. Both sea grasses showed positive sign for the presence of Alkaloids and Glycosides which are used as antispasmodic and antibacterial components in medical field. Glycosides are known to lower the blood pressure to some extent[24].

Table 1: Phytochemicals of the methanol extract of seagrasses *Cymodocea rotundata and Syringodium isoetifolium*

Phyto -constitutents	*Cymodocea rotundata*	*Syringodium isoetifolium*
Steroids	Present	Negative
Terpenoids	Present	Moderately Present
Tannins	Present	Present
Saponins	Present	Present
Phenols	Present	Moderately Present
Flavonoids	Present	Absent
Glycosides	Present	Present
Phytosterols	Negative	Negative
Benedict's test (Reducing sugar)	Positive	Moderately Present
Hager's test (Alkaloids)	Present	Present

The antimicrobial activity of the methanolic extract of *Cymodocea rotundata and Syringodium isoetifolium* against human pathogens was determined by measuring the diameter of zone of inhibition expressed in millimeter (mm) [25] and is represented in Table 2. Sea grasses showed varied in the exploitation of antimicrobial activity of zone of inhibition from 6- 17 mm against all the tested microorganisms. The degree of antibiotic property depends upon the several factors such as age of the plant, duration of storage, temperature, preparation of the media and pH [26]. *Cymodocea rotundata* showed maximum activity against the gram positive *Staphylococcus aureus* (17 mm), gram negative *Escherichia coli* (15 mm) and in Fungi *Aspergillus niger* (15 mm), minimum activity against gram positive *Bacillus subtilis* (14mm), *Streptococcus faecalis*(13mm), *Salmonella enteric*(8mm),gram negative *Shigella boydii*(8mm), *Vibrio cholera* (8mm) and in Fungi *Fusarium*(10mm), *Aspergillus fumigates*(8mm) and no activity against gram negative *Pseudomonas aeruginosa* and Yeast *Candida albicans*. The antimicrobial activity performed by the extract of *C.rotundata* on various microorganisms in the present study might be due to the presence of promising quantity of the phytoconstituents such as saponins[27,28,29,30,31], tannins[32,33], phenolic[34,35,36] components and flavonoids[37,38,39] which are having the antimicrobial potentials as said by several authors whereas *Syringodium isoetifolium* showed maximum activity against gram positive *Staphylococcus aureus* (15mm), gram negative *Escherichia coli* (8mm)and Fungi *Aspergillus niger* (12mm), minimum activity against gram positive *Streptococcus faecalis* (10 mm), *Bacillus subtilis* (6mm), gram negative *Vibrio cholera* (6mm) and in Fungi *Fusarium* (8mm), *Aspergillus fumigates* (6mm) and no activity against gram negative *Shigella boydii*, *Pseudomonas aeruginosa* and Yeast *Candida albicans*. When compared to the antimicrobial activity of C.rotundata the antimicrobial property of S.isoetifolium is limited and restricted to certain organisms in the present study and this may be because of lacking of or presence of traces of saponins,tannins, phenolic compounds and flavanoids which are responsible for antimicrobial action. The difference further observed in the antimicrobial effect of the sea grass extracts studied against both gram positive and gram negative bacteria in the present studymay be due to differences in permeability barriers. In gram negative species outer membrane is fairly effect barrier for the extract and also active compounds persist in the sea grass [15]. The more susceptibility of gram positive bacteria to the sea grass extract was due to the differences in their cell wall structure and their composition [40]. In gram negative bacteria the outer membrane act as barrier to many environmental substances including antibiotics [41]. The presence of thick murine layer in the

cell wall of gram negative bacteria which act as a barrier and prevent the entry of foreign particles may be the reason for the poor performance of the studied extracts in the present study[42]. The difference in antifungal activity is due to the potential differences in the susceptibility of condia, germinated conidia and hyphae to antifungal compound[15].

Table 2 : Invitro antimicrobial activity of the methanol extract of seagrasses *Cymodocea rotundata and Syringodium isoetifolium* against the selected pathogens.

Human pathogens	Zone of Inhibition (mm)			
	Positive control (Streptomycin)	**Negative control (DMSO)**	***Cymodocea rotundata***	***Syringodium isoetifolium***
Gram positive bacteria				
Staphylococcus aureus	20±0.5	NS	17±0.5	15±0.3
Streptococcus faecalis	18±0.3	NS	13±0.3	10±0.4
Salmonella enteric	22±0.8	NS	8±0.4	6±0.6
Bacillus subtilis	20±0.6	NS	14±0.5	10±0.5
Gram negative bacteria				
Escherichia coli	22±0.5	NS	15±0.4	8±0.2
Shigella boydii	20±0.3	NS	8±0.4	NS
Pseudomonas aeruginosa	22±0.3	NS	NS	NS
Vibrio cholera	21±0.7	NS	8±0.5	6±0.3
Fungi	**(Ketoconazole)**	**(DMSO)**		
Aspergillus niger	18±0.6	NS	15±0.6	12±0.6
Aspergillus fumigates	12±0.4	NS	8±0.7	6±0.8
Candida albicans	12±0.7	NS	NS	NS
Fusarium oxysporum	12±0.6	NS	10±0.5	8±0.5

Values are the mean of the triplicates with ± SD. Zone of inhibition is given in diameter (mm)

The inhibition zone obtained by methanolic extracts were compared with standard antibiotics, Streptomycin and Ketoconazole and it is well marked in the table 2 and it showed that the performance of the standard antibiotics against the studied microbes are very effective. When compared to standard antibiotics the performance of the C.rotundata was good against almost all organisms studied whereas the extract of S.isoetifolium showed a poor performance. The antimicrobial index was calculated using the values of zone of inhibition and is represented in Table 3. *Cymodocea rotundata* showed a high antimicrobial index against the gram positive organism such as*Staphylococcus aureus* (85%), Streptococcus faecalis (72%) and Bacillus subtilis (70%). Gram negative organism*Escherichia coli* (68%) and antifungal activity against Fungi *Aspergillus niger*(83%) whereas *Syringodium isoetifolium*showed high antimicrobial index against one gram positive organism such as*Staphylococcus aureus* (75%), one gram negative organism *Escherichia coli* (36%) and antifungal activity against one Fungi *Aspergillus niger* (67%).

Table 3: Antimicrobial activity index of the methanol extract of seagrasses *Cymodocea rotundata and Syringodium isoetifolium*

Human pathogens	*Cymodocea rotundata*	*Syringodium isoetifolium*
Gram positive organisms		
Staphylococcus aureus	85%	75%
Streptococcus faecalis	72%	56%
Salmonella enteric	36%	27%
Bacillus subtilis	70%	50%
Gram negative organisms		
Escherichia coli	68%	36%
Shigella boydii	40%	-
Pseudomonas aeruginosa	-	-
Vibrio cholera	38%	29%
Fungi		
Aspergillus niger	83%	67%
Aspergillus fumigates	67%	50%
Candida albicans	-	-
Fusarium	10%	8%

CONCLUSION

From the results it can be concluded that the methanol extract of sea grass*Cymodocea rotundata* gave better performance than*Syringodium isoetifolium*.It is evident that the gram positive organism is more sensitive than the gram negative organism. Hence the sea grass*Cymodocea rotundata*may be used as a source of extraction of new antibiotic compounds in future. The result from this study form a basis for further studies of the potent sea grass so as to isolate the compounds responsible for the antimicrobial activity.

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