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Antibacterial Activity and Preliminary Phytochemical Analysis of Sea Grass *Cymodocea rotundata*

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Abstract: Antibacterial activities of the solvent extracts of sea grass *Cymodocea rotundata* against 10 human pathogens were investigated. The ethanol extract showed best activity. In the case of phytochemical analysis the ethanol and methanol extracts showed positive activity with phytoconstituents such as tannins, saponins, resins, proteins, acidic compounds, reducing sugars, terpenoids, cardiac glycosides and alkaloids but phenols, steroids, catachols and flavanoids showed negative activity.

Key words: *Cymodocea rotundata* · Antibacterial Activity · Phytochemical Analysis

INTRODUCTION

Sea grass is an angiosperm that lives in marine or brackish environment. It represents a unique flora adapted to rigorous salinity, immersion, occasional desiccation, anchorage on the seabed and hydrophilic pollination. Habitats of sea grasses are known to be highly productive and play an important ecological role as nursery grounds for fish and crustaceans such as shrimps, as food source and shelter for many organisms and in recycling of nutrients [1]. Sea grass leaves support sessile invertebrates such as bryzoans, chidarian, sponges and tunicates that compete with algae for space. Marine plants have long been recognized as producers of biological active substances. It has been suggested that sea grasses produce secondary metabolites that have a defensive role against marine pathogens [2]. Sea grasses are one of the prominent and specialized groups of marine flora which are poorly known in India compared to mangroves. Marine derived chemicals often possess quite novel structure and this in turn leads to pronounced biological activity. *Cymodocea* sp. is used as a tranquillizer for babies as soothing help during pregnancy and against cough and malaria [3]. Coastal people use the rhizomes of *Cymodocea* sp. commonly known as sea sugarcane as food for the preparation of salad [4]. Calorific value of sea grasses *E. acoroides* and *Cymodocea rotundata* are comparable to that of potato and sweet potato [5].

Marine plants and animals are well known to have developed symbiotic relationship with numerous microbes. The importance of bacterial symbiosis is growing in recognition that may be true producers of many compounds isolated from sea grasses, seaweeds, coral, sponges and other marine invertebrates [6]. Studies on leaf extracts suggest that phenolic constituents of eelgrass may inhibit amphipod grazing and microbial growth [7]. The tannin cells are specialized for the production of phenolic compounds, which play defensive roles against microorganisms and herbivorous [8-10]. Chemical constituents of several sea grasses have been described including the antibiotic flavone glycoside from *T. testudium* [2], one sugar derivative from *Ruppia maritime* [11], phenolic compounds from *P. oceanica* [12, 13], diterpens from *R. maritima* [14] and steroids and fatty acids from *Zostera japonica* [15-17]. Only a few studies have been carried out on sea grass activity such as antibacterial, antiviral, antifungal and antialgal and also the phytochemical studies. To date little information exists describing the antibacterial and phytochemical analysis of sea grass. Hence the goals of this study were to determine the antibacterial activity of the sea grass C. *rotundata* against 10 human pathogens and to analyze the phytoconstituents present in it.

MATERIALS AND METHODS

Fresh C. *rotundata* was collected during the low tide period from Gulf of Mannar Coast of Tuticorin, India. The samples were brought to the laboratory in plastic bags and washed with seawater twice and then with distilled water to remove potential contaminants and epiphytes. The leaves were separated and were dried in the shade.

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The dried leaves were milled to a fine powder which was stored in a refrigerator for further use. About 20 gm of the powdered sea grass was soaked in 1: 4 w/v ratio in solvents such as methanol, ethanol, butanol, water and acetone and kept in a mechanical shaker for 1 week. The extract was filtered through Whatmann filter paper [18]. The filtrate was evaporated and dried at 55 - 60°C [19] and the dried material was kept in the refrigerator till use [20]. The concentrates were reconstituted with their respective extracts. The extracts for the analysis of phytoconstituents were prepared in the same way using same extraction procedure as given above.

Ten human pathogens; *V. cholerae*, *B. cereus*, *P. vulgaris*, *E. coli*, *S. dysenteriae*, *S. paratyphi, Shigella sp.*, *S. mutants*, *S. aureus* and *P. fluorescens* were obtained from the Christian Medical College Hospital, Vellore. All the bacterial pathogens were grown on nutrient agar and maintained at 4°C.

Antibacterial Activity: Antibacterial activity of the prepared C. *rotundata* extracts was analyzed using well diffusion technique. The wells with 5mm diameter were punched with a sterile cork borer [18] on to the Muller Hinton agar plates that was previously inoculated with the bacterial cultures. The wells were filled with 50 µl of different solvent extracts. Plates were held in the refrigerator for 2 hours and then incubated at 37°C for 24 hours. Streptomycin sulphate was used as a positive control and wells containing the solvent alone were used as negative control. Antibacterial activities were evaluated by measuring the zone of inhibition in millimeters.

Phytochemical Analysis: The phytochemicals such as tannins, saponins, phenols, resins, proteins, acidic compounds, reducing sugar, steroids, terpenoids, cardiac glycosides, catachols, alkaloids and flavanoids analysis were carried out by the standard method [21] using concentrated crude extracts from 3 different solvents such as methanol, ethanol and acetone.

RESULTS

The antibacterial activity of C. *rotundata* extracts against 10 human pathogens is presented in the Table 1. The extracts obtained using ethanol showed maximum activity against *Shigella* (7mm) and *P. fluorescens* (7mm) when it was compared to the positive control streptomycin sulphate (9mm)for these two pathogens. This was followed *by E. coli* (5mm) and *S. aureus* (4mm) which showed moderate activity *B. cereus, P. vulgaris* S. *paratyphi* (3mm) showed low activity. No activity was seen against *V. chlorae* in any of the solvents. The butanolic extract showed maximum activity against *S. aureus* (6mm) followed by *P. vulgaris* (5mm) showed good activity. The zone of inhibition found for the positive control for all the pathogens in the butanol extracts was 8mm. *Shigella, S. mutants*, *P. fluorescens* (4mm) showed moderate activity. *S. dysenteriae* (3mm), *B. cereus* (2mm) showed poor activity. In the methanolic extract moderate activity was seen against *S. dysenteriae*, *S. paratyphi*, *S. aureus*. (4mm). *P. fluorescens*, *B. cereus* (3mm), *P. vulgaris*, *Shigella*, *S. mutants* (2mm) *E. coli* (1mm) showed poor activity. The acetone extract showed moderate activity against *E. coli* (5mm), *S. aureus* (4mm),

Table 1: Antimicrobial Activity of sea grass *C.rotundata* against human pathogens (50µl per well)

		Solvents Used					
S. No		Pathogens	Methanol	Ethanol	Butanol	Acetone	Water
1.	*V. chlorae*	-	-	-	-	-	-
2.	*B. cerus*	++	++	+	++	-	-
3.	*P. vulgaris*	+	++	+++	++	-	-
4.	*E. coli*	+	+++	+	+++	+	-
5.	*S. dysentriae*	++	++	++	++	-	-
6.	*S. paratyphi*	++	++	-	++	-	-
7.	*Shigella* sp.	+	+++	++	+	+++	-
8.	*S. mutants*	+	++	++	++	++	-
9.	*S. aureus*	++	++	+++	++	+++	-
10.	*P. fluorescence*	++	+++	++	++	+	-

- No zone of inhibition
+ 1-2 mm zone of inhibition
++ 3-4 mm zone of inhibition
+++ 5-7 mm zone of inhibition

Table 2: Phytochemical analysis of sea grass *C. rotundata*

Phytoconstituents	Methanol	Ethanol	Acetone
Tannins	=	=	=
Saponin	=	=	×
Phenols	×	×	×
Resins	=	=	=
Proteins	=	=	=
Acidic Compounds	=	=	=
Reducing Sugar	=	=	=
Steroids	×	×	×
Terpenoids	=	=	×
Cardiac Glycoside	=	=	×
Catachols	×	×	×
Alkaloids	=	=	=
Flavanoids	×	×	×

= - Present, × - Absent

S. dysenteriae (4mm). *B. cereus*, *P. vulgaris*, *S. paratyphi*, *S. mutants*, *P. fluorescens* (3mm), *Shigella* (1mm) exhibited poor activities when it was compared with the positive control which had zone of inhibition (10mm). The water extracts did not show any activity with all the test organisms which shows very poor activity. In the present investigation three different solvents were used as the qualitative tests for *C. rotundata* (Table 2).

DISCUSSION

Research on bioactive compounds from marine organisms has provided a broad and better support of marine natural products research throughout the past quarter century. The emergence of resistant bacteria has created a serious concern and an urgent need for the discovery of new antibacterial agents [22, 23]. Marine organisms collected from the southeast coast of India have been shown to posses a number of biological activities [24]. Our present findings is consistent with some earlier reports [25] and it showed that ethanol and methanol extractions of the sea grasses *Halophila ovalis* and *Halodule pinafolia* showed better zone of inhibition against bacterial pathogens that other tested extracts. Butanolic extraction also showed good activity which is in agreement with fractionation using butanol of the mangrove *Sonneratia casealaris* [26]. The antifouling potential of some marine organisms against *Bacillus* and *Pseudomonas* sp. was reported [27] Regarding the acetone extract our results coincide with the report of acetone extract of *Halophila ovalis* and *Zostera capensis* which showed less activity compared to the other solvents [3]. The variation of antibacterial activity of the extracts might be due to distribution of antimicrobial substances, which varied from species to species [28]. The water extract showed no activity against any of the tested organisms. Aqueous and ethanol extract of *Heracleum Sphondylium* showed antimicrobial activities against Gram-positive and Gram-negative bacteria, [20]. The susceptibility of the microorganisms towards the antibiotic depends upon the mechanism of action of the compound and the differences in the cell wall structure of both types of bacteria.

The phytochemical analysis of *C. rotundata* revealed the presence of tannins, saponins, proteins, resins, reducing sugar, acidic compounds, alkaloids, cardiac glycosides and terpenoids [20]. Phytoconstituents like phenolics (tannin and phenol) have been implicated as antioxidants in the scavenging of radicals like NO and H_2O_2 in algae and terrestrial plants [29, 30]. The phytochemical compounds *viz.,* glycoside, saponins, tannins, flavonoids, terpenoides and alkaloids have antimicrobial activity [31]. The antibacterial activity exhibited by the marine plant parts could be due to the presence of phytochemicals like alkaloids, tannins, flavonoids and sugars present in the plant extracts [32]. Tannins form irreversible complexes with proline rich proteins, resulting in the inhibition of cell protein synthesis of bacteria [20, 33]. The preliminary phytochemical studies of the active fraction of root extracts of *C. serrulata* had variety of phytochemical constituents, namely alkaloids, carboxylicacid, coumarins, flavonoids, phenols, saponins, xanthoprotein, protein, steroids, tannins and sugar [34].

The sea grass *Cymodocea rotundata* has variety of biologically active molecules which can be used as a source of antibiotics. Further purification of active compounds and structural elucidation can be used for drug discovery.

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REFERENCES

1. Matthew Richmond, D., 1997. A guide to the seashores of eastern Africa and the Western Indian. Ocean Islands, Sida/ Department for Research Cooperation, SAREC.

2. Jensen, P.R., K.M. Kensin, D. Porter and W. Fenical, 1998. Evidence that a new antibiotic flavone glycoside chemically defends the seagrass Thalassia testudium against zoosporic fungi. Appl. Environ. Microbiol., 64: 1490-1496.
3. Sreenath Kumar, C., D.V.L. Sarada, T.P. Gideon and R. Rengasamy, 2008. Antibacterial activity of three South Indian seagrasses, Cymodoceaserrulata, Halophila ovalis and Zostera capensis. World J. Microbiol. Biotechnol., 24: 1989-1992.
4. Kannan, L. and Thangaradjou, 2005. Seagrasss ecosystem: importance and need for conservation. National symposium on marine plants, their chemistryand utilization.Souvenir Seaweed Research Utilization Association. Suganthi Devadason Marine Research Institute, Tuticorin., pp: 76-78.
5. Pradheeda, M., E. Dilipan, E.P. Nobi, T. Thangaradjou and K. Sivakumar, 2011. Evalution of seagrasses for their nutritional value. Indian J. Geo-marine Sci., 40(1): 105-111.
6. Fenical, W., 1993. Chemical studies of marine bacteria: Developing a new resource Chemical Review, 93: 1673-1683.
7. Harrison, P.G., 1982. Control of microbial growth and of amphipod grazing by water soluble compounds from the leaves of Zostera Marina. Marine Biol., 67: 225-230.
8. Tempel, A.S., 1982. Tannin measuring technique. A review, J. Chemical Ecol., 8: 1289-1298.
9. Cariello, L. and L. Zanetti, 1979. Distribution of chicoric acid during leaf development of Posiodonia oceanica, Marine Botany, 22: 359-360.
10. Kuo, J. and A.J. Mc Comb, 1989. Seagrass taxonomy structure and development, In: Larkum, A.W.D. Mc A.J. Comb and S.A. Sheperds, (Eds), Biology of seagrass, Aquatic Plant Studies, Elsevier Publication, France, 2: 66-73.
11. Aquino, R.S., A.M. Landeira-Fernandez, A.P. Valente, L.R. Andrade and O.P.A.S. Moura˜, 2005. Occurrence of sulfated galactans in marine angiosperms: evolutionary implications. Glycobiol., 15: 11-20.
12. Todd, J.S., R.C. Zimmerman, P. Crews and R.S. Alberte, 1993. The antifouling activity of natural and synthetic phenol acid sulphate esters. Phytochemistry, 34: 401-404.
13. Bushmann, P.J. and M.S. Ailstock., 2006. Antibacterial compounds in estuarine submersed aquatic plants. J. Exp. Mar. Biol. Ecol., 331: 141-150.
14. Della Greca, M., A. Fiorentino, M. Isidori, P. Monaco and A. Zarrelli, 2000. Antialgal ent -labdane diterpenes from Ruppiamaritima. Phytochemistry, 55: 909-913.
15. Gillan, F.T., R.W. Hogg and E.A. Drew, 1984. The sterol and fatty acid compositions of seven tropical seagrasses from North Queensland, Australia. Phytochemistry, 23: 2817-2821.
16. Sanina, N.M., S.N. Goncharova and E.Y. Kostetsk., 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. Phytochemistry, 65: 721-730.
17. Kuo, J. and A.J. Mc Comb, 1989. Seagrass taxonomy structure and development, In: Larkum, A.W.D. Mc A.J. Comb and S.A. Sheperdn, (Eds), Biology of seagrass, Aquatic Plant Studies, Elsevier Publication, France, 2: 66-73.
18. Adomi, P.O., 2006. Antibacterial activity ofaqueous and ethanol extracts of the stem bark of Alstonia boonei and Morindalucida. Scientific Research and Essay, 1(2): 50-53.
19. Lima-Filho, J.M., F.F. Ana, M.F. Sissi and M.M. Vania, 2002. Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian coast. Braz. J. Microbiol., 33(4).
20. Ergene, A., P. Guler, S. Tans, S. Miric, E. Hamzaoglu and A. Duran, 2006. Antibacterial and antifungal activity of Heracleum sphondylium subsp. artvinense, Afirican J. Biotech., 5: 1087-1089.
21. Sofowora, A., 1931. Medicinal Plans and Traditional Medicine in Africa. Spectrum Book. Ibadan.
22. Davis, J., 1994. Inactivation of antibiotics and the dissemination of resistance genes. Sci., 26: 375-381.
23. Spratt, B.G., 1994. Resistance to antibiotics mediated by target alterations. Sci., 264: 388-393.
24. Ely, R., T. Supriya and C.G. Naik, 2004. Antimicrobial activity of marine organisms collected off the coast of South East India. J. Exp. Mar. Bio. Ecolo., 309: 121-127.
25. Umamaheshwari, R., G. Thirumaran and P. Anantharaman, 2009. Potential Antibacterial Activities of Seagrasses from Vellar Estuary; South East Coast of India. Advances in Biological Res., 3(3-4): 140-143.
26. Prabha Devi, Solimabi W. LD'Souza, S. Sonak, S.Y. Kamat and S.Y.S. Singbal, 1997. Screening of Some Marine Plants for Activity against Marine Fouling Bacteria.Botanica Marina, 40: 87-91.

27. Bhosale, S.H., V.L. Nagle and T.G. Jagtab, 2002. Antifouling potential of some marine organisms from India against species of Bacillus and Pseudomonas. Marine Biotechnol., 4: 111-8.
28. Lustigman, B. and C. Brown, 1991. Antibiotic production by marine algae isolated from the New Work, New Jersey Coast. Bulletin of Environmental Contamination and Toxicol., 49: 329-335.
29. Gulcin, I., O. Irfan Kufrevioglu, M. Oktay and M.E. Buykokuroglu, 2004. Antioxidants, antimicrobial antiulcer and algesic analgesic activities of nettle (*Urtica diocia* L.). J. Ethnopharmacol., 90: 205-215.
30. Badami, S., S.R. Rai and B. Suresh, 2005. Antioxidant activity of Aporosa lindleyana root. J. Ethnopharmacol., 101: 180-184.
31. Okeke, M.I., C.U. Iroegbu, E.N. Eze A.S. Okoli and C.O. Esimone, 2001. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity. J. Ethnopharmacol., 78: 119-127.
32. Fennel, C.W., K.L. Lindsey, J.L. McGaw, G.I. Stafford, E.E. Elgorashi, M.O. Grace and V. Staden, 2004. Assessing African medicinal plants for efficacy and safety. Pharmacological screening and tocxicology. J. Ethnopharmacol., 94: 205-217.
33. Scalbert, A., 1991. Antimicrobial properties of tannins. Phytochem., 30: 3875-3883.
34. Ravikumar, S. and K. Kathiresan, 1993. Influence of tannins, amino acids and sugar on fungi of marine halophytes. Mahasagar., 26(1): 21-25.