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Antibacterial activity of aqueous extract from selected macroalgae of southwest coast of India

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ABSTRACT

Aqueous extract of seven species of marine macroalgae were screened for their antimicrobial potency against ten pathogenic bacterial strains. *Ulva fasciata*, *Gracilaria corticata*, *Sargassum wightii* and *Padina tetrastromatica* showed significantly higher activity against 70% of the tested bacterial isolates. The maximum zone of inhibition was noted for the red alga *G.corticata* against *Proteus mirabilis* (17mm) and brown alga *P. tetrastromatica* against the pathogens *Staphylococcus aureus* and *Vibrio harveyi* (15mm). The general trend of inhibitory activity was higher towards Gram negative bacteria.

Introduction

Marine organisms are source material for structurally unique natural products with pharmacological and biological activities (Faulkner, 2001; Da Rocha *et al*., 2001; Schwartsmann *et al.*, 2001). Among the marine organisms, the macroalgae (seaweeds) occupy an important place as a source of biomedical compounds (Manilal *et al*., 2010; Selvin and Lipton, 2004). About 2400 natural products have been isolated from macroalgae belonging to the classes Rhodophyceae, Phaeophyceae and Chlorophyceae (Faulkner, 2001). The antimicrobial activity was regarded as an indicator to detect the potent pharmaceutical capacity of macroalgae for its synthesis of bioactive secondary metabolites (Gonzalez *et al.*, 2001; Smit, 2004). The compounds derived from macroalgae are reported to have broad range of biological activities such as antibacterial (Chakraborty *et al*., 2010a; Manilal *et al*., 2009; Selvin *et al*., 2004) anticoagulant (Lipton and Jose, 2006; Farias *et al*., 2000) and antifouling activity (Selvin and Lipton, 2002; Marechal et *al*., 2004). The antimicrobial agents such as Chlorellin derivatives, acrylin acid, halogenated aliphatic compounds, phenolic inhibitors and more recently Guaiane sesquiterpenes and labdane diterpernoids were also detected from macroalgae (Chakraborty *et al*., 2010b; Espeche *et al*., 1984).

Screening of all classes of marine algae for their antibiotic value is recorded in the literature by a number of scientists from India and abroad (Kandhasamy and Arunachalam, 2008). Efforts were made by several researchers to bring out bioactive substances from macroalgae. Selvi and Selvaraj (2000) reported the antibacterial activity of some Indian seaweeds. Padmakumar (2002) studied the seasonal

variations of antimicrobial activity in marine algae from Kanyakumari coast. Vanitha *et al*. (2003) indicated the antibacterial action of seaweeds against human upper respiratory tract pathogens. The antibacterial activity of methanol extracts of fifty six seaweeds collected from South African coast, belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae were reported by Vlachos *et al*. (1999). Considering the scenario of the availability on very few records on the antibacterial activity of aqueous extracts of macroalgae, the present study was made to examine the efficacy of aqueous extracts of selected marine macro algal species collected from southwest coast of India against clinical and fish pathogens.

Materials and Methods

Collection of the macroalgae

The marine macroalgae viz. *Ulva fasciata* and *Codium tomentosum* (Chlorophyceae), *Sargassum wightii, Dictyota dichotoma* and *Padina tetrastromatica* (Phaeophyceae) and *Gracilaria corticata* and *Hypnea musciformis* (Rhodophyceae) were collected during low tide period from Mulloor (Kerala), Muttom and Kanyakumari Coast (Tamil Nadu). The month and localities of algae collected are given in Table - 1.

Extract preparation

For assessing antibacterial activity, 50 g each of fresh macroalgal samples were taken and washed with filtered seawater. The epiphytes and other extraneous matter were removed. After removing the water content, algae samples were ground with 50 ml of phosphate buffer pH 7 in a mixer grinder. The extract was filtered under vacuum, quantified and stored in refrigerated condition.

Test bacteria and bioassay

For antibacterial assay, bacterial cultures viz., *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus, Streptococcus sp., Pesudomonas aeruginosa, Escherichia coli, Micrococcus luteus, Proteus mirabilis, Vibrio alginolyticus, Vibrio fischeri, Vibrio harveyi* and *Klebsiella pneumoniae* were used. The axenic cultures were obtained from the Institute of Microbial Type Culture collection (MTCC) Chandigarh, CMFRI, Vizhinjam Research Centre and Scudder Microbiology Laboratory, Nagercoil. The *in vitro* antibacterial activity was determined by using the standard disc diffusion method (Bauer *et al*., 1966).

Agar disc diffusion method was followed for antibacterial susceptibility test (CLSI, 2005). A weighed aliquot of the water extract diluted in sterile saline water (20µl) was transferred to sterile 6mm Whatman No – 1

Table 1. *Macroalgal species and their collection sites*

Classes	Macroalgae	Month of collection	Place of collection
Chlorophyceae	*Ulva fasciata*	April, 2005	Mulloor Coast
	Codium tomentosum	February, 2006	Kanyakumari Coast
Phaeophyceae	*Sargassum wightii*	April, 2005	Mulloor Coast
	Padina tetrastromatica	October, 2006	Muttom Coast
	Dictyota dichotoma	February, 2006	Kanyakumari Coast
Rhodophyceae	*Gracilaria corticata*	January, 2006	Muttom Coast
	Hypnea musciformis	February, 2006	Kanyakumari Coast

filter paper disc. After air drying, the disc was placed on the Mueller Hinton agar (Hi Media) plates inoculated with each of the previously mentioned microorganisms. The disc soaked with similar quantity of phosphate buffer was used as the control. After 24 h of incubation at 37^0C, the inhibition zones were measured. Duplicates of each sample were kept and the mean of the zone of these replicates were recorded.

Results

The aqueous extracts prepared individually from seven different macroalgae belonging to three classes (Chlorophyceae, Phaeophyceae and Rhodophyceae) showed various degrees of activity against clinical and fish pathogenic bacteria (Table-2 to 4). The extracts of *U. fasciata* (Chlorophyceae), *G.corticata* (Rhodophyceae), *S. wightii* and *P. tetrastromatica* (Phaeophyceae) showed higher activity against 70% of the tested bacterial isolates. *Codium tomentosum* (Chloropyceae) and *H. musciformis* (Rhodophyceae) extracts showed moderate activity while *D. dichotoma* extract had lesser activity towards clinical pathogens and the fish pathogen *V. alginolyticus.*

Comparison among the members of same class and antibacterial activity

Members belonging to the same class showed various degrees of antibacterial activity against clinical and fish pathogenic bacteria. Among the two members of Chlorophyceae, extracts of *Ulva fasciata* showed broad inhibitory spectrum against clinical pathogens than *Codium tomentosum* (Table-2) . However, the extract of *C. tomentosum* produced maximum inhibitory zone diameters of 10, 11 and 12mm at 0.1and 1.0% concentrations against *M.luteus , E. coli and V. fischeri* . No inhibition could be noted for *S. aureus, Streptococcus sp. and K. pneumoniae* which were inhibited by *U. fasciata* of same class. Also the extract of *U. fasciata* showed equal inhibitory action towards Gram positive and Gram negative bacteria. But *C. tomentosum* showed better activity towards Gram negative than Gram positive bacteria.

The *members of* Phaeophyceae namely *Padina tetrastromatica* and *Sargassum wightii*, showed higher antibacterial activity than *Dictyota dichotoma* belonging to the same class. *Sargassum wightii* showed highest antibacterial activity against clinical pathogens namely *P.aeruginosa, K. pneumoniae* and *E. coli* and fish pathogen *V. alginolyticus.* It was more active against Gram negative than Gram positive bacteria. It produced maximum inhibitory zones at 0.1 and 1.0% concentration against those pathogens. Moderate activity was noted against other tested bacteria. *P.tetrastromatica* strongly inhibited the growth of *S. aureus, P. mirabilis, P. aeruginosa, E. coli, V. harveyi* and *V. fischeri* (14 to 15mm diameter at 50 and 100% concentrations and 8 and 10mm at 0.1 and1.0% concentrations) respectively. The extract of *D. dichotoma* showed poor activity than other two members of the same class. It produced maximum inhibitory zone of 9, 10 and 12 mm at higher concentrations against pathogens namely *B. subtilis, P. mirabilis* and *V.alginolyticus.* Other pathogens which were strongly inhibited by other two members of the same class were not inhibited by *D.dichotoma*. Also it had poor activity towards Gram negative bacteria than Gram positive bacteria.

Among the members of Rhodophyceae, the aqueous extracts of *G.corticata* showed highest antibacterial activity than *H. musciformis. Gracilaria corticata* aqueous extract strongly inhibited the growth of *Proteus mirabilis, P.aeruginosa,*

Table 2. *Zone of inhibition (mm) of aqueous extract of green algae against selected bacteria*

Test organisms	Gram+ve/ Gram-ve	*Ulva fasciata*					*Codium tomentosum*				
		0.1%	1%	10%	50%	100%	0.1%	1%	10%	50%	100%
Clinical pathogens											
Staphylococcus aureus	+	9	9	9	9	10	-	-	-	-	-
Streptococcus sp.	+	9	9	10	10	10	-	-	-	-	-
Bacillus subtilis	+	7	7	7	8	9	-	-	8	8	9
Micrococcus luteus	+	10	10	7	-	-	11	9	7	7	6
Proteus mirabilis	-	7	8	8	8	9	-	-	-	8	8
Pseudomonas aeruginosa	-	8	8	10	10	11	7.5	8	9	10	10
Klebsiella pneumoniae	-	7	9	9	9	10	-	-	-	-	-
Escherichia coli	-	+	+	+	7	9	12	10	9.5	8	7
Fish pathogens											
Vibrio fischeri	-	7	8	9	9	9	9	10	8	9	10
Vibrio alginolyticus	-	8	9	9	9	9	9	8.5	7.5	7	6
Vibrio harveyi	-	8	9	10	9	8	9	10	11	12	13

Table 3. *Zone of inhibition (mm) of aqueous extract of brown algae against selected bacteria*

Test organisms	Gram+ve/ Gram-ve	*Sargassum wightii*					*Padina tetrstromatica*					*Dictyota dichotoma*				
		0.1%	1%	10%	50%	100%	0.1%	1%	10%	50%	100%	0.1%	1%	10%	50%	100%
Clinical pathogens																
Staphylococcus aureus	+	-	7	7	7	8	10	11	14	14	15	-	-	-	-	-
Streptococcus sp.	+	-	-	-	7	8	-	8	-	-	-	-	-	+	+	+
Bacillus subtilis	+	8	9	9	9	10	-	8	9	9	10	8	8	9	10	12
Micrococcus luteus	+	8	8	10	-	-	-	-	-	-	-	-	+	+	+	+
Proteus mirabilis	-	-	10	8	8	7	8	8	9	10	12	-	8	9	9	10
Pseudomonas aeruginosa	-	10	11	11	12	12	8	10	13	14	14	-	-	-	-	-
Klebsiella pneumoniae	-	12	11	11	10	-	-	-	-	8	8	-	-	+	+	+
Escherichia coli	-	8	11	12	13	13	8	11	12	13	13	-	-	-	-	-
Fish pathogens																
Vibrio fischeri	-	7	9	10	-	-	-	-	-	-	-	-	-	-	-	-
Vibrio alginolyticus	-	10	9	8.5	8	-	-	7	8	9	9	-	7	9	9	10
Vibrio harveyi	-	7	7	7	7	8	10	12	13	14	15	-	-	-	-	-

Table 4. *Zone of inhibition (mm) of aqueous extract of red algae against selected bacteria*

Test organisms	Gram+ve/ Gram-ve	*Gracilaria corticata*					*Hypnea musciformis*				
		0.1%	1%	10%	50%	100%	0.1%	1%	10%	50%	100%
Clinical pathogens											
Staphylococcus aureus	+	9	10	10	10	11	-	-	-	7	10
Streptococcus sp.	+	8	8	8	9	9	-	-	-	-	-
Bacillus subtilis	+	-	-	7	8	10	-	-	-	-	-
Micrococcus luteus	+	9	9	10	-	-	9	10	10	11	12
Proteus mirabilis	-	13	14	15	16	17	8	10	10	11	13
Pseudomonas aeruginosa	-	10	10	11	11	12	9	10	10	11	12
Klebsiella pneumoniae	-	8	8	10	11	12	-	-	-	-	-
Escherichia coli	-	7	8	8	9	10	-	-	8	9	10
Fish pathogens											
Vibrio fischeri	-	7	8	10	10	10	7	7	7	8	8
Vibrio alginolyticus	-	7	9	11	12	12	-	-	-	-	-
Vibrio harveyi	-	7	8	10	11	11	7	7	7	7	8

- No activity; + Mild activity

K. pneumoniae and V.alginolyticus. It produced the maximum inhibitory zone of 16 and 17mm diameter at 50 and 100% concentration against *P. mirabilis* and 11 and 12mm diameter at 1.0 and10% concentrations against *S. aureus, P. aeruginosa, K.pneumoniae and V.alginolyticus*. But aqueous extract of *H. musciformis* produced a maximum inhibitory zone of 12 and 13mm diameter at 100% concentration towards *P.aeruginosa, P. mirabils and M. luteus.* It strongly inhibited the growth of Gram negative bacteria than Gram positive bacteria.

Correlation between taxonomy and antibacterial activity

Aqueous extracts of the Phaeophyceae *i.e., S. wightii* and *P. tetrastromatica* showed the highest antibacterial activity against all the tested pathogens. *G. corticata* (Rhodophycea) also showed higher antibacterial activity as well as produced larger inhibition zone diameter against *P. mirabilis* than other extracts. The extracts from Chlorophycean members tested had moderate activity. The inhibition by the extract of *U. fasciata, C.tomentosum* and *H. musciformis* were low compared to other members. *Ulva fasciata, P.tetrastromatica, S. wightii* showed a broad spectrum of antibacterial activity inhibiting the growth of both Gram positive and Gram negative tested organism (Table - 2 to 4). Extract of *C. tomentosum* did not exhibit antibacterial activity against all the tested Gram positive (no activity in S. *aureus* and *Streptococcus* sp.) and Gram negative (no activity in *K.pneumoniae*) bacteria. The extract of *D.dichotoma* inhibited the growth of *B. subtilis, P. mirabilis* and *V. alginolyticus.* It showed the least antibacterial activity against the tested organisms.

Discussion

Different solvent systems were used earlier to extract bioactive principles from macroalgae with concomitant changes in the antibacterial activities (Thirupurasundari *et al*., 2008). The solvents such as acetone, benzene, butanol (Vanitha *et al*., 2003; Prakash *et al*., 2005), ethanol (Selvi *et al*., 2001) were used to extract antimicrobial compounds from macroalgae. The present study revealed the effectiveness of the aqueous extract of different macroalgae against clinical and fish pathogens. The aqueous extracts prepared from seven macroalgal samples showed varying degrees of activity against tested pathogens, including the Gram positive and Gram negative bacteria. Among the algal samples screened for antibacterial activity, the aqueous extract of *U.fasciata* (Chlorophyceae), *G. corticata* (Rhodophyceae*), S. wightii* and *P.tetrastromatica* (Phaeophyceae) showed higher activity against most of the tested organisms. This is in contrary to the earlier findings of Selvi *et al.* (2001) wherein they reported lowest antibacterial activity in aqueous extract of *U. fasciata* and *G. corticata.*

The results of the present study revealed that aqueous extract of *H. musciformis* from lowest concentration (10%) showed antibacterial activity against *P.aeruginosa.* Veeragurunathan *et al*. (2008) reported highest antibacterial activity (12mm at 75µl) of ethanolic extract of *H. musciformis* against *E.coli*. However, as observed by Selvi and Selvaraj (2000) ethanol extract of *Hypnea sp*. had no antibacterial activity against *P. aeruginosa* even at the tested higher concentration of 200µg/disc. In the present study, aqueous extract of *H. musciformis* showed highest antibacterial activity of 13 mm and 12 mm diameter at 100% and 11 mm at 50% concentration against *P. mirabilis*, *M. luteus* and *P. aeruginosa* which is in agreement with the earlier findings of Jose *et al*. (2008). But in *E. coli*, maximum zone of 10 mm diameter was noticed only when extract was not diluted. These differences could be due to the different solubility behavior of secondary metabolites which could be influenced by seasonal and geographical distribution of the species as indicated by Padmakumar (2002).

The aqueous extract of *P. tetrastromatica* inhibited most of the tested clinical and fish pathogens except *M. luteus*. Kandhasamy and Arunachalam (2008) pointed out that the methanol extract of *P.tetrastromatica* inhibited the growth of *K.pneumoniae, E. aerogens, M. luteus, S.aureus, P. aeruginosa* and *B. subtilis* except *E. coli* and *S. faecalis.* In contrary, the aqueous extract of *P. tetrastromatica* in the present study inhibited the growth of *E. coli* effectively. An inhibitory zone of 13 and 14mm diameter was recorded at 50% of the raw extract against *E. coli* and *S. aureus* respectively. The clinical pathogen *P. aeruginosa* responsible for causing the nosocomial infections was inhibited effectively by the aqueous extract of *P. tetrastromatica* at all the tested concentrations and produced the maximum inhibitory zone to the tune of 14mm. However, resistance by *P. aeruginosa* was reported against twenty macroalgal extracts tested (Selvi *et al*., 2001).

The water extract of *D. dichotoma* exhibited minimum inhibition towards most of the pathogens tested. Similar results were also reported by Selvi *et al*. (2001) and Salvador *et al*. (2007). The aqueous extract *of D.dichotoma* did not show inhibitory effect on *Streptococcus sp., S. aureus, E. coli, P.aeruginosa, S. typhimurium* and *K.pneumoniae*. The maximum inhibition of *D.dichotoma* was towards the Gram positive bacteria *B. subtilis* in the undiluted extract as also observed by Selvi *et al*. (2001). The order Dictyotales is known to produce biologically active compounds such as Dictyterpenoids

(Suzuki *et al.,* 2002). The larger zone of inhibition by *D. dichotoma* against a few pathogenic strains of bacteria in the present study could be attributed to the presence of such active compounds. The separation and further purification of such active compounds is a necessity when considering the application of such biologically important drugs in the era of increased antibiotic resistance.

It could be noticed from the present investigation that the aqueous extract of *S. wightii, P. tetrastromatica* and *D. dichotoma* (Phaeophyceae) were more effective as an antibacterial agent when compared to *G. corticata* and *H. musciformis* (Rhodophyceae). This is evidence from the observation that the aqueous extracts of the brown algae effectively inhibited most of the pathogens with the maximum zone of 15 mm produced against *S. aureus.* The greater antibiotic activity of the brown algae against pathogens was supported by the recent findings of Veeragurunathan and Geetha (2009). Nair (2005) reported that both the aqueous and ethanol extract of *G. corticata* could not inhibit *B. subtilis* and *K. pneumoniae*. But the present study revealed that both these pathogens were inhibited by all the tested dilutions of the aqueous extract of *G. corticata*, with maximum inhibitory zone in the undiluted extract.

Apart from these, the results of the present study has brought to light that Gram negative organisms were more susceptible to the aqueous extract of the algae used. In contrast, Taskin *et al*. (2001) and Tuney *et al*. (2006) reported that Gram positive bacteria were more effectively controlled by the extracts of the algae used in their study compared to Gram negative bacteria. The more susceptibility of a particular group of bacteria was due to the difference in their cell wall structure and their composition (Paz *et al*., 1995). Although the outer membrane of Gram negative bacteria acts as a barrier to many environmental substances including antibiotics (Tortora *et al*., 2007), the higher susceptibility noticed against the algal extracts gives a promising indication of developing a potent drug from these marine natural sources to be used in combating the infections due to such pathogens. Though the exact reason for the high sensitivity observed in Gram negative strains need to be revealed through further research work, it could be opinioned that the compounds in macroalgae may be of phenolic nature, which solubilized the lipopolysaccharide layer of its cell wall, leading to the entry of the inhibitory molecules. The Gram positive bacteria characterized by a thick peptidoglycan layer in its outer cell wall might have resisted the entry of the inhibitory molecules, hence attributed to the less susceptibility.

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