Research Journal of Environmental Toxicology

ISSN 1819-3420

Academic Journals Inc.

www.academicjournals.com

Research Journal of Environmental Toxicology 6 (3): 101-109, 2012
ISSN 1819-3420 / DOI: 10.3923/rjet.2012.101.109

Influence of Bioactive Compounds from Seaweeds and its Biocidal and Corrosion Inhibitory Effect on Mild Steel

[1]S. Roseline Kantida, [1]K.R.T. Asha and [2]S. Sujatha
[1]Department of Biotechnology, Malankara Catholic College, Mariagiri, Kaliakkavilai, 629153, Kanyakumari District, Tamil Nadu, India
[2]International Centre for Bioresources Management, Malankara Catholic College, Mariagiri, Kaliyakkavilai-629153, Tamil Nadu, India

Corresponding Author: S. Sujatha, International Centre for Bioresources Management, Malankara Catholic College, Mariagiri, Kaliyakkavilai-629153, Tamil Nadu, India

ABSTRACT
Microbial corrosion is recognized as one of the major problem in various fields during favorable environment for biological growth that causes fouling and corrosion problems. In the present study, an attempt has been made to evaluate the biocidal and corrosion inhibitory effect of three seaweed extracts such as *Turbinaria, Jania* and *Gelidiella* on mild steel. Among the three seaweed extracts *Turbinaria ornate* showed a highest biocidal effect (84%) followed by *Gelidiella acerosa* (73%) and *Jania adhaerens* (71%) mainly this biocidal efficiency was revealed to be concentration dependent. The antibacterial activity of the seaweed extracts were tested against *Bacillus* sp., *Micrococcus* sp., (Gram+ve, g+ve) *Klebsiella* sp., *Pseudomonas* sp., (Gram-ve, g-ve). All the three seaweeds showed superior antibacterial activity. *Pseudomonas* was found to relatively resistant to all the three seaweeds. The seaweed extracts found to inhibit corrosion of mild steel for both seawater and 0.5 N HCl. Whenever, concentration of seaweed extracts increased the corrosion inhibition efficiency also been increased. While, 90.7% corrosion inhibition was achieved seawater added with *Turbinaria* extracts. Relatively *Gelidiella* sp., showed least inhibitory effect in seawater. Moreover, in seawater the *Turbinaria* extracts showed peak corrosion inhibitory activity range was 76.49% in acid medium. Meanwhile, minimum and maximum antifouling activity also observed on seaweed extracts such as *Jania adhaerens* and *Gelidiella acerosa*. The results indicated that marine seaweed could provide an important source of natural antibacterial, anti fouling as well as anticorrosion activity possessed bioactive compounds and that the interaction between marine microbial floras cannot easily be generated.

Key words: Corrosion, seaweed, antibacterial activity, micro fouling, *Gelidiella, Turbinaria, Jania*

INTRODUCTION
The importance of different seaweeds in pharmacology is known, the development of antimicrobial, antifungal and antiviral substances from seaweeds is still in an initial stage of research and development. According to Tiller and Booth (1982) and Bianco *et al.* (2008), bacterial corrosion stands for the several structure of deterioration, which supplied to significant victims to the economy. This requires at least a minimum basic understanding in the fields of chemistry, biochemistry, metallurgy and microbiology. By definition it refers to the degradation of metallic structures resulting in the activity of a variety of organisms, which either produce aggressive

metabolites or tender the environment corrosive or able to participate directly in the electrochemical reactions occurring on the metal surfaces (Miao and Qain, 2005).

A large variety of microorganisms were mainly influenced for corrosion has been described by Tiller and Booth (1982). Again, Miao and Qain (2005) published about that the general corrosion effect mainly depends upon the abiotic factors and its microbial activity. In addition microbial corrosion for instance is a more serious risk in injection water systems and it associated with soil on oil field equipments and pipelines. Beside, it has major influences on catholically protected structures and it also creates some problems of blockage in filters and pipelines described by several authors (Brinkhoff *et al*., 2004; Fakoya *et al*., 2011; Shah *et al*., 2008).

The antibiotic activity of the algae is not widespread but also variable with different strains or collections of the same organism (Wefky and Ghobrial, 2008). Bernard and Pesando (1989) described the seasonal variation have also been reported the wide spread occurrence of the phenomenon, together with its variability, suggests that most algal antibiotics are common metabolites that under some conditions accumulates in sufficient quantities is inhibitory to micro organisms (Erwan *et al*., 2008). Hence in the present study, three seaweeds were screened for antifouling as well as corrosion inhibitor compounds. There have been several methods in practice for external surface and use of chemical biocide for cooling water systems. Most of the methods are responsible for today's environmental pollution therefore it has become urgent need to develop eco friendly method for the control of biofouling. Keeping in mind the present study has been made to identify the biocide and corrosion inhibitors from natural product like seaweeds. Therefore the present study has been carried out with these objectives.

MATERIALS AND METHODS

This study was conducted from July 2004 February 2005. Live and healthy samples *Turbinaria*, *Jania* and *Gelidiella* were collected from the Kurusadai Island in the gulf of Manner Mandapam. Seaweeds were thoroughly washed with seawater to remove all epiphytes, shells etc. and allowed to dry in the shadow place for about 3-4 days. The dried samples were than brought, to the laboratory, thoroughly washed with distilled water to remove the surface salts and dries on blotting paper to remove the excess moisture before preparation of the extracts all the samples were ground in to a fine powder prior to solvent extraction.

Preparation of experimental extract: Each 20 g of seaweed powder of Turbinaria, *Jania* and *Gelidiella* species was taken in Whatman thimbles and placed in 250 mL capacity Soxhlet extractor. About 250 mL of distilled ethanol was taken in a flask to which the extractor was fitted and heated thermostatically controlled hot plate (Fig. 1). The extraction was carried out repeatedly until the solvent becomes colorless in the extractor. Then the extract collected in the flask was concentrated to dryness and the content was weight out. Again the content was resuspended in ethanol of known quality for further experimental study.

Characterization and identification of bacterial isolates: The characterization of bacteria up to generic level was done according to the key described in Bergey's manual of determination.

For characterization of bacterial strains a loopful of bacterial culture was inoculated into sterile nutrient broth for THB and K-medium for MNOB. They were incubated overnight at 37°C. The fresh overnight broth culture was subjected to microscopic physiological and biochemical tests for the characterization and identification:

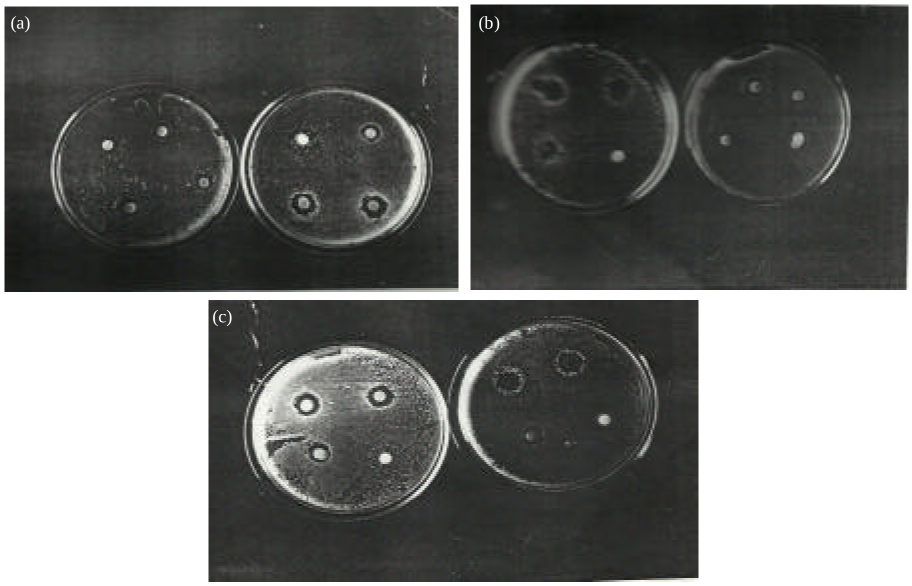

Fig. 1(a-c): Antibacterial activity of (a) *Jania* (b) Seaweed *Gelidiella* and (c) *Turbinaria* sp. seaweed extract on *Klebsiella* and *Micrococcus* sp.

- Different tests were performed as per the key given in Bergey's manual for gram positive cocci, pigment production, catalyses glucose fermentation etc.
- For gram positive spore formers methyl red, Voges-Proskauer hydrolysis of starch case in carbohydrates fermentation etc.
- For gram-positive rods catalyzed oxidize nitrate reduction test etc.

Corrosion inhibition efficiency of seaweed extract in hydrochloric acid medium: Mild steel coupons of the dimensions 50×20 nm were cut from a sheet pickled, rinsed polished and degreased. Test solution of 0.5 N. Hydrochloric acid was prepared using Annular grade HCL and double distilled water. Ethanolic extract of the following seaweed were used as inhibitor substance. *Turbinaria* (2, 4 and 6 mg), *Jania* (1, 2 and 3 mg), *Gelidiella* (3, 6 and 9 mg).

The prepared mild steel coupons were exposed to three different concentrations of seaweed extract in 0.5 N HCL blank was maintained simultaneously. The experiment was terminated after 2 h of exposed period. Weight loss measurements were carried as described earlier. The percentage of Inhibition Efficiency (IE) was calculated using the following equation:

$$\text{IE (\%)} = \frac{W - W_1}{W} \times 100$$

Where, W and W_1 are weight loss without and with inhibitors, respectively.

RESULTS

Effect of seaweed extracts on bacterial population in seawater: Table 1 showed the another seaweed of *Jania* of treated with two different concentration 0.5 and 1 mL of extract showed total

Table 1: Effect of seaweed extracts on bacterial population in seawater

Seaweeds	Extracts concentration (mg)	Bacterial population density (CFU mL^{-1})	Percentage
Control	0.0	170×10^2	TNTC
Turbinaria ornata	1.0	40×10^2	83.5
	2.0	28×10^2	76.5
Jania adhaerens	0.5	68×10^2	60.0
	1.0	49×10^2	71.7
Gelidiella acerosa	1.5	120×10^2	29.5
	3.0	45×10^2	72.6

TNTC: Too numerous to count

Table 2: Effect of three seaweed extract and its antibacterial activity on four different bacterial species by disc method

		Seaweed (mm)					
		Turbinaria (mg)		*Jania* (mg)		*Gelidiella* (mg)	
Bacterial strains	Control	1	2	0.5	1	1.5	3
Bacillus	Nil	3	10	3	1.0	1.5	3
Klebsiella	Nil	2	6	Nil	5.5 m	12.0	15
Micrococcus	Nil	4	9	8	5.0	5.0	9
Pseudomonas	Nil	Nil	3	2	10.0	8.0	13

percentage 66% and respectively. Further more the third experimental seaweed extract also record he following bacterial colony sub 29 (120×10^2 CFU mL^{-1}) 73% (45×10^2 CFU mL^{-1}). From the study depicted when the concentration of the seaweed extract were increased total number of bacterial count also been decreased. While the total three experimental seaweed extract *Turbinaria* showed the highest total bacterial population than the other two seaweed extracts (Table 1).

Antibacterial activity of seaweed extract: In the present study antibacterial activities of three extracts were tested against *Bacillus* sp., *Klebsiella* sp., *Micrococcus* sp. and *Pseudomonas* sp. the results of the antibacterial activity are represented inhibition zone (diameter) in Table 2.

Among the three seaweeds the ethanolic extract of *Gelidiella* sp. showed relatively higher antibacterial activity when tested against *Bacillus* sp. (15 mm). *Klebsiella* (5 mm) and *Micrococcus* sp. (13 mm). However *Pseudomonas* sp. was found to be sensitive. To the extract of *Jania* sp., this produced inhibition zone of 0.5 mm. Generally, it was observed that the diameter of inhibition zone was found to be concentration dependent. It was interesting to observed that among the 4 bacterial strains tested. *Pseudomonas* was found to be relatively resistant to all the three seaweed extracts used in the present study (Fig. 1).

Corrosion inhibition by seaweed extracts on mild steel in seawater: The corrosion rate and percentage inhibition of corrosion by seaweed extraction mild steel are shown in Table 3. In the present study mild steel coupons were exposed to seawater with and without seaweed extract for 7 days. The concentration rate of mild steel exposed to seawater (control) was found to be 0.0355 mdd. While the corrosion rate of the mild steel coupon exposed to seawater containing 4 and 6 mg of *Turbinaria* seaweed extract decreased range about 11.26% (0.0315 mdd) 53.39 (0.0166 mdd) and 90.70% (0.033 mdd), respectively. In the seawater added with *Jania* extract of 1,2 and 3 mg the corrosion of mild steel was inhibited by about 45% (0.0195 mdd) 59.9% (0.0153 mdd) and 69.92% (0.0109 mdd) respectively. When the *Gelidiella* sp. extract was added to seawater as high as 76% inhibition was recorded.

Table 3: Inhibitory effect of seaweed extracts on corrosion of mild steel in seawater

	Extract concentration (mg)	Corrosion rate (mdd)	Inhibition (%)
Control	0	0.0355	-
Turbinaria ornata	2	0.315	11.26
	4	0.0166	53.39
	6	0.0033	90.70
Jania adhaerens	1	0.0195	45.07
	2	0.0153	56.90
	3	0.0109	69.92
Gelidiella acerosa	3	0.011	40.56
	6	0.0181	49.01
	9	0.0085	76.05
Seaweeds			
Control	0	0.0355	-
Turbinaria ornata	2	0.315	11.26
	4	0.0166	53.39
	6	0.0033	90.70
Jania adhaerens	1	0.0195	45.07
	2	0.0153	56.90
	3	0.0109	69.92
Gelidiella acerosa	3	0.011	40.56
	6	0.0181	49.01
	9	0.0085	76.05

Table 4: Corrosion inhibition efficiency of seaweed extract on mild steel in 0.5 N HCl

Seaweeds	Extracts concentration (mg)	Inhibition (%)
Turbinaria ornata	2	15
	4	23
	6	79.49
Jania adhaerens	1	35.47
	2	38.32
	3	78.32
Gelidiella acerosa	3	16.91
	6	64.22
	9	78.81

Corrosion inhibition by seaweed extracts on mild steel in acid medium: The corrosion inhibition efficiency of seaweed extract on mild steel in 0.5 N HCL has been determined by weight loss method and the data are presented in Table 4. The results reveal that an increase in inhibition efficiency with increasing of the extract concentration (inhibitor) is evident in all the cases tested with three various different seaweed extracts.

Almost all the three seaweed extracts used in the present study excreted similar inhibitory effect. The ethanolic extract (6 mg) obtained from *Turbinaria* sp. excreted the highest inhibition efficiency of 79.8% while 78.32 and 78.8% inhibition efficiency were recorded for the mild steel treated with he extract of *Jania* sp. (3 mg) and *Gelidiella* sp. (9 mg). From the result it could be understood that the extract of *Jania* sp. contain highly effective inhibitor compound because its inhibition efficiency of 78.81% is obtained even at lower concentration.

Effect of seaweed extracts on micro fouling: Mild steel coupons were allowed to get fouled by microorganisms in seawater with and without adding of seaweed extract. Subsequently after 7 days

Table 5: Effect of seaweed extracts on Micro fouling on mild steel

Seaweeds	Extracts concentration (mg)	Population density (CFU CM^{-1})	Percentage
Control	0	170×10	-
Turbinaria ornata	2	126×10	25.8
	4	76×10	55
	6	42×10	75
Jania adhaerens	1	94×10	44
	2	73×10	57
	3	49×10	71
Gelidiella acerosa	3	-	-
	6	120×10	29
	9	40×10	76

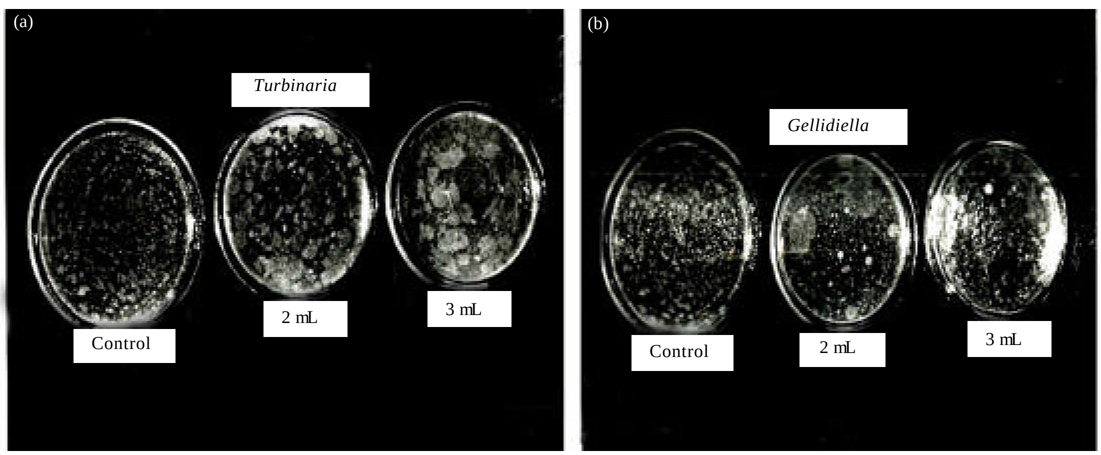

Fig. 2(a-b): Effect of two seaweed extract of (a) *Turbinaria* and (b) *Gelidiella* and its microfouling activity in mild steel

of exposure numerical density of heterotrophic bacteria was enumerated and the values are shown in Table 5. The bacterial population in biofilm was found decreased drastically with seaweed extracts when compared to these values observed in control. The numerical density recorded in con troll system was (70×10^{2} CFU CM^{-1}). In the system treated with *Turbinaria* extract in population were found to vary from 42×10^{2}-12×10^{2} CFU CM^{-2}). Similarly the bacterial population recorded in the treated with *Jania* sp. and *Gelidiella* sp. extract were in the range of 49×10^{2} to 94×10^{2} CFU CM^{-2} and 40×10^{2} to 120×10^{2} CFU CM^{-2}, respectively (Fig. 2).

DISCUSSION

In the present study antibacterial activity of seaweed extracts increased with increasing concentration. Similar observation was made by Brinkhoff *et al.* (2004). Seaweed is in the kingdom Protista and may belong to one of several groups of multicellular algae the red algae green algae and brown algae. Moreover, this observation denoted brown and red seaweed were screened for antibacterial compounds, which existed good results when tested against *Bacillus* sp. This observation extended support to the work of Cacamese *et al.* (1981) and Afaq-Husain *et al.* (1992) who reported that brown and red algae extracts showed higher antibacterial activity against

Bacillus sp. and *E. coil*. Similarly, Rao and Karmarkar (1986) and Rao *et al.* (2005) reported that red, brown algae exerted greater antibacterial activity than green algae.

As observed in the present study reported that the seaweed extract posses inhibitory effect against gram positive bacteria and gram negative bacteria except *Pseudomonas* reported by Brinkhoff *et al.* (2004). The degree of antibiotic property depends upon the suitable solvent used for the extraction. Previously the similar findings also conformed by Kumar and Rengasamy (2000a, b) besides there are several factors such as age of the plants duration of storage, temperature, preparation of the media and pH, which would indirectly, affect the degree of the antibacterial activity. Many of the biocides, commonly used in various experiments are reported to be effective in the control of organisms when they are suspended in aqueous medium i.e., when they are occurring as single cells or as small clumps of cells in the medium when compared to that in biofilm. Previously, Vidyavathi and Sridhar (1991) and Miao and Qain (2005) have been reported Antibacterial activity of six seaweeds extract has been shown to vary with respect to seasons and location. Similarly the extract of seaweed collected during post monsoon season was more active than the other season (Rao *et al.*, 2005; Cacamese *et al.*, 1981) have reported the Antimicrobial and antiviral activities of some marine algae from each algae from each algae. They proved that different fractions obtained from seaweeds exhibited antibacterial activity against gram positive and gram negative test organism Bernard and Pesando (1989) the solvents acetone ethanol have been used to obtain crude from *Sargassum johnstonii* and the efficiency of the solvent is tested using gram positive and gram(-ve) bacteria. The experiment lipid from samples preserved as listed above were extracting divided in to two fractions. These two fractions were tested for the activity against microorganisms. In an experiment made by De-Campos-Takaki *et al.* (1988) different concentration of the fractions isolated, from seaweeds were tested for their antibacterial activity. Seaweeds can also be classified by uses as well as them efficiency towards the natural system (as food, medicine, fertilizer, industrial, etc.) depicted by Rao and Parekh (1981).

Furthermore, Cacamese *et al.* (1981) have reported lipid extracts of more than twenty algae from eastern Sicily were tested for antimicrobial activity against Tobacco Mosaic Virus. The term of bioactive compound' is an expression in common use and includes compounds, which at low concentrations, may be either beneficial or harmful to living organisms reported by Arunkumar *et al.* (2010). Some of them mainly belonging to dictyotales were found to be activity besides the fraction A and B isolated from the selected sp. of seaweeds have been found to be active against various tested microorganisms. Different activities and metabolic profiles were found in *Laurencia obtusa*. Previously, Vidyavathi and Sridhar (1991) have been investigated extracts of 25 Seaweeds from Indian coast have been put through abroad biological screen anti protozoal anti fertility activities and wide range of significant biological activity was obtained in seaweeds the most promising activity been 100% anti fertility.

CONCLUSION

Along with the three experimental seaweeds the ethanolic extract of *Gelidiella* sp., showed relatively elevated antibacterial activity when tested against *Bacillus* sp., (15 mm), *Klebsiella* (5 mm) and *Micrococcus* sp. (13 mm). However *Pseudomonas* sp., was found to be sensitive. Furthermore, it could be understood that the extract of *Jania* sp., contains a highly effective inhibitor compound obtained even at lower concentration because its inhibition efficiency of 78.81%. Subsequently, the bacterial population in biofilm was found drastically decreased compared with observed in control. From this study clearly shows an enhanced important source of natural antibacterial, anti fouling as well as anticorrosion activity present in the experimental seaweeds.

ACKNOWLEDGMENT

Authors were deeply gratitude to the College Correspondent Rev. Fr. Premkumar, MSW, Malankara Catholic College, Mariagiri for his strongly encouragement and his kind support for the preparation of this manuscript.

REFERENCES

Afaq-Husain, S., M. Shameel and R. Khan, 1992. Phycochemical investigations on four species of *Hypnea* (Gigartinales: Rhodophyta). Bot. Mar., 35: 141-146.

Arunkumar, K., S.R. Sivakumar and R. Rengasamy, 2010. Review on bioactive potential in seaweeds (Marine Macroalgae): A special emphasis on bioactivity of seaweeds against plant pthogens. Asian J. Plant Sci., 9: 227-240.

Bernard, P. and D. Pesando, 1989. Antibacterial and Antifungal Activity of Extracts from the Rhizomes of the Mediterranean *Seagrass posidonia oceanica*. Bot. Mar., 32: 85-88.

Bianco, E.M., R. Rogers, V.L. Teixeira and R.C. Pereira, 2008. Antifoulant diterpenes produced by the brown seaweed *Canistrocarpus cervicornis*. J. Applied Phycol., 21: 341-346.

Brinkhoff, T., G. Bach, T. Heidorn, L. Liang, A. Schlingloff and M. Simon, 2004. Antibiotic production by a *Roseobacter* clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. Applied Environ. Microbiol., 70: 2560-2565.

Cacamese, S., R. Azzolina, G. Furnari, M. Cormali and S. Grasso, 1981. Antimicrobial and antiviral activities of some marine algae from Eastern Sicily. Bot. Mar., 24: 365-367.

De-Campos-Takaki, G.M., M.B.S. Diu, M.L. Koening and E.C. Pereira, 1988. Screening of marine algae from Brazilian northeastern coast for antimicrobial activity. Bot. Mar., 31: 375-377.

Erwan, P., H. Claire, D. Eric, V.G. Benoit and S.P. Valerie, 2008. Anti-microfouling activities in extracts of two invasive algae; *Grateloupla turuturu* and *Sargassum muticum*. Bot. Mar., 3: 202-208.

Fakoya, K.A., F.G. Owodeinde, S.L. Akintola, M.A. Adewolu, M.A. Abass and P.E. Ndimele, 2011. An exposition on potential seaweed resources for exploitation, culture and utilization in West Africa: A case study of Nigeria. J. Fish. Aquat. Sci., 6: 37-47.

Kumar, K.A. and R. Rengasamy, 2000a. Antibacterial activities of seaweed extracts/fractions obtained through a TLC profile against the phytopathogenic bacteria *Xanthomonas oryzae* pv. *Oryzae*. Bot. Mar., 43: 417-421.

Kumar, K.A. and R. Rengasamy, 2000b. Evaluation of antibacterial potential of seaweeds occurring along the coast of Tamil Nadu, India against the plant pathogenic bacterium *Xanthomonas oryzae* pv. *Oryzae* (Ishiyama) Dye. Botanica Marina, 43: 409-415.

Miao, L. and P.Y. Qain, 2005. Antagonistic antimicrobial activity of marine fungi and bacterial isolated from marine biofilm and seawater of Hong Kong. Aquat. Microb. Ecol., 38: 231-238.

Rao, D., J.S. Webb and S. Kjelleberg, 2005. Competitive interactions in mixed-species biofilms containing the marine bacterium *Pseudoalteromonas tunicate*. Applied Environ Microbiol., 71: 1729-1736.

Rao, P.P.S. and S.M. Karmarkar, 1986. Antibacterial Substances from Brown Algae II. Efficiency of Solvents in the Evaluation of Antibacterial Substances from *Sargassum johnstonii* Setchell et Gardner. Bot. Mar., 29: 503-508.

Rao, P.S. and K.S. Parekh, 1981. Antibacterial activity of Indian seaweed extracts. Bot. Marina, 24: 577-582.

Shah, M.M.R., M.Y. Hossain, M. Begum, Z.F. Ahmed and J. Ohtomi *et al*., 2008. Seasonal variations of phytoplanktonic community structure and production in relation to environmental factors of the Southwest Coastal waters of Bangladesh. J. Fish. Aquatic Sci., 3: 102-113.

Tiller, A.K. and G.H. Booth, 1982. Polarization studies of mild steel in cultures of sulphate-reducing bacteria. Trans. Faraday Soc., 58: 110-115.

Vidyavathi, N. and K.R. Sridhar, 1991. Seasonal and geographical variations in the antimicrobial activity of seaweeds from the Mangalore coast of Indian. Bot. Mar., 34: 279-284.

Wefky, S. and M. Ghobrial, 2008. Studies on the bioactivity of different solvents extracts of selected marine macroalgae against fish pathogens. Res. J. Microbiol., 3: 673-682.