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ANTIFOULING POTENTIAL OF SELECTED MACROALGAE FROM THE GULF OF MANNAR, INDIA

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Abstract: Marine macroalgae are mostly free from fouling organisms in the marine environment. They defend themselves against fouling directly through the production of antifouling compounds, or indirectly through regulating the attachment of micro and macrofoulers. In this study, the methanol extracts of 43 species of macroalgae, collected from the Gulf of Mannar and Palk Bay regions of India, were evaluated for antifouling activity against the epiphytic diatoms (*Navicula subinflata*, *Nitzschia palea*) as well as cyprid larvae of *Balanus amphitrite*. It was found that extracts of 23 species of macroalgae exhibited antidiatom activity and 16 extracts inhibited settlement property of barnacle larvae. The algae, *Asparagopsis taxiformis* showed the highest inhibitory effect on both the test diatoms with inhibition of 11.5±1.11mm and 14.25±2.38mm zones respectively. Meanwhile, *Liagora erecta* and *Acanthophora spicifera* exhibited highest activity against the diatom, *Navicula subinflata*. The assay using cyprid larvae revealed that 16 algal extracts showed antisettlement activity and the highest was recorded by *Liagora erecta* (84.80±1.33% inhibition), with an IC_{50} of 77.45µg ml^{-1}. The observations in the present study revealed that ten species of macroalgae have both micro and macrofouling property. The Principal Component Analyses (PCA) was also employed to assess the antifouling activity of algae.

Key Words: antifouling, microfouling, macrofouling, antidiatom, antibarnacle, antisettlement

INTRODUCTION

Both living and non-living submerged surfaces in marine environment are facing the process of undesirable accumulation of microorganisms and subsequently by the macroorganisms. This natural phenomenon is termed as biofouling. It is one of the more serious problems currently facing worldwide maritime domains. It has many adverse effects on mankind, especially creating roughness in ship's hull, reducing efficiency and speed, and it also causes detrimental effect to aquaculture operations. In the efforts to avoid marine fouling, antifouling paints are used which are highly toxic and contains unselective agents. Biocide-based antifouling paints containing compounds such as Irgarol 1051, diuron, Sea-Nine 211, chlorothalonil, dichlofluanid, tributyltin and zinc pyrithione are the most frequently used booster biocides worldwide, and some of these have also been found to accumulate in coastal waters at levels that are deleterious for marine organisms (Omae 2003; Konstantinou and Albanis 2004; Bellas 2006; Thomas and Brooks 2010). The International Maritime Organisation (IMO) called for a stepwise reduction of the use of organotin and other compounds by 2003 and complete prohibition by 2008. New antifouling strategies, involving chemical, physical and mechanical mechanisms, are therefore needed to replace these toxic agents (Bers and Wahl 2004).

Most of the marine sessile organisms including macroalgae are rarely fouled than the other species in the same habitat, which is indicating their defence mechanisms against fouling. This antifouling mechanisms are due to the presence of their secondary metabolites, and it has been proven that macroalgae produce secondary metabolites with antibacterial, antifungal, antialgal, and antimacrofouling properties (Padmakumar and Ayyakkannu, 1997; Hellio *et al.,* 2001; 2002; Fusetani 2004; Tsoukatou *et al.,* 2007; Cassano *et al.,* 2008; Culioli *et al.,* 2008; Mokrini *et al.,* 2008 and Plouguerne *et al.*, 2010). Screening for antifouling effect of these secondary metabolites on both micro and macrofouling process may leads to the production of new nontoxic and eco-friendly antifouling compounds.

The chemical defence of marine macroalgae against a microfouler, the epiphytic diatom has previously been studied (Hellio *et al.*, 2002; 2004; Amsler *et al.,* 2000; Lam *et al.,* 2008, Plouguerne *et al.,* 2010). However such studies on antifouling properties of algae from Indian waters are rather limited. Macroalgae were also reported with anti-macrofouling properties, especially against mussel (Cho *et al.,* 2001) and barnacle (Dobretsov *et al.,* 1999; Nylund and Pavia 2003) and a combined study of antifouling strategies of Indian macroalgae against both the micro and macro foulers are scanty and hence an attempt was made to explore the same. In our continuing search for nontoxic marine derived antifouling compounds, we have carried out the screening for antifouling property of 43 species of macroalgae collected from 7 different coastal areas of Gulf of Mannar and Palk Bay region, India, against two species of epiphytic diatoms, *Navicula subinflata* and *Nitzschia palea* and cyprid larvae of *Balanus amphitrite*. Thus an effort was made to study both anti micro and macro fouling properties of macroalgae.

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MATERIAL AND METHODS

Collection sites

Different species of marine macroalgae were collected by hand picking and snorkeling from the intertidal and sub tidal areas of different sampling stations like Mandapam (9° 17' N : 79° 8' E), Krusadai Island (9 ° 15' N : 79 ° 12' E), Pamban (9° 15' N : 79° 12' E), Rameswaram (9° 17' N : 79° 8' E), Keelakarai (9° 15' N : 79° 12' E), Pudumadam (9° 15' N : 79° 12' E) and Hare Island (9° 11' N : 79° 04' E), Gulf of Mannar region, India.

Extraction of macroalgae

After collection, the algae were rinsed with fresh water to remove extraneous matters. The cleaned samples were air dried in the shade and subsequently at 40°C for 24h in the oven. The samples were powdered and extraction was carried out with known quantity methanol by soaking the known weight of material at room temperature. The extracts thus obtained were filtered and concentrated by evaporating to dryness under reduced pressure at a temperature of 40±°C using Rotary Vacuum Evaporator (Buchi). The dried extracts were kept frozen at -20 ° C until further use.

Isolation of fouling diatoms

Different species of fouling microalgae (diatoms) (*Navicula subinflata*, and *Nitzschia palea*) were chosen as test organisms. They were isolated from submerged rock and surface of macroalgae, from Vizhinjam (8° 22' N: 76° 59' E) coast. The rocks and macroalgae were first washed with seawater to remove loosely associated organisms. The surfaces of objects were gently rubbed to release attached microalgae into the seawater. The algal suspension was spread plated onto the soft agar supplemented with f/2 seawater medium and incubated at room temperature under fluorescent light for 7 days. The microalgal colonies thus developed were repeatedly purified by surface spreading on the f/2 seawater agar medium.

Antidiatom assay

The agar well diffusion assay was used to screen crude methanol (MeOH) extracts of macroalgae for antidiatom activity. Agar plates were prepared with 20 ml of f/2 medium with 1.5% agar-agar. A suspension 0.2 ml of specific diatom taken from the logarithmic growth phase was aseptically transferred on to the centre of the agar plate and then spread evenly over the entire surface with a sterile glass spreader. After drying of the agar plate, a well of 7 mm diameter was cut in the middle of the agar plate. The crude MeOH extract was diluted in MeOH: H_2O (1:1) and various concentrations i.e., 100 µg and 250 µg were prepared. An aliquot of the extract at appropriate concentration was transferred into the agar well and incubated. Inhibitory activity could be seen as a clear zone around the wells after 5-7 days of incubation of the agar plates at room temperature and under illumination. The size of the inhibition zone was measured in millimeters from the edge of the well to the edge of the zone of growth. A negative control was conducted using sterile seawater and MeOH alone for each experiment. As positive control of tributyl tinoxide (TBTO) used in antifouling paints was also run simultaneously. All the experiments were repeated in four times and the standard error (SE) was calculated for each concentration.

Antisettlement assay

The test samples (extracts of marine organisms) were dissolved in methanol (MeOH) and aliquots of the solution at appropriate concentration was applied to wells of polystyrene tissue culture plates (6x4 wells, each well 3ml) and air dried. Negative control (filtered sea water) and positive control (TBTO) were maintained. To each well 2 ml of filtered seawater and five 1 day-old cyprids were added. Cyprids were collected from laboratory reared brood stock of adult barnacles, *Amphibalanus amphitrite* which was maintained in natural seawater using methods modified from Rittschof *et al.,* (1984), as described in detail by Head *et al.,* (2003). The plates were kept in the dark for 24 h to 48 h at 25°C, and the numbers of larvae that attached and metamorphosed, died, and live were counted under a microscope. The assay was repeated four times with two different concentrations (100 µg and 250 µg) and the IC_{50} value of all the species were calculated by using probit scale analysis method.

Statistical analysis

Data presented are as mean values (± SE) of treatments. Principal Component Analyses (PCA) was employed to assess the antidiatom and antisettlement activity of active species as per Boyacioglu (2008).

RESULTS

Antidiatom activity of macro algae

Out of the 43 species of marine macroalgae tested against two fouling diatoms i.e., *Navicula subinflata* and *Nitzschia palea*, 23 species (53.48%) exhibited antidiatom activity (Table 1) Only 8 species (18.60%) i.e., *Hypnea valentiae*, *Cheilosporum spectabile*, *Boergesenia forbesii, Liagora erecta*, *Laurencia obtusa*, *Acanthophora spicifera*, *Asparagopsis taxiformis* and *Bryopsis plumosa* were found to be active against both *N. subinflata* and *N. palea*. Among this, *A. taxiformis* showed highest inhibitory effect (11.5±1.11mm and 14.25±2.38mm), on both the test diatoms. *L. erecta* and *A. spicifera* exhibited comparatively higher activity against the diatom, *N. subinflata.*

Table 1: Antidiatom activity of macroalgal extracts against two species of diatoms. Data are in mean ± SE of inhibition zone in 'mm', where n=4; TR = inhibition zone >1mm.

DIVISION	SPECIES	Diatoms			
		Navicula subinflata		*Nitzschia palea*	
		100 µg /well	250 µg / well	100 µg /well	250 µg / well
CYANOPHYTA	*Lyngbya majuscula*	TR	0.75±0.43	0.0	0.0
	Acanthophora spicifera	4.75±1.90	7.50±0.50	2.75±0.82	5.25±0.44
	Amphiroa fragilissima	0.0	TR	0.0	0.0
	Asparagopsis taxiformis	7.75±1.63	11.5±1.11	10.25±0.43	14.25±2.38
	Centroceras clavulatum	0.0	0.0	0.0	0.0
	Champia parvula	0.0	0.0	0.0	2.12±0.54
	Cheilosporum spectabile	2.37±0.41	4.37±0.41	1.00±0.00	1.37±0.41
RHODOPHYTA	*Portieria hornemannii*	TR	4.00±0.70	0.0	0.0
	Gracilaria edulis	0.0	0.0	0.0	0.0
	Gracilaria sjoestedtii	0.0	TR	0.0	0.0
	Hypnea musciformis	TR	4.00±0.70	0.0	0.0
	Hypnea valentiae	2.75±0.43	5.00±0.00	0.0	TR
	Laurencia obtusa	5.50±0.35	11.62±0.41	3.00±0.00	5.00±0.41
	Liagora erecta	5.00±0.00	11.50±1.11	2.00±0.00	3.0±0.00
	Gelidiella acerosa	0.0	0.0	0.0	0.0
	Gracilaria corticata	0.0	0.0	0.0	0.0
	Laurencia papillosa	0.0	0.0	0.0	0.0
	Dictyopteris delicatula	0.0	0.0	0.0	0.0
	Dictyota bartayresiana	10.75±1.11	21.75±1.08	0.0	0.0
	Dictyota dichotoma	0.0	0.0	0.0	0.0
	Hydroclathrus clathratus	0.0	3.00±1.22	0.0	0.0
	Padina gymnospora	0.0	0.0	0.0	0.0
	Sargassum ilicifolium	0.0	0.0	0.0	0.0
	Sargassum longifolium	0.0	0.0	0.0	0.0
	Sargassum tenerrimum	0.0	0.0	0.0	0.0
PHAEOPHYTA	*Sargassum wightii*	0.0	0.0	0.0	0.0
	Spatoglossum asperum	0.0	0.0	0.0	0.0
	Turbinaria conoides	2.50±0.50	4.00±0.00	0.0	0.0
	Turbinaria decurrens	0.0	0.0	0.0	0.0
	Turbinaria ornata	0.0	0.0	0.0	0.0
	Zonaria crenata	TR	5.00±2.00	0.0	0.0
	Boergesenia forbesii	1.00±0.00	3.00±0.00	1.75±0.25	5.00±0.70
	Bryopsis plumosa	2.37±0.41	3.62±0.41	5.00±0.70	9.00±1.87
	Caulerpa cupressoides	0.0	0.0	0.0	0.0
	Caulerpa peltata	0.0	TR	0.0	0.0
	Caulerpa sertularioides	TR	4.00±1.00	0.0	0.0
CHLOROPHYTA	*Cladophora vagabunda*	0.0	0.0	0.0	0.0
	Codium arabicum	0.0	0.0	0.0	0.0
	Enteromorpha intestinalis	0.0	0.0	0.0	0.0
	Halimeda gracilis	0.0	0.0	1.75±0.25	5.00±0.70
	Halimeda macroloba	0.0	0.0	0.0	0.0
	Halymenia floresia	0.0	TR	0.0	0.0
	Ulva reticulata	1.37±0.41	2.75±0.55	0.0	0.0

Fifteen species (34.88%) *viz.*, *Lyngbya majuscula, Hypnea musciformis, Gracilaria sjoestedtii, Amphiroa fragilissima, Champia parvula, Portieria hornemannii, Dictyota bartayresiana, Zonaria crenata, Turbinaria conoides, Hydroclathrus clathratus, Caulerpa peltata, Caulerpa sertularioides, Halymenia floresia, Halimeda gracilis* and *Ulva reticulata*, showed antialgal activity against any one of the fouling microalgae. Out of these fifteen species, *D. bartayresiana* showed the highest inhibitory activity (21.75±1.08 mm) against *N. subinflata* alone. Among the two species of fouling diatoms, *N. subinflata* was more sensitive than *N. palea,* as most of the algal extracts inhibited the growth of *N. subinflata*. Among the four class of marine algae screened, the extracts of Rhodophyceae were comparatively more effective in inhibiting the growth of fouling diatoms.

Principal Component analysis (PCA) of data on antidiatom activity yielded a total of 4 principal components (PCs). Out of the 4 PCs, only the first 2 PCs had the Eigen value above one. This indicates that all the data could be represented by these two PCs. The first two PCs together explained the 98.30 % (PC1 = 67.62 %; PC2 = 30.68 %) of variability of the data. The fig.1 illustrates the scores plot of these PCs, which denoted the association of the various macroalgal species with respect to antidiatom activity. It was observed that all species forms two major groups. The activity of *H. musciformis*, *C. sertularioides*, *Z. crenata*, *U. reticulata* and *H. clathratus* are closely associated with

each other. *T. conoides* and *H. valentiae* were associated with each other and this cluster is nearer to the *H. valentiae* and *H. musciformis* group. The activity of *C. parvula*, *L. majuscula*, *G. sjoestedtii* and *C. peltata* were closely associated and positively correlated with each other. Antidiatom activity of *H. gracilis* was positively and somewhat distinctly correlated with the *C. parvula*, *L. majuscula*, *G. sjoestedtii* and *C. peltata*. Among all the tested species, *D. bartayresiana* and *A. taxiformis* were highly distinct from all other. The negative scores of the *L. obtusa*, *L. erecta* and *D. bartayresiana* indicated the good inhibition effect on the *N. subinflata* and *N. palea* at studied concentrations. The *L. obtusa* and *L. erecta* shown activity against both the diatom whereas *D. bartayresiana* had a very good inhibition activity on *N subinflata,* which made this species as an outlier as well as away from the *L. obtusa* and *L. erecta* group. *A. taxiformis* was observed distinct and on outlier from all other because of it's higher activity against both *N. subinflata* and *N. palea*. Similarly ~~the~~ *B. plumosa* and *B. forbesii* extracts also showed higher inhibitory activity on *N. palea* than *N. subinflata* and hence these two species are correlated with at higher distinct.

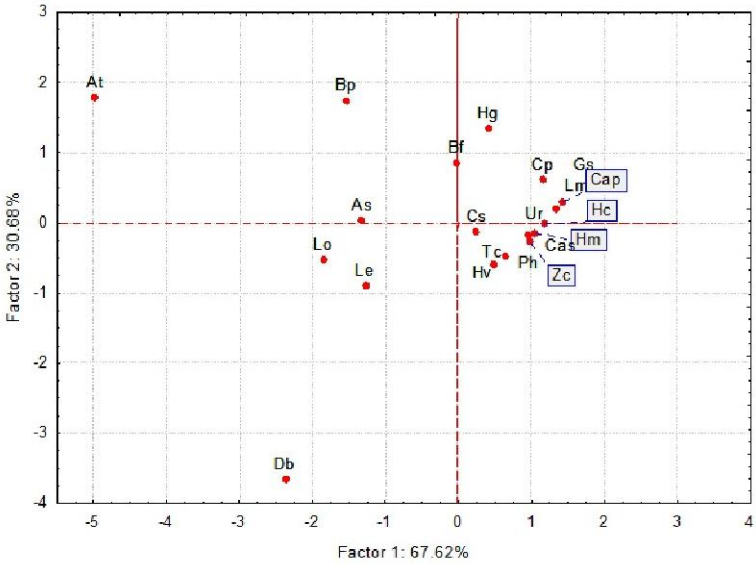

Figure 1: The scores plot of PCs (principal components) of antidiatom activity of macroalgal species obtained by PCA (Principal Compenent Analysis), where species are represented as Hm- *Hypnea musciformis*, Cas- *Caulerpa sertularioides*, Zc- *Zonaria crenata*, Ur- *Ulva reticulata*, Hc- *Hydroclathrus clathratus*, Tc- *Turbinaria conoides*, Hv- *Hypnea valentiae*, Cp- *Champia parvula*, Lm- *Lyngbya majuscula*, Gs- *Gracilaria sjoestedtii*, Cap- *Caulerpa peltata*, Hg- *Halimeda gracilis*, Db- *Dictyota bartayresiana*, At- *Asparagopsis taxiformis*, Lo- *Laurencia obtusa*, Le- *Liagora erecta*, Db- *Dictyota bartayresiana*, Bp- *Bryopsis plumosa* and Bf- *Boergesenia forbesii*.

Antisettlement property of macroalgae

Sixteen species of macroalgae showed antimacrofouling properties (Fig. 2) against cyprid larvae of *Amphibalanus amphitrite*. The highest antisettlement activity was exhibited by the red alga *L. erecta* (84.80±1.33%) at 250 µg/ml concentration. But at 100µg/ml concentration both *B. plumosa* and *L. erecta* showed 52% inhibition. *B. plumosa* and *L. majuscula* exhibited about 70% inhibition on the attachment of barnacle cyprids at 250 µg/ml concentration. Three species, *Z. crenata* (63.4±1.8%), *P. hornemanii* (63.4±2.2%), and *D. dichotoma* (60.0±1.1%) had comparatively better inhibitory effect. Further, the following species, *A. taxiformis*, *C. cupressoides*, *C. clavulatum*, *C. arabicum*, *H. gracilis*, *H. macroloba*, *H. floresia*, *L. obtusa*, *S. wightii* and *S. ilicifolium* showed moderate inhibition on the balanus cyprids. All the remaining 27 species didn't inhibit cyprid settlement. IC_{50} values of all species are represented on Fig.3. *L. erecta* showed the lowest IC_{50} (77. 45 µg ml^{-1}).

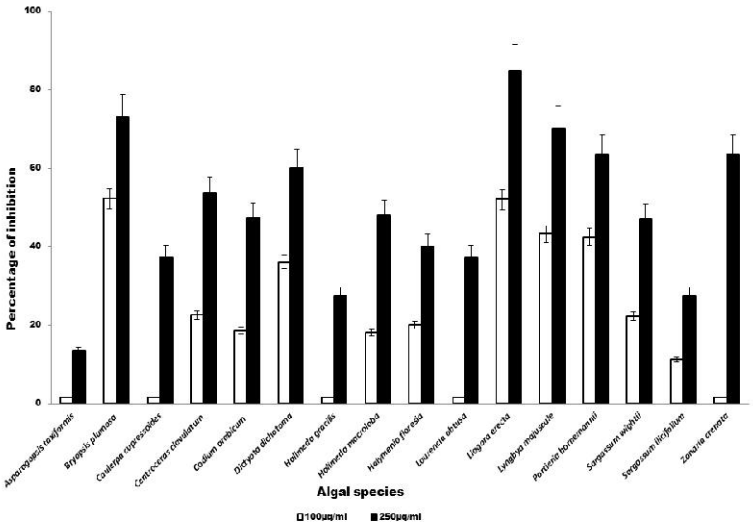

Figure 2: Anti settlement activity of macroalgae against cyprid larvae of *Amphibalanus amphitrite*, data are mean ± SE where n=4.

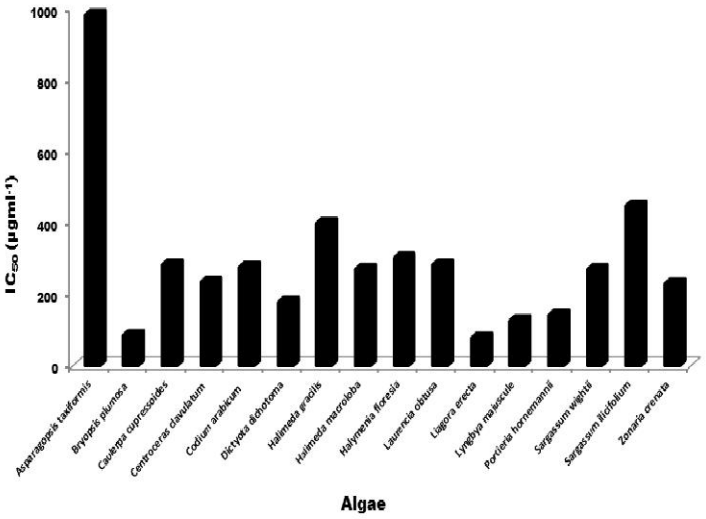

Figure 3: Settlement inhibition (IC_{50}) of algal extracts

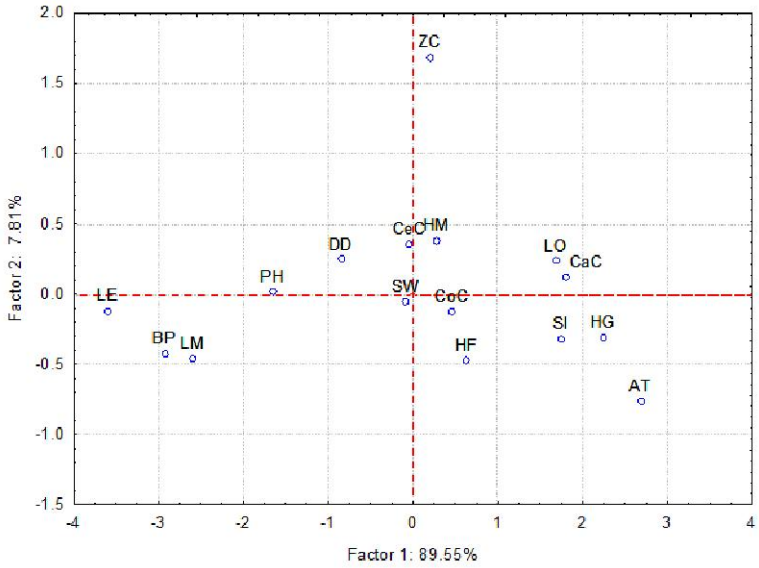

Figure 4: The scores plot of PCs (principal components) of antisettlement activity of macroalgal species obtained by PCA (Principal Compenent analysis) where species representing are, LO- *Laurencia obtusa*, CaC- *Caulerpa cupressoides*, *Codium arabicum* CoC, SW- *Sargassum wightii*, CeC- *Centroceras clavulatum*, HM- *Halimeda macroloba*, SI- *Sargassum ilicifolium*, HG- *Halimeda gracilis*, HF- *Halymenia floresia*, ZC- *Zonaria crenata*, LE- *Liagora erecta*, BP- *Bryopsis plumosa* and LM- *Lyngbya majuscula.*

The PCA of the results on settlement assay (Fig. 4) yielded a total of 4 PCs; in the midst of 4 PCs only 1 PC had the Eigen value above 1. The first PC alone explains the 89.55% of variability of the data. In scores plot observed a lesser similarity between the species. Activity of *L. obtusa* and *C. cupressoides* were formed one group and were positively correlated with each other. With respect to antisettlement activity, these two species were nearer to the activity of *C. arabicum*, *S. wightii*, *C. clavulatum*, *H. macroloba*, *S. ilicifolium*, *H. gracilis* and *H. floresia*. Because of high antissettlement effect, *Z. crenata*, *L. erecta*, *B. plumosa* and *L. majuscula* came as an outlier. *B. plumosa* and *L. majuscula* had shown similar activity and were nearer to the *L. erecta*. Because of higher inhibition at higher concentration *L. erecta* was slightly away from the *B. plumosa* and *L. majuscula*. *Z. crenata* showed higher inhibition only at higher concentration that is the reason why it is away from the *B. plumosa, L. majuscula* and *L. erecta,* and which made it distant from other highly active species.

DISCUSSION

Macroalgae produce a wide range of secondary metabolites (Da Gama *et al.*, 2002; Aguila-Ramierz *et al.*, 2012; Kumar *et al.*, 2012) and it could be used for developing new antifouling agents (De Nys and Steinberg 2002). The main objective of this study was to evaluate the antifouling activity of different species of seaweeds. The strong antidiatom activity of eight species (18.60%) of algae *viz.*, *Hypnea valentiae*, *Cheilosporum spectabile*, *Boergesenia forbesii*, *Liagora erecta*, *Laurencia obtusa*, *Acanthophora spicifera*, *Asparagopsis taxiformis* and *Bryopsis plumosa* on both the test diatoms, *Navicula subinflata* and *Nitzschia palea*, indicates that these algae produce antifouling (antidiatom) compounds and which may not have species specific inhibitory effect on fouling diatoms. The highest inhibitory effect of *A. taxiformis* on both the test diatoms indicated the antimicroalgal compounds of these algae have better inhibitory action. Few studies have addressed the capacity of macroalgae to deter diatom attachment. There were no previous reports on antidiatom activity for these algae, except *L. obtusa.* This species was reported with strong antifouling activity, including its antialgal property (Da Gama *et al.*, 2002). Studies on *L. johnstonii* have shown their antifouling activity against microalgae (Aguila-Ramierz *et al.*, 2012) and some brominated sesquiterpenes, halogenated sesquiterpenes, halogenated acetogenins and bromoalenes were also isolated from *Laurencia* species (Vairappan *et al.*, 2008)

The inhibitory effect of fifteen species (34.88%) viz., *Lyngbya majuscula, Hypnea musciformis, Gracilaria sjoestedtii, Amphiroa fragilissima, Champia parvula, Portieria hornemannii, Dictyota bartayresiana, Zonaria crenata, Turbinaria conoides, Hydroclathrus clathratus, Caulerpa peltata, Caulerpa sertularioides, Halymenia floresia, Halimeda gracilis* and *Ulva reticulata* had against single species of fouling diatom, indicated the species specific inhibition. There are no previous reports on antidiatom activity of these 15 algal species. The higher inhibitory effect of *D. bartayresiana* against *N. subinflata* was supported with the previous findings.

Dictyota sp. are rich source of bioactive secondary metabolites known to deter feeding by herbivores such as fish, urchins, amphipods and crabs (Hay and Steinberg 1992; Aguila-Ramierz *et al.,* 2012). Orhan *et al.,* (2003) reported that the ethanol extract of *D. dichotoma* had most potent antialgal activity against both the microalgae, *Nitzschia* sp. and *Chlorella* sp. The Brazilian *D. menstrualis* have the diterpenes, pachydictyol A and (6R)-6-hidroxydichotoma-3, 14-diene-1, 17-dial as the two major secondary metabolites (Pereira *et al.,* 2000). Diterpene alcohols of *D. menstrualis* have antimacrofouling activity (Schmitt et al., 1995). Faulkner (1984; 1986; 1987; 1988) reported that *Dictyota* species such as *D. divaricata, D. furcellata, D. indica* and *D. lineris* are chemically defended from reef herbivores. All these investigations support the present finding on *Dictyota* sp.

Antimicroalgal activity of *Ulva lactuca* (Hellio *et al.,* 2002) and of *Ulva* sp. (Lam *et al.,* 2008) were previously reported. But there is no report on antidiatom compounds from this species, while benzene, diethyether and petroleum ether extracts from *U. reticulata* were found to exhibit antimicrobial activity. The antimicroalgal effect of the extracts of *U. reticulata* observed in the present study may be due to the presence of such antimicrofouling compounds. It has been reported that the epiphytic bacterial community of the *U. lactuca* regulate fouling activity of the pinnate diatom, *Cylindrotheca fusiformis* (Kumar *et al.,* 2012).

Biological activities like antibacterial and antifungal activities varied with the species belonging to different divisions of algae (Rao and Parekh 1981); Padmakumar and Ayyakkannu (1997) as well as Zheng (2001) reported that the species belonging to Rhodophyta showed the highest antimicrobial activity, whereas Caccamese and Azzolina (1979) and Pesando and Caram (1984) found that the highest antimicrobial activity was exhibited by the species representing Phaeophyta. The reason for this was not explained by these workers but it was suggested that more species have to be screened before coming to a definite conclusion. In the present study, the species belonging to Rhodophyta (46.15%) showed the strongest activity against both the test diatoms, which agrees with the findings of Rao and Parekh (1981), Padmakumar and Ayyakkannu (1997), Zheng *et al.,* (2001) and Salvador *et al.,* (2007).

The results of antimacrofouling effect of sixteen species (37.20%) out of the forty three species of macroalgae suggest that all these sixteen species may produce antifouling compounds, which chemically deter settlement of barnacle cyprids. The inhibition shows dose dependent activity. The red alga *L. erecta* showed highest antisettlement effect at 250 µg ml^{-1} concentration, followed by the green alga *B. plumosa*. These two species exhibited highest inhibitory activity with IC_{50} values 77.45µg ml^{-1} and 84.92 µg ml^{-1} respectively. There is no previous report on antibarnacle activity of *L. erecta*. The antifouling activity of *L. majuscula* has been reported in earlier studies. A study on the recruitment of coral larvae in Guam observed that the presence of *L. majuscula* significantly reduced the larval survival and recruitment (Kuffner and Paul 2004). Similar studies showed that larvae of *Porites asteroids* avoided settling near *L. polychroa* and *L. confervoides* on recruitment tiles and *L. majuscula* negatively impacted survival and recruitment of larvae of octocoral (Kuffner *et al.,* 2006). Lyngbyatoxin A is an indole alkaloid that was first isolated from *L. majuscula* (Cardellina *et al.,* 1979). It is inferred that the inhibitory activity observed in *L. erecta*, *B. plumosa* and *L. majuscula* may be due to the presence of antifouling compounds in these algae.

Dictyota spp. was studied widely for antisettlement effect on different invertebrate larvae (Da Gama *et al.,* 2006). Schmitt *et al.,* (1995; 1998) reported that *D. menstrualis*, which contains dictyols, reduced the settlement and normal development of a common fouling bryozoan species, *Bugula neritina*. Raveendran and Limna Mol, (2009) reviewed on the antibarnacle activity of the compounds dictyol E, pachydictyol A and dictyodial isolated from *D. menstrualis* and diterpene from *D. pfaffii*. *Zonaria crenata* inhibited the barnacle settlement with the IC_{50} of 229.13 µg ml^{-1}. The *Z. toumefortii* was earlier reported to inhibit byssal attachment of the mussel *Perna perna* by Da Gama *et al.,* (2006). These previous findings support the antifouling activity of *D. menstrualis* and *Z. crenata* observed. The remaining ten species, *Asparagopsis taxiformis*, *Caulerpa cupressoides, Centroceras clavulatum, Codium arabicum, Halimeda gracilis, Halimeda macroloba, Halymenia floresia, Laurencia obtusa, Sargassum wightii* and *Sargassum ilicifolium* showed moderate anticyprid effect. The antifouling effect of *A. taxiformis* against common fouling sanil, *Limnea truncatula* (80% foot repellency at 150mg l^{-1}) was reported by Manilal *et al.,* (2010). Yang *et al.,* (2007) reported a moderate antisettlement activity of *Centroceras clavulatum* against the pediveliger larvae of the mussel *Mytilus* sp. The antimussel (*Perna perna*) activity of *C. cupressoides*, *Halimeda* sp, *Sargassum* sp., *Codium* spp. and *L. obtusa* were also reported (Da Gama *et al.,* 2002; 2003; 2006). Steinberg *et al.,* (1998) suggested that the compounds, palisol and palisadin A isolated from *L. obtusa* caused antibarnacle activity. Antibarnacle (Hellio *et al.,* 2004) and antimussel activity (Da Gama *et al.,* 2006) of *Sargassum* spp. shows the presence of antifouling compounds in these brown algae. The present finding and the reports available

confirms the presence of antifouling, especially macrofouling compounds in marine algae. The remaining 20 species of algae didn't show any antifouling activity but exists free of any fouling organisms in the natural environment, which may be due to the physical sloughing of surface layers (Johnson and Mann 1986; Keats *et al.,* 1997). Thus even though relatively few investigations have focused on antifouling effect of algal metabolites, the results of present study suggest that production of antisettlement substances may be common among seaweeds.

CONCLUSIONS

Overall, the present investigation demonstrates that most of the marine macroalgal species are enriched with bioactive compounds which plays a crucial role to prevent biofouling. Further investigations on potent species can lead to the development of better eco-friendly antifouling compounds.

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