Pharmacogn J. 2017; 9(6): 815-820
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com | www.journalonweb.com/pj | www.phcog.net

Original Article

Bioactive Propensity of Macroalgae from the Andaman & Nicobar Islands

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ABSTRACT

Aims: Marine macroalgae are the rich source of biologically active metabolites and potential source for development of novel biotechnological products. The present study was made to explore the metabolically active compounds from the macroalgae of the Andaman & Nicobar Islands. **Methods and Material:** Different solvents such as methanol (MeOH), ethyl acetate (EtoAc), butanol (BuOH) and aqueous (H_2O) extracts of nine macroalgae were tested for antimicrobial, antibiofilm and cytotoxicity (brine shrimp larvae). **Results:** Out of the 36 extracts 27 extracts showed antimicrobial activity against the human pathogens and 14 extracts revealed antibiofilm activities. The three EtoAc extracts of *Sargassum ilicifolium,* MeOH *extract of Sargassum* sp. and MeOH extract of *Padina tetrastromatica* showed inhibition against 8 pathogenic bacteria. Also, aqueous extract of *Padina tetrastromatica* (71.82 %) and BuOH extract of *Dictyosphaeria cavernosa* (71.58 %) exhibited higher antibiofilm nature. The highest cytotoxic effect was exhibited by species *Actinotrichia fragilis* and all its four extracts significantly ($P<0.01$) inhibited the brine shrimp larvae, among this aqueous extract showed the lowest LC_{50} value, 31.7 µg/ml, followed by EtoAc extract, 89.33 µg/ml. **Conclusion:** It was observed that different species have different kind of bioactive nature.

Key words: Bioactivity, Antimicrobial, Antifouling, Antibiofilm, Cytotoxic Effect, Marine Macro Algae.

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History
- Submission Date: 28-06-2017;
- Review completed: 28-07-2017;
- Accepted Date: 18-08-2017

DOI : 10.5530/pj.2017.6.127

Article Available online
http://www.phcogj.com/v9/i6

INTRODUCTION

Marine organisms inhabit in a variety of ecosystems where the ecological processes provide support to sustain the life with adaptive interactions. Studies on ecological interactions of marine organisms and their products have taken momentum in recent years with the growing recognition of their importance due to biomedical potential to promote human welfare. Research and utilization of marine algae also increased markedly in recent years. Totally about 8,950 species (6000 Rhodophyceae, 1750 Phaeophyceae and 1200 Chlorophyceae) of marine algal species are hitherto recorded from the seas and oceans. Out of which only 221 species are utilized commercially. These include 145 species for food and 110 species for phycocolloid production. They form one of the major living renewable resources of the ocean.[1] Approximately 841 species of marine algae are found in both the intertidal and deepwater regions of Indian Coast.[2] Marine algae have been reported to possess a wide array of bioactive properties[3-11] and many species have been isolated with antibacterial, antiviral, antifungal, cytotoxic and antioxidant properties. More than 2400 marine natural products have been reported from the subtropical and tropical populations of seaweeds.[12-13] Biologically active compounds in algal extract are polysaccharides, proteins, pigments, polyphenols etc.[14] Sulfated polysaccharides inhibit activity of many bacterial species as well as viruses.[15] Laminarin, a polysaccharides found in brown algae can act as a prebiotic and it has also antiviral and antibacterial properties.[16] Many macroalgae were widely studied for their antibacterial activity against pathogenic bacteria[6,17-21] and still the investigations are going on. A number of studies have demonstrated the cytotoxic activity of macroalgal secondary metabolites from different geographical region.[22-26] The brine shrimp cytotoxic assay is very useful for the isolation of biogenic compounds from plant extracts[27] and it is considered as a convenient probe for preliminary assessment of toxicity. Antifouling nature of macroalgae has also been studied widely and reports suggest that most of the marine macroalgae are potential source of antifouling compounds.[5,8,28] Hence, the present study was aimed to screen the bioactive potentials including antimicrobial, antibiofilm and cytotoxic activity of macroalgae from the Andaman and Nicobar Islands.

MATERIALS AND METHODS

Collection of algae and extract preparation

The marine macroalgae belonging to Chlorophyceae (*Caulerpa scalpeliformis*, *Dictyosphaeria cavernosa* and *Codium* sp.), Phaeophyceae

Cite this article: Deepa S, Venkateshwaran P, Vinithkumar NV, Kirubagaran R. Bioactive Propensity of Macroalgae from the Andaman & Nicobar Islands. Pharmacog J. 2017;9(6):815-20.

(*Sargassum ilicifolium*, *Sargassum* sp. and *Padina tetrastromatica)* and Rhodophyceae (*Amphiro anceps*, *Actinotrichia fragilis* and *Gracilaria* sp.) were collected from Wandoor (11°35′44.56″N; 92°36′26.63″E), North Bay (11°42′30.80″N; 92°44′44.42″E), Science centre (11°39′15.76″N; 92°45′25.96″E) Carbyn's cove (11°38′28.65″N; 92°44′48.93″E) and Shastri Nagar (6°48′8.65″N; 93°53′20.69″E) of the Andaman and Nicobar Islands. The collected algae were cleaned with fresh water to remove the epiphytes and other extraneous matters and shade dried. The dried algae were ground to fine powder using a blender and mixed with solvents such as methanol (MeOH), ethyl acetate (EtoAc), butanol (BuOH) and water (H_2O) and kept for 48 hours at room temperature. The solvent mixture was filtered with Whatman No.1 filter paper and concentrated in a rotary vacuum evaporator (Buchi, Germany) at 40°C under reduced pressure. The residual extract was lyophilised and preserved at 4°C until further analysis.

Antibacterial assay

The antibacterial assay was performed by the disc diffusion method against 12 human pathogens viz., *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424), *Proteus mirabilis* (MTCC 1429), *Vibrio cholerae* (MTCC 3905), *Escherichia coli* (MTCC 443), *Shigella flexneri* (MTCC 1457), *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 2616), *Streptococcus pneumoniae* (MTCC 655), *Enterococcus faecalis* (MTCC 439), *Klebsiella pneumoniae* (MTCC 3384) and *Salmonella typhi* (MTCC 3216). Briefly, What man No. 1 filter paper disc (5 mm Ø) was impregnated with 200 µg/disc of each extract and placed on Muller Hinton Agar plates (Hi-media, India) pre inoculated with the above human pathogens. The inoculated plates (triplicate) with extract loaded disc were incubated for 24 hrs at 37° C and the discs soaked with respective solvents used as negative control while kanamycin and streptomycin were as positive control under static condition. The assay was carried out in triplicate. The zone of inhibition which appeared around the disc after the completion of incubation period were measured in millimeter and recorded.

Anti-biofilm assay

The anti-biofilm assay was carried out as described by the modified methods of O'Toole and Kolter (1998).[29] Briefly, 2 µL of aliquots (5 mg/1mL) of extract were coated to each well of a 96-well microtiter plate and 200 µL *Vibrio azureus* culture (18 hrs old) added to each well. Along with the test extracts respective solvent and marine broth were also added in separate wells as control. The microtiter plates with test samples were incubated at 28±2°C for 24 hrs. After the incubation, the plates were washed with sterile water to remove planktonic *Vibrio* cells and the wells were stained with 200 µL of 0.1% crystal violet for 20 min. Finally, 200 µL of ethanol was added to the well to release the stain and the extent of biofilm staining was determined by measuring the absorbance of the resulting solution at 590 nm in a multimode micro plate reader (TECAN M200, Austria). All the assay were carried out in triplicate.

Brine shrimp cytotoxicity assay

The brine shrimp cytotoxic assay was performed using freshly hatched free swimming nauplii of *Artemia salina*. The assay system was prepared with 2 ml of filtered seawater containing specific concentration of extracts (100 to 600 µg) in a cavity block (embryo cup) with 20 nauplii. The experiment was carried out with test and control (solvent and filtered sea water) for 24 hrs, under constant illumination. The percentage of mortality was recorded after the incubation period and LC_{50} of the test extract was determined using probit scale method.[30]

Statistical analysis

A one –way ANOVA test was performed on the cytotoxic activity results to investigate significant differences among the effect of extracts.

RESULTS

The four different solvent extracts prepared individually from nine different macro algae belonging to three classes (Chlorophyceae, Phaeophyceae and Rhodophyceae) showed various degree of activity against human pathogens (Table 1). Of these 36 extracts, 27 showed antimicrobial activity against at least one of the human pathogens. Further, 3 extracts i.e. EtoAc extract of *Sargassum ilicifolium,* MeOH extract of *Sargassum* sp. and MeOH extract of *Padina tetrastromatica* showed antimicrobial effect against 8 pathogenic bacteria. The EtoAc and BuOH extract of *Sargassum* sp. and EtoAc of *Padina tetrastromatica* showed inhibition against 7 pathogens (Table 1). Similarly, aqueous extract of *Sargassum* sp. exhibited inhibition activity against 6 pathogens and MeOH extract of *Sargassum ilicifolium* and EtoAc extract of *Actinotrichia fragilis* inhibited 5 pathogens. The BuOH extract of *Sargassum ilicifolium*, BuOH extract of *Padina tetrastromatica,* EtoAc of *Amphiroa anceps* and MeOH extract of *Actinotrichia fragilis* exhibited activity against 4 bacteria. The rest of the active extracts showed activity only against 3 or less than 3 pathogens. Altogether, the highest inhibition zones (5 mm) were exhibited by MeOH extract of *Codium* sp. and EtOAc fraction *Amphiroa anceps* against the pathogen *Shigella flexneri.* However, 9 extracts did not show antimicrobial activity against any of the tested pathogenic bacteria as presented in Table 1.

The antibiofilm activity against *Vibrio azureus* was analyzed and the results showed that out of the 36 extracts, 14 had inhibitory effect (Figure 1). The percentage of biofilm inhibition was calculated with control (OD value of marine broth with specific solvent), the aqueous extract of *Padina tetrastromatica* (71.82 %) and BuOH extract of *Dictyosphaeria cavernosa* (71.58 %) showed higher inhibition. The other 12 extracts, BuOH extract of *Sargassum* sp. was observed with above 50% of inhibition and aqueous extract of *Dictyosphaeria cavernosa* (46.66%), *Codium* sp. (MeOH) (39.07%), *Caulerpa scalpeliformis* (BuOH) (37.42%), *Padina tetrastromatica* (MeOH) (28.83%), aqueous extract of *Amphiroa anceps* (26.41%), *Sargassum ilicifolium* (BuOH) (24.11%), *Codium* sp. (18.49 %) (EtoAc), *Actinotrichia fragilis* (BuOH) (17.01%), *Amphiroa anceps* (EtoAc) (16.89 %), *Amphiroa anceps* (MeOH) (14.92 %) and *Padina tetrastromatica* (BuOH) (10.21%), had inhibitory effect against biofilm formation.

The brine shrimp cytotoxic assay results are represented in figure 2 based on the mortality percentage of *Artemia salina* at different concentrations of extracts. The 50% lethal concentration (LC_{50} value) was calculated and all the species were evaluated by one way ANOVA with different extracts and concentrations. The highest toxic effect was exhibited by all its four extracts of *Actinotrichia fragilis* which was highly significant ($P<0.01$). Among this, aqueous extract showed the lowest LC_{50} value of 31.7 µg/ml followed by the EtoAc extract, (89.33 µg/ml) (Figure 3). Three extracts of *Dictyosphaeria cavernosa* ($P<0.05$) showed LC_{50} values with less than 200 µg/ml (EtoAc extract -138 µg/ml, MeOH -190.5 µg/ml and BuOH -199.5 µg/ml). Two extracts of *Padina tetrastromatica* (MeOH and EtoAc) showed LC_{50} values of 166 µg/ml and 173.8 µg/ml respectively. Similarly, MeOH extract of *Caulerpa scalpeliformis* (LC_{50} values 195 µg/ml), *Gracilaria* sp. (BuOH) (LC_{50} - 128.9 µg/ml), *Codium* sp. (LC_{50} - 129.4 µg/ml) and aqueous extract of *Sargassum* sp. (LC_{50} - 195.9 µg/ml) also exhibited LC_{50} below 200 µg/ml. Among this, the extracts of *Caulerpa scalpeliformis* ($P<0.01$) and *Sargassum* sp. ($P<0.01$) were significantly inhibited. All extracts of *Sargassum ilicifolium* and *Amphiroa anceps* had LC_{50} values with more than 200 µg/ml.

DISCUSSION

The bioactive compounds produced by marine macroalgae are mostly used for its self-defence and these molecules have tremendous probabili-

Table 1: Antibacterial activity of macrolagae from Andaman & Nicobar Islands against human pathogens

Sl. No	Species name	Solvents	Zone of inhibition (mm) in diameter											
			S. a	P. a	P. m	V. c	E. c	S. f	M. l	B. s	S. p	E. f	K. p	S. t
1	*Caulerpa scalpeliformis*	MeOH	0.5	-	-	-	-	3	-	0.5	-	-	-	-
		EtoAc	0.5	-	-	-	-	2.5	-	-	-	-	-	-
		BuOH	-	-	-	-	-	-	-	-	-	-	-	-
		H_2O	-	-	-	-	-	-	-	-	-	-	-	-
2	*Dictyosphaeria cavernosa*	MeOH	-	-	-	-	-	-	-	-	-	-	-	-
		EtoAc	-	-	-	-	-	-	-	-	-	-	-	-
		BuOH	-	-	-	-	-	-	-	-	-	-	1	-
		H_2O	-	-	-	-	-	-	-	-	-	-	1	-
3	*Codium* sp.	MeOH	1	-	-	-	-	5	1	-	-	-	-	-
		EtoAc	1	-	-	-	-	3.5	1	-	-	-	-	-
		BuOH	-	-	-	-	-	3	-	-	-	-	-	-
		H_2O	-	-	-	-	-	1	0.5	-	-	-	-	-
4	*Sargassum ilicifolium*	MeOH	0.5	-	1	-	-	-	-	-	2	2	3	-
		EtoAc	1	-	1	-	3	1	1	-	1	2	2	-
		BuOH	2	-	1.5	-	-	-	-	-	-	2	1	-
		H_2O	-	-	-	-	-	-	-	-	-	-	-	-
5	*Sargassum* sp.	MeOH	2.5	2	2	2	2	2	-	-	2	1	-	-
		EtoAc	1	1	1	1	1	0.5	-	-	1	-	-	-
		BuOH	1	1.5	1	0.5	1	1	-	-	1	-	-	-
		H_2O	0.5	1	0.5	1	1	-	-	-	1	-	-	-
6	*Padina tetrastromatica*	MeOH	-	-	1	-	2	-	1	2	1	0.5	1	1
		EtoAc	-	-	1	-	1	-	2	1	2	-	1	1
		BuOH	-	-	1	-	-	-	1	1	-	-	-	1
		H_2O	-	-	-	-	-	-	-	-	-	-	-	-
7	*Amphiroa anceps*	MeOH	0.5	-	-	-	-	4	-	-	1	-	-	-
		EtoAc	0.5	-	-	-	-	5	-	-	1	-	-	0.5
		BuOH	-	-	-	-	-	2	-	-	0.5	-	-	-
		H_2O	-	-	-	0.5	-	2.5	-	-	-	-	-	0.5
8	*Actinotrichia fragilis*	MeOH	-	-	-	-	2	-	-	-	2	4	2	-
		EtoAc	-	1	2	-	1	-	-	-	-	2	1	-
		BuOH	-	-	-	-		-	-	-	-	-	-	-
		H_2O	-	-	-	-	0.5	-	-	-	-	-	1	-
9	*Gracilaria* sp.	MeOH	-	-	-	-	-	1	0.5	-	-	-	-	-
		EtoAc	1	-	-	-	-	1	-	-	-	-	-	-
		BuOH	-	-	-	-	-	-	-	-	-	-	-	-
		H_2O	-	-	-	-	-	-	-	-	-	-	-	-

ties for usage in pharmaceutical industry. The present study disclosed the presence of potential bioactive compounds in the screened macroalgae and most of the species have at least one of the bioactive effects, such as antimicrobial, antibiofilm and cytotoxic effect.

The four solvent extracts of all the nine macroalgae displayed different degree of antimicrobial activities against different pathogenic bacteria. Three extracts viz. EtoAc extract of *Sargassum ilicifolium,* MeOH extract of *Sargassum* sp., and MeOH extract of *Padina tetrastromatica* inhibited the eight pathogenic bacteria. The inhibitory effect of the methanol extract of *Padina tetrastromatica* also reported previously[31] and pointed out that the methanol extract (10 µl from 20 mg/1 ml) of *P. tetrastromatica* with the same concentration of the present study inhibited the growth of six human pathogens such as *K. pneumonia, E. aerogens, M. luteus, B. subtilis, S. aureus and P. aeruginosa,* while reports on ethanol extract (1 ml of aliquots) of *P. tetrastromatica* showed antibacterial activity against *B. subtilis*, *E.coli, K. pneumoniae*, *S. typhimuruim S. aureus* and *P. vulgaris*.[32]

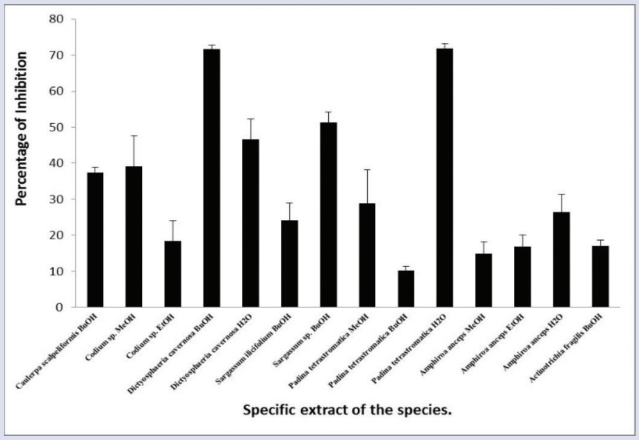

Figure1: Percentage of antibiofilm activity of active extracts of macroalgae. Data are mean (n = 3) ± SD and percentage of inhibition calculated with the OD values of broth and specific solvent controls.

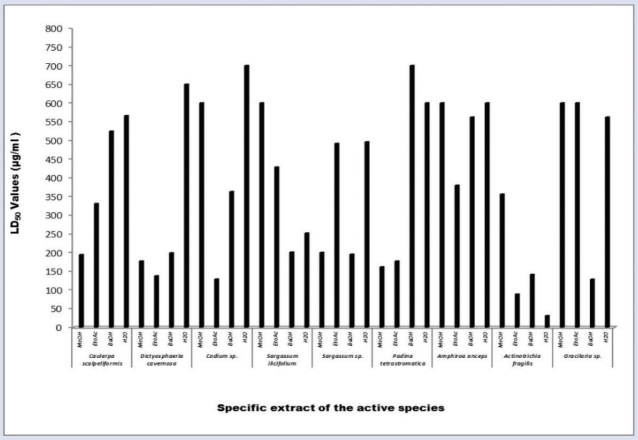

Figure 3: LC_{50} values of different solvent extracts of macroalgae, MeOH-methanol extract, EtoAc- ethyl acetate extract, BuOH- Butanol extract and H_2O – aqueous extract of macroalgae on freshly hatched nauplii of *Artemia salina*.

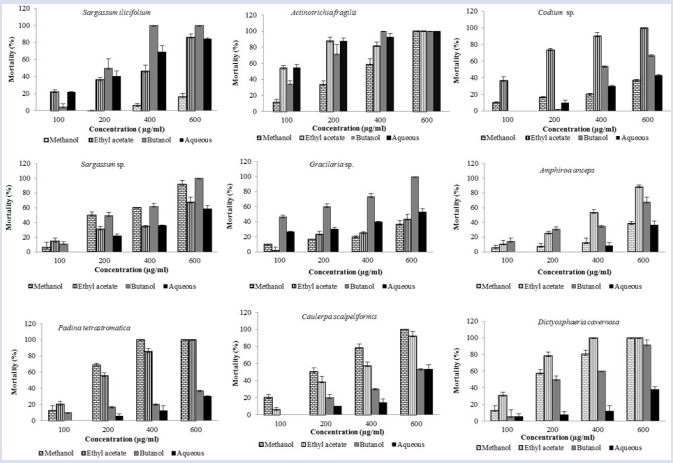

Figure 2: Percentage (Mean ± SE) mortality of brine shrimp larvae against macroalgal extracts after 24 hrs exposure at different concentrations. *Sargassum ilicifolium* , *Actinotrichia fragilis* (**P<0.01), *Codium* sp., *Sargassum* sp. (**P<0.01), *Gracilaria* sp., *Amphiroa anceps* (*P<0.05), *Padina tetrastromatica*, *Caulerpa scalpeliformis* (**P<0.01), *Dictyosphaeria cavernosa* (*P<0.05){* less significant & ** highly significant}.

The antibacterial activities of *Sargassum* species are widely studied and most of the reports are supporting the present study (200 *µg* /disc). Rizvi[33] reported its inhibitory effects of *Sargassum ilicifolium* (200 *µg)* against *Shigella dysenteriae*, while Rebecca *et al*[34] examined 50 µl to 100 µl of 5 gm of powdered sample of *Sargassum ilicifolium* against human pathogen like *Escherichia coli, Salmonella* sp. and *Klebsiella* sp. Antibacterial activity of *S.dentifolium and S.hystrix*[35] against human pathogens and *S. latifolium*,[36] against shrimp pathogens were studied at 50 mg/ml concentration. Devi *et al*.[37] and Seenivasan *et al*.[38] were screened antibacterial effect of *S. wightii* against human pathogens using 75 and 20 µl of 5 mg crude extracts respectively.

Biofilm formation (microfouling) is the initial step of biofouling followed by the attachment of macrofoulers such as spores of macroalgae and larvae of invertebrates. Marine natural products or extracts with antifouling activities have been isolated from a wide number of seaweeds.[7,39-42] Microfouling by bacteria can be prevented by two methods, one by bactericidal activity of natural or synthetic products or by inhibiting the biofilm formation. The bioassay study in the present work revealed that the tested fouling bacteria (*Vibrio azureus*) is highly sensitive to aqueous extract of *P. tetrastromatica* and BuOH extract of *D. cavernosa* (above 70% inhibition) and BuOH extract of *Sargassum* sp (above 50%). So it is evident that these algae possess better antibiofilm metabolites. The antibiofilm nature of *Ulva reticulata, Sargassum wightii* and *Halimeda macroloba* has also been reported.[43] Recently, Yuvaraj and Arul[44] reported the antibiofilm activity of ten macroalgal extracts among this *Sargassum wightii* and *Hypnea musiformis* were prominent in inhibiting biofilm.

Many of the secondary metabolites of macroalgae are well known for their cytotoxic activity. The brine shrimp cytotoxicity test has been used as a bioassay for a variety of toxic substances[45] and it is extrapolated for cell-line toxicity and anti-tumour activity as well. The cytotoxic property of plant material is due to the presence of anti-tumour compounds.[46] In the present study, the higher cytotoxic profile of all solvent extracts of *Actinotrichia fragilis* and other macro algae *Dictyosphaeria cavernosa* and *Padina tetrastromatica* indicated the presence of high amount of cytotoxic substances. The previous inventions also reported on high cytotoxic profiles of macroalgae like *Stoechospermum marginatum, S. swartzii, S. binderi, Spatoglossum asperum, Stokeyia indica* and *Caulerpara racemosa*,[46] *Acrosiphonia orientali, P. tetrastromatica* and *G. corticata*[25] *L. brandenii*[26] and *H. musiformis* and *U. fasciata*.[23] The *Caulerpa* sp. is well known for its cytotoxic effect because of compounds like caulerpin and caulerpenyne in *Caulerpa cupressoides,*[47] *Caulerpa racemosa*[48] and *Caulerpa taxifolia*.[49] Fischel *et al*.[50] showed that caulerpenyne caused early and marked shift into S phase followed by a blockage in the G_2/M phase in the cell cycle. The comparatively high inhibitory activity shown by the extracts of *Caulerpa peltata* in this study might be due to the presence of the cytotoxic compounds caulerpenyne or caulerpin and further evaluation is required to confirm this.

The extraction process of bioactive compounds were varied by researchers and the most used solvents are acetone, benzene, butanol,[51-52] ethanol,[43,53] methanol[26] and water.[19] In our study an approach was made to analyse the effectiveness of the four solvents (MeOH, EtoAc, BuOH and H_2O).

MeOH was found to be effective solvent in dissolving the bioactive compounds than other solvents.

CONCLUSION

The present study revealed the bioactivity of algae from Andaman & Nicobar Islands and it showed that few species had antimicrobial activity and some algae have antibiofilm as well as cytotoxic effect. Altogether, bioactivity of nine tested macroalgae, *Sargassum ilicifolium, Sargassum* sp., *Padina tetrastromatica* and *Actinotrichia fragilis* have more potent bioactive compounds. Further studies should be undertaken to characterise the active components residing in the algae and to evaluate the effect of each compounds on microbes can be useful for developing ecologically significant bioactive compounds and are effective in biopharmaceuticals.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided by the Earth System Sciences Organization (ESSO), Ministry of Earth Sciences (MoES) Government of India, to conduct this research. We thank Director, ESSO-National Institute of Ocean Technology (NIOT), MoES, Chennai, India, for his constant encouragement and support to do this work. We are thankful Dr. M. Vijaykumaran, Consultant, ESSO-NIOT, Chennai, for the critical review. We also thank the scientific and supporting staffs of ANCOST, ESSO- NIOT Port Blair, India, for their timely help during this research work.

CONFLICT OF INTEREST

No conflict of interest are declared.

ABBREVIATIONS USED

MeOH: methanol; EtoAc: ethyl acetate; BuOH: butanol; H_2O: aqueous; LC_{50}: 50% lethal concentration; ANOVA: Analysis of variance.

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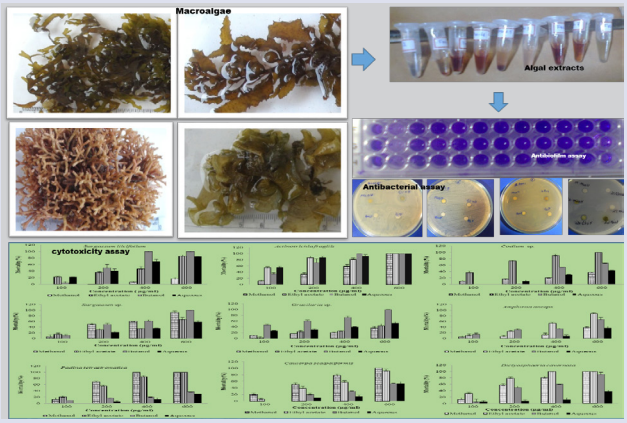
GRAPHICAL ABSTRACT



SUMMARY

- Andaman and Nicobar Islands, are biodiversity hot spot, have diverse marine macroalgal resources and it could be utilized for the isolation of bioactive metabolites. The present study, investigated the bioactive nature of macro algae and it emphasis that further work required to disclose the efficient natural pharmaceutical product.

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Cite this article: Deepa S, Venkateshwaran P, Vinithkumar NV, Kirubagaran R. Bioactive Propensity of Macroalgae from the Andaman & Nicobar Islands. Pharmacog J. 2017;9(6):815-20.