Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.1.20133D254

Evaluation of antibacterial activity of bioactive compounds obtained from the seaweed *Chondrococcus hornemanni* on ichthyopathogenic bacteria affecting marine ornamental fish

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PEER REVIEW

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Comments

This is a good work where the authors evaluated the antibacterial activity against Ichthyo pathogens using seaweed. The results are interesting and show the effective antibacterial activities.
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ABSTRACT

Objective: To investigate antibacterial effects of extracts from the seaweed *Chondrococcus hornemanni* (*C. hornemanni*) on bacterial pathogens of marine ornamental fish.

Method: Methanol extract obtained from *C. hornemanni* showed a broad and high antibacterial activity against four fish pathogens including *Providencia rettgeri*, *Aeromonas hydrophila*, *Vibrio alginoticus* and *Vibrio parahaemolyticus*. The crude extract obtained from the dried seaweeds was fractionated and purified using column chromatography. Purified extracts were analyzed with Fourier transform infrared spectroscopy (FTIR) for identifying the functional groups. Phytoconstituents of the active fraction were further identified by means of gas chromatography and mass spectrometric (GC-MS) analysis.

Result: The first fraction of the extracts showed effective inhibitory activity against *Aeromonas hydrophila* and *Vibrio parahaemolyticus* at a concentration of 100 μL. However, *Vibrio alginolyticus* and *Providencia rettgeri* had shown a moderately lesser inhibitory response to the extract.

Conclusion: Hence, it is concluded that extracts of seaweed *C. hornemanni*, contain potential bioactive compounds with a considerable antibiotic activity.

KEYWORDS

Seaweed, Fish pathogen, Antibacterial activity, FTIR analysis, GC-MS analysis

1. Introduction

The trade in live marine ornamentals including corals, fish and other reef-associated organisms for keeping in marine aquaria is expanding. The overall annual trade in live marine ornamentals has been estimated between US $28 million to US $44 million globally[1,2]. More than 1 400 marine species are traded globally each year[3]. The live aquatic trade is often affected by bacterial diseases causing severe losses in revenue. Considerable mortality in the wild and cultured marine ornamental fish is caused by bacterial pathogens such as *Aeromonas* sp., *Vibrio* sp., *Pseudomonas* sp. and *Enterobacter* sp.[4,5]. Such problems in the farms are usually dealt through prevention of disease outbreaks or failing which by attacking the causative agent with appropriate drugs or chemicals. The use of antimicrobial agents in aquaculture practices has increased in the recent past[6].

Seaweeds are marine algae, which are primitive non-flowering plants without roots, stem or leaves. They produce an assortment of secondary metabolites characterized by a multitude of biological activity. Seaweeds are considered a rich resource of bioactive compounds. Compounds with antiviral, antifungal and antibacterial activity have been

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Foundation Project: Supported by the Centre for Marine Living Resources and Ecology (Ministry of Earth Sciences), Kochi for financial assistance (Grant No. MoES/10MLR/2007).

Article history:
Received 15 May 2013
Received in revised form 20 Jun, 2nd revised form 23 Jun, 3rd revised form 29 Jun 2013
Accepted 20 Jul 2013
Available online 28 Aug 2013

detected in green, brown and red algae[7,8]. These algae contain phenolic compounds, terpenoids, phlorotannins, steroids, amino acids, halogenated alkanes and ketones, cyclic polysulphides, fatty acids and acrylic acid. The antimicrobial activity of seaweeds has been studied by Taskin *et al.* and Rajasulochana *et al*[9,10]. Many products that have been developed using marine algae are being used in numerous fields. Phytocolloids of seaweed origin, such as alginate, carrageen and agar are widely used in medicine[11,12].

In the present study, the antibacterial activity of solvent-extracts obtained from the seaweed *Chondrococcus hornemanni* (*C. hornemanni*) on bacterial fish pathogens of marine ornamental fish was investigated.

2. Materials and methods

2.1. Sample collection and preparation

Healthy specimens of *C. hornemanni* were collected while snorkeling along the Coast of Agatti Island, Lakshadweep, southwest coast of India. The collected samples were washed thoroughly with clean seawater to remove extraneous materials. They were placed in plastic bags with water to prevent evaporation and brought to the laboratory. In the laboratory, the seaweeds were dried under shade until the required weight was achieved. They were then ground to a powder using a pestle and mortar. The powdered sample was then stored for further study.

2.2. Extraction of the crude compounds

About 30 g of dried seaweed powder was placed in 100 mL of methanol and placed in a shaker at 35 °C. The shaker was operated at 120 r/min for 24 h to allow satisfactory extraction of active compounds from the dried seaweed sample. At the end of this period, the extract was filtered using Whatman No. 1 filter paper, fitted to a suction pump. Subsequently the filtrate was centrifuged at 5 000 r/min for 20 min. The supernatant was filtered through a filter of 0.2 mm, millipores and was concentrated on a rotary evaporator under vacuum at a low temperature. The crude extract thus obtained was stored in the refrigerator until required[13].

2.3. Purification of crude extract

The methanol extract of one gram of seaweed was applied in a silica gel column (230-400 mesh), packed with hexane and eluted with a mixture of hexane and chloroform (9:1 to 1:9 and 100% chloroform). In addition, chloroform and methanol (9:1 to 1:9 and 100% methanol) were used as an eluent.

2.4. Test microorganisms

Strains of *Aeromonas hydrophila* (*A. hydrophila*), *Vibrio alginoticus* (*V. alginoticus*), *Vibrio parahaemolyticus* (*V. parahaemolyticus*) and *Providencia rettgeri* (*P. rettgeri*) were obtained from the marine ornamental fish hatchery of the Centre of Advanced Study in Marine Biology, Annamalai University, Tamil Nadu, India. These were inoculated in Muller-Hinton broth and incubated at 30 °C for 24 h. The culture broths obtained in this manner were used in the present study.

2.5. Agar diffusion method

The agar diffusion test was used to measure the antibacterial effect of the seaweed extracts on the selected bacteria. The selected pathogenic bacteria were cultured in Muller-Hinton broth at 30 °C. Wells of size 6 mm were made on Muller-Hinton agar plates using gel puncture. The plates were inoculated with the bacterial broth and incubated for 24-28 h at 35 °C. Using a micropipette, 100 (mg/mL) of the sample of seaweed extract was poured in to each well. The positive control was maintained with chloramphenicol and the negative control was maintained using only the solvent.

2.6. Fourier transform-infrared spectroscopy (FTIR) analysis

The seaweed samples were analyzed using FTIR spectroscopy. The unutilized balance seaweed samples were encapsulated in KBr at a ratio of 1:100. The IR spectra were collected using a Shimadzu spectrometer within the range of 500-5 000 cm^{-1}.

2.7. GC-MS analysis

A sample of the extracted fraction was subjected to GC-MS (Perkin Elmer) analysis. Phytoconstituents of the sample were analyzed using Perkin Elmer Clarus 500 series gas chromatographic system and capillary column. Elite-5ms (5% phenyl, 95% dimethylpolysiloxane-Column length: 30 m Column id: 250 μm) was used with helium at a 1 mL/min as the carrier gas and the GC oven temperature was programmed at 70 °C @ 6 °C to 150 °C (2 min) @ 6 °C to 290 °C (5 min). The split ratio was adjusted to 1:20 and injection volume was 2 ìl. The injection and detector temperature was 250 °C. The GC-MS electron ionization mode was 70 eV. Mass range was from m/z 45-450 amu.

3. Results

3.1. Screening for antibacterial activity

The seaweed extracts showed a significant antimicrobial activity against all four pathogenic bacteria tested (Table 1). The first fraction of the extracts showed effective inhibitory activity against *A. hydrophila* (20 mm) and *V. parahaemolyticus* (19 mm) at a concentration of 100 mg/mL. However, *V. alginolyticus* (16 mm) and *P. rettgeri* (15 mm) had shown a relatively lower inhibitory response to the extract (Figure 1). The antibacterial

Table 1
GC-MS analysis of active compounds of *C. hornemanni*.

S. No.	Retention time	Name of the compound	Molecular formula	Molecular weight	Peak area %
1	14.11	Butyrophenone	$C_{10}H_{12}O$	148.00	2.5173
2	16.46	Cyclopropane,1-chloro-2,2-dimethyl-3-(3,3-dimethyl-1-butynyl)-	$C_{11}H_{17}Cl$	184.00	0.9787
3	19.59	Adamantane	$C_{10}H_{16}$	136.23	3.5806
4	24.00	9-(methylthio)-	$C_{16}H_{26}S$	250.00	1.3350
5	24.53	5,5,10,10Tetrachlorotricyclo[7.1.0.0(4,6)]decane	$C_{10}H_{12}Cl_4$	272.00	26.6201
6	26.28	Isoamyl acetate,3-methylbut-1-yl ethanoate	$C_7H_{14}O_2$	130.19	11.5688
7	28.25	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one,Camphor	$C_{10}H_{16}O$	152.23	41.8878
8	30.42	Germacrene A	$C_{15}H_{24}$	204.00	1.6711

activity is attributed to the presence of bioactive compounds present in the seaweed *C. hornemanni*.

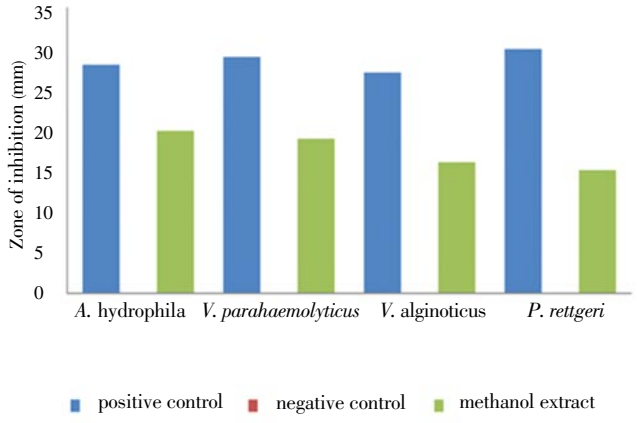

Figure 1. Antibacterial activity shown by purified fractions of *C. hornemanni* against bacterial strains.

3.2. Characterization of functional groups

The methanol extract of the seaweed *C. hornemanni* was subjected to FTIR analysis. The functional groups present in the extracts were determined based on these results. The assimilation bands associated with CH3 and CH2 aliphatic compound formation occur between 2945.30 and 2850.79 cm^{-1}. The region from 2345.44 cm^{-1} represents the phosphines stretch. The CH3 aliphatic compounds and OH carboxylic acids appear at 1440.83 cm^{-1}. The P-O-C in organophosphorus compounds occurs approximately at the range of 1033 cm^{-1}. The bands related to C≡C-H alkynes appear at 661.58 cm^{-1} (Figure 2).

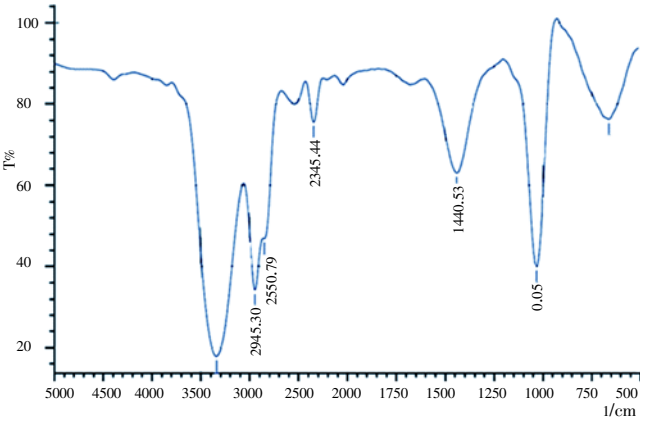

Figure 2. Functional group and class of compounds absorbed in the infrared wave number.

3.3. Characterization of active compounds

Based on the GC-MS results, eight bioactive compounds were identified from the partially purified fractions of the seaweed *C. hornemanni* (Figure 3). The results are given in Table 1. These eight compounds were found to be the active molecules responsible for the inhibition of bacteria on which the bioactivity of the seaweed extract was assessed.

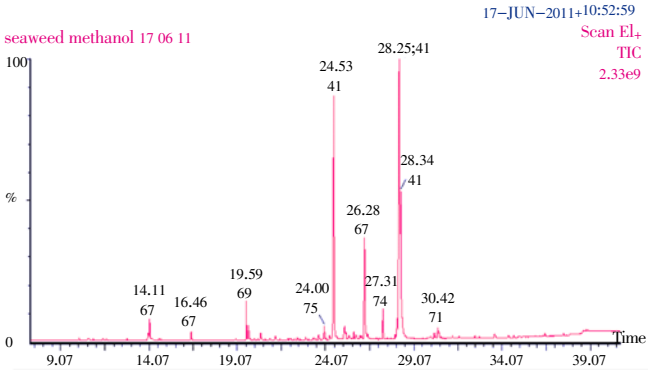

Figure 3. GC-MS chromatogram of the active fraction of *C. hornemanni*.

4. Discussion

Much attention is being paid towards plant extracts and biologically active compounds isolated from marine resources in the present. Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. Most of the secondary metabolites produced by seaweeds have bacteriocidal or the antimicrobial compounds derived from seaweeds consist of diverse groups of bacteriostatic properties terpenols, sterols, polysaccharides, dibutenolides peptides and proteins metabolites. Compounds with antibacterial activity have been detected in green, brown and red algae[14]. Lipid-soluble extracts from marine macroalgae have been investigated for their antibacterial properties. Several different organic solvents have been used to screen algae for antibacterial activity[15]. Similar to the results found in the current study, methanol deemed to be the best solvent for extracting the bioactive compounds.

A high antimicrobial activity of seaweed extracts has been reported against both Gram negative and Gram-positive bacteria[16,17]. Rajasulochana *et al.*[18] have reported that the chloroform extract of *Enteromorpha compressa* and

Chaetomorpha antennina had a moderate bactericidal and fungal activity against *Staphylococcus aureus, Bacillus subtills, bacteria Escherichia coli, Proteus vulgaris* and two fungal species *Aspergillus niger, Candida albicans*. Zbakh *et al.*[19] have investigated the antibacterial activity of extracts from 20 species of algae prepared through methanol extraction against three pathogenic bacteria. Umamaheswari *et al.*[20] observed the antibacterial activity of marine macro alga Chaetomorpha aerea, the maximum antibacterial potential was recorded from the ethanol extract against *Pseudomonas aeruginosa*, and the minimum was noted in methanol extracts against *Micrococcus* sp. and *Salmonella typhii*. In the present investigation, the purified fraction of methanol extracts of *C. hornemanni* has shown a high inhibitory activity against four fish pathogenic bacteria, such as, *Entrobacter* sp., *A. hydrophila, V. alginoticus, V. parahaemolyticus, Proteus proteus, Pseudomonas flurescens, Entrobacter* sp., *Flavobacterium* sp., *Edwarsiella tarda*, and *micrococcus*. Ghanthikumar *et al.*[21] have reported that the antibacterial activity of the extracts from 7 algal species prepared by methanol and toluene extracts against pathogenic bacteria *viz.*, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus vulgaries, Salmonella typhimurium and Klebsiella pneumoniae*. Karthikaidevi *et al.*[22] reported that antibacterial activity of three species of marine macro algae *Codium adherens, Ulva reticulata and Halimeda tuna* from the Gulf of Mannar coast. The maximum activity was noted in the methanol, Ethanol and Acetone extracts against *Klebsiella pneumonia, Staphylococcus aureus, Enterococci* sp., *Pseudomonas aeruginos* and *V. parahaemolyticus*.

Sasidharan *et al.*[23] predicted the C-OH deformation vibration with contribution of O-C-O symmetric stretching vibration of carboxylate group, at range of 1 415, 1 315, 1 090 and 1 033 cm^{-1}. Robic *et al.*[24] studied the infrared spectrum of ulvan showed strong absorbances at about 1 650, 1 250, and 1 070 cm^{-1} and small ones at about 1 400, 850 and 790 cm^{-1}. Carboxylate groups show two bands: an asymmetrical stretching b and near 1 650 cm^{-1} and a weaker symmetric stretching band near 1 400 cm^{-1}, and sulfate esters show a major band at about 1 250 cm^{-1}. In the present study, the absorption bands associated with CH_3 and CH_2 aliphatic compounds, PH phosphines stretch, CH_3 aliphatic compounds and OH carboxylic acids, P-O-C in organophosphorus compounds and C≡C-H alkynes are indicated in the methanol extracts

Manilal *et al.*[25] studied the characterization and compound identification using GC-MS for the active fraction and they reported that the fatty acids exhibited antimicrobial activity against different species of bacterial pathogens from the red algae such as *Laurencia brandenii*. In the present study eight major peaks were observed in GCMS analysis pointing at 1,7,7-Trimethylbicyclo, tetrachlorotricyclo, isoamyl acetate, adamantane, butyrophenone, germacrene A and 9-(methylthio), cyclopropane.

The methanol extracts of *C. hornemanni* showed significant antimicrobial activity against four fish pathogens. This can be attributed to the presence of trimethylbicyclo, tetrachlorotricyclo, isoamyl acetate, adamantane, butyrophenone, germacrene A, 9-(methylthio) and cyclopropane in the extracts. Hence, it is concluded that extracts of seaweed *C. hornemanni*, contain potential bioactive compounds with a considerable antibiotic activity. These compounds can be utilized for the development of natural antibiotics where multi drug-resistant pathogenic bacteria are involved.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are thankful to the authorities of Annamalai University for providing facilities and the Centre for Marine Living Resources and Ecology (Ministry of Earth Sciences), Kochi for financial assistance (Grant No. MoES/10MLR/2007).

Comments

Background

In the present scenario, marine ornamental fish culture is a leading business but in some situation there may be the heavy mortality of fishes will occur due to the disease outbreak by fish pathogens. Hence, the authors screened the methanolic extract of the seaweed *C. hornemanni*against the pathogens which was also isolated from marine ornamental fishes. The results suggested that the various compound present in the seaweed might be responsible for the antibiotic activity against *P. rettgeri*, *A. hydrophila*, *V. alginoticus* and *V. parahaemolyticus*.

Research frontiers

The study was performed to determine the antibiotic action of the seaweed *C. hornemanni* against fist pathogens from marine ornamental fish hatchery. The bioactive compounds such as trimethylbicyclo, tetrachlorotricyclo, isoamyl acetate, adamantane, butyrophenone, germacrene A, 9-(methylthio) and cyclopropane were identified as responsible for the antibacterial activity.

Related reports

Some authors such as Rajasulochana *et al.*, Zbakh *et al.*, Umamaheswari *et al.* and Ghanthikumar *et al.* had reported about the antibacterial activity against the pathogens[18-21]. But, there was no more report regarding the antibacterial activity against ornamental fish Ichthyo pathogenic bacteria, including the Seaweed *Chondrococcus hornemanni*.

Innovations and breakthroughs

The first innovation is the selection of bacterial pathogens, which is very important for the aquaculture due to the rapid development of multiple drug resistant pathogens. The second one is the identification of eight major compounds such as 1,7,7-trimethylbicyclo, tetrachlorotricyclo, isoamyl acetate, adamantane, butyrophenone, germacrene a, 9-(methylthio) and cyclopropane in a single seaweed.

Applications

It may be significant to know about the antibiotic activity of the *C. hornemanni* extract. So, it might be very helpful in ornamental fish aquaculture to grow more fishes without any bacterial infections.

Peer review

This is a good work were the authors evaluated the antibacterial activity against Ichthyo pathogens using seaweed. The results are interesting and show the effective antibacterial activities.

References

[1] Dhayanithi NB, Ajith Kumar TT, Kathiresan K. Effect of neem extract against the bacteria isolated from marine fish. *J Environ Biol* 2010; **31**: 409-412.

[2] Robert S, Pomeroya, Michael D, Pido B, John Francisco A, Pontillas C, et al. Evaluation of policy options for the live reef food fish trade in the province of Palawan, Western Philippines. *Mar Policy* 2008; **32**: 55-65.

[3] Whittington RJ, Chong R. Global trade in ornamental fish from an Australian perspective: The case for revised import risk analysis and management strategies. *Prev Vet Med* 2007; **81**: 92-116.

[4] Dhaneesh KV, Ajith Kumar TT, Vinoth R, Shunmugaraj T. Influence of brooder diet and seasonal temperature on reproductive efficiency of clownfish amphiprion sebae in captivity. *Recent Res Sci Technol* 2011; **3**(2): 95-99.

[5] Kolanjinathan K, Ganesh P, Govindarajan M, Antibacterial Activity of ethanol extracts of seaweeds against fish bacterial pathogen. *Eur Rev Med Pharmacol Sci* 2009; **13**: 173-177.

[6] Ganeshamurthy R, Ajith kumar TT, Dhayanithi NB. Effect of secondary metabolites of the seaweed (*Halimeda micronesia*) at lakshadweep islands against aquatic pathogens. *Int J Pharm Biol Sci* 2012; **3**(2): 213-220.

[7] Vallinayagam K, Arumugam R, Ragupathi Raja Kannan R, Thirumaran G, Anantharaman P. Antibacterial activity of some selected seaweeds from Pudumadam coastal regions. *Global J Pharmacol* 2009; **3**(1): 50-52.

[8] Vijayabaskar P, Shiyamal V. Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar Biosphere Reserve. *Adv Biol Res* 2011; **5**(2): 99-102.

[9] Taskin E, Ozturk M, Taskin E, Kurt. Antibacterial activities of some marine algae from the aegean Sea (Turkey). *Afr J Biotechnol* 2007; **6**(24): 2746-2751.

[10] Gupta S, Rajauria G, Abu-Ghannam N. Study of the microbial diversity and antimicrobial properties of Irish edible brown seaweeds, Inter. *J Food Sci Tech* 2010; **45**: 482-489.

[11] Manilal A, Sujith S, Sabarathnam B, Seghal Kiran G, Selvin J, Shakir C, et al. Antifouling potentials of seaweeds collected from the southwest coast of India. *World J Agric Sci* 2010; **6**(3): 243-248.

[12] Taskin E, Taskin E, Ozturk M. Antibacterial activities of some seaweeds from northern cyprus against some food-related pathogens. *Asian J Biol Sci* 2012; **5**: 250-256.

[13] Manilal A, Sujith S, Selvin J, Shakir C, SeghalKiran G. Antibacterial activity of *Falkenbergia hillebrandii* (Born) from the Indian coast against human pathogens. *Int J Exp Bot* 2009; **78**: 161-166.

[14] Kayalvizhi K, Vasuki S, Anantharaman P, Kathiresan K. Antimicrobial activity of seaweeds from the gulf of mannar. *Int J Pharm Appl* 2012; **3**(2): 306-314.

[15] Jeyanthi Rebecca L, Dhanalakshmi V, Chandra Shekhar. Antibacterial activity of *Sargassum Ilicifolium* and *Kappaphycus alvarezii*. *J Chem Pharma Res* 2012, **4**(1): 700-705.

[16] Prasanna Latha D, Hema Latha KPJ. Antimicrobial activity of the chloroform extracts of the chlorophycean seaweeds *Enteromorpha compressa* and *Chaetomorpha antennina*. *Int Res J Microbiol* 2011; **2**(8): 249-252.

[17] Yuvaraj N, Kanmani P, Satishkumar R, Paari KA, Pattukumar V, Arul V. Extraction, purification and partial characterization of cladophora glomerata against multidrug resistant human pathogen acinetobacter baumannii and fish pathogens. *World J Fish Mar Sci* 2011; **3**(1): 51-57.

[18] Rajasulochana P, Dhamotharan R, Krishnamoorthy P, Murugesan S. Antibacterial activity of the extracts of marine red and brown algae. *J Am Sci* 2009; **5**(3): 20-25.

[19] Zbakh H, Chiheb H, Bouziane H, Motilva V, Sanchez, Riadi H. Antibacterial activity of benthic marine algae extracts from the mediterranean coast of morocco. *J Microbiol Biotechnol Food Sci* 2012; **2**(1): 219-228.

[20] Umamaheswari R, Thirumaran G, Anantharaman P. Antibacterial activity of marine macro alga (*Chaetomorpha aerea*) collected from *Vellar estuary*. *Environ Ecol* 2006; **24**(2): 280-282.

[21] Ghanthikumar S, Vanila D, Masillamani A, Kanthasamy R. Antibacterial activity of some seaweeds. *Appl Biol Biotechnol* 2012; **1**(1): 1-5.

[22] Karthikaidevi G, Manivannan K, Thirumaran G, Anantharaman P, Balasubaramanian T. Antibacterial properties of selected green seaweeds from Vedalai coastal waters; Gulf of Mannar marine biosphere reserve. *Global J Pharmacol* 2009; **3**: 107-112.

[23] Sasidharan S, Darah I, Kassim M. *In vitro* antioxidant properties and FTIR analysis of two seaweeds of Gulf of Mannar. *Asian Pac J Trop Biomed* 2011; **1**(Suppl 1): 66-70.

[24] Robic A, Bertrand D, Sassi JF, Lerat Y, Lahaye M. Determination of the chemical composition of ulvan, a cellnwall polysaccharide from *Ulva* spp. (Ulvales, Chlorophyta) by FT IR and chemometrics. *J Appl Phycol* 2009; **21**: 451-456.

[25] Manilal A, Sujith S, Sabarathnam B, Seghal Kiran G, Selvin J, Shakir C, et al. Biological activity of the red alga *Laurencia brandenii*. *Acta Bot Croat* 2011; **70**: 81-90.