Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016J5-185

Bioactive compounds and antifungal activity of three different seaweed species *Ulva lactuca*, *Sargassum tenerrimum* and *Laurencia obtusa* collected from Okha coast, Western India

Megha Barot, Nirmal Kumar J.I.*, Rita N. Kumar

P.G. Department of Environmental Science and Technology, Institute of Science and Technology for Advanced Studies and Research, Vallabh Vidyanagar-388 120, Gujarat, India

ARTICLE INFO

Article history:
Received 23 Sep 2015
Received in revised form 22 Dec 2015
Accepted 26 Jan 2016
Available online 14 Apr 2016

Keywords:
Antifungal activity
Bioactive compounds
Crude extract
Gas chromatography-mass spectrometer
Seaweeds

ABSTRACT

Objective: To evaluate bioactive compounds responsible for antifungal activity from seaweeds of Okha coast, Western India.
Methods: Each species were extracted with different solvents with increasing polarity: hexane, ethyl acetate, chloroform and methanol using Soxhlet apparatus. The antifungal activity was determined by agar diffusion plate method by using fluconazole, ketoconazole and amphotericin B as standards. Gas chromatography-mass spectrometer analysis was done for identification of bioactive compounds present in crude extract.
Results: The gas chromatography-mass spectrometer analysis of all the extracts revealed the presence of steroids, fatty acids and esters compounds. Among the three species, the maximum crude extract yield (53.46%) and the largest inhibition zone (36 mm) were recorded in methanol extract of *Ulva lactuca*, whereas the minimum crude extract yield and inhibition zone were recorded in chloroform extract of the same species as 0.5% and 10 mm, respectively. Methanol and ethyl acetate extract showed the maximum antifungal activity and the major important compounds like steroids, fatty acids and esters were detected with higher amount in all the extracts.
Conclusions: The present study revealed that the different seaweed extracts showed moderate to significant antifungal activity against the strains tested as compared with the standard fungicides, and polar solvents methanol and ethyl acetate were comparatively efficient for extraction of different metabolites that are responsible for antifungal activity.

1. Introduction

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae[1-3]. Numerous substances were identified as antimicrobial agents from algae: chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds, phenolic inhibitors, *etc*. [4]. Many researchers suggested that the crude extracts of Indian seaweeds are active against Gram-positive bacteria[5]. Decreased efficiency and resistance of pathogen to antibiotics has demanded the development of new alternatives[6]. Steroids and fatty acid esters of *Acanthophora spicifera* were reported to exhibit potent antitumor and antibacterial activity against human cancer cell lines and microorganisms[7]. Algae and their extracts are the source of biologically active compounds. Their beneficial properties for humans, animals and plants were recognized in the past and are appreciated nowadays, in the development of new biotechnological products[8] due to the finding of antibacterial and antifungal activities in many species of marine algae from different parts of the world and the isolation of some active compounds from them[9,10]. Compounds with cytostatic,

*Corresponding author: Nirmal Kumar JI, P.G. Department of Environmental Science and Technology, Institute of Science and Technology for Advanced Studies and Research, Vallabh Vidyanagar-388 120, Gujarat, India.
Tel/Fax: +91 02692 234955
E-mail: nirmalkumar@istar.edu.in
Foundation Project: Supported by University Grant Commission (UGC-F No. 42-415), New Delhi, India.
The journal implements double-blind peer review practiced by specially invited international editorial board members.

antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae[11,12]. The seaweed species *Chaetomorpha* spp., *Cladophora fascicularis, Ulva lactuca* Linnaeus (*U. lactuca*), *Caulerpa racemosa, Caulerpa sertularioides, Valoniopsis pachynema, Sargassum ilicifolium, Sargassum polycystum* and *Porphyra vietnamensis* of Bet Dwarka, Okha coast are rich in minerals, metabolites and pigments[13,14]. Therefore the present study aims to investigate the bioactive compounds possibly responsible for antifungal activity of three different seaweed species *U. lactuca*, *Sargassum tenerrimum* J.G. Agardh (*S. tenerrimum*) and *Laurencia obtusa* Lamouroux (*L. obtusa*) collected from Okha coast, Western India.

2. Materials and methods

2.1. Sample collection and processing

The seaweed species *U. lactuca*, *S. tenerrimum* and *L. obtusa* were collected during November 2014 from Okha situated at 22°28' N and 69°5' E at the mouth of Gulf of Kutch on the north-western part of Saurashtra, Gujarat, Western India. The species were picked with hand and immediately washed with seawater to remove the foreign particles, sand particles and epiphytes. Then they were kept in an ice-box and immediately transported to the laboratory and washed thoroughly with tap water to remove the salts on the surface of the sample. After that, the species were identified according to the methods described by Jha *et al*.[15]. They were spread on blotting paper to remove excess water. The air-dried samples were placed in an oven at 50 ˚C, pulverized in the grinder and sieved through a screen with mesh size of 0.5 mm. Then, the powdered material was kept in airtight plastic bottles at room temperature.

2.2. Preparation of extract

The three seaweed species were evaluated for the important biochemical composition as well as their bioactive properties. The seaweed sample (3.0 g) was extracted in solvents with increasing polarity: hexane, ethyl acetate, chloroform and methanol (1:10; w/v) for 24–48 h, using Soxhlet apparatus as described by Zakaria *et al*.[16]. The resulting extracts were concentrated to dryness under reduced pressure using rotary evaporator (40–50 ˚C). All concentrated extracts were then air-dried in fume hood and stored at 4 ˚C. Analytical grade solvents (Sigma, USA) were used in the present study.

2.3. Antifungal assay

The seaweed extracts were further tested for antifungal activity using the agar diffusion plate method[17]. Each seaweed extract was tested against two fungal species *Aspergillus niger* (*A. niger*) (NCBI accession No. KC545848) and *Penicillium janthinellum* (*P. janthinellum*) (NCBI accession No. KC545842) which have petroleum oil-degrading capacity and isolated from petrol spill site used for antifungal assay[18]. In this assay, 0.1 mL of fungal spore suspension grown for 3 days on 10 mL of nutrient dextrose agar was thoroughly mixed with 25 mL of melted potato dextrose agar (Himedia, India) and was poured into sterilized Petri plates. When the agar solidified, 4 wells of 6 mm diameter were made on each of the seeded plates. These wells were filled with 50, 100 and 200 μL of the test samples and solvent alone in the fourth cup was kept as control. The standard fluconazole (10 μg), ketoconazol (10 μg) and amphotericin B (20 μg) commercial antifungal discs were used for comparison. The Petri plates were incubated at 28 ˚C for 2–4 days and all the culture plates were examined from 24 h onwards.

2.4. Bioactive compound analysis by gas chromatography-mass spectrometer (GC-MS) method

The crude extracts of seaweed samples were subjected to GC-MS (AutoSystem XL GC, PerkinElmer, USA) at Sophisticated Instrumentation Centre for Applied Research and Testing, Vallabh Vidhyanagar, Gujarat. GC-MS analysis was performed using GC apparatus attached to a PE-5MS fused silica capillary 5% phenyl/95% methyl polysiloxane column (30 mm × 50 mm, 0.25 μm film thickness, PerkinElmer, USA). The column temperature was initially 80 ˚C, held for 5 min, then increased at 10 ˚C/min from 80 ˚C to 290 ˚C and detector temperature was set at 250 ˚C. Helium at 1 mL/min was used as the carrier gas. Samples (1 μL) were injected in the split mode (1:40). Mass spectral condition was run in electron impact mode through a PerkinElmer turbo MS as follows: ionization energy 70 eV; scan rate 1.6 scans/s; interscan delayed 0.01 s; source temperature 250 ˚C; mass unit ranged from 30 to 650 *m/z*; solvent delayed 3 min. Mass spectra of the chromatographic peaks were compared by spectra in the Wiley NIST/EPA/NIH Mass Spectral Library 2005[19].

3. Results

The yields of 2.7% followed by 2.5% and 1.9% of hexane extract were recorded in *L. obtusa*, *U. lactuca* and *S. tenerrimum*, respectively. Besides, yields of 0.8%, 3.6% and 4.2% of ethyl acetate extract were found in *S. tenerrimum*, *L. obtusa* and *U. lactuca*. Moreover, the yields of chloroform extracts of *U. lactuca*, *S. tenerrimum* and *L. obtusa* were 0.5%, 2.0% and 2.5%, whereas yields of methanol extract of *S. tenerrimum*, *L. obtusa* and *U. lactuca* were 2.70%, 36.06% and 53.46%, rerspectively. Among all the extracts, the maximum yield was recorded in methanol extract of *U. lactuca* as 53.46% and the minimum yield was noticed in the same species as 0.5% in chloroform extract. Methanol extract showed the maximum yield for all the three species compared to

other solvent extracts. The yield of extract indicated the efficiency of solvent for extraction of different biochemical compounds.

3.1. Antifungal activity

For methanol extract, the maximum antifungal activity was showed by *U. lactuca* (36 mm), followed by *S. tenerrimum* (35 mm) against *A. niger* at 200 µL, but at the same concentration, the minimum zone of inhibition was observed in *P. janthinellum* by *L. obtusa* (17 mm) followed by *U. lactuca* (21 mm) (Table 1). The ethyl acetate extract of *S. tenerrimum* at 200 µL exhibited highest activity (20 mm) against *P. janthinellum* followed by *L. obtusa* (17 mm), while at 200 µL the minimum zones of inhibition 11 mm and 14 mm were measured in *U. lactuca* and *L. obtusa* against *A. niger* (Table 2). Moreover, the chloroform extract of *S. tenerrimum* showed the highest activity (30 mm) against *P. janthinellum* at 200 µL, and the lowest activity at the same concentration was observed in *U. lactuca* (10 mm) against *A. niger* (Table 3). On the other hand, the hexane extract of *U. lactuca* showed the maximum activity (15 mm) against *A. niger* at 200 µL, while at the same concentration, the minimum activity (12 mm) against *P. janthinellum* was showed by *L. obtusa* (Table 4). The inhibition zone was increased with increase in amount of extract. All the extract showed the maximum activity at 200 µL. Chloroform and hexane extracts did not show much more significant antifungal activity.

Table 1
Zone of inhibition by methanol extract of three tested seaweeds. mm.

Extracts	*A. niger*			*P. janthinellum*		
	50 µL	100 µL	200 µL	50 µL	100 µL	200 µL
U. lactuca	15	20	36	13	19	21
S. tenerrimum	19	23	35	13	15	25
L. obtusa	18	20	25	13	14	17

Table 2
Zone of inhibition by ethyl acetate extract of three tested seaweeds. mm.

Extracts	*A. niger*			*P. janthinellum*		
	50 µL	100 µL	200 µL	50 µL	100 µL	200 µL
U. lactuca	NA	10	11	11	13	15
S. tenerrimum	11	12	15	10	17	20
L. obtusa	11	13	14	11	13	17

NA: Not appeared.

Table 3
Zone of inhibition by chloroform extract of three tested seaweeds. mm.

Extracts	*A. niger*			*P. janthinellum*		
	50 µL	100 µL	200 µL	50 µL	100 µL	200 µL
U. lactuca	9	9	10	11	NA	NA
S. tenerrimum	11	NA	12	15	17	30
L. obtusa	10	12	18	10	12	13

NA: Not appeared.

Table 4
Zone of inhibition by hexane extract of three tested seaweeds. mm.

Extracts	*A. niger*			*P. janthinellum*		
	50 µL	100 µL	200 µL	50 µL	100 µL	200 µL
U. lactuca	8	13	15	NA	NA	NA
S. tenerrimum	9	10	13	NA	10	13
L. obtusa	NA	NA	NA	NA	9	12

NA: Not appeared.

The standard fungicides fluconazole, ketoconazole and amphotericin B showed inhibition zone of 10, 17 and 18 mm against *A. niger* and inhibition zone of 12, 20 and 19 mm against *P. janthinellum*, respectively. There was no inhibition zone showed by the control against *A. niger* or *P. janthinellum*. The present investigation revealed that methanol extract was the most active fraction against both of the tested fungi *A. niger* and *P. janthinellum*. Considering the sensitivity of the microorganisms, it was noted that *P. janthinellum* was more sensitive to different extracts than *A. niger*. However, results revealed that all of the tested extracts showed higher antifungal effects at higher concentration than the standards fluconazole, ketoconazole and amphotericin B.

3.2. GC-MS analysis

GC-MS analysis of the crude extracts of three seaweed species was carried out in order to investigate the presence of bioactive compounds, which might be responsible for antifungal potency (Tables 5–8). The results showed that methanol and ethyl acetate extract contained important bioactive compounds mainly steroids, fatty acids and esters of fatty acids. In addition, some high molecular weight hydrocarbons like cyclohexane, 1,2-benzendicarboxylic acid, hexane, dodecane and octane were observed (Tables 5–8).

Table 5
Composition of methanol extract of selected seaweed species.

Species	Compound name	RT (min)	Area (%)	Molecular weight (g/mol)
U. lactuca	Palmitic acid	18.12	48.79	256.42
	Squalene	25.27	2.07	410.71
S. tenerrimum	Myristic acid	15.58	2.77	228.37
	Methyl palmitate	17.73	37.38	270.45
	Stearic acid methyl ester	19.25	3.47	298.50
	Methyl oleate	19.42	19.95	296.48
	Arachidonic acid	20.89	3.85	304.46
	Erucic acid methyl ester	22.84	1.32	352.59
	Arachidonic acid methyl ester	20.84	7.25	318.50
	Stigmasterol	32.94	4.59	412.69
	15-hydroxyprogesterone	36.16	1.63	330.46
L. obtusa	Squalene	25.27	10.52	410.71
	Tetradecanol	16.63	2.36	214.39
	Palmitic acid methyl ester	17.70	8.55	270.45
	DTB	17.78	4.82	206.32
	Palmitic acid	18.10	15.70	256.42
	Beta sitosterol	29.15	5.16	414.70

DTB: 2,6-Di-*tert*-butylphenol (antioxidant and UV stabilizer). RT: Retention time.

The chloroform extract showed only two fatty acids like palmitic and stearic acid with hydrocarbons like dibutyle phthalate, 1,2 benzendicarboxylic acid and squalene (precursor of sterol) (Table 7) while hexane extract contained palmitic acid, stigmasterol and some hydrocarbons (Table 8). 2,6-Di-*tert*-butylphenol (antioxidant and UV stabilizer) and 15-hydroxyprogesterone were detected only from methanol extract of *L. obtusa* and *S. tenerrimum* respectively. Fatty acids like palmitic acid (saturated fatty acid), myristic acid (saturated fatty acid), arachidonic acid (essential polyunsaturated

omega 6 fatty acid), stearic acid (saturated fatty acid), erucic acid (mono unsaturated omega 9 fatty acid), oleic acid and linolenic acids (omega 3 fatty acid) were recorded from GC-MS analysis of different extracts; while different sterols were also detected, such as stigmasterol, 15-hydroxyprogesterone, beta sitosterol and fucosterol. In terms of peak area, palmitic acid was detected as a major compound in all the studied species. Moreover, palmitic acid, methyl oleate, stearic acid methyl ester, stigmasterol, arachidonic acid methyl ester, squalene, beta sitosterol, 2,6-Di-*tert*-butylphenol and tetradecanol were recorded as major compounds in methanol extract in terms of peak area (Table 5).

Table 6
Composition of ethyl acetate extract of selected seaweed species.

Species	Compound name	RT (min)	Area (%)	Molecular weight (g/mol)
U. lactuca	Cyclohexane	18.76	8.79	84.16
	1,2-benzendicarboxylic acid	19.08	1.20	166.13
	Oleic acid methyl ester	21.01	2.40	296.50
	Stearic acid	21.49	0.40	284.47
	Erucic acid	23.47	1.59	338.57
	Squalene	26.84	1.03	410.71
	Stigmasterol	34.74	4.42	412.69
S. tenerrimum	Fucosterol	32.87	12.29	412.69
	Oleic acid	19.81	6.83	282.46
	Stearic acid	19.99	1.36	284.47
	Linolenic acid	21.26	0.73	278.43
	Squalene	25.28	0.83	410.71
L. obtusa	Myristic acid	16.07	2.39	228.37
	Palmitic acid	18.23	36.42	256.42
	Methyloleate	19.40	0.75	310.51
	Oleic acid	19.81	7.49	282.46
	Erucic acid	19.85	2.14	338.57
	Stearic acid	20.01	2.86	284.47
	Beta sitosterol	29.13	1.54	414.70

RT: Retention time.

Table 7
Composition of chloroform extract of selected seaweed species.

Species	Compound name	RT (min)	Area (%)	Molecular weight (g/mol)
U. lactuca	Dibutyle pthalate	18.61	1.45	278.34
	1,2 benzendicarboxylic acid	19.09	5.38	166.13
S. tenerrimum	Palmitic acid	19.61	1.62	256.42
	Stearic acid	21.48	0.36	284.47
L. obtusa	Palmitic acid	19.61	1.18	256.42
	Stearic acid	21.50	0.46	284.47
	Squalene	26.84	0.51	410.71

RT: Retention time.

Table 8
Composition of hexane extract of selected seaweed species.

Species	Compound name	RT (min)	Area (%)	Molecular weight (g/mol)
U. lactuca	Hexane	24.29	0.50	168.00
	Octane	26.66	8.36	242.00
	Cyclohexylamine	36.82	6.94	141.00
S. tenerrimum	Palmitic acid	19.61	0.82	256.42
	Stigmasterol	34.30	8.57	412.69
L. obtusa	Dodecane	40.09	4.71	226.00
	Cyclohexylamine	36.78	6.44	141.00

RT: Retention time.

4. Discussion

Plant substances continue to serve as important source of drugs for the world population and several plant-based drugs are in clinical use. The metabolic and physiological capacity of marine organisms allows them to survive in complex habitat types and provides a great potential for production of secondary metabolites which are not found in terrestrial environments. Thus, marine algae are among the richest sources of known and novel bioactive compounds[20].

In the present study, the highest extraction yield was recorded for the methanol extract of *U. lactuca* (53.46%); whereas the lowest yield was also recorded in chloroform extract of the same species (0.5%). As compared to the results of the present study, Wang *et al.*[21] showed considerable variations in extraction yield among different seaweed species such as green, red and brown algae. From the studied seaweed species, the extraction yields of methanol extracts were higher than those of other solvent extracts with decrease in polarity which indicated that most of the soluble components in seaweeds were high in polarity. Herrero *et al.*[22] also reported that the polarity of solvents significantly affects the extract yield of *Spirulina* microalgae wherein the highest yield was recorded in ethanol extract as compared with other higher and lower polarity solvents extracts. The application of serial exhaustive extractions involves successive extraction with solvents of increasing polarity from non polar to a more polar solvent in order to ensure that a wide polarity range of compounds could be extracted. Green[23] has successfully applied terrestrial plants, and it is worth investigating the case of seaweed extracts. Rajauria *et al.*[24] noticed that extraction by using 60% aqueous methanol for *Himanthalia elongata* produced a much higher yield (6.8%) as compared to extraction by 100% methanol (1.2%).

The results from the present research revealed that the strongest antifungal activity was exhibited by the methanol extract and the least by the chloroform and hexane extract. Stronger antifungal activity was found in methanol extract of *U. lactuca* compared to other extracts suggesting that a particular solvent is required for extracting antimicrobial substances within the seaweeds. Manilal *et al.*[25] and Rangaiah *et al.*[26] reported that methanol extract exhibited higher antimicrobial activity than *n*-hexane and ethyl acetate extracts, which is in close to our results. This variation in the results may be due to difference in species used, time and place of sample collection. It is worthy to mention that using solvents such as chloroform and hexane, inhibitory activity was not observed clearly in the same species. This result could be related to the presence of bioactive metabolites in this species of algae, which are not soluble in one solvent but might be soluble in the other one. Karthikaidevi *et al.*[27] obtained the similar results and suggested

that a particular solvent is required to extract some antimicrobial substances within the algae, and therefore the inhibitory activity will increase when several solvents are used in the extraction.

The antifungal effects of extracts were comparable to the standard antifungal agent: fluconazole, ketoconazole and amphotericin B, and were found to be active against both the fungal strain tested. In the present investigation, it was observed that the maximum inhibition zone was found in polar solvent extracts like methanol, ethyl acetate and chloroform extracts. Thinakaran and Sivakumar[28] recorded that fungal mycelial growth was strongly inhibited by methanol and ethyl acetate extracts, which is in agreement with the present investigation. Salem *et al*.[29] also reported that ethyl acetate was the best solvent for isolation of antimicrobial compounds from the tested marine algae followed by methanol. The steroids, fatty acids, esters of fatty acids and other hydrocarbons were recorded in polar solvents like methanol and ethyl acetate rather than less polar solvent like hexane. This indicates that polarity of the solvent is associated with extraction of important compounds that are responsible for antifungal activity. Previous reports on the most effective solvent for the extraction of antimicrobials have various results. González del Val *et al*.[30] selected methanol as solvent for extraction of antimicrobial compounds for red, green and brown seaweeds. Shanmughapriya *et al*.[31] found methanol: toluene (3:1) as the best solvent for extracting antimicrobials from fresh algae. The same author also reported that ethanolic extracts had no antibacterial activity. Whereas, Khallil *et al*.[32] reported that chloroform, ethanol and cyclohexane extracts had antifungal potential; whereas acetone and ethyl acetate extracts exhibited the lowest antifungal activity, which is in contrast with the present study. Their study also revealed that the tested fungi exhibited variable responses to the tested seaweed extracts depending upon the applied solvent, tested seaweed and fungal species.

Plaza *et al*.[33] also identified several volatile compounds in ethanol extracts of brown seaweeds *Himanthalia elongata*. The compounds include fatty acids, alkanes, phenols and compounds such as phytol (2-hexadecen-1-ol, 3,7,11,15-tretramethyl) and neophytadiene. The compounds 1,2-benzene dicarboxylic acid, bis-(2-ethylhexyl) ester and fatty acids have been evaluated against many microbes as antimicrobial agents[34,35]. Lauric, palmitic, linolenic, oleic, stearic and myristic acids are known to be potential antibacterial and antifungal agents[36,37]. Similarly in the present study, these compounds were found in methanol and ethyl acetate extract of all the three species. Antimicrobial activity has usually been attributed to long-chain unsaturated fatty acids (C16-C20), including palmitoleic, oleic, linoleic and linolenic acids, while long chain saturated fatty acids, including palmitic and stearic acids were less effective[38]. Steroid may serve as an intermediate for the biosynthesis of downstream secondary natural products and it is believed to be a biosynthetic precursor for cardenolides in plants. Marine algae have been shown to be good source of unsaponifiable, nontoxic sterols that have medicinal value[39,40]. In the present study palmitic acid (saturated fatty acid), myristic acid (saturated fatty acid), arachidonic acid (essential polyunsaturated omega 6 fatty acid), stearic acid (saturated fatty acid), erucic acid (mono unsaturated omega 9 fatty acid), oleic acid and linolenic acids (omega 3 fatty acid) were recorded and different sterols such as stigmasterol, 15-hydroxyprogesterone, beta sitosterol and fucosterol were also found in methanol and ethyl acetate extract which might be responsible for the highest antifungal activity in the present study. Further, detailed analysis is required to evaluate spectral composition, effectiveness and potential use of seaweed compounds for medicinal purposes.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

One of the authors would like to thank University Grant Commission, New Delhi for financial support. We are also thankful to Sophisticated Instrumentation Centre for Advanced Research and Testing, Vallabh Vidyanagar, Gujarat for GC-MS analysis.

References

[1] Yuan YV, Carrington MF, Walsh NA. Extracts from dulse (*Palmaria palmata*) are effective antioxidants and inhibitors of cell proliferation *in vitro*. *Food Chem Toxicol* 2005; **43**: 1073-81.

[2] Bansemir A, Blume M, Schroder S, Lindequist U. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture* 2006; **252**: 79-84.

[3] Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT-Food Sci Technol* 2008; **41**: 1067-72.

[4] Espeche ME, Fraile ER, Mayer AMS. Screening of Argentine marine alage from antimicrobial activity. *Hydrobiologia* 1984; **117**: 525-8.

[5] Rao PS, Parekh KS. Antibacterial activity of Indian seaweed extracts. *Bot Marina* 1981; **24**: 577-82.

[6] Smith P, Hiney MP, Samuelsen OB. Bacterial resistance to antimicrobial agents used in fish farming: a critical evaluation of method and meaning. *Annu Rev Fish Dis* 1994; **4**: 273-313.

[7] Han L, Shi D, Xu F, Yuan Z, Sun J, Shi J. [Bioactive sterols from red alga *Acanthophora spicifera* Boergesen]. *Zhongguo Zhong Yao Za Zhi* 2009; **34**: 60-3.

[8] Chojnacka K, Saeid A, Witkowska Z, Tuhy Ł. Biologically active compounds in seaweed extracts-the prospects for the application. *Open Conf Proc J* 2012; **3**(Suppl 1-M4): 20-8.

[9] Hornsey IS, Hide D. The production of antimicrobial compounds by British marine algae. I. Antibiotic producing marine algae. *Br Phycol J* 1974; **9**: 353-61.

[10] Reichelt JL, Borowitzka MA. Antimicrobial activity from marine algae: results of a large-scale screening programme. *Hydrobiologia* 1984; **116**: 158-68.

[11] Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 2003; **66**: 1022-37.

[12] Lindequest U, Schweder T. Marine biotechnology. In: Rehm HJ, Reed G, editors. *Biotechnology*. Weinheim: Wliey-VHC; 2001, p. 441-84.

[13] Nirmal Kumar JI, Kumar RN, Patel K, Viyol S, Bhoi R. Nutrient composition and calorific value of some seaweeds from Bet Dwarka, West Coast of Gujarat, India. *Our Nat* 2009; **7**: 18-25.

[14] Nirmal Kumar JI, Kumar RN, Bora A, Amb MK, Chakraborthy S. An evaluation of the pigment composition of eighteen marine macroalgae collected from Okha Coast, Gulf of Kutch, India. *Our Nat* 2009; **7**: 48-55.

[15] Jha B, Reddy CRK, Thakur MC, Rao MU. *Seaweeds of India: the diversity and distribution of seaweeds of the Gujarat coast*. Dordrecht: Springer Publication; 2009, p. 216.

[16] Zakaria NA, Ibrahim D, Sulaiman SF, Supardy NA. Assessment of antioxidant activity, total phenolic content and *in-vitro* toxicity of Malaysian red seaweed, *Acanthophora spicifera*. *J Chem Pharm Res* 2011; **3**: 182-91.

[17] Kavanagh F. *Analytical microbiology: Volume II*. New York: Academic Press; 1992, p. 241-3.

[18] Khan SR, Nirmal Kumar JI, Kumar RN, Patel JG. Baseline study for bioremediation of diesel contaminated soil site of Anand, Gujarat, India. *Int Res J Chem (Irjc)* 2014; **4**; 37-53.

[19] Manilal A, Sujith S, Kiran GS, Selvin J, Panikkar MVN. Evaluation of seaweed bioactives on common aquatic floral and faunal weeds of shrimp ponds. *Thalassas* 2010; **27**: 47-56.

[20] Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep* 2007; **24**: 31-86.

[21] Wang T, Jónsdóttir R, Ólafsdóttir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem* 2009; **116**; 240-8.

[22] Herrero M, Martín-Álvarez PJ, Señoráns FJ, Cifuentes A, Ibáñez E. Optimization of accelerated solvent extraction of antioxidants from *Spirulina platensis* microalga. *Food Chem* 2005; **93**; 417-23.

[23] Green RJ. Antioxidant activity of peanut plant tissues [Dissertation]. Raleigh: North Carolina State University; 2004.

[24] Rajauria G, Jaiswal AK, Abu-Ghannam N, Gupta S. Antimicrobial, antioxidant and free radical-scavenging capacity of brown seaweed *Himanthalia elongata* from Western Coast of Ireland. *J Food Biochem* 2013; **37**: 322-35.

[25] Manilal A, Sujith S, Selvin J, Shakir C, Kiran GS. Antibacterial activity of *Falkenbergia hillebrandii* (Born) from the Indian coast against human pathogens. *Phyton* 2009; **78**: 161-6.

[26] Rangaiah SG, Lakshmi P, Manjula E. Antimicrobial activity of seaweeds *Gracillaria, Padina* and *Sargassum* sps. on clinical and phytopathogens. *Int J Chem Anal Sci* 2010; **1**(6): 114-7.

[27] 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**(2): 107-12.

[28] Thinakaran T, Sivakumar K. Antifungal activity of certain seaweeds from Puthumadam coast. *Int J Res Rev Pharm Appl Sci* 2013; **3**(3): 341-50.

[29] Salem WM, Galal H, Nasr El-deen F. Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). *Afr J Microbiol Res* 2011; **5**: 2160-7.

[30] González del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I, et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol* 2001; **4**: 35-40.

[31] Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran GS, Natarajaseenivasan K. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Ann Microbiol* 2008; **58**(3): 535-41.

[32] Khallil AM, Daghman IM, FadyAA. Antifungal potential in crude extracts of five selected brown seaweeds collected from the Western Libya Coast. *J Microbiol Creat* 2015; **1**(1): 103.

[33] Plaza M, Herrero M, Cifuentes A, Ibañez E. Innovative natural functional ingredients from microalgae. *J Agric Food Chem* 2009; **57**: 7159-70.

[34] Kavitha A, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y. Production of bioactive metabolites by Nocardia levis MK-VL_113. *J Appl Microbiol* 2009; **49**: 484-90.

[35] Smaoui S, Mathieu F, Elleuch L, Coppel Y, Merlina G, Karray-Rebai I, et al. Taxonomy, purification and chemical characterization of four bioactive compounds from new *Streptomyces* sp. TN256 strain. *World J Microbiol Biotechnol* 2012; **28**: 793-804.

[36] McGaw LJ, Jäger AK, van Staden J. Isolation of antibacterial fatty acids from *Schotia brachypetala*. *Fitoterapia* 2002; **73**(5): 431-3.

[37] Seidel V, Taylor PW. *In vitro* activity of extracts and constituents of *Pelagonium* against rapidly growing mycobacteria. *Int J Antimicrob Agent* 2004; **23**: 613-9.

[38] Zheng HF, Li XL, Jin ZY, Sun JB, Li ZL, Xu WX. Effects of unsaturated fatty acids on calcium-activated potassium current in gastric myocytes of guinea pigs. *World J Gastroenterol* 2005; **11**(5): 672-5.

[39] Rajasulochana P, Dhamotharan R, Krishnamoorthy P. Primary phytochemical analysis of *Kappaphycus* sp. *J Am Sci* 2009; **5**(2): 91-6.

[40] Sanchez-Machado DI, Lopez-Hernandez J, Paseiro-Losada P, Lopez-Cervantes J. An HPLC method for the quantification of sterols in edible seaweeds. *Biomed Chromatogr* 2004; **18**: 183-90.