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Full Length Research Paper

Evaluation of *in vitro* antimicrobial property of seaweed (*Halimeda tuna*) from Tuticorin coast, Tamil Nadu, Southeast coast of India

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The seaweed (*Halimeda tuna*) was examined for antibacterial and antifungal activity *in vitro* using the well diffusion method, minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration. The activity was against 10 bacterial strains (*Staphylococcus aureus, Salmonella typhimurium, Salmonella paratyphi, Klebsiella oxytoca, Escherichia coli, Proteus mirabillis, Lactobacillus vulgaris, Pseudomonas* sp.*, Klebsiella pneumonia* and *Vibrio cholerae*) and nine fungal strains (*Aspergillus niger, Aspergillus flavus, Alternaria alternaria, Candida albicans, Epidermophyton floccossum, Trichophyton mentagrophytes, Trichophyton rubrum, Pencillium* sp. and *Rhizopus* sp.). The methanolic extracts in the present study exhibited a broad spectrum of antimicrobial activity compared to the ethanolic and chloroform extracts. Results of the present study confirm the potential use of seaweed extracts as a source of antimicrobial compound.

Key words: *Halimeda tuna*, minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration, antimicrobial activity.

INTRODUCTION

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterised by a broad spectrum of biological activities. Compounds with antioxidant, antiviral, antifungal and antimicrobial activities have been detected in brown, red and green algae (Yuan et al., 2005; Bansemir et al., 2006; Chew et al., 2008). Seaweeds are rich and varied source of bioactive natural products and have been studied as potential biotical and pharmaceutical agents. They are used in traditional remedies in many parts of the world. Extracted substances from seaweeds have antibacterial actions and other properties include antifungal activities and growth inhibition of plants (Abdussalam, 1990; Scheuer, 1990; Rizvi and Shameel, 2003; Su et al., 1973; Burkholder and Sharma, 1969; Chapman, 1980; Arasaki and Arasaki, 1983; Abbott, 1988).

Numerous substances were identified as antimicrobial agents from algae: chlorellin derivatives, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds, phenolic inhibitors, etc (Espeche et al., 1984). Antibacterial activity was, however, found to vary with season (Moreau et al., 1984). Therefore, the aim of the present investigation was to evaluate the antimicrobial activity of the seaweed *Halimeda tuna* against different human pathogenic bacterial and fungal strains.

MATERIALS AND METHODS

Collection, extraction and preparation of sample

Fresh plants of *H. tuna* (Bryopsidales, Chlorophyta) (J.Ellis and Solander) J.V.Lamouroux 1816 were collected from the intertidal region (Lat. 8° 40′ to 8° 55′ N and Long. 78° 0′ to 78° 15′ E) on the

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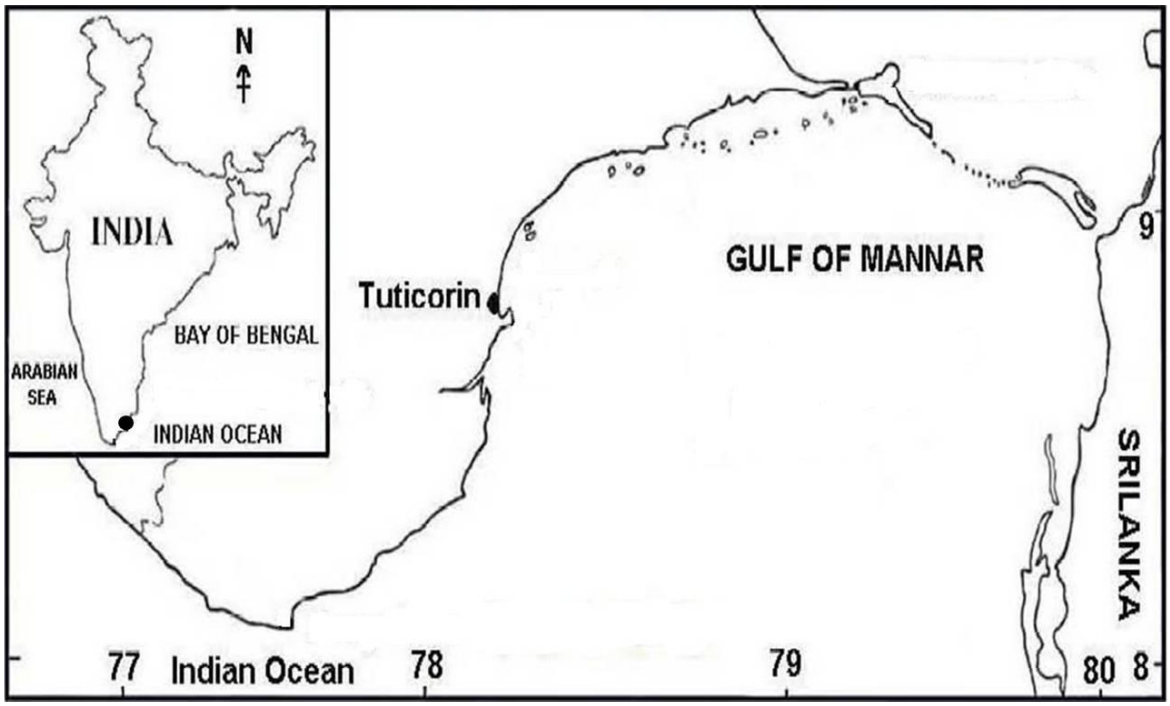

Figure 1. Map showing the sampling area.

Tuticorin, Tamil Nadu, Southeast coast of India (Figure 1). The plants were cleaned of epiphytes and extraneous matter, and the necrotic parts were also removed. Afterward, the plants were washed with seawater and then in fresh water and then transported to the laboratory in sterile polythene bags at 0°C temperature.

Samples were rinsed with sterile distilled water and were then shade-dried, cut into small pieces and powdered in a mixer grinder. The seaweed powdered samples were extracted (10 g/100 ml) in chloroform, ethanol and methanol thrice by soaking overnight at room temperature. The extracts from three consecutive soaking were pooled and evaporated under reduced pressure. The residues (crude extracts) obtained were finally dried under rotary vacuum evaporator (Rotavapor® R-210/R-215, Lark, Chennai), and the crude extracts were tested for their antibacterial activity against the pathogens used in the present investigation.

Aqueous extract

The powdered sample was mixed with distilled water and filtered. The filtrate was evaporated in vacuo at 40°C using a rotary evaporator.

Microorganisms used

The 10 bacterial strains used were: *Staphylococcus aureus, Salmonella typhi, Salmonella paratyphi, Klebsiella oxytoca, Escherichia coli, Proteus mirabillis, Lactobacillus vulgaris, Pseudomonas* spp*., Klebsiella pneumoniae* and *Vibrio cholerae*. The stock culture was maintained on nutrient agar medium at 4°C. Nine fungal pathogens namely *Aspergillus niger, Aspergillus flavus, Alternaria alternaria, Candida albicans, Epidermophyton floccossum, Trichophyton mentagrophytes, Trichophyton rubrum, Pencillium* sp. and *Rhizopus* sp*.* were also used. The stock culture was maintained on Sabouraud dextrose agar (SDA) medium at 4°C. These bacterial and fungal strains were isolated and obtained from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

In vitro antibacterial activity was determined by using Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB), while the *in vitro* antifungal activity was determined by using SDA, all of which were obtained from Himedia Ltd., Mumbai.

Preparation of inocula

Twenty-four hour old culture of selected bacteria was mixed with physiological saline and the turbidity was corrected by adding sterile physiological saline until a Mac Farland turbidity standard of 0.5 (106 colony forming units (CFU/ml). The isolates were sub cultured on Sabouraud dextrose agar and incubated at 35°C for seven to 14 days. The growth was scraped aseptically, crushed and macerated thoroughly in sterile distilled water and the fungal suspension was standardized spectrophotometrically to an absorbance of 0.600 at 450 nm.

Antibacterial and antifungal assays

The antibacterial and antifungal susceptibility test was followed by well diffusion method (Reinheimer et al., 1990). Petri plates were prepared by pouring 15 ml of Mueller Hinton agar for bacteria and Sabouraud dextrose agar for fungi and allowed to solidify. After swabbing the bacterial pathogens on the Muller Hinton agar plates, as well as the fungal pathogens on Sabouraud dextrose agar, 0.1 ml of seaweed extract was poured into the wells and the plates were incubated at 37°C for 24 h and 28°C for 72 to 96 h (fungi). The extract inhibiting the growth of pathogen was assessed based on the inhibition zone around the well and the results were recorded.

Table 1. Antibacterial activity of *Halimeda tuna* extracts.

Bacteria strain	Inhibiting zone diameter in mm (mean ± SD)								
	Chloroform		Ethanol		Methanol		Aqueous		CIP
	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL	25 µL
Staphylococcus aureus	6 ± 1.63	12 ± 1.25	5 ± 1.25	9 ± 1.25	8 ± 1.63	16 ± 1.25	4 ± 1.25	8 ± 1.25	18 ± 5.65
Salmonella typhimurium	3 ± 0.82	3 ± 1.70	2 ± 1.25	4 ± 1.25	4 ± 1.63	7 ± 1.25	ND*	2 ± 1.63	10 ± 3.45
S. paratyphi	2 ± 1.25	4 ± 1.25	6 ± 1.25	11 ± 1.63	6 ± 1.63	12 ± 1.63	ND*	2 ± 0.82	14 ± 5.22
Klebsiella oxytoca	4 ± 1.63	8 ± 1.25	3 ± 1.63	6 ± 1.25	4 ± 1.63	8 ± 1.25	2 ± 1.25	3 ± 1.63	9 ± 2.92
K. pneumoniae	4 ± 1.25	7 ± 1.25	5 ± 1.25	9 ± 1.25	3 ± 1.63	6 ± 1.25	2 ± 1.63	4 ± 2.05	14 ± 4.98
Escherichia coli	7 ± 1.63	12 ± 1.25	5 ± 1.25	101.63 ±	7 ± 1.63	15 ± 1.63	3 ± 1.63	5 ± 1.25	16 ± 5.00
Proteus mirabillis	2 ± 1.25	4 ± 2.05	2 ± 1.25	4 ± 1.25	5 ± 1.25	9 ± 1.63	1 ± 1.25	2 ± 1.25	12 ± 4.79
Lactobacillus vulgaris	5 ± 1.63	10 ± 1.63	7 ± 1.25	13 ± 1.63	6 ± 1.63	11 ± 1.25	4 ± 1.25	6 ± 1.25	16 ± 4.98
Pseudomonas spp.	8 ± 1.25	15 ± 1.25	9 ± 1.63	16 ± 1.25	10 ± 1.63	17 ± 1.63	5 ± 1.63	7 ± 1.25	18 ± 5.10
Vibrio cholerae	3 ± 1.63	6 ± 1.63	2 ± 0.82	4 ± 1.25	5 ± 1.63	10 ± 1.63	ND*	2 ± 1.25	14 ± 5.43

*ND, Antibacterial activity was not detected; *CIP, ciprofloxacin antibacterial control.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of the plant extract was tested by the two-fold serial dilution method. The test extract was dissolved in 5% dimethyl sulfoxide (DMSO) to obtain 1000 µg/ml stock solutions. Then 0.5 ml of stock solution was incorporated into 0.5 ml of Mueller Hinton agar for bacteria and Sabouraud dextrose broth for mycelial fungi to get a concentration of 500 µg/ml, and serially diluted by double technique to achieve 250, 125, 62.5 and 31.25 µg/ml, respectively. Next, 50 µL of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and not the plant extract. The culture tubes were incubated at 37°C for 24 h (bacteria) and 28°C for 72 to 96 h (mycelia fungi). The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation was determined as MIC.

Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

All the tubes used in the MIC study that did not show any growth of the bacteria and fungi after the incubation period were first diluted (1:4) in fresh Mueller-Hinton broth for bacteria and Sabouraud dextrose broth for mycelial fungi and then subcultured onto the surface of the freshly prepared Mueller-Hinton agar (for bacteria) and Sabouraud dextrose agar (for fungi) plates and incubated in biological oxygen demand (BOD) incubators at 37°C for 24 h (bacteria) and 28°C for 72 to 96 h (mycelia fungi). The MBC and MFC were recorded as the lowest concentration of the extract that did not permit any visible bacteria and fungal colony growth on the appropriate agar plate after the period of incubation.

RESULTS

In the present investigation, extracts of a marine algal species were tested against the bacterial and fungal pathogens by well diffusion method. A total of 19 microorganisms which consisted of 10 bacteria and nine fungi were tested. When the chloroform, ethanol, methanol and aqueous extracts were assayed against the test organisms, the zones of inhibition obtained was between 2 to 20 mm. Methanolic extracts showed exhibited broad spectrum of antimicrobial activity compared to other ethanolic and choloroform extract. The results of preliminary screening tests are summarized in Tables 1 and 2, which revealed that *H. tuna* species possessed antibacterial and antifungal activity.

MIC values of 31.25 to 500 µg/ml were obtained for the chloroform and ethanol extract, 15.62 to 250 µg/ml were obtained for the methanol extract in the tests with the bacterial agents, while the range of 15.62 to 250 µg/ml were obtained for the methanol extract with the fungi agents. Moreover, 15.62 to 500 µg/ml and 31.25 to 500 µg/ml obtained for the ethanol extract and chloroform extract was recorded against the fungal isolates. On the other hand, the MIC values obtained in antibacterial assays using aqueous extract were 125 to 500 µg/ml, while the values recorded in the antifungal assays were 62.5 to 250 µg/ml.

The MBC of the chloroform extracts showed

Table 2. Antifungal activity of *Halimeda tuna* extracts.

Fungal strain	Inhibiting zone diameter in mm (mean ± SD)								
	Chloroform		Ethanol		Methanol		Aqueous		KET
	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL	50 µL	100 µL	25 µL
Aspergillus niger	9 ± 1.25	13 ± 1.25	8 ± 1.25	12 ± 3.69	11 ± 3.98	14 ± 4.63	5 ± 1.25	8 ± 1.25	15 ± 1.25
A. flavus	4 ± 1.25	8 ± 1.25	3 ± 1.63	7 ± 2.26	5 ± 2.72	10 ± 4.22	2 ± 1.25	4 ± 1.25	12 ± 1.25
Alternaria alternaria	7 ± 1.25	11 ± 1.25	8 ± 0.82	12 ± 3.66	9 ± 3.38	13 ± 4.23	5 ± 1.63	9 ± 1.63	13 ± 1.25
Candida albicans	8 ± 1.25	12 ± 1.25	11 ± 1.25	14 ± 4.78	9 ± 3.67	13 ± 3.84	4 ± 1.25	6 ± 1.25	15 ± 1.25
Epidermophyton floccossum	6 ± 1.25	10 ± 1.63	7 ± 1.25	11 ± 3.88	12 ± 3.61	16 ± 5.69	4 ± 0.82	7 ± 1.25	16 ± 0.82
Trichophyton mentagrophytes	5 ± 0.82	9 ± 1.25	4 ± 1.63	9 ± 3.08	8 ± 2.65	11 ± 4.37	3 ± 1.63	5 ± 1.25	13 ± 1.25
T. rubrum	3 ± 1.63	7 ± 1.63	6 ± 1.25	10 ± 4.11	7 ± 2.24	11 ± 3.57	2 ± 1.25	4 ± 1.63	12 ± 1.25
Pencillium sp.	4 ± 1.25	8 ± 1.25	5 ± 1.25	9 ± 3.30	5 ± 2.62	9 ± 2.76	2 ± 1.25	3 ± 1.63	12 ± 1.25
Rhizopus sp.	6 ± 1.25	10 ± 1.25	4 ± 1.25	9 ± 2.70	5 ± 3.34	9 ± 3.50	3 ± 1.25	5 ± 1.25	14 ± 1.63

*KET, Ketoconazole antifungal control.

that with the exception of the antibacterial assays against *S. aureus, S. typhimurium, S. paratyphi, K. oxytoca, E. coli*, all the extracts exhibited a MBC at a concentration of 500 µg/ml, while the aqueous extracts had a MBC value ranging from 250 to 500 µg/ml (Table 3). On the other hand, MFC of the methanol extracts showed that with the exception of the antifungal assays against *A. niger, A. flavus, A. alternaria, C. albicans, E. floccossum, T. mentagrophytes, T. rubrum* and *Pencillium* sp., the MFC of the ethanol extracts showed that with the exception of the antibacterial assays against *A. niger, A. flavus, A. alternaria, C. albicans* and *E. floccossum,* and the chloroform extracts showed that with the exception of the antibacterial assays against *A. niger, A. flavus, A. alternaria, C. albicans, E. floccossum* and *T. mentagrophyte*, all the extracts exhibited a MFC at a concentration of 500 µg/ml, while aqueous extracts had a MFC value ranged between 250 to 500 µg/ml (Table 4).

DISCUSSION

Some volatile compounds released from species and herb extracts show wide antimicrobial activities against fungi (Sindhu et al., 2009) and bacteria (Bandyopadhyay et al., 2007). Seaweeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae (Ganesan et al., 2008). In the present study, results show that all solvent extracts and aqueous extracts of *H. tuna* gave favorable results against all the tested microorganisms with MIC values between 15.62 and 500 µg/ml. The present study reveals that the seaweed extracts of *H. tuna* was very effective against *S. aureus, S. typhimurium, S. paratyphi, K. oxytoca, E. coli, A. niger, A. flavus, A. alternaria, C. albicans, E. floccossum,* than the other strains tested. The algal extracts such as *Enteromorpha ramulosa* (Smith) Carmichael and *Dictyopteris membranacea* (Stackhouse) Batters were active against Gram-positive and Gram-negative bacteria (González et al., 2001).

In the present study, fungal strains were more susceptible to the extracts than the bacterial strains. Among all the extracts used for the present study, the methanol extract showed a higher antibacterial and antifungal activity than that of other extracts and aqueous extract. This may be due to the solvent to extract the different constituents having antimicrobial activity. Kandhasamy and Arunachalam (2008) studied *in vitro* antibacterial activities of seaweeds belonging to Chlorophyceae (*Caulerpa racemosa* and *Ulva lactuca*), Rhodophyceae (*Gracillaria folifera* and *Hypneme muciformis*) and Phaeophyceae (*Sargassum myricocystum*, *Sargassum tenneerimum* and *Padina tetrastomatica*); they

Table 3. Minimum Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Halimeda tuna* extracts.

MIC µg/ml				MBC µg/ml			
Chloroform	**Ethanol**	**Methanol**	**Aqueous**	**Chloroform**	**Ethanol**	**Methanol**	**Aqueous**
31.25	31.25	15.62	125	62.5	62.5	31.25	250
62.5	62.5	31.25	125	125	250	62.5	250
62.5	125	31.25	250	250	250	62.5	500
125	125	62.5	250	250	250	125	500
125	125	62.5	250	250	250	125	500
125	250	62.5	500	250	500	125	500
250	250	125	500	500	500	250	500
250	250	125	500	500	500	500	500
500	500	250	500	500	500	500	500
500	500	250	500	500	500	500	500

Table 4. Minimum Inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of *Halimeda tuna* extracts.

MIC µg/ml				MFC µg/ml			
Chloroform	**Ethanol**	**Methanol**	**Aqueous**	**Chloroform**	**Ethanol**	**Methanol**	**Aqueous**
31.25	15.62	15.62	62.5	62.5	62.5	31.25	250
62.5	62.5	31.25	125	125	250	125	500
31.25	62.5	31.25	125	250	250	62.5	500
125	125	62.5	250	250	250	125	500
125	125	62.5	250	250	250	125	500
125	250	62.5	500	250	500	125	500
250	250	125	500	500	500	250	500
250	250	125	500	500	500	250	500
500	500	250	500	500	500	500	500

were studied against both Gram negative and Gram-positive pathogenic bacteria. Methanolic extracts of all the seaweed extracts exhibited broad spectrum of antibacterial activity. The crude aqueous extracts of the whole plant of *Parthemia iphionoids* showed limited antibacterial activity against *E. coli*, *S. aureus*, *S. typhimurium* and *Bacillus cereus* (Afifi et al., 1991). Methanol was the most effective solvent for extracting antibacterial compounds from the selected seaweeds (Vlachos et al., 1996). Previous reports on the most effective solvent for the extraction of antimicrobials have been varied; Gonzalez et al. (2001) selected methanol as solvent for extraction of antimicrobial compounds from red, green and brown seaweeds, Shanmughapriya et al. (2008) found methanol: toluene (3:1) as the best solvent for extracting antimicrobials from fresh algae, while Plaza et al. (2009) found significant differences in the antimicrobial activity depending on the solvent used. The higher zone of inhibition was recorded at 1000 µg/ml concentration than 500 µg/ml concentration of all the extracts. When the disc dosage level increases, the inhibitory effect also increased. Similar observations were made by Chattopadhyay et al. (2001, 2002) while studying the antimicrobial activities of *Alstonia macrophylla* and *Mallotus peltatus* leaves.

The overall antimicrobial activity assessed from the above results indicates the presence of active constituents in the extractions of seaweed which showed better antimicrobial activity against pathogens used. Differences between the results of the present investigation and results of other studies may be due to the production of bioactive compounds related to the seasons, method, organic solvents used for extraction of bioactive compounds and differences in assay methods. Finally, it can be concluded from the study that the extracts of *H. tuna* species used in the present investigation shows better antibacterial activity against pathogens used. They are potential sources of bioactive compounds and should be investigated for natural antibiotics. Hence, further research should be made to identify and purify these antibacterial substances.

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REFERENCES

Abbott IA (1988). Food and products from algae. In: Algae and Human Affairs. (Eds.): Lembi C.A. and Waaland J.R., Cambridge University Press, Cambridge, pp. 135-147.

Abdussalam S (1990). Drugs from Seaweeds. Med.Hypo. 32:33-35.

Afifi FU, Al-Khalil S, Abdul-Haq BK, Mahanesh A, Al-Eisawi DM, Sharf M, Wong LK, Chiff PLS (1991). Antifungal flavonoids from *Varthemia iphionoides*. Phys. Res. 5:173-175.

Arasaki S, Arasaki T (1983). Vegetables from the Sea. Japan Publ. Inc., Tokyo. 96:251-223.

Bandyopadhyay M, Chakraborty R, Raychaudhuri U (2007). A process for preparing a natural antioxidant enriched dairy product (Sandesh). LWT 40(5):842-851.

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

Burkholder PR, Sharma GM (1969). Antimicrobial agents from the sea. Lloydia 32:466-483.

Chapman VJ, Chapman DJ (1980). Seaweeds and their uses. Chapman and Hall, London. 3rd Edition. 9: p. 334.

Chattopadhyay D, Arunchalam G, Mandal AB, Sur TK, Mandal SC, Bhattacharya SK (2002). Antimicrobial and anti-inflammatory activity of folklore: *Mallotus peltatus* leaf extract. J. Ethnopharmacol. 82:229-237.

Chattopadhyay D, Maiti K, Kundu AP, Bhadra R, Mandel SC, Mandal AB (2001). Antimicrobial activity of *Alstonia macrophylla*: a folklore of bay islands. J. Ethnopharmacol. 77:49-55.

Chew YL, Lim YY, Omar M, Khoo KS (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT. 41:1067-1072.

Espeche ME, Fraile ER, Mayer AMS (1984). Screening of Argentine marine alage from antimicrobial activity. Hydrobiologia 117:525-528.

Ganesan P, Kumar CS, Bhaskar N (2008). Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. Bioresour. Technol. 99:2717-2723.

Gonzalez del Val A, Platas G, Basilio A (2001). Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). Int. Microbiol. 4:35-40.

Kandhasamy M, Arunachalam KD (2008). Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. Afr. J. Biotechnol. 7(12):1958-1961.

Moreau J, Pesando D, Caram B (1984). Antifungal and antibacterial screening of Dictyotales from the French Mediterranean coast. Hydrobiologia 116/117:521-524.

Plaza M, Santoyo S, Jaime L, García-Blairsy Reina G, Herrero M, Senoráns FJ, Ibánez E (2009). Screening for bioactive compounds from algae. J. Pharma. Biom. Ana. article in press.

Reinheimer JA, Demkow MR, Condioti MC (1990). Inhibition of coliform bacteria by lactic cultures. Austr. J. Dairy Technol. pp. 5-9.

Rizvi MA, Shameel M (2003). Biological activity and elementology of benthic algae from Karachi coast. Pak. J. Bot. 35(5):717-729.

Scheuer PJ (1990). Some marine ecological phenomena: Chemical Basis and Biomedical Potential. Science 248:173-177.

Shanmughapriya S, Manilal A, Sujith S, Selvin J, Kiran GS, Natarajaseenivasan K (2008). Antimicrobial activity of seaweeds extracts against multiresistant pathogens. Ann. Microbiol. 58(3):535-541.

Sindhu R, Suprabha GN, Shashidhar S (2009). Optimization of process parameters for the production of a-amylase from *Penicillium janthinellum* (NCIM 4960) under solid state fermentation. Afr. J. Microbiol. Res. 3(9):498-503.

Su KL, Abul-Hajj Y, Staba EJ (1973). Antimicrobial effects of aquatic plants from Minnesota. Lloydia. *PMID:* 4576314 [PubMed - indexed for MEDLINE]. 36(1):80-87.

Vlachos V, Critchely AT, Von Holy A (1996). Establishment of a protocol for testing antimicrobial activity in Southern African macroalgae. Microbios. 88:115-123.

Yuan YV, Carrington MF, Walsh NA (2005). Extracts from dulse (*Palmaria palmata*) are effective antioxidants and inhibitors of cell proliferation *in vitro*. Food Chem. Toxicol. 43:1073-1081.