African Journal of Biotechnology Vol. 10(39), pp. 7684-7689, 27 July, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.491
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Antioxidant and antibacterial activities of *Cladophora glomerata* (L.) Kütz. in Caspian Sea Coast, Iran

Saeid Soltani*, Sara Saadatmand, Ramzanali Khavarinejad and Taher Nejadsattari

Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran.

Accepted 19 May, 2011

***Cladophora* is one of the largest filamentous green-algal genus and has a widespread distribution in Caspian Sea Coast. This study aimed at assaying the antimicrobial and antioxidant activities of *Cladophora glomerata* in South of Caspian sea. The antioxidant activity of the extract was investigated, including the total phenolic contents (3077 ± 105 mg gallic acid equivalent g^{-1} of extract), total flavonoid contents (595 ± 23 mg quercetin equivalent gl^{-1} of extract), scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals (920 ± 42 µg ml^{-1} as IC_{50} value), iron chelating activity (IC_{50} = 971 ± 39 µg ml^{-1}), scavengers of nitric oxide (0.20 ± 0.01 mg ml^{-1} as IC_{50}) and reducing power. There were significant differences between the extract and vitamin C ($P<0.001$). Furthermore, antimicrobial activities of the hydroalcoholic extracts of five different gram negative and positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Proteus mirabilis* were investigated. The extract was primarily screened for their possible antimicrobial effects using disc diffusion methods. The potential antibacterial activities at different concentrations of the extract were elucidated. The extract displayed a variable degree of antimicrobial activities on different bacteria. Among the gram positive bacteria, the *S. aureus* (with wider zones of inhibition) was found to be more sensitive than *B. subtilis*. Among the gram negative bacteria, *Salmonella enteritidis* was found to be more resistant than *Proteus vulgaris*. Our findings suggest the possibility of using the *Cladophora glomerata* as a novel source of natural antimicrobial and antioxidant agents for pharmaceutical industries.**

Key words: *Cladophora glomerata*, antibacterial activity, antioxidant activity, flavonoid contents, phenolic contents.

INTRODUCTION

Bioactive natural products are widely distributed in the plant kingdom, and extract from different plants as well as red, green and brown macro and micro algae can be used as natural products (Iliopoulou et al., 2002; Metzger et al., 2002; Soobrattee et al. 2005). Biological activities in algal bioactive natural compounds have wide effects such as antibacterial (Stirk et al., 2007), antifungal (Volka and Furkert, 2006), antitumor (Jiao et al., 2009) and antioxidant activities. Antioxidant activity studied in some red, brown and green algae demonstrated that antioxidative properties of extract vary in different seaweeds and is proportional to the content of antioxidative compound (Zubia et al., 2007). In fact, the antioxidant activity in algae acts by several processes and compounds such as lipophilic scavengers (carotenoids), enzymatic scavengers (catalase, superoxide dismutase and peroxidase), polyphenols and antioxidative molecules (ascorbic acid, tocopherols, carotenoids, chlorophyll related compounds, bromophenols, polysaccharides, etc.) (Mittler, 2002; Le Tutour et al., 1998; Rupérez et al., 2002; Yuan et al., 2005), and they have effects such as anti-inflammatory, hepato-protective and skin protective. The algal total antioxidant compounds changes with respect to seasonal factors and geographic regions. Antibacterial activity has been the most widely investigated in green, red and brown seaweeds for new classes of antibiotics with novel structures that are effective against human pathogens

*Corresponding author. E-mail: ketaban@gmail.com. Fax: +98-123-2263127.

Abbreviations: DPPH, Diphenyl-2-picryl hydrazyl; **BHA,** butylhydroxyanisole; **BHT,** butylhydroxytoluene; **EDTA,** ethylenediaminetetraacetic acid.

(Vlachos et al., 1999; Abourriche et al., 1999; Kajiwara et al., 2006).

In the shallow coastal zone of the southern Caspian Sea, especially, filamentous algae *Cladophora* sp. are dominant benthic plants. This alga is predominantly found attached to rocky and stony shores or mixed with other genera (such as *Enteromorpha* sp.). In this study, we determined the total phenolic and flavonoid contents, the 2,2-diphenyl-1-picrylhydrasyl (DPPH) radical scavenging activity, metal chelating activity, nitric oxide-scavenging activity, reducing power and antibacterial activity on several gram positive and negative bacteria (*Salmonella typhimurium*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus mirabilis*) of *Cladophora glomerata* extract in order to understand the usefulness of this alga as a foodstuff as well as a medicine.

MATERIALS AND METHODS

Ferrozine, linoleic acid, trichloroacetic acid (TCA), DPPH, potassium ferricyanide and hydrogen peroxide were purchased from Sigma Chemicals Co. (USA). Gallic acid, quercetin, butylated hydroxyanisole (BHA), ascorbic acid, sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, EDTA, ferric chloride, Muller Hinton Agar, Nutrient broth and DMSO were purchased from Merck (Germany). All other chemicals were of analytical grade.

Collection and preparation of sample

Samplings were carried out in the southern coast of the Caspian Sea in the city of Sari Mazandaran, Iran, in summer 2010. Samples of *C. glomerata* were collected manually from the rock. The harvested macroalgae were stored in plastic bags for transportation to the laboratory. Voucher specimen of species were pressed and stored in 5% formalin for identification according to Burrows (1991) and Leliaert and Coppejans, (2003). Voucher (No. 121) was deposited in the herbarium (Islamic Azad University, Qaemshahr, Iran). Biomass was rinsed with fresh water to eliminate other materials such as sand, shells, etc. The macroalgae were stored in the laboratories and dried at 50°C under ventilation in an oven and ground in a blender.

Collection and preparation of algal extracts

Dried materials were coarsely ground before extraction. 5 g of dried materials were extracted by maceration with 70% ethanol (1 h sonication, filtered; repeated 2 times). The extract was then separated from the sample residue by filtration through Whatman No.1 filter paper. The resultant extracts were concentrated in a rotary evaporator under reduced pressure until a crude solid extract was obtained, which were then freeze-dried for complete solvent removal (0.9 g).

Determination of total phenolic compounds and flavonoid content

Total phenolic compound content was determined by the Folin-Ciocalteau method (McDonald et al., 2001). Extract (0.5 ml, 1.6 mg ml^{-1}) was mixed with 2.5 ml of 0.2 N Folin-Ciocalteau reagent for 5 min and 2.0 ml of 75 g l^{-1} sodium carbonate was then added. The absorbance of the reaction was measured spectrophotometrically (Perkin elmer Wellesley, MA) at 760 nm after 2 h of incubation at room temperature (r.t.). Results were expressed as gallic acid equivalents. Total flavonoids were estimated according to the method of Chang et al. (2002). Briefly, 0.5 ml solution of extract in methanol (1.6 mg ml^{-1}) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water and left at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer. Total flavonoid content was calculated as quercetin from a calibration curve.

DPPH radical-scavenging activity

The stable DPPH was used for the determination of free radical scavenging activity of the extract (Koleva et al., 2002). Different concentrations of extract were added at an equal volume, to the methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Determination of metal chelating activity

The ability of the *C. glomerata* extract to chelate ferrous ions was estimated according to Danis et al. (1994). Different concentration of the extract was added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixtures was then shaken vigorously and left to stand at room temperature for 10 min. The absorbance of the solutions was measured at 562 nm. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated as:

$$[(A_0 - A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the control and A_1 is the mixture containing the extract or the absorbance of a standard solution. EDTA was used as a standard.

Assay of nitric oxide-scavenging activity

For the experiment, sodium nitroprusside (10 mM, 1 ml), in phosphate-buffered saline, was mixed with different concentration of the extract dissolved in water and incubated at room temperature for 150 min. The same reaction mixture, without the extract but with an equivalent amount of water, served as the control. After the incubation period, 0.5 ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546 nm. Quercetin was used as the positive control (Sreejayan and Rao, 1997).

Reducing power determination

Fe (III) reduction is often used as an indicator of electron-donating activity, an important mechanism for phenolic antioxidant action. The reducing power of the extracts was determined according to the method of Yildirim and Mavi, (2001). Different amounts of each extract (25 to 800 µg ml^{-1}) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixtures were incubated for 20 min at 50°C. 2.5 ml of trichloroacetic acid (10%) was added to the mixture to stop the reaction, and then centrifuged at 3000 rpm for

10 min. The supernatant of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Vitamin C was used as the positive control.

Microorganisms used and determination of antibacterial activity

Five bacterial strains (gram positive and negative) were selected for the study. The gram positive species were *B. subtilis* and *S. aureus*, while the gram negative species were *P. mirabilis*, *P. aeruginosa* and *S. typhimurium*. Each bacterial strain was incubated in nutrient broth at 37°C overnight (14 h), and test bacterial solutions were prepared with the same broth to give a concentration 1.5 x 10^8 CFU ml^{-1}. Suspensions of microorganisms were transferred onto the surface of Muller Hinton Agar media and spread evenly over the entire surface of the plates. Blank discs (6.4 mm, Padtan Teb, Iran) impregnated with 20 µl of a serial 20-fold dilution of extract compounds (100, 50, 25, 12.5, 6.25, 3.125, 1.565 mg ml^{-1}) were prepared using 50% DMSO. The plates spread with bacteria were incubated at 37°C for 24 h. After incubation, the inhibition zones formed around the disks were measured (Androw 2001). Gentamycin disc (10 µg), cefalexin disc (30 µg) and tetracycline disc (30 µg) were used as the positive control.

Statistical analysis

The experimental results were expressed as means ± SD. All measurements were replicated three times. The data were analyzed by analysis of variance ($P<0.05$) and the means were separated by Duncan's multiple range test. The IC_{50} values were calculated from linear regression analysis

RESULTS AND DISCUSSION

Determination of total phenolic compounds and flavonoid content

Total phenol compounds were reported as gallic acid equivalents by reference to standard curve ($y = 0.0054x + 0.0628$, $r^2 = 0.987$). The total phenolic content was 3077 ±105 mg gallic acid equivalent g^{-1} of extract. The total flavonoid content was 595 ± 23 mg quercetin equivalent g^{-1} of extract, by reference to standard curve ($y = 0.0063x$, $r^2 = 0.999$). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources. There are different amounts of phenols and polyphenolic compounds in the Iranian medicinal plants, for example in aerial parts of *Delphinium elbursense*, the total phenolics was 52.24 ± 1.7 mg gallic acid equivalent g^{-1} of extract and total flavonoid content was 17.26 ± 0.6 mg quercetin equivalent g^{-1} of extract powder (Ebrahimzadeh et al., 2010b). In this alga, there were high amounts of phenols and polyphenolic compounds. Increasing the levels of flavonoids in the daily diet may decrease the impact or occurrence of certain human diseases because they interact with various biological systems and show anti-inflammatory, hypolipidemic, hypoglycemic and anti-oxidant activities (Middleton et al., 2000).

DPPH radical-scavenging activity

DPPH is a free radical that accepts an electron or hydrogen radical to become a stable molecule and a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Ebrahimzadeh et al., 2010a). IC_{50} for the DPPH radical-scavenging activity was 920 ± 42 µg ml^{-1}. The IC_{50} values for ascorbic acid, quercetin and BHA were 5.05 ± 0.1, 5.28 ± 0.2 and 53.96 ± 3.1 µg ml^{-1}, respectively. Phenol and flavonoid contents of this alga may have led to its good DPPH-scavenging activity. The correlation between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables.

Fe^{2+} chelating activity

Iron chelators mobilize tissue iron by forming soluble and stable complexes that are then excreted in the feces and/or urine. Chelation therapy reduces iron-related complications in human and thereby improves quality of life and overall survival in some diseases such as thalassemia major (Grazul and Budzisz, 2009). Deferoxamine and deferiprone are clinically useful iron chelators. But many adverse effects may occur after administration (Porter, 1997). There is an urgent need to identify other chelators with acceptable degree of tolerability (Porter, 1997). So, many researches focused on some natural product, especially flavonoids that possess direct influence on iron ions level within tissues (Grazul and Budzisz, 2009). Ferrozine can quantitatively form complexes with iron ions. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. In this assay, both extract and EDTA interfered with the formation of iron ions and ferrozine complex, suggesting that it has chelating activity and captures iron ions before ferrozine. The extract showed weak iron chelating activity with IC_{50} = 971 ± 39 µg ml^{-1}. EDTA showed very powerful activity (IC_{50} = 18 ± 1.5 µg ml^{-1}).

Reducing power of extracts

In reducing power assay, the presence of antioxidants in the sample would result in the reduction of Fe^{3+} to Fe^{2+} by donation of an electron. The amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 1

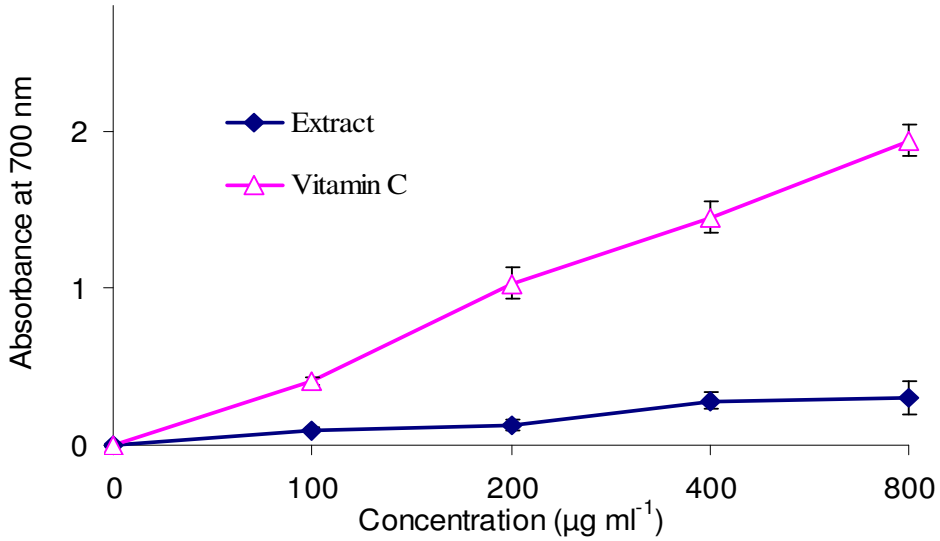

Figure 1. Reducing power of *C. glomerata*.

Table 1. Phenol and flavonoids contents and antioxidant activities of *C. glomerata* extracts. Results are means ± SD.

Extract	Total phenol content (mg g^{-1})	Total flavonoid content (mg g^{-1})	DPPH free radical scavenging IC_{50} (µg ml^{-1})[a]	Nitric oxide scavenging IC_{50} (µg ml^{-1})[b]	Fe^{2+} chelating IC_{50} (µg ml^{-1})[c]
C. glomerata	3077±105	595±23	920±42	0.20±0.01	971±39

[a]IC_{50} of BHA, vitamin C and quercetin were 53.96 ± 3.1 5.05 ± 0.1 and 5.28 ± 0.2 µg ml^{-1}, respectively. [c]EDTA was used as control (IC_{50} = 18 ± 1.5 µg ml^{-1}).

shows the dose-response curves for the reducing power of the extract. The reducing power of the extract also increased with an increase in concentration. There were significant differences between the extract and vitamin C ($P<0.001$).

Assay of nitric oxide-scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates NO which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of NO compete with oxygen, leading to reduced production of nitrite ions. NO has been associated with a variety of physiologic processes in the human body since it was identified as a novel signal molecule. It also participates in pathogenic pathways underlying a large group of disorders such as muscle diseases, inflammatory bowel disease, primary headaches, stroke and neurodegenerative disorders such as Alzheimer disease (Aliev et al., 2009; Moncada et al., 1991). So, using the herbal remediation as a NO scavenger may be useful. In scavengers of nitric oxide, percentage of inhibition was increased with increasing concentration of the extract. The IC_{50} was 0.20 ± 0.01 mg ml^{-1} vs. quercetin with IC_{50} = 20 ± 1 µg ml^{-1} (Table 1).

Determination of antibacterial activity

The inhibitory effects of the concentrations of *C. glomerata* extract on the growth of various gram positive and negative bacteria using disc diffusion method is shown in Table 2. The extract showed activity against gram positive as well as gram negative bacteria and inhibitory effects were augmented with increase in extract concentrations. The *Cladophora* extract displayed variable degrees of antimicrobial activity on different bacteria. The *S. aureus* was found to be more sensitive among the gram positive bacteria, and was more sensitive (widest zones of inhibition) than *B. subtilis*. Among the gram negative bacteria, *S. typhimurium* was found to be more resistant than *P. mirabilis*. In general, the gram negative bacteria were more resistant than the gram positive bacteria. Studies by other researchers revealed same type of results. The *P. aeruginosa* was found to be the most resistant among all the bacteria (without zones of inhibition).

Conclusions

This work represents the screening of antioxidant and antibacterial activities of the *C. glomerata* extract. This

Table 2. Antibacterial activity of *Cladophora glomerata* extract.

Bacteria name	Inhibition zone (mm)						
	100 mg ml^{-1}	50 mg ml^{-1}	25 mg ml^{-1}	12.5 mg ml^{-1}	7.25 mg ml^{-1}	3.125 mg ml^{-1}	1.565 mg ml^{-1}
Salmonella typhimurium	7.7	6.3	-	-	-	-	-
Staphylococcus aureus	22.5	21.8	18	13.8	8.7	-	-
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Bacillus subtilis	11.5	9.2	8.2	-	-	-	-
Proteus mirabilis	15	13.8	12	-	-	-	-

- No activity.

species which was collected from the coast of Caspian sea, showed good but different levels of antioxidant activities in some models studied. The extracts had weak reducing power and nitric oxide scavenging activity but high amount of phenols and polyphenolic compounds. Phenols and polyphenolic compounds were in very good amount and higher than those in some plants such as *D. elbursense* (Ebrahimzadeh et al., 2010b). DPPH-scavenging activity showed potent activity. Also, the *C. glomerata* extract showed more potent antibacterial activity against *S. aureus* and *P. mirabilis* than *S. typhimurium* and *B. subtilis*. In *P. aeruginosa*, the extract showed antibacterial activity. Identification of the antioxidant compounds of this extract will lead to their evaluation in considerable commercial potential in medicine, food production and in the cosmetic industry.

ACKNOWLEDGEMENTS

This research was partially supported by a grant from the Research Council of Islamic Azad University, Science and Research branch, Tehran, Iran. The authors are very grateful to Dr Ebrahimzadeh, of the Pharmaceutical Sciences Research Center, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran, for the help in this research.

REFERENCES

Abourriche A, Charrouf M, Berrada M, Bennamara A, Chaib N, Francisco C (1999). Antimicrobial activities and cytotoxicity of the brown alga *Cystoseira tamariscifolia*. Fitoterapia, 70: 611-614.

Aliev G, Palacios HH, Lipsitt AE (2009). Nitric oxide as an initiator of brain lesions during the development of Alzheimer disease. Neurotox. Res., 16: 293-305.

Androw JM (2001). BSAC Standardized disc suceptibility testing method. J. Antimicrob. Chemother., 7(5): 48-57.

Burrows EM (1991). Seaweeds of the British Isles volume 2 Chlorophyta. Natural history museum publications London.

Chang C, Yang M, Wen H, Chern J (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10: 178-182.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B (2010a). Antihypoxic and antioxidant activity of *Hibiscus esculentus* seeds. Grasas Aceites., 61: 30-36.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Eslami B, Dehpour AA (2010b). Biological and pharmacological effects of *Delphinium elbursense*. Afr. J. Biotechnol., 9(34): 5548-5555.

Danis TCP, Madeira VMC, Almeida MLM (1994). Action of phenolic derivates (acetoaminophen, salycilate and 5-amino salycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophy., 315: 161-169.

Grazul M, Budzisz E (2009). Biological activity of metal ions complexes of chromones, coumarins and flavones. Coord. Chem. Rev., 253: 2588-2598.

Iliopoulou D, Vagias C, Harvala C, Roussis V (2002). C_{15} acetogenins from the red alga *Laurencia obtusa*. Phytochemistry, 59(1): 111-116.

Jiao L, Li X, Li T, Jiang P, Zhang L, Wu M, Zhang L (2009). Characterization and anti-tumor activity of alkali-extracted polysaccharide from *Enteromorpha intestinalis*. Int. Immunopharmacol., 9: 324-329.

Kajiwara T, Matsui K, Akakabe Y, Murakawa T, Arai C (2006). Antimicrobial browning-inhibitory effect of flavor compounds in seaweeds. J. Appl. Phycol., 18: 413-422.

Koleva II, Van Beek TA, Linssen JPH (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis, 13: 8-17.

Leliaert F, Coppejans E (2003). The marine species of *Cladophora* (Chlorophyta) from the South African East Coast. Nova Hedwigia, 76: 45-82.

Le Tutour B, Benslimane F, Gouleau MP, Gouygou JP, Saadan B, Quemeneur F (1998). Antioxidant and pro-oxidant activities of the brown algae, *Laminaria digitata*, *Himanthalia elongata*, *Fucus vesiculosus*, *Fucus serratus* and *Ascophyllum nodosum*. J. Appl. Phycol., 10: 121-129

McDonald S, Prenzler PD, Autolovich M, Robards K (2001). Phenolic content and antioxidant activity of olive extracts. Food Chem., 73: 73-84.

Metzger P, Rager MN, Largean C (2002). Botryolins A and B, two tetramethylsqualene triethers from the green microalga *Botryoccus braunii*. Phytochemistry, 59: 839-843.

Middleton JE, Kandaswami C, Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer, Pharmacol. Rev., 52: 673-839.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7(9): 405-410.

Moncada A, Palmer RMJ, Higgs EA (1991). Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacol. Rev., 43: 109-142.

Porter JB (1997). A risk-benefit assessment of iron-chelation therapy. Drug. Saf., 17: 407-421.

Stirk WA, Reinecke DL, Staden J (2007). Seasonal variation in antifungal, antibacterial and acetylcholinesterase activity in seven South African seaweeds. J. Appl. Phycol., 19: 271-276.

Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T (2005) Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. Res., 579: 200-213.

Sreejayan N, Rao MNA (1997) Nitric oxide scavenging by curcuminoids. J. Pharmacol., 49: 105-107.

Vlachos V, Critchley AT, Holy A (1999). Differential antibacterial activity of extracts from selected Southern African macroalgal thalli. Bot. Mar., 42: 165-173.

Volka RB, Furkert FH (2006). Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. Microbiol. Res., 161: 180-186.

Yildirim A, Mavi A (2001) Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem., 49: 4083-4089.

Yuan YV, Bone DE, Carrington MF (2005). Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated in vitro. Food Chem., 91: 485-494.

Zubia M, Robledo D, Freile-Pelegrin Y (2007). Antioxidant activities in tropical marine macroalgae from the Yucatan Peninsula, Mexico. J. Appl. Phycol., 19: 449-458.