Research Article

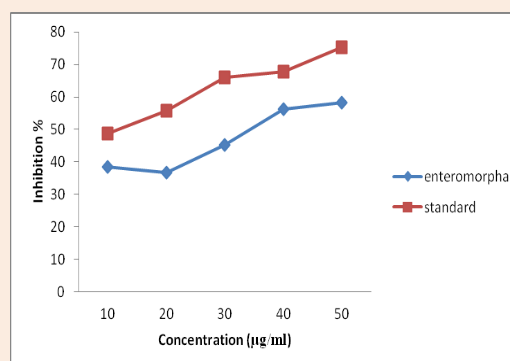
Studies on Free Radicals Scavenging Potential of Seaweed *Enteromorpha sp.*

R. Subashini* and R. Prasanth

Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar 608002, Tamil Nadu, India

Abstract

Enteromorpha sp is one of the filamentous green-algal genus and has a widespread distribution in Caspian Sea Coast. **Objectives**: The current study was initiated to explore the free radical potential of Enteromorpha. **Methods**: The green seaweed, Enteromorpha sp was collected from Mandapam coastal region, Rameswaram, Tamilnadu. The collected seaweed was extracted by using two solvents namely, ethanol and water. The antioxidants activity of Enteromorpha was determined by free radical scavenging assays such as DPPH, ABTS, Superoxide anion, Hydroxyl ion and Nitric oxide radical assay. **Results**: The ethanolic extract of Enteromorpha sp has exhibited better scavenging activity when compared to the aqueous extract. **Conclusion**: The findings evidently showed that the scavenging activity which revealed that the antioxidant properties might be due to the presence of Phenolic compounds.



Keywords: Enteromorpha sp, Free radical scavenging, Antioxidants

***Correspondence**
R.Subashini,
Email: dr.r.subashini@gmail.com

Introduction

Seaweed is the most accessible marine resource of the coastal zone that occupies potential importance source of biochemical compound. As more than 70% of the world's surface is covered by oceans, the wide diversity of marine organisms offer a rich source of natural products [1]. Marine environment contains a source of functional materials, including polyunsaturated fatty acids, polysaccharides, essential minerals and vitamins, antioxidants, enzymes and bioactive peptides. Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. A marine algae in human consumption has been documented since 600 BC.

Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers have revealed that marine algal originated compounds exhibit various biological activities [2]. While marine algae have traditionally formed part of the oriental diet, especially in Asian-pacific region; their major use in Western countries has traditionally concentrated on the extraction of compounds used by pharmaceutical, cosmetics, and food industries [3]. Nowadays, the field of marine natural products becomes more sophisticated. Hence, most consumers prefer additive-free foods or a safer approach like the utilization of more effective antioxidants of natural origin.

ROS, along with reactive nitrogen species (collectively labelled RS) have been identified as agents in various pathogenic diseases and deleterious clinical conditions related to human health. These include cancer, cardiovascular disease, atherosclerosis, hypertension, ischemia / re-perfusion, diabetes mellitus, hyperoxaluria, neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, rheumatoid arthritis and ageing [4-12]. There is ample evidence that reactive oxygen species (ROS) generated in the human body can cause oxidative damages associated with many

degenerative diseases such as atherosclerosis, coronary heart diseases, aging and cancer [13]. Recently, various phytochemicals like polyphenols, which are widely distributed in plants, have been reported to act as free radical scavengers [14]. Marine plants like seaweeds also contain high amount of polyphenols.

Pharmaceutical importance of seaweed is well known all over the world and extensive efforts were given to bring out substances from algae. There are a number of reports regarding the medicinal importance of seaweeds belonging to Phaeophyceae, Rhodophyceae and Chlorophyceae from all over the world [15-16]. The green algae genus Enteromorpha has great potential for commercial exploitation because of its abundant and varied chemical composition, and quality and concentration of basic nutriments for other living organisms [17-18]. According to those naturally developed antioxidants such as seaweed, green algae and plants evolved to prevent the excess oxidative stress of cells [19]. Hence, the present study was undertaken to evaluate the free radical potential of Green algae, *Enteromorpha sp*.

Materials and Methods

Chemicals

Methanol, ethanol, acetone, and hexane were at HPLC grade of purity and it was purchased were obtained from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore, India. Folin-ciocalteu's phenol reagent, dpph (1, 1-Diphenyl-2-pikryl-hydrazin) and sodium carbonate purchased from Hi-media Laboratories Mumbai, India. All other chemicals and solvents used were of analytical grade.

Collection of seaweed

The algae biomass was collected from Mandapam Coast, Rameswaram. It was harvested by hand from sea water and washed after with fresh water to remove the salt and sand. After that the biomass was left in freezer for 24 hours, at -70°c. It was performed the lyophilisation and after that, the dry biomass was kept in freezer at -20°C for preservation until the next step.

Preparation of the extract

All samples were brought to the laboratory in plastic bags containing sea water to prevent evaporation. Few collected seaweeds were preserved for identification. Algae samples were cleaned such that epiphytes and necrotic parts were removed. Samples were rinsed with sterile water and shade dried for 7-14 days and ground thoroughly to powder in a kitchen-type blender. The extraction of the sample was carried out with solvents namely: ethanolic and water by macerating the samples in the respective solvents (1:10, w/v) on a rotary shaker at 150 rpm at room temperature (25-30˚c) for 72 hours. The extracts from three consecutive soakings were pooled and filtered using filter paper (whatmann no.4); the obtained filtrate was evaporated and the residues (crude extracts) obtained were suspended in the DMSO to a final concentration of 100 µg/ml; the extracts were stored at -20˚c.

Free radical scavenging activity of seaweed

DPPH radical scavenging activity

The free radical scavenging activity of the seaweed extracts was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) following the method of Blois [20]. This method is based on the reduction of stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical antioxidants in a methanolic solution. In the presence of antioxidants the purple colour of the DPPH radical solution changes to a bright yellow and the intensity of this can be monitored spectrophotometrically. Used as a reagent, DPPH evidently offers a convenient and accurate method for the titration of oxidizable groups of natural or synthetic antioxidants. 0.1 mM solution of DPPH in methanolic was prepared and 1 mL of this solution was

added to 3 mL of seaweed extracts at different concentrations (50, 100, 250, 500 and 1000µg). After 10 minutes, absorbance was measured at 517 nm. All the measurements were measured in triplicates. The percentage scavenging was calculated as follows.

$$\text{Radical scavenging activity (\%)} = [(A_0\text{-}A_1/ A_0) \text{ x100}]$$

Where A_0= Absorbance of control; A_1= Absorbance of sample

ABTS radical scavenging assay

The free radical scavenging activity was also determined by ABTS (2, 2'azino bis (3-ethylbenzo thiazoline-6-sulphonic acid) diammonium salt) radical cation decolourization assay [21]. ABTS was generated by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium per sulphate under darkness at room temperature for 16 hours. The solution was diluted with 50% ethanolic and the absorbance at 734 nm was measured. The ABTS radical cation scavenging activity was assessed by mixing 5ml ABTS solution (absorbance of 0.7 ± 0.05) with 0.1 mL seaweed extract (50, 100, 250, 500 and 1 000µg). The final absorbance was measured at 743 nm with spectrophotometer. The percentage of scavenging was calculated by the following formula,

$$\text{Radical scavenging activity (\%)} = ((A_o\text{-}A_1) / A_o) \text{ x } 100$$

Where A_o is absorbance of control and A_1 is absorbance of sample.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}- Ascorbate EDTA H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao [22]. The hydroxyl radicals attack deoxyribose that eventually results in TBARS formation. The reaction mixture contained in a final volume of 1.0mL, 100µL of 2-deoxy-2-ribose (28mM in potassium phosphate-potassium hydroxide buffer, pH 7.4), 500µL solutions of various concentrations of extracts (50, 100, 250, 500 and 1000 µg) and standard in KH_2PO_4-KOH buffer (20mM, pH 7.4), 200 µL of 1.04mM ethylene diamine tetra acetic acid and 200 µL of 200 µM Ferric chloride, 100 µL of 10 mM hydrogen peroxide and 100 µL of 1.0mM ascorbic acid was incubated at 37°C for 1 hour. The free radical damage imposed on the substrate, deoxyribose was measured as TBARS by the method of Yuan et al. 1.0 mL of thiobarbituric acid (1%) and 1.0 mL of trichloroacetic acid (2.8%) were added to the test tubes and were incubated at 100°C for 30 minutes. After cooling, absorbance was measured at 535nm against control containing deoxyribose and buffer. The percentage scavenging was determined by the comparing the result of the test compound and control using the formula,

$$\text{Radical scavenging activity (\%)} = [(A_o\text{-}A_1/ A_o) \text{ x } 100]$$

Where A_o= Absorbance of control; A_1= Absorbance of sample.

Superoxide anion radical scavenging assay

Measurement of superoxide anion scavenging activity of the extracts was done based on the method described by the slightly modified method of Nishimiki [23]. About 1 mL of nitro blue tetrazolium (NBT) solution (156M NBT in 100mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468M in 100mM phosphate buffer, pH 7.4) and 0.1 mL of sample at various concentrations (50, 100, 250, 500 and 1 000g in distilled water) were mixed and the reaction was started by adding 100 L of phenazine metho sulphate (PMS) solution (60M PMS in 100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance at 560nm was measured against blank samples. The percentage scavenging value was determined as follows.

$$\text{Radical scavenging activity (\%)} = [(A_o\text{-}A_1/ A_o) \text{ x100}]$$

Where A_o = Absorbance of control; A_1 = Absorbance of sample.

Nitric oxide radical scavenging assay

Nitric oxide radicals generated from sodium nitroprusside solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by the Griess reaction [24]. 2 mL of sodium nitroprusside (10 mm) was mixed with 1 mL of the test extracts (50, 100, 250, 500 and 1000µg) in phosphate buffer (pH 7.4). The mixture was incubated at 25° C for 150 minutes. To 0.5 mL of the incubated solution, 1 mL of sulphanilic acid reagent (0.33% sulphanilamide in 20% acetic acid) was added and allowed to stand for 5 minutes for completing diazotization. 1mL of 0.1% napthyl ethylene diamine dihydrochloride was added and incubated at room temperature for 30 minutes.

Absorbance was read at 540nm and percentage scavenging was calculated as follows

$$\text{Radical scavenging activity (\%)} = [(A_o - A_1 / A_o) \times 100]$$

Where A_o = Absorbance of control; A_1 = Absorbance of sample.

Results

Free radical scavenging of *Enteromorpha sp* (ethanolic and aqueous extract)

DPPH radical scavenging activity

Figure 1.1 & 1.2 shows the effect of seaweed extracts (*Enteromorpha sp*) on DPPH radical was compared with standard. The scavenging effect increases with the concentration of standard and samples. All the concentration of *Enteromorpha sp* (ethanolic extract) showed higher activity than the aqueous extract.

ABTS cation scavenging activity

Figure 2.1 & 2.2 shows the effect of seaweed extracts on ABTS cation was compared with standard. The scavenging effect increases with the concentration of standard and samples. All the concentration of *Enteromorpha sp* (ethanolic extract) showed higher activity than the aqueous extract.

Hydroxyl scavenging activity

Figure 3.1 & 3.2 shows the effect of seaweed extracts on Hydroxyl scavenging activity was compared with standard. The scavenging effect increases with the concentration of standard and samples. All the concentration of *Enteromorpha sp* (ethanolic extract) showed elevated activity than the aqueous extract.

Superoxide anion radical scavenging activity

Figure 4.1 & 4.2 shows the effect of seaweed extracts on Superoxide scavenging assay was compared with standard. It indicates that *Enteromorpha sp* (ethanolic extract) exhibited the maximum Superoxide scavenging activity which is drastically higher than the aqueous extract.

Nitric oxide radical scavenging activity

Figure 5.1 & 5.2 shows the effect of seaweed extracts on Nitric oxide scavenging assay was compared with standard. It indicates that *Enteromorpha sp* (ethanolic extract) exhibited the maximum Superoxide scavenging activity which is drastically higher than the aqueous extract.

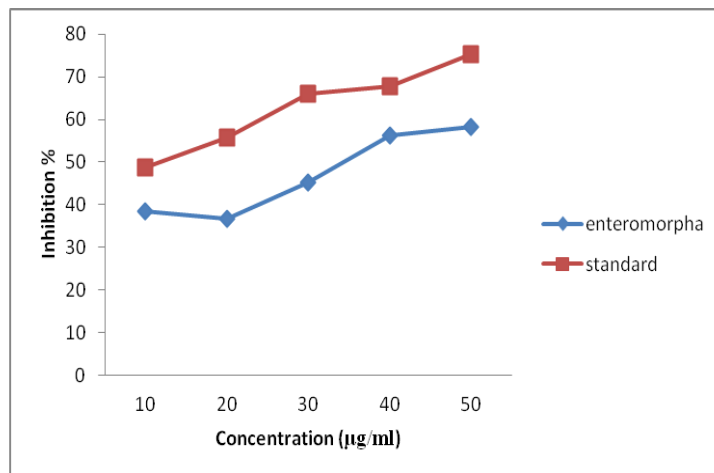


Figure 1.1 Shows the DPPH scavenging activity of *Enteromorpha* (ethanolic extract) compared with standard

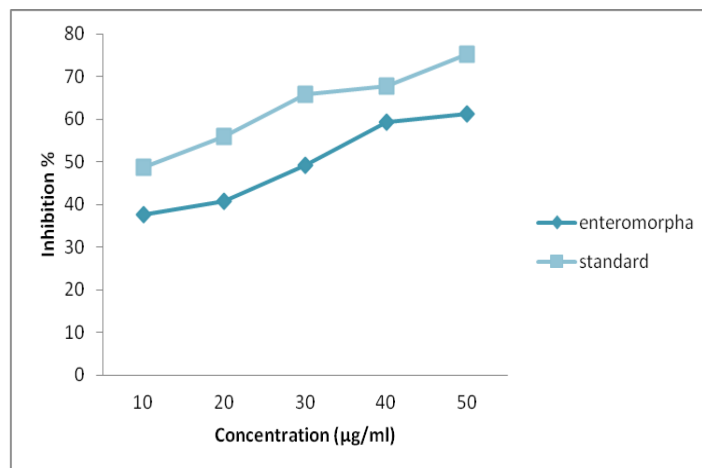


Figure 1.2 Shows the DPPH scavenging activity of *Enteromorpha* (aqueous extract) compared with standard

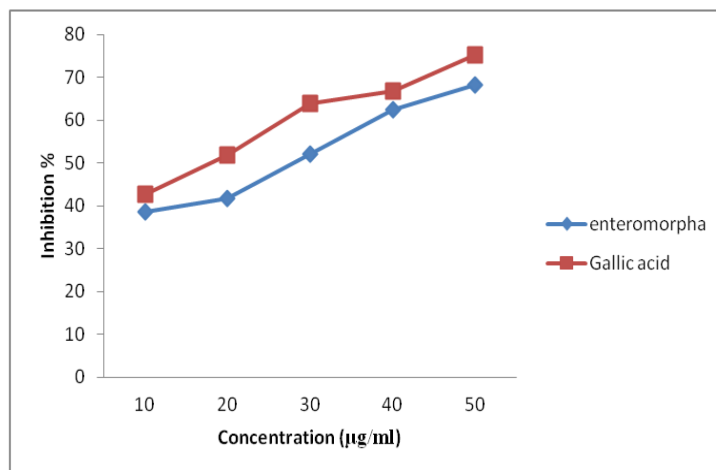


Figure 2.1 Shows ABTS scavenging activity of *Enteromorpha sp* (ethanolic extract) compared with standard

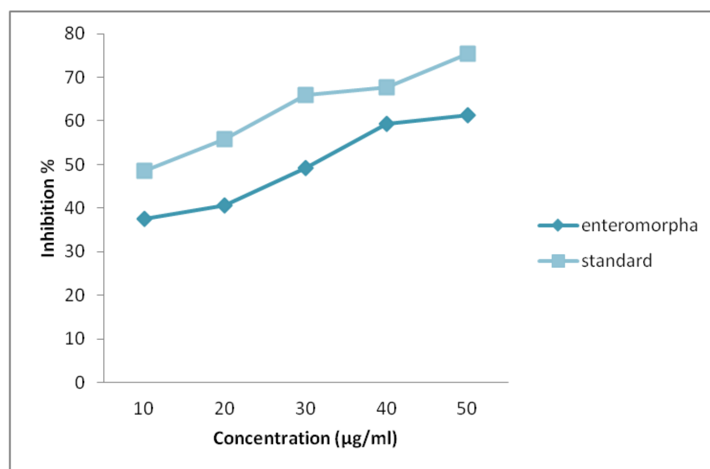


Figure 2.2 Shows ABTS scavenging activity of *Enteromorpha sp* (aqueous extract) compared with standard.

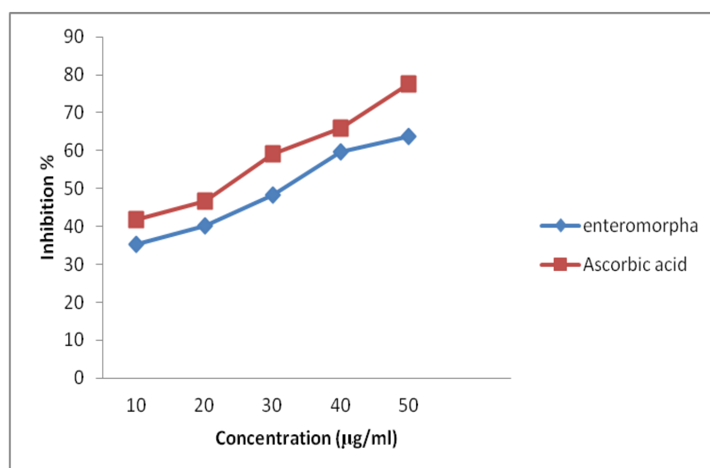


Figure 3.1 Shows the Hydroxyl scavenging activity of *Enteromorpha sp* (ethanolic extract) compared with standard.

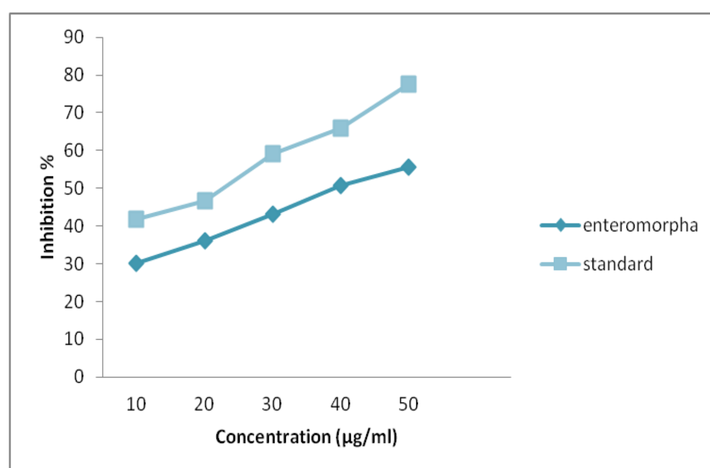


Figure 3.2 Shows the Hydroxyl scavenging activity of *Enteromorpha sp* (aqueous extract) compared with standard.

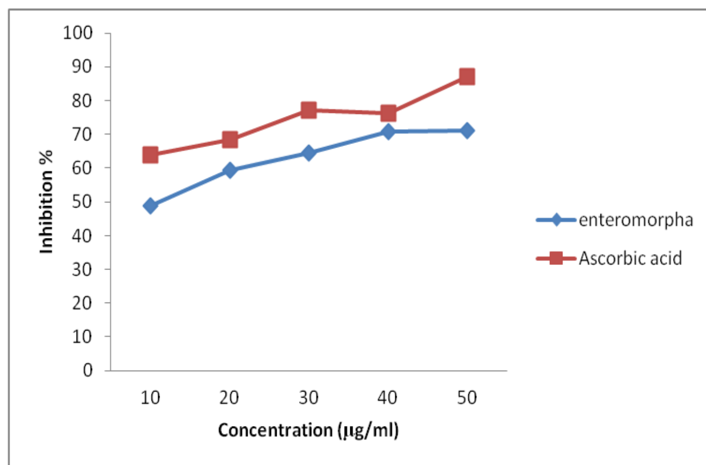


Figure 4.1 Shows the Superoxide anion radical scavenging activity of *Enteromorpha* (ethanolic extract) compared with standard

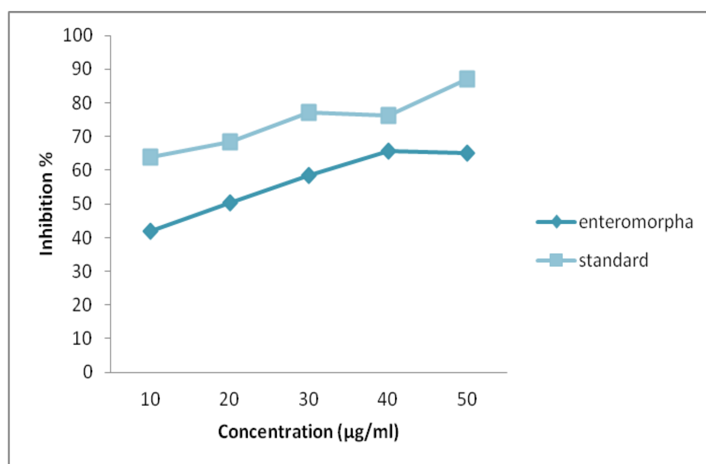


Figure 4.2 Shows the Superoxide anion radical scavenging activity of *Enteromorpha sp* (aqueous extract) compared with standard

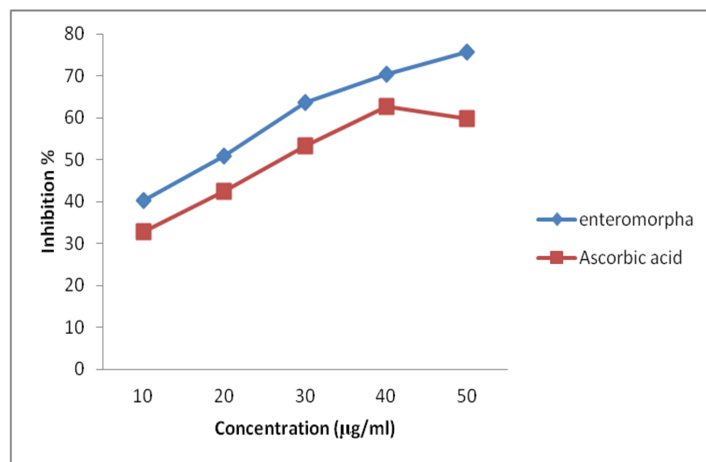


Figure: 5.1 Shows the Nitric oxide scavenging activity of *Enteromorpha* (ethanolic extract) compared with standard

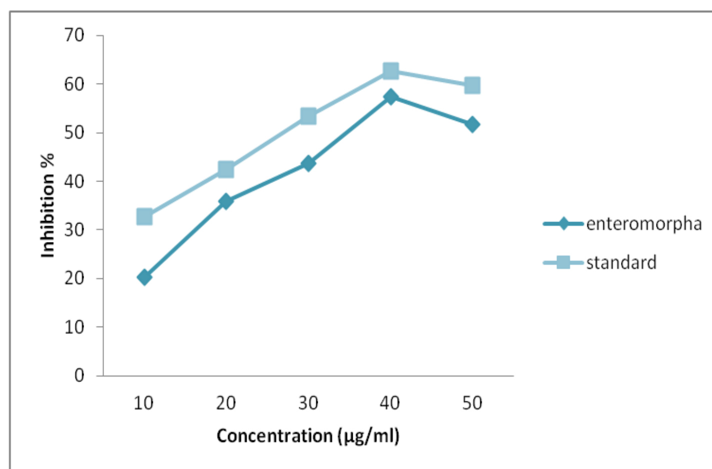


Figure 5.2 Shows the Nitric oxide scavenging activity of *Enteromorpha* (aqueous extract) compared with standard

Discussion

Seaweeds are low in fat but contain vitamins and bioactive compounds like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants [25]. Dietary natural antioxidants are reported to help in preventing aging and other diseases. There are some evidences that seaweeds contain compounds with a relatively high antioxidant and antiproliferative activity. Many metabolites isolated from marine algae have been shown to possess bioactive efforts [26-28]. Among the different compounds with functional properties, antioxidants are the most widely studied. Moreover the important role of antioxidants in human health has been demonstrated thus increasing the interest in such products and their demand by consumers.

Naturally seaweeds are contains novel antioxidant compounds which control the free radical formation from metabolic reaction. Phenols are very important seaweed constituents because of their scavenging ability due to their hydroxyl groups [29]. It was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation [30].

The antioxidant activity of several naturally occurring compounds have been known for decades. Recently, many types of seaweed have been considered as source of reactive oxygen species inhibitors. They can be used as food additive and can also provide protection against tissue oxidation [31]. In our previous communication, we have reported that Nelumbo nucifera leaf extract (NNE) posses free radical scavenging and antioxidant properties in ISO-induced rats [32]. This study revealed that the Seaweed polyphenols possess antioxidant activity to scavenge free radicals.

Antioxidant compounds scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus reduce the level of oxidative stress and slow or prevent the development of complications associated with oxidative stress related diseases [33]. Many synthetic antioxidants have shown toxic and mutagenic effects, which have shifted attention towards naturally occurring antioxidants. A great number of naturally occurring substances like seaweeds have been recognized to have antioxidant abilities [34].

Our findings showed that the data may contribute to a rational basis for the use of antioxidant rich marine algal extracts in the therapy of diseases related to oxidative stress. In addition, the results signify that bioactive compounds might be major contributors to the *antioxidant* activities of Enteromorpha Sp. From the results it was quite evident that, the ethanolic extracts of *Enteromporpha sp* (seaweed) has elevated antioxidant activity when compared to aqueous extract.

Conclusion

The finding of the current report appear useful for further research aiming to isolate, identify and characterize the specific phenolic compounds in *Enteromorpha sp* for its industrial and pharmaceutical purposes. In the future, these marine algae derived compounds will be used more often in pre-clinical studies for drug discovery.

References

[1] Kim SK, Wijesekara I. Development and biological activities of marine derived bioactive peptides: A review. J Funct Foods 2010; 2: 1-9.
[2] Matanjun P, Mohamed S, Mustapha NM, Muhammad K, Ming CH. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. J Appl Phycol 2008; 20(4): 367-373.
[3] Ordonez EG, Escrig AJ, Ruperez P. Dietary fibre and physicochemical properties of several edible seaweeds from northwestern Spanish coast. Food Research International. 2010; 43: 2289–2294.
[4] Cerutti PA. Prooxidant states and tumor promotion. Science. 1985; 227: 375-381.
[5] Borek C. Molecular mechanisms in cancer induction and prevention. Environ. Health Perspect. 101 Suppl. 1993; 3:237-245.
[6] Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress, and aging. Free Radic. Biol. Med. 2000; 29: 222-230.
[7] Park DS, Lee KH, Kim HC, Ahn MJ, Moon CJ, Ko MS, Lee KK, Go GM, Shin TK. Effects of *Callophyllis japonica* powder on carbon tetrachloride-induced liver injury in rats. Orient. Pharm. Exp. Med. 2005; 5:231-235.
[8] Kang KA, Bu HD, Park DS, Go GM, Jee Y, Shin T, Hyun JW. Antioxidant activity of ethanolic extract of *Callophyllis japonica*. Phytother. Res. 2005; 19: 506-510.
[9] Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol. 2007; 39:44-84.
[10] Veena CK, Josephine A, Preetha SP, Varalakshmi P. Beneficial role of sulfated polysaccharides from edible seaweed *Fucus vesicuolosus* in experimental hyperoxaluria. Food Chem.2007; 100:1552-1559.
[11] Hercberg S, Galan P, Preziosi P, Alfarez MJ, Vazquez C. The potential role of antioxidant vitamins in preventing cardiovascular diseases and cancers. Nutrition. 1998; 14:513-520.
[12] Halliwell B. The wanderings of a free radical. Free Radic. Biol. Med. 2009; 46:531-542.
[13] Caroline Delma, Karthikeyan Ramalingam, Vijayabaskar Pandian, Albert Baskar, Ignacimuthu Savarimuthu, Balasubramanian Thangavelu, et al. Abstract A5: Inhibition of tumor cell migration and angiogenesis by sulphated polysaccharides from Sargassum wightii (Greville). Cancer Prevention Res 2008; 1: doi: 10.1158/1940-6207.PREV-08-A5.
[14] Lekameera R, Vijayabaskar P, Somasundaram ST. Potential antioxidant activity of brown alga Loboohora varie. Seaweed Res Utiln 2007; 29(1&2): 55-61.
[15] Kolanjinathan K, Ganesh P, Govindarajan M. Antibacterial activity of ethanolic extracts of seaweeds against fish bacterial pathogens. Eur Rev Med Pharmacol Sci 2009; 13: 173-177.
[16] 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.
[17] Hoppe HA, Levring T, Tanaka Y. Marine Algae in Pharmaceutical Science. Walter de Gruyter and Co., Berlin. 1979.
[18] Chapman VJ, Chapman DJ. Seaweeds and Their Uses. Chapman and Hall, London. 1980.
[19] O'Sullivan AM, O'Callaghan YC, O'Grady MN, Queguineur B, Hanniffy D, Troy DJ, et al. In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. Food Chem. 2010; 126: 1064-1070.
[20] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181: 1199-1200.
[21] Re R, Pellagrini N, Proteggente A, Pannala A, Yang M, Evans CR. Antioxidant activity applying an improved ABTS radical cation decolorizing assay. Free Radical. 1999; 26: 1231-1237.

[22] Kunchandy and Rao. Antioxidant properties of dried kayamo-nori, a brown alga Scytosiphon lomentaria. Food Chem 1990; 89: 617-622.
[23] Nishimiki M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun, 1972; 46: 849-853.
[24] Gulcin I. Antioxidant and antiradical activities of L- Carnitine. Life Sciences. 2006; 78: 803-811.
[25] 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: 1067-1072.
[26] Oh., Kim and Lee. Antimicrobial activities of the bromophenols from the red alga *Odonthalia corymbifera* and some synthetic derivatives. Bioorganic and Medicinal Chem. Lett. 2008; 18: 104-108.
[27] Venkateswarlu, G., K. Panchagnula, L. Aditya and V.S. Gottumukkala. Synthesis, structural revision and biological activities of 4-chloroaurone, a metabolite of marine brown alga *Spatoglossum variabile*. Tetrahedron. 2007; 63: 6909-6914.
[28] Yang, L., L.S. Peng and S. Zhou. Lactones from a brown alga endophytic fungus (No. ZZF36) from the South China Sea and their antimicrobial activities. Bioorganic and Med. Chem. Lett. 2006; 16: 4205-4208.
[29] Wanga T, Jonsdottir R, Olafsdottir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chemistry 2009; 116: 240-248.
[30] Yuan VY, Bone ED, Carrington FM. Antioxidant activity of Dulse (Palmaria palmate) extract evaluated in vitro. Food Chemistry 2005; 91: 485-494.
[31] Alekseyenko TV, Zhanayeva SY, Vendiktova AA, Zvyagintseva TN, Kuznetsova TA, Besednova N. Antitumor and antimetastatic activity of fucoidan, a sulfated polysaccharide isolated from the Okhotsk sea Fucus evanescens brown alga. Bull Exp Biol & Med 2007; 147: 730-732.
[32] Subashini R, Rajadurai M. Evaluation of cardioprotective efficacy of *Nelumbo nucifera* leaf extract on isoproterenol-induced myocardial infarction in Wistar rats. Int J Pharma Biosci 2011; 2:285-94.
[33] Wu XJ, Hansen C. Antioxidant capacity, phenolic content, polysaccharide content of Lentinus edodes grown in Whey permeate based submerged culture. J Food Sci 2008; 73: 434-438.
[34] Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R. Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian balm (Dracocephalum moldavica L) extract. Food Science and Techn. 2007; 40: 239-248.

© 2014, by the Authors. The articles published from this journal are distributed to the public under "**Creative Commons Attribution License**" (http://creativecommons.org/licenses/by/3.0/). Therefore, upon proper citation of the original work, all the articles can be used without any restriction or can be distributed in any medium in any form.

Publication History

Received	29th	Sep 2014
Revised	10th	Oct 2014
Accepted	18th	Oct 2014
Online	30th	Oct 2014