Vol 5 | Issue 1 | 2014 | 36-41.

e - ISSN 2249-7544
Print ISSN 2229-7464

INTERNATIONAL JOURNAL
OF
PHYTOPHARMACY RESEARCH
www.phytopharmacyresearch.com

ANTIOXIDANT ACTIVITIES OF SOME SELECTED SEAWEEDS FROM TUTICORIN COAST, TAMILNADU, INDIA

C. Parthiban, C. Saranya, S. T. Somasundaram, and P. Anantharaman*

CAS in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai – 608 502, Tamilnadu, India.

ABSTRACT

The antioxidant potential of the acetone and ethanol extract of three seaweeds (*Enteromorpha compressa, Turbinaria conoides and Gelidiella acerosa*) were evaluated by total antioxidant activity assay, DPPH radical scavenging assay, hydrogen peroxide radical scavenging assay and ferric reduction assay. Amongst the seaweeds, the acetone extract of *T. conoides* was exhibited the maximum antioxidant activity in the following assays such as total antioxidant activity assay (222.68±2.09 mg ascorbic acid/g), DPPH radical scavenging assay (47.24±1.31%), hydrogen peroxide radical scavenging assay (54.29±1.30%) and ferric reduction assay (2.67±0.003). The higher phenolic content (12.45±0.20mg gallic acid equivalents/g) was also recorded in acetone extract of *T. conoides.* Over all, the acetone extract of *T. conoides* was found to yield better antioxidant activity than the other tested seaweeds and this may be attributed with the higher phenolic content of *T. conoides.* However, further studies still needed to identify the compounds responsible for the antioxidant activity of T. *conoides* for its future application in the field of medicine.

Keywords: Antioxidant activity, *Enteromorpha compressa*, *Turbinaria conoides*, *Gelidiella acerosa*, DPPH radical scavenging, Phenolic content.

INTRODUCTION

The most important and biologically significant free radicals are the radical derivatives of oxygen called Reactive Oxygen Species (ROS). These free radicals contain two unpaired electrons in their outer shell. The most common ROS include the super oxide anion ($O_2^{\cdot -}$), the hydroxyl radical (OH.), Nitric Oxide (NO.), singlet oxygen (O_2) and hydrogen peroxide (H_2O_2). Hydroxyl radicals, though short lived, are the most damaging radicals within the body. Hydrogen peroxide is produced *in vivo* by many reactions and it can be converted to the highly damaging hydroxyl radical or catalyzed and excreted harmlessly as water. If hydrogen peroxide is not converted into water, singlet oxygen is formed. Singlet oxygen, though not a free radical, can cause further reactions and act as a catalyst for free radical formation.

Antioxidant activity is intensively focused due to the currently growing demand from the pharmaceutical industry where there is interest in anti-aging and anti carcinogenic natural bioactive compounds, which possess health benefits [1]. Therefore antioxidants of natural origin are much preferred [2]. The development of safe and effective antioxidants received much attention in recent time. The search for powerful but non-toxic antioxidants from natural sources, especially edible or medicinal plants, is continuing for several years. Recently, the health effects especially the suppression of active oxygen species by phytochemicals like tea; spices and herbs received much attention as natural antioxidants.

Recently, there is a growing interest on the discovery of natural antioxidants, mainly for 2 reasons: (I) there is epidemical and clinical evidence suggesting that consumption of vegetables and fruits reduces the risk of developing chronic disease, e.g., cancer; (II) phytochemicals are generally safer than synthetic chemicals Dastmalchi et al. 2007 [3]. Principal source of antioxidant chiefly include those of herbs, spices, and medicinal plants. There are reports that seaweeds are also rich sources of antioxidant compounds [4, 5]. Seaweeds provide for an excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins, and minerals [6].

The use of seaweed as food and medicine prior to 2000BC found mention in ancient Chinese medicinal literature [7]. Seaweeds also have a number of secondary metabolites that serve as chemical defense mechanisms against herbivores and fouling [8, 9]. It is thus highly probable that algae have the potential to provide an alternative source of leads in solving many biomedical problems, including oxidative damage [10].

Corresponding Author**: P. Anantharaman Email:-** drparaman64@gmail.com

Phenolic compounds play an important role in the antioxidative properties of many plant derived antioxidants [11, 12, 13] and phenolic substances were also reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions. The antioxidant effect of naturally occurring phenolic components has previously been studied in relation to the prevention of coronary diseases and cancer, as well as for age- related degenerative brain disorders [14, 15].

The Gulf of Mannar located along the South East coast of India is adorned with plentiful seaweed resources. But, seaweeds are mainly utilized for agar and algin production. Seaweeds have not found a place in the dietary habits of people in India. But, the rich nutrients and health benefits associated with the seaweeds make them the best low cost supplementary food for the people especially the poor. Hence, these studies were undertaken to investigate the biochemical composition and evaluate the antioxidant potential of the seaweeds such as *Enteromorpha compressa, Turbinaria conoides and Gelidiella acerosa.*

MATERIALS AND METHODS

Collection of Sample

Three seaweed species (*Enteromorpha compressa, Turbinaria conoides and Gelidiella acerosa*) were collected in the coastal area of Tuticorin during March and April 2012. After epiphyte and sand removal, the seaweed samples were washed with clean seawater and fresh water, dried in the shade, sealed in small polyethylene plastic bags, and stored in a medical refrigerator at −20 °C.

ANTIOXIDANT ASSAY

Preparation of extract

Each sample weighed 10 gm was transferred into a conical flask. The seaweed powders were extracted with Acetone and Ethanol in a soxhlet extractor for six hours. The extracts were then concentrated under reduced pressure and the resultant residue was stored in dark at 4ºC until further use.

Evaluation of antioxidant activity

The lyophilized seaweed extracts were dissolved in distilled water at a concentration of 10 mg ml^{-1}. The free radial scavenging activity of the seaweed extracts was evaluated using standard procedures and Gallic acid was used as the reference compound. All analysis were run in triplicates and averaged.

Total polyphenolic compound:

Phenolic contents of crude extracts were estimated by the method of Taga et al. (1984) [16]. Briefly 100 µl of aliquot sample was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin Ciocalteau's phenol reagent was added and the reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Phenolic content are expressed as Gallic acid equivalent per gram).

Total Antioxidant activity

Total antioxidant activity was measured following the method of Prieto et al. (1999) [17]. To 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2359 g of ammonium molybdate (4 mM solution) were mixed together in 250 ml distilled water and labeled as Total Antioxidant Capacity (TAC) reagent. 300 µl of extract was dissolved in 3 ml of TAC reagent. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Reducing Power

Reducing power of different crude extract was determined by the method prescribed by Oyaizu (1986) [18]. 1.0 ml of different solvent extract containing different concentration of samples was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50ºC for 20 min. After incubation, 2.5 ml of Trichloroacetic acid (10% of TCA) was added and centrifuged at 650 rpm for 10 min. From the layer, 2.5 ml solution was mixed with 2.5 ml of distilled water at 0.5 ml of ferric chloride (0.1%). Absorbance of all the solution was measured at 700 nm. Increased absorbance indicates increased reducing power.

Hydrogen peroxide radical scavenging assay

The ability of seaweed extract to scavenge hydrogen peroxide was determined by following the standard procedure [19]. Hydrogen peroxide 10 mM solution was prepared in the phosphate buffer saline of 0.1 M, pH 7.4. 1 ml (0.25 mg) of the extract was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer against a blank (without hydrogen peroxide) after 10 min of incubation at 37ºC. The percentage of scavenging of hydrogen peroxide was calculated using the following formula

% scavenging (H_2O_2) = (A_0-A_1/A_0) X 100

A_0 - absorbance of control

A_1 – Absorbance of sample).

DPPH radical scavenging activity

The Scavenging effects of crude methanol extract and fractions were determined by the method of Yen and Chen (1995) [20]. Briefly, 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The Scavenging effect (%) was calculated by using the formulae given by Duan et al. (2006) [4].

Scavenging effect (%) = [1-(A sample-A blank)/A control] × 100
A control - absorbance of the control (DPPH solution without sample)
A sample - absorbance of the test sample (DPPH solution +Test sample)
A sample blank - absorbance of the sample only (sample without DPPH solution).

RESULTS

Total Phenolic content

The acetone (12.451±0.20 mg gallic acid equivalents g^{-1}) and ethanolic extract (10.32±0.18 mg gallic acid equivalents g^{-1}) of brown seaweed *Turbinaria conoides* showed higher phenolic content than the all other seaweeds used in this experiment (Fig. 1). The minimum total phenolic content was noticed in ethanol extract (5.04±0.28 mg gallic acid equivalents g^{-1}) of green seaweed *Enteromorpha compressa*.

Total Antioxidant activity

Higher antioxidant activity (222.68±2.09 mg ascorbic acid g^{-1}) was observed in acetone extract of *Turbinaria conoides* (231.70±2.64 mg ascorbic acid g^{-1}) followed by ethanolic extract of *T. conoides* (180.50±1.94 mg ascorbic acid g^{-1}). The minimum activity was noticed in ethanol extract of *E. compressa* (129.42±3.07 mg ascorbic acid g^{-1}). The results are presented in Fig. 2.

Reducing power ferric reducing antioxidant power (FRAP)

The maximum (3.676±0.003) reducing power value was observed in acetone extracts in 1 ml concentration of *Turbinaria conoides* and minimum (1.11±0.002) was obtained from ethanol extracts of *Gelidiella acerosa* (Figs. 3, 4).

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide radical scavenging inhibition was more than 54.29% in acetone extract of brown algae *Turbinaria conoides* followed by ethanolic extract (48.52%). Lower inhibition rate of 33.99% was observed in ethanolic extract of *E. compressa* (Fig. 5).

DPPH radical scavenging assay

The higher DPPH radical scavenging activity was observed in the acetone extracts of *Turbinaria conoides* (47.24±1.31%). The minimum radical scavenging activity was observed in ethanol extract of *Gelidiella acerosa* (25.38±0.93%). The results are presented in Fig. 6.

Fig 1. Total phenolic content of selected seaweeds from Tuticorin coast

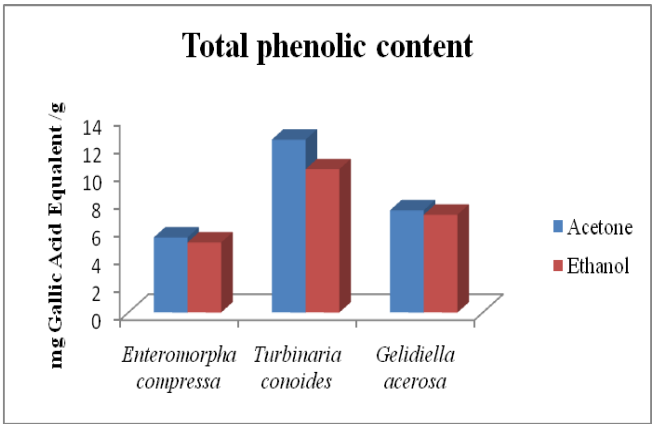


Fig 2. Total antioxidant activity of selected seaweeds from Tuticorin coast

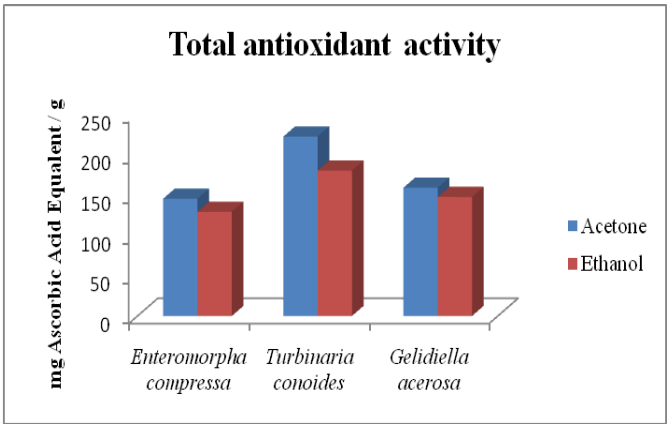


Fig 3. Reducing power of acetone extract of selected seaweeds from Tuticorin coast

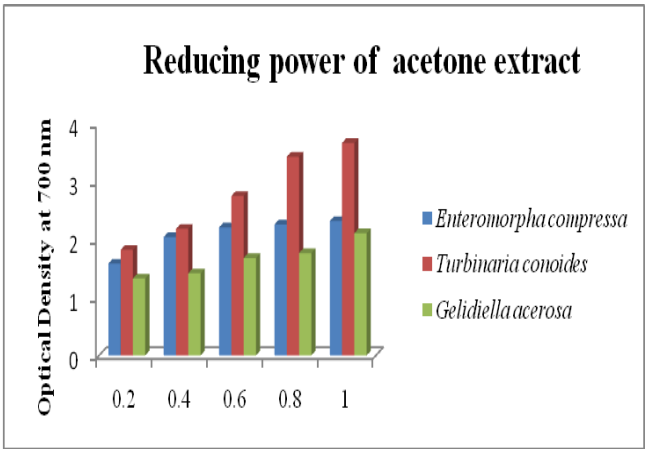


Fig 4. Reducing power of ethanol extract of selected seaweeds from Tuticorin coast

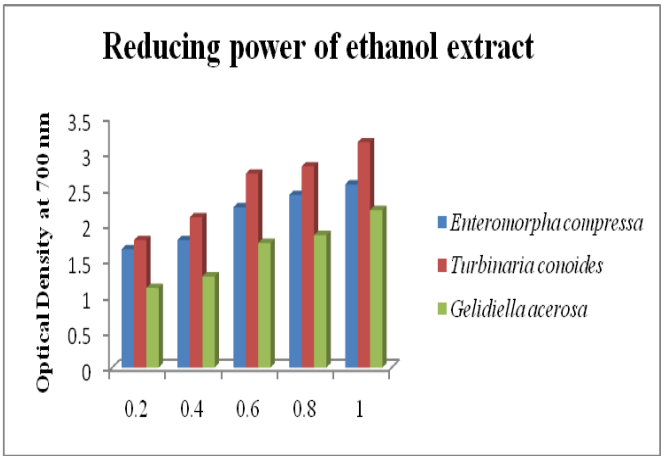


Fig 5. Hydrogen peroxide scavenging activity of selected seaweeds from Tuticorin coast

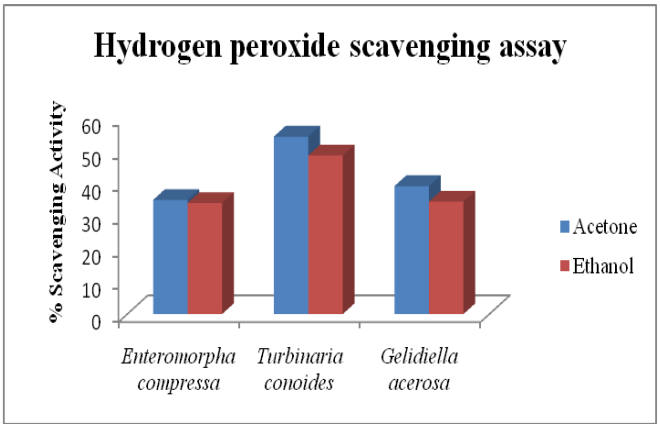
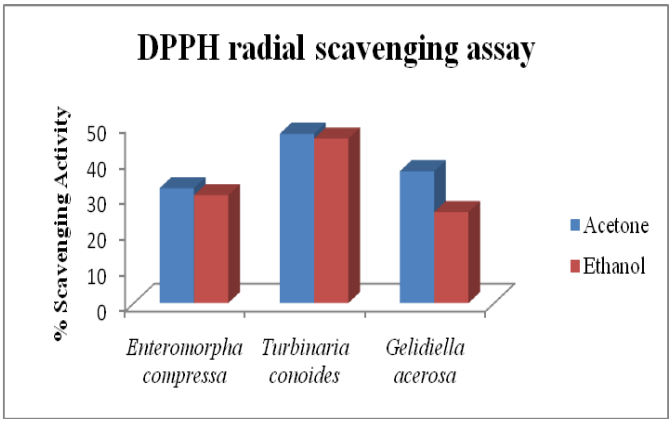


Fig 6. DPPH radical scavenging activity of selected seaweeds from Tuticorin coast



DISCUSSION

Total Phenolic content

The phenolic content of seaweeds was evaluated using the Folin-Ciocalteu method. The variation in phenolic content was quite large. The minimum total phenolic content was noticed in ethanol extract (1.04±0.03 mg gallic acid equivalents g^{-1}) of red seaweed *Hypnea musciformis*. Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties. A number of studies focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers [21-23]. Earlier reports revealed that seaweed extracts, especially polyphenols, have antioxidant activity [5, 24, 25]. The brown seaweed *Dictyota dichotoma* showed higher phenolic contents than all the seaweeds tested. Jimenez-Escrig et al. (2001) [26] also reported similar findings that brown seaweeds contain higher phenolic content than the red seaweeds. Earlier reports revealed that phenolic compounds are one of the most effective antioxidants in brown algae [27].

Total Antioxidant activity

The total antioxidant activity of seaweeds was evaluated and the results are presented as mg ascorbic acid/g (Fig. 2). In phosphor molybdenum method, molybdenum VI (Mo^{6+}) is reduced to form a green phosphate/Mo^{5+} complex. Kumaran and Karunakaran (2007) [28] have reported total antioxidant activity in the range of 245 to 376 mg ascorbic acid equivalents g^{-1} in *Phyllanthus* species. Ye et al. (2009) [29] have noticed higher antioxidant activity (30.50 µmol $FeSo_4$ mg^{-1}) in ethanol extract of brown seaweed *Sargassum pallidum*. Ganesan et al. (2008) [30] noticed higher activity (32.01 mg ascorbic acid equivalent g^{-1}) in ethyl acetate fraction of red seaweed *A. spicifera*. It has been reported that solvents used for extraction have dramatic effect on the chemical species [31].

Reducing Power Ferric Reducing Antioxidant Power (FRAP)

In the Reducing power assay (Figs. 3 and 4), antioxidants in the sample reduce ferric (III) to ferrous (II) in a redox-linked colourimetric reaction [32] that involves single electron transfer. The reducing power indicates that the antioxidant compounds are electron donors and reduce the oxidized intermediate of the lipid peroxidation process, so that they can act as primary and secondary antioxidants [20]. The reducing power of the extracts increased with the increasing the concentration of extracts. Same trend has also been reported by Kumaran and Karunakaran (2007) [28] in methanolic extracts of *Phyllanthus* species.

Hydrogen peroxide radical scavenging activity

H_2O_2 scavenging activities (% inhibition) of acetone and ethanol extracts of seaweeds are presented on Fig. 5. The effect of extracts in scavenging hydrogen peroxide radicals to prevent oxidative degradation of substrate was determined.

DPPH radical scavenging assay

The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. When a DPPH solution is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical form of DPPH is obtained with simultaneous change of the violet colour to pale yellow [33]. Hence DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds [34]. Ganesan et al. (2008) [30] also noticed higher percentage DPPH radical scavenging activity in methanol extract of brown seaweed *T. conoides.*

CONCLUSION

In the present study, the extracts from *T. conoides* were found to possess strong antioxidant activity. The antioxidant mechanisms of seaweed extracts may be attributed to their free radical-scavenging ability. In addition, phenolic compounds appear to be responsible for the antioxidant activity of seaweed extracts. On the basis of the results obtained, seaweeds can be used for a variety of beneficial chemo-preventive effects. However, further studies on the antioxidative components of seaweed extracts and more in vivo evidence are required.

ACKNOWLEDGEMENTS

Authors are thankful to higher authorities of CAS in Marine Biology, Annamalai University, Parangipettai for providing with facilities and support. Authors are also thankful to University Grant Commission, Govt. of India, New Delhi for providing financial support throughout the study period.

REFERENCES

1. Matsukawa R, Dubinsky Z, Kishimoto E, Masak K, Masuda Y. A comparison of screening methods for antioxidants activity in seaweeds, *J Appl Phycol*, 9, 1997, 29-35.
2. Gülçin I, Büyükokuroglu MF, Oktay M, Küfrevioglu ÖI. On the in vitro antioxidant properties of melatonin. *J Pineal Res*, 33, 2002, 167–171.
3. 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. *LWT- Food Sci Technol*, 40, 2007, 239-248.
4. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem*, 95, 2006, 37-43.
5. Kuda T, Tsunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *J Food Comp Anal*, 18, 2005, 625-633.
6. Bhaskar N, Miyashita K. Lipid composition of *Padina tetratomatica* (*Dictyotales, Pheophyta*), a brown sea weed of the west coast of India. *Ind J Fish*, 52, 2005, 263-268.
7. Abbott J. Sharing the city: community participation in urban management. Earthscan Publication, London, 1996.
8. De Nys R, Dworjanyn SA, Steinberg PD. A new method for determining surface concentrations of marine natural products on seaweeds. *Mar Ecol Prog Ser*, 162, 1998, 79-87.
9. De lara Isassi G, Alvarez-Hernandez S, Collado-Vides L. Ichtyotoxic activity of extracts from Mexico marine macroalgae. *J. Appl. Phycol*, 12, 2000, 45-52.
10. Ruberto G, Baratta MT, Kaabeche M. Chemical composition and antioxidant activity of essential oils from Algerian Origanum glandulosum Desf. *Flavour Frag J*, 17, 2002, 251–254.
11. Canadanovic-Brunet JM, Djilas SM, Cetkovic GS, Tumbas VT, Mandic AI, Canadanovic VM. Antioxidant activities of different *Teucrium montanum* L extracts. *Int J Food Sci Technol*, 41, 2006, 667-673.
12. Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO. Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron-a Garcinia kola seed extract. *Food Chem Toxicol*, 38, 2000, 535–541.
13. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables – the millennium's health. *Int J Food Sci Technol*, 36, 2001, 703–725.
14. Stoclet JC, Chataigneau T, Ndiaye M, Oak MH, El Bedoui J, Chataigneau M, Schini-Kerth VB. Vascular protection by dietary polyphenols. *Eur J Pharma*, 500, 2004, 299-313.
15. Stevenson DE, Hurst RD. Polyphenolic phytochemicals-just antioxidants or much more. *Cell Mol Life Sci*, 64, 2007, 2900-2916.
16. Taga MS, Miller EE, Pratt DE. Chia seeds as a source of natural lipid antioxidants. *J Amer Oil Chem Soc*, 61, 1984, 928-931.
17. Prieto P, Pineda M, Aguilar M. Spectrophometeric quatitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*, 269, 1999, 337-341.
18. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. *Japn J Nutri*, 44, 1986, 307-315.
19. Gulçin G, Beydemir O. Kufrevio. Evaluation of the *in vitro* antioxidant properties of extracts of broccoli (*Brassica oleracea* L). *Italian J Food Sci*, 16, 2004, 17-30.
20. Yen GH, Chen HY. Antioxidant activity of various tea extract in relation to their antimutagenicity. *J Agri Food Chem*, 43, 1995, 27-32.
21. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Rad Res*, 22, 1995, 375-383.
22. Sugihara A, Arakawa T, Ohnishi M, Furuno K. Anti and pro-oxidative effects of flavonoids on metal induced lipid hydroperaxidase-dependant lipid peraxidation in cultured hepatocytes located with α-linolic acid. *Free Rad Bio Med*, 27, 1999, 1313-1323.
23. Kahkonen MP, Hopia AI, Vourela HJ, Rauha J, Pihlaja K, Kujala TH, Heinonen M. Antioxidant activity plant extracts containing phenolic compounds. *J Agri Food Chem*, 47, 1999, 3954-3962.
24. Yan X, Li X, Zhou C, Fan X. Fucoxanthin as the major antioxidant in Hijikia fusiformis, common edible seaweed. *Biosci Biotechnol Biochem*, 63, 1999, 605-607.
25. Lim SN, Cheung PCK, Ooi VEC, Ang PO. Evaluation of antioxidative activity of extracts from brown seaweed, *Sargassum siliquastrum*. *J Agri Food Chem*, 50, 2002, 3862-3866.
26. Jimenez- Escrig A, Jimenez-Jimenez I, Pulido R, Saura-Calixto F. Antioxidant activity of fresh and processed edible seaweeds. *J Sci Food Agri*, 81, 2001, 530-534.
27. Nagai T, Yakimoto T. Preparation and functional properties of beverages made from sea algae. *Food Chem*, 81, 2003, 327-332.

28. Kumaran A, Karunakaran RJ. In vitro antioxidant properties of methanol extracts of five Phillanthus species from India. *LWT-Food Sci Technol*, 40, 2007, 344-352.
29. Ye H, Zhou C, Sun Y, Zhang X, Liu J, Hu Q, Zeng X. Antioxidant activities in vitro of ethanol extract from brown seaweed *Sargassum pallidum*. *Eur Food Res Technol*, 230(1), 2009, 101-109.
30. Ganesan P, Kumar CS, Bhaskar N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Biores Technol*, 99, 2008, 2717-2723.
31. Yuan YV, Carrington MF, Walsh NA. Extracts from dulse (*Palmaria palmata*) are effective antioxidants and inhibitors of cell proliferation in vitro. *Food Chem Toxicol*, 43, 2005, 1073–1081.
32. Li YF, Guo CJ, Yang JJ, Wei JY, Xu J, Cheng S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem*, 96, 2006, 254–260.
33. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for the Antioxidant activity, Song Kla nakarin. *J Sci Technol*, 26(2), 2004, 211-219.