International Journal of Biology Research
ISSN: 2455-6548
Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 3; Issue 1; January 2018; Page No. 319-323

Biosynthesis of silver nanoparticles using *Sargassum tenerrimum* and evaluation of their antioxidant activity

A Mohamed Ismail[1*], M Parthasarathy[2], H Sheik Jahabar Ali[3]

[1] Associate Professor & Head, Department of Biotechnology, Edayathangudy. G.S. Pillay Arts & Science College, Nagapattinam, Tamil Nadu, India

[2, 3] Biotechnology Research Laboratary, Department of Biotechnology, Edayathangudy. G. S. Pillay Arts & Science College, Nagapattinam, Tamil Nadu, India

Abstract
The synthesis of Silver nanoparticles using *Sargassum tenerrimum* (powder or extract) is demonstrated here. The rapid formation of stable silver nanoparticles has been found using *S. tenerrimum* extract in aqueous medium at normal atmospheric condition. The antioxidant activity was determined by means of the Total phenolic content, Total antioxidant (TAA), De-oxyribose radical scavenging activity and DPPH radical scavenging test using different fractions of methanol extract. Result from these three methods indicated that the antioxidant activity of *S. tenerrimum* of methanol extract were time and concentration dependent. TPC exhibited higher activity in the methanolic extraction (6.26±0.45 mg GAE/g). TAA of methanolic extract were noted higher activity (0.77±0.17). The Deoxy- ribose radical scavenging activity (3.02±0.02% inhibition) of total methanolic extract and fractions of *S. tenerrimum.* The results showed that there was highest DPPH scavenging activity (1.53±0.44 %) hexane. In FT-IR analysis the major peaks were 3354.21 (Alcohol phenol O-H stretch) and 2972.31 (Alkyl C-H stretch).

Keywords: *Sargassum tenerrimum,* silver nanoparticles, antioxidant activity, TPC, TAA, deoxy- ribose radical scavenging activity, DPPH, FT-IR

Introduction

Seaweeds are marine macro algae are potential renewable resource in the marine environment. About 6000 species of seaweeds have been identified and are grouped into different classes viz., green (chlorophytes), brown (phaeophytes) and red (rhodophytes) algae. The total global seaweed production of the year 2004 was 15 million metric tones of which nearly 15-20% is contributed by Indian Ocean region (FAO 2006). Seaweed harvest across Indian coast is about 100000 metric tones (wet weight) (Dhargalkar and Pereira, 2005). Seaweeds provides for an excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals (Bhaskar and Miyashita, 2005; Florence, 1999; Nisizawa, 1988) [2, 18]. In Asian countries, Japanese are the main consumers of seaweed with an average of 1.6kg (dry weight) per year per capita (Fujiwara-Arasaki, *et al*., 1984) [8]. However in India seaweeds are exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan and not as culinary item or for recovering beneficial biomolecules.

Seaweeds belong to a group of plants known as algae nutrient and chemical composition. Like other plants, seaweeds contain various inorganic and organic substances which can benefit human health (Kuda *et al.*, 2002) [14]. 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) [21, 1, 3].

Antioxidant activity has become a hot topic and the subject of intensive investigation due to the ever increasing demand by the food and pharmaceutical industries to develop natural bioactive anti-aging and anti-carcinogenic compounds that demonstrate measurable health benefits. Antioxidant from biosources have created deep interest among researchers, food manufactures, and cosumers due to their protective role against dreadful diseases such as coronary heart disease and cancer (Loliger, 1991). The search for novel antioxidants bimolecule with high phenolic content has become of their important issue, become of their inhibitor role in on mutagenesis and carcinogenesis in human beings. Antioxidative substances obtained from natural source, such as seed oil, grains, beans, vegetables, fruits, leafwaxes, burk, roots, species and hulls, have already been investigated (Fujimoto *et al.*, 1985; Guiry and Blunden 1991; Gordon *et al*.,1993; Dush, 1999) [7, 10]. However, there are very few studies in the literature on antioxidant activity associated to sulfated polysaccharides from seaweeds.

The term phenolic compounds describes several hundred molecules found in edible plants that possess on their structure a benzenic ring substituted by at least, one hydroxyl group (Manach *et al.*,2004) [16].These phenolic compounds are commonly found in plants, including seaweeds (Duan *et al.*,2006) [4]. Polyphenols represent a diverse class of

compounds including flavonoids (i.e. flavones, flavonols, flavanones, flavonols, chalcones and flavan-3-ols), ligins, tocopherols, tannis and phenolic acids (Shukla *et al.*, 1997) [20]. Flavonoids, the largest group of phenolic compounds are known to contain a broad spectrum of chemical and biological activities including antioxidants and free radicals scavenging properties (Kahkonen *et al.*, 1999). Flavonoids include flavonols, flavones, catechins, proanthocyanidins, anthocyanidins and isoflavonoids (Ndhlala *et al.*, 2007). Phenolic compounds are important in plant defence mechanisms against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet (UV) radiation (Harborne, 1994; Herrmann, 1989) [12] the objective of present study was to analysis the *invitro* antioxidant activities of brown alga *S. tenerrimum*.

Materials and Methods

Collection and processing of seaweeds

The collected seaweeds were washed with seawater to remove all the epiphytes and sand particles then washed thoroughly in fresh water (3 - 4 times) to remove the salts and extraneous materials. Morphologically distinct thallus of seaweeds was placed separately in new polythene bags and were kept in an icebox containing slush ice and transported to the laboratory. Then the seaweeds were spread on blotting paper to remove excess water and shadow dried for few days and then in an oven at 60ºC until constant weight obtained. Then they were cut into small pieces and made into powder for analysis for activities.

Preparation of the seaweed extracts

Finely dry ground algae of the investigated *S. tenerrimum* (50g) were extracted using methanol solvents in a soxchlet extractor. The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator. The dry extracts were stored at -18ºC until they were used in the experiment.

Preparation of seaweed extracts and fractions

First extraction of seaweed was prepared by pouring methanol into the bottle containing 50 g of seaweed powder at the ratio of 10:1 (v/w), the mixture was flushed with kept under orbital shaking incubator at room temperature (29 ± 2 ºC) for 24 h under dark condition. Then the methanol extract were purified using diffenent solvents like hexane, chloroform, acetone and aqueous extract. Then the methanol extract and their fractions of were pooled together and evaporated under reduced pressure using rotary flash evaporator. The fraction of each sample was weighed and then dissolved in 90% aqueous methanol for fractionation. Methanol fraction was further fractionated into different solvent fractions as per Duan *et al.* (2006) [4]. Briefly, first fractionation was carried out with 100 ml hexane. Methanol fraction was collected and chloroform fraction finally water, methanol, ethanol ratio from (1:6:3) phase was evaporated under reduced pressure to give a semisolid. Then semisolid portion was dissolved in 200 ml distilled water and further fractionated with Resulting fractions including aqueous were evaporated to dryness. Dried fractions were dissolved in methanol and stored in colored vials for further analysis. Extracts used for all experiments were at the concentration activity estimations.

Fig 1: Fractions obtained through silica gel chromatography

Total phenolic content

The total phenolic content of the extract was estimated by the Folin-ciocalteu method (Malick Singh, 1980.). Two hundred microlitres of diluted sample were added to 1ml of 1:10 diluted Folin-ciocalteu reagent. After 4 minutes, 800µl of saturated sodium carbonate (75g) was added after 2 h of incubation at room temperature. The absorbance at 765 nm was measured using the perkin Elmer lamba 25UV – Vis spectrophotometer. The results were expressed as milligrams of gallic acid equivalents (GAE)/g dry weight of *S. tenerrimum*, and calculated as mean value±SD (n=3)

Determination of total antioxidant activity (Prieto *et al.* 1992)

Briefly 0.3ml of sample solution (0.1 mg /ml) was mixed with 3.0ml of reagent solution (0.6M sulfuric acid 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95˚C for 90 min under water bath. Absorbance of all the sample mixture was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalent of ascorbic acid. A calibration curve of ascorbic acid was prepared and the total antioxidant activity was standardized against ascorbic acid equivalents /g of sample on a dry weight basis.

Reducing Power

The Reducing power of *S. tenerrimum* was determined according to the method of (Oyaizu, 1986). Ten mg of *S. tenerrimum* extract in 1ml of distilled water was mixed with phosphate buffer (2.5ml, 0.2M, PH 6.6) and potassium ferric cyanide 2.5 ml of (1%). The mixture was incubated at 50ºC for 20 minutes. A portion (2.5ml) of trichloro acetic acid (10%) was added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and ferric chloride 0.5ml, (0.1%) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

De-oxyribose radical scavenging activity

De-oxyribose non- site specific hydroxyl radical scavenging activity of crude extracts was determined according to the method, Henug *et al.* (1997), briefly 2.0ml aliquots of sample were added to the test containing reaction mixture of 2.0 ml $FeSo_4$, $7H_2O$ (10mM), 0.2ml EDTA (10mM) and 0.2ml de-oxyribose (10mM). The volume was made upto 1.8ml with phosphate buffer (0.1M, PH7.4) and to that 0.2ml H_2O_2 (10mM) was added. The mixture was incubated at 37^0 c under dark for 4 h. After Incubation 1ml of TCA (2.8%) and TBA (1%) were added to the mixture, and then left to stand under boiling water bath for 10 min. After treatment absorbance was measured at 532nm. If the mixture was turbid, the absorbance was measured after filtration, scavenging activity (1%) was calculated using the equation given by Heo *et al.*, 2005.

DPPH scavenging activity determination

The free radical scavenging activity of *S. tenerrimum* was determined by bloi's method (Blois, 1958). One ml of the extract solution were added into 0.1mM of 1,1-diphenyl-2-picryl-hydrazil (DPPH) methanol solution. After 30 min of incubation, the absorbance was measured at 517 nm.

FT-IR analysis of seaweed extracts

FT-IR spectroscopy of *S. tenerrimum* Methonal extracts were tested using Perkin Elmer- FT-IR instrument. 5mg extract mixed 10 mg of KBr separately and then compressed to prepare a salt disc. The disc was subjected to FT-IR spectroscopy analysis in the frequency range of 400-4000 cm-1.

Results

The methanolic extract of *S. tenerrimum were* purified with different fractions like chloroform, acetone and aqueous. The Total phenolic content (TPC) exhibited higher activity than methanol extraction maximum (6.26±0.45 mg GAE/g) and minimum value hexane fraction of (0.81±0.05mg GAE/g) following order by methanol, acetone, chloroform, aqueous and hexane fractions of different based on their polarity the results were observed. Fig- 2. The Total antioxidant activity of methanolic extract were noted higher activity (0.77±0.17) and of aqueous fraction (0.15±0.01) were recorded the minimum activity Fig- 3.

Concentration dependency of antioxidant activity was investigated as a function of reducing power as shown in Fig 4. The reducing capacity of various methanolic extract and their fractions behaved in a dose dependent manner of different solvent (0.24±0.01 to 2.37±0.04 mg/mL), the Methanol extract was maximum activity (2.37±0.04 mg/mL) and minimum in Hexane fraction (0.24±0.01).

The Deoxy- ribose radical scavenging activity (3.02±0.02% inhibition) of total methanolic extract and fractions of brown seaweed (*S. tenerrimum)* were presented in Fig 5. The deoxyribose assay is that it involves the hydroxyl radical which is the most active reactive. Lower inhibition rate of 1.32±0.06% was observed in Hexane fraction of *S. tenerrimum*.

S. tenerrimum were assessed by the DPPH assay the activity shown in the Fig-6. The results were highest DPPH scavenging activity (1.53±0.44 %) hexane fraction and the minimum activity were represented the aqueous fraction (0.94±0.11%).

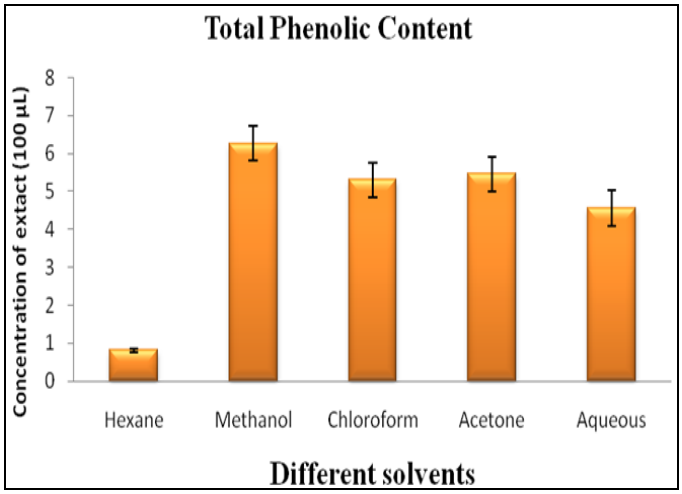

Fig 2: Total Phenolic content

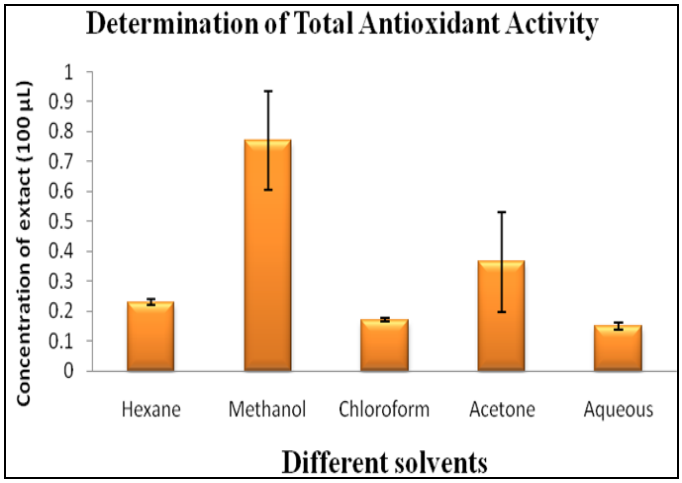

Fig 3: Determination of total antioxidant activity

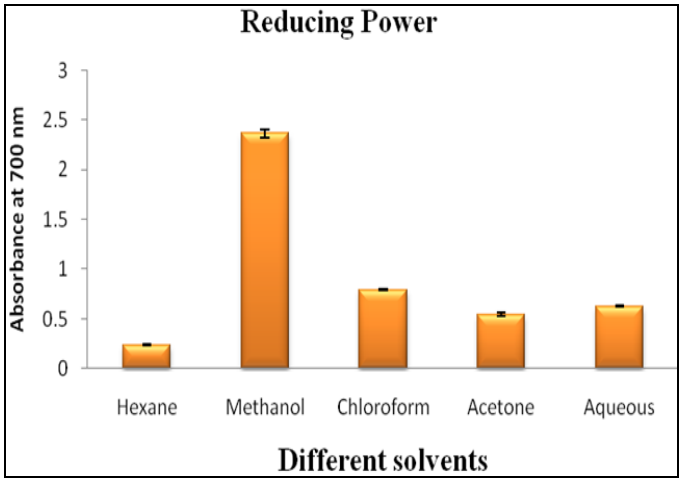

Fig 4: Reducing power

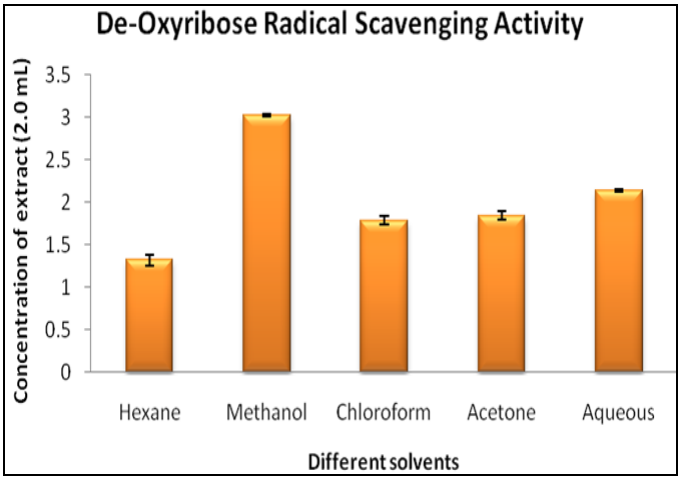

Fig 5: De-oxyribose radical scavenging activity

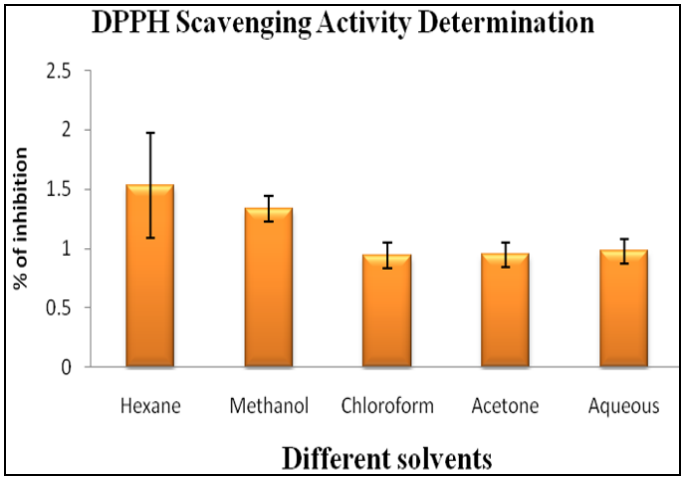

Fig 6: DPPH scavenging activity determination

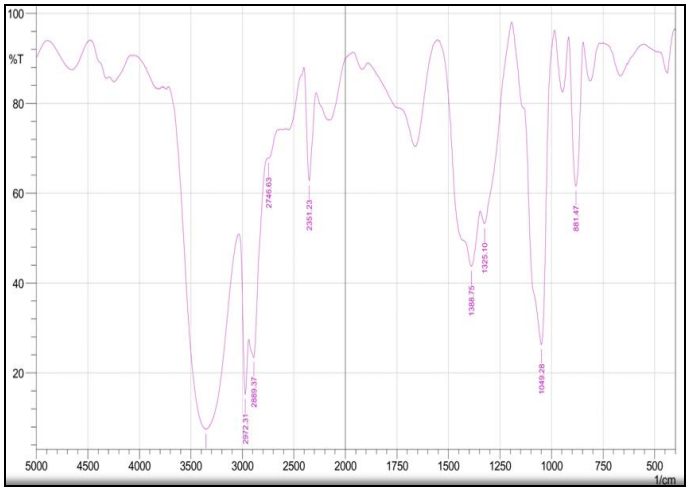

Fig 7: FT-IR analysis of *S. tenerrimum*

Table 1: Shows the FT-IR spectral functional assigments of *S. tenerrimum*

S. No	Peak	Functional groups
1	881.47	C-N stretch Nitro NO_2 compounds
2	1049.28	S=O in alkyl sulfoxides
3	1325.1	S=O stretches in covalent sulfates
4	1388.75	CH_3 sym deformation Methyl
5	2351.23	RNH3 $^+X^-$ stretch, Amine hydrohalides
6	2746.63	RNH3 $^+X^-$ stretch, Amine hydrohalides
7	2889.37	CH stretches in alkenes (Methylene CH_2)
8	2972.31	Alkyl C-H stretch
9	3354.21	Alcohol phenol O-H stretch

The representative IR spectra studies in the mid-infrared region (4000-400 cm-1) for methanolic extracts of *S. tenerrimum* shown in Fig.7 and Table 1. The methanolic extract of FT-IR spectral data were observed 9 peak position were assigned. According to the crude extract spectrum reveals the following functional groups were highly related to antioxidant properties. In FT-IR analysis the major peaks were 3354.21 (Alcohol phenol O-H stretch) and 2972.31 (Alkyl C-H stretch).

Summary and Conclusion

The present study chosen seaweed to investigate the *in vitro* antioxidant properties and its functional assignments using FT- IR analysis. The total phenolic content (TPC) of three different extracts (hexane, methanol, chloroform, acetone and aqueous) of *S. tenerrimum* was expressed as milligrams GAE/g. The maximum total phenolic content were registered Methanol extraction maximum (6.26±0.45 mg GAE/g) and minimum value hexane extract of (0.81±0.05mg GAE/g), the Total antioxidant activity of total methanolic extract the higher activity of (0.77±0.17) and fractions of *S. tenerrimum* are presented. Lowest value of aqueous extract (0.15±0.01).

The reducing power activities were investigated as a function of (0.24±0.01 to 2.37±0.04 mg/mL), the methanol extract was maximum activity (2.37±0.04 mg/mL) and hexane extracts showed better reducing power than (0.24±0.01). The Deoxyribose scavenging activity (3.02±0.02% inhibition) of tmethanolic extract and fractions of brown seaweed (*S. tenerrimum)* are presented. Lower inhibition rate of 1.32±0.06% was observed in hexane fraction of *S. tenerrimum*. The evaluation of radical scavenging activity of seaweed material were *S. tenerrimum was* assessed by the DPPH assay by different solvent used, determined the activity were highest DPPH scavenging activity (1.53±0.44 %) among

the minimum activity were (0.94±0.11%) seaweed as a good source of natural antioxidants.

The representative IR spectra studies in the mid-infrared region (4000-400 cm-1) for methanolic extract of *S. tenerrimum*. The methanolic extract of FT-IR spectral data were observed 9 peak position were assigned. According to the crude extract spectrum reveals the following functional groups were highly related to antioxidant properties. In FT-IR analysis the major peaks were 3354.21 (Alcohol phenol O-H stretch) and 2972.31 (Alkyl C-H stretch).

It can be concluded that seaweeds or marine macroalgae can be utilized as a source of natural antioxidant compounds as their crude extracts and fractions exhibit antioxidant activity. The results indicate that different solvent fractions obtained from total (methanolic) extract exhibit higher antioxidant activities as compared to the total extract. The findings of this work are useful to further research to identify, isolate and characterize the specific compound which is responsible for higher antioxidant activity. Bioactive compounds found in seaweeds await a major breakthrough for a variety of food/medical applications as they have the potential for application of such compounds as natural antioxidants in different food/ pharmaceuticals products.

References

1. Bansemir A, Blume M, Schroder S, Lindequist U. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture. 2006; 252:79-84.
2. Bhaskar N, Miyashita K. Lipid composition of *Padina tetratomatica* (Dictyotales, Phaeophyta), brown seaweed of the west Coast of India. Ind J of Fish. 2005; 52:263-268.
3. Chew YL, Lim YY, Omar M, Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT. 2008; 41:1067-1072.
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 Chemistry. 2006; 95:37-43.
5. Duh PD. antioxidant activity of burdock (Arctium Lappa Linne): its scavenging effect on free radical and activr oxygen. Journal of the American oil chemists society. 1998; 75:455-461.
6. Fleurence J. Seaweed proteins: Biochemical, nutritional aspects and potential uses. Trends in Food Sci and Technol. 1999; 10:25-28.
7. Fujimoto K, Ohmura H, Kaneda T. Screening test for antioxygenic compounds from marine algae and fractionation from *Eisenia bicyclis* and *Undaria pinnatifida*. Bull Jpn Soc Fish. 1985; 46:1125-1130.
8. Fujiwara-Arasaki T, Mino N, Kuroda M. The protein value in human nutrition of edible marine algae in Japan. Hydrobiol. 1984; 116:513-516.
9. Gorden GC, Yen P, Duh D, Tsai CL. Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem. 1993; 41:67-70.
10. Guiry M, Blunden G. Seaweeds resources in Europe: uses and potential. Jhon Wiley, Londen, 1991.
11. Harbourne JB. The Flavonoids: Advances in Research since 1986. London UK: Chapman and Hall, 1994.
12. Herrmann K. Occurrence and content of hydroxyciinnamic and hydroxybenzoic compounds in food. CRC Critical Reviews in Food Science and Nutrition. 1989; 28:315-347.
13. Kuda TT, Sunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J of Food Composition and Analysis. 2007; 18:625-633.
14. Kuda T, Taniguchi E, Nishizawa M, Araki Y. Fate of water-soluble polysaccharides in dried *Chorda filum* a brown alga during water washing. Journal of Food Composition and Analysis. 2002; 15:3-9.
15. Malick CP, Singh MB. Plant enzymology and histoenzymology. Kalyani, New Delhi, 1980.
16. Manach C, Scalbert A, Morand C, Rémés C, Jiménez L. Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition. 2004; 79:727-747.
17. Marinova EM, Yanishlieva NV. Antioxidative activity of extracts from selected species of the family *Lamiaceae* in sunflower oil. *Food Chem.* 1997; 58:245-248.
18. Nisizawa K. In DJ Mchaugh (Ed.). Production and utilization of products from commercial seaweeds (Vol. 299, pp. 147). Rome: FAO, 1988.
19. Nisizawa K. In DJ Mchaugh (Ed.). Production and utilization of products from commercial seaweeds. 1998; 229:147.
20. Shukla VKS, Wanasundara PKJPD, Shahidi F. Natural antioxidants fromoilseeds. In: Shahidi, F. (Eds), Natural antioxidants: Chemistry, Health Effects, and Applications. AOCS Press, Champaign, IL, 1997, 97-132).
21. Yuan YV, Carrington MF, Walsh NA. Extracts from dulse (*Palmaria palmata*) are effective antioxidants and inhibitors of cell proliferation *in vitro*. Food and Chemical Toxicology. 2005; 43:1073-1081.