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Research Article

Antioxidant activity, Total Phenolic and Flavonoid Contents of Selected Commercial Seaweeds of Sabah, Malaysia

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Abstract

Kappaphycus alvarezii (An edible type of red seaweed) is commonly used in South-East Asia as raw material for domestic industries and for export. In the state of Sabah, Malaysia, this species has been commercialized by local people. This study was conducted to evaluate the phytochemicals and antioxidant activity of selected commercial seaweeds available in Sabah market. Three varieties of *Kappaphycus alvarezii*, 'giant', locally known as white and purple seaweeds, 'tambalang hijau', locally known as green seaweed and 'green flower' seaweeds were used. All samples were extracted using 80% methanol. Giant (white seaweed) was found to have the highest total phenolic and total flavonoid contents with the values of 49.04±6.05mg GAE/100g dried sample and 15.54±1.68mg CE/100g dried sample; respectively. Giant (white seaweed) also displayed the highest free radical scavenging (DPPH and ABTS assays) and ferric reducing activity as compared to other seaweeds. These findings suggested that Sabah commercial seaweeds contain an acceptable amount of phytochemicals which has a potential as a natural antioxidant that might be beneficial for human health.

1. INTRODUCTION

Marine algae have recently received significantly attention for their potential as natural antioxidants. They are suggested to have antioxidative defense system in their cells[1] due the absence of oxidative damage in structural components[2] and have the ability to prevent the oxidation during storage when exposed to combination of light and oxygen[3,4,5]. Previous literature reported the potential antioxidant compounds such as some pigments (i.e. fucoxanthin, astaxanthin, carotenoid) and polyphenols (i.e. phenolic acid, flavonoid, tannins), that are widely distributed in seaweeds and are known to exhibit higher antioxidative activities, which have been reported through various methods (i.e. ROS scavenging activity and the inhibition of lipid peroxidation)[6, 7, 8, 9, 10].

Seaweeds have been used as food in Asian diet over centuries. They contain carotenoids, dietary fibers, proteins, essential fatty acids, vitamins and minerals[11]. Fresh and dry seaweeds are widely consumed by people especially living in the coastal areas. In food manufacturing, seaweeds have been developed as processed food products[12,13]. There are many types of seaweeds available commercially in Sabah market. The most popular is *Kappaphycus alvarezii*, an edible type of seaweed which is classified under division of Rhodophyta (red seaweed). It is highly demanded for its cell wall polysaccharide which is the most important source of kappa carrageenan[14]. At least six varieties of *Kappaphycus alvarezii* are farmed around Sabah[15]. Currently, nutraceutical research is interested in the presence of antioxidant substances in fresh and processed foods. Thus, the aim of this study is to determine the antioxidant activity, total phenolic and flavonoid content of selected commercial seaweeds of Sabah, Malaysia.

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2. MATERIALS AND METHODS

2.1 Plant Materials and Sample Preparation

The dry seaweeds were purchased from Kota Kinabalu market in Sabah, Malaysia. The drying process of the seaweeds is not specifically known but according to the locals, the seaweeds were dried using different drying methods which produces different colour of seaweeds (Fig. 1a, b, c & d). One of the samples was mixed with an artificial green dye to give more attractive colour (Fig. 1d). After purchased, the seaweeds were washed with distilled water to remove dirt and excess salt. The samples were then cut into appropriate size and kept in the oven at 40^{o}C for 24 h. The samples were then grounded to a fine powder and passed through a 0.5 mm sieve to get a uniform powder. The powdered seaweeds were collected in air tight container and kept in -20^{o}C for further analysis.

2.2 Extraction

Samples (1 g) were extracted for 2 h with 80% methanol (Fisher, Loughborough, Leicestershire, UK) with a ratio of 1:10 at room temperature on an orbital shaker (Jeio Tech, SK-71, Geum Cheon-Gu, Seoul, Korea) set at 200 rpm[16]. The mixture was then centrifuged at 1400 x g for 20 min and the supernatant was decanted into a 15 ml vial. The pellet was re-extracted under identical conditions. The supernatants were combined and used for measurement of total antioxidant activity, total phenolic and total flavonoid contents.

2.3 DPPH Free-Radical Scavenging Assay

The scavenging activity of the extract was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, St. Louis, MO, USA) assay described by Mensor method[17] with some modification. An aliquot (2.5 ml) of extracts was mixed with 1 ml of 0.3mM of DPPH in absolute methanol (Fisher, Loughborough, Leicestershire, UK). The mixture was shaken vigorously and kept in the dark for 30 min at room temperature. The absorbance of the mixture was

measured at 518 nm using a spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland) and the free-radical scavenging activity was calculated as follows:
Scavenging effect (%) = [1- {absorbance of sample/absorbance of control}] x 100
The scavenging percentage of all samples was plotted. The final result was expressed as an EC_{50} value (the concentration of sample producing 50% scavenging of the DPPH radical; mg/ml).

2.4 FRAP (Ferric reducing/antioxidant power) Assay
This procedure was conducted according to Benzie and Strain method[18] with slight modification. The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s- triazine (TPTZ) (Sigma Aldrich, St. Louis, MO, USA) solution and 20 mM $FeCl_3.6H_2O$ (Fisher, Loughborough, Leicestershire, UK) in a 10:1:1 ratio prior to use and heated to 37°C in a water bath. The blank reading was measured by adding 200 µl of this working solution to the microtiter plate and read at 593 nm using spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland). The sample reading was measured by adding 3 ml of this working solution with 100 µl of extracts and 300 µl of distilled water and left for 4 min before read at 593 nm using spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland). Ferrous sulphate, in the concentration of 0 to 1000 µM, was used as standard and for calibration. The results were expressed as the concentration of the antioxidant having a ferric reducing ability (mM ferric reduction to ferrous in 1g of dried sample).

2.5 ABTS Decolorization Assay
2,2`-azino-bis-(3-ethyl-benzothiazoline-6-sulphonic acid) (Sigma Aldrich, St. Louis, MO, USA) assay was carried out according to Re method[19] with slightly modification. Working ABTS solution (7 mM) and potassium persulfate (2.45 mM) (Sigma Aldrich, St. Louis, MO, USA) were added into a beaker, and the mixture was allowed to stand in the dark for 15 to 18 h to generate an ABTS free radical cation solution. The mixture was diluted with 80% methanol (Fisher, Loughborough, Leicestershire, UK) in order to obtain absorbance of 0.7±0.2 units at 734 nm. An aliquot (200 µl) of extracts was mixed with 2 ml of ABTS working solution and shaken for 45 sec before measured at 734 nm using spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland). Ascorbic acid, with the concentration of 100 mg/ml was used as standard and calibration. The result was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dried sample.

2.6 Determination of Total Phenolic Content
Total phenolic content was determined using Folin-Ciocalteu reagent (Fisher, Loughborough, Leicestershire, UK) as described by Velioglu[16] with slightly modification. An aliquot (100 µl) of extracts was mixed with 0.75 ml of Folin-Ciocalteu reagent (Fisher, Loughborough, Leicestershire, UK) (previously diluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min. To this was added 0.75 ml of sodium bicarbonate (60 g/l) (Sigma Aldrich, St. Louis, MO, USA) solution. The mixture was incubated at room temperature and kept in the dark for 90 min. The absorbance of the mixture was measured at 725 nm using spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland). A standard curve was plotted using different concentration of gallic acid (Sigma Aldrich, St. Louis, MO, USA) and the amount of total phenolics was calculated as gallic acid equivalents (GAE) in mg/100g of dried sample [20].

2.7 Determination of Total Flavonoid Content
Total flavonoid content was determined according to Aluminium Chloride Calorimetric Assay [21]. An aliquot (1 ml) of extracts was mixed with 4 ml of distilled water. At 0 min, 0.3 ml of 5% sodium nitrite (Sigma Aldrich, St. Louis, MO, USA) was added to the mixture. After 5 min, 0.6 ml of 10% $AlCl_3.6H_2O$ (Sigma Aldrich, St. Louis, MO, USA) was added to the mixture. After 6 min, 2 ml of 1M NaOH (Sigma Aldrich, St. Louis, MO, USA) was added to the mixture. The mixture was mixed well and the absorbance was measured at 510 nm using a spectrophotometer (Thermo Fisher Scientific Oy Ratastie 2, FI-01620 Vantaa, Finland). A standard curve was plotted using different concentration of catechin (Sigma Aldrich, St. Louis, MO, USA) and the amount of total flavonoid content was measured as catechin equivalents (CE) in mg/100g 0f dried sample.

2.8 Statistical Analysis
All experiments were carried out in 3 replicates in 3 independent experiments. The results were presented as mean±standard deviation (SD) using IBM SPSS Statistics 21 software. The data were statistically analysed by one-way ANOVA and Duncan post-hoc test. The level of statistical significance was set at $p < 0.05$. Pearson's correlation analysis was done to correlate the phytochemicals content and antioxidant potential in the samples.

3. RESULTS AND DISCUSSION

3.1 Scavenging Activity on 2, 2-Diphenyl-1-picrylhydrazyl Radical
DPPH is a compound that possesses a nitrogen free radical and is readily destroyed by a free radical scavenger[22]. DPPH assay was used to test the ability of the antioxidative compounds functioning as proton scavengers or hydrogen donors[23]. This assay has been extensively used for screening antioxidants such as polyphenols and anthocyanins from marine algae[10, 24, 25, 26]. In this study, the 'giant' white seaweed displayed the highest scavenging effects, while the 'green flower' seaweed displaying the lowest scavenging effects among the samples tested (Fig. 2).
The EC_{50} was determined to quantify the radical scavenging effects (Table 1). The lowest value of EC_{50} indicates strongest ability of the extract as DPPH scavengers. The 'giant' white seaweed displayed the lowest EC_{50} with value of 18.2±1.11mg/ml, followed by 'tambalang hijau' green seaweed and 'giant' purple seaweed with values of 60.93±1.01mg/ml and 81.13±6.18mg/ml, respectively. The EC_{50} for 'green flower' were not detected and this could be due to the destruction of some antioxidant bioactive compounds in the seaweed during the drying process.

3.2 Ferric Reduction Based on FRAP Assay
In FRAP assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound[10, 27]. The presence of antioxidant in the sample causes the conversion of the Fe^{3+}/ferricyanide complex into the ferrous form in a redox-linked colourimetric reaction[28] that involves single electron transfer. Table 1 shows that 'giant' white seaweed displayed the highest ability for reducing Fe^{3+} to Fe^{2+}, followed by 'tambalang hijau' green seaweed, 'giant' purple seaweed and 'green flower' seaweed. The ability of the seaweed extracts to reduce the ferric-cyanide complex into the ferrous form could be contributed by the phenolic compounds in the seaweeds.

3.3 ABTS Scavenging Assay
The mechanism of ABTS scavenging involves the direct production of the blue/green $ABTS^{\bullet-}$ chromophore though the reaction between ABTS and $K_2S_2O_8$. Radical-scavenging capacity of seaweed extracts might be mostly related to their phenolic hydroxyl group[4]. Table 1 shows that 'giant' white seaweed displayed the highest scavenging activity, followed by 'tambalang hijau' green seaweed, 'giant' purple seaweed and 'green flower' seaweed.

3.4 Total Phenolic Content
Phenolic compounds are large and diverse group of molecules, which includes many different families of aromatic secondary metabolites in plants[29]. It has been reported to have several biological activities including antioxidant properties. Earlier reports revealed that marine seaweed extracts, especially their polyphenols, have antioxidant activity[6, 30].
The major active compounds in different seaweed extracts have been reported to be phlorotannins and fucoxanthin[6, 31]. These phenolic compounds could assist the seaweeds to overcome oxidative stress as well as play a putative adaptive role in defense against grazers, such as marine herbivores[32] due to their plasticity characteristics[22].
The result of this study shows that the total phenolic content was highest in 'giant' white seaweed, followed by 'giant' purple seaweed, 'tambalang hijau' green seaweed and 'green flower' seaweed (Table 2). According to Fayaz [33], *Kappahycus alvarezii* contains ascorbic acid and polyphenols, which are hydrophilic which might also contribute to the antioxidant activity of the sample.

3.5 Total Flavonoid Content

Flavonoids are crucial antioxidants since they have high redox potential, which allows them to act as reducing agent, hydrogen donors and singlet oxygen quenchers, as well as having metal chelating potential[16]. The result of this study shows that the total flavonoid content was highest in 'giant' white seaweed, followed by 'tambalang hijau' green seaweed, 'giant' purple seaweed and 'green flower' seaweed (in Table 2).

3.6 Relation between Antioxidant Activity and Content of Phytochemicals

In this study, strong correlation between ABTS and FRAP assays (R^2=0.925) indicated that the compounds present in the seaweed extracts capable of reducing ABTS radical were also able to reduce ferric ions. According to Pulido[34], the ferric ion reducing ability of antioxidant was correlates with the results from other methods used to estimate antioxidant capacity. Matanjun [4] also reported high correlation between ABTS and FRAP assays.

Previous studies shows that there was a significant correlation between antioxidant activity and phenolic compounds in seaweeds[4,24,35]. Many seaweeds species contain polyphloroglucinol phenolics (phlorotannins)[4, 36, 37] and in this study the antioxidant activity of seaweed extracts could be due to these compounds.

The total phenolics in seaweed extracts shows a strong positive correlation with reducing power (R^2=0.744) and ABTS (R^2=0.817), but weak correlation with DPPH free radical-scavenging activity (R^2=0.194). The total flavonoids in seaweed extracts also shows a positive correlation with reducing power (R^2=0.940) and ABTS (R^2=0.889), but negative correlation with DPPH free radical-scavenging activity (R^2=-0.250). The lower correlation between DPPH values and the phenolic contents in the seaweed extracts indicated that not only the phenolic compounds were involved in the antioxidant activity through this pathway but there could be some effects involving other active compounds.

Table 1: Antioxidant properties of extracts of commercial seaweeds (*Kappaphycus alvarezii*), assessed by three different assays

Samples	DPPH Assay[1]	FRAP Assay[2]	ABTS Assay[3]
'Giant' White seaweed	18.2±1.11^a	3.74±0.08^a	0.515±0.04^a
'Giant' Purple seaweed	81.13±6.18^b	0.89±0.03^b	0.236±0.01^b
'Tambalang Hijau' Green seaweed	60.93±1.01^c	1.32±0.12^c	0.333±0.02^c
'Green flower'	ND	0.75±0.13^b	0.139±0.01^d

Values are presented as mean±SD (n = 3) which, with different letters (within column), indicate significant difference ($p < 0.05$).
[1]DPPH free radical scavenging activity was expressed as EC_{50} (mg/ml).
[2]FRAP was expressed as mM ferric reduction to ferrous in 1 g of dried sample.
[3]ABTS was expressed as mg ascorbic acid equivalent antioxidant capacity (AEAC) in 1 g of dried sample.

Table 2: Content of total phenolics and total flavonoids in extracts of commercial seaweeds (*Kappaphycus alvarezii*)

Samples	Total Phenolics[4]	Total Flavonoids[5]
'Giant' White seaweed	49.04±6.05^a	15.54±1.68^a
'Giant' Purple seaweed	36.35±6.30^{ab}	5.41±0.97^b
'Tambalang Hijau' Green seaweed	30.96±1.33^a	7.72±2.89^b
'Green flower'	16.47±4.96^c	4.64±1.18^b

Values are presented as mean±SD (n = 3) which, with different letters (within column), indicate significant difference ($p < 0.05$).
[4]Total phenolic content was expressed as mg gallic acid equivalents in 100 g of dried sample (mg GAE/100g).
[5]Total flavonoid content was expressed as mg catechin equivalents in 100 g of dried sample (mg CE/100g).

Figure 1a: *Kappaphycus alvarezii* var. giant, locally known as white seaweed. The white seaweed was produced by put it inside a clear plastic bag and placed it under the sun until the seaweeds turn into yellow-white colour. Then, the seaweed was placed under the sun until a constant weight was obtained. This drying technique is called as a 'sauna-dried'.

Figure 1b: *Kappaphycus alvarezii* var. giant, locally known as purple seaweed. The purple seaweed was produced by hanging it under the sun until the seaweed turn to purplish colour. The seaweed was dried under the sun until a constant weight was obtained.

Figure 1c: *Kappaphycus alvarezii* var. tambalang hijau, locally known as green seaweed. The green seaweed was produced by drying it under the sun until a constant weight was obtained.

Figure 1d: *Kappaphycus alvarezii* var. green flower. This seaweed was dried using the same method in fig. 1a. However, an artificial dye colour (usually used in food) was mixed with this seaweed.

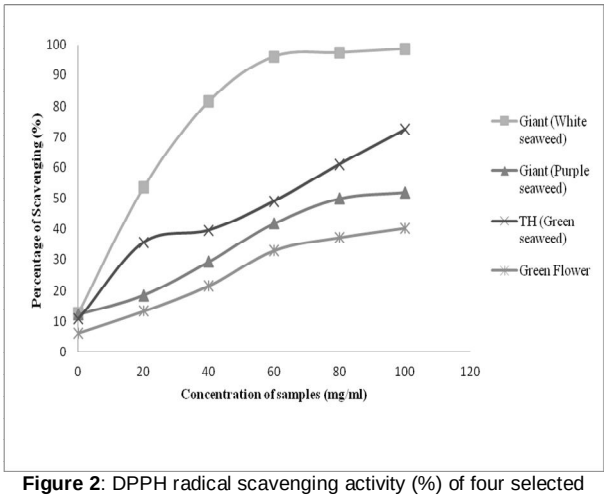

Figure 2: DPPH radical scavenging activity (%) of four selected commercial seaweeds (*Kappaphycus alvarezii*) of Sabah, Malaysia (n = 3).

4. CONCLUSION

In conclusion, our investigation found that these commercial seaweeds contain an acceptable amount of phenolic and flavonoid content. Thus, these commercial seaweeds could be consumed as functional foods due to the presence of antioxidant substances in the seaweeds.

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REFERENCES

1. Jiménéz-Escrig A, Jiménéz-Jiménéz I, Pulido R and Saura-Calixto F. Antioxidant activity of fresh and processed edible seaweeds. *J. Sci Food Agric*, 2001, 81:530-534.
2. Matsukawa R, Dubinsky Z, Kishimoto E, Masaki K, Matsuda Y, Takeuchi T, Chihara M, Yamamoto Y, Niki E and Karube I. A comparison of screening methods for antioxidant activity in seaweeds. *J Appl Phycol*, 1997, 9:29-35.
3. Ramarathnam N, Osawa T, Ochi H and Kawakashi S. The contribution of plant food antioxidants to human health. *Trends Food Sci Technol*, 1995, 6:75-82.
4. Matanjun P, Mohamed S, Mustapha NM, Muhammad K and Ming CH. Antioxidant activities and phenolics content of eight species of seaweeds from North Borneo. *J Appl Phycol*, 2008, 20:367-373.
5. Yongthang M, Hutadilok-Towatana N and Phromkunthong W. Antioxidant activities of four edible seaweeds from the Southern Coast of Thailand. *Plant Foods Human Nutr*, 2009, 64:218-223.
6. Yan X, Chuda Y, Suzuki M and Nagata T. Fucoxanthin as the major antioxidant in *Hijika fusiformis*, a common edible seaweed. *Biosci Biotechnol Biochem*, 1999, 63:605-607.
7. Athukorala Y, Lee KW, Song CB, Ahn CB, Shin TS, Cha YJ, Shahidi F and Jeon YJ. Potential antioxidant activity of marine red alga *Grateloupia filicina* extracts. *J Food Lipids*, 2003a, 10:251-265.
8. Athukorala Y, Lee KW, Shahidi F, Heu MS, Kim HT, Lee JS and Jeon YJ. Antioxidant efficacy of extracts of an edible red alga *Grateloupia filicina* in linoleic acid and fish oil. *J Food Lipids*, 2003b, 10:313-327.
9. Heo SJ, Cha SH, Lee KW and Jeon YJ. Antioxidant activities of Chlorophyta and Phaeophyta from Jeju Island. *Algae*, 2005, 20(3):251-260.
10. Luo HY, Wang B, Yu CG, Qu YL and Su CL. Evaluation of antioxidant activities of five selected brown seaweeds from China. *Med Plants Res*, 2010, 4(18):2557-2565.
11. Jeeva S, Marimuthu@Antonisamy J, Domettila C and Mahesh M. Preliminary phytochemical studies on some selected seaweeds from Gulf of Mannar, India. *Trop Biomed*, 2012, S30-S33.
12. Mabeau S and Fleurence J. Seaweeds in food products: biochemical and nutritional aspects. *Trends Food Sci Technol*, 1995, 6:103-107.
13. Ismail A and Tan SH. Antioxidant of selected commercial seaweeds. *J Mal Nutri*, 2002, 8(2):167-177.
14. Suresh Kumar K, Ganesan K and Subba Rao PV. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty – An edible seaweed. *Food Chem*, 2008, 107:289-295.
15. Phang SM, Yeong HY, Lim PE, Adibi RMN and Gan KT. Commercial varieties of *Kappaphycus* and *Eucheuma* in Malaysia. *J Sci Msia*, 2010, 29(3):214-224.
16. Velioglu YS, Mazza G, Gao L and Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J Agric Food Chem*, 1998, 46:4113-4117.
17. Mensor LI, Menezes FS, Leitao GG, Reis AS, Dos Santos T, Coube CS and Leitao SG. Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, 2001, 15:127-130.
18. Benzie IF and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': The FRAP Assay. *Anal Biochem*, 1996, 239: 70-76.
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radical Bio Med*, 1999, 26: 231-1237.
20. Sabeena KHF and Jacobsen C. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chem*, 2013, 138:1670-1681.
21. Zhishen J, Mengcheng T and Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*, 1999, 64:555-559.
22. Chew YL, Lim YY, Omar M and Khoo KS. Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT*, 2008, 41:1067-1072.
23. Singh N and Rajini PS. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem*, 2004, 85:611-616.
24. Duan XJ, Zhang WW, Li XM and Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem*, 2006, 95:37-43.
25. Wang BG, Zhang WW, Duan XJ and Li XM. In vitro antioxidative activities of extract and semi-purified fractions of the marine red alga, *Rhodomela confervoides* (Rhodomelaceae). *Food Chem*, 2009, 113:1101-1105.
26. Chandini SK, Ganesan P and Bhaskar N. In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chem*, 2008, 107:707-713.
27. Barros L, Baptista P and Ferreira ICFR. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity

measured by several biochemical assays. *Food Chem, Toxicol*, 2007, 45:1731-1737.
28. Li YF, Guo CJ, Yang JJ, Wei JY, Xu J and Cheng S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem*, 2006, 96:254-260.
29. Waterman PG and Mole S. Analysis of phenolic plant metabolites. Blackwell Scientific Publications, Oxford, 1994.
30. Kuda T, Tsunekawa M, Goto H and Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula Japan. *J Food Comp Anal*, 2005, 18:625-633.
31. Yan K, Nagata T and Fan X. Antioxidant activities in some common seaweeds. *Plant Foods Human Nutr*, 1998, 52:253-262.
32. Altena V and Steinberg PD. Are differences in the responses between North American and Australasian marine herbivores to phlorotannins due to differences in phlorotannin structure? *Biochem Systematics and Ecology*, 1992, 20:493-499.
33. Fayaz M, Namitha KK, Chidambara Murthy KN, Mahadeva Swamy M, Sarada R, Khanam S., Subbarao PV and Ravishankar GA. Chemical composition, iron bioavaibility and antioxidant activity of *Kappaphycus alvarezii* (Doty). *J Agricul and Food Chem*, 2005, 53:792-797.
34. Pulido R, Bravo L and Saura-Calixto F. Antioxidant activity of dietary polyphenols determined by ferric reducing antioxidant power assay. *J Food Agric Food Chem*, 2000, 48:3396-3402.
35. Nagai T and Yukimoto. Preparation and functional properties of beverages made from sea alga. *Food Chem*, 2003, 81:327-332.
36. Pavia H and Aberg P. Spatial variation in polyphenolic content of *Ascophyllum nodosum* (Fucales, Phaeophyta). *Hydrobiology*, 1996, 326/327:199-203.
37. Nakamura T, Nagayama K, Uchida K and Tanaka R. Antioxidant activity of phlorotannins from the brown alga *Eisenia bicyclis*. *Fish Sci*, 1996, 62:923-926.