INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
10–429/MMI/2011/13–1–95–99
http://www.fspublishers.org

Full Length Article

Antioxidant Activity of some Seaweed from the Gulf of Thailand

WALAILUCK BOONCHUM1, YUWADEE PEERAPORNPISAL, DUANGTA KANJANAPOTHI†, JEEREPORN PEKKOH, CHAYAKORN PUMAS, UTAN JAMJAI†, DOUNGPORN AMORNLERDPISON‡, THIDARAT NOIRAKSAR¶ AND PANMUK VACHARAPIYASOPHON

Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
†Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand
‡Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Chiang Mai, Thailand
¶Institute of Marine Science, Burapha University, Bangsaen, Chon Buri, Thailand
[1]Corresponding author's e-mail: aom_amm_1@hotmail.com

ABSTRACT

Four species of seaweed, *Sargassum binderi* Sonder, *Amphiroa* sp., *Turbinaria conoides* (J. Agardh) Küzting and *Halimeda macroloba* Decaisne, were collected from the Gulf of Thailand. Seaweeds were extracted with water or ethanol and examined for phenolic compounds and antioxidant activities by measuring the scavenging activity of both ABTS and DPPH radicals. In general, the aqueous extracts (AE) showed higher antioxidant activities and phenolic contents than ethanolic extracts (EE). Therefore, AE were chosen for three additional assays: superoxide anion scavenging assay, anti-lipid peroxidation in liver homogenate and reducing power. *T. conoides* extract showed the highest antioxidation activity in all assays. Therefore, the dried *T. conoides* had a potential to antioxidative agent in nutraceutical products. © 2011 Friends Science Publishers

Key Words: Aqueous extracts; Ethanolic extracts; Phenolic compound; Superoxide radical; *Turbinaria conoides*

INTRODUCTION

Humans are impacted by many free radicals both from inside our body and surrounding environments, particularly reactive oxygen species (ROS). ROS can be generated by several metabolic pathways in cells (endogenous sources). ROS constitutes superoxide (O_2^-), hydroxyl ($HO^\bullet$) and hydrogen peroxide (H_2O_2). Furthermore, free radicals also produced by other several other pathways (exogenous sources), ionizing radiation, UV light, cigarette smoke, industrial waste and pollutants. The ROS and free radicals can be reduced by the defensive activity of antioxidants present in tissues (Halliwell & Cross, 1994; Cervantes-Cervantes *et al*., 2005). The steady state levels of ROS are maintained in cells by the activity of antioxidant defense system. However, under stress conditions this delicate balance is disturbed and caused enhanced production of ROS (Betteridge, 2000). These ROS can damage essential bio-molecules: proteins, DNA and lipids and caused various human diseases for instance, atherosclerotic (Wu *et al.*, 1998). Lipid oxidations frequently occur in our body, especially in cell membrane phospholipids. The high level of polyunsaturated fatty acid and methylene groups in their double bonds make them sensitive to oxidation, which causes the free radical chain reaction (Valko *et al.*, 2004). Malondialdehyde (MDA) is the most important aldehyde product from lipid peroxidation. Therefore, MDA has been used to reflect lipid peroxidation levels in clinical diagnosis (Fukunaka *et al.*, 1995).

Natural antioxidants are found in some vegetables, fruits and a variety of other foods (Moon & Shibamoto, 2009). Specifically, many researchers reported the finding of various antioxidants present in seaweeds, for example polysaccharides, dietary fibers, minerals, proteins, amino acids, vitamins, polyphenols and carotenoids (Burtin, 2003). Seaweed produces various types of antioxidant to counteract environmental stresses (Lesser, 2006). Therefore, seaweed is a potential source of novel antioxidants. In addition, natural antioxidants are more acceptable than synthetic antioxidants as these antioxidants do not contain chemical contaminants and display a variety of beneficial functions. Thus, natural antioxidants are considered safe for use as ingredients in medicine, dietary supplements, nutraceuticals and cosmetics with the objective of improving consumer health, reducing the effects of harmful diseases and other broader aspects of immune system function (Shahidi, 2009). The antioxidant activity of several seaweeds has been reported from research conducted in various countries, such as Malaysia (Matanjun *et al.*, 2008), Indonesia (Santoso *et al.*, 2004), India (Chandini *et al.*, 2008), Korea (Heo *et al*., 2005) and Japan (Matsukawa *et al.*, 1997). Recently, the seaweed from the southern coast of Thailand (Yangthong *et al.*, 2009) and the Andaman Sea (Amornlerdpison *et al.*, 2007; Peerapornpisal *et al.*, 2010) were studied for their

To cite this paper: Boonchum, W., Y. Peerapornpisal, P. Vacharapiyasophon, J. Pekkoh, C. Pumas, U. Jamjai, D. Amornlerdpison, T. Noiraksar and D. Kanjanapothi, 201x. Antioxidant activity of some seaweed from the gulf of Thailand. *Int. J. Agric. Biol*., 13: 95–99

antioxidant activities. However, seaweeds found on the east coast of the Gulf of Thailand have not yet been investigated. Thus, it is likely that seaweeds from the east coast of the Gulf of Thailand would contain antioxidative agents worth investigating. This study was aimed to evaluate the antioxidative potential of four seaweeds: Division *Pheaophyta*: *Sargassum binderi* Sonder and *Turbinaria conoides* (J. Agardh) Küzting, Division *Rhodophyta*: *Amphiroa* sp. and Division *Chlorophyta*: *Halimeda macroloba* Decaisne, which are all considered common species found along the east coast of the Gulf of Thailand. These four seaweeds may be seen by locals as less than valuable, but also may present many inconveniences for local fisherman. Once studied, if they show promising antioxidant activities, the seaweed extract can be used as antioxidative agents in cosmeceutical or nutraceutical products which can increase the value of an otherwise worthless weed.

MATERIALS AND METHODS

Sample collection: Four species of seaweeds were collected from various parts of the Gulf of Thailand in May 2007. *Sargassum binderi* Sonder was collected from Salak Phet Bay, Koh Chang, Trat Province. *Amphiroa sp.* was collected from Yang Bay, Laem Sing District, Chanthaburi Province. *Turbinaria conoides* (J. Agardh) Küzting was collected from Nang Rong Beach, Samae San Sub-district, Chonburi Province and *Halimeda macroloba* Decaisne was collected from Khao Ma Jor, Samae San Sub-district, Chonburi Province.

Preparation of extract from seaweeds: Fresh seaweeds were clean from epiphytes, salt and sand before being dried at 55°C for 48 h. Dried seaweed was ground into a fine powder. Preparations of extract were modified from Senevirathne *et al.* (2006). Dried seaweed (100 g) was soaked in 1000 mL of distilled water or ethanol and kept in a shaking incubator at 25°C for 3 days and the suspension was then filtered through Whatman No. 1 filter paper. The re-extraction process was repeated 3 times. The solvent was pooled, evaporated and lyophilized. The dried extract was dissolved in distilled water or ethanol for different assays.

Antioxidant Activities Screening of Aqueous and Ethanolic Seaweed Extracts

ABTS (2, 2'-azino-bis3-ethylbenzthiazoline-6-sulfonic) radical cation decolorization assay: The ABTS radical anion scavenging assay was carried out by the method of Re *et al.* (1999) with some modifications. The ABTS reagent was prepared by mixing 5 mL of 14 mM ABTS with 5 mL of 4.9 mM $K_2S_2O_8$. The mixture was kept in the dark at room temperature for 16 h. The absorbance was adjusted with distilled water to 0.700 ± 0.02 at 734 nm. To determine the scavenging activity, 1 mL ABTS reagent was added to 10 µL of different concentrations of seaweed extract and absorbance was measured at 734 nm at 3 min interval of. Trolox was used as standard. Percentage inhibition of the sample was calculated by the following equation:

$$\% \text{ Inhibition} = [\ (A_0 - A_1)/A_0\ ] \times 100$$

A_0 expresses the absorbance of control; A_1 expresses the absorbance of the tested seaweed extract. The ABTS radical anion scavenging assay was expressed as trolox equivalent antioxidant capacity (TEAC) and defined as mg of trolox equivalents per 1 g of sample.

DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) radical-scavenging assay: The DPPH radical-scavenging activity assay was performed after Hou *et al.* (2001) with some modifications. A 1.2 mL extract of different concentrations were added to 0.1 mL of 1 M Tris-HCl buffer (pH 7.9) and mixed with 1.2 mL of 5 mM DPPH in MeOH and kept in the dark at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. Gallic acid, a phenolic organic acid, was used as a standard. The decrease of absorbance at 517 nm was calculated as the percentage of inhibition by the same equation of the ABTS assay, expressed as gallic acid equivalent (GAE) and defined as mg of gallic acid equivalents per 1 g of sample.

Determination of total phenolic content: The concentration of phenolic compounds was measured by the Folin-Ciocalteu method (Chandler & Dodds, 1983). Aqueous extracts (AE) and the ethanolic extracts (EE) 250 µL each of seaweed was mixed with 1250 µL deionized water, 250 µL ethanol and 125 µL of the Folin-Ciocalteu reagent. The mixture was incubated at room temperature for 5 min and then 250 µL of 5% Na_2CO_3 was added. The mixture was kept in the dark at room temperature for 1 h. Absorbance was measured at 725 nm. The content of phenolic compounds was standardized with gallic acid and defined as mg of gallic acid equivalents per 1 g of sample.

Anti-lipid peroxidation in liver homogenate: Lipid peroxide formation was measured (lipid peroxidation assay) by the method of Hattori *et al.* (1993). The reaction mixtures contained 0.5 mL of 10 mg protein/mL rat liver homogenate, 0.1 mL of 150 mM Tris–HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, 0.05 mL of 4 mM $FeCl_2$ and 0.05 mL of different concentrations of seaweed AE. The mixture was incubated at 37°C for 1 h. After the incubation, 0.9 mL of distilled water and 2 mL of 0.6% TBA were added and then the samples were shaken vigorously. The mixture was heated for 30 min in a boiling water bath at 100°C and cooled drown to room temperature. Five milliliter of *n*-butanol was added and the mixture shaken vigorously. The *n*-butanol layer was separated by centrifugation at 3000×*g* for 10 min. The organic layer was taken and its absorbance at 532 nm was measured and calculated as the percentage of inhibition by the same equation of ABTS assay. The inhibition was expressed as trolox equivalent antioxidant capacity (TEAC) and defined as mg of trolox equivalents per 1g of sample.

Superoxide radical scavenging activity: Superoxide radical scavenging activity was carried out by the method of Nikishimi *et al.* (1972). Superoxide radicals were

determined by the phenazine metrosulphate (PMS)–NADH superoxide generating system containing a 0.5 mL of seaweed extract, 0.5 mL of 2.52 mM nitroblue tetrazolium (NBT), 0.5 mL of 624 µM b-nicotinamide adenine dinucleotide (NADH) and 0.5 mL of 120 µM PMS were added. The NBT, NADH and PMS solutions were prepared in 0.1 M sodium phosphate buffer (pH 7.4) and various concentrations of the AE. The mixture was incubated at room temperature for 5 min and the absorbance read at 560 nm. The decrease of absorbance at 560 nm was calculated as percentage of inhibition of NBT using the same equation of ABTS assay and expressed as gallic acid equivalent (GAE) and defined as mg of gallic acid equivalents per 1 g of sample.

Reducing power: The reducing power was measured by an ability to put forth electrons. It was determined according to the method of Oyaizu (1986). A 120 µL AE was mixed with 290 µL of 0.2 M phosphate buffer (pH 6.6) and 290 µL of 1% potassium ferricyanide, incubated at 50°C for 20 min before 2.5 mL of 10% TCA was added. The mixture was centrifuged at 3000×*g* for 10 min. One milliliter of supernatant was mixed with 1 mL distilled water and 0.2 mL of 0.1% ferric chloride and incubated at room temperature for 15 min. The absorbance was measured at 700 nm. The reducing power at 700 nm was calculated as percentage of inhibition and expressed as gallic acid equivalent (GAE), which defined as mg of gallic acid equivalents per 1 g of sample.

Statistical analysis: All assays were done in triplicate. All data are expressed as means ± standard deviation. Data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by one-way ANOVA with Turkey's b test. The data were calculated by computer programs: Microsoft Excel and SPSS version 17.0.

RESULTS

Antioxidant activities and total phenolic content of aqueous and ethanolic seaweed extracts: Four species of seaweed were extracted with water and ethanol. In genaral, the AE provided higher extraction yields than the EE. Among the AE of four seaweeds, *S. binderi* had the highest extraction yield of 12.25% followed by *T. conoides* (6.41%), *Amphiroa* sp. (2.94%) and *H. macroloba* (2.52%), respectively. Among the EE, the extraction yields which were found in *T. conoides*, *H. macroloba* and *S. binderi*, were 3.11%, 2.27% and 1.14%, respectively. However, EE of *Amphiroa* sp. was too low to be analyzed.

ABTS IC_{50} of AE and EE of four seaweeds are presented in Table I. The AE of *T. conoides* and *Amphiroa* sp. showed significantly higher levels of ABTS scavenging activity ($p > 0.05$). Furthermore, all AE samples showed higher activity than EE samples. In addition, *T. conoides* was significantly observed to have the highest TEAC in terms of standard equivalent. Data showed that AE of all four seaweeds contained higher DPPH radical scavenging activity than the EE (Table I). The AE of *T. conoides* showed the highest scavenging activity of DPPH radicals, followed by AE of *S. binderi*, *H. macroloba*, *Amphiroa* sp. and EE of three seaweeds, respectively. The highest phenolic content of the AE was found in *T. conoides*, while *H. macroloba* presented the highest phenolic content level of EE (Table I). Base on ABTS assay, DPPH assay and total phenolic content. The AE of seaweed showed higher antioxidant activities than EE. Therefore, the AE of four seaweeds was selected for further study on anti-lipid peroxidation, superoxide radical scavenging activity and reducing power.

Antioxidant activities and reducing power of aqueous seaweed extract: From Table II, our results indicated that the extract of *H. macroloba* and *T. conoides* contained high anti-lipid peroxidation activity with low IC_{50}. However, the AE of *S. binderi* showed the highest TEAC (mg trolox/g dry weight) due to its highest extraction yield. In our results, the AE of *T. conodies* showed a promising superoxide radical scavenging activity with significantly the lowest IC_{50} and highest GAE, followed by *S. binderi*, *H. macroloba* and *Amphiroa* sp., respectively (Table II). The absorbance value of 1.000 at 700 nm was defined for its potential of reducing power and results of GAE. In this experiment, all four seaweeds showed reducing power, with the highest activity observed for *T. conoides* (Table II).

DISCUSSION

The extraction yield in AE was higher than EE. This indicated that most extract dissolved in high polarity solvent more than in low polarity solvent. Similarly, Matanjun (2008) reported that more polar compounds were found in seaweed extracts and increasing solvent polarity increased the extraction yield. From the results of ABTS radical cation decolorization assay and DPPH radical scavenging assay, the extract of four seaweeds both aqueous and ethanolic extract, especially, the AE of *T. conoides*, could eliminate the free radicals by acted as free radical scavengers (Molyneux, 2004) or by donating a hydrogen atom to the molecule (Re *et al.*, 1999).

Polyphenolics contain reducing properties as hydrogen or electron-donating agents, thus seen as potential free-radical scavengers (antioxidants) (Rice-Evans *et al.*, 1997). Polyphenolic compounds are natural antioxidants which are found mostly in plants (Moon & Shibamoto, 2009) and seaweeds. In AE, the results indicated strong correlation between the antioxidant activity (ABTS, DPPH) and total phenolic content, which are in agreement with studies of Nagai and Yukimoto (2003), Duan *et al.* (2006) and Dudonné *et al.* (2009).

Our results revealed that the AE was better than EE as a source of antioxidants. Previous reports also found that AE of seaweed contained higher antioxidant activity than EE (Kuda & Ikemori, 2009). The high antioxidant activities of AE may be due to the difference in polyphenolic

Table I: IC_{50} and standard equivalent of scavenging activities and total phenolic content of aqueous and ethanolic extracts

Seaweed		*Amphiroa* sp.		*Halimeda macroloba*		*Sargassum binderi*		*Turbinaria conoides*	
		Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
ABTS radical cation depolarization activity	IC_{50} (mg/mL)	8.026 ± 0.092[a]	ND	14.397 ± 0.164[b]	17.554 ± 1.479[b]	15.164 ± 0.092[b]	36.627 ± 3.754[c]	5.290 ± 0.088[a]	96.242 ± 1.643[d]
	mg trolox/g dry weight	0.011 ± 0.000[d]	ND	0.005 ± 0.000[c]	0.004 ± 0.000[b]	0.024 ± 0.000[e]	0.001 ± 0.000[a]	0.036 ± 0.001[f]	0.001 ± 0.000[a]
DPPH radical scavenging activity	IC_{50} (mg/mL)	7.827 ± 0.120[c]	ND	0.837 ± 0.002[b]	20.147 ± 0.000[d]	0.841 ± 0.010[b]	45.047 ± 0.000[e]	0.128 ± 0.002[a]	113.944 ± 0.000[f]
	mg gallic acid/g dry weight	0.045 ± 0.000[b]	ND	0.096 ± 0.000[c]	0.004 ± 0.003[a]	0.461 ± 0.005[d]	0.001 ± 0.000[a]	1.589 ± 0.031[e]	0.001 ± 0.000[a]
Total phenolic content	mg gallic acid/g dry weight	0.085 ± 0.003[b]	ND	0.077 ± 0.001[b]	0.369 ± 0.007[e]	0.267 ± 0.002[d]	0.063 ± 0.004[a]	1.116 ± 0.011[f]	0.192 ± 0.001[c]

Table II: IC_{50} and standard equivalent of antioxidant activities and reducing power of aqueous extracts

Characteristics	Seaweed	*Amphiroa* sp.	*Halimeda macroloba*	*Sargassum binderi*	*Turbinaria conoides*
Anti-lipid peroxidation activity	IC_{50} (mg/mL)	328.012 ±23.461[c]	155.590 ±16.129[a]	218.318 ±8.511[b]	155.795 ±0.495[a]
	mg trolox/g dry weight	0.010 ±0.001[a]	0.018 ±0.002[b]	0.062 ±0.002[d]	0.046 ±0.000[c]
Superoxide radical scavenging activity	IC_{50} (mg/mL)	ND	50.552 ±0.341[c]	9.224 ±0.070[b]	2.066 ±0.066[a]
	mg gallic acid/g dry weight	ND	0.121 ±0.070[a]	3.207 ±0.024[b]	7.502 ±0.236[c]
Reducing power	Concentration at OD700 = 1 (mg/mL)	69.204 ±0.710[d]	14.323 ±0.051[b]	15.594 ±0.072[c]	2.136 ±0.003[a]
	mg gallic acid / g dry weight	0.022 ±0.000[a]	0.092 ±0.000[b]	0.410 ±0.002[c]	1.569 ±0.002[d]

Data are expressed as the mean ± standard deviation (SD) of three replicates. Different letters represent the statistical comparisons between groups by using ANOVA and post hoc Turkey's b test ($p<0.05$) ND, no detectable

compound pattern. Many hydrophillic polyphenolic compounds were reported in seaweed, for example epigallocatechin gallate (Santoso *et al*., 2004), epicatechin (Takeshi *et al*., 2005) and phlorotannins (Targett & Arnold, 1998), which are strong antioxidant components. Moreover, the antioxidant activities in aqueous extract were not the result of only phenolic compounds. The activities were caused by other hydrophilic compounds, for example peptides, fucoidan and Maillard reaction products (Kuda & Ikemori, 2009). The hydrophobic phenolic compounds in seaweed did not contain antioxidant potential. This phenomenon could be explained from the observation that the EE of *H. macroloba*, which contained high phenolic content but showed very low antioxidant activity.

In this study, we found that the AE of *T. conoides* showed low anti-lipid peroxidation activity, which did not correspond with its phenolic content. However, the anti-lipid peroxidation activity was carried out in hydrophobic condition; the active substances in AE itself are high polarity molecules and could not react well with the non- or low-polar free radical molecules in lipid peroxidation pathway (Matsukawa *et al.*, 1997). The AE of *T. conoides* showed a high reducing power to perform electron donating ability. In addition, it also performed high hydrogen atom donating ability from ABTS and DPPH assay so that the active substances in the AE of *T. conoides* could behave as primary and secondary antioxidants (Zhu *et al*., 2002). The AE of *T. conoides* was consistent with broad antioxidant activities via both single electron transfer and hydrogen atom transfer system (Prior *et al.*, 2005). Interestingly, the AE performed higher potential than EE. Water extract is non toxic, easy to use and waste handle both for common people and in commercial production (Matu & Staden 2003).

In conclusion, both AE and EE of all seaweeds from the east cost of the Gulf of Thailand showed to have antioxidant activities and high amounts of total phenolics. From this study, the dried *T. conoides* could be applied as healthy tea or use as antioxidative agent in nutraceutical products after pass safety and toxicity test.

Acknowledgement: Walailuck Boonchum is thankful to the Commission on Higher Education, Thailand for a scholarship under the program of Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree. This research was also supported by a grant from the National Research Council of Thailand (NRCT) under management of the Institute for Science and Technology Research and Development, Chiang Mai University. We thank the Graduate School of Chiang Mai University and Department of Biology, Faculty of Science, Chiang Mai University for their support. Thanks to Prasan Saengpaiboon, Rambhai Barni Rajabhat University, Chantaburi Province, for help with seaweeds collections and initial identification.

REFERENCES

Amornlerdpison, D., Y. Peerapornpisal, C. Rujjanawate, T. Taesotikul, M. Nualchareon and D. Kanjanapothi, 2007. Antioxidant activity of *Padina minor* Yamada, *KMITL Sci. Tech. J*., 7: 1–7

Betteridge, D.J., 2000. What is oxidative stress? *Metabolism*., 49: 3–8

Burtin, P., 2003. Nutritional value of seaweeds. *Electron. J. Environ. Agric. Food Chem*., 2: 498–503

Cervantes-Cervantes, M.P., J.V. Calderón-Salinas, A. Albores and J.L. Muñoz-Sánchez, 2005. Copper increases the damage to DNA and proteins caused by reactive oxygen species. *Biol. Trace Elem. Res*., 103: 229–248

Chandler, S.F. and J.H. Dodds, 1983. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasodine in callus cultures of *Solanum laciniatum*. *Plant Cell Rep*., 2: 205–208

Chandini, S.K., P. Ganesan and N. Bhaskar, 2008. *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chem*., 107: 707–713

Duan, X., W. Zhang, X. Li and B. Wang, 2006. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. *Food Chem*., 95: 37–43

Dudonné, S., X. Vitarc, P. Coutiére, M. Woillez and J. Mérillon, 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD and ORAC assays. *J. Agric. Food Chem*., 57: 1768–1774

Fukunaka, K., K. Takama and T. Suzuki, 1995. High-performance lipid chromatographic determination of plasma malondialdehyde level without a solvent extraction procedure. *Anal. Biochem*., 230: 20–23

Halliwell, B. and C.E. Cross, 1994. Oxygen-derived species: their relation to human disease and environmental stress. *Environ. Health Perspect*., 102: 5–12

Hattori, M., X. Yang, H. Miyashrio and T. Nabma, 1993. Inhibitory effects of monomeric and dimeric phenylpropanoids from mace on lipid peroxidation *in vivo* and *in vitro*. *Phytother. Res*., 7: 395–401

Heo, S., E. Park, K. Lee and Y. Jeon, 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresour. Technol*., 96: 1613–1623

Hou, W., Y. Chen, H. Chen, Y. Lin, L. Yang and M. Lee, 2001. Antioxidant activities of trypsin inhibitor, a 33 kDa root storage protein of sweet potato (*Ipomoea batatas* (L.) Lam cv. Tainong 57). *J. Agric. Food Chem*., 49: 2978–2981

Kappus, H., 1992. Oxidative Stress in Chemical Toxicity. *In*: Csomós, G. and J. Fehér, (eds.), *Free Radicals and the Liver*, p: 13. Springer-Verleg, Berlin

Kuda, T. and T. Ikemori, 2009. Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macroalgal beach-casts in the Noto Peninsula, Ishikawa. *Japanese Food Chem*., 112: 575–581

Lesser, P.M., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. *Annu. Rev. Physiol*., 68: 253–278

Matanjun, P., S. Mohamed, N.M. Mustapha, K. Muhammad and C.H. Ming, 2008. Antioxidant activities and phenolics content of eight species of seaweeds from north Borneo. *J. Appl. Phycol*., 20: 367–373

Matsukawa, R., Z. Dubinsky, E. Kishimoto, K. Masaki, Y. Masuda, T. Takeuchi, M. Chihara, Y. Yamamoto, E. Niki and I. Karube, 1997. A comparison of screening methods for antioxidant activity in seaweeds. *J. Appl. Phycol*., 9: 29–35

Matu, E.N. and J. van Staden, 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *J. Ethnopharmacol*., 87: 35–41

Molyneux, P., 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J. Sci. Technol*., 26: 211–219

Moon, J. and T. Shibamoto, 2009. Antioxidant assays for plant and food components. *J. Agric. Food Chem*., 57: 1655–1666

Nagai, T. and T. Yukimoto, 2003. Preparation and functional properties of beverages made from sea algae. *Food Chem*., 81: 327–332

Nikishimi, M., N. Rao and K. Yagi, 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. *Biochem. Biophys. Res. Commun*., 46: 849–854

Oyaizu, M., 1986. Studies on product of browning reaction prepared from glucose amine. *Japanese J. Nutr*., 44: 307–315

Peerapornpisal, Y., D. Amornlerdpison, U. Jamjai, T. Taesotikul, Y. Pongpaibul, M. Nualchareon and D. Kanjanapothi, 2010. Antioxidant and anti-inflammatory activities of brown marine alga, *Padina minor* Yamada. *Chiang Mai J. Sci*., 37: 507–516

Prior R.L., X. Wu and K. Schaich, 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem*., 53: 4290–4302

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biol. Med*., 26: 1231–1237

Rice-Evans, C.A., N.J. Miller and G. Paganga, 1997. Antioxidant properties of phenolic compounds. *Trends Plant Sci*., 2: 152–159

Santoso, J., Y. Yoshie and T. Suzuki, 2004. Polyphenolic compounds from seaweeds: Distribution and their antioxidative effect. *Dev. Food Sci*., 42: 169–178

Senevirathne, M., S. Kim, N. Siriwardhana, J. Ha, K. Lee and Y. Jeon, 2006. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging, metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci. Tech. Int*., 12: 27–38

Shahidi, F., 2009. Nutraceuticals and functional foods: whole versus processed foods. *Trends Food Sci. Tech*., 20: 376–387

Takeshi, S., Y. Yumiko and S. Joko, 2005, Mineral components and anti-oxidant activities of tropical seaweeds. *J. Ocean University China*, 4: 205–208

Targett, N.M. and T.M. Arnold, 1998, Predicting the effects of brown algal phlorotannins on marine herbivores in tropical and temperate oceans. *J Phycol.*, 34: 195–205

Valko, M., M. Izakovic, M. Mazur, C.J. Rhodes and J. Telser, 2004. Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell. Biochem*., 266: 37–56

Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol*., 39: 44–84

Wu, Y., C. Hong, S. Lin, P. Wu and M. Shiao, 1998. Increase of vitamin E content in LDL and reduction of atherosclerosis in cholesterol-fed rabbits by a water-soluble antioxidant-rich fraction of *Salvia miltiorrhiza*. *Arterioscler. Thomb. Vasc. Biol*., 18: 481–486

Yangthong, M., N. Hutadilok-Towatana and W. Phromkunthong, 2009. Antioxidant activities of four edible seaweeds from the southern coast of Thailand. *Plant Foods Human Nutr*., 64: 218–223

Zhu, Q.Y., R.M. Hackman, J.L. Ensunsa, R.R. Holt and C.L. Keen, 2002. Antioxidative activities of Oolong tea. *J. Agric. Food Chem*., 50: 6929–6934

(Received 10 August 2010; Accepted 03 September 2010)