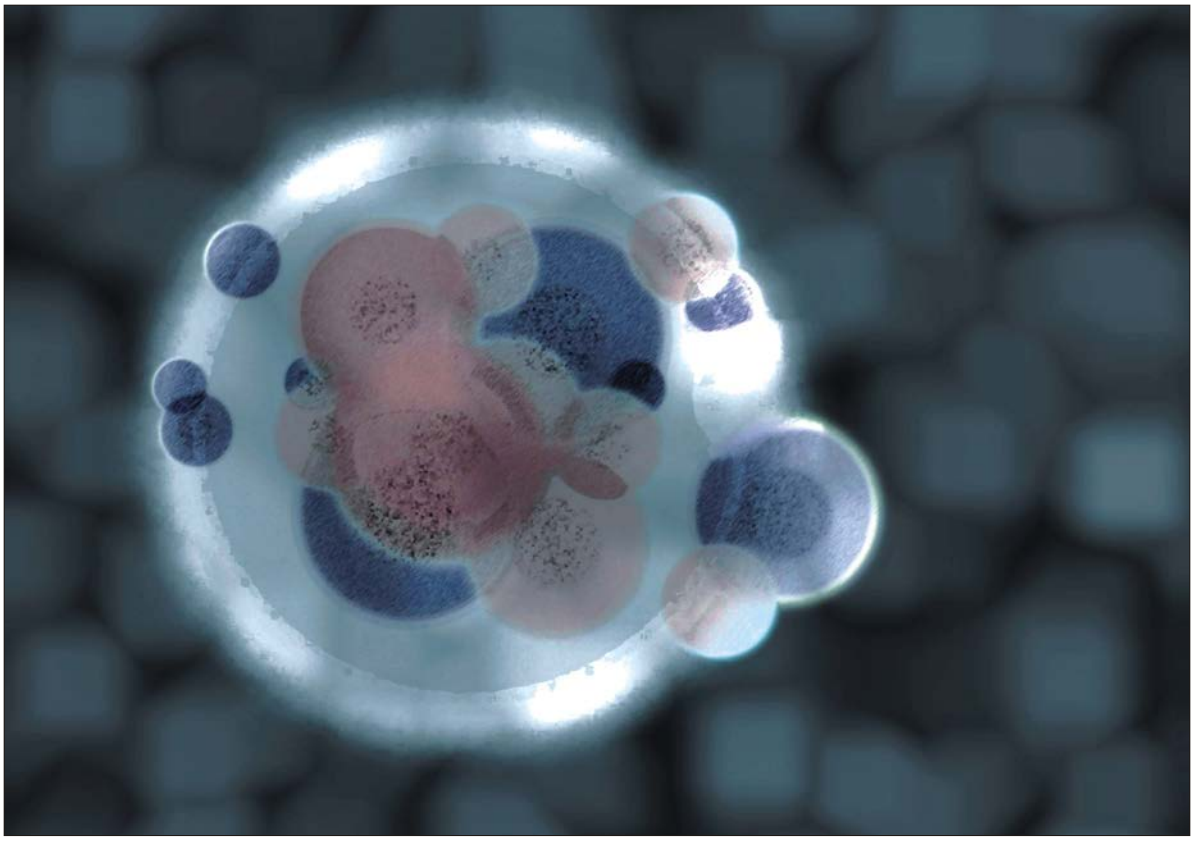

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 11 (4): 288-298, 2011
ISSN 1727-3048 / DOI: 10.3923/jbs.2011.288.298
© 2011 Asian Network for Scientific Information

Free Radical Scavenging, Metal Chelating and Singlet Oxygen Quenching Activity of Fractionated Brown Seaweed *Sargassum hystrix* Extract

[1]Siti A. Budhiyanti, [2]Sri Raharjo, [2]Djagal W. Marseno and [1]Iwan Y.B. Lelana
[1]Department of Fisheries, Faculty of Agriculture,
[2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology,
Gadjah Mada University, Jl Flora, Bulaksumur, Yogyakarta 55281, Indonesia

Abstract: The objective of the study was to isolate phenolic compound from the *Sargassum hystrix* crude extract by sequential solvent extraction using ethyl acetate, dichloromethane and butanol. *In vitro* antioxidant activity of fractionated cytoplasmic and membrane bound extracts were investigated. In addition to total phenolic content, the antioxidant activity were studied using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, metal chelating ability and Singlet Oxygen Quenching (SOQ) activity. Singlet oxygen quenching activity was examined using linoleic acid as substrate, containing 100 ppm erythrosine as a photosensitizer. The results showed that the membrane bound fractions had higher total phenolic compound, radical scavenging activity, ferrous ion-chelating ability and singlet oxygen quenching activity than cytoplasmic fractions ($p<0.05$). Among the eight fractions from membrane bound and cytoplasmic extracts isolated by differential solvent extraction, aqueous and butanol fractions of membrane bound extracts showed the highest DPPH radical scavenging, metal chelating ability and singlet oxygen quenching activity. The IC_{50} and metal chelating activity of aqueous fractions were 0.27±0.02 mg mL^{-1} and 51.53±6.63%, respectively and butanol fractions were 0.33±0.03 mg mL^{-1} and 44.75±3.33%, respectively. The butanol and aqueous fractions could act as SOQ at 75 ppm and 80 ppm, respectively. These results suggested that butanol and aqueous fractions were the most potent radical scavenger, metal chelator and singlet oxygen quencher.

Key words: *Sargassum hystrix,* antioxidant activity, free radical scavenger, metal chelator, singlet oxygen quencher

INTRODUCTION

Brown algae have attracted an emerging interest mainly for their bioactive substances which have great chances to be used as antioxidant (Nagai and Yukimoto, 2003; Nakai *et al*., 2006). Antioxidant compounds play an important role against various diseases (e.g., chronic inflammation, atherosclerosis, cancer and cardiovascular disorders) and aging processes (Kohen and Nyska, 2002; Mudgal *et al*., 2010). Moreover, interest in employing antioxidants from natural sources is considerably enhanced by consumer preference for natural products and concern about the potential toxic effects of synthetic antioxidant (Safer and Nughamish, 1999; Odukoya *et al*., 2007; Tibiri *et al*., 2007; Zubia *et al*., 2007; Hasan *et al*., 2010; Annegowda *et al*., 2010).

Earlier report revealed that phenolic compounds were one of the most effective antioxidant in brown algae (Nagai and Yukimoto, 2003). Many studies showed that phlorotannins were the main phenolic compounds detected in brown algae (Koivikko, 2008). Phlorotannin is a group of phenolic compounds which are formed by the polymerization of phloroglucinol (1,3,5 trihydroxybenzene) monomer units and synthesized in the acetate-malonate pathway in marine alga (Ragan and Glombitza, 1986; Arnold and Targett, 2000). Phenolic substances in brown algae are found in physodes, membrane-bound vesicles. It has been suggested that phlorotannins become components of brown algal cell walls when physodes fuse with cell membrane and the phlorotannins are secreted from cells, complexing finally with alginic acid (Schoenwaelder and Clayton, 1998; Koivikko, 2008). As the secondary roles, phenolic compounds are important in plant defense mechanism against invading bacteria and other types of environmental stress, such as wounding and excessive light or ultraviolet radiation (Amsler, 2008). In addition, phlorotannin are known to release, i.e., exude,

Corresponding Author: Siti A. Budhiyanti, Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Jl Flora, Bulaksumur, Yogyakarta 55281, Indonesia Tel: +62-274-551218 Fax: +62-274-551218

into the surrounding seawater (Swanson and Druehl, 2002; Koivikko, 2008). Therefore, Koivikko *et al.* (2005) divided phlorotannin into three parts, there are soluble phlorotannin from algal matrix or cytoplasmic phlorotannin, cell-wall bound phlorotannin that attached to the membrane or cell wall and exuded phlorotannin.

Phlorotannins isolated and purified from several brown algae have been reported to possess strong antioxidant activity which may be associated with their unique molecular skeleton (Ahn *et al.*, 2007). The multifunctional antioxidant activity of polyphenols is highly related to phenol rings which act as electron traps to scavenge peroxy and hydroxyl radicals. Antioxidant activity of phenolic acids and their derivatives depends on the number and position of hydroxyl groups bound to the aromatic ring, the binding site and the type of substituent (Sroka and Cisowski, 2003). Marine algae, like other photosynthesizing plants, are exposed to a combination of light and oxygen that leads to the formation of free radicals and other strong oxidizing agents. However, the absence of oxidative damage in the structural components of macroalga (i.e., polyunsaturated fatty acids) and their stability to oxidation during storage suggest that their cells have protective antioxidative defense systems (Matsukawa *et al.*, 1997; Zubia *et al.*, 2007). Phlorotannins from brown algae have up to eight interconnected rings. They are therefore more potent free radical scavenger than other polyphenols derived from terrestrial plants, including green tea catechins which only have three to four rings (Cox *et al.*, 2010). It was recognized that polyphenols can act as antioxidants by radical scavenging (Sroka and Cisowski, 2003), singlet oxygen quenching (Mukai *et al.*, 2005) and metal chelation mechanism (Andjelkovic *et al.*, 2006).

Antioxidative properties of seaweed extracts have been studied in several geographic regions but only a few studies have been performed on tropical seaweed species (Anggadiredja *et al.*, 1997; Lim *et al.*, 2002; Santoso *et al.*, 2004; Zubia *et al.*, 2007; Chandini *et al.*, 2008). Lack of information about the antioxidant activity of tropical macroalgae is surprising since these species are expected to develop a very effective antioxidant defense system due to the strong UV radiation in the tropical environment (Zubia *et al.*, 2007; Matanjun *et al.*, 2008). In fact, previous studies have demonstrated that UV radiation induces the promotion of antioxidant defense in macroalgae (Aguilera *et al.*, 2002; Bischof *et al.*, 2002). The genus *Sargassum*, kind of brown algae, had been studied extensively showing high antioxidant potential in vitro (Anggadiredja *et al.*, 1997; Matsukawa *et al.*, 1997; Yan *et al.*, 1998; Lim *et al.*, 2002; Santoso *et al.*, 2004; Heo *et al.*, 2005; Kim *et al.*, 2005; Park *et al.*, 2005; Cho *et al.*, 2007; Zubia *et al.*, 2007, 2008; Kuda *et al.*, 2005) but there are no publications on the antioxidant activities of *Sargassum* from Yogyakarta, Indonesia, especially on cytoplasmic and membrane bound extract.

The coastlines of Gunung Kidul, Yogyakarta, Indonesia has abundant resource of seaweed, especially brown algae *Sargassum* sp. but little effort has been made to explore the antioxidant potential of seaweed harvested from this area. Previous research screened many spesies of seaweed commonly found in the coastal waters of the Gunung Kidul and Jepara Indonesia for their antioxidant activity. The study showed that cytoplasmic and membrane bound extract from *Sargassum hystrix* had the highest antioxidant activity compared to other spesies. In addition, the membrane bound had higher antioxidant activity than cytoplasmic extract. Therefore the aim of the present study was to isolate phenolic compound from the crude cytoplasmic and membrane bound extract by sequentially solvent extraction with ethyl acetate, dichloromethane butanol and water. In addition to total phenolic content, the antioxidant activity of crude and fractionated extracts were studied using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferrous ion-chelating ability and singlet oxygen quenching activity.

MATERIALS AND METHODS

Plant material: The brown algae *Sargassum hystrix* were collected from the coastal area of Gunung Kidul (8°8'1" S; 110°33'16" E), Yogyakarta, Indonesia in October 2009. The washed seaweed was stored at -20°C until used. The seaweed was dried with oven at 55°C for 4 h before used.

Chemicals: 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, ethylene diamine tetra acetic acid (EDTA), 3-(2-pyridyl)-5,6-di (p-sulfophenyl)-1,2,4-triazine disodium salt (ferrozine), linoleic acid, ammonium thiocyanate, tocopherol and phloroglucinol were purchased from Sigma-Aldrich Co. Methanol, ferro chloride, butanol, ethyl acetate, dichloromethane were purchased from E. Merk (Darmstadt, Germany).

Preparation of seaweed extracts: Crude extracts of *Sargassum* sp. were prepared using the modified method from Lim *et al.* (2002), Haider *et al.* (2009) and Ye *et al.* (2009). Ten grams of dried seaweed were immersed in 100 mL methanol (MeOH) and shaked for 1 h at 40°C, followed by centrifuging the extract to collect the supernatant. The extraction was repeated three times. The supernatant were transferred to a conical flask and then washed with chloroform in a separatory funnel to remove

pigments. The extracts were evaporated with rotary vacuum evaporator until all solvent had evaporated. The extracts were called as cytoplasmic extract. The residues from the extractions of cytoplasmic extract were dried (30 min, 60°C) with oven. The dried residue (200 mg) were extracted with 8 mL of 1 M sodium hydroxide (NaOH), stirred for 2 h and neutralized with H_3PO_4 (Koivikko *et al*., 2005; Koivikko, 2008). The extracts were called as membrane bound extract. The two extracts were evaporated and freeze dried, then dissolved in distilled water. The extracts were partitioned sequentially with three different solvents, ethyl acetate (EtOAc), dichloromethane (DCM) and n-butanol (n-BuOH) to fractionate the polar and nonpolar compounds in the methanol crude extract. The resulting four extract evaporated to dryness in a rotary evaporator. They were kept in the dark and stored at 4°C prior to analysis. The each fraction and crude methanol extract were determined for total phenolic compound, free radical scavenging activity, ferrous ion chelating ability and singlet oxygen quenching activity.

Antioxidant assay

Total Phenolic Content (TPC): Total phenolic content was determined spectrophotometrically by Follin-Ciocalteau method (Chandini *et al*., 2008). Total content of phenolic compounds was calculated based on a standard curve of phloroglucinol. The phenolic content was expressed as g of Phloroglucinol Equivalents (PGE) per 100 g of extract (Zubia *et al*., 2007). This analysis was made in triplicate for each extract.

Free Radical Scavenging Activity (RSA): The free radical scavenging activity was determined according to the method of Chandini *et al*. (2008) and Samchai *et al*. (2009) with slightly modification. One milliliter seaweed extracts with various concentration was mixed with 2 mL of 0.08 mM methanolic solution of DPPH. The mixture was then vortexed and left for 30 min at room temperature in the dark and the absorbance was measured at 517 nm. BHT was used as the positive control.

A curve of extract concentration against % DPPH scavenging activity was made to estimate the concentration of extract needed to scavenge 50% of radicals. This value was known as IC_{50} (Inhibition Concentration) and expressed in terms of mg mL^{-1} (Senevirathne *et al*., 2006). As a blank was one milliliter of methanol mixed with 2 mL of 0.08 mM methanolic solution of DPPH. The result was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = [1-(\frac{A_{sample}}{A_{blank}})\times 100\%]$$

Ferrous Ion-Chelating (FIC) ability: The ferrous ion-chelating ability was determined according to the method of Ye *et al*. (2009) and Wang *et al*. (2009). EDTA was used as the positive control. The result was expressed as percentage of chelating ability (% chelating ability), using following equation:

$$\text{Ferrous ion-chelating ability (\%)} = [1-\frac{(A_{sample}-A_{blank})}{A_{control}}]\times 100\%$$

$FeCl_2$ solution substituted by distilled water was used as blank and the sample substituted by distilled water was used as negative control.

Singlet Oxygen Quenching (SOQ) activity of seaweed extracts on erythrosine sensitized photooxidation: The procedure was according to Suryanto *et al*. (2004). The various concentration of seaweed extracts were mixed with 1% linoleic acid and 100 ppm erythrosine as photosensitizer in methanol solution. The bottles were sealed air-tight with teflon septa and placed in the light box. The light intensity was 4000 lux at room temperature. Peroxide values were determined for 6 h according to the method from Chapman and Mackay (1949).

Statistical analysis: The test for antioxidant activity and total phenol content were carried out in triplicate. The data was recorded as Mean±SD. The means of all parameters were examined for significance by Analysis of Variance (ANOVA) with Duncan's significant difference post-hoc test using Microsoft Excel 2007. The p-value of less than 0.05 was considered significant. A linier and non linier regression analysis was used to determine correlation coefficient between total phenolic content and IC_{50}; total phenolic content and ferrous ion-chelating ability; IC_{50} and SOQ.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC): The cytoplasmic and membrane bound extract were sequentially separated into four fractions, including EtOAc, DCM and BuOH-soluble fractions and the final aqueous residue, by liquid-liquid partition. The total phenolic contents of the fractionated extracts were determined from regression equation of standard curve y = 3.91x+0.002 (Fig. 1) and expressed as gram of Phloroglucinol Equivalents (PGE) per 100 g of extract.

There were significant differences ($p<0.05$) in total phenolic compound among the crude extract and its fractions. The total phenolic content revealed that fractionated membrane bound extracts showed higher yields than fractionated cytoplasmic extracts. The amount

varied from 0.15-4.78 g PGE/100 g dried extract for cytoplasmic extract and 1.16-18.58 g PGE/100 g dried extract for membrane bound extract. The results were higher than Zubia *et al.* (2008) and Haider *et al.* (2009) who reported a phenolic content of *Sargassum* species from Karachi Pakistan, Tahiti and Qingdao, China. In fact, the production of antioxidant compounds, like phenolics, are influenced by several extrinsic factors (herbivory pressure, irradiance, depth, salinity, nutrients, tidal cycle, etc.) and intrinsic factors (type, age and reproductive stage) (Amsler and Fairhead, 2005; Connan *et al.*, 2006; Connan *et al.*, 2007). Therefore, phenolic content of seaweeds could be subjected to great intra specific variation, even at very small scales.

The phenolic contents of fractionated seaweed extracts were significantly different among the fractions ($p<0.05$). The EtOAc fraction which was intermediate polarity solvent showed the highest phenolic content among cytoplasmic extracts, as the result from Mokbel *et al.* (2006) and Okpuzor *et al.* (2009). On the contrary, the aqueous fraction which was the highest polarity solvent showed the highest phenolic content among membrane bound extract. It indicated that the phenolic compounds from membrane bound extracts were more soluble in polar solvent than cytoplasmic extract.

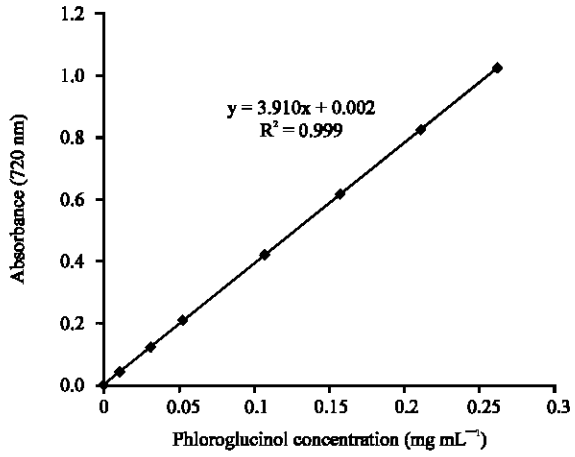

Fig. 1: Standard curve of phloroglucinol for determination of total phenolic content

Free Radical Scavenging Activity (RSA): The parameter used to measure the free radical scavenging activity was IC_{50}. The DPPH radical-scavenging activity in the study was reported after 30 reaction times for all samples evaluated. The less IC_{50} showed the higher antioxidant activity of plant fractions (Maisuthisakul *et al.*, 2007; Uddin *et al.*, 2008).

The presented data in Table 1 indicated that IC_{50} were in the range of 0.27 to 3.98 mg mL^{-1} for membrane bound and 1.59 to 15.92 mg mL^{-1} for cytoplasmic extracts. The activities of these fractions were observed to be significantly ($p<0.05$) higher than that of crude extract, except DCM fractions. Among the four different polarity fractions isolated from cytoplasmic extract by solvent partition, the EtOAc fraction appeared to posses the highest antioxidant activity ($p<0.05$) with IC_{50} value of 1.59±0.04 mg mL^{-1}. The aqueus, n-BuOH and DCM revealed only moderate and low activities. It indicated that compounds with strongest radical-scavenging activity in cytoplasmic extract had medium polarity. The results were similar with the research from Kang *et al.* (2004), Duan *et al.* (2006) and Wang *et al.* (2009) that reported EtOAc fraction contained phlorotannin compound, had the highest antioxidant activity compared with DCM, n-BuOH and aqueus. On the contrary, the aqueous and n-BuOH fractions had the highest antioxidant activity among membrane bound extract, with IC_{50} value of 0.27±0.02 and 0.33±0.03 mg mL^{-1}.

The results indicated that the fractions with high polarity solvents exhibited the highest antioxidant activity than low and medium polarity solvent. The active component as an antioxidant in the membrane bound fraction seemed to be more soluble in polar solvents than the cytoplasmic fraction (Duan *et al.*, 2006). It was probably caused by high molecular weight fractions within the extracts. The high molecular weight fractions from the kelps *S. kjellmanianum* and *Ecklonia cava* containing polymers such as tetrafuhalol, dieckol,

Table 1: Total phenolic content, IC50 RSA DPPH and ferrous ion-chelating ability of fractionated cytoplasmic and membrane bound extract

	Total phenolic content (g PGE/100 g dried extract)		IC50 RSA DPPH (mg mL^{-1})		Ferrous ion-chelating ability (%)	
Fractions	Cytoplasmic	Membrane bound	Cytoplasmic	Membrane bound	Cytoplasmic	Membrane bound
Crude Extract	1.21±0.14^{b}	9.95±0.85^{c}	13.86±1.17^{b}	1.01±0.08^{b}	0.84±0.11^{c}	12.65±1.41^{d}
EtOAc	4.78±0.31^{a}	11.12±0.94^{b}	1.59±0.04^{e}	0.55±0.04^{c}	14.67±2.89^{b}	18.99±1.43^{c}
DCM	0.15±0.03^{d}	1.16±0.34^{d}	15.92±0.22^{a}	3.98±0.14^{a}	11.29±0.62^{b}	6.97±2.18^{e}
Bu-OH	1.42±0.31^{b}	11.13±1.93^{b}	4.45±0.39^{c}	0.33±0.03^{d}	12.61±2.96^{b}	44.75±3.33^{b}
AQU	0.69±0.19^{c}	18.58±3.61^{a}	2.75±0.11^{d}	0.27±0.02^{d}	17.40±0.23^{b}	51.53±6.63^{b}
Control-BHT			0.10±0.02^{f}	0.10±0.02^{e}		
Control-EDTA 2 ppm					95.00±0.12^{a}	95.00±0.12^{a}

Each value is expressed as mean±SD of three replicates. Values in the same column followed by different letter are significantly different ($p<0.05$)

Table 2: Correlation and determination coefficient of fractionated cytoplasmic and membrane bound extract

		Correlation coefficient (r)		Determination coefficient (R^2)	
Treatments	Fractions	Linier	Non linier	Linier	Non linier
Total phenolic content vs. IC_{50}	Cytoplasmic	-0.59	-0.71	0.35	0.50
	Membrane bound	-0.88	-0.99	0.78	0.99
Total phenolic content vs ferrous ion-chelating ability	Cytoplasmic	ns	ns	0.04	0.04
	Membrane bound	0.81	0.87	0.65	0.76
IC_{50} vs. SOQ	Cytoplasmic	0.76	0.81	0.58	0.66
	Membrane bound	0.85	0.93	0.73	0.86

phlorofucofuroeckol and 6-6' bieckol were more polar and conferring greater free radical quenching activity than low molecular weight fractions containing phloroglucinol and eckol (Kim *et al*., 2004; Nakamura *et al*., 1996; Yan *et al*., 1996). Nevertheless, the results of this fractionation had a lower antioxidant activity than the commercial antioxidant BHT.

There was significant negative correlation ($p<0.05$) observed between total phenolic content and the IC_{50} of cytoplasmic and membrane bound fractions, as shown in Table 2. These results indicated that the higher phenol concentration, the less IC_{50} or higher the ability to bind DPPH. Positive correlation between phenolic contents and antioxidant activities have often been reported in *Sargassum* spesies (Kang *et al*., 2003; Kim *et al*., 2005; Connan *et al*., 2006; Nakai *et al*., 2006; Zhang *et al*., 2007; Zubia *et al*., 2008).

The IC_{50} of the membrane bound fractions were 3-14 times stronger than the scavenging activity of cytoplasmic extract. The fractionated membrane bound extracts also exhibited relatively higher DPPH radical scavenging activities than *Sargassum* from Mexico waters (Zubia *et al*., 2007). It was probably caused by the function of the cell walls and membranes as an effective optical barrier attenuating incident UV radiation before reaching intracellular organelles and biomolecules (Holzinger and Lutz, 2006). Phenolic compounds were assumed to protect algal thalli from photodestruction by UV radiation (Pavia and Toth, 2000) and to exhibit free-radical scavenging properties (Nakai *et al*., 2006; Connan *et al*., 2006). In addition, Pavia *et al*. (1997) showed that the phlorotannin content in *A. nodosum* increased when seaweeds were exposed to increase levels of UV-B radiation in a field experiment and posses a high antioxidant activity.

The coefficient of determination (R^2) of total phenolic content and IC_{50} is presented in Table 2. The R^2 value indicated that scavenging effect of fractions was not limited to phenolic compound. The activity may also come from the presence of other compounds, such as protein or peptides, ascorbic acid, low-molecular-weight polysaccharides, fucoidan, maillard reaction products and mycosporines-like amino acids (Kuda *et al*., 2005, 2006; Kuda and Ikemori, 2009; Wang *et al*., 2009; Zubia *et al*., 2008). Among those isolated from *Sargassum* species are meroterpenoids from S. *siliquastrum* (Jang *et al*., 2005), plastoquinones from *S. micracanthum* (Iwashima *et al*., 2005) and some aromatic compounds from *S. thunbergii* (Seo *et al*., 2007).

Ferrous Ion-chelating (FIC) ability: The ability of cytoplasmic and membrane bound extract as the ferrous ion-chelating was shown in Table 1. Metal chelating ability of seaweed fractions were tested at a concentration 2.15 mg mL^{-1} for cytoplasmic extract and 0.81 mg mL^{-1} for membrane bound extract. The fractions from membrane bound showed higher ferrous ion-chelating ability than cytoplasmic extracts. The activity was in the range of 11.29-17.40% for cytoplasmic and 6.97-51.53% for membrane bound. The highest activity was found in aqueus and butanol extract. Nevertheless, when compared with the positive control 2 ppm EDTA, the results of fractionation had a lower activity. Andjelkovic *et al*. (2006) reported that the ability of phenolic compounds to chelate iron were far lower than that of EDTA.

The ferrous ion chelating ability had positive correlation with total phenolic compound of membrane bound fractions but no significant correlation was observed on cytoplasmic fractions (Table 2). The coefficient of determination (R^2) of cytoplasmic and membrane bound extract are presented in Table 2. The higher determination coefficient in membrane bound was probably caused that phenolic compounds in membrane bound were potent ferrous ion chelator and could form complexes with metal ions, as protection against toxic metal ions (Ragan and Glombitza, 1986; Toth and Pavia, 2000; Chew *et al*., 2008; Senevirathne *et al*., 2006). Metal chelating potency of phenolic compounds are dependent upon their unique phenolic structure and the number and location of the hydroxyl groups (Santoso *et al*., 2004; Andjelkovic *et al*., 2006). The metal chelating ability of polyphenols is related to the presence of ortho-dihydroxy polyphenols (Khokhar and Apenten, 2003).

In contrast, the results of cytoplasmic fractions were not as good as membrane bound fractions as metal chelator. It was in agreement with the findings of Saiga *et al.* (2003) and Wang *et al.* (2009), that explained ferrous ion-chelating capacity and phlorotannin did not appear to be very effective metal chelator. The components such as polysaccharides, proteins or peptides in the extracts have also been reported to possess the abilities to chelate metal ions. In addition, the study conducted by Toth and Pavia (2000) showed that other compounds such as polysaccharides, e.g., alginates, fucoidan and or phytochelatins were more effective than phlorotannins for detoxification and resistance to copper accumulation in *A. nodosum*. The ferrous binding capacities of a quantity of dietary fibers, such as carrageenan, agar, alginate and fucoidan may have caused the decrease of ferrous ion in the assay system in this study (Kuda *et al.*, 1998, 2005).

Singlet Oxygen Quenching (SOQ) activity of fractionated seaweed extracts on erythrosine sensitized photooxidation: Photooxidation occurs when there is light, triplet oxygen and photosensitizer (Min and Boff, 2002). Type I photo-sensitized reaction involves the formation of superoxide anion and other radicals due to the transfer of hydrogen atoms or electrons by interaction of triplet sensitizer with molecular or other components. Type II reaction involves the generation of singlet oxygen by the energy transfer from an excited triplet sensitizer to a triplet oxygen. The photochemical processes in the food systems are dependent on the types and concentration of sensitizers and substrates in the system (Jung *et al.*, 1999; Suryanto *et al.*, 2004). Erythrosine is efficient photochemical sensitizers for the formation of singlet oxygen (Pan *et al.*, 2005). The formation of singlet oxygen by erythrosine as photosensitizer accelerated lipid peroxidation (Yang *et al.*, 2002). The antioxidant as singlet oxygen quencher is capable to capturing singlet oxygen, so that unsaturated fatty acids damage becomes obstructed. The damage or oxidation of linoleic acid in the system was determined by measuring the peroxide values for 6 h (Jung *et al.*, 1999; Pan *et al.*, 2005; Suryanto *et al.*, 2004). There are few antioxidants that can be used for the protection of foods from the photosensitized oxidation. These are ascorbic acid, ascorbyl palmitate, carotenoids and tocopherols (Jung *et al.*, 1999).

The effect of fractionated *S. hystrix* as antioxidant on photooxidation of linoleic acid system is presented in Fig 2a-h. Photooxidation was performed under accelerated condition using fluorescent lights with an intensity of 4000 lux and samples were exposed to light for up to 6 h at room temperature, with erythrosine as a sensitizer. Intense light exposure in photooxidation induced an increased rate of peroxide formation in the oil (Mukai *et al.*, 2005).

Figure 2 showed the effects of different concentration of fractionated cytoplasmic and membrane bound extract on erythrosine-sensitized photooxidation of linoleic acid during 6 h, with linoleic acid system without antioxidant as control. As the illumination time increased, the peroxide value of the control increased, resulting in peroxide value of 6.96 meq kg^{-1} oil after 6 h. The presented data showed that the addition of antioxidants could inhibit peroxide formation rate compared to the control ($p<0.05$). These proved that antioxidants derived from the fractionation of extract could act as a Singlet Oxygen Quencher (SOQ). The results indicated that the fractions have great potential for the protection of numerous foods from light-induced deterioration.

Linoleic acid system without sensitizer (Control-WS) did not show a significant increase in peroxide value ($p>0.05$) and proved that without sensitizer, singlet oxygen could not be generated. Min and Boff (2002) stated that singlet oxygen can be generated from triplet oxygen in the presence of sensitizer and light. However, the SOQ activities of the fractions were not as good as 100 ppm tocopherol as positive control ($p<0.05$).

Figure 2a and b showed that the EtOAc fraction significantly ($p<0.05$) inhibited peroxide value formation compared to the control, with minimum inhibitory concentration were 1900 and 270 ppm for cytoplasmic and membrane bound extract, respectively. Figure 2c and d showed that minimum inhibitory concentration of DCM fractions were 4200 and 400 ppm for cytoplasmic and membrane bound extract, respectively. The minimum inhibitory concentration of n-BuOH and aqueus extract were presented at Fig. 2e-h. The results showed that the n-BuOH extracts could significantly ($p<0.05$) inhibit peroxide formation compared to the control at 2770 ppm and 75 ppm for cytoplasmic and membrane bound, respectively. The aqueus extract could significantly ($p<0.05$) inhibit peroxide formation at 4800 ppm and 80 ppm for cytoplasmic and membrane bound, respectively.

To evaluate SOQ activity among the fractions, the minimal concentration of each fraction that inhibited peroxide formation was collected (Table 3). The data indicated that the fractions from membrane bound extract had higher activity as SOQ than cytoplasmic extract. In

Table 3: Minimum concentration of fractionated cytoplasmic and membrane bound extract as Singlet Oxygen Quencher

Extract	Fractions	Minimum concentration as SOQ (ppm)
Cytoplasmic	Ethyl acetate	1900
	Dichloromethane	4200
	Butanol	2770
	Aqueous	4800
Membrane bound	Ethyl acetate	270
	Dichloromethane	400
	Butanol	75
	Aqueous	80

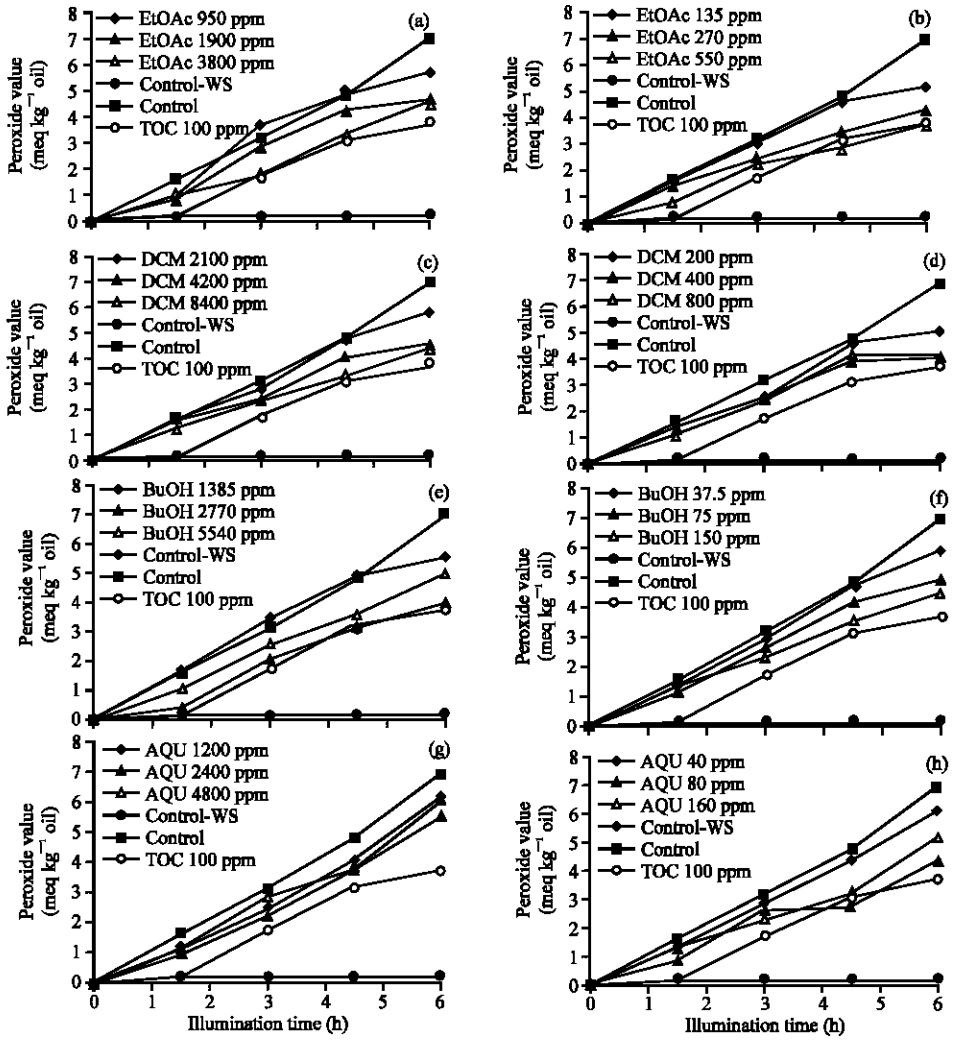

Fig. 2(a-h): Effect of fractionated cytoplasmic (left column) and membrane bound (right column) extract from *S. hystrix* on the erythrosine-sensitized photooxidation of linoleic acid in methanol during 6 h fluorescent light illumination (4000 lux) at room temperature EtOAc: Ethyl acetate, DCM: Dichloromethane, BuOH: Butanol, AQU: Aqueous, TOC: Tocopherol, WS: Without sensitizer

the membrane bound extract, SOQ activity was found in the range of 75-400 ppm with the highest activity at n-BuOH and aqueous fractions. In the cytoplasmic extract, SOQ can be seen in the range of 1900-4800 ppm with the highest activity at EtOAc. These indicated that EtOAc isolated from cytoplasmic extract and n-BuOH and aqueous fraction isolated from membrane bound extract were the most effective fractions as SOQ. In membrane bound fraction, as the polarity of the extracting solvent increased, the antioxidative activity of the extract also increased. This result indicated that the antiphotooxidative components in the membrane bound fractions had strong polar properties and easily extracted with highly polar solvent.

A significant positive correlation was found between the IC_{50} and minimum concentration of fractionated cytoplasmic and membrane bound extracts, as shown in Table 2. The results were supported by Mukai *et al.* (2005) that found free radical scavenging and singlet oxygen quenching activity correlate to each other.

The determination coefficient (R^2) between IC_{50} and minimum inhibitory concentration as SOQ is presented in Table 2. The results showed that SOQ activity might also come from the presence of other brown seaweed carotenoid compounds, such as fucoxanthin, fucoxanthinol and halocynthiaxanthin (Sachindra *et al.*, 2007).

CONCLUSION

The fractions from fractionated membrane bound extracts were the source of more potent antioxidants as radical scavenger, ferrous-ion chelator and singlet oxygen quencher than that of cytoplasmic fractions, with the highest antioxidant activity were aqueous and butanol fraction, respectively. The present findings appear useful in leading to further experiments on the identification and characterization specific compounds that are responsible for the relatively high antioxidant activities in the fractions of *S. hystrix*.

ACKNOWLEDGMENT

The authors wish to thank to The Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia for providing research fund through Doctorate Dissertation Research Grant 2010.

REFERENCES

Aguilera, J., A. Dummermuth, U. Karsten, R. Schriek and C. Wiencke, 2002. Enzymatic defences against photooxidative stress induced by ultraviolet radiation in Arctic marine macroalgae. Polar Biol., 25: 432-441.

Ahn, G.N., K.N. Kim, S.H. Cha, C.B. Song and J. Lee *et al*., 2007. Antioxidant activities of phlorotannins purified from *Ecklonia cava* on free radical scavenging using ESR and H_2O_2-mediated DNA damage. Eur. Food Res. Technol., 226: 71-79.

Amsler, C.D and V.A. Fairhead, 2005. Defensive and sensory chemical ecology of brown algae. Adv. Bot. Res., 43: 1-91.

Amsler, C.D., 2008. Algal Chemical Ecology. Springer-Verlag, Berlin, Germany, ISBN-13: 9783540929987, pp: 313.

Andjelkovic, M., J.V. Camp, B.D. Meulenaer, G. Depaemelaere, C. Socaciu, M. Verloo and R. Verhe, 2006. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. Food Chem., 98: 23-31.

Anggadiredja, J., R. Andyani and H. Muawanah, 1997. Antioxidant activity of *Sargassum polycystum* (Phaeophyta) and *Laurencia obtuse* (Rhodophyta) from Seribu islands. J. Applied Phycol., 9: 477-479.

Annegowda, H.V., C. Ween Nee, M.N. Mordi, S. Ramanathan and S.M. Mansor, 2010. Evaluation of phenolic content and antioxidant property of hydrolysed extracts of *Terminalia catappa* L. leaf. Asian J. Plant Sci., 9: 479-485.

Arnold, T.M. and N.M. Targett, 2000. Evidence for metabolic turnover of polyphenolics in tropical brown algae. J. Chem. Ecol., 26: 1393-1408.

Bischof, K., D. Hanelt, J. Aguilera, U. Karsten, B. Vogele, T. Sawall and C. Wiencke, 2002. Seasonal variation in ecophysiological patterns in macroalga from an Arctic fjord. I. Sensitivity on photosynthesis to ultraviolet radiation. Mar. Biol., 140: 1097-1106.

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.

Chapman, R.H. and J. Mackay, 1949. The estimation of peroxides infats and oils by the ferric thiocyanate method. J. Am. Oil Chem. Soc., 26: 360-363.

Chew, Y.L., Y.Y. Lim, M. Omar and K.S. Khoo, 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT-Food Sci. Technol., 41: 1067-1072.

Cho, S.H, S.E. Kang, J.Y. Cho, A.R. Kim, S.M. Park, Y.K. Hong and D.H. Ahn, 2007. The antioxidant properties of brown seaweed (*Sargassum siliquastrum*) extracts. J. Med. Food, 10: 479-485.

Connan, S., F. Delisle, E. Deslandes and E.A. Gall, 2006. Intra-thallus phlorotannin content and antioxidant activity in phaeophyceae of temperate waters. Bot. Mar., 49: 34-46.

Connan, S., E. Deslandes and E.A. Gall, 2007. Influence of day-night and tidal cycles on phenol content and antioxidant capacity in three temperate intertidal brown seaweeds. J. Exp. Mar. Biol. Ecol., 349: 359-369.

Cox, S., N. Abu-Ghannam and S. Gupta, 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int. Food Res. J., 17: 205-220.

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

Haider, S., Z. Li, H. Lin, K. Jamil and B.P. Wang, 2009. *In vivo* study of anti allergenicity of ethanol extracts from *Sargassum tenerrimum*, *Sargassum cervicorne* and *Sargassum graminifolium* turn. Eur. Food Res. Technol., 229: 435-441.

Hasan, N.S., Z.H. Amom, A.I. Nor, N. Mokhtarrudin, N.M. Esa and A. Azlan, 2010. Nutritional composition and *in vitro* evaluation of the antioxidant properties of various dates extracts (*Phoenix dactylifera* L.) from libya. Asian J. Clin. Nutr., 2: 208-214.

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

Holzinger, A. and C. Lutz, 2006. Algae and UV irradiation: Effects on ultrastructure and related metabolic functions. Micron, 35: 190-207.

Iwashima, M., J. Mori, X. Ting, T. Matsunaga and K. Hayashi *et al*., 2005. Antioxidant and antiviral activities of plastoquinones from the brown alga *Sargassum micracanthum* and a new chromene derivative converted from the plastoquinones. Biol. Pharm. Bull., 28: 374-377.

Jang, K.H., B.H. Lee, B.W. Choi, H.S. Lee and J. Shin, 2005. Chromenes from the brown alga *Sargassum siliquastrum*. J. Nat. Prod., 68: 716-723.

Jung, M.Y., J.P. Kim and S.Y. Kim, 1999. Methanolic extract of *Coptis japonica* Makino reduces photosensitized oxidation of oils. Food Chem., 67: 261-268.

Kang, H.S., H.Y. Chung, J.Y. Kim, B.W. Son, H.A. Jung and J.S. Choi, 2004. Inhibitory phlorotannins from the edible brown alga Ecklonia stolonifera on total Reactive Oxygen Species (ROS) generation. Arch. Pharm. Res., 27: 194-198.

Kang, K.Y. Park, H.J. Hwang, S.H. Kim, J.G. Lee and H.C. Shin, 2003. Antioxidative properties of brown algae polyphenolics and their perspectives as chemopreventive agents against vascular risk factors. Arch. Pharm. Res., 26: 286-293.

Khokhar, S. and R.K.O. Apenten, 2003. Iron binding characteristics of phenolic compounds: Some tentative structure-activity relations. Food Chem., 81: 133-140.

Kim, J.A., J.M. Lee, D.B. Shin and N.H. Lee, 2004. The antioxidant activity and tyrosinase inhibitory activity of phlorotannins in *Ecklonia cava*. Food Sci. Biotechnol., 13: 476-480.

Kim, S.J., S. Woo, H. Yun, S. Yum and E. Choi *et al*., 2005. Total phenolic contents and biological activities of Korean seaweed extracts. Food. Sci. Biotechnol., 14: 798-802.

Kohen, R. and A. Nyska, 2002. Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions and methods for their quantification. Toxicol. Pathol., 30: 620-650.

Koivikko, R., 2008. Brown algal phlorotannins: Improving and applying chemical methods. University of Turku, Turku, Finland, pp: 1-61. http://www.doria.fi/bitstream/handle/10024/36054/AI381.pdf?sequence=1

Koivikko, R., J. Loponen, T. Honkanen and V. Jormalainen, 2005. Contents of cytoplasmic, cell-wall-bound and exudes phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. J. Chem. Ecol., 31: 195-209.

Kuda, T. and T. Ikemori, 2009. Minerals, polysaccharides and antioxidant properties of aqueus solutions obtained from macroalgal beach-coasts in the Noto Peninsula, Ishikawa, Japan. Food Chem., 112: 575-581.

Kuda, T., H. Goto, M. Yokoyama and T. Fujii, 1998. Fermentation of dietary fiber in dried products of brown algae and their effects on fecal microflora and levels of plasma lipid in rats. Fish. Sci., 64: 582-588.

Kuda, T., M. Tsunekawa, H. Goto and Y. Araki, 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J. Food Composition Anal., 18: 625-633.

Kuda, T., T. Hishi and S. Maekawa, 2006. Antioxidant properties of dried product of haba-nori, an edible brown algae, *Petalonia binghamiae* (J. Agaradh) Vinogradova. Food Chem., 98: 545-550.

Lim, S. N., P.C.K. Cheung, V.E.C. Ooi and P.O. Ang, 2002. Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. J. Agric. Food Chem., 50: 3862-3866.

Maisuthisakul, P., M. Suttajit and R. Pongsawatmanit, 2007. Assesment of phenolic content and free radical scavenging capacity of some Thai indigenous plants. Food Chem., 100: 1409-1418.

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. Applied Phycol., 20: 367-373.

Matsukawa, R., Z. Dubinsky, E. Kishimoto, K. Masak and Y. Masuda *et al*., 1997. A comparison of screening methods for antioxidants activity in seaweeds. J. Applied Phycol., 9: 29-35.

Min, D.B. and J.M. Boff, 2002. Chemistry and reaction of singlet oxygen in foods. Compr. Rev. Food Sci. Saf., 1: 58-72.

Mokbel, M.S., Y. Watanabe, F. Hashinaga and T. Suganuma, 2006. Purification of the antioxidant and antimicrobial substance of ethyl acetate extracts from Buntan (*Citrus grandis* Osbeck) fruit peel. Pak. J. Biol. Sci., 9: 145-150.

Mudgal, V., N. Madaan, A. Mudgal and S. Mishra, 2010. Dietary polyphenols and human health. Asian J. Biochem., 5: 154-162.

Mukai, K., S. Nagai and K. Ohara, 2005. Kinetic study of the quenching reaction of singlet oxygen by tea catechins in ethanol solution. Free Radical Biol. Med., 39: 752-761.

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

Nakai, M., N. Kageyama, K. Nakahara and W. Mild, 2006. Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*. Mar. Biotechnol., 8: 409-414.

Nakamura, T., K. Nagayama, K. Uchida and R. Tanaka, 1996. Antioxidant activity of phlorotan-nins isolated from the brown algae *Eisenia bicyclis*. Fish. Sci., 62: 923-926.

Odukoya, O.A., S.I. Inya-Agha, F.I. Segun, M.O. Sofidiya and O.O. Ilori, 2007. Antioxidant activity of selected Nigerian green leafy vegetables. Am. J. Food Technol., 2: 169-175.

Okpuzor, J., H. Ogbunugafor and G.K. Kareem, 2009. Antioxidative properties of ethyl acetate fraction of *Globimetula braunii* in normal albino rats. J. Biol. Sci., 9: 470-475.

Pan, X., H. Ushio and T. Ohshima, 2005. Effects of molecular configurations of food colorants on their efficacies as photosensitizers in lipid oxidation. Food Chem., 92: 37-44.

Park, P.J., S.J. Heo, E.J. Park, S.K. Kim, H.G. Byun, B.T. Jeon and Y.J. Jeon, 2005. Reactive oxygen scavenging effect of enzymatic extracts from *Sargassum thunbergii*. J. Agric. Food Chem., 53: 6666-6672.

Pavia, H. and G.B. Toth, 2000. Influence of light and nitrogen on the phlorotannin content of the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. Hydrobiologia, 440: 299-305.

Pavia, H., G. Cervin, A. Lindgren and P. Aberg, 1997. Effects of UV-B radiation and simulated herbivory on phlorotannins in the brown alga *Ascophyllum nodosum*. Mar. Ecol. Prog. Ser., 157: 139-146.

Ragan, M.A. and K.W. Glombitza, 1986. Phlorotannins, Brown Algal Polyphenols. In: Progress in Phycological Research, Round, F.E. and D.J. Chapman (Eds.). Biopress Ltd., Bristol, UK., pp: 129-241.

Sachindra, N.M., E. Sato, H. Maeda, M. Hasokawa, Y. Niwano, M. Kohno and K. Miyashita, 2007. Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolits. J. Agric. Food Chem., 55: 8516-8522.

Safer, A.M. and A.L. Nughamish, 1999. Hepatotoxicity induced by the antioxidant food additive Butylated Hydroxytoluene (BHT) in rats: An electrol microscopical study. Histol. Histopathol., 14: 391-406.

Saiga, A., S. Tanabe and T. Nishimura, 2003. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. J. Agric. Food Chem., 51: 3661-3667.

Samchai, S., P. Seephonkai, A. Sangdee, A. Puntumchai and U. Klinhom, 2009. Antioxidant, cytotoxic and antimalarial activities from crude extracts of mushroom *Phellinus linteus*. J. Biol. Sci., 9: 778-783.

Santoso, J., Y. Yumiko and S. Takeshi, 2004. Antioxidant activity of methanol extracts from Indonesian seaweeds in an oil emulsion model. Fish. Sci., 70: 183-188.

Schoenwaelder, M.E.A and M.N. Clayton, 1998. Secretion of phenolic substances into the zygote wall and cell plate in embryos of *Hormosira* and *Acrocarpia* (Fucales, Phaeophyceae). J. Phycol., 34: 969-980.

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. Technol. Int., 12: 27-38.

Seo, Y., K.E. Park and T.J. Nam, 2007. Isolation of a new chromene from the brown alga *Sargassum thunbergii*. Bull. Korean Chem. Soc., 28: 1831-1833.

Sroka, Z. and W. Cisowski, 2003. Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food Chem. Toxicol., 41: 753-758.

Suryanto, E., H. Sastrohamidjojo, S. Raharjo and Tranggono, 2004. Singlet oxygen quenching effect of andaliman (*Zanthoxylum acanthopodium* DC) extracts in light-induced lipid oxidation. Indonesian Food Nutr. Prog., 11: 48-55.

Swanson, A.K. and L.D. Druehl, 2002. Induction, exudation and the UV protective role of kelp phlorotannins. Aquat. Bot., 73: 241-253.

Tibiri, A., O. Rakotonandrasana, G.O. Nacoulma and J.T. Banzouzi, 2007. Radical scavenging activity, phenolic content and cytotoxicity of bark and leaves extracts of *Entada africana* Guill. and Perr. (Mimosaceae). J. Boil. Sci., 7: 959-963.

Toth, G. and H. Pavia, 2000. Lack of phlorotannin induction in the brown seaweed *Ascophyllum nodosum* in response to increased copper concentration. Mar. Ecol. Prog. Ser., 192: 119-126.

Uddin, S.N., M.E. Ali and M.N. Yesmin, 2008. Antioxidant and antibacterial activities of *Senna tora* roxb. Am. J. Plant Physiol., 3: 96-100.

Wang, T., R. Jonsdottir and G. Olafsdottir, 2009. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem., 116: 240-248.

Yan, X, X. Li, C. Zhou and X. Fan, 1996. Prevention of fish oil rancidity by phlorotannins from *Sargassum kjellmanianum*. J. Applied Phycol., 8: 201-203.

Yan, X., T. Nagata and X. Fan, 1998. Antioxidative activities in some common seaweeds. Plant Foods Hum. Nutr., 52: 253-262.

Yang, W.T., J.H. Lee and D.B. Min, 2002. Quenching mechanism and kinetics of α-tocopherol and β-carotene on the photosensitising effect of synthetic food colorant FD and C Red No. 3. J. Food Sci., 67: 507-510.

Ye, H., C. Zhou, Y. Sun, X. Zhang, J. Liu, Q. Hu and X. Zeng, 2009. Antioxidant activities *in vitro* of ethanol extract from brown seaweed *Sargassum pallidum*. Eur. Food Res. Technol., 230: 101-109.

Zhang, W.W., X.J. Duan, H.L. Huang, Y. Zhang and B.G. Wang, 2007. Evaluation of 28 marine algae from the qingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and subfractions derived from *Symphyocladia latiuscula* (Rhodomelaceae). J. Applied Phycol., 19: 97-108.

Zubia, M., D.Y. Robledo and Y. Freile-Pelegrin, 2007. Antioxidant activities in tropical marine macroalgae from The Yucatan Peninsula, Mexico. J. Applied Phycol., 19: 449-458.

Zubia, M., C. Payri and E. Deslandes, 2008. Alginate, mannitol, phenolic compounds and biological activities of two range-extending brown algae, *Sargassum mangarevense* and *Turbinaria ornata* (Phaeophyta: Fucales), from Tahiti (French Polynesia). J. Applied Phycol., 20: 1033-1043.