International Food Research Journal 22(5): 1977-1984 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Antioxidant activity of red algae *Kappaphycus alvarezii* and *Kappaphycus striatum*

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Article history

Received: 7 July 2014
Received in revised form: 9 March 2015
Accepted: 17 March 2015

Keywords

Red seaweeds
TPC
DPPH free radical scavenging assay
TEAC
Solvent

Abstract

Antioxidants in seaweeds have attracted increasing interest for its role in protecting human health. Therefore, the aim of this study was to assess the Total phenolic content (TPC) values and antioxidant activities in red seaweeds *Kappaphycus alvarezii* and *Kappaphycus striatum* of different solvent extracts. Total phenolic content (TPC) and antioxidant activities (DPPH scavenging assay and Trolox equivalent antioxidant capacity assay, TEAC) for both *K. alvarezii* and *K. striatum* extracts were determined using different solvents at different concentrations (ethanol: 50%, 70%, 100%; acetone: 50%, 70%, 100%; methanol: 50%, 70%, 100%). The TPC value was measured using the Folin-Ciocalteu's method. The antioxidant activities were measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and Trolox Equivalent Antioxidant Capacity (TEAC) assay. The highest TPC value of *K. alvarezii* antioxidant extract was obtained by 50% ethanol extracts while for *K. striatum* obtained by 50% methanol extract. The highest percentage of DPPH free radical inhibition for *K. alvarezii* was shown by 50% acetone extract while *K. striatum* was shown using 50% methanol extract. The highest TEAC value for *K. alvarezii* was shown by 50% acetone while *K. striatum* extract was shown by 50% ethanol extract. The TPC values and antioxidant activities of all solvent extracts of K. striatum were significantly higher ($p<0.05$) than *K. alvarezii* antioxidant extracts. The TPC values showed strong correlation ($r = 0.797$) with TEAC values for *K. alvarezii* antioxidant extract ($p<0.01$). The TEAC values also showed strong correlation ($r = 0.735$) with percentage of DPPH free radical inhibition for *K. alvarezii* ($p<0.01$). The TPC value, DPPH free radical scavenging assay and TEAC assay for *K. striatum* extracts showed strong correlation ($r > 0.8$) with each other ($p<0.01$). In summary, *K. striatum* showed better antioxidant activity and higher TPC value than *K. alvarezii*.

Introduction

Seaweed has been used widely in South Asian countries for multipurpose application such as food, animal feeds, fertilizers and others (Dhargalkar and Verlecar, 2009). Seaweed is a primitive plant which grows extensively in shallow marine water and estuaries. Generally, seaweed is categorized into three categories which are red algae, brown algae and green algae scientifically known as Rhodophyceae, Phaeophyceae and Chlorophyceae respectively. These classifications are determined due to their pigments, morphological and anatomical characteristics (Manivannan *et al.*, 2009).

Seaweed is also known as macroalgae. According to McHugh (2003), macroalgae which macroscopic in size and can be seen clearly without microscope is different from microalgae (Cyanophyceae) which is microscopic in size, mostly unicellular, and also known as blue green algae. Microalgae sometimes bloom and pollute the marine and the rivers.

Seaweeds are the source of hydrocolloid such as carrageenan, agar and alginate. Hydrocolloids are commercially used in food product as thickeners and gelling agents. Seaweeds also have been used as vegetables by the coastal area inhabitants (McHugh, 2003). Seaweeds also have been utilized as pasta ingredients and it has been accepted by the consumers. Pasta containing seaweed posses more nutrition because it has high content of bioactive components such as fucoxanthin and fucosterol (Prabhasankar *et al.*, 2009). Generally, seaweeds contain good nutritional content with potentially source of proteins, carbohydrate, fibers, minerals, vitamins and even it has low lipid content. Therefore, seaweeds can be a potential interesting new source

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of natural substances with biological activities that being utilized as functional ingredients (Plaza *et al.*, 2008).

Among all substances contain in seaweed, antioxidant has been a major attraction. Seaweeds are exposed to free radical and strong oxidizing agents due to reaction between sunlight and oxygen. However, seaweeds structural component does not experience any oxidative damage (Matsukawa *et al.*, 1997). This hypothesized that seaweeds are capable to generate essential defence mechanisms against oxidation. Therefore seaweeds are considered as an important source of antioxidant substances that may also correspond to protect human body against reactive oxygen species (Plaza *et al.*, 2008).

A variety of dietary antioxidants can be considered as effective agents to reduce oxidative stress which can give a major impact in development of diseases including cancer. Generation of reactive oxygen species and other free radical by ultraviolet radiation are obstructed by antioxidants to prevent oxidative stress. Excessive reactive oxygen species is associated with carcinogenesis due to DNA damage and mutation (Khan *et al*., 2008).

There are a lot of methods to determine the antioxidant activities such as ferrous ion chelating (FIC) assay, beta carotene bleaching (BCB) assay, ferric reducing antioxidant power (FRAP) assay, DPPH free radical scavenging assay, Trolox equivalent antioxidant capacity (TEAC) assay, deoxyribose radical scavenging activity, hydroxyl radical scavenging assay, superoxide anion scavenging assay (Chew *et al*., 2008; Matanjun *et al*., 2008; Kumar *et al*., 2008; Yangthong *et al.*, 2009; Candrawinata *et al.*, 2014) and many more. This present study chose DPPH free radical scavenging assay and TEAC assay because TPC act as free radical scavenger. The main mechanism of food antioxidant also is the free radical scavenging (Pokorny *et al*., 2001). DPPH free radical scavenging assay is a rapid, simple, cheap and widely used methods to measure the capacity of a substance as a hydrogen donor or scavenge free radical. It is used to assess the antioxidant activities in food. DPPH is a stable dark purple free radical (Kedare and Singh, 2011). The addition of DPPH with hydrogen donor substance will reduce the stable DPPH free radical hence will cause the purple decolorization (Matsukawa *et al*., 1997) to produce yellow DPPH-H (Lu *et al*., 2010).

In Malaysia, Malaysian Department of Fisheries (2013) reported that the production of seaweed has increased from 138 855 metric tonnes in 2009 to 138 897 metric tonnes in 2010 that made a production value of seaweed is RM 28.2 million. So, seaweed has become an economically important natural resource for Sabah and Malaysia. This study chose *Kappaphycus* sp. because it is the main seaweed in Malaysia and cultivated on a large scale at Sabah coastal area (Ahemad *et al*., 2006). *Kappaphycus* sp. is sold in Sabah local markets as salad, soups and pudding (Phang, 2010). Recently, *Kappaphycus* sp. has been widely utilized in food, health beverages, cosmetics and anticancer nutraceutical (Phang *et al*., 2010) due to its antioxidant content and other nutritive compounds (Cornish and Garbary, 2010).

A lot of study has been done to assess the antioxidant activities in *Kappaphycus alvarezii* extract (Chew *et al*., 2008; Matanjun *et al.*, 2008; Ganesan *et al*., 2008; Kumar *et al*., 2008). However, there are no study has been done to assess the antioxidant activities in *Kappaphycus striatum*. Therefore, the aim of this study was to evaluate the antioxidant activities of these two seaweeds using different concentrations of different solvents.

Materials and Methods

Sample preparation

Red seaweeds *K. alvarezii* and *K. striatum* samples washed with distilled water to remove sand and salt. Then, the samples were dried in oven at 60°C for 5 hours until it reached 7% of moisture. Dried samples were ground up into a powder and were filtered using a mesh with a diameter of 250 µm to get homogenous samples.

Extraction of antioxidant

The extraction of antioxidant was based on the method of Chew *et al.* (2008) with some modifications. *K. alvarezii* and *K. striatum* powder was extracted by ethanol, methanol and acetone solvent. Each solvent has three concentrations (50%, 70% and 100%). Seaweed powder was mixed with solvent in the ratio of 1 g powder to 5 mL solvent. The mixed sample was shaken continuously on an orbital shaker for 72 hours. Then, the extracts were centrifuged for 10 minutes at 10845 x g (Top Refrigerated Centrifuge, Hermle Z323K, Germany). After the extracts were filtered using filter paper (Sartorius Grade 292), the supernatants were kept in airtight amber bottle and stored in -20°C for further analysis.

Total phenolic content

Total phenolic content (TPC) analysis in each extracts was measured using Folin-Ciocalteu methods (Kahkonen *et al*., 1999) with some modifications. The Folin-Ciocalteu's phenol reagent (Merck, Germany) (5 mL) and 7.5% w/v Na_2CO_3 (Sigma,

Germany) (4 mL) were added to sample extracts (1 mL). The mixture was incubated in the dark for 2 hours in room temperature for the reaction to occur. The reaction mixture's absorbant was measured at 765 nm wavelength using spectrophotometer (EPOCH Microplate Reader Spectrophotometer, Vermont, USA). Gallic acid (Sigma, Germany) in the concentration of 0 to 400 ppm was used as standard and calibration. All samples were done in triplicate. TPC value was expressed in mg Gallic Acid Equivalents (GAE)/100 g sample.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

Antioxidant capacity to scavenge 2, 2-diphenil-1picrylhydrazil (DPPH) free radical was determined based on DPPH free radical scavenging assay method (Chew *et al*., 2008) with some modification. 100 µL samples extracts or methanol (as control) was added into 96-well microtiter plate. Then 200 µL DPPH reagent (Sigma, Germany) (0.1 mM) was added into the sample extract or control. The mixture was then incubated in the dark for 1 hour in room temperature. Later the absorbance of the mixture was measured by spectrophotometer (EPOCH Microplate Reader Spectrophotometer, Vermont, USA) at 517 nm wavelength. All samples and control were done in triplicate. The unit for this method is percentage of radical scavenging activity. The scavenging activity was determined by using the following formula:

$$\text{radical scavenging activity (\%)} = \frac{(\text{Control absorbance} - \text{Sample absorbance}) \times 100}{\text{Control absorbance}}$$

Trolox equivalent antioxidant capacity

Antioxidant capacity of seaweeds extracts was measured using Trolox Equivalent Antioxidant Capacity (TEAC) method (Re *et al*., 1999) with some modification. In this method, the $ABTS^{+}$ radical cation was generated from the reaction of the mixture between 7 mM ABTS (Sigma, Germany) and 2.45mM potassium persulfate (Sigma, Germany) with the ratio was 1:1. The mixture was allowed to react in the dark for 16 hours in room temperature. Then, the mixture was diluted with methanol until the absorbance at 734 nm wavelength reached 0.70 ± 0.05 using spectrophotometer (EPOCH Microplate Reader Spectrophotometre, Vermont, USA). Later, 200 µL mixture was added with 20 µL sample extracts and the mixture was added into 96-well microtiter plate. The mixture of $ABTS^{+}$ and sample extracts was allowed to react for 10 minutes in dark. Then, the mixture absorbance was measured by spectrophotometer at 734 nm. Trolox (Sigma, Germany), the vitamin E analogue in the concentration of 0 to 300 µM was used as standard and calibration. All the measurements were done in triplicate. The results are expressed in µmolTrolox Equivalent/100g sample.

Statistical analysis

Data was analyzed by Statistical Package for Social Science (SPSS, version 15.0) software. Two way ANOVA was used to analyze for significant differences among species, solvent types and solvent concentration of *K. alvarezii* and *K. striatum* extracts. Independent T-test has been used to determined the differences of TPC value and the antioxidant activities (DPPH free radical scavenging activity and TEAC assay) between seaweeds species. Pearson correlation was used to correlate the TPC value with antioxidant activities (DPPH free radical scavenging activity and TEAC assay). Strong correlation is defined by the r = 0.71 - 1.00, moderate correlation has the r value = 0.51 - 0.70, weak correlation is defined as r = 0:01 to 0:50 and r = 0.00 is defined as no correlation.The significant value for the data analyzed was set at $p \leq 0.05$.

Results and Discussion

Lipid peroxidation and generation of free radicals often occur in biological and food systems. In biological systems, antioxidants as part of the defense mechanism can prevent oxidative damage (Madhavi *et al*., 1996) and free radical generation by prooxidative from environment such as ultraviolet radiation, cigarette smoke and air pollutants (Khan *et al*., 2008).

This current study extracted the red seaweeds antioxidant using ethanol, acetone and methanol at 3 different concentration (50%, 70% and 100%) for 72 hours to determine the total phenolic content and antioxidant activity. Antioxidant extraction using solvents for 72 hours could maximize the phenolics yield compare to 24 hours extraction (Matanjun *et al*., 2008). Other than the time of extraction, the type of solvent has been widely used to ensure the efficiency of extraction of plant bioactive components (Musa *et al*., 2011).

Solvent extraction is a process designed to isolate soluble antioxidant compounds through diffusion from the solid matrix (plant tissue) using liquid matrix (solvent) (Musa *et al.*, 2011). The solvent used to extract the antioxidants are methanol, ethanol and acetone whether in singly or in combination with water (Kahkonen *et al*., 1999; Lim *et al.*, 2007; Tachakittirungrod *et al.*, 2007). Different

Table 1. Total phenolic content (TPC) values in *Kappaphycus alvarezii* and *Kappaphycus striatum* extracts (Mean ± Standard Deviation)

Solvents (% v/v)	Red Seaweeds (mg GAE/100 g wet weight sample)	
	Kappaphycus alvarezii	***Kappaphycus striatum***
Ethanol (50%)	17.32 ± 1.20^{a,B}	90.38 ± 0.72^{c,A}
Ethanol (70%)	14.55 ± 1.09^{b,c,B}	45.81 ± 0.60^{e,A}
Ethanol (100%)	6.74 ± 0.23^{d,B}	22.6 ± 0.09^{g,A}
Acetone (50%)	14.82 ± 0.76^{b,B}	96.31 ± 2.00^{b,A}
Acetone (70%)	11.83 ± 1.07^{c,B}	67.51 ± 0.33^{d,A}
Acetone (100%)	11.80 ± 0.28^{c,B}	21.55 ± 0.56^{g,A}
Methanol (50%)	14.18 ± 0.44^{b,c,B}	107.48 ± 1.34^{a,A}
Methanol (70%)	12.51 ± 0.56^{c,B}	94.18 ± 0.49^{b,A}
Methanol (100%)	7.51 ± 0.16^{d,B}	32.63 ± 1.02^{f,A}

Different capital letters at the same row shows significant differences (p≤0.05) between red seaweeds species.
Different small letters at the same column shows significant differences (p≤0.05) between solvent extracts

Table 2. Percentage of DPPH scavenging activity by *Kappaphycus alvarezii* and *Kappaphycus striatum* extracts (Mean ± Standard Deviation)

Solvents (% v/v)	Red Seaweeds (%DPPH scavenging activity)	
	Kappaphycus alvarezii	***Kappaphycus striatum***
Ethanol (50%)	27.65 ± 0.24^{b,B}	53.55 ± 2.19^{b,A}
Ethanol (70%)	23.84 ± 1.44^{c,B}	45.16 ± 1.32^{c,A}
Ethanol (100%)	18.34 ± 0.57^{d,B}	25.58 ± 0.48^{d,A}
Acetone (50%)	35.63 ± 0.41^{a,B}	53.83 ± 1.17^{a,b,A}
Acetone (70%)	34.80 ± 1.49^{a,B}	50.76 ± 0.28^{b,A}
Acetone (100%)	19.21 ± 0.52^{d,A}	12.29 ± 0.57^{e,B}
Methanol (50%)	26.50 ± 0.44^{b,B}	56.63 ± 1.34^{a,A}
Methanol (70%)	24.12 ± 0.35^{c,B}	46.49 ± 0.48^{c,A}
Methanol (100%)	19.35 ± 0.78^{d,B}	45.44 ± 0.42^{c,A}

Different capital letters at the same row shows significant differences (p≤0.05) between red seaweeds species.
Different small letters at the same column shows significant differences (p≤0.05) between solvent extracts.

polarities of organic solvents greatly influence the selection of a particular solvent for the extraction of bioactive compounds (Musa *et al*., 2011). Plant extracts contains different classes of phenolic, which have different solubility in different solvents. Phenolic compounds in plant extracts commonly associated with other molecules such as proteins, polysaccharides, terpenes, chlorophyll and inorganic compounds. Therefore, a suitable solvent is required to extract phenolic compounds from those molecules (Tatiya *et al.*, 2011).

The TPC value of *K. alvarezii* and *K. striatum* antioxidant extracts showed in Table 1. The TPC values of *K. alvarezii* antioxidant extracts were in the range of 6.74 – 17.32 mgGAE/100 g wet weight sample. The highest TPC value found in 50% ethanol extract (p≤ 0.05) and the lowest TPC value found in 100% ethanol extracts (p≤ 0.05). The TPC values of *K. striatum* antioxidant extracts were in the range of 21.55 – 107.48 mgGAE/100 g wet weight sample. The highest TPC value of *K. striatum* extracts found in 50% methanol extract (p≤ 0.05) while the lowest TPC value of *K. striatum* extracts found in 100% acetone extract (p≤ 0.05).

The results presented in Table 1 showed that there was an increase of total phenolic content (TPC) value with an increased of solvent polarity. This finding is different from a study which has been done by Chew *et al.* (2008). The study found that 50% methanol extract of *K. alvarezii* gives an 8-fold higher value of TPC i.e. 115 mgGAE/100g sample than in the present study i.e. 14.18 mgGAE/100g wet weight sample. The TPC values of *K. striatum* extracts also exhibit a higher value than the TPC values of *K. alvarezii* (p≤ 0.05) based on the independent T-Test. According to Chew *et al.* (2008), the high TPC value may be due to the presence of both hydrophilic and hydrophobic antioxidants. The TPC values also varied among different species of seaweeds (Ganesan *et al*., 2008).

Table 2 shows the result of the percentage of DPPH free radical scavenging activity of both seaweeds extracts. The percentages of DPPH free radical scavenging activity by *K. alvarezii* extracts were ranged between 18.34 and 35.63%. The 50% acetone extract showed the highest percentage of DPPH free radical scavenging activity (p≤ 0.05) while 100% ethanol extract showed the lowest DPPH free radical scavenging activity percent (p≤ 0.05). The present study found that *K. alvarezii* had 1.6-fold higher scavenging activities (19.35%) compared to study conducted by Ganesan *et al.* (2008). The study found that the percentage of scavenging activity of *Eucheuma* sp. (former name of *K. alvarezii*) using methanol extracts is 11.9%. Meanwhile, the percentages of DPPH free radical scavenging by *K. striatum* extracts ranged between 12.29% and 56.63%. The highest percentage of DPPH free radical scavenging activity was shown by 50% methanol extract (p≤ 0.05). The lowest DPPH free radical scavenging activity percent was shown by 100% acetone antioxidant extract (p≤ 0.05). The differences of radical scavenging activity between the seaweeds extracts were probably due to differences in the chemical composition of each extract which can provide significant changes in antioxidant activity

Table 3. Antioxidant capacity values of *Kappaphycus alvarezii* and *Kappaphycus striatum* extracts (Mean ± Standard Deviation)

Solvents (% v/v)	Red Seaweeds (µmol TE/100 g wet weight sample)	
	Kappaphycus alvarezii	*Kappaphycus striatum*
Ethanol (50%)	$46.59 \pm 0.54^{b,B}$	$111.79 \pm 0.71^{a,A}$
Ethanol (70%)	$45.44 \pm 0.60^{b,B}$	$90.12 \pm 0.24^{c,A}$
Ethanol (100%)	$27.98 \pm 1.72^{d,B}$	$67.50 \pm 1.67^{d,A}$
Acetone (50%)	$52.74 \pm 3.93^{a,B}$	$105.79 \pm 1.49^{b,A}$
Acetone (70%)	$38.29 \pm 1.92^{c,B}$	$86.67 \pm 2.02^{c,A}$
Acetone (100%)	$27.10 \pm 0.73^{d,B}$	$55.99 \pm 0.36^{e,A}$
Methanol (50%)	$35.99 \pm 2.35^{c,B}$	$111.63 \pm 0.99^{a,A}$
Methanol (70%)	$29.96 \pm 1.85^{d,B}$	$106.15 \pm 0.60^{b,A}$
Methanol (100%)	$14.01 \pm 0.69^{e,B}$	$85.75 \pm 3.82^{c,A}$

Different capital letters at the same row shows significant differences ($p \leq 0.05$) between red seaweeds species.
Different small letters at the same column shows significant differences ($p \leq 0.05$) between solvent extracts

(Samarth *et al*., 2008).

Table 3 shows the antioxidant capacity exhibited by *K. alvarezii* and *K. striatum* extracts by using TEAC assay. The TEAC assay in this study followed the improved technique for the $ABTS^{\bullet+}$ generation (Re *et al*., 1999). This decolourization technique is used to measure the hydrophilic and hydrophobic antioxidant capacity. Antioxidant extracts which having capacities to inhibit ABTS free radical show the mechanism of action as a hydrogen donor. The substance will exterminate the oxidation process by conversion of free radical to a more stable product (Tachakittirungrod *et al*., 2007).

The result of this current study (46.59 µmol TE/100g wet weight sample) is not in agreement to the earlier study done to *K. alvarezii* extract (Matanjun *et al*., 2008). The TEAC value of *K. alvarezii* extract from that study was 1.63 mM TE/mg sample. In TEAC assay, we found that 50% acetone extract of *K. alvarezii* exhibit the highest TEAC value ($p \leq 0.05$) which was similar to the results obtained from DPPH scavenging assay. Meanwhile 50% ethanol extract exhibit the highest TEAC value ($p \leq 0.05$) of *K. striatum* antioxidant extract. According to Matanjun *et al.* (2008), radical scavenging capacity of red seaweed extracts may be mostly related to their phenolic hydroxyl groups. Seaweed contains a novel antioxidant compound that controls free radical formation from metabolic reactions. The most important constituent in seaweeds is phenol because it is capable to scavenge radical through the hydroxyl group (Subashini and Howe, 2014). Therefore, the results of this study showed *K. alvarezii* extracts

Table 4. Pearson correlation between the TPC values, percentages of DPPH scavenging free radical activity and TEAC values for *K. alvarezii* and *K. striatum* extracts

	K. alvarezii			*K. striatum*		
	TPC	**DPPH**	**TEAC**	**TPC**	**DPPH**	**TEAC**
TPC	1			1		
DPPH	0.562**	1		0.804**	1	
TEAC	0.797**	0.735**	1	0.921**	0.916**	1

**Correlation is significant at $p \leq 0.01$

using 50% acetone and *K. striatum* extract using 50% ethanol gave the high TEAC value.

Two way ANOVA indicated that there were significance interaction between samples species, types of solvents and solvent concentration for all analysis where $F(4, 36) = 211.019$, $p \leq 0.001$ for TPC analysis, $F(4, 36) = 91.764$, $p \leq 0.001$ for DPPH free radical scavenging activity and $F(4, 36) = 26.212$, $p \leq 0.001$ for TEAC assay. The results show that among of all solvents, ethanol-water, acetone-water and methanol-water is a better solvent for the extraction of phenolic than non-aqueous solvents such as 100% ethanol, 100% acetone and 100% methanol. This may be due to the phenolic often produced in higher amounts in the more polar solvent (Tatiya *et al*., 2011).

This study also found that 100% solvent extract exhibit a low value of antioxidant activities. This result might be due the low polarity of the solvents. The antioxidant activities values rose with the increasing of water to solvent. Musa *et al*. (2011) also found that antioxidants extracts using 50% solvent-water provides the high antioxidant activity. The high values of antioxidant activities in 50% solvent-aqueous extracts may be due to the high content of TPC. According to Samarth *et al*. (2008), phenolic in plants is one of the main groups of compounds which act as the main antioxidant free radical scavengers. Phenolics also soluble in solvents those are less polar than water. It is suggested to extract the phenolic using aqueous ethanol, acetone and methanol (Waterman and Mole, 1994). The increasing of polarity up to 50% of aqueous solvent may increase the solubility of antioxidant substances (Musa *et al*., 2011) and produce high antioxidant capacity (Tachakittirungrod *et al*., 2007). Therefore, the attachment of phenolic compounds to sugar or protein, saponins, tannins, glycosides, organic acids, salts, and mucus could be extracted (Cho *et al*., 2007).

This study also found that most of *K. striatum* extracts had greater antioxidant properties compared to *K. alvarezii* extracts ($p \leq 0.05$). This indicated that different species of seaweeds exhibited different

antioxidant activities using different solvents. Verzelloni *et al.* (2007) reported that not all vegetables, fruits and their derivatives of the same class contain similar phenolic composition. This factor may have contributed to the different levels of phenolic compounds and antioxidant activities in *K. alvarezii* and *K. striatum* though they are of the same genus.

Based on Table 4, this present study found similar result with study done by Piluzza and Bullitta (2011). There was a significant correlation between TPC value and antioxidant activities of both red seaweeds extracts. However the correlation between TPC value and TEAC value is stronger (r = 0.797) than the correlation between TPC value and the percentage of DPPH scavenging activity (r = 0.562) of *K. alvarezii* extracts. According to Matanjun *et al.* (2008), the moderate correlation between TPC value and the percentage of DPPH scavenging activity (r = 0.562) indicated that phenolics compound is not the only substance that involved in antioxidant activities in *K. alvarezii* extracts. Meanwhile the correlations between TPC value and antioxidant activities are strong ($r \geq 0.8$) in *K. striatum* extracts. The strong correlations between the TPC value and antioxidant activities in *K. striatum* extracts support the hypothesis that the phenolic substances significantly contribute to antioxidant activities in the study plant (Cai *et al*., 2004).

In this study also found that there are strong positive correlations between DPPH free radical scavenging assay and TEAC assay for both seaweeds extracts. It is similar to a previous study (Miliauskas *et al*., 2004) who found a high correlation between DPPH free radical scavenging assay and TEAC assay. This strong positive correlation between percentage of DPPH scavenging activity and TEAC value of *K. alvarezii* and *K. striatum* (r = 0.735 and r = 0.916 respectively) might be due to the similar mechanism of free radical scavenging activity (Leong and Shui, 2002).

The results obtained from the experimental data shows that there is a correlation between TPC and antioxidant activities. However, there are studies showing antioxidant activity does not only depend on the phenolic content but it may be due to the presence of other antioxidants (Matanjun *et al*., 2008) in *K. alvarezii.* This indicates that *K. alvarezii* contain complex mixtures of various types of bioactive compounds, and the contribution compounds other than phenolic compound cannot be ignored (Song *et al*., 2010). In addition, the different classes of phenolic compounds have different antioxidant capacity (Tatiya *et al*., 2011). Other compounds may also affect the measured antioxidant activity, in which the composition of different compounds has different activities (Garcia-Alonso *et al*., 2004). However, the antioxidant activities in *K. striatum* probably contributed mostly by phenolic compound.

Conclusion

TPC values and the antioxidant activities increase as the polarity of the extraction solvents increased. The *K. striatum* antioxidant extracts showed a higher TPC values and antioxidant activities than *K. alvarezii* antioxidant extracts. There were strong correlation between TPC values with TEAC assay and also DPPH free radical scavenging assay with TEAC assay. The TPC values and antioxidant activities varied between seaweeds species. However, the components of antioxidant in the seaweeds extracts are still not clear. Therefore, the analysis of identification of antioxidant components is in progress.

Acknowledgements

The author acknowledges Associate Prof. Dr. Ahmad Bin Ismail and his student, Farah Zainee for the identification of *K. alvarezii* and *K. striatum* sample. Special thanks to Toxicology Lab members, Faculty of Health Sciences, National University of Malaysia for the technical assistance and laboratory facilities.

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