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Antioxidant Activity of Selected Commercial Seaweeds

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ABSTRACT

This study aims to evaluate the antioxidant activity (total antioxidant and free radical scavenging activities) of seaweeds commercially available in the Malaysian supermarket. Four types of seaweeds namely Nori (*Porphyra* sp.), Kumbu (*Laminaria* sp.), Wakame (*Undaria* sp.) and Hijiki (*Hijikia* sp.) were used in the study. The extracts were prepared with water and ethanol, respectively. The β-carotene bleaching and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays were used to determine antioxidant properties of seaweeds by measuring the decrease in absorbance at 470 and 517 nm. In water extract, Kumbu showed the highest total antioxidant activity of 63% compared with other samples. Kumbu, Nori and Hijiki exhibited higher radical scavenging activity than Wakame when extracted with water. Wakame exhibited the highest antioxidant and free radical scavenging activities in ethanolic extract with 58% and EC_{50} = 0.42 mg/ml respectively. The results of ANOVA analysis show significant differences ($p<0.05$) in the means of total antioxidant and free radical scavenging activities of the seaweeds. The results showed that processed commercial seaweeds exhibited varying degrees of antioxidant properties.

INTRODUCTION

Free radicals are responsible for aging and causing various human diseases. A study shows that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases. By donating hydrogen radicals, the primary radicals are reduced to nonradical chemical compounds and are then converted to oxidize antioxidant radicals (Jadhav *et al*., 1995; Yamaguchi *et al*., 1998). This action helps in protecting the body from degenerative diseases. Epidemiological studies have shown the beneficial effects of diets rich in vegetables, fruits and grain products in reducing the risk of cardiovascular disease and certain cancers (Beecher, 1999). The principal agents responsible for the protective effects could be the presence of antioxidant substances that exhibit their effects as free radical scavengers, hydrogen-donating com-pounds, singlet oxygen quenchers and metal ion chelators (Okawa *et al*., 2001).

Seaweeds have become a major food ingredient in products especially in Japan, Korea and China. Boukhari & Sophie (1998) reported that most Europeans and Americans use processed seaweeds as additives in their food preparation. In Asia, seaweeds have been used for centuries in the preparation of salads, soups and also as low-calorie foods (Jiménez-Escrig & Sánchez-Muniz, 2000). Although most Malaysians exhibit little interest in consuming seaweeds, it is consumed by small pockets of the population along the coastal areas of Peninsular Malaysia and East Malaysia (Norziah & Ching, 2000).

Seaweeds belong to a group of marine plants known as algae. The estimated range of seaweeds is probably around 45,000 species (Bequette & France, 1997). Most seaweeds are divided into three categories based on their colours such as red (4,500 species), green (900 species) and brown (1,000 species). It has been used as food, fertilizer and for medicinal purposes for a long time. Like other plants, seaweeds contain various kinds of inorganic and organic substances which probably benefit human health. It has been reported that seaweeds contain high levels of minerals, vitamins, essential amino acids, indigestible carbohydrates, and dietary fiber (Jiménez-Escrig & Goni, 1999). In food manufacturing, seaweeds have been developed as raw or semi-processed food products (Mabeau & Fleurence, 1995). Currently of interest in the field of nutritional sciences is the presence of antioxidant substances in fresh and processed foods.

There are many types of seaweeds available commercially in the Malaysian market. Only four types are commonly used for food preparation in the Japanese restaurants in Malaysia namely Nori (*Porphyra* sp.), Kumbu (*Laminaria* sp.), Wakame (*Undaria* sp.) and Hijiki (*Hijikia* sp.). To our knowledge, there is a dearth of study on the antioxidant activity of commercial seaweeds. Therefore, the aim of this study is to determine total antioxidant and free radical scavenging activities of imported seaweeds available commercially in Malaysia. In addition, this study will also comparatively evaluate the antioxidant properties of these seaweeds with commercial antioxidants α-tocopherol (vitamin E) and butylated hydroxytoluene (BHT).

MATERIALS AND METHODS

Seaweeds

Four types of seaweeds namely Nori, Kumbu, Wakame and Hijiki were purchased from a supermarket at Kuala Lumpur, Malaysia. The samples were randomly selected off the shelf and were 100% seaweed. The species, taxonomy and processing conditions of the seaweeds were not known. All commercial seaweeds used in this study were not locally produced.

Chemicals

Linoleic acid, DPPH, BHT, vitamin E and β-carotene were purchased from Sigma Chemical Co (St Louis, MO, USA). All chemicals used were of analytical grade.

Preparation of sample

The dried samples were cut into small pieces and ground into fine powder using a dry grinder. The ground samples were sieved to get uniform particle size, then kept in an air-tight container and stored in a freezer (–20^{o}C) until further analysis.

Extraction of sample

Each ground sample was weighed and transferred into a beaker. Water or ethanol was added in the ratio of 1:10 and stirred for 1 h with the aid of a magnetic stirrer. The extraction mixture was left to sediment for at least 1 h before the extract was separated from the residue by filtration

through Whatman No. 1 filter paper. The residue was re-extracted twice, and the two extracts were combined. The water extract was lyophilised using a freeze dryer (Virtis Co., Inc., New York), and the residual solvent of ethanolic extract was removed under reduced pressure at 40°C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). Extracts were produced in duplicates and used to assay the antioxidant activity.

Measurement of antioxidant activity

β-Carotene bleaching assay

Total antioxidant activity of seaweed extracts and standards (vitamin E and BHT) was measured according to the method of Velioglu *et al*. (1998) and Lu & Foo (2000). One mililitre of β-carotene solution (0.2 mg/ml chloroform) was pipetted into a round-bottom flask (50ml) containing 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. The mixture was then evaporated at 40°C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 ml of distilled water. The distilled water was added slowly to the mixture with vigorous agitation to form an emulsion.

Five ml aliquots of the emulsion were transferred into different test tubes containing 0.2 ml of samples in 80% methanol at 1 mg/ml. The tubes were then gently mixed and placed at 45°C in a water bath for 2 h. Absorbance of the samples was measured at 470 nm using a Spectronic® Genesys™ 5 spectrophotometer (Milton Roy Company, New York) at initial time (t=0) against a blank, consisting of an emulsion without β-carotene. Standards at the same concentration with samples were used as comparison. 0.2 ml of 80% methanol in 5 ml of the above emulsion was used as the control. The measurement was carried out at 15 min intervals. All determinations were performed in triplicate.

Antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using a slightly modified version of the formula from Jayaprakasha *et al*. (2001). According to Jayaprakasha, Singh & Sakariah (2001), the time for A_t and A^o_t were at 180 min. In this method, as the absorbance was measured at 120 min, A_t and A^o_t were at 120 min.

$$AA = \left(1 - \frac{(A_0 - A_t)}{(A^\circ_0 - A^\circ_t)}\right) \times 100$$

where A_0 and A^o_0 are the absorbance values measured at initial time of the incubation for samples and control respectively, while A_t and $A½_t$ are the absorbance values measured in the samples or standards and control at t = 120 min.

Free radical scavenging assay

Effect of seaweed extracts on DPPH radical was measured based on the method modified by Lu & Foo (2000) and Lai, Chou & Chao (2001). An aliquot of 200 µl of seaweed extract (0.62 - 4.96 mg/ml), vitamin E (0.04 - 1.28 mg/ml) or BHT (0.04 - 1.28 mg/ml) were mixed with 800 µl

of 100 mM Tris - HCl buffer (pH 7.4). The mixture was then added to 1 ml of 500 μM DPPH. This was made up to a DPPH final concentration of 250 μM. The mixture was shaken vigorously and left to stand at room temperature for 20 min in a dark room. Absorbance at 517 nm was measured using a UV-Vis spectrophotometer until the reading reached a plateau. The capability of seaweeds extracts to scavenge the DPPH radical was calculated by using the following equation:

Scavenging effect (%)

$$= 1 - \left(\frac{\text{Absorbance of sample at 517 nm}}{\text{Absorbance of control at 517 nm}} \right) \times 100$$

EC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of seaweed extracts, which is defined as the amount of antioxidant necessary to decrease the initial DPPH radical concentration by 50%. Triplicate measurements were carried out and their activity was calculated by the percentage of DPPH scavenged.

RESULTS

Comparison of total antioxidant activity of water and ethanolic extracts of seaweeds

Figures 1 and 2 show the comparative β-carotene bleaching rates of the control, standards and seaweed extracts. It shows a decrease in absorbance of β-carotene in the presence of different seaweed extracts due to the oxidation of β-carotene and linoleic acid. This indicates that all tested seaweeds possessed antioxidant capacity.

As shown in Figure 3, mean total antioxidant activity of water extracts of Kumbu, Nori, Hijiki and Wakame was 54%, 51%, 46% and 31%, respectively. Mean total antioxidant activity of Kumbu in water extract was slightly higher than that of Nori. Standards exhibited a stronger antioxidant activity compared to seaweed extracts. Results of ANOVA analysis show a significant difference ($p < 0.05$) between the means of total antioxidant activity of water extracts of different seaweeds and standards. However, the activities of Nori and Kumbu did not show any significant difference ($p > 0.05$) (Figure 3).

Wakame, Hijiki, Nori and Kumbu ethanolic extracts showed mean total antioxidant activity of 57%, 55%, 17% and 3%, respectively (Figure 4). The highest antioxidant activity among the seaweeds was observed in Wakame while Kumbu showed very low antioxidant activity. There was a significant difference ($p<0.05$) between the means of total antioxidant activity among the ethanolic extracts of the samples. But there was no significant difference ($p > 0.05$) between Wakame and Hijiki.

For Kumbu, its water extract exhibited a mean total antioxidant activity that was 17 times higher than that of ethanolic extract. It is clearly shown that water extract of Kumbu corresponds to the highest ability of antioxidant activity compared to an ethanolic extract (Figures 3 and 4).

Significant differences ($p<0.05$) were observed in the means of total antioxidant activity of Kumbu between water and ethanolic extracts.

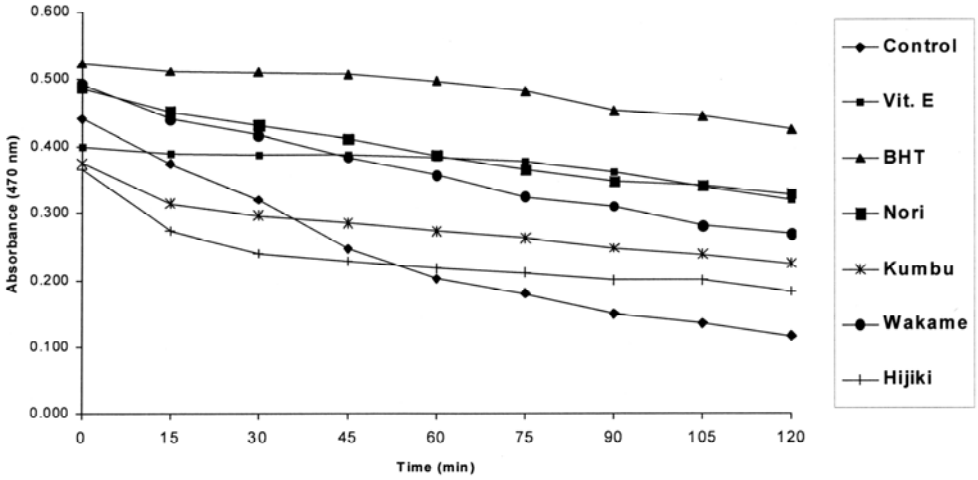

Figure 1. Antioxidant activity of seaweeds water extract compared with vitamin E and BHT at 1 mg/ml using a β-carotene bleaching assay

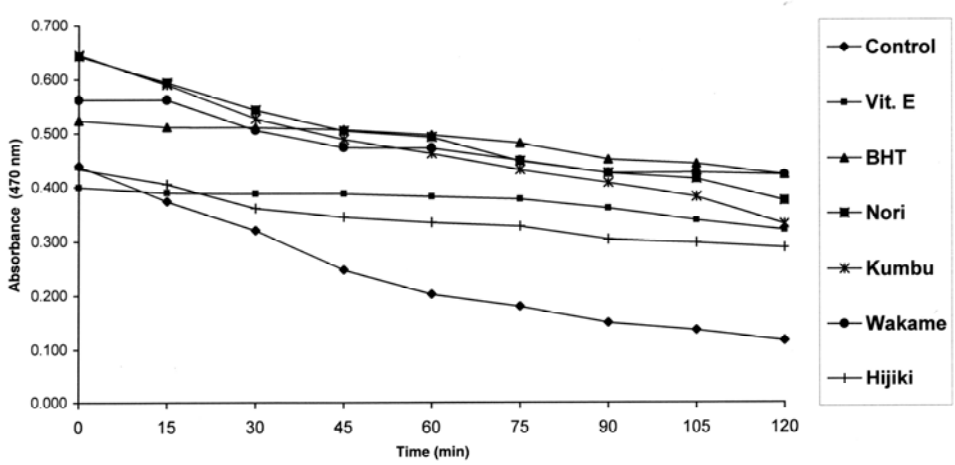

Figure 2. Antioxidant activity of seaweeds ethanolic extract compared with vitamin E and BHT at 1 mg/ml using a β-carotene bleaching assay

Comparison of free radical scavenging activity of water and ethanolic extracts of seaweeds

The dose-response curve for the free radical scavenging activity of studied samples of water and ethanolic extracts, and standards at different concentrations are presented in Figures 5 and 6. The scavenging activity of all samples on the DPPH radical was found to be strongly dependent on concentration. In general, the scavenging effects on the DPPH radical increased sharply with increasing concentration of all the samples and standards to a certain extent and then slowly increased.

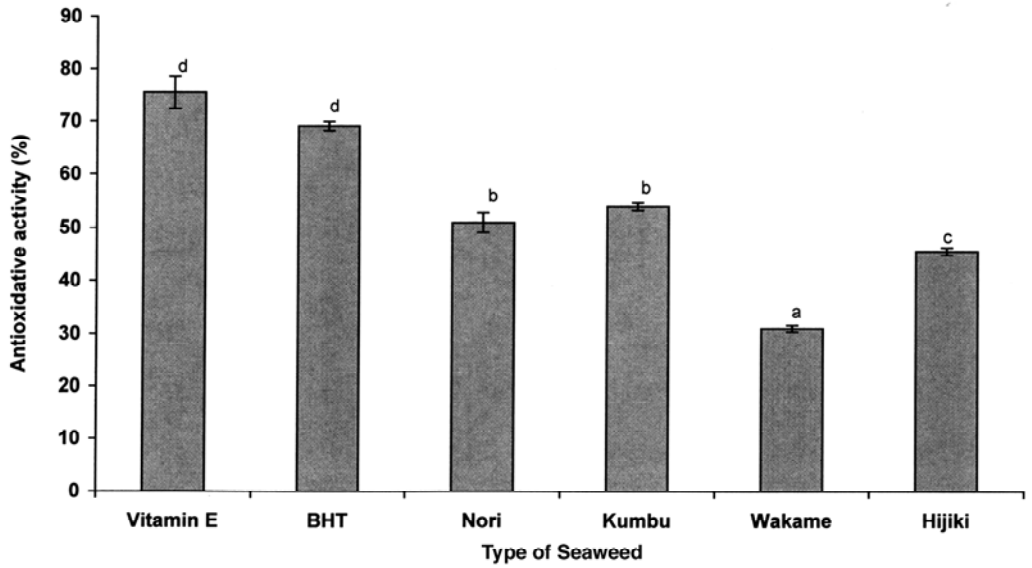

Figure 3. Mean total antioxidant activity of seaweed water extract, vitamin E and BHT. a, b, c, d, e: values with the same letter are not significantly different at $p>0.05$. Antioxidant activity was measured using a β-carotene bleaching assay

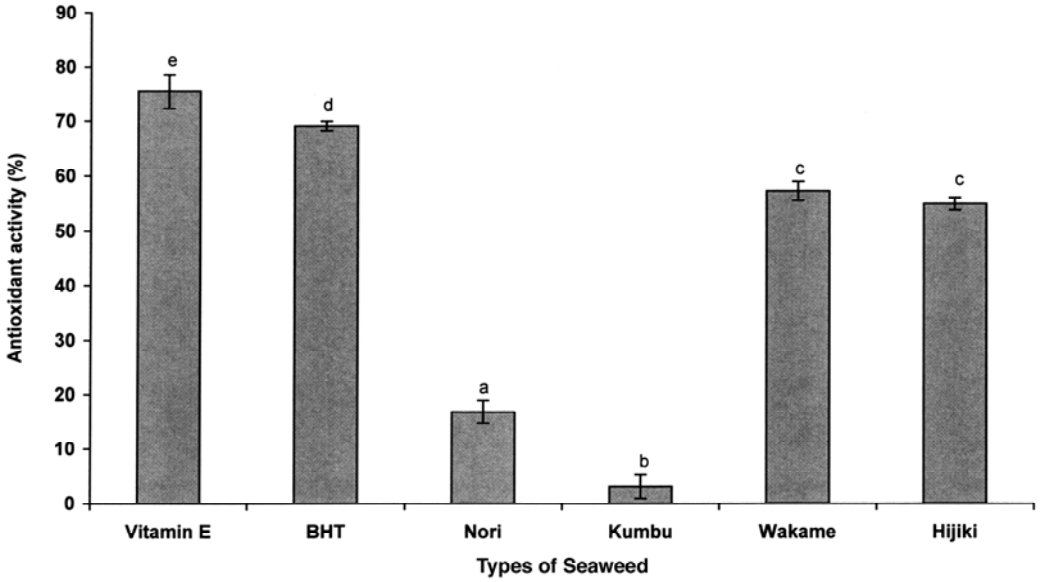

Figure 4. Mean total antioxidant activity of seaweed ethanolic extract, vitamin E and BHT. a, b, c, d, e: values with the same letter are not significantly different at $p>0.05$. Antioxidant activity was measured using a β-carotene bleaching assay

Table 1 shows the comparison of the mean concentration for 50% free radical scavenging activity (EC_{50}) of water and ethanolic extracts of seaweeds against 250 μM DPPH radical. The EC_{50} of vitamin E and BHT were 0.09 mg/ml and 0.08 mg/ml respectively, which is stronger than other water and ethanolic extracts (Figure 5). The EC_{50} values of water extracts of Nori, Kumbu, Wakame and Hijiki were 0.55, 0.58, 0.66 and 0.57 mg/ml, respectively. For ethanolic extracts, the EC_{50} values of Nori, Kumbu, Wakame and Hijiki were 0.67, 0.86, 0.42 and 0.47

mg/ml respectively. The highest mean EC_{50} value was found in ethanolic extract of Wakame (Table 1). Thus, Wakame exhibited the highest ability of free radical scavenging activity among the studied seaweeds. The results of ANOVA analysis reveal a significant difference ($p < 0.05$) between the mean of EC_{50} values of water and ethanolic extracts of Wakame; however the lowest value was found in water extract of Kumbu.

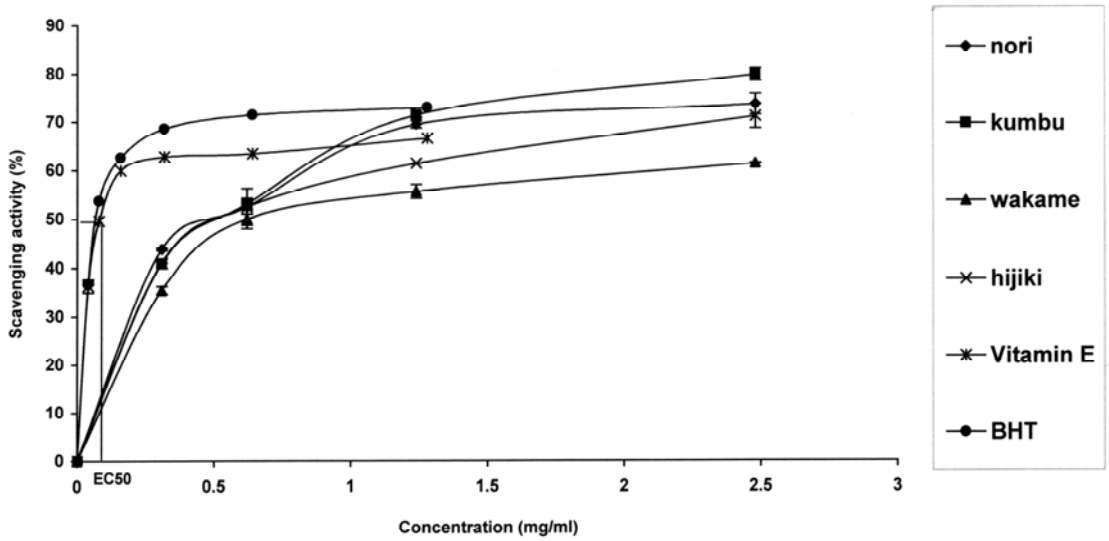

Figure 5. Scavenging activity of water extract of different seaweeds, vitamin E and BHT on DPPH• radical

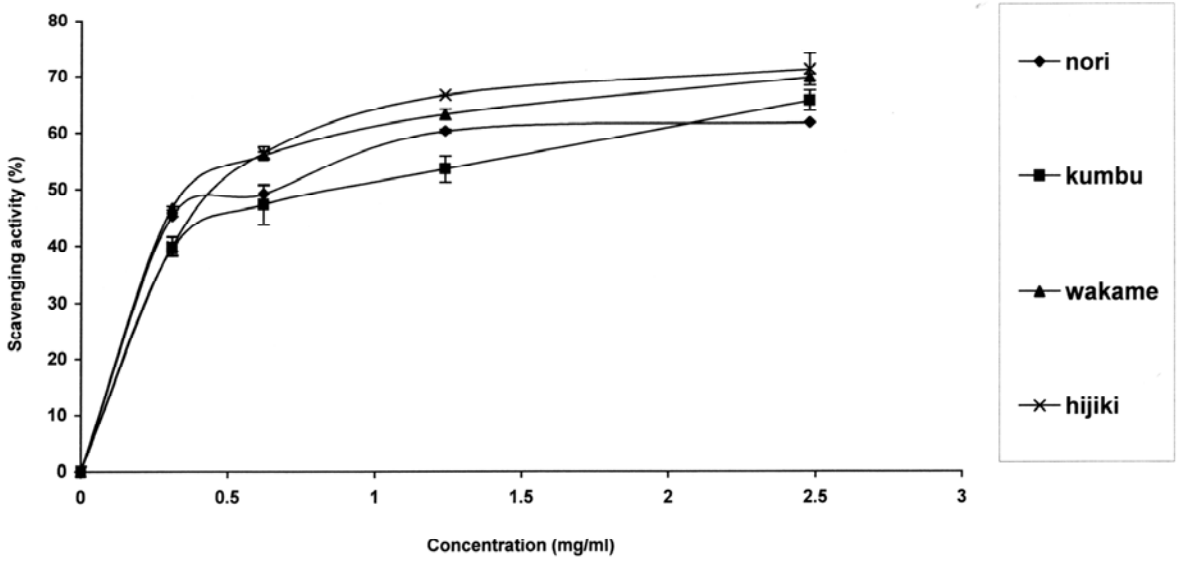

Figure 6. Scavenging activity of ethanolic extract of different seaweeds on DPPH• radical

For kumbu, obviously ethanolic extract corresponded to the least free radical scavenging potency. The ANOVA analysis shows no significant difference ($p > 0.05$) between the mean of EC_{50} values of the two extracts (Table 1). Extracts of Hijiki in ethanol which had mean EC_{50} values of 0.46 mg/ml showed the strongest ability as DPPH radical scavengers. However, there

was no significant difference ($p>0.05$) in the means of EC_{50} values of the two different extracts. The finding presented evidence of the significant difference ($p < 0.05$) in the means of free radical scavenging activity between ethanolic extracts of Kumbu and Wakame, Kumbu and Hijiki. However, no significant difference ($p > 0.05$) was observed between Nori and Kumbu, Wakame and Hijiki.

Table 1. Free radical scavenging activity (EC_{50}) of water and ethanolic extracts of different seaweeds

Seaweed	Extract	EC_{50} (mg/ml) *
Nori	Water	$0.55^{b} \pm 0.06$
	Ethanol	$0.67^{bc} \pm 0.06$
Kumbu	Water	$0.51^{b} \pm 0.01$
	Ethanol	$0.86^{b} \pm 0.33$
Wakame	Water	$0.66^{b} \pm 0.08$
	Ethanol	$0.42^{c} \pm 0.00$
Hijiki	Water	$0.57^{b} \pm 0.08$
	Ethanol	$0.47^{cd} \pm 0.06$

* Values are expressed as mean ± standard deviation of three replicate measurements. Different letters indicate significant difference at the level of $p<0.05$. Comparison was made between two extracts of the respective seaweeds. The EC_{50} value is defined as the amount of extract necessary to decrease the initial DPPH radical concentration by 50%.

DISCUSSION

According to Jayaprakasha *et al*. (2001), the bleaching mechanism of β-carotene is a free radical mediated phenomenon resulting from the formation of hydroperoxides from linoleic acid. In the absence of antioxidant, β-carotene will undergo rapid discoloration. Linoleic acid will become a free radical with a hydrogen atom abstracted from one of its diallylic methylene groups. The radical formed then attacks the highly unsaturated β-carotene molecules. When β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and orange colour, which can be monitored spectrophotometrically. The presence of antioxidants in the different extracts can protect the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system.

In order to provide additional data on antioxidant potential of the ability of the seaweed extracts to act as free radical scavengers or hydrogen donors, DPPH radical scavenging activity assay was carried out.

The mean total antioxidant and free radical scavenging activities of Nori and Kumbu were shown to decrease in the order of water > ethanolic extracts, while the activity for Wakame and Hijiki

was in the order of ethanolic > water extracts. The difference is probably due to the characteristics of the antioxidant components extracted from the seaweeds matrix. This study found different seaweed extracts of the studied samples to show varying degrees of free radical scavenging activity. DPPH radical scavenging activity was seen to increase as the concentration increased from 0.31 to 1.24 mg/ml for all samples and 0.04 to 1.28 mg/ml for standards. These findings are in agreement with the results obtained by Lai & Foo (2001).

These results also supported by the study of Duffy & Power (2001) who described different samples in different solvents to give different antioxidant potentials. Previous studies reported that ethanolic extracts of licorice samples displayed high antioxidant potential compared to water extracts. However, other ethanolic Chinese plant extracts showed little antioxidant potential (Duffy & Power, 2001).

In water extract, the mean total antioxidant activity was in the order of Kumbu > Nori > Hijiki > Wakame. However, the mean of EC_{50} values decreased in the order of Nori > Hijiki > Kumbu > Wakame, while the mean total antioxidant activity and EC_{50} of ethanolic extract were in the order of Wakame > Hijiki > Nori > Kumbu. The order of total antioxidant activity of water extract was contradictory to the results obtained for ethanolic extract. According to Marinova & Yanishlieva (1997), antioxidant activity of extracts is strongly dependent on the types of solvent used due to compounds with different polarity exhibiting differing rates of antioxidant potential. In addition, apolar solvents were found to be the most suitable solvents for extracting polyphenols from water (Moure *et al*., 2001). In a study carried out by Moller *et al*. (1999), the water extract of dittany (*Origanum dictamnus)* was found to have an unexpectedly high amount of phenolic compounds compared to organic solvents. Ethanolic and water extracts are the most widely employed solvents due to their more hygienic characteristics (Moure *et al*., 2001). In addition, besides the type of solvents that can affect antioxidant potential of a sample, the effects of extraction pH has also been reported. Moure *et al*. (2001) reported that oat fibre extract give higher antioxidant activity at pH 10 compared to pH 6. Thus, it can be assumed that each seaweed possesses different antioxidant potential in different extraction mediums. The results obtained on total antioxidant activity of ethanolic extract was supported by the findings from DPPH radical scavenging activity. This finding is in agreement with the previous results reported by Lu & Foo (2000) where flavonoids from apple showed the highest antioxidant activity using β-carotene bleaching method and DPPH scavenging assay. The difference in the DPPH radical scavenging activity of each sample in different extracts implies that the extracting solvent used would affect the radical scavenging potency. This may be due to the different polarities of each antioxidant compound group present in the seaweeds (Marinova & Yanishlieva, 1997).

CONCLUSION

This study showed that Nori, Kumbu, Wakame and Hijiki possessed varying degrees of antioxidative activity in different extraction mediums. Ethanolic extracts of Nori and Kumbu exhibited lower antioxidant activity based on total antioxidant and free radical scavenging activities compared to their water extract. Kumbu possessed the highest antioxidant activity among water extracts. For ethanolic extract, Wakame and Hijiki exhibited the highest

antioxidant activity. Although their antioxidant activity was lower than that of commercial antioxidants (vitamin E and BHT) other potential benefits of seaweeds which can contribute to human health should be explored in future studies.

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