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Food Chemistry 91 (2005) 485–494
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity of dulse (*Palmaria palmata*) extract evaluated in vitro

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Received 22 December 2003; received in revised form 6 April 2004; accepted 6 April 2004

Abstract

Palmaria palmata (dulse) is traditionally consumed as a snack food and garnish; but, little is known about its potential as a source of antioxidants. A 1-butanol soluble fraction extracted from dulse exhibited ·OH scavenging activity ± EDTA (non-site and site specific activity) in a deoxyribose assay. EC_{50} concentrations of dulse extract to quench DPPH· and $ABTS^{\cdot+}$ free radicals were 12.5 and 29.5 mg/ml. Dulse extract inhibited ($p < 0.05$) conjugated diene production in a linoleic acid emulsion at 24, 48 and 52 h, 38 °C; and inhibited ($p = 0.044$) thiobarbituric acid reactive substances (TBARS) production at 52 h. One milligram dulse extract exhibited reducing activity = 9.68 μg L-ascorbic acid and total polyphenol content = 10.3 μg gallic acid; the dulse extract did not chelate transition metal ions. The antioxidant activity of the dulse extract was associated with aqueous/alcohol-soluble compounds characterized by phenolic functional groups with reducing activity.

Keywords: Antioxidant activity; *Palmaria palmata*; Dulse; Hydroxyl radical; DPPH; ABTS; Linoleic acid emulsion, Free radical scavenger

1. Introduction

Interest in natural sources of antioxidant molecules in the food and beverage and cosmetic industries has resulted in a large body of research in recent years characterizing the mechanisms of action of well known lipid- and water-soluble antioxidants such as the tocopherols and ascorbic acid, respectively, as well as antioxidants isolated from herbs, spices, oilseeds, green and black teas, citrus fruits, grapes and alcoholic beverages (Shahidi, 1997; Thompson, Robb, Serraino, & Cheung, 1991). Fruits, vegetables and oilseeds are increasingly recognized as sources not only of vitamins, minerals, soluble and insoluble dietary fibre, but also phenolic compounds including phenolic acids, flavonoids, isoflavones and lignans (Shahidi, 1997; Thompson et al., 1991). A currently underused potential source of dietary fibre and antioxidant molecules in North America are seaweeds, which are also referred to as sea vegetables (Lahaye, Michel, & Barry, 1993; Mabeau & Fleurence, 1993). Seaweeds are an ubiquitous component of diets in Asia, in particular China, Japan, Korea and Indonesia (Anggadiredja, Andyani, & Hayati, 1997; Chan, Cheung, & Ang, 1997; Yan, Nagata, & Fan, 1998; Yan, Chuda, Suzuki, & Nagata, 1999; Nakayama, Tamura, Kikuzaki, & Nakatani, 1999); to a lesser extent in Europe (e.g. France, Iceland, Ireland and Norway) (Mabeau &

Abbreviations: $ABTS^{\cdot+}$, 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) cation free radical; BHA, butylated hydroxyanisole; CD, conjugated dienes; DPPH·, 1,1-diphenyl-2-picrylhydrazyl stable free radical; EC_{50}, effective concentration for 50% reduction of the free radical; EGC, epigallocatechin; GSH, glutathione; MAA, mycosporine-like amino acid; MDA, malondialdehyde; ·OH, hydroxyl radical; ROS, reactive oxygen species; TBARS, 2-thiobarbituric acid reactive substances; TCA, trichloroacetic acid.

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doi:10.1016/j.foodchem.2004.04.039

Fleurence, 1993; Le Tutour, 1990; Le Tutour et al., 1998; Karsten & Wiencke, 1999) and the Canadian Maritimes (Morgan, Wright, & Simpson, 1980; Bird & McLachlan, 1992). Dietary seaweeds include marine algae from brown (Phaeophyta), green (Chlorophyta) and red (Rhodophyta) taxonomies such as the Laminariales, Ulvales and Porphyridiales, respectively (Anggadiredja et al., 1997; Le Tutour, 1990; Yan et al., 1998). These seaweeds are commonly used as wrappers such as the Japanese sushi seaweed 'Nori' or Korean 'Kim' derived from roasted or toasted *Porphyra tenera*; condiments and seasonings in soups and salads such as 'Hijiki' (*Hijikia fusiformis*), 'Wakame' (*Undaria pinnatifida*) or 'Makonbu' (*Laminaria japonica*); as well as health food snacks such as 'Dulse' (*Palmaria palmata*).

Within the food ingredient industry, seaweeds have been traditionally viewed as sources of soluble and insoluble dietary fibres such as gums (i.e. carrageenans, agar), storage polysaccharides (i.e. β-1,3-glucan) and floridean starch (Han, Lee, & Sung, 1999; Lahaye et al., 1993; Mabeau & Fleurence, 1993). However, in recent years researchers have also focussed on the nutritional value of proteins from edible seaweeds (Chan et al., 1997), the fermentation of soluble fibre from dulse producing short-chain fatty acids (Lahaye et al., 1993) as well as the anticarcinogenic effects of dietary kelp and other seaweeds against mammary (Maruyama, Watanabe, & Yamamoto, 1991; Teas, 1983; Teas, Harbison, & Gelman, 1984; Yamamoto, Maruyama, & Moriguchi, 1987) and intestinal carcinogenesis (Reddy, Numoto, & Choi, 1985; Yamamoto & Maruyama, 1985). One of the mechanisms thought to contribute to the inhibitory effect of dietary kelp against chemically induced mammary carcinogenesis is the enhancement of antioxidant enzyme activity (i.e. glutathione peroxidase) and reduction in lipid peroxidation in livers of treated rats (Maruyama et al., 1991).

Edible seaweeds are known to contain labile antioxidant molecules such as ascorbate and glutathione (GSH) when fresh (Morgan et al., 1980; Burritt, Larkindale, & Hurd, 2002); as well as more stable molecules including carotenoids (α- and β-carotene, fucoxanthin (Morgan et al., 1980; Yan et al., 1998)), mycosporine-like amino acids (mycosporine-glycine and – taurine (Dunlap, Masaki, Yamamoto, Larsen, & Karube, 1997; Nakayama et al., 1999; Sekikawa, Kubota, Hiraoki, & Tsujino, 1986)), catechins (e.g. catechin, epigallocatechin, epigallocatechin gallate (Yoshie, Wang, Petillo, & Suzuki, 2000)), phlorotannins (e.g. phloroglucinol, eckol (Jiménez-Escrig, Jiménez-Jiménez, Pulido, & Saura-Calixto, 2001)) and the tocopherols (α-, γ-, δ-tocopherols (Morgan et al., 1980)). Thompson and coworkers (1991) reported that the fecal fermentation of the Japanese processed seaweeds Mekuba and Hijiki, resulted in the production of the mammalian lignans enterolactone (EL) and enterodiol (ED). The mammalian lignan precursor, secoisolariciresinol diglycoside (SDG) as well as ED and EL have been reported to exhibit antioxidant activity in vitro (Kitts, Yuan, Wijewickreme, & Thompson, 1999) and have also been demonstrated to influence antioxidant enzyme activity in vivo (Yuan, Rickard, & Thompson, 1999). Thus, it is noteworthy that extracts from selected brown, green and red alga harvested in France (Le Tutour, 1990; Le Tutour et al., 1998), Spain (Jiménez-Escrig et al., 2001), Indonesia (Anggadiredja et al., 1997), Korea (Han et al., 1999), China (Lim, Cheung, Ooi, & Ang, 2002; Yan et al., 1998) and Japan (Matsukawa et al., 1997; Sekikawa et al., 1986; Yan et al., 1999) have been reported to demonstrate antioxidant activity by a variety of in vitro methodologies. This evidence suggests a potential for protective effects of seaweed against lipid oxidation in foods and oxidative stress in target tissues and LDL in vivo. There is a paucity of data in the literature, however, about the antioxidant potential of North American seaweeds, in particular, *P. palmata* (order Palmariales, family Palmariaceae), which is also known as dulse. Dulse is harvested on the east coast of Canada (New Brunswick) and the USA (Maine); it is then sun-dried and consumed as a snack food or condiment with other foods such as soups and salads. Therefore, the objective of the present study was to characterize the antioxidant activity of dulse using a variety of in vitro methods to assess the hydroxyl, stable free radical and radical cation scavenging ability of this red seaweed variety. The ability of the dulse to inhibit lipid oxidation in a linoleic acid emulsion as a food system model was also evaluated in this study.

2. Materials and methods

2.1. Materials

Dulse (*P. palmata*) (Atlantic Mariculture Ltd., Grand Manan, New Brunswick, Canada) was purchased from a local grocery store. Linoleic acid, 1-butanol, ethyl acetate, hexane, methanol, ethanol, chloroform, butylated hydroxyanisole (BHA), malondialdehyde (MDA), $CuSO_4$ were purchased from Fisher Scientific (Mississauga, Ont., Canada). 1,1-diphenyl-2-picrylhydrazyl (DPPH•); 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) diammonium salt (ABTS); 2-thiobarbituric acid (TBA), 2-deoxy-D-ribose, L-ascorbic acid, 30% H_2O_2, monolaurate Tween 20, $FeCl_3 \cdot 6H_2O$, $K_3Fe(CN)_6$, potassium persulfate, gallic acid, Folin–Ciocalteau's phenol reagent, EDTA-Na_2 dihydrate, trichloroacetic acid (TCA) were purchased from Sigma–Aldrich Canada (Oakville, ON). Water (H_2O) used in all assays was purified using an E-pure Barnstead system (VWR Canlab, Mississauga, ON). All solvents used were of ACS or HPLC grades. Sample absorbances were

read using a Lambda 20 UV/Vis Spectrometer (Perkin–Elmer, Norwalk, CT).

2.2. Preparation of dulse extract

Two hundred and fifty grams ground, freeze-dried dulse was extracted with methanol overnight 3 X at room temperature, filtered and concentrated by rotary evaporation (Buchi R-200V, Brinkmann Instruments, Mississauga, ON), 40 °C. The concentrated extract was further extracted with hexane and the lower methanol phase then extracted with H_2O + ethyl acetate. The lower H_2O–methanol layer was further washed with 1-butanol and the upper butanol layer concentrated by rotary evaporation to obtain a light brown residual powder. The dulse extract was solubilized in 0.1% ethanol for use in assays.

2.3. Deoxyribose assay to assess site-specific and non-site specific $^{\cdot}OH$ radical scavenging activity

Non-site specific $^{\cdot}OH$ radical scavenging activity of dulse extract (0–5 mg/ml) was determined according to the deoxyribose method of Halliwell, Gutteridge, and Aruoma (1987) in the presence of 100 μM EDTA. $FeCl_3 \cdot 6H_2O$ and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 μM EDTA, 1 mM H_2O_2, 100 μM L-ascorbic acid, 100 μM $FeCl_3 \cdot 6H_2O$ in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Following incubation at 38 °C, 1 h, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture which was then heated in a boiling water bath for 15 min. Once samples were cooled, the absorbances were read at 532 nm. The percent inhibition of hydroxyl radical was calculated as follows:

$$\%\text{Inhib} = \frac{(\text{Abs. 532 nm Control} - \text{Abs. 532 nm sample})}{\text{Abs. 532 nm Control}} \times 100.$$

Site-specific $^{\cdot}OH$ radical scavenging activity of dulse extract was performed as described above except that the EDTA was replaced by an equivalent volume of buffer.

2.4. DPPH$^{\cdot}$ stable free radical scavenging activity

DPPH$^{\cdot}$ radical scavenging activities of dulse extract, ascorbic acid and BHA (controls) were determined according to Shimada, Fujikawa, Yahara, and Nakamura (1992) with modifications. 0.2 mM DPPH$^{\cdot}$ (in 50% ethanol) was mixed with dulse extract (10–40 mg/ml in 0.1% ethanol), ascorbic acid (0.1–1 mM) or BHA (0.1–0.5 mM in 0.1% ethanol) and the absorbance read at 523 nm. Sample absorbances were followed for 15 min up to 100 min until a steady state was reached. The antioxidant capacity of test compounds was expressed as EC_{50}, the concentration necessary for 50% reduction of DPPH$^{\cdot}$.

2.5. ABTS$^{\cdot+}$ radical cation scavenging activity

The ABTS$^{\cdot+}$ radical cation scavenging activity of dulse extracts, ascorbic acid or BHA (as controls) was determined according to Re et al. (1999). Briefly, 5.0 ml 7.0 mM ABTS was reacted with 88.0 μl 140 mM potassium persulfate overnight in the dark to yield the ABTS$^{\cdot+}$ radical cation. Prior to use in the assay, the ABTS$^{\cdot+}$ radical cation was diluted with 50% ethanol for an initial absorbance of ≈ 0.700 (1:88 ratio) at 734 nm, with temperature control set at 30 °C. Free radical scavenging activity was assessed by mixing 1.0 ml diluted ABTS$^{\cdot+}$ radical cation with 10 μl of test antioxidant and monitoring the change in absorbance at 0, 0.5, 1 min and again at 5 min intervals until a steady state was achieved. The antioxidant capacity of test compounds was expressed as EC_{50}, the concentration necessary for 50% reduction of ABTS$^{\cdot+}$.

2.6. Inhibition of lipid oxidation in a linoleic acid emulsion

A linoleic acid emulsion was prepared by homogenizing 2.5 ml linoleic acid + 22.5 ml H_2O + 125 μl Tween 20 on ice 2× 10 s (Powergen 700, Fisher Scientific). The emulsion was incubated at 38 °C with shaking and aliquots removed at 0, 1, 4, 6, 24, 30, 48 and 52 h followed by phase separation using $CHCl_3$:CH_3OH 2:1 and 2-thiobarbituric acid reactive substances (TBARS) measured in the aqueous fraction at 532 nm according to Coupland et al. (1996). The organic layer was taken for conjugated diene determination at 233 nm according to Recknagel and Glende (1984). Inhibition of lipid oxidation by dulse extract was determined as described above.

2.7. Reducing activity

The reducing activity of the dulse extract was evaluated according to the method of Yen and Chen (1995) with modifications. L-ascorbic acid was prepared in degassed H_2O for use as the standard to quantify reducing activity. To 0.5 ml of reducing agent were added the following: 1.25 ml 0.2 M phosphate buffer, pH 6.6 + 1.25 ml 1% $K_3Fe(CN)_6$ followed by incubation at 50 °C in a water bath for 20 min. Samples were then cooled and mixed with 1.25 ml 10% TCA and an 1.25 ml aliquot removed to a fresh tube. For the final reaction, the sample aliquot was mixed with 1.25 ml of H_2O + 0.25 ml 0.1% $FeCl_3 \cdot 6H_2O$ and left to react at room temperature for 10 min. Sample absorbances were read at 700 nm. Reducing activity of the dulse extract was expressed as ascorbic acid equivalents from the ascorbic acid calibration curve.

2.8. Transition metal ion chelation

Chelation of transition metal ions by dulse extract was evaluated according to Miranda et al. (2000) with modifications. Briefly, the chelation control contained 0.9 ml 10 mM phosphate buffer, pH 7.4, 50 μl 1.0 mM $CuSO_4$ and 50 μl 1.0 mM EDTA. Transition ion spectra were scanned from 200 to 900 nm. Test samples contained 0.9 ml 10 mM phosphate buffer, pH 7.4, 50 μl 1.0 mM $CuSO_4$ and 50 μl 20 mg/ml dulse extract.

2.9. Total polyphenols

The polyphenol content of dulse extract was quantified according to the method of Taga, Miller, and Pratt (1984) with modifications. Aliquots of test samples (100 μl) were mixed with 2.0 ml 2% Na_2CO_3 and allowed to sit at room temperature for 2 min. At this time, 100 μl 50% Folin–Ciocalteau's phenol reagent was added, and the reaction tube allowed to sit at room temperature for a further 30 min prior to reading the absorbance at 720 nm. Gallic acid was used as the standard for a calibration curve (Sellés et al., 2002). Polyphenol content of the dulse extract was expressed as gallic acid equivalents.

2.10. Statistics

All data are expressed as means ± SEM. One-way analysis of variance (ANOVA; SPSS 8.0 for Windows; SPSS Inc., Chicago, IL) was used to test for differences between sample treatments. Student's *t*-test for independent samples was used to test for differences at a $p \leqslant 0.05$ significance level where appropriate.

3. Results

Table 1 summarizes the site-specific and non-site-specific hydroxyl radical scavenging activity of the dulse extract using the deoxyribose assay. The dulse extract was effective at quenching the hydroxyl radicals generated in both the site- and non-site-specific assays, exhibiting a concentration dependence in both cases. The effectiveness of the dulse extract in inhibiting deoxyribose degradation due to hydroxyl radical (·OH) damage was greater in the site-specific assay (i.e. in the absence of EDTA) compared to the non-site-specific results.

Dulse extract also exhibited free radical scavenging activity against the stable free radical DPPH· and the $ABTS^{\cdot+}$ radical cation (Table 2). The EC_{50} concentration of the dulse extract required to scavenge 50% of the DPPH· stable free radical was approximately 212- and 500-fold greater than that required for L-ascorbic acid and BHA, respectively. The length of time required to achieve a steady state absorbance for ascorbic acid, BHA and dulse extract in the DPPH· assay was 15, 50 and 105 min, respectively. The EC_{50} concentration of the dulse extract required to quench 50% of the $ABTS^{\cdot+}$ radical cation was approximately 157- and 172-fold greater than that required for L-ascorbic acid and BHA, respectively (Table 2). The length of time required to achieve a steady state absorbance for ascorbic acid, BHA and dulse extract in the $ABTS^{\cdot+}$ radical cation assay was 20, 35 and 105 min, respectively.

In the linoleic acid emulsion model, the dulse extract was effective at inhibiting the production of lipid oxidation products such as TBARS (Figs. 1a and 2a) and conjugated dienes (CD, Figs. 1b and 2b). The 2 ml dulse extract treatment inhibited ($p = 0.044$) the production of TBARS after 52 h incubation compared to the control sample; however, the 1 ml dulse extract treatment did not inhibit TBARS production (Figs. 1a and 2a). The 2 ml dulse extract treatment was also able to inhibit

Table 2
DPPH· and $ABTS^{\cdot+}$ stable free radical scavenging activity of dulse, *P. palmata*, extract[a]

Antioxidant	DPPH· EC_{50} (mg/ml)[b]	$ABTS^{\cdot+}$ EC_{50} (mg/ml)[b]
L-ascorbic acid	0.059	0.188
BHA[c]	0.025	0.171
Dulse extract	12.5	29.5

[a] DPPH· = 1,1-diphenyl-2-picrylhydrazyl; $ABTS^{\cdot+}$ = 2,2′-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) cation free radical.
[b] EC_{50} = concentration of antioxidant required to quench 50% of the stable free radical.
[c] BHA = butylated hydroxyanisole.

Table 1
Site-specific and non-site-specific hydroxyl radical scavenging activity of dulse, *P. palmata*, extract[a]

Dulse extract (mg/ml)		Abs. 532 nm		% Inhibition[b]	
		Non-site specific[c]	Site-specific[d]	Non-site specific[c]	Site-specific[d]
Control	0	0.624 ± 0.024	0.313 ± 0.006	0	0
Dulse	1	0.478 ± 0.018	0.249 ± 0.006	18.2 ± 1.8	20.3 ± 1.7
	2	0.449 ± 0.019	0.210 ± 0.006	25.4 ± 1.9	32.8 ± 2.4
	5	0.369 ± 0.029	0.185 ± 0.008	31.4 ± 5.6	40.7 ± 2.0

[a] Values represent means ± SEM, $n = 6$.
[b] $\%\text{Inhibition} = \frac{(\text{Abs. 532 control} - \text{Abs. 532 sample})}{\text{Abs. 532 control}} \times 100$.
[c] Non-site specific hydroxyl radical scavenging occurs in the presence of EDTA.
[d] Site-specific hydroxyl radical scavenging occurs in the absence of EDTA.

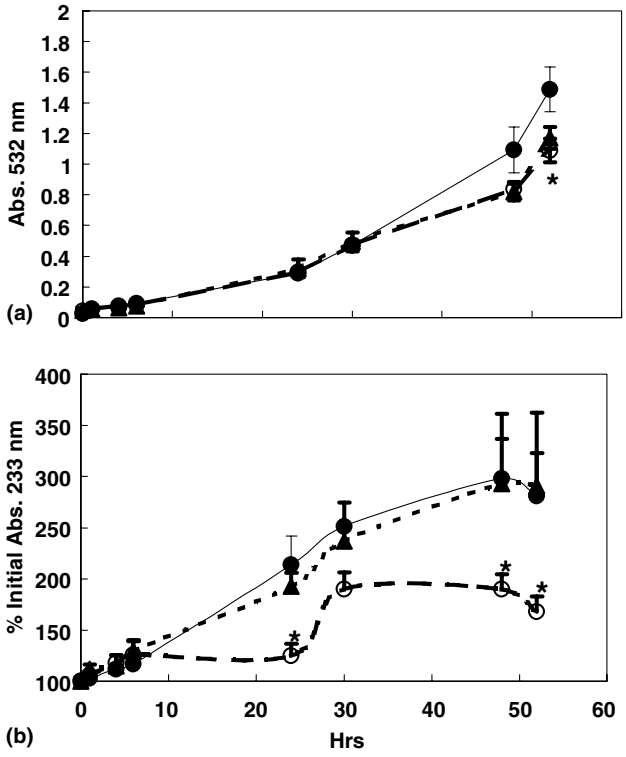

Fig. 1. Influence of dulse extract on lipid oxidation parameters of a linoleic acid emulsion incubated with shaking at 38 °C. (a) TBARS; (b) conjugated dienes. (●), control; (▲) 1 ml Dulse extract; (○) 2 ml Dulse extract. * indicates a significant difference ($p < 0.05$) between control and 2 ml Dulse extract samples by Student's *t*-test.

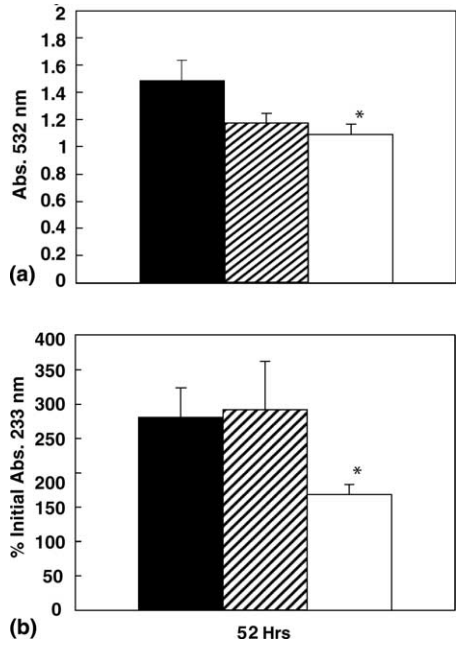

Fig. 2. Influence of dulse extract on lipid oxidation of a linoleic acid emulsion incubated with shaking at 38 °C, at 52 h. (a) TBARS; (b) conjugated dienes. (■), control; (▨), 1 ml Dulse extract; (□), 2 ml Dulse extract. * indicates a significant difference ($p < 0.05$) between control and 2 ml Dulse extract samples by Student's *t*-test.

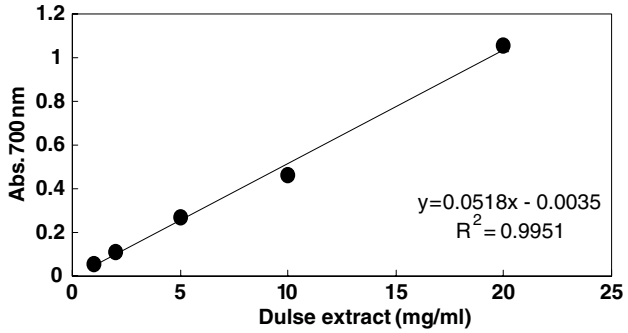

Fig. 3. Calibration curve for the reducing activity of the dulse extract.

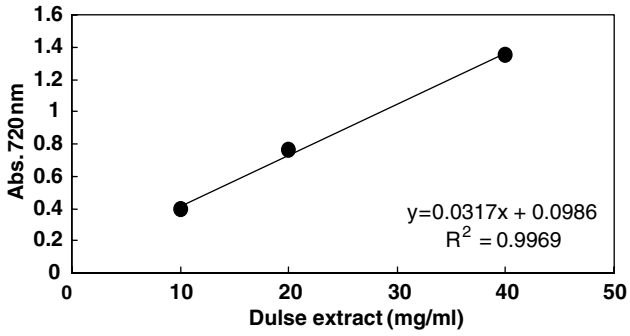

Fig. 4. Calibration curve for the total polyphenol content of the dulse extract.

($p < 0.05$) the production of conjugated dienes in the linoleic emulsion at 24, 48 and 52 h compared to the control emulsion (Figs. 1b and 2b). The 1 ml dulse extract treatment was not effective against CD production in the linoleic acid emulsion model.

The reducing activity of the dulse extract was expressed as L-ascorbic acid equivalents after confirming the linearity of the response of the assay using the extract (Fig. 3). One milligram of the dulse extract exhibited reducing activity equivalent to 9.68 μg L-ascorbic acid. The total polyphenol content of the dulse extract was expressed as gallic acid equivalents following confirmation of the linearity of the response of the assay using the extract (Fig. 4). One milligram of the dulse extract exhibited a total polyphenol content equivalent to 10.3 μg gallic acid. The dulse extract did not exhibit transition metal ion chelation activity when incubated with Cu^{2+} in the present study (data not shown).

4. Discussion

This study is the first to evaluate the antioxidant activity of *P. palmata* (dulse) in a comprehensive manner employing a variety of in vitro methods. We report that an extract from dulse was active in scavenging $^{\cdot}OH$ radicals in a deoxyribose assay, as well as quenching the stable free radical $DPPH^{\cdot}$ and the $ABTS^{\cdot+}$ radical cation

in separate model systems. The ability of the dulse extract to scavenge reactive oxygen species (ROS) and free radicals was further confirmed by the inhibition of lipid peroxidation indices in a linoleic acid emulsion model system in the present study. The antioxidant potential of dulse can be attributed to the fact that this intertidal red alga is known to be exposed to not only wide-variations in photosynthetically available radiation (PAR; 400–700 nm) and more specifically, UV-B (280–320 nm) and UV-A (320–400 nm) irradiation depending upon the season, depth in the water and water turbidity (Aguilera, Bischof, Karsten, & Hanelt, 2002; Karsten & Wiencke, 1999) but also, dehydration due to daily tidal fluctuations (Burritt et al., 2002). Therefore, these marine organisms require endogenous antioxidant protection against cell and organelle membrane damage due to phospholipid membrane damage and subsequent leakiness (Burritt et al., 2002) as well as UV-induced lipid oxidation (Aguilera et al., 2002; Karsten & Wiencke, 1999).

In the present study, the dulse extract exhibited weak reducing activity, approximately 100-fold weaker than L-ascorbic acid. Fresh dulse has been reported to contain ≈ 200–500 μg ascorbic acid/g wet wt. (Morgan et al., 1980). However, the drying and storage of dulse is known to severely reduce the vitamin C and the carotenoid content (β-carotene 30 μg/g dry wt.; α-carotene 80 μg/g dry wt. (Morgan et al., 1980)). Nevertheless, the dulse extract was effective in scavenging $^{\cdot}OH$ radicals to prevent the degradation of the deoxyribose substrate in a dose-dependent manner herein. The ability of the dulse extract to inhibit deoxyribose degradation (% inhibition) was slightly greater for the site-specific (in the absence of EDTA) versus the non-site specific (in the presence of EDTA) version of the deoxyribose assay at the highest concentration of dulse extract. Thus, the dulse extract was able to prevent deoxyribose damage associated with the direct binding of iron to deoxyribose and the subsequent attack by $^{\cdot}OH$ radicals generated via the Fenton reaction. Other workers investigating the red alga *Stictosiphonia arbuscula*, harvested in New Zealand, reported that ascorbate and GSH levels in fresh specimens were ≈ 2.5–2.8 and 0.69–0.71 μmol/g wet wt., respectively (Burritt et al., 2002). Interestingly, water depth seemed to influence the ability of *S. arbuscula* to maintain GSH reserves upon dessication/ dehydration, in that specimens from deeper waters exhibited reductions in total GSH at 24 and 48 h of dessication, whereas specimens from shallow waters only experienced a decrease in GSH after 48 h. Total content of combined reduced and oxidized ascorbate did not change in *S. arbuscula* with dessication; but the concentration of reduced ascorbate did decline at 12 h and remained low at 48 h of dessication (Burritt et al., 2002). Therefore, low molecular weight water-soluble antioxidant molecules are likely most important to the oxidative status of alga prior to harvest, and are found in low concentrations in the dehydrated product used in prepared foods.

The ability of the dulse extract to reduce the DPPH$^{\cdot}$ radical by donating an electron or hydrogen atom was several hundred fold weaker than both L-ascorbic acid and BHA in the present study. However, the activity of the dulse extract took place over a sustained period of time compared to the rapid acting L-ascorbic acid and intermediate BHA. This ability to reduce and quench free radicals over a longer period of time may have benefits for extending the shelf-life of processed foods during distribution and storage. The DPPH$^{\cdot}$ assay has been used by many researchers to evaluate the free radical scavenging activity of antioxidant molecules and plant extracts despite the fact that this chromogen does not solubilize easily; albeit, it does generate strongly coloured solutions with methanol and ethanol as solvents (Blois, 1958; Han et al., 1999; Matsukawa et al., 1997; Yan et al., 1999; Yan et al., 1998). Pellegrini, Re, Yang, and Rice-Evans (1999) and coworkers (Re et al., 1999) reported that the decolorization of the ABTS$^{\cdot+}$ radical cation also reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate this radical species. The ABTS$^{\cdot+}$ radical cation is generated from the reaction of ABTS with potassium persulfate overnight in water, followed by dilution with ethanol (Re et al., 1999). Similar to DPPH$^{\cdot}$ above, the dulse extract exhibited weaker ABTS$^{\cdot-}$ radical cation scavenging activity than both L-ascorbic acid and BHA. However, again, the duration of the antioxidant activity for the dulse extract occurred over a longer duration of time compared to the shorter reaction times for L-ascorbic acid and BHA. These differences in kinetic behaviour of antioxidant compounds in the DPPH$^{\cdot}$, and by extension the ABTS$^{\cdot+}$, free radical model systems is thought to be related to the reaction stoichiometry of the number of electrons available to inactivate the free radical (Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002). Slow reacting compounds such as butylated hydroxytoluene (BHT), or the closely related BHA and dulse extract used in the present study, are hypothesized to have a more complex reaction mechanism involving one or more secondary reactions in the quenching of the DPPH$^{\cdot}$ (Koleva et al., 2002) and thereby also, ABTS$^{\cdot+}$ free radicals.

Extracts of red alga from the Rhodomelaceae and Bangiaceae families have been reported to exhibit weak DPPH$^{\cdot}$ quenching activity when obtained using water (Han et al., 1999; Matsukawa et al., 1997), ethanol (Matsukawa et al., 1997) or methanol (Yan et al., 1998, 1999) as solvents. On the other hand, chloroform, ethyl acetate and acetone extracts from several Rhodomelaceae genera have been reported to exhibit strong DPPH$^{\cdot}$ quenching activity in vitro (Yan et al., 1998). Thus, the particular solvent used to extract the seaweed

material will have a dramatic effect on the chemical species recovered. For example, the non-polar fraction from a chloroform:methanol extraction of *P. tenera* exhibited a significant protective effect against lipid oxidation associated with phospholipids such as, phosphatidyl choline and phosphatidyl ethanolamine (Kaneda & Ando, 1971). Whereas, Nakayama et al. (1999) reported that hexane, chloroform and methanol extracts of *Porphyra yezoensis* exhibited antioxidant activities attributed to the presence of β-carotene, chlorophyll analogues (pheophytin a and chlorophyll a) and amino compounds (leucine, phenylalanine and the mycosporine-like amino acid, usujirene), respectively. The dulse extraction and solvent washing protocol used in the present study has been reported to yield an *n*-butanol soluble fraction from an initial methanol extract of plant material containing flavonoid and hydroquinone glycosides (Zhang et al., 1997, Zhang, Ide, Otsuka, Hirata, & Takeda, 1998a, 1998b) as well as glycosylated lignan derivatives such as (+)-isolariciresinol 3a-*O*-sulphate (Zhang et al., 1998a, 1998b). As well, Yoshie et al. (2000) identified several catechins following the acid hydrolysis of methanol extracts of Japanese red alga. Methanol is the preferred solvent for non-glycosylated flavonoids which are considerably less water-soluble than the respective glycones. More recently, Jiménez-Escrig et al. (2001) determined that the EC_{50} values for DPPH$^{\cdot}$ free radical scavenging by brown and red alga aqueous/organic extracts were positively correlated with the total polyphenol content of these extracts. In the present study, 1 mg of the dulse extract exhibited a total polyphenol content approximating 10.3 μg gallic acid equivalents indicating the presence of compounds with phenolic functional groups in the extract.

The antioxidant activity of compounds is often described by the ability to delay the onset of autoxidation by scavenging ROS, or the ability to act as chain-breaking antioxidants to inhibit the propagation phase of lipid autoxidation (Nawar, 1996). The production of both TBARS and CD in the linoleic acid emulsion was inhibited by the dulse extract herein, with the strongest effects observed with the higher dose of dulse extract after 24 h of incubation. Measurement of the increase in CD absorbance is associated with the resonance stabilization and shift in double bond position upon the formation of isomeric hydroperoxides during lipid autoxidation (Nawar, 1996). As such, this index of lipid oxidation is known to correlate with the degree of oxidation only in the early stages of the reaction. The UV-absorbance due to CD formation can subsequently be seen to decrease as hydroperoxides begin to decompose, as observed in the present study, prior to increasing once again as decomposition products begin to form (Puhl, Waeg, & Esterbauer, 1994). TBARS formation reflects primarily the production of dialdehyde products such as malondialdehyde (MDA) from fatty acids containing at least two, or three or more double bonds (Nawar, 1996). MDA is produced when there is a double bond β to the carbon bearing a peroxy function, which enables the formation of closed five-membered ring structure prior to decomposition, which releases one molecule of MDA (Nawar, 1996). Therefore, the absence of an effect of the dulse extract on TBARS production prior to 52 h incubation corroborates the CD data indicating that hydroperoxide decomposition and therefore, a substantial level of MDA production likely began to occur in the 48–52 h time period in the present study. The efficacy of the dulse extract to inhibit oxidation of the linoleic acid emulsion is a reflection of the complexity of the extract composition (i.e. aqueous vs. hydrophobic nature of compounds) as well as potential interactions between the extract and emulsion components (i.e. oil:water or lipid:air interfaces (Koleva et al., 2002)). These interactions form the basis of whether or not the 'polar paradox' can be observed in the present study. The polar paradox occurs in emulsions when non-polar compounds have strong antioxidant activity in an emulsion due to the concentration of the antioxidant at the lipid:air interface, thereby ensuring strong protection of the emulsion against oxidation (Koleva et al., 2002). Conversely, polar compounds exhibit weak antioxidant activity in emulsions due to the dilution of these compounds in the aqueous phase. Moreover, the opposite antioxidant profile is observed in bulk lipid or oil systems (Koleva et al., 2002). These phenomena no doubt help to explain the variability in the efficacy of different red alga extracts to prevent lipid oxidation in vitro. For example, Le Tutour (1990) reported that organic extracts of *P. tenera* and dulse improved the stability of sunflower oil stored at 75 °C; whereas methanol extracts of *Laurencia obtusa* (Rhodomelaceae) were relatively weak in protecting a linoleate emulsion against oxidation (Anggadiredja et al., 1997). Moreover, linoleic acid emulsions are noted to have different properties compared to oil-in-water emulsions due to the potential for linoleic acid to influence the degree of dissociation of organic acid antioxidants (Koleva et al., 2002). The efficacy of the dulse extract in the present study to inhibit CD formation at the highest concentration was relatively stronger, compared to the inhibition of TBARS formation in the linoleic acid emulsion. Thus, the use of more than one method to evaluate lipid oxidation and antioxidant activity was instrumental in determining the efficacy of the dulse extract as an antioxidant in an emulsion system. These findings also potentially help explain the mechanism underlying the reduction in hepatic lipid peroxidation (MDA production) of rats treated with the breast carcinogen 7,12-dimethylbenz[α]anthracene and fed on diets containing 2% kelp (*Laminaria religiosa*) over 14 or 210 days (Maruyama et al., 1991).

The dulse extract in the present study has been demonstrated to contain a mixture of potential antioxidant compounds which possess reducing activity and/or phenolic functional groups soluble in alcohol solvents including 1-butanol, methanol and aqueous ethanol (used to solubilize the dulse extract for use in the assays). Plant-derived polyphenolic flavonoids are well known to exhibit antioxidant activity through a variety of mechanisms including scavenging of ROS, inhibiting lipid peroxidation as well as chelating metal ions (Shahidi, 1997). However, the dulse extract did not appear to demonstrate transition metal ion chelation activity in the present study when incubated with $CuSO_4$ at pH 7.4. Generally, compounds with structures containing two or more of the following functional groups: –OH, –SH, –COOH, $–PO_3H_2$, C═O, $–NR_2$, –S– and –O– in a favourable structure–function configuration will have chelation activity (Lindsay, 1996). Thus, molecules including organic acids such as citric, malic, tartaric, oxalic, succinic, lipoic and phytic acid (Lindsay, 1996) as well as the flavonoid quercetin and its glycoside, rutin are noted to chelate transition metal ions (Afanas'ev, Dorozhko, Brodskii, Kostyuk, & Potapovitch, 1989). On the other hand, while flavonoids are characterized as phenolic compounds with functional groups associated with the chelation of metal ions, there are numerous flavonoids, such as the prenylated and non-prenylated chalcones and flavanones found in beer and hops, which do not chelate copper ions in vitro (Miranda et al., 2000). The structure of the chalcone flavonoids studied by these workers, such as xanthohumol, xanthogalenol and chalconaringen, included an open C-ring in the carbon skeleton. Moreover, from structure–activity studies, Miranda and coworkers (2000) reported that a B-ring catechol moiety is key for copper ion chelation. The absence of any copper ion chelation by the prenylated and non-prenylated chalcones and flavanones was associated with the absence of 3′,4′-dihydroxy substituents on the B-ring of the above compounds. Thus, the dulse extract in the present study could potentially contain non-chelating phenolic flavonoid compounds. In previous studies, Japanese red alga have been reported to contain low amounts of epigallocatechin (EGC; from 0.70 up to 16 mg/g dry wt.) and catechins (0.036 up to 2.17 mg/g dry wt. (Yoshie et al., 2000)). As well, Takano, Nakanishi, Uemera, and Hirata (1979) isolated and determined the structure of several UV-absorbing mycosporine-like amino acids (MAAs) from the red alga *P. tenera*. Several MAAs, characterized by a glycine residue sidechain, an aromatic methyl ester core and a hydroxylated sidechain function, have also been identified in dulse harvested from Japanese and Norwegian waters (Karsten & Wiencke, 1999; Sekikawa et al., 1986). These intracellular alcohol- and water-soluble compounds are thought to act as UV-absorbing sunscreens, in that concentrations of MAAs were lowest in dulse samples from deeper waters (3–10 m) and increased when dulse was transplanted to shallower depths (Karsten & Wiencke, 1999). Taken together, this evidence suggests that the dulse extract in the present study likely contained a mixture of compounds with phenolic functional groups, potentially including MAAs.

In summary, a 1-butanol soluble fraction derived from a methanol extract of *P. palmata* (dulse) exhibited $^{\cdot}OH$ radical scavenging activity both in the presence and absence of EDTA in a deoxyribose assay, as well as the ability to quench $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ free radicals in vitro. For the latter two radical species, the dulse extract exhibited antioxidant activity over a prolonged period of time, which may be beneficial in applications to extend product shelf life. The efficacy of the dulse extract in quenching ROS and free radical species was confirmed by the inhibition of CD and TBARS products of lipid autoxidation in a linoleic acid emulsion as well. The antioxidant potential of the dulse extract was further demonstrated through its reducing activity and total polyphenol content. Further studies will elucidate the identity of the antioxidant molecules in the dulse extract.

Acknowledgements

This research was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to Y.V.Y. and an NSERC undergraduate student research award to D.E.B.. Funds were also provided by the Faculty of Community Services, Ryerson University and the Ontario Work Study Program. The authors thank K. Zhong and K. Harrison for their technical assistance.

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