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SCIENCE DIRECT
Food and Chemical Toxicology 44 (2006) 1144–1150
www.elsevier.com/locate/foodchemtox

Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds

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Received 17 January 2006; accepted 1 February 2006

Abstract

Dietary *Laminaria* and *Porphyra* sp. have been reported to reduce the risk of intestinal or mammary cancer in animal studies. Algal anticarcinogenicity may involve effects on cell proliferation and antioxidant activity. Thus, in the present study, we evaluated the effect of red alga, dulse (*Palmaria palmata*) and three kelp (*Laminaria setchellii*, *Macrocystis integrifolia*, *Nereocystis leutkeana*) extracts on human cervical adenocarcinoma cell line (HeLa cells) proliferation using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The 1-butanol soluble fractions from the methanol extracts of these algae were also evaluated for reducing activity and total polyphenol content. After 72 h incubation, HeLa cell proliferation was inhibited ($p < 0.05$) between 0% and 78% by *P. palmata*; 0% and 55% by *L. setchellii* and 0% and 69% by *M. integrifolia* and *N. leutkeana* at 0.5–5 mg/mL algal extract. Algal extract reducing activities were as follows: *P. palmata* > *M. integrifolia* > *L. setchellii* > *N. leutkeana*; and total polyphenol contents were: *P. palmata* > *M. integrifolia* = *N. leutkeana* > *L. setchellii*. The antiproliferative efficacy of these algal extracts were positively correlated with the total polyphenol contents ($p < 0.05$), suggesting a causal link related to extract content of kelp phlorotannins and dulse polyphenols including mycosporine-like amino acids and phenolic acids.

Keywords: *Laminaria setchellii*; *Macrocystis integrifolia*; *Nereocystis leutkeana*; *Palmaria palmata*; Cell proliferation; HeLa cells; Antioxidant activity

1. Introduction

Edible seaweeds, including algae from the *Protista* orders: *Laminariales* (brown), *Chlorophyta* (green) and *Rhodophyta* (red) have a long history of use in the diets of Pacific and Asian cultures compared to those of Europe, and the Canadian and US Maritimes. These seaweeds include red algae in Japanese and Korean cuisine ['Nori' or 'Kim', 'Laver' (*Porphyra tenera*)] or European/North American diets ['Dulse' (*Palmaria palmata*)]; brown kelps in Japanese cuisine ['Hijiki' (*Hijikia fusiformis*), 'Wakame' (Sea Mustard, *Undaria pinnatifida*), 'Makonbu' (Sea Tangle, *Laminaria japonica*)] or Chinese cuisine ['Hai dai' (*Laminaria* sp.)]; green algae in Hawaiian cuisine ['Limu palahalaha' (*Ulva* sp.)]. Within the traditional Japanese diet, seaweeds are commonly used as sushi wrappings, seasonings, condiments and vegetables and can thus constitute between 10% and 25% of food intake by most Japanese (Skibola, 2004; Teas, 1981). On the other hand, Japanese migrants in Hawaii and populations of most Western (i.e. North American and European) cultures have very low to zero seaweed intakes (Teas, 1981). When considered in combination with international diet-related chronic disease incidences, a significant environmental (i.e. dietary) difference between populations varying in seaweed consumption is revealed. For example, breast cancer rates are 42.2 and 13.1 one yr prevalence cases/100,000 in Japan and China vs 125.9 and 106.2 cases in North America (NA) and Europe; and prostate cancer rates are 10.4 and 0.7 one yr prevalence cases in Japan and China vs 117.2 and 53.1 cases in NA and Europe (Pisani et al., 2002).

The epidemiological data are supported by rodent model studies demonstrating protective effects of dietary

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0278-6915/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2006.02.002

kelps and other red and green algae against mammary (Funahashi et al., 2001; Yamamoto et al., 1987; Teas et al., 1984), intestinal (Lee and Sung, 2003; Yamamoto and Maruyama, 1985) and skin carcinogenesis (Yamamoto et al., 1986; Higashi-Okai et al., 1999). Moreover, cell culture and cell-free studies have begun to elucidate the mechanisms underlying the potential anticarcinogenic effects of seaweed constituents, such as the antimutagenicity of kelp and red algal extracts against breast and colon cancer inducers (Reddy et al., 1984) as well as aflatoxin B_1 and *N*-methyl-*N*′-nitro-*N*-nitroguanidine (Cho et al., 1997); inhibition of hyaluronidase activity (Shibata et al., 2002) and anti-inflammatory and -proliferative activities by a variety of red algal (Bergé et al., 2002) and kelp extracts (Ellouali et al., 1993). An algal antioxidant-mediated mechanism was hypothesized as a contributing factor in the inhibition of mammary carcinogenesis by dietary kelp in the presence of enhanced antioxidant enzyme activity and reduced lipid peroxides in livers of treated rats (Maruyama et al., 1991). Moreover, antioxidant and/or antimutagenic effects of dietary seaweeds have been observed in rodent model studies of colon and skin carcinogenesis, wherein treated animals exhibited suppression of tumor initiation (Lee and Sung, 2003; Higashi-Okai et al., 1999). Indeed, seaweeds are noted to contain not only labile antioxidants (i.e. ascorbate, glutathione) when fresh (Morgan et al., 1980; Indergaard and Minsaas, 1991; Kakinuma et al., 2001; Burritt et al., 2002), but also, more stable molecules such as carotenoids (Morgan et al., 1980; Okai et al., 1996; Yan et al., 1999), mycosporine-like amino acids (Nakayama et al., 1999) and a variety of polyphenols (e.g. catechins, phlorotannins; Nakamura et al., 1996; Yoshie et al., 2000).

We recently reported that dulse extracts were not only effective hydroxyl and stable free radical scavengers, but also inhibitors of lipid oxidation and cell proliferation in vitro (Yuan et al., 2005a,b). The antioxidant activity of *P. palmata* extracts may be associated with the presence of a unique class of secondary metabolites, the mycosporine-like amino acids (MAAs) which function as UV-absorbing sunscreen molecules in the *Rhodophyta* (Yuan et al., 2005b; Karsten and Wiencke, 1999). The MAAs, such as mycosporine-glycine, palythine and palythene, consist of glycine residue and hydroxylated sidechain functions on an aromatic methyl ester core. Algal tissue levels of these metabolites have been reported to have a positive correlation with UV exposure during growth (Karsten and Wiencke, 1999). It is noteworthy that UV sunscreen protection is not unique to the *Rhodophyta* however, as *Phaeophyceae*, such as the *Laminariales*, are known to synthesize UV-inducible polyphenols, i.e. phlorotannins, which absorb in the UVB range (280–320 nm; Swanson and Druehl, 2002). Moreover, kelp tissue levels of phlorotannins have been reported to increase during short-term exposure of *Macrocystis integrifolia* to UVA (320–400 nm) and UVB irradiation (Swanson and Druehl, 2002). While several workers have reported antioxidant activity of phlorotannin-containing extracts from a variety of kelps from China and Spain (Jiménez-Escrig et al., 2001; Yan et al., 1996), it is not known whether kelp extracts also influence the proliferation of cancer cell lines.

The evidence outlined above suggests a protective role for edible seaweeds against oxidative stress and cell proliferation. However, there is little data elucidating the antioxidant and anticarcinogenic activities of NA algae such as the red alga, *P. palmata* (order *Palmariales*, family *Palmariaceae*) and the brown kelps, *Laminaria setchellii* (*Laminariales*, *Laminariaceae*), *M. integrifolia* and *Nereocystis leutkeana* (*Laminariales*, *Lessionaceae*). These edible seaweed varieties are harvested and packaged for retail sale from the East (Grand Manan Island, New Brunswick; *P. palmata*) and West Coasts of Canada (Vancouver Island, British Columbia; all three kelps). Therefore, the objectives of the present study were to evaluate the antioxidant potential of extracts from these algae, and to assess the effect of these extracts on epithelial cancer cell (HeLa cells) proliferation in vitro.

2. Materials and methods

2.1. Materials

Certified organic (Organic Crop Improvement Association International) dulse (*P. palmata*) harvested in Spring 2001 was provided by Atlantic Mariculture Ltd. (Dark Harbour, Grand Manan Island, New Brunswick, Canada). Several kelps (*L. setchellii*, *M. integrifolia* and *N. leutkeana*) were purchased from Canadian Kelp Resources (Bamfield, Vancouver Island, BC). HeLa cells were obtained from American Type Culture Collection (ATCC CCL-2, Manassas, VA). Solvents (1-butanol, ethyl acetate, hexane, methanol, ethanol and chloroform) were purchased from Fisher Scientific (Mississauga, ON). L-Ascorbic acid, gallic acid, Folin–Ciocalteau's phenol reagent and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma–Aldrich Canada (Oakville, ON). Cell culture medium components (Minimum essential medium (MEM), L-glutamine, sodium bicarbonate, non-essential amino acids, sodium pyruvate, foetal bovine serum (FBS) and phosphate buffered saline (PBS)) were purchased from Invitrogen Corporation (Burlington, 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 seaweed extracts

Two-hundred-and-fifty grams ground, freeze-dried samples of each sun-dried seaweed were extracted with 3 L methanol overnight 3 × at room temperature, filtered and concentrated down to approx. 200 mL by rotary evaporation (Buchi R-200 V, Brinkmann Instruments, Mississauga, ON), 40 °C, as described by Yuan et al. (2005a). After transfer to a separatory funnel (1 L volume), the concentrated extracts were washed with an equal volume of hexane twice. After separation of the phases at room temperature, the lower methanol phase was extracted with 100 mL H_2O + 150 mL ethyl acetate. The lower H_2O–methanol layer was then extracted with 200 mL 1-butanol in another separatory funnel and the upper butanol layer removed and concentrated by rotary evaporation, 50–55 °C, to obtain a light brown residual powder. The seaweed extracts were solubilized in 21.7 mM ethanol for use in the assays.

2.3. Reducing activity

The reducing activities of the seaweed extracts were evaluated according to Yen and Chen (1995) with modifications (Yuan et al., 2005a). L-Ascorbic acid was prepared in degassed H_2O for use as the standard to quantify reducing activity. Sample absorbances were read at 700 nm. Reducing activities of the seaweed extracts were expressed as ascorbic acid equivalents from the ascorbic acid calibration curve. Each seaweed extract was assayed in quadruplicate.

2.4. Total polyphenols

The polyphenol content of each seaweed extract was quantified according to the method of Taga et al. (1984) as modified by Yuan et al. (2005a). Sample absorbances were read at 720 nm. Gallic acid was used as the standard for a calibration curve; the total polyphenol contents of the seaweed extracts were expressed as gallic acid equivalents. Each seaweed extract was assayed in quadruplicate.

2.5. Cell proliferation studies

HeLa cells were grown in 75 cm^2 flasks in MEM with 2 mM L-glutamine, 17.8 mM $NaHCO_3$, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate and 10% FBS. Cells were seeded into 96-well plates at a density of 5×10^3 per well and allowed to attach overnight in 300 μL medium incubated at 37 °C, 5% CO_2.

The seaweed extracts in 21.7 mM ethanol were each sonicated (38.5–40.5 kHz; VWR Ultrasonic bath model 75HT, VWR International, Mississauga, ON) for 30 min and sterile-filtered prior to addition to plated cells. Seaweed extracts were added at a final concentration of 0.5, 1, 2 or 5 mg/mL of medium, and the cells left to incubate in the seaweed extract-containing medium for 72 h at 37 °C and 5% CO_2 (Yuan et al., 2005b). A set of solvent controls (21.7 mM ethanol) were included in each microtitre plate. After incubation, traces of seaweed extract were removed by washing the cells twice with 200 μL PBS and applying 100 μL of fresh medium plus 10 μL of 12 mM MTT dissolved in PBS to determine the effects of the algal extracts on cell proliferation (Mosmann, 1983). Cells were then incubated for 4 h at 37 °C, 5% CO_2. To solubilize the product of MTT cleavage, 100 μL of isopropanol containing 0.04 N HCl was added to each well and thoroughly mixed using a multichannel pipettor. Within 1 h of HCl-isopropanol addition, the absorbance at 570 nm, with a reference wavelength of 630 nm, was read using a Multiskan Ascent Microplate Reader (Thermo Labsystems, Franklin, MA). The percent inhibition of cell proliferation was calculated as follows:

$$\%\ \text{Inhibition} = \frac{(\text{Abs. 570 nm Control} - \text{Abs. 570 nm sample})}{\text{Abs. 570 nm Control}} \times 100$$

Each concentration of the respective algal extracts was assayed in triplicate.

It is unlikely that the ethanol contents of the dulse and kelp extracts were influenced by the sonication treatment above, since not only is ethanol considered an extremely weak hydrogen donor and acceptor which will not ionize in solution to yield ^{-}OH or H^{+} ions, but also that ethanol solutions have been reported to be stable to prolonged sonication (40 kHz, 1 wk) resulting in neither alteration to the concentration nor the generation of free radicals in solutions as analyzed by GC and electron spin resonance (Haseba et al., 1993). Similarly, the chemical profile of the dulse and kelp extracts would not be expected to be influenced by sonication, as other workers (Sališová et al., 1997; Albu et al., 2004) have reported that ethanolic extracts of antioxidant and pharmaceutically active compounds from plant materials were stable, during sonication over extended periods of time (15–45 min up to12 h) at ambient and elevated temperatures (20, 30 and 50 °C), as determined by GC and HPLC.

2.6. Statistics

All data are expressed as means ± SEM. One-way analysis of variance (ANOVA; SPSS 10.0 for Windows; SPSS Inc., Chicago, IL) was used to test for differences between different treatment concentrations as well as between seaweed species. Where differences did exist, the source of the differences at a $p \leqslant 0.05$ significance level was identified by the Student–Newman–Keuls multiple range test. The relationships between algal reducing activities or total polyphenol contents and inhibition of cell proliferation were determined using correlation coefficients (SPSS).

3. Results

Table 1 summarizes the reducing activity and total polyphenol content of the dulse and kelp extracts. The reducing activity (expressed as ascorbic acid equivalents) of the red alga, dulse extract was greater than that of any of the brown kelps (Table 1). Within the kelps, *M. integrifolia* had the greatest reducing activity, followed by *L. setchellii* and then, *N. leutkeana*. The reducing activity of *M. integrifolia* was 1.50-fold greater than that of *N. leutkeana*. The total polyphenol content (expressed as gallic acid equivalents) of the dulse extract was 3.24-fold greater than that of the *M. integrifolia* and *N. leutkeana* extracts, which were both greater in total polyphenol content than the extract from *L. setchellii* (Table 1).

The dulse and kelp extracts inhibited HeLa cell proliferation in a dose-dependent manner during the 72 h incubation period (Fig. 1). For the dulse extract, after 72 h incubation, inhibition of HeLa cell proliferation was greatest ($p < 0.001$) with the 2.0 and 5.0 mg/mL dulse extract treatments and least with the 0.5 mg/mL dose. For the *L. setchellii* extract, after 72 h incubation, inhibition of HeLa cell proliferation at 5.0 mg/mL was greater ($p = 0.002$) than with the 0.5, 1.0 and 2.0 mg/mL doses. Inhibition of HeLa cell proliferation after 72 h incubation was greater ($p < 0.001$) with the 5.0 mg/mL *M. integrifolia* extract treatment, compared to the 0.5, 1.0 and 2.0 mg/mL treatments for this kelp variety. Similarly, HeLa cell proliferation was greater ($p = 0.012$) with the 5.0 mg/mL *N. leutkeana* extract treatment compared to the 0.5, 1.0 and 2.0 mg/mL treatment levels. There were no differences in HeLa cell proliferation between the dulse and kelp algal species at the 0.5 mg/mL treatment level (Fig. 1). The dulse extract exhibited greater inhibitory effects on HeLa cell proliferation at 1 mg/mL ($p = 0.002$) and 2.0 mg/mL ($p = 0.011$) concentrations compared to the brown kelps. However, there were no differences in HeLa cell proliferation between dulse and

Table 1
Reducing activity and total polyphenol content of seaweed extracts[a]

	Reducing activity (μg ascorbic acid equivalents)	Polyphenols (μg gallic acid equivalents)
Seaweed extract		
P. palmata	4.48	12.8
L. setchellii	2.57	1.84
M. integrifolia	3.35	3.95
N. leutkeana	2.23	3.94

[a] Data are expressed as equivalents for 1 mg of each individual seaweed extract; each seaweed extract was assayed in quadruplicate for reducing activity and polyphenol content.

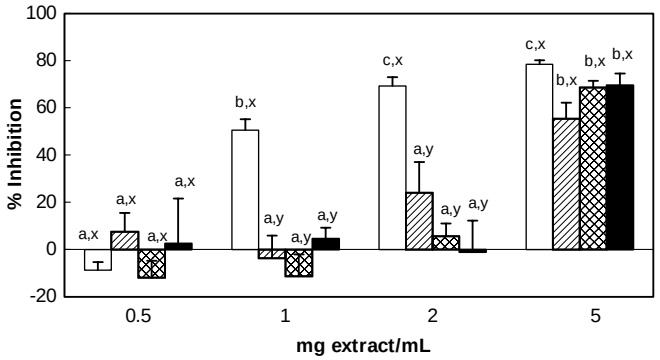

Fig. 1. Influence of seaweed extracts on inhibition of cell proliferation of HeLa cells after 72 h incubation. (□) *P. palmata*; (▨) *L. setchellii*; (▩) *M. integrifolia*; (■) *N. leutkeana*. [a,b,c] indicates a significant difference ($p < 0.05$) between concentrations of extract for an individual seaweed species; [x,y] indicates a significant difference ($p < 0.05$) between seaweed species at each concentration of extract.

kelp algal species at the highest dose of 5.0 mg/mL after 72 h incubation.

Correlation coefficient analyses showed a positive relationship between algal extract polyphenol content and inhibition of HeLa cell proliferation. The trend was strongest at the 1 mg/mL concentration of dulse and kelp extracts: $r = 0.953$, $p = 0.024$, but weaker at 2 mg/mL ($r = 0.857$, $p = 0.071$) and 5 mg/mL of dulse and kelp extracts ($r = 0.853$, $p = 0.074$). There were no significant relationships between algal extract reducing activity and inhibition of HeLa cell proliferation.

4. Discussion

The present study is the first to report on the antiproliferative activities of extracts from the edible kelp species, *L. setchellii* (Oar weed, Split laminaria), *M. integrifolia* (Macro or Giant kelp) and *N. leutkeana* (Bull kelp) using an epithelial adenocarcinoma cell line (HeLa cells) model. We extend these findings by reporting the reducing activity and total polyphenol content of 1-butanol soluble extracts of these brown kelps compared to the red alga *P. palmata* (dulse). Previous work from this laboratory indicated that 1-butanol soluble extracts from dulse were effective hydroxyl radical scavengers as well as stable free radical quenchers and inhibitors of lipid peroxidation (Yuan et al., 2005a). More recently, we reported that the antiproliferative activity of dulse extracts likely reflected the bioactivity of the polyphenol metabolites of this alga (Yuan et al., 2005b). A requirement for endogenous antioxidant capacity in algae is implicit, due to the fact that algae, as intertidal organisms, require protection against UV irradiation (Swanson and Druehl, 2002; Aguilera et al., 2002a,b; Karsten and Wiencke, 1999) and the effects of dessication from daily tidal fluctuations (Burritt et al., 2002). This antioxidant protection encompasses enzymatic defenses (e.g. superoxide dismutase (SOD); Aguilera et al., 2002a,b); bioactive molecules including L-ascorbic acid, glutathione (GSH) and carotenoids (Morgan et al., 1980; Burritt et al., 2002) as well as secondary metabolites such as the phlorotannins (e.g. phloroglucinol polymers; Yan et al., 1996) in brown kelp and the mycosporine-like amino acids in red algae (Nakayama et al., 1999).

While fresh algal specimens have been reported to contain several hydrophilic, but labile, antioxidant molecules such as L-ascorbate (Aguilera et al., 2002b; Burritt et al., 2002; Indergaard and Minsaas, 1991; Morgan et al., 1980) and GSH (Burritt et al., 2002; Kakinuma et al., 2001), sun drying and subsequent storage of algae will considerably decrease the levels of these labile antioxidants (Burritt et al., 2002; Jiménez-Escrig et al., 2001). There is also considerable variability in tissue antioxidant levels between algal orders. For example, *Rhodophyta*, such as *P. palmata and Stictosiphonia arbuscula*, have been reported to contain between 0.2 and 0.5 mg ascorbate/g wet wt (or 200 mg/100 g dry wt; Aguilera et al., 2002b; Burritt et al., 2002; Indergaard and Minsaas, 1991), whereas *Phaeophyceae*, such as *Laminariales*, contain only trace amounts to 0.17 mg ascorbate/g wet wt (or 11 mg/100 g dry wt; Aguilera et al., 2002b; Indergaard and Minsaas, 1991). Algal tissue levels of GSH are similarly variable between orders, with *Rhodophyta* containing approx. 0.22 mg GSH/g wet wt (or between 21 and 200 mg/100 g dry wt) and *Phaeophyceae* containing between 19 and 3082 mg GSH/100 g dry wt (Burritt et al., 2002; Kakinuma et al., 2001). In the present study, the 1-butanol soluble extracts from all three varieties of kelp exhibited weaker reducing activities compared to the red alga, dulse. These differences are likely attributable to not only the reduced tissue levels of L-ascorbate reported in *Phaeophyceae* versus *Rhodophyta*, but also the variable GSH levels between these algae, thereby influencing the low molecular weight reducing agents and reducing activities herein.

Another distinguishing characteristic between the *Phaeophyceae* and *Rhodophyta* which can be expected to contribute to differences in the reducing activity of the algal extracts in the present study is the polyphenol profile of these algal orders. The presence of polyphenolic functional groups was demonstrated in both the dulse and kelp extracts, with the dulse extract exhibiting between 6.96 and 3.24-fold more polyphenols than the kelps. The algal solvent extraction protocol used in the present study is noted to yield a 1-butanol soluble fraction, from an initial methanol extract, which contains flavonoid and hydroquinone glycosides including lignan derivatives such as (+)-isolariciresinol 3a-*O*-sulphate from terrestrial plant material (Zhong et al., 1997, 1998). Similarly, other workers have isolated and identified a number of polyphenolic compounds such as catechins (flavan-3-ols), flavonols and flavonol glycosides in methanol extracts of Japanese red and brown algae (Yoshie et al., 2000; Yoshie-Stark et al., 2003). Thus, while we have not yet identified the specific chemical compounds in our algal extracts, the literature can provide some valuable insights for this future work. The polyphenols of terrestrial plants (i.e. gallo- or

condensed tannins) are largely based on polymers of 4-8 linked flavan-3-ols, with some esterified to gallic acid; whereas, the polyphenols of algae such as the brown kelps (i.e. phlorotannins) are polymers of phloroglucinol (1,3,5-trihydroxybenzene) which can account for between 1% and 10% of the dry weight of these seaweeds (Ragan and Glombitza, 1986). Lipid oxidation studies have reported that phlorotannin-containing extracts from the kelps *Sargassum kjellmanianum* and *Ecklonia cava* were effective antioxidants; the high molecular weight fractions containing polymers such as dieckol, phlorofucofuroeckol and 6-6′ bieckol conferring greater protection than low molecular weight fractions containing phloroglucinol and eckol (Kim et al., 2004; Nakamura et al., 1996; Yan et al., 1996). Moreover, the stable free radical quenching activity of phloroglucinol was considerably less than that of polymers such as phlorofucofuroeckol (Kim et al., 2004). Jiménez-Escrig et al. (2001) reported that extracts from *Laminariales* exhibited not only stable free radical scavenging activity, but also ferric ion reducing activity, albeit, the reducing activity was lower than that of the red alga *Porphyra umbilicalis*. This latter evidence may relate to the low levels of free phloroglucinol in kelps (Nakamura et al., 1996; Ragan and Glombitza, 1986). Indeed, the low reducing activities and total polyphenol contents of the kelp 1-butanol soluble extracts in the present study likely reflected the oxidation and polymerization of phlorotannins which form brown-coloured high molecular weight compounds such as phycophaein (Ragan and Glombitza, 1986). The brown to black colouration of kelps is associated with phlorotannins and their oxidation products. Thus, it was not surprising that the polyphenol contents of the 1-butanol soluble extracts from the three kelps studied herein were quite low, given that during the isolation and purification of phlorotannins from kelps, Ragan and Glombitza (1986) recommend that only fresh or frozen material be used, as opposed to air- or oven-dried algae. The edible kelps used in the present study had been sun-dried prior to packaging for sale as a health-food ingredient and snack, thus air-oxidation had likely taken place with our kelp samples. This evidence suggests that the oxidation of phlorotannins, as well as the tendency of these pigments to bind to tissue protein, likely reduced the total polyphenols extractable from these edible kelps in the present study.

The 1-butanol soluble extracts of *P. palmata* likely contain the alcohol- and water-soluble MAAs which function as UV-absorbing sunscreens identified in *Rhodophyta* such as *P. tenera* (Takano et al., 1979), *P. yezoensis* (Nakayama et al., 1999) as well as *P. palmata* harvested in Norway (Karsten and Wiencke, 1999; Aguilera et al., 2002a) and Japan (Sekikawa et al., 1986). The MAAs are synthesized via the shikimic acid pathway from 3-dehydroquinic acid and 4-deoxygadusol intermediates; the latter compound has also been reported to possess antioxidant activity (Dunlap et al., 1997). Moreover, the shikimic acid pathway is also responsible for the synthesis of various phenolic acids as products of aromatic amino acid (i.e. phenylalanine and tyrosine) metabolism: quinic acid is initially converted to 3-dehydroquinic acid which undergoes elimination of one water molecule to yield 3-dehydroshikimic acid and finally protocatechuic acid by a 1,2 elimination of a proton and C-5 hydroxyl group. It is noteworthy that phenolic acids such as caffeic acid have been reported in *Rhodophyta* such as *P. yezoensis* and *Chondrus* sp. but rarely in *Phaeophyceae* such as *U. pinnatifida* (Yoshie-Stark et al., 2003). Thus, the polyphenol profile of the 1-butanol soluble extract of *P. palmata* in the present study likely represents a mixture of constituents including shikimic acid pathway derivatives such as MAAs and phenolic acids.

The dulse and kelp extracts exhibited dose-dependent inhibition against the proliferation of the human cervical epithelial adenocarcinoma HeLa cell line in the present study. The EC_{50} values calculated for *P. palmata*, *L. setchellii*, *M. integrifolia* and *N. leutkeana* extract inhibition of HeLa cell proliferation after 72 h incubation were 2.30, 4.53, 4.11 and 4.10 mg/mL, respectively. Moreover, the antiproliferative effects of the dulse and kelp extracts were positively correlated with the total polyphenol contents of the extracts. Thus, it is noteworthy that flavan-3-ols, as a class of polyphenols, have been reported to inhibit telomerase activity in cell free studies as well as leukemia and HT-29 colon adenocarcinoma cells (Naasani et al., 1998). Telomerase reverse transcriptase activity is required for the maintenance of chromosomal telomeres to prevent eventual cell death, and is present in 80–90% of human cancers but absent from normal cells. Moreover, the effect of the flavan-3-ols was dose-dependent and appeared to be due to competitive inhibition at the telomerase substrate binding site (Naasani et al., 1998). Cho et al. (1997) have also reported that methanol extracts of various kelps including *L. japonica* and the red algae *P. tenera* and *Gelidium amansii* exhibited dose-dependent inhibition of the growth of human gastric (AGS) and HT-29 colon cancer cells. An in vivo study by Funahashi et al. (2001) reported that a cold water extract of *L. japonica*, administered in place of drinking water to DMBA-treated rats, reduced the incidence of mammary tumours. Moreover, the *L. japonica* water extract induced apoptosis in several human breast cancer cell lines, potentially related to inhibition of SOD activity by iodine, or effects of other compounds such as polyphenols (Funahashi et al., 2001). A role for algal polyphenols as anticarcinogens and antiproliferative agents is further supported by antitumour promotion activity against ornithine decarboxylase induction by tumour promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in BALB/c 3T3 fibroblasts with 75–87% inhibition by *Laminariales* sp. and 92% inhibition by *P. tenera* methanol extracts (Okai et al., 1994). Similarly, in the present study, the antiproliferative effects of 1-butanol soluble extracts of the kelps *L. setchellii*, *M. integrifolia* and *N. leutkeana* ranged between 55% and 69% inhibition at the highest dose (5 mg/mL), which were lower than the 78% inhibition for the *P. palmata* extract. Thus, the greater total polyphenol

content of the red algal *P. palmata* extract in the present study compared to those of the brown kelps, *L. setchellii*, *M. integrifolia* and *N. leutkeana*, likely contributed to the greater antiproliferative effects against HeLa cells herein, potentially involving the mechanisms discussed above.

In conclusion, the greater reducing activity of the dulse extract reflected the generally greater L-ascorbate content of *Rhodophyta* compared to *Phaeophyceae*. The lower total polyphenol contents of the kelp extracts were associated with the oxidation and polymerization of the phlorotannins in these sun-dried algae, which in turn influenced the proliferation of HeLa cells herein. The greater antiproliferative effects of the dulse extract compared to the kelp extracts, likely reflects the quantity as the well as the bioactivity of shikimic acid pathway derived polyphenol secondary metabolites in the former, and the monomeric and polymeric phlorotannins in the latter. Further work will identify the antioxidant and antiproliferative molecules in Canadian dulse and kelp extracts to further our understanding of the bioactivity of these under utilised seaweeds for application to the North American diet. Moreover, studies of the bioactivity of algal polyphenols such as the MAAs or phlorotannins will contribute to the body of knowledge about marine-derived compounds and their use in the processed and/or functional food industries.

Acknowledgements

This research was funded by a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant. The authors thank M. Carrington and M. De Jesus for their technical support.

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