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Antioxidant Potential of Two Red Seaweeds from the Brazilian Coasts

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ABSTRACT: In this work, in vitro antioxidant activity of two Brazilian red seaweeds, *Gracilaria birdiae* and *Gracilaria cornea*, was characterized. The total phenolic content, the radical-scavenging activity and the antioxidant activity were determined in two solvent extracts of the algae. Liquid chromatography−mass spectrometry (LC−MS/MS) allowed identification of important antioxidant compounds. The ethanol extract of *G. birdiae* was found to have the highest value of total phenolic content: 1.13 mg of gallic acid equiv (GAE)/g of extract. The radical-scavenging activity of *G. birdiae* and *G. cornea* extracts has been evaluated at different extract concentrations; the IC_{50} values of ethanolic extracts of *G. cornea* and *G. birdiae* were 0.77 and 0.76 mg mL^{-1}, respectively, while for methanolic extracts, the IC_{50} values of *G. cornea* and *G. birdiae* were 0.86 and 0.76 mg mL^{-1}, respectively. The antioxidant activities of these two seaweeds' extracts as assessed by the β-carotene−linoleic acid assay were equally high, achieving values of β-carotene oxidation inhibition of up to 40%. Finally, in the methanolic extracts, LC−MS/MS allowed identification in both algae of two important antioxidants: apigenin and gallic acid.

KEYWORDS: antioxidant, seaweeds, phenolic content

INTRODUCTION

Antioxidants have multiple functions in biological systems, including the defense against oxidative damage and participation in the major signaling pathways of cells. One major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species.[1] Free radicals are responsible for aging, and their presence in excess constitutes the cause of various human diseases. Different studies show that antioxidant substances which scavenge free radicals play an important role in the prevention of free radical-induced diseases.[2]

Several synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and butylated hydroxyquinone (TBHQ) are commercially available and currently used; however, concerns on their safety and toxicity are hindering their use by the food industry.[1,3] Therefore, research on alternative antioxidants from natural origins has drawn increasing attention, particularly in the most recent years during which the consumer's awareness on food quality and safety issues has improved very significantly. Great effort has been focused on e.g. medicinal plants for the extraction of natural and low-cost antioxidants that can replace synthetic additives.[4]

Many researchers have reported the existence of various types of antioxidants in different kinds of higher plants.[5,6] More recently, reports have revealed seaweeds to be a rich source of antioxidant compounds.[7−9]

The seaweeds are considered a traditional diet in different regions of the globe, particularly in Asian countries. Their chemical composition shows that they are foods low in calories, with high concentrations of minerals, vitamins and protein, rich in fiber and with relatively high concentrations of polyunsaturated fatty acids and different antioxidants.[10−12]

During their life cycle, algae are exposed to large amounts of light and high concentrations of oxygen; this combination favors the generation of free radicals, as well as other powerful oxidizers. It is suggested that the absence of oxidative damage in the structural components of the algae and their stability against adverse conditions are due to the presence of antioxidants.[13]

Phenolic compounds such as flavonoids, phenolic acids, and tannins are considered to be major contributors to the antioxidant capacity of plants. Phenolics or polyphenols have received considerable attention because of their physiological functions, including antioxidant, antimutagenic and antitumor activities.[14] Phenolic compounds are commonly found in edible brown, green and red seaweeds, whose antioxidant properties have been correlated to their phenolic content.[10,15]

Brazilian coasts and seas, with their vast extension, are very rich in unexploited or underexploited biological resources. Some local communities are now being supported by governmental and nongovernmental projects that aim at providing them with means of improving their life standards while practicing a sustainable exploitation of such resources. Those means include the cultivation and collection of algae in the northeast coast of the country. Such algae are already commercialized, but the aim is to improve their added value by giving them a different use. In that line, this work aims at investigating the antioxidant properties of two different red seaweed extracts from *G. birdiae* and *G. cornea*, collected in the northeast Atlantic coast of Brazil.

MATERIALS AND METHODS

Marine Algae Collection and Preparation of the Extracts.

Specimens of red seaweeds *Gracilaria birdiae* (*Gb*) were cultivated and collected on the coast of Brazil (Fleixeiras Beach, Trairi, Ceará).[16]

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Specimens of red seaweeds *Gracilaria cornea* (*Gc*) were collected on the coast of Brazil (Pacheco Beach, Caucasia, Ceará).

Upon collection, both algae were cleaned of epiphytes, rinsed with tap water and stored at −20 °C before utilization. A sample of each of the algae (10 g) was extracted with 100 mL of either ethanol (purity 99.8%, Riedel-de Haën, Germany) or methanol (purity 99.8%, Riedel-de Haën, Germany) using a Soxhlet extractor for 6 h (20 °C). After 6 h, the mixtures were filtered, the extracts were concentrated in a rotary evaporator at 40 °C and the extraction yields (%) were determined. The extracts were stored at −20 °C until further use. All the experiments were conducted in triplicate.

Antioxidant Activity. *DPPH Radical-Scavenging System.* The DPPH-scavenging potential of different extracts was measured, based on the scavenging ability of stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals by *Gb* and *Gc* antioxidants. The ability of extracts to scavenge DPPH radicals was determined according to Blois.[17] Briefly, 1 mL of methanolic solution of DPPH (1 mM) (Sigma-Aldrich, USA) was mixed with 1 mL of extract solution (containing 0.0001−5.0 mg mL^{-1} of dried extract). The mixture was then vortexed vigorously (1 min) and left for 30 min at 20 °C in the dark. A control sample was prepared following the same procedure, but using 1 mL of distilled water instead of 1 mL of extract solution. The absorbance was measured at 517 nm, and the results were expressed in terms of % DPPH-scavenging activity relative to the control sample. All experiments were carried out in triplicate.

β-Carotene−Linoleic Acid Assay. The antioxidant activity of extracts was also evaluated by the β-carotene−linoleic model system as described by Shon et al.[6] with some modifications. First, β-carotene (0.2 mg) (Sigma-Aldrich, USA) was dissolved in 1.0 mL of chloroform (Riedel-de Haën, Germany); 0.02 mL of linoleic acid (Fluka, United Kingdom) and 0.2 mL of Tween 80 (Acrõs organics, USA) was subsequently added, and the mixture was left standing at 20 °C for 15 min. After evaporation of the chloroform in a rotary evaporator at 40 °C, 50 mL of oxygen-saturated distilled water at 25 °C was added and the mixture was vortexed vigorously (1 min) to form an emulsion (β-carotene−linoleic acid emulsion). The necessary wells of a 96-well microtiter plate (polystyrene) were charged with 50 μL of each sample and 200 μL of the emulsion per well. The micro plate was placed on a horizontal shaker up 100 rpm (during 1 min) and incubated at 50 °C. The initial absorbance (A_0) was immediately measured at 470 nm using an Elisa (Bio-Tek, USA). A second absorbance value (A_{120}) was recorded 120 min after incubation at 50 °C. A blank, without β-carotene, was also prepared and charged in the microtiter plate for background subtraction. The results were expressed in terms of the percentage antioxidant activity relative to the control sample.[6] All experiments were carried out in triplicate.

Analysis of Phenolic Content. *Total Phenolic Concentration.* The concentration of total phenolic in the extracts was measured by an Elisa (Bio-Tek, USA), based on a colorimetric oxidation/reduction reaction, as described by Skerget et al.[18] To 0.5 mL of diluted extract (20 mg in 10 mL of distilled water), 2.5 mL of Folin−Ciocalteau (Panreac, Spain) reagent (diluted 1:10 with distilled water) and 2 mL of Na_2CO_3 (75 $g \cdot L^{-1}$) (Panreac, Spain) were added. This solution was incubated for 30 min at 25 °C and then cooled. The absorbance was measured at 760 nm. A blank solution was also prepared, where 0.5 mL of distilled water replaced the 0.5 mL of diluted extract, and the procedure was repeated for absorbance measurements.

Identification of Phenolic Compounds Using LC−MS/MS. LC Analysis. LC analysis was carried out on a Prostar 210 LC pump (Varian, CA, USA). Solvent A was 100% methanol and solvent B was 100% water with 0.1% formic acid (w/v). Solvents were filtered prior to use through an FA 0.22 μm filter (Millipore, USA). The mobile phase was prepared daily, degassed using an in-line degasser (MetaChem) and delivered at a flow rate of 0.4 mL/min at isocratic mode 50% B and 50% A. Samples were injected on a reversed phase−phase column of 25 cm length with 5 mm C18 coated particles (Supelcosil LC-18, Supelco Inc., Bellefonte, PA, USA) to perform the chromatographic separation, using a 20 mL loop.

Table 1. Yield of Total Extract (Expressed as % (w/w) of Seaweed on a Dry Weight Basis) of *Gracilaria birdiae* and *Gracilaria cornea*

	yield (%)	
seaweed	ethanol extract[a]	methanol extract[a]
Gracilaria birdiae	4.97 ± 0.52 a	4.87 ± 0.66 a
Gracilaria cornea	4.92 ± 0.61 a	4.72 ± 0.57 a

[a] Different letters indicate a statistically significant difference (Tukey test $p < 0.05$).

Mass Spectra Acquisition. The operating parameters of the ESI source were all optimized with regard to maximum signal intensity as follows: the nebulizing gas pressure was 40 psi; the drying gas pressure was 20 psi; the drying gas temperature was 200 °C; the housing temperature was 55 °C; the needle voltage was −4.3 kV at negative ion mode and 5.5 kV at positive ion mode; the shield voltage was −550 V at negative ion mode and 400 V at positive ion mode.

The experimental results from LC−MS/MS show that gallic acid and apigenin were easily deprotonated at the electrospray ion source at 20 eV under negative mode to form negative molecular ions $[M - H]^-$ of 169 and 269 Da, respectively. The collision energy used in the MS/MS detector was set at 15 eV, originating 125 mz and 151 mz, respectively, as product ions. Gallic acid was identified using gallic acid standard (Sigma-Aldrich, USA) on the 169.0 → 125.0 mz transition, and the apigenin was tentatively identified on the 179.0 → 135.0 mz transition as reported in Luján et al.[19]

Statistical Analyses. The Tukey test (α = 0.05) was used to determine any significance of differences between specific means (SigmaStat, trial version, 2003, USA).

RESULTS AND DISCUSSION

Extraction and Yield. Solvent extraction is the most commonly used method in sample preparations from plants. The total yield values of both ethanolic and methanolic extracts of the two red seaweeds under analysis are given in Table 1. Ethanolic and methanolic extractions led to similar extraction yields in both *G. birdiae* and *G. cornea* ($p < 0.05$) seaweeds. This is particularly relevant in the case of ethanol, once the use of this solvent is allowed in the food industry (while methanol is not). These values are within the range of values found in the literature. Ganesan et al.[15] observed yields of 5.01, 3.98 and 2.85% for methanolic extracts of red seaweeds *Acanthophora spicifera*, *Euchema kappaphycus* and *Gracilaria edulis*, respectively. Chandini et al.[20] observed similar values for Indian brown seaweed methanolic extracts: *Padina tetrastomatica* (12.31%), *Turbinaria conoides* (5.76%) and *Sargassum marginatum* (5.45%).

Antioxidant Activity. *DPPH Radical-Scavenging System.* This assay was used to test the ability of the antioxidant compounds present in the algal extracts to function as proton radical scavengers or hydrogen donors.[21] The inhibition of radical-scavenging activity by *G. birdiae* and *G. cornea* ethanolic and methanolic extracts was evaluated at different extract concentrations, and the results are illustrated in Figure 1a expressed as percentage reduction of the initial DPPH absorption by the tested extract. The results indicate that the ethanolic extracts of *Gb* exhibit the best performance showing a high scavenging

activity (close to 60%, at concentration of 5 mg mL^{-1}) followed by *Gc* extracts with the same solvent. Although the results indicate that these values are lower than those obtained with BHT (a strong chemical radical-scavenger), at a similar concentration, the scavenging activities are still very significant considering that this are extracts derived from natural resources.

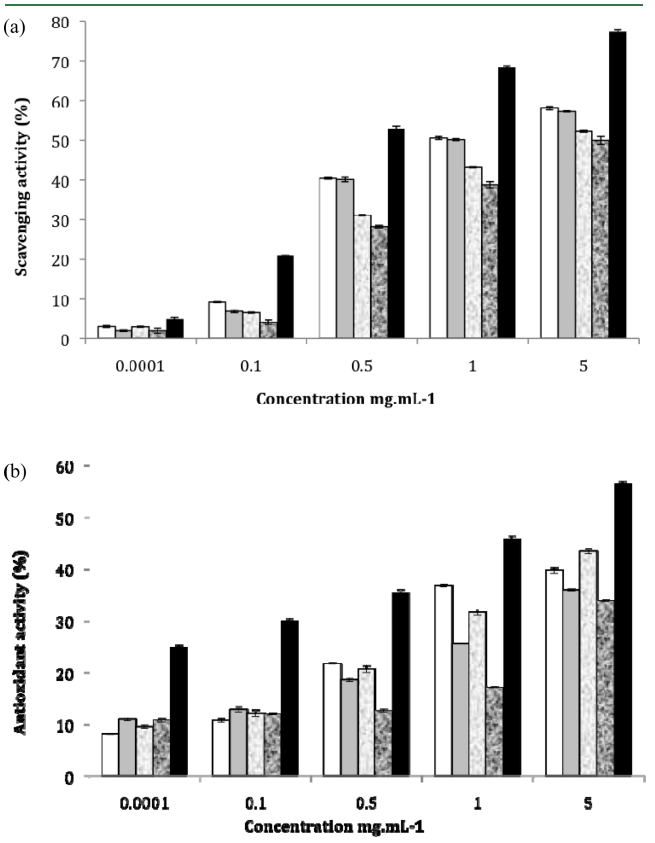

Figure 1. Antioxidant and scavenging activities of different solvent extracts of *Gracilaria birdiae* (*Gb*) and *Gracilaria cornea* (*Gc*) determined by DPPH radical-scavenging activity (a) and β-carotene−linoleic acid assay (b) (white bars) *Gb*-ethanol; (gray bars) *Gc*-ethanol; (light gray mottled bars) *Gb*-methanol; (dark gray mottled bars) *Gc*-methanol; (black bars) BHT. Data are expressed as mean ± standard deviation ($n = 3$).

Table 2 shows the values of IC_{50} for the methanolic and ethanolic extracts of the seaweeds against the DPPH radical. The IC_{50} values for the ethanolic extracts of *Gc* and *Gb* were 0.77 and 0.76 mg mL^{-1}, respectively. For methanolic extracts, the IC_{50} values of *Gb* and *Gc* were 0.76 and 0.86 mg mL^{-1} respectively. For comparison, the IC_{50} of BHT was 0.48 mg mL^{-1}.

Siriwardhana et al.[22] and Lu and Foo[23] reported a high correlation between DPPH radical-scavenging activity and total polyphenolics. Components such as low molecular weight polysaccharides, pigments, proteins or peptides, also influence the antioxidant activity.[22] Devi et al.[24] state that it is possible that the antioxidant activity of seaweed extracts can be the result of the existence of phenolic compounds.

Nevertheless, the presented results indicate that these seaweeds, widely distributed in Brazil, exhibited similar scavenging activities than closely related seaweeds used in other studies.[2] The extracts of *Gb* and *Gc* showed a better radical-scavenging activity than did the ethanol and methanol extracts of *Kappaphycus alvarezzi* with IC_{50} values of 3.03 and 4.28 mg mL^{-1}, respectively.[1] Especially when compared with the values of IC_{50} for many other species and extraction procedures/solvents (see Table 2), the IC_{50} values found for *Gc* and *Gb* are very interesting and point at the possibility of using these extracts as antioxidants e.g. in the food industry.

β-Carotene−Linoleic Acid Assay. The bleaching mechanism of β-carotene is a free radical mediated event resulting in the formation of hydroperoxides from linoleic acid. In the absence of an antioxidant, β-carotene will undergo rapid discoloration.[25] 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. Therefore, the orange colored chromophore of β-carotene would be degraded and the results could 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.[26] Hydroperoxides formed in this system will be neutralized by the antioxidants from the extracts. Therefore, the degradation rate of β-carotene depends on the

Table 2. Comparison of IC_{50} DPPH Scavenging Activity of Different Algal Extracts

seaweed	solvent	IC_{50} DPPH[a] (mg mL^{-1})	refs
Kappaphycus alvarezzi	ethanol	3.03	Kumar et al.[1]
Kappaphycus alvarezzi	methanol	4.28	Kumar et al.[1]
Padina antillarum	50% methanol (v/v)	0.34	Chew et al.[25]
Caulerpa racemosa	50% methanol (v/v)	14.30	Chew et al.[25]
Kappaphycus alvarezzi	50% methanol (v/v)	11.80	Chew et al.[25]
Porphyra sp	ethanol	0.67	Ismail and Hong[2]
Laminaria sp	ethanol	0.86	Ismail and Hong[2]
Undaria sp	ethanol	0.42	Ismail and Hong[2]
Hijikia sp	ethanol	0.47	Ismail and Hong[2]
Ecklonia cava	70% methanol (v/v)	0.02	Senevirathne et al.[33]
Gracilaria birdiae	ethanol	0.76 ± 0.084 a	this work
Gracilaria birdiae	methanol	0.76 ± 0.072 a	this work
Gracilaria cornea	ethanol	0.77 ± 0.091 a	this work
Gracilaria cornea	methanol	0.86 ± 0.074 a	this work

[a] Different letters indicate a statistically significant difference (Tukey test $p < 0.05$).

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antioxidant activity of the extracts. In our work, two solvents (ethanol and methanol) were used to prepare the *G. birdiae* and *G. cornea* extracts. In the linoleic emulsion system, the oxidation of β-carotene was effectively inhibited by all extracts of *G. birdiae* and *G. cornea* in different concentrations (Figure 1b), achieving values of β-carotene oxidation inhibition of up to 40%. Again, these are very good values when compared to other systems described in the literature.

Total Phenolics: Quantification and Characterization. Devi et al.[24] state that it is possible that the antioxidant activity of seaweed extracts can be the result of the existence of phenolic compounds. The contents of total phenolics in ethanol and methanol extracts of the red seaweeds *G. birdiae* and *G. cornea* are presented in Table 3. For both algae, no significant difference was observed for methanolic and ethanolic extracts. However, it was possible to determine a significantly higher total phenolics content for *G. birdiae* than that found for *G. cornea*.

LC−MS/MS determination allowed identifying the presence of gallic acid (by comparison with pure standard—results not shown—as mentioned in Materials and Methods), a well-known phenolic antioxidant compound, in both *G. birdiae* and *G. cornea* methanolic extracts (Figure 2a). Although a precise quantification was out of the scope of the present work, a rough comparison of peak heights (peak indentified by the arrows in Figure 2a) shows that the amount of gallic acid in *G. birdiae* is *ca.* 5-fold that in *G. cornea*.

Moreover, it was also possible to tentatively identify apigenin in both algae extracts (see Materials and Methods). This phenolic compound is a nonmutagenic bioflavonoid, playing an important role in cancer prevention and treatment.[27] Figure 2b shows apigenin peaks (identified by the arrows) in methanolic extracts of *G. birdiae* and *G. cornea*. Again, although no precise quantification was performed, a comparison of peak heights allows concluding that *G. birdiae* extracts have *ca.* twice the amount of apigenin as *G. cornea* extracts.

A series of polyphenolic compounds such as catechins and flavonols have been identified from methanol extracts of red and brown algae.[28−30] However, the identification of apigenin in seaweeds is a novelty and indicates that these algae may be a new source of phenolic antioxidants and potential candidates for the development of a prophylactic agent of biological origin.

Correlation between Phenolic Content and Antioxidant Activity in *G. birdiae* and *G. cornea*. Literature reports on a correlation between phenolic content and antioxidant activity are not new.[22−24,31] The antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors, and free radical quenchers; phenolics can also act as metal chelators therefore preventing the catalytic function of metals in the process of initiating radicals.[32] However, components such as low molecular weight polysaccharides, pigments, proteins or peptides can also influence the antioxidant activity.[22]

To investigate if the antioxidant activity of the studied algae extracts was due to the presence of phenolic compounds, correlations were established between phenolic content and antioxidant activities (DPPH and β-carotene). Figure 3 shows a linear relationship between antioxidant activity and total phenolics content. This confirms literature reports and may indicate that these compounds are responsible for the antioxidant activity or at least are involved in the reactions leading to such effect.

Many synthetic antioxidants have shown toxic and mutagenic effects, which have shifted attention toward naturally occurring antioxidants. A great number of naturally occurring substances like seaweeds have been recognized to have antioxidant abilities. In the present work, ethanolic and methanolic extracts of *G. birdiae* and *G. cornea* exhibited excellent scavenging effects as evaluated by DPPH assay and β-carotene linoleic acid assay, while also demonstrating the presence of phenolic compounds in the algae. Moreover, the characterization of the phenolics present in the extracts showed the presence of gallic acid and apigenin, both valuable compounds. It can be concluded that these seaweeds can be used as a source of natural antioxidants. The

Table 3. Total Phenolic Content (mg of gallic acid equiv/g of extract) of Ethanolic and Methanolic Extracts of *Gb* and *Gc*

seaweed	solvent	total phenolics[a]
Gracilaria birdiae	ethanol	1.13 ± 0.03 a
	methanol	1.06 ± 0.07 a
Gracilaria cornea	ethanol	0.88 ± 0.03 b
	methanol	0.89 ± 0.07 b

[a] Different letters indicate a statistically significant difference (Tukey test $p < 0.05$).

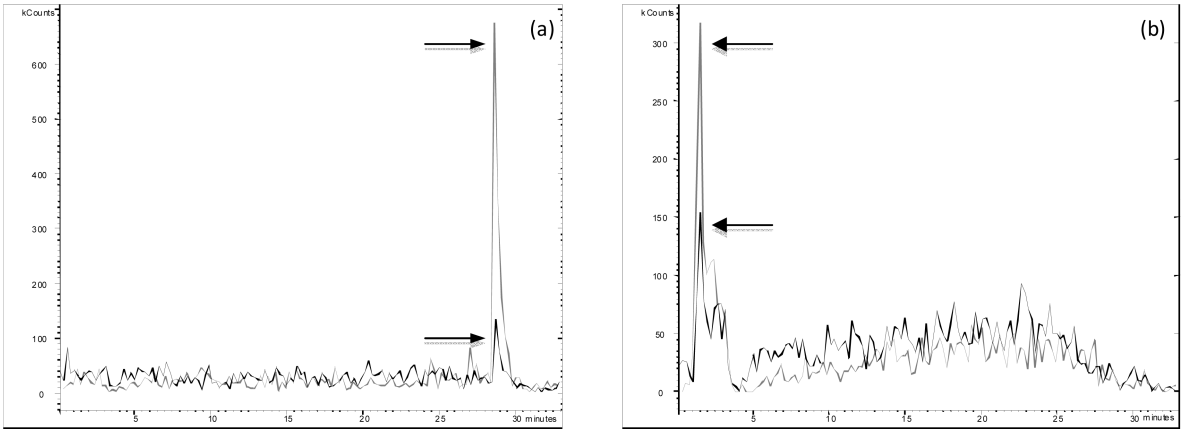

Figure 2. Spectra for methanolic extracts of *G. birdiae* (gray line) and *G. cornea* (black line). Identification of gallic acid on 169.0 → 125.0 mz transition (the arrows indicate the gallic acid peak obtained for each methanolic extract) (a) and tentative identification of apigenin on 179.0 → 135.0 mz transition (b) (the arrows indicate the apigenin peak obtained for each methanolic extract).

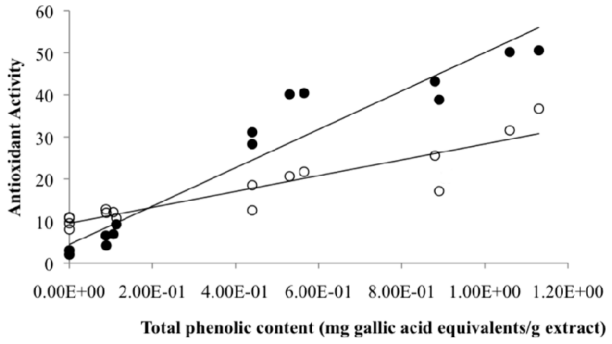

Figure 3. Correlation between phenolic content and antioxidant activity and scavenging activity of *Gracilaria birdiae* (*Gb*) and *Gracilaria cornea* (*Gc*) samples as determined by β-carotene−linoleic acid assay ($y = 45.631x + 4.4212$; $R^2 = 0.84$) (closed circles) and DPPH radical-scavenging activity ($y = 18.86x + 9.4822$; $R^2 = 0.92$) (open circles).

findings of the current report are useful for further research aiming at isolating specific phenolic compounds responsible for the antioxidant activity of *G. cornea* and *G. birdiae*, thus enabling the Brazilian communities to produce a high added-value product.

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REFERENCES

(1) Kumar, K. S.; Ganesan, K.; Subba Rao, P. V. Antioxidant potential of solvent extracts of *Kappaphycus alvarezii* (Doty) Doty—An edible seaweed. *Food Chem.* **2008**, *107*, 289–295.

(2) Ismail, A.; Hong, T. S. Antioxidant activity of selected commercial seaweeds. *Malays. J. Nutr.* **2002**, *8*, 167–177.

(3) Ito, N.; Hirose, M.; Fukushima, S.; Tsuda, H.; Shirai, T.; Tatematsu, M. Studies on antioxidants: Their carcinogenic and modifying effects on chemical carcinogenesis. *Food Chem. Toxicol.* **1986**, *24*, 1071–1082.

(4) Atmani, D.; Chaher, N.; Berboucha, M.; Ayouni, K.; Lounis, H.; Boudaoud, H.; Debbache, N.; Atmani, D. Antioxidant capacity and phenol content of selected Algerian medicinal plants. *Food Chem.* **2009**, *112*, 303–309.

(5) Larson, R. A. The antioxidants of higher plants. *Phytochemistry* **1988**, *27*, 969–978.

(6) Shon, M. Y.; Kim, T. H.; Sung, N. J. Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellius of Hymenochaetaceae) extracts. *Food Chem.* **2003**, *82*, 593–597.

(7) Keyrouz, R.; Abasq, M. L.; Le Bourvellec, C.; Blanc, N.; Audibert, L.; ArGall, E; Hauchard, D. Total phenolic contents, radical scavenging and cyclic voltammetry of seaweeds from Brittany. *Food Chem.* **2011**, *126* (3), 831–836.

(8) Wang, T.; Jónsdóttir, R.; Ólafsdótti, G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem.* **2009**, *116* (1), 240–248.

(9) Ganesan, K.; Suresh Kumar, K.; Subba Rao, P. V. Comparative assessment of antioxidant activity in three edible species of green seaweed, Enteromorpha from Okha, Northwest coast of India. *Innovative Food Sci. Emerging Technol.* **2011**, *12* (1), 73–78.

(10) Jiménez-Escrig, A.; Goni, C. I. Nutritional evaluation and physio-logical effects of edible seaweeds. *Arch. Latinoam. Nutr.* **1999**, *49* (2), 114–120.

(11) Risso, S.; Escudero, C.; Estevao, S.; De Portela, M. L.; Fajardo, M. A. Chemical composition and seasonal fluctuations of the edible green seaweed, *Monostroma undulatum*, Wittrock, from the Southern Argentina coast. *Arch. Latinoam. Nutr.* **2003**, *53* (3), 306–311.

(12) Cardozo, K. H. M.; Guaratini, T.; Barros., M. P.; Falcão, V. R.; Tonon, A. P.; Lopes, N. P.; Campos, S.; Torres, M. A.; Souza, A. O.; Colepicolo, P.; Pinto, E. Metabolites from algae with economical impact. *Comp. Biochem. Physiol., Part C: Pharmacol., Toxicol. Endocrinol.* **2007**, *146*, 60–78.

(13) Burritt, D.; Larkindale, J.; Hurd, C. L. Antioxidant metabolism in the intertidal red seaweed *Stictosiphonia arbuscula* following desiccation. *Planta* **2002**, *215* (5), 829–838.

(14) Othman, A.; Ismail, A.; Ghani, N. A.; Adenan, I. Antioxidant capacity and phenolic content of cocoa beans. *Food Chem.* **2007**, *100*, 1523–1530.

(15) Ganesan, P.; Kumar, C. S.; Bhaskar, N. Antioxidant properties of methanol extract and its solvent fractions obtained from selected Indian red seaweeds. *Bioresour. Technol.* **2008**, *99*, 2717–2723.

(16) Maciel, J. S.; Chaves, L. S.; Souza, B. W. S.; Teixeira, D. I. A.; Freitas, A. L. P.; Feitosa, P. A.;Structural characterization of cold extracted fraction of soluble sulfated polysaccharide from red seaweed *Gracilaria birdiae*. *Carbohydr. Polym.* **2008**, *71*, 559–565.

(17) Blois, M. S. Antioxidant determination by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200.

(18) Skerget, M.; Kotnik, P.; Hadolin, M.; Rizner-Hras, A.; Simonic, M.; Knez, Z Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem.* **2005**, *89*, 191–198.

(19) Luján, R. J.; Capote, F. P.; Marinas, A.; de Castro, M. D. L. Liquid chromatography/triple quadrupole tandem mass spectrometry with multiple reaction monitoring for optimal selection of transitions to evaluate nutraceuticals from olive-tree materials. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 855–864.

(20) Chandini, S. K.; Ganesan, P.; Bhaskar, N. In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chem.* **2008**, *107*, 707–713.

(21) Singh, N.; Rajini, P. S. Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem.* **2004**, *85*, 611–616.

(22) Siriwardhana, N.; Lee, K. W.; Kim, S. H.; Ha, J. W.; Jeon, Y. J. ntioxidant activity of *Hizikia fusiformis* on reactive oxygen species scavenging and lipid peroxidation inhibition. *Food Sci. Technol. Int.* **2003**, *9*, 339–347.

(23) Lu, Y. R.; Foo, L. Y. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem.* **2000**, *68*, 81–85.

(24) Devi, K. P.; Suganthy, N.; Kesika, P.; Pandian, S. K. Bioprotective properties of seaweeds: *In vitro* evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. *BMC Complementary Altern. Med.* **2008**, *8*, 38.

(25) Jayaprakasha, G. K.; Singh, R. P.; Sakariah, K. K. Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem.* **2001**, *73*, 285–290.

(26) Chew, Y, L.; Lima, Y. Y.; Omar, M.; Khoo, K. S. Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT Food Sci. Technol.* **2008**, *41*, 1067–1072.

(27) Wang, W.; VanAlstyne, P. C.; Irons, K. A.; Chen, S.; Stewart, J. W.; Birt, D. F. Individual and Interractive effects of apigenin analogs on G2/M cell-cycle arrest in Human colon carcinoma cell lines. *Nutr. Cancer* **2004**, *48* (1), 106–114.

(28) Santoso, J.; Yoshie, Y.; Suzuki, T. The distribution and profile of nutrients and catechins of some Indonesian seaweeds. *Fisheries Science* **2002**, *68* (Suppl.), 1647–1648.

(29) Yoshie, Y.; Wang, W.; Petillo, D.; Suzuki, T. Distribution of catechins in Japanese seaweeds. *Fish. Sci.* **2000**, *66* (5), 998–1000.

(30) Yoshie-Stark, Y.; Hsieh, Y. P.; Suzuki, T. Distribution of flavonoids and related compounds from seaweeds in Japan. *J. Tokyo Univ. Fish.* **2003**, *89*, 1–6.

(31) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117.

(32) Wu, X., J.; Hansen, C. Antioxidant capacity, Phenolic content, Polysaccharide content of *Lentinus edodes* grown in whey permeate-based submerged culture. *J. Food Sci.* **2008**, *73* (1), M1–M8.

(33) Senevirathne, M.; Kim, S.-H.; Siriwardhana, N.; Ha, J.-H.; Lee, K.-W.; Jeon, Y.-J. *Food Sci. Technol. Int.* **2006**, *12* (1), 27–38.