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In vitro evaluation of antioxidant activity of methanolic extracts obtained from seaweeds endemic to the coast of Ceará, Brazil

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ABSTRACT. The aim of the present research was to evaluate the antioxidant activity of six species of marine algae (*Caulerpa cupressoides*, *Ulva fasciata*, *Amansia multifida*, *Cryptonemia crenulata*, *Dictyota dichotoma* and *Sargassum vulgare*) collected in 2011 at Paracuru Beach, Ceará State, Brazil. Methanol extracts were prepared from oven-dried algae and used to measure total phenolic content (TPC) using the Folin-Ciocalteu colorimetric method. *In vitro* antioxidant activity of the algal extracts (12.5 to 100 μg mL^{-1}) was analyzed by DPPH radical scavenging, ferric-reducing antioxidant power (FRAP) and ferrous ion chelation (FIC). BHA and EDTA were used as positive controls. Based on the standard curve of gallic acid, TPC values ranged from 10.49 to 19.94 mg gallic acid equivalents (GAE) g^{-1} of extract. DPPH results of algal extracts at all concentrations tested remained between 53.96 and 64.96%, lower than the positive control (BHA). All species showed little FRAP activity (less than 0.1) when compared to BHA. FIC above 20% was detected only in the extract from *U. fasciata* (100 μg mL^{-1}), while all the others had activities below 17%. The activity of EDTA ranged from approximately 48 (12.5 μg mL^{-1}) to 98% (100 μg mL^{-1}). These results allow us to consider the algae species studied as potentially promising sources of natural antioxidants.

Keywords: algal extracts, phenolic compounds, oxidative stress, natural antioxidants.

Avaliação da atividade antioxidante *in vitro* de extratos metanólicos obtidos de algas marinhas endêmicas da costa do Ceará, Brasil

RESUMO. O presente trabalho teve como objetivo avaliar a atividade antioxidante de seis espécies de algas marinhas (*Caulerpa cupressoides*, *Ulva fasciata*, *Amansia multifida*, *Cryptonemia crenulata*, *Dictyota dichotoma* e *Sargassum vulgare*), coletadas em 2011, na Praia do Paracuru, São Gonçalo do Amarante, Ceará, Brasil. Os extratos metanólicos foram preparados com alga desidratada e neles determinados o conteúdo fenólico total (CFT) pelo método colorimétrico de Folin-Ciocalteu. A atividade antioxidante *in vitro* dos extratos algáceos (12,5 a 100 μg mL^{-1}) foram analisadas através do sequestro do radical DPPH, do poder de redução do ferro (FRAP) e da habilidade de quelação do íon ferroso (FIC). BHA e EDTA foram utilizados como controles positivos. Com base na curva padrão de ácido gálico, CFT variou de 10,49 a 19,94 mg de ácido gálico equivalente (AGE) g^{-1} por extrato. Os resultados de DPPH, em todas as concentrações testadas, ficaram entre 53,96 e 64,96% e foram inferiores aos do controle positivo (BHA). Todas as espécies estudadas apresentaram baixos valores de FRAP (< 0,1), quando comparados aos do BHA. FIC superior a 20% foi detectado apenas no extrato de *Ulva fasciata* (100 μg mL^{-1}), enquanto todos os outros tiveram atividades inferiores a 17%. A atividade do EDTA variou, aproximadamente, de 48 (12,5 μg mL^{-1}) a 98% (100 μg mL^{-1}). Estes resultados permitem considerar as espécies de macroalgas estudadas como fontes potencialmente promissoras de antioxidantes naturais.

Palavras-chave: extratos algáceos, compostos fenólicos, estresse oxidativo, antioxidantes naturais.

Introduction

Marine macroalgae (seaweeds) have a long traditional use as human food particularly in countries bordering the Pacific Ocean, such as China, Japan, Korea and the Philippines, because of their characteristic taste and nutritional value. Nutritionally, they are generally poor in fat, but rich in polyunsaturated fatty acids (PUFAs), and present low digestible carbohydrates, but high fiber content, as well as a moderate level of proteins. In addition, they contain minerals, both water-soluble and fat-soluble vitamins (Ito & Hori, 1989; Lordan, Ross, & Stanton, 2011).

Marine algae present a great taxonomic diversity. Since they live in habitats that can vary drastically,

they have developed diverse defense strategies and are capable of producing a wide variety of secondary metabolites (biologically active) (Plaza, Cifuentes, & Ibáñez, 2008, Lordan et al., 2011).

ROS are relatively unstable molecules derived from molecular oxygen by electron addition, and RNS refers to nitric oxide (generated by nitric oxide synthetases) and its derivatives, e.g., hydroxyl radical (HO^-), hydrogen peroxide (H_2O_2), nitric oxide (NO^-), dinitrogen trioxide (N_2O_3) and peroxynitrite ($ONOO^-$). Accumulation of excessive ROS/RNS can oxidize cellular components, including lipids, proteins, and DNA, leading to impaired function and, eventually, cell death (Jones, 2008; Johnson, Wilson-Delfosse, & Mieyal, 2012).

Thus, seaweeds are able to generate the necessary compounds to protect themselves from external stressors, such as pollution and UV radiation, suggesting that the algae, much like photosynthesizing plants, have antioxidant mechanisms and compounds that act as antioxidant agents (Gupta & Abu-Ghannam, 2011).

Food industry consumes a wide range of algae (Cardozo et al., 2007) and considers a good antioxidant that compound that efficiently inhibits lipid peroxidation, a process that causes rancidity in foodstuffs. Antioxidants have been defined as substances that, when present at low concentrations compared to an oxidizable compound such as DNA, proteins, lipids or carbohydrates, delay or prevent oxidative damage caused by the presence of ROS (Halliwell & Aruoma, 1991; Halliwell, Aeschbach, Löliger, & Aruoma, 1995).

Until recently, several synthetic antioxidants, including butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *t*-butyl hydroquinone (TBHQ), were commonly used in the food industry. However, the use of these products has been restricted because they are now suspected of having carcinogenic effects (Kumar, Ganesan, & Rao, 2008). As a result, interest in naturally occurring antioxidants from bioresources, such as seaweeds, has received considerable attention during the last few years, as they are generally believed to be good candidates for the production of safe biologically active substances (Cornish & Garbary, 2010; Ganesan, Kumar, & Rao, 2011; Souza et al., 2011). In marine algae, the Japanese scientists Fujimoto and Kaneda (1980) investigated the presence of potentially antioxidant active substances for the first time. Screening test for antioxygenic compounds from marine algae carried out with 21 species of marine algae revealed that more than half of them showed antioxygenic effects. These authors searched for substituents of toxic synthetic food additives after observing that the red algae *Porphyra tenera*, produced in considerable amounts and preserved by drying in thin films, remained with no detectable rancid odor even after a long storage. After 30 years of the initial study, the interest in marine algae as a source of natural antioxidants has only grown worldwide (Cardozo et al., 2007; Vijayabaskar & Shiyamala, 2011).

In Brazil, there are relatively few researches in this area despite both the existence of the long Brazilian coastline and the diversity of algal species. Antioxidant activity has been found in several Brazilian algal species, for example, *Gracilaria birdiae* and *G. cornea* collected at Flecheiras Beach (Ceará) (Souza et al., 2011); *Amansia multifida* and *Meristiella echinocarpa* collected at Paracuru Beach (Ceará) (Alencar et al., 2014); *G. domingensis* and *G. birdiae* from Cotovelo Beach (Natal) (Guaratini, Lopes, Marinho-Soriano, Colepicolo, & Pinto, 2012); 26 species of green, red and brown marine algae from the States of Rio de Janeiro and Espírito Santo (Martins et al., 2013); and 11 species of green, red and brown marine algae from the coast of Natal (Costa et al., 2010).

The biotechnological importance of the algal species *Caulerpa cupressoides* (Vahl) C. Agardh (Caulerpaceae), *Ulva fasciata* Delile (Ulvaceae) (Chlorophyta), *Amansia multifida* J. V. Lamouroux (Rhodomelaceae), *Cryptonemia crenulata* (Rhodophyta), *Dictyota dichotoma* (Hudson) J. V Lamouroux (Dictyotaceae) and *Sargassum vulgare* C. Agardh (Sargassaceae) (Ochrophyta) has been recognized (Rodrigues et al., 2011; Rodrigues et al., 2012; Dore et al., 2013; Mendes et al., 2014; Rivanor et al., 2014; Shao, Pei, Fang, & Sun, 2014; Sujatha, Singh, Vohra, Kumar, & Sunitha, 2015). In this study, the antioxidant potential of these species was evaluated on the basis of total phenolic content (TPC) and the measurement of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, ferric-reducing antioxidant power (FRAP) and ferrous ion chelating activity (FIC).

Material and methods

Seaweed collection

Green marine algae (*C. cupressoides* (Vahl) C. Agardh and *U. fasciata* Delile), red marine algae (*A. multifida* J. V. Lamouroux and *C. crenulata* (J. Agardh) J. Agardh) and brown marine algae (*D. dichotoma* (Hudson) J. V Lamouroux and *S. vulgare* C. Agardh) were collected at Paracuru Beach, São Gonçalo do Amarante, Ceará State, Brazil, in August 2011, during low tides, under the authority of the new Brazilian Biodiversity

Authorization and Information System (Sisbio) (Number 33913-1) operated by the Brazilian Institute of Environment and Natural Resources (Ibama).

Samples of algae were transported to the Marine Natural Products Laboratory, cleaned for removal of macroscopic epiphytes, washed in distilled water, blotted to remove excess water and then dried in an air-forced oven at 35°C for 15 hours. Afterwards, dried algae were stored at room temperature in sealed dark containers for protection against light and then transformed into a fine powder used for extract preparation just before analysis.

The identification was carried out by Professors A. H. Sampaio and K. M. S. Pires-Cavalcante from the Departamento de Engenharia de Pesca of the Universidade Federal do Ceará (UFC). Voucher specimens were deposited in the Herbário Prisco Bezerra of the Departamento de Biologia - UFC with the sequence of numbers from 53168 to 53173.

Seaweed extract preparation

Algal extracts (1:20, w v^{-1}) were prepared with methanol (MeOH), shaken at 20°C for 1 hour, and filtered. The residues were re-extracted twice and finally combined to optimization of the total biomass.

A 1 mg mL^{-1} MeOH extract was used for TPC analyses. *In vitro* antioxidant activities were measured for DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, ferric-reducing antioxidant power (FRAP), and ferrous ion chelating activity (FIC), using 100, 50, 25 and 12.5 μg mL^{-1} dilutions of 1 mg mL^{-1} MeOH extract.

The synthetic antioxidant butylated hydroxyanisole (BHA) was used as positive control for the DPPH and the FRAP assays, and ethylenediamine tetraacetic acid (EDTA), a metal chelating agent, was used as positive control for the FIC assay.

Determination of TPC

TPC was determined using the Folin-Ciocalteu method described by Kumar, Ganesan, and Rao (2008). Briefly, 200 µL of the extract solution (1 mg mL^{-1}) was mixed with 100 µL of Folin-Ciocalteu reagent, 400 µL of distilled water, and 300 µL of 20% sodium carbonate (Na_2CO_3). The mixture was left in the dark at room temperature for 30 min., after which a 200 µL aliquot of the mixture was transferred to each well of a 96-well microplate. The absorbance was then read at 760 nm using a Biochrom Asys UVM 340 microplate reader (Cambridge, UK). Gallic acid was used as a standard, and the quantification of TPC in the samples was expressed as mg gallic acid equivalents (GAE) per gram of extract.

DPPH radical scavenging activity

The DPPH scavenging activity of the diluted extracts (100, 50, 25 and 12.5 μg mL^{-1}) was measured according to the method described by Duan, Zhang, Li, and Wang (2006). The absorbance of sample, sample blank and control was measured at 517 nm, after 30 min. incubation in the dark at room temperature, using a Biochrom Asys UVM340 microplate reader (Cambridge, UK). The sample consisted of the mixture of DPPH methanolic solution (0.16 mM, 1 mL) with each diluted extract (1 mL). The sample blank was prepared with 2 mL of each diluted extract, while the control was prepared with 2 mL of DPPH methanolic solution (0.16 mM). The percentage of DPPH scavenging activity was calculated using the expression below, where Abs is absorbance at 517 nm, according as equation 1.

DPPH scavenging activities

$$(\%) = \left[1 - \frac{\left(\mathrm{Abs}_{\mathrm{sample}} - \mathrm{Abs}_{\mathrm{sample\,blank}}\right)}{\mathrm{Abs}_{\mathrm{control}}}\right] \times 100\% \qquad (1)$$

Ferric-reducing antioxidant power assay (FRAP)

The ferric-reducing antioxidant powers of the diluted extracts (100, 50, 25 and 12.5 μg mL^{-1}) were determined using the method described by Ganesan, Kumar, and Bhaskar (2008). Briefly, 1 mL of each diluted extract was mixed with 2.5 mL 0.2 M phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide (1%, w v^{-1}). The mixture was then incubated at 50°C for 20 min. After cooling, 2.5 mL trichloroacetic acid (10%, v w^{-1}) were added to the mixture. Next, a 2.5 mL aliquot of the upper layer of the solution was mixed with 2.5 mL distilled water and 0.5 mL $FeCl_3$ (0.1%, w v^{-1}). After 10 min incubation at room temperature, the absorbance was read at 700 nm, using a Biochrom Asys UVM340 microplate reader (Cambridge, UK). Increased absorbance of the reaction mixture indicated increased reducing power.

Ferrous ion chelating activity (FIC)

FIC of the diluted extracts (100, 50, 25 and 12.5 μg mL^{-1}) was determined according to Wang, Jónsdóttir, and Ólafsdóttir (2009). The absorbance of sample, blank and control was measured at 562 nm, after 10 min. incubation at room temperature, using a Biochrom Asys UVM340 microplate reader (Cambridge, UK). The sample consisted of 1 mL of each diluted extract, 1.35 mL

distilled water, 50 μL 2 mM $FeCl_2$ and 100 μL 5 mM ferrozine. Distilled water was used for both the sample blank and the control test tube instead of ferrozine and the diluted extract, respectively. The percentage of ferrous ion chelating activity was calculated using the expression below, where Abs is absorbance at 562 nm, according as equation 2.

Ferrousion chelating activity

$$(\%) = \frac{\left[\mathrm{Abs}_{\mathrm{control}} - \left(\mathrm{Abs}_{\mathrm{sample}} - \mathrm{Abs}_{\mathrm{sample\ blank}}\right)\right]}{\mathrm{Abs}_{\mathrm{control}}} \times 100\% \quad (2)$$

Statistical analysis

All data are presented as mean ± standard deviation. The mean values were calculated on the data taken from three independent solutions/extracts prepared on the same day. Data of both the total phenolic content of algal extracts (1 mg mL^{-1}) and the *in vitro* antioxidant activity (DPPH scavenging activity, FRAP and FIC) of diluted algal extracts (100, 50, 25 and 12.5 μg mL^{-1}) were separately compared using one-way analysis of variance (ANOVA), followed by Tukey's HSD (Honestly Significant Difference) test in the case of null hypothesis rejection. The statistical significance was achieved when $p < 0.05$.

Results and discussion

TPC values

The results of TPC of the MeOH algal extracts (1 mg mL^{-1}) are illustrated in Figure 1 for each algal species studied from their respective dehydrated matter. The extracts of *D. dichotoma*, *A. multifida*, *C. cupressoides*, and *S. vulgare* were found to have the highest TPC values ($p < 0.05$), while the seaweeds *C. crenulata* and *U. fasciata* had the lowest ones.

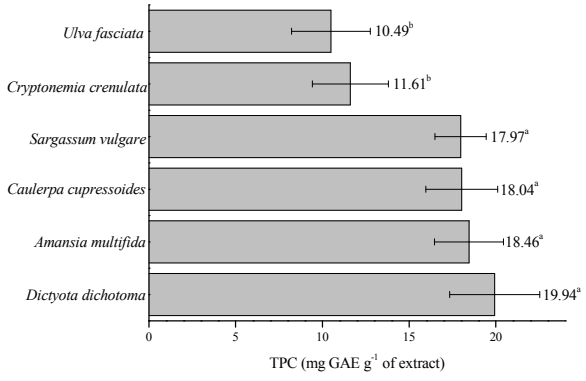

Figure 1. TPC values of the MeOH extracts (1 mg mL^{-1}) obtained from marine algae. Values are the mean with the error bars representing standard deviation, n = 3. Similar lowercase letters - no statistically significant difference ($p > 0.05$); different lowercase letters - statistically significant difference ($p < 0.05$).

High levels of TPC in brown marine algae are expected, considering reports indicating they are rich sources in fucoxanthin (D'Orazio et al., 2012) and phlorotannins (Wang et al., 2012). Similar results were also described by Wang et al. (2009) in brown algae *Fucus vesiculosus*, *F. serratus*, *Laminaria hyperborea*, *L. saccharina*, *L. digitata*, and *Alaria esculenta* (TPC varying from 13.8 to 30 g phloroglucinol equivalents (PGE) per 100 g of extract), red algae *Palmaria palmata* and *Chondrus crispus*, and the green algae (*Ulva lactuca*) with values ranging from 0.4 to 24.2 g PGE per 100 g of extract. The TPC of seven species of brown marine algae (*Dictyopteris australis*, *D. delicatula*, *Padina tetrastromatica*, *Sargassum marginatum*, *Spatoglossum aspermum*, *S. variabile*, and *Stoechospermum marginatum*) ranged from 13.19 to 25.29 mg GAE per gram of methanolic extract (Vinayak, Sabu, & Chatterji, 2011), whose values were very similar to the methanolic extracts from the brown seaweeds *D. dichotoma* and *S. vulgare* analyzed in this study.

Regarding the green and red algae, they do not contain a large amount of TPC in comparison with brown algae, although higher values (mg AGE 100 g^{-1} dry alga) have been found in green algae when compared with red species. The results demonstrated by Farvin and Jacobsen (2013) for the green algae *Enteromorpha intestinalis* (265.8) and *Ulva lactuca* (236.5) were higher than those for the red ones *Palmaria palmata* (76.9), *Gracilaria vermiculophylla* (95.2), *Mastocarpus stellatus* (102.5) and *Chondrus crispus* (113.6). These findings are consistent with the results of the present study, wherein the extract from the green marine algae *C. cupressoides* contained higher content than the red marine algae *C. crenulata*, with the exception of extract from the green marine algae *U. fasciata* which was lower than that of the red marine algae *A. multifida*.

Methanol extracts of the green marine algae showed lower TPC in comparison to *Enteromorpha spirulina* extract prepared with the same solvent (Cox, Abu-Ghannam, & Gupta, 2010). On the other hand, methanol extracts of the red marine algae exhibited antioxidant activities higher than those reported by Souza et al. (2011) in *Gracilaria birdiae* and *G. cornea*.

These conflicting differences at the level of TPC among species of algae may be related to numerous factors representing both endogenous and exogenous sources, including age of the plant, reproductive stage and/or herbivory (Farvin & Jacobsen, 2013).

DPPH radical scavenging activity

The values of percent DPPH scavenging activities of diluted algal extracts at the concentrations of 100, 50, 25 and 12.5 μg mL^{-1} are shown in Table 1. These values were compared with those of the well-known powerful synthetic antioxidant BHA, which was used in this study as positive control and had scavenging activity ranging from approximately 91 (12.5 μg mL^{-1}) to 97% (100 μg mL^{-1}), values that were 50-60% more active than the algal extracts tested. For the lowest tested concentrations (25 and 12.5 μg mL^{-1}), all algal extracts exhibited activity roughly between 54 and 58%, showing no significant difference. In general, at concentrations of 100 and 50 μg mL^{-1}, brown and red algal extracts exhibited higher activity than green algal extracts ($p < 0.05$). The algal extracts with higher DPPH radical scavenging activity indicate their hydrogen-donating ability (Alves, David, David, Bahia, & Aguiar, 2010; Mishra, Ojha, & Chaudhury, 2012).

Values of DPPH scavenging activity reported here are higher than those cited for a variety of seaweeds. For example, extracts of the green marine algae *C. cupressoides* and *U. fasciata* at 12.5 μg mL^{-1} showed DPPH scavenging activities 2-fold higher than 95% ethanol extract of *Enteromorpha prolifera* at 130 μg mL^{-1} (Cho, Lee, Kang, Won, & You, 2011). Similarly, the extracts of both red (*A. multifida* and *C. crenulata*) and brown marine algae (*Dictyota dichotoma* and *Sargassum vulgare*) analyzed in the present study, even at the lowest tested concentration, also presented higher DPPH scavenging activities than the extracts of *Gracilaria edulis*, *Chondrococcus hornemanni*, *Hypnea pannosa* and *Jania rubens* at 100 μg mL^{-1}, which varied from 5 to 25% (Devi, Suganthy, Kesika, & Pandian, 2008) and those of *Ascophyllum nodosum*, *Laminaria hyperborea*, *Pelvetia canaliculata*, *Fucus vesiculosus* and *F. serratus* at 5 mg mL^{-1} (O'Sullivan et al., 2011).

Ferric-reducing antioxidant power assay (FRAP)

Antioxidant components act as electron donors, reducing ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The determination of the amount present in solution involves a redox-linked colorimetric reaction, which measures the intensity of the Prussian blue pigment. The darker the color is, the higher the reducing activity (Soltani, Saadatmand, Khavarinejad, & Nejadsattari, 2011). The reducing power of a compound may serve as a significant indicator of its potential for use as an antioxidant.

As listed in Table 2, the absorbance of diluted extracts for all species (100, 50, 25 and 12.5 μg mL^{-1}) ranged from 0.060 to 0.087, representing only a small fraction of values observed for the positive control at the same concentrations. Statistical significance was, however, observed in all tested concentrations ($p < 0.05$) among all algal species based on individual absorbance. On the other hand, results of reducing power reported here are much lower than those cited for a variety of seaweeds, such as *Enteromorpha compressa* (Ganesan et al., 2011), *Kappaphycus alvarezii* (Kumar et al., 2008), and *Turbinaria conoides* (Devi et al., 2011).

Ferrous ion chelating activity (FIC)

As determined in the present study (Table 3), the values of percent FIC activities ranged, respectively, from 10.98 to 21.61, 8.63 to 16.24 and 8.93 to 13.28 in the methanolic extracts of green, red and brown marine algae analyzed. At concentrations of 100, 25 and 12.5 μg mL^{-1}, all algal extracts exhibited activities with statistically significant difference. However, at 50 μg mL^{-1}, FIC activities showed no statistically significant difference. The EDTA activity for ferrous ion chelating at tested concentrations was much higher and varied roughly from 48 (12.5 µg mL^{-1}) to 98% (100 µg mL^{-1}). These results do not allow the definition of any relationship with algal species and/or extract concentration.

Table 1. DPPH radical scavenging activity (%) of the diluted MeOH extracts (100, 50, 25 and 12.5 μg mL^{-1}) obtained from marine algae.

	Antioxidant activity ± SD			
Species	100 μg mL^{-1}	50 μg mL^{-1}	25 μg mL^{-1}	12.5 μg mL^{-1}
Positive control	F = 8.616	F = 8.280	F = 5.178	F = 0.361
	$p < 0.05$			
Chlorophyta				
Caulerpa cupressoides	58.70 ± 0.78^{d}	57.98 ± 0.66^{c}	57.11 ± 1.37	56.06 ± 1.22
Ulva fasciata	59.29 ± 0.96^{cd}	58.06 ± 0.40^{c}	56.55 ± 0.71	55.93 ± 0.20
Rhodophyta				
Amansia multifida	63.79 ± 1.76^{ab}	60.48 ± 0.23^{a}	57.16 ± 1.43	55.81 ± 1.01
Cryptonemia crenulata	60.32 ± 0.60^{bcd}	58.87 ± 1.10^{bc}	57.82 ± 0.10	57.11 ± 0.80
Ochrophyta				
Dictyota dichotoma	63.53 ± 0.93^{abc}	59.21 ± 0.17^{abc}	55.86 ± 3.63	54.51 ± 5.21
Sargassum vulgare	64.96 ± 2.97^{a}	59.67 ± 0.35^{ab}	56.23 ± 3.53	53.96 ± 5.89
BHA	96.74 ± 0.06	96.49 ± 0.04	95.48 ± 0.15	91.06 ± 0.03

Values are the mean ± standard deviation (SD), n = 3. Similar lowercase letters in the same column - no statistically significant difference ($p > 0.05$); different lowercase letters in the same column - statistically significant difference ($p < 0.05$).

Table 2. Ferric-reducing antioxidant power assay (FRAP) of the diluted MeOH extracts (100, 50, 25 and 12.5 μg mL^{-1}) obtained from marine algae.

Species	Antioxidant activity ± SD			
	100 μg mL^{-1}	50 μg mL^{-1}	25 μg mL^{-1}	12.5 μg mL^{-1}
Positive control	F = 6.658	F = 10.140	F = 12.190	F = 6.931
	$p < 0.05$			
Chlorophyta				
Caulerpa cupressoides	0.074 ± 0.005^{ab}	0.073 ± 0.003^{a}	0.073 ± 0.003^{a}	0.071 ± 0.003^{ab}
Ulva fasciata	0.080 ± 0.005^{a}	0.072 ± 0.004^{a}	0.072 ± 0.004^{ab}	0.070 ± 0.003^{ab}
Rhodophyta				
Amansia multifida	0.066 ± 0.002^{b}	0.061 ± 0.003^{b}	0.060 ± 0.001^{c}	0.061 ± 0.004^{c}
Cryptonemia crenulata	0.087 ± 0.006^{a}	0.076 ± 0.001^{a}	0.074 ± 0.004^{a}	0.073 ± 0.002^{a}
Ochrophyta				
Dictyota dichotoma	0.075 ± 0.002^{ab}	0.069 ± 0.003^{a}	0.065 ± 0.003^{bc}	0.064 ± 0.002^{bc}
Sargassum vulgare	0.081 ± 0.006^{a}	0.073 ± 0.002^{a}	0.072 ± 0.002^{ab}	0.069 ± 0.004^{abc}
BHA	0.433 ± 0.006	0.266 ± 0.008	0.190 ± 0.019	0.137 ± 0.011

Values are the mean ± standard deviation (SD), n = 3. Similar lowercase letters in the same column - no statistically significant difference ($p > 0.05$); different lowercase letters in the same column - statistically significant difference ($p < 0.05$).

Table 3. Ferrous ion chelating activity (FIC) (%) of the diluted MeOH extracts (100, 50, 25 and 12.5 μg mL^{-1}) obtained from marine algae.

Species	Antioxidant activity ± SD			
	100 μg mL^{-1}	50 μg mL^{-1}	25 μg mL^{-1}	12.5 μg mL^{-1}
Positive control	F = 22.054	F = 1.983	F = 5.178	F = 5.564
	$p < 0.05$			
Chlorophyta				
Caulerpa cupressoides	13.22 ± 0.88^{bc}	12.00 ± 1.54	11.03 ± 1.61^{ab}	10.98 ± 1.22^{ab}
Ulva fasciata	21.61 ± 1.86^{a}	13.51 ± 1.64	12.93 ± 1.46^{a}	12.65 ± 1.61^{a}
Rhodophyta				
Amansia multifida	12.63 ± 0.62^{c}	11.22 ± 1.46	8.63 ± 0.81^{b}	11.59 ± 1.36^{ab}
Cryptonemia crenulata	16.24 ± 1.80^{b}	12.03 ± 1.21	10.98 ± 0.98^{ab}	9.22 ± 0.92^{b}
Ochrophyta				
Dictyota dichotoma	13.28 ± 0.92^{bc}	12.19 ± 1.19	10.07 ± 0.87^{ab}	8.93 ± 0.74^{b}
Sargassum vulgare	12.39 ± 1.31^{c}	10.07 ± 1.31	10.04 ± 0.12^{ab}	9.19 ± 0.40^{b}
EDTA	98.05 ± 1.22	93.79 ± 2.88	69.21 ± 1.69	48.42 ± 1.48

Values are the mean ± standard deviation (SD), n = 3. Similar lowercase letters in the same column - no statistically significant difference ($p > 0.05$); different lowercase letters in the same column - statistically significant difference ($p < 0.05$).

The extracts of three species of green marine algae (*Enteromorpha compressa*, *E. linza* and *E. tubulosa*), at the concentration of 200 μg mL^{-1}, showed FIC of approximately 15% (Ganesan et al., 2011). Similar results were observed in the present study, but with 2 to 16 times less concentrated methanolic extracts. This suggests that the ferrous ion chelating ability of our extracts was more efficient than that of the genus *Enteromorpha*.

Both aqueous and 70% acetone extracts of the red marine algae *Chondrus crispus* and *Palmaria palmata* at 5 mg mL^{-1} showed FIC values ranging from 30 to 95% (Wang et al., 2009). These results were higher than those obtained for *A. multifida* and *C. crenulata*, which were from 50 (100 µg mL^{-1}) to 400 (12.5 µg mL^{-1}) times less concentrated.

Among the methanol extracts of brown seaweeds analyzed by Vinayak et al. (2011), *Dictyopteris australis* showed FIC approximately 16% higher than the extracts of *Dictyota dichotoma* and *Sargassum vulgare* at 100 mg L^{-1}. All extracts of the other species assessed in that study, including *Dictyopteris delicatula*, *Padina tetrastromatica*, *Sargassum marginatum*, *Spatoglossum aspermum*, *S. variabile* and *Stoechospermum marginatum*, showed FIC between 2.5 and 10% lower than the extracts analyzed in the present work.

For all cases mentioned above, it has been noted that the highest FIC values were observed with the positive control (EDTA) when compared to algal extracts.

Because of the presence of different antioxidant components in the algal extracts, it is relatively difficult to measure each antioxidant component separately. Therefore, several methods have been developed and applied in recent years to screen and evaluate the total antioxidant activity of such extracts. In the present study, the evaluation of TPC was combined with DPPH radical scavenging, ferric-reducing antioxidant power (FRAP), and ferrous ion chelation (FIC) assays and successfully used to determine the antioxidant activity of the algal extract solutions (1 mg mL^{-1}) and dilutions (100, 50, 25 and 12.5 μg mL^{-1}) for six seaweed species endemic to the coastal waters of Ceará State, Brazil.

Antioxidant activity registered in these species may be due to the presence of several different

compounds. Further studies must be conducted to isolate such compounds aiming their application as substitutes for the synthetic antioxidants. It is important to mention that for the production of any natural compound, one must take into consideration its viability in terms of environmental preservation. For this reason, these compounds must be either extracted from cultivated algae or obtained by chemical synthesis. Besides the *Caulerpa cupressoides* antioxidant potential showed herein, this species also revealed anti-inflammatory (Rodrigues et al., 2012; Rivanor et al., 2014) and antithrombotic (Rodrigues et al., 2011) actions. Antitumor, anticoagulant, anti-inflammatory, antilithiatic, and/or antiviral activities have been registered in algal extracts of *U. fasciata* (Shao et al., 2014), *Sargassum* spp. (Dore et al., 2013; Sujatha et al., 2015) and *Cryptonemia seminervis* (Mendes et al., 2014).

Conclusion

The methanolic extracts from the marine algae *Caulerpa cupressoides*, *Ulva fasciata*, *Amansia multifida*, *Cryptonemia crenulata*, *Dictyota dichotoma* and *Sargassum vulgare* exhibit lower antioxidant activity when compared to positive controls. As they do not pose risk to human health, the antioxidant activities of these algal species may be considered of chemical importance. Nonetheless, they are still promising sources of natural antioxidants, which can be used in either food, cosmetic and pharmaceutical, based on our *in vitro* evaluation by TPC combined with DPPH radical scavenging, FRAP, and FIC assays. Therefore, they deserve deeper studies for further application as substitutes for synthetic antioxidants.

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