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Antioxidant potential and cytotoxic activity of two red seaweed species, *Amansia multifida* and *Meristiella echinocarpa*, from the coast of Northeastern Brazil

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ABSTRACT

Natural antioxidants found in marine macroalgae are bioactive compounds known to play an important role in the prevention of diseases associated with aging cells protecting them against the oxidative damage. The purpose of this study was to evaluate the antioxidant and cytotoxic activity of ethanolic extracts of two species of red seaweeds, *Amansia multifida* and *Meristiella echinocarpa*. *In vitro* antioxidant activity was determined by DPPH radical scavenging assay, ferric-reducing antioxidant power (FRAP) assay, ferrous ion chelating (FIC) assay, β-carotene bleaching (BCB) assay and total phenolic content (TPC) quantification. Cytotoxicity was evaluated with the brine shrimp *Artemia* sp. lethality test. The TPC values observed in the present study indicated that both species *A. multifida* and *M. echinocarpa* are rich in phenolic compounds, reaching values of 45.40 and 28.46 mg gallic acid equivalent (GAE) g^{-1} of ethanolic extract, respectively. DPPH radical scavenging and ferrous ion chelating showed values of 60% and 17%, respectively. Both seaweed extracts inhibited β-carotene oxidation by approximately 40%. None of the algal extracts were potentially cytotoxic. The results have showed that extracts of both species of marine red algae exhibit antioxidant potential and low toxicity. They are sources of natural antioxidant compounds.

Key words: antioxidant, bioactive compounds, cytotoxic, phenolic compounds, Rhodophyta.

INTRODUCTION

Produced endogenously in cellular tissues, reactive oxygen species (ROS), such as the hydroxyl radical ($OH^{\cdot}$), superoxide radical (O_2^{-}), hydroperoxyl radical ($HO_2^{\cdot}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$), can cause oxidative damage to DNA, protein, lipids and other cell components frequently associated with extreme chronic conditions such as atherosclerosis, aging, arthritis, diabetes, pulmonary

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dysfunction, muscular dystrophy, ischemia, tissue damage and neurological disorders (Vinayak et al. 2011a, b, Wang et al. 2011, Yangthong et al. 2009).

In addition to damaging cell components, ROS can cause dietary oils and fats to degrade, producing a rancid taste and smell and consequent loss of quality and food safety associated with the formation of potentially toxic secondary metabolites (Andreo and Jorge 2006, Oliveira et al. 2009).

Asian countries have a long-standing tradition of culinary use of seaweeds. They are used as extracts and/or powders and can be home made or produced industrially. In the West, macroalgae have generally been used indirectly by the food industry in the form of agar, carrageenan and alginate due to their important gelling, emulsifying and thickening properties (Mohamed et al. 2012). However, over the past few years table consumption has grown considerably around the world, awareness of the nutritional value of macroalgae has become more widespread. Seaweeds have few calories and are rich in provitamin A, vitamin B, C and E, minerals (calcium, magnesium, phosphorous, potassium, sodium, iron and iodine) and dietary fiber (Matanjun et al. 2009, Patarra et al. 2011, Pires et al. 2008, Pires-Cavalcante et al. 2011, Sousa et al. 2008).

Marine macroalgae are also rich in bioactive compounds with anti-inflammatory, antimicrobial, antitumoral, antiviral and antioxidant activities (Mayer et al. 2011). Secondary metabolites such as carotenoids, tocopherols, terpenes and phenolic compounds can be considered natural antioxidants, with several potential applications in the food industry (Pires et al. 2008, Pires-Cavalcante et al. 2011, Sousa et al. 2008). Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) contain a phenolic structure allowing them to donate a hydrogen atom to a free radical, thereby regenerating the acylglycerol molecule and interrupting the oxidation process of the free radical. However, phenolic compounds can turn into free radicals which in turn can stabilize or promote health-threatening oxidation reactions, usually associated with excessive use. Toxicological studies in rodents have shown that phenolic compounds can also produce carcinogenic effects. BHA induces gastrointestinal hyperplasia while TBHQ causes basal cell hyperplasia and a reduction in hemoglobin levels (Andreo and Jorge 2006, Ramalho and Jorge 2006).

Red seaweeds are arguably the largest producers of secondary metabolites in marine environment. Among them, the genus *Laurencia* represents a particularly impressive and extensively researched source of bioactive compounds (Teixeira 2013). To our knowledge, in contrast, no studies on the bioactive compounds of the red seaweed species *Amansia multifida* and *Meristiella echinocarpa* have been published so far. The purpose of the present study was, therefore, to evaluate the antioxidant and cytotoxic activity of ethanolic extracts of these two algal species and their possible use.

MATERIALS AND METHODS

Standards and Reagents

The reagents used to determine antioxidant activity (methanol, linoleic acid, ferric chloride, potassium ferricyanide, anhydrous sodium carbonate, trichloroacetic acid, chloroform and anhydrous monobasic potassium phosphate) were purchased from Vetec (Brazil), p.a. grade. Gallic acid (G7384), ferrous chloride (37287-0), Folin-Ciocalteu reagent (F9252), Tween 40 (P1504), ferrozine (P9762), 2,2-diphenyl-1-picryl-hydrazyl (D913-2) and the synthetic antioxidants butylated hydroxyanisole (B1253), butylated hydroxytoluene (47168) and ~95% hydrated quercetin (33795-1) were purchased from Sigma Aldrich (USA).

Collection of Algae and Preparation of Extracts

Specimens of the red seaweed species *Amansia multifida* J. V. Lamouroux and *Meristiella echinocarpa* (Areschoug) D. P. Cheney & P. W. Gabrielson

were collected in August 2010 at Paracuru (a coastal town in São Gonçalo do Amarante, Ceará, Northeastern Brazil) during low tide, under the authorization SISBIO Number 33913-1 given by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA). The algae were transported to the Marine Natural Products Laboratory of the Federal University of Ceará (UFC) and washed with distilled water in order to remove epiphytes and other organisms. Their identification was carried out by Professor Alexandre Holanda Sampaio and Doctor Kelma Maria dos Santos Pires-Cavalcante. Voucher specimens were deposited at the Herbarium Prisco Bezerra of the Biology Department of the Federal University of Ceará (UFC) with numbers 53175 and 53177, respectively, for *A. multifida* and *M. echinocarpa*.

Macroalgae *in natura* were lyophilized, then ground. The lyophilized sample was submitted to extraction with 70% ethanol at 1:20 (m/v), then reextracted twice using the same solvent (Alencar et al. 2012). The ethanolic extracts of *A. multifida* and *M. echinocarpa* were concentrated by reduced-pressure distillation and used to quantify total phenolic content (TPC) and determine antioxidant activity by the methods DPPH, FIC, FRAP and BCB.

Total Phenolic Content (TPC)

The TPC of the ethanolic extracts of *A. multifida* and *M. echinocarpa* was determined with the Folin-Ciocalteu method (Kumar et al. 2008). Distilled water, Folin-Ciocalteu reagent and 20% Na_2CO_3 were added to 200 µL of algal extract (1 mg mL^{-1}). Following 30 min of incubation in the dark, at room temperature, absorbance was measured at 760 nm using a microplate reader (Biochrom Asys UVM 340). The calculation of TPC was based on a previously generated gallic acid standard curve, and the results were expressed in mg gallic acid equivalent (GAE) g^{-1} of extract.

DPPH Radical Scavenging Assay

The ability of the seaweed extracts to scavenge DPPH radicals was evaluated with the method of Duan et al. (2006). The sample corresponded to the tubes where a methanolic solution containing 0.16 mM DPPH was added to seaweed extracts at different concentrations (1 to 100 µg mL^{-1}). The results were compared against a blank (seaweed extract only) and a control (0.16 mM DPPH solution only). The sample, blank and control were all incubated in the dark, at room temperature, for 30 min and the absorbance was read at 517 nm using a microplate reader (Biochrom Asys UVM 340). BHT, BHA and quercetin were used as positive controls. The percentage of DPPH radical scavenging effect was calculated with the following equation:

$$\text{Scavenging effect (\%)} = \left[1 - \frac{(Abs_{sample} - Abs_{blank})}{Abs_{control}}\right] \times 100\%$$

Ferrous Ion Chelating (FIC) Assay

The ferrous ion chelating (FIC) power of the extract was determined with the method described by Wang et al. (2009). The sample corresponded to the tubes where distilled water, 2 mM ferrous chloride ($FeCl_2$) and 5 mM ferrozine were added to seaweed extracts at different concentrations (1 to 100 µg mL^{-1}). Distilled water and 2 mM ferrous chloride ($FeCl_2$) were added to seaweed extracts at different concentrations (1 to 100 µg mL^{-1}) and used as a blank. The control was prepared with distilled water, 2 mM ferrous chloride ($FeCl_2$) and 5 mM ferrozine only. The sample, blank and control were all incubated at room temperature for 10 min and the absorbance was read at 562 nm using a microplate reader (Biochrom Asys UVM 340). BHT, BHA and quercetin were used as positive controls. FIC activity was calculated with the following equation:

$$\text{Ferrous ion chelating activity (\%)} = \frac{\left[Abs_{control} - (Abs_{sample} - Abs_{blank})\right]}{Abs_{control}} \times 100\%$$

Ferric-Reducing Antioxidant Power (FRAP)

Ferric-reducing antioxidant power (FRAP) was determined with the method described by Ganesan et al. (2008). Initially 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide were added to seaweed extracts at different concentrations (1 to 100 μg mL^{-1}). The samples were then incubated at 50°C for 20 min. After cooling at room temperature, 10% trichloroacetic acid was added. An aliquot was mixed with distilled water and 0.1% ferric chloride. Ten minutes later the absorbance was read at 700 nm using a microplate reader (Biochrom Asys UVM 340). BHT, BHA and quecertin were used as positive controls. Greater absorbance indicated greater FRAP.

β-Carotene Bleaching (BCB) Assay

The coupled oxidation of β-carotene and linoleic acid was determined with the method described by Chew et al. (2008). Linoleic acid and Tween 40 were added to the β-carotene solution in chloroform (100 μg mL^{-1}). The chloroform was then evaporated and oxygen-saturated ultrapure water was added to the residue. The β-carotene/linoleic acid emulsion was shaken vigorously and aliquots of this emulsion were added to the extracts. The absorbance was read at 470 nm immediately after the emulsion was prepared ($Abs_{initial}$) and after 1 hour of incubation in a water bath at 50°C (Abs_{1h}). BHT, BHA and quercetin were used as positive controls. The percentage AOA was calculated with the following formula:

$$\text{Antioxidant activity (\%)} = \left(\frac{Abs_{1h}}{Abs_{initial}} \right) \times 100\%$$

Cytotoxic Activity by Brine Shrimp Lethality Test

The sensitivity of the brine shrimp nauplii (*Artemia* sp.) was previously tested with the method of Veiga and Vital (2002) using sodium dodecyl sulfate as standard toxin to make sure the nauplii were not too sensitive or too resistant to produce reliable results.

The 70% ethanolic extracts of *A. multifida* and *M. echinocarpa* were suspended in seawater until reaching a stock solution concentration of 1 mg mL^{-1}. The assays were performed in triplicate with final concentrations of 5, 10, 50, 100 and 500 μg mL^{-1}, using acrylic plates with 24 wells, each of which contained 10 stage-II nauplii. Wells with seawater and nauplii were used as negative control. The lethal potential of the seaweed extracts was evaluated after 24 hours and 48 hours by counting the dead nauplii in each well. Mortality below 50% was considered non-cytotoxic; mortality higher than 50% but below 75% was considered mildly cytotoxic; and mortality higher than 75% was considered highly cytotoxic. Based on the results, the percentage of dead nauplii for each concentration and the concentration required to induce 50% lethality (CL_{50}) were calculated.

Statistical Analysis

The percentage values obtained for DPPH, FIC and BCB with each concentration (1, 2, 10, 20 and 100 μg mL^{-1}) of the ethanolic extracts of *A. multifida* and *M. echinocarpa* were converted into absolute values, submitted to angular transformation and compared with Student's *t* test for independent data.

The TPC values obtained for the ethanolic extracts of *A. multifida* and *M. echinocarpa* (1 mg mL^{-1}), and the FRAP values obtained for each concentration of the ethanolic extracts of both algae were also analyzed with Student's *t* test for independent data, but since they were not measured as percentages, no previous transformation was needed.

The seaweed extracts and the positive controls were compared by single-factor variance analysis, complemented with Dunn's test in case of rejection of the null hypothesis.

The assumptions of normality and homoscedasticity were met in all cases. The results were expressed as average ± standard deviation (n = 5). All analyses were performed with the software BioEst 4.0 (Ayres et al. 2005). The level of statistical significance was set at 5%.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

Found in higher plants and marine macroalgae, phenolic compounds are a very diversified and heterogeneous group of biologically active molecules with important functions in metabolism and cell physiology. Studies have shown that phenolic compounds can have antioxidant, anti-inflammatory, antitumoral and antiviral activities, with positive effects on human health (Novoa et al. 2011, Thomas and Kim 2011, Wijesingher and Jeon 2012).

TPC was quantified for extracts of *A. multifida* and *M. echinocarpa* based on the gallic acid standard curve. The existence of a correlation (r = 0.9972, $p < 0.05$) between absorbance (760 nm) and concentration (0.0002 to 0.1 mg gallic acid mL^{-1}) made quantification possible ($y = 0.0509 + 27.160x$, $n = 11$).

The two seaweed species differed significantly ($p < 0.001$) with regard to TPC, with *A. multifida* yielding almost 1.6 times higher TPC values than *M. echinocarpa.*

Over the past years, interest in phenolic compounds in seaweeds has grown considerably. However, according to recent literature, brown and green seaweeds tend to yield higher TPC levels than red seaweeds (Kumar et al. 2011, Wang et al. 2009). Table I shows TPC values for a number of marine macroalgal species.

The TPC values observed in the present study are higher than those of almost all red macroalgal species evaluated so far, and higher than those of some species of green and brown macroalgae, indicating that the red seaweed species *A. multifida* and *M. echinocarpa* are rich in phenolic compounds.

Variation in TPC values of marine macroalgae could be influenced by extrinsic factors (such as herbivory pressure, irradiance, depth, salinity and nutrients), by intrinsic factors (morphology, age and reproductive stage), but also by the type of solvent used in the extraction of the phenolic compounds (Chew et al. 2008, Ganesan et al. 2011, Lann et al. 2012).

TABLE I
Total phenolic content (TPC) of a number of marine algal species extracted with varying solvents compared to the TPC values of the ethanolic extracts of *Amansia multifida* and *Meristiella echinocarpa* prepared for the present study.

Algae	Solvent	TPC (mg GAE g^{-1} extract)	Reference
Phaeophyta			
Alaria crassifolia	ethanol	7.21 ± 0.80	Airanthi et al. (2011)
Dictyopteris australis	methanol	13.37 ± 0.14	Vinayak et al. (2011a)
D. delicatula	methanol	21.34 ± 0.43	Vinayak et al. (2011a)
Dictyota dichotoma	methanol	35.23 ± 5.65	Matanjun et al. (2008)
Eisenia bicyclis	ethanol	1.87 ± 0.70	Airanthi et al. (2011)
Kjellmaniella crassifolia	ethanol	8.01 ± 0.78	Airanthi et al. (2011)
Padina tetrastromatica	methanol	25.29 ± 0.44	Vinayk et al. (2011a)
Padina spp.	methanol	33.11 ± 3.96	Matanjun et al. (2008)
Sargassum marginatum	methanol	13.19 ± 0.32	Vinayak et al. (2011a)
Spatoglossum aspermum	methanol	14.13 ± 0.05	Vinayak et al. (2011a)
S. variable	methanol	14.85 ± 0.09	Vinayak et al. (2011a)
S. polycystum	methanol	45.16 ± 3.01	Matanjun et al. (2008)
Stoechospermum marginatum	methanol	20.04 ± 0.38	Vinayak et al. (2011a)
Chlorophyta			
Caulerpa lentilifera	methanol	42.85 ± 1.22	Matanjun et al. (2008)
C. peltata	methanol	38.93 ± 0.63	Vinayak et al. (2011b)

Different letters indicate statistically significant difference ($p < 0.05$).

TABLE I (continuation)

Algae	Solvent	TPC (mg GAE g^{-1} extract)	Reference
Chlorophyta			
C. taxifolia	methanol	24.09 ± 0.65	Vinayak et al. (2011b)
C. racemosa	methanol	23.12 ± 0.49	Vinayak et al. (2011b)
C. racemosa	methanol	40.36 ± 1.05	Matanjun et al. (2008)
Chlorodesmis fastigiata	methanol	7.32 ± 0.35	Vinayak et al. (2011b)
Codium elongatum	methanol	7.41 ± 0.28	Vinayak et al. (2011b)
Rhodophyta			
Gracilaria birdie	ethanol	1.13 ± 0.03	Souza et al. (2011)
G. birdie	methanol	1.06 ± 0.07	Souza et al. (2011)
G. cornea	ethanol	0.88 ± 0.03	Souza et al. (2011)
G. cornea	methanol	0.89 ± 0.07	Souza et al. (2011)
Eucheuma cottonii	methanol	22.50 ± 2.78	Matanjun et al. (2008)
E. spinosa	methanol	15.82 ± 1.24	Matanjun et al. (2008)
Halymenia durvillae	methanol	18.90 ± 1.03	Matanjun et al. (2008)
Amansia multifida	ethanol	45.40 ± 2.99[a]	present study
Meristiella echinocarpa	ethanol	28.46 ± 2.79[b]	present study

Different letters indicate statistically significant difference ($p < 0.05$).

DPPH Radical Scavenging Assay

DPPH is commonly used as a free radical to evaluate antioxidant compounds capable of reducing DPPH by donating a hydrogen atom, thereby forming the non-radical DPPH-H (Cho et al. 2011). The ethanolic extracts of *A. multifida* and *M. echinocarpa* at 1, 10 and 100 μg mL^{-1} differed significantly with regard to their ability to scavenge the DPPH radical. The positive controls (BHT, BHA and quercetin) also differed significantly from the seaweed extracts (Fig. 1).

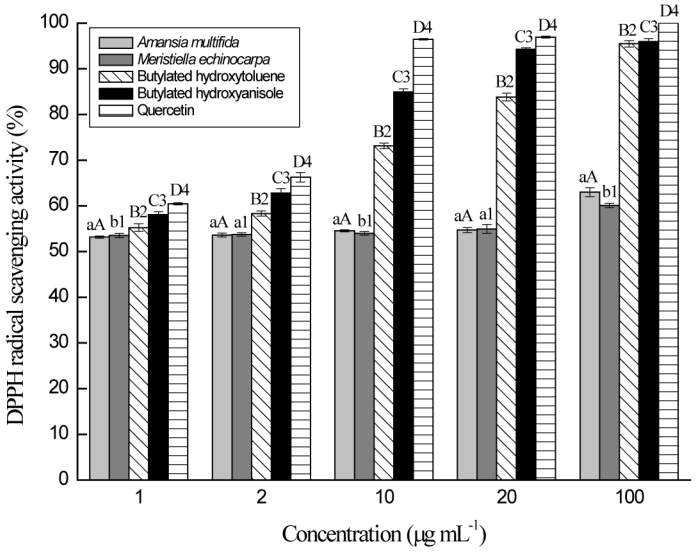

Figure 1 - Scavenging activity (%) of DPPH of ethanolic extract of red marine algae *Amansia multifida* and *Meristiella echinocarpa* and positive controls (BHT, BHA and quercetin). The results are expressed as average ± SD (n = 5). Small letters compare the algal extracts (two-sample *t* test) in the same concentration. Capital letters compare the extract of *A. multifida* with the positive controls (ANOVA following Dunn) in the same concentration. Arabic numerals compare the extract of *M. echinocarpa* with the positive controls (ANOVA following Dunn) in the same concentration. Same symbols indicate no statistically significant difference ($p > 0.05$) and different symbols indicate statistically significant difference ($p < 0.05$) among the concentrations.

Even at the lowest concentration (1 μg mL^{-1}), the extract displayed over 50% DPPH activity. This is higher than the values reported for the green macroalgae *Enteromorpha prolifera* (44%) at 250 μg mL^{-1} (Cho et al. 2011). *Enteromorpha compressa*, *E. tubulosa* and *E. linza* (~45%) at 1.5, 2.5 and 3.0 mg mL^{-1}, respectively (Ganesan et al. 2011). With extracts of the red macroalgae *Polysiphonia urceolata* and *Kappaphycus alvarezii*, activity was 20.9% at 10 μg mL^{-1} and ~45% at 2 mg mL^{-1} (Duan et al. 2006, Kumar et al. 2008). Further studies should be carried out using these extracts at lower concentrations to observe their antioxidant activity.

Like phenolic compounds, other bioactive compounds such as vitamin E, provitamin A carotenoids and sulfated polysaccharides are considered excellent antioxidants, capable of efficiently scavenging free radicals (Pires et al. 2008, Pires-Cavalcante et al. 2011, Sousa et al. 2008, Souza et al. 2012).

Ferrous ion Chelating (FIC) Assay

The ability of the antioxidant compounds to bind to metal ions was evaluated by FIC assay. Extracts of *A. multifida* displayed smaller FIC activity than extracts of *M. echinocarpa* at all concentrations, and smaller activity than the positive controls (BHT, BHA and quercetin) at all concentrations except 100 μg mL^{-1} at which the extract was comparable to BHT ($p > 0.05$). Extracts of *M. echinocarpa* presented significantly greater activity than BHT and BHA at all concentrations ($p < 0.05$), but no significant difference was observed between extracts at 10 and 20 μg mL^{-1} and quercetin (Fig. 2). These differences could be due to various factors that need to be studied. There is no explanation as of yet for this behavior, but it is most likely related to physiological aspects such as the chemical composition of each species.

The extracts of *A. multifida* and *M. echinocarpa* at 100 and 20 μg mL^{-1}, respectively, presented approxi-

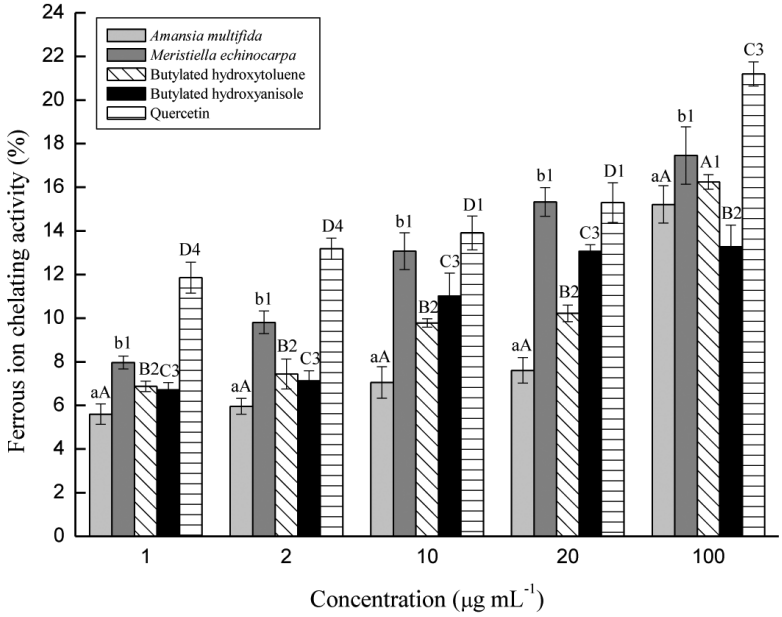

Figure 2 - Chelating ability (%) of ferrous ion of ethanolic extract of red marine algae *Amansia multifida* and *Meristiella echinocarpa* and positive controls (BHT, BHA, and quercetin). The results are expressed as average ± SD (n = 5). Small letters compare the algal extracts (two-sample *t* test) in the same concentration. Capital letters compare the extract of *A. multifida* with the positive controls (ANOVA following Dunn) in the same concentration. Arabic numerals compare the extract of *M. echinocarpa* with the positive controls (ANOVA following Dunn) in the same concentration. Same symbols indicate no statistically significant difference ($p > 0.05$) and different symbols indicate statistically significant difference ($p < 0.05$) among the concentrations.

mately 15% activity. This is comparable to the values reported by Ganesan et al. (2011) for methanolic extracts of the green macroalgae *Enteromorpha linza* and *E. tubulosa* at 500 μg mL^{-1}. Nguyen et al. (2011) reported FIC values below 10% for extracts of the green seaweed species *Caulerpa lentillifera* at 100 μg mL^{-1}.

A study published by Wang et al. (2009) showed that phenolic compounds are not strong metal chelating agents. Other compounds in the extracts, such as dietary fiber (agar, carrageenan and alginate) are also known for their ability to chelate metals. Their activity is evidenced by their inhibitory effect on the absorption of ferrous ions (Vinayak et al. 2011b).

Ferric-Reducing Antioxidant Power

Ferric-reducing power is an important indicator of the antioxidant potential of a compound or an extract (Vinayak et al. 2011b). Thus, the ethanolic extracts of *A. multifida* and *M. echinocarpa* were evaluated by ferric reducing/antioxidant power assay to determine their ability to reduce Fe^{3+} to Fe^{2+}. The ability to reduce ferric ions indicates that the antioxidant compounds are electron donors and could reduce the oxidized intermediate of lipid peroxidation processes, thus acting as primary and secondary antioxidants (Matanjun et al. 2008, Yen and Chen 1995).

Absorbance increased as the concentration of the extracts rose from 1 to 100 μg mL^{-1}. In decreasing order, the observed variation of optical density was: quercetin (0.076 – 0.532), BHA (0.072 – 0.406), BHT (0.072 – 0.384), *A. multifida* (0.045 – 0.059) and *M. echinocarpa* (0.041 – 0.05) (Fig. 3). Despite the low values observed at 100 μg mL^{-1}, the extracts of *A. multifida* and *M. echinocarpa* displayed greater reduction power than the values reported for extracts of four species of red macroalgae (*Gracilaria edulis*, *Chondrococcus hornemanni*, *Hypnea pannosa* and *Jania rubens*), four species of brown macroalgae (*Turbinaria conoides*, *Padina gymnospora*, *Dictyota dichotoma* and *Sargassum wightii*) and one species of green macroalgae (*Caulerpa lentillifera*) (Devi et al. 2008, Nguyen et al. 2011), all at the same concentration. In addition, Kumar et al. (2011) observed low values (~0.04) for the extracts of three species of red seaweeds (*Kappaphycus alvarezii*, *Gracilaria dura* and *G. salicornia*). Other authors have reported low ferric-reducing power for extracts of green, red and brown seaweeds at low concentrations (Chandini et al. 2008, Ganesan et al. 2008).

In the present study, ethanolic extracts of *A. multifida* presented low reduction activity regardless of the concentration. Thus, even at 100 μg mL^{-1}, the reduction power of the extract was approximately 9, 7 and 6.5 times smaller than that of quercetin, BHT and BHA, respectively. Similar results were obtained for extracts of *M. echinocarpa*, where reduction power was approximately 11, 8 and 8 times smaller than that of quercetin, BHT and BHA, respectively.

β-Carotene Bleaching (BCB) Assay

The seaweed extracts tested at 1 to 100 μg mL^{-1} inhibited β-carotene oxidation by approximately 40%. The corresponding values for the positive controls were 63-92.8% (BHT), 65.2-84.4% (BHA) and 63.2-85.5% (quercetin) (Fig. 4). The BCB assay makes it possible to evaluate the ability of a compound to prevent β-carotene oxidation by protecting it against free radicals generated during linoleic acid peroxidation. The antioxidant compounds found in seaweed extracts can neutralize linoleate and thereby prevent β-carotene degradation (Chew et al. 2008).

At 20 μg mL^{-1}, our extracts displayed a moderate level of antioxidant activity, inhibiting β-carotene oxidation by 41.7%. This matches the findings of Souza et al. (2011) who found methanolic extracts of the red seaweed species *Gracilaria birdiae* and *G. cornea* to inhibit β-carotene degradation by ~40%. The antioxidant activity observed for extracts of *A. multifida* and *M. echinocarpa* was greater than that reported for extracts of nine species of

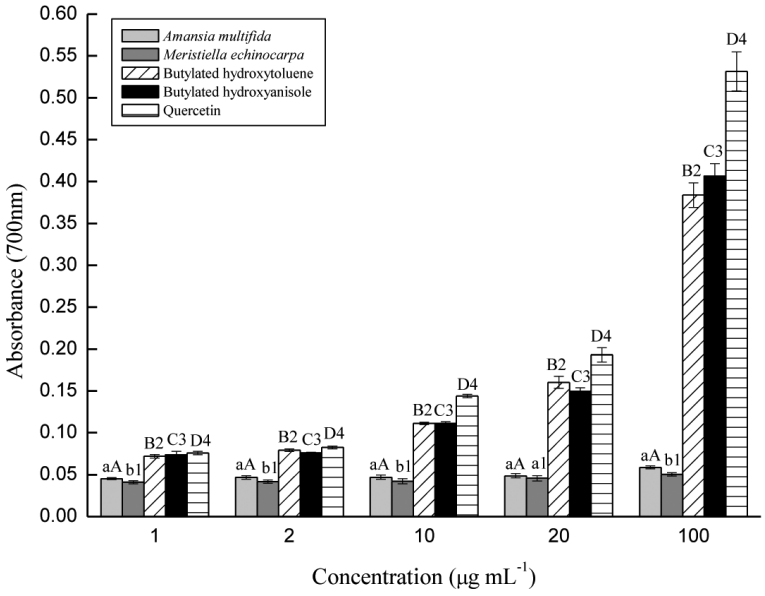

Figure 3 - Reducing power (Abs_{700nm}) of ethanolic extract of red marine algae *Amansia multifida* and *Meristiella echinocarpa* and positive controls (BHT, BHA, and quercetin). The results are expressed as average ± SD (n = 5). Small letters compare the algal extracts (two-sample *t* test) in the same concentration. Capital letters compare the extract of *A. multifida* with the positive controls (ANOVA following Dunn) in the same concentration. Arabic numerals compare the extract of *M. echinocarpa* with the positive controls (ANOVA following Dunn) in the same concentration. Same symbols indicate no statistically significant difference ($p > 0.05$) and different symbols indicate statistically significant difference ($p < 0.05$) among the concentrations.

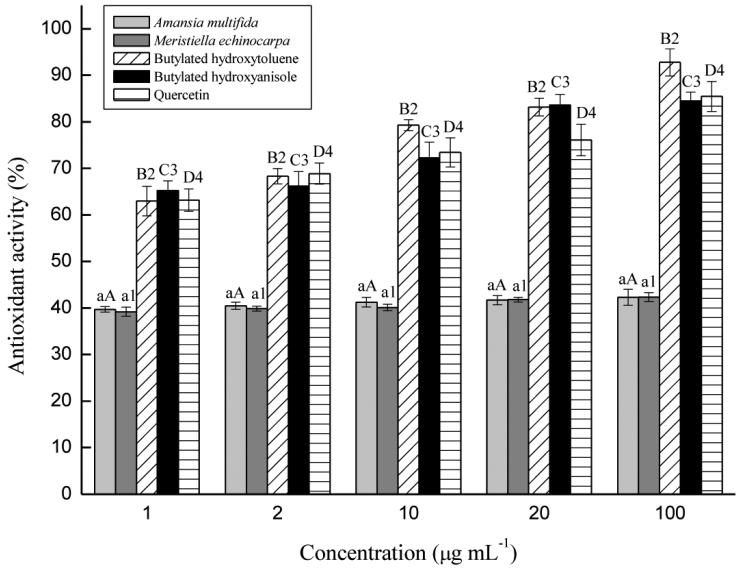

Figure 4 - β-carotene bleaching assay (%) of ethanolic extract of red marine algae *Amansia multifida* and *Meristiella echinocarpa* and positive controls (BHT, BHA and quercetin). The results are expressed as average ± SD (n = 5). Small letters compare the algal extracts (two-sample *t* test) in the same concentration. Capital letters compare the extract of *A. multifida* with the positive controls (ANOVA following Dunn) in the same concentration. Arabic numerals compare the extract of *M. echinocarpa* with the positive controls (ANOVA following Dunn) in the same concentration. Same symbols indicate no statistically significant difference ($p > 0.05$) and different symbols indicate statistically significant difference ($p < 0.05$) among the concentrations.

brown macroalgae at 50 μg mL^{-1}: *Alaria esculenta* (24.6%), *Asperococcus bullosos* (5.6%), *Bifurcaria bifurcata* (2.7%), *Cystoseria tamariscifolia* (38.8%), *Desmarestia ligulata* (-28.1%), *Dictyota dichotoma* (-24.1%), *Fucus cerenoides* (4.3%), *Fucus serratus* (16.8%) and *Saccorhiza polyschides* (23.6%) (Zubia et al. 2009).

Cytotoxic Activity by Brine Shrimp Lethality Test

The toxicity of the extracts was evaluated after 24 hours of exposure. However, the concentration required to induce 50% mortality (CL_{50}) could only be determined after 48 hours.

At 24 hours, the percentage of dead brine shrimp was calculated for each concentration. Ethanolic extracts of *A. multifida* caused an average mortality of 3.3, 6.7 and 66.7% at 50, 100 and 500 μg mL^{-1}, respectively, with no statistically significant difference between 50 and 100 μg mL^{-1}. In comparison, ethanolic extracts of *M. echinocarpa* caused an average mortality of 13.3, 40, 67 and 90% at 10, 50, 100 and 500 μg mL^{-1}, respectively (Table II).

The CL_{50} values obtained after 48 hours of exposure were 484.2 μg mL^{-1} and 281.9 μg mL^{-1} for the ethanolic extracts of *A. multifida* and *M. echinocarpa*, respectively.

TABLE II
Cytotoxic activity of ethanolic extracts of the red algal species *Amansia multifida* and *Meristiella echinocarpa* evaluated with the brine shrimp lethality test, using seawater as a negative control.

Algae / Negative control	Concentration (μg mL^{-1})	After 24 h	After 48 h
A. multifida	5	0 ± 0*	0 ± 0*
	10	0 ± 0*	10.0 ± 0*
	50	3.3 ± 5.8*	16.7 ± 5.8*
	100	6.8 ± 5.8*	36.7 ± 5.8*
	500	66.7 ± 5.8**	73.3 ± 11.6***
M. echinocarpa	5	0 ± 0*	23.3 ± 15.3*
	10	13.3 ± 5.8*	23.3 ± 5.8*
	50	40.0 ± 0*	80.0 ± 10.0***
	100	66.7 ± 11.6**	100 ± 0***
	500	90.0 ± 0***	100 ± 0***
Seawater	-	0 ± 0*	0 ± 0*

* Non-cytotoxic activity; ** Moderately cytotoxic; *** Highly cytotoxic.

Toxicity increased over time. Thus, the greatest percentages of dead brine shrimp at each concentration were registered after 48 hours of exposure for ethanolic extracts of both *A. multifida* and *M. echinocarpa*. The concentrations of 5 and 10 μg mL^{-1} did not differ significantly with regard to the percentage of dead brine shrimp (Table II).

Extracts of *M. echinocarpa* were more lethal than extracts of *A. multifida* at both 24 and 48 hours, suggesting that lethality against *Artemia* sp. was dose-dependent. The highest lethality of the extracts of *M. echinocarpa* was observed in this study but the causes need to be further investigated.

In a study by Ara et al. (1999), the toxicity of 22 ethanolic extracts was evaluated using *Artemia salina*, but only six extracts presented significant levels of lethality. These included five species of brown macroalgae (*Spatoglossum asperum*, *Stokeyia indica*, *Stoechospermum marginatum*, *Sargassum swartzii* and *S. binderi*) and one species of green macroalga (*Caulerpa racemosa*), with the respective CL_{50} values 443, 507, 612, 928, 735 and 929 μg mL^{-1}. Aqueous extracts of *S. indica* and *C. racemosa* were the most effective (CL_{50} below 70 μg mL^{-1}). Cytotoxicity could be due to the polarity of the different compounds.

When ethanolic extracts of the red seaweed species *Solieria robusta*, *Jania capillacea* and *Botryocladia leptopoda* were submitted to the brine shrimp lethality test, CL_{50} values could not be estimated after 24 hours of exposure at concentrations below 1,000 μg mL^{-1} (Ara et al. 1999).

Manilal et al. (2009) showed that secondary metabolites extracted from the red seaweed species *Laurencia brandenii* have potential cytotoxic activity against *Artemia salina.* This supports the reliability of the brine shrimp lethality test for preliminary studies on bioactive compounds derived from seaweeds.

Natural extracts and bioactive compounds previously isolated from a number of sources have been studied as biotechnological tools in search for new compounds with applications in different fields of Biotechnology, such as Pharmacology and Food Science (Pangestuti and Kim 2011, Freitas et al. 2012). The lethality test based on *Artemia salina* has been used as a preliminary test in combination with investigations of possible biological activities of these compounds, including antitumoral activity (Bagya et al. 2011, Pervin et al. 2006), antibiotic activity (Parvin et al. 2012, Pereira et al. 2008), molluscicidal activity (Patel et al. 2008) and antioxidant activity (Ganesan et al. 2008, Souza et al. 2011). In this respect, the brine shrimp lethality test has been very useful in preliminary evaluations of bioactive compounds with potential importance to Biotechnology. In view of the correlation between toxicity against *Artemia* and growth inhibition in certain cancer cell lineages, as demonstrated by researchers from the National Cancer Institute (USA), the brine shrimp lethality test is considered a valuable pretest in antitumor drug research (Anderson et al. 1991).

CONCLUSION

Ethanolic extracts of the marine red macroalgae *Amansia multifida* and *Meristiella echinocarpa* were shown to have significant antioxidant activity using DPPH, FIC and BCP. No significant activity was detected for FRAP. All the assays carried out in the present work showed that both algae are interesting source of natural antioxidant compounds. In addition, the low cytotoxic activity observed for these species make them potential tools for utilization in the food industry. The differences observed between them should be matter of further studies as, until now, no information about these species are available in literature.

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RESUMO

Antioxidantes naturais encontrados em macroalgas marinhas são substâncias bioativas conhecidas por desempenhar um papel importante na prevenção de doenças associadas com o envelhecimento, protegendo as células de danos oxidativos. O objetivo deste estudo foi avaliar as atividades antioxidante e citotóxica de extratos etanólicos de duas espécies de algas vermelhas, *Amansia multifida* e *Meristiella echinocarpa*. A atividade antioxidante *in vitro* foi determinada pela capacidade de sequestro do radical DPPH, o poder de redução do ferro (FRAP), o poder de quelação do íon ferroso (FIC), o teste de oxidação acoplada β-caroteno/ ácido linoleico (BCB), bem como a quantificação do conteúdo fenólico total. A citotoxicidade foi avaliada pelo teste de letalidade contra *Artemia* sp. Os valores das substâncias fenólicas observados no presente estudo indicaram que as espécies *A. multifida* e *M. echinocarpa* são ricas em substâncias fenólicas, atingindo valores de 45,40 e de 28,86 mg de ácido gálico equivalente (AGE) g^{-1} de extrato etanólico, respectivamente. A capacidade de seqüestro do radical DPPH e o poder de quelação do

íon ferroso foram 60% e 17%, respectivamente. Ambos os extratos algáceos inibiram a oxidação do β-caroteno em torno de 40%. Nenhum dos extratos algáceos foi potencialmente citotóxico. Os resultados mostraram que os extratos destas espécies de rodófitas apresentam potencial antioxidante e baixa toxicidade. Elas são fontes de compostos antioxidantes naturais.

Palavras-chave: antioxidante, substâncias bioativas, citotóxico, compostos fenólicos, Rhodophyta.

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