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Antioxidant activity and related chemical composition of extracts from Brazilian beach-cast marine algae: opportunities of turning a waste into a resource

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Abstract

Beach-cast marine algae are a potential biomass for several biofunctional products and occur in large volumes in some coastal regions due to natural current processes or drifting algae like pelagic *Sargassum*. Antioxidant activity and chemical composition of methanolic and aqueous extracts from fifteen beach-cast marine algae from the Brazilian Coast were evaluated. In general, the highest antioxidant activities were found in extracts from brown macroalgae followed by the extracts of red algae, and the lowest activities were detected in the green beach-cast algae. The concentrations of phenolic compounds and carbohydrates exhibited a positive correlation with the antioxidant activities of the tested extracts. To the best of our knowledge, this is one of the few worldwide studies concerning beach-cast seaweeds and antioxidant activity and related antioxidant metabolites. This study suggests that these algae are potential sources for obtaining extracts with antioxidant properties, rich in phenolic compounds and sulfated carbohydrates. Beach-cast macroalgae are unused biomass and the beneficial utilization of this biomass for prospection of natural products and functional foods is suggested.

Keywords Beach-cast seaweeds · Chlorophyceae · Functional properties · Natural products · Phaeophyceae · Phenolic compounds · Rhodophyceae · Sulfated polysaccharides

Introduction

Seaweeds have been used in many countries as food and traditional medicine since ancient times (Pérez-Lloréns et al. 2020). Macroalgae are classified into three groups, Phaeophyta (brown algae), Rhodophyta (red algae), and Chlorophyta (green algae), mainly inhabiting the marine environment under continuous abiotic factor changes. These stressing conditions can result in the synthesis and accumulation of active metabolites against oxidative stress (Maschek and Baker 2008), some of them with high-value antioxidants for a broad variety of applications, especially in food.

The incorporation of macroalgae in the human diet with antioxidant properties has attracted the interest of food industries and other commercial sectors due to the substantial benefit to prevent oxidation processes (e.g., oxidation food damage) and oxidative stress–related diseases (e.g., neurodegenerative, inflammatory, cardiovascular diseases, and aging) (Peñalver et al. 2020). In addition, macroalgae are low in calories and can be added to meat products, such as burgers, frankfurters, sausages, steaks among others (Peñalver et al. 2020). The incorporation of macroalgae or byproducts can acts as antioxidants, preserving the quality of products and as fat substitutes, developing low fat products (Gullón et al. 2020).

According to Ferdouse et al. (2018) in 2017, 30.4 million tonnes of seaweed was collected, being 29.4 million from aquaculture and 1.1 million harvested from field. Brazil has short participation in this amount; however, Brazilian littoral has great potential for collecting beach-cast marine algae.

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The beach-cast marine algae are often those organisms that naturally come off the substrate and arrive and accumulate at the beaches driven by currents, winds, and tides (do Nascimento Santos et al. 2013). In recent years, several studies have been carried out in floating seaweed clumps, especially on ecological approaches, which depend on the currents and which are also beach-cast material.

Tons of macroalgae are deposited on the beaches of certain regions along the Brazilian coast and could be used in several commercial sectors, but are often burned or buried by local governments due to the bad smell caused by the deterioration of the organic matter, which affects tourism and keeps away users of coastal environments. By contrast, beach-cast seaweeds can be reused due to their high nutritional quality and chemical diversity and converted into applications for several sectors, production of packaging, supplementation, fertilizers, food, feed, nutraceuticals, cosmetics, and even for the discovery of new drugs among others. Nowadays, beach-cast algae are already sold to supplement nutrition, and as fertilizers, some companies manufacture nutritional supplements as brown seaweed flour that can be incorporated into diets for cattle and poultry (Wischnat 2013). Beach-cast algae have the advantage of being a renewable, economically viable and abundant resource. The use of this resource is an eco-efficient production that favors society and the environment, generating usable resources that were previously seen as limited and "garbage," in addition to mitigating pollution in coastal regions and encouraging socioeconomic development in a sustainable way.

The worldwide perspective of population growth indicates accelerated and continuous increase in the next decades, which should enhance the general demand for food. The United Nations (2019) expect an increase of 2 billion people by 2050, especially in developed countries, promoting, consequently, the increase of marketable supplies and demands in the search for expansion alternatives of several industrial sectors, especially food industry. Regarding this scenario, beach-cast seaweeds represent a potential raw material that can contribute with biofunctional and bioactive properties with wide application in food (Peñalver et al. 2020).

Therefore, the main objective of this study was to analyze the antioxidant potential and chemical composition of crude extracts of beach-cast marine algae from the Brazilian coast, in order to highlight potential new natural biomass that can be used as matrix for several ingredients for the food industry and other applications.

Material and methods

Biological material and extraction procedure

Thirteen selected species and two mixtures (Mix) of beach-cast macroalgae were collected from the northeast (Ceará and Paraíba states) and southeast (Espírito Santo State) of the Brazilian coast. Identity, localization, herbarium voucher, and collection date are summarized in Table 1, and general habit is shown in Supplementary Materials, Fig. S1. The collected material was cleaned of macroepiphytes, washed in abundant tap water, with the water excess removed by manual centrifugation followed by air drying in shadow. In the laboratory, the pre-dried material was oven dried at 40 °C until constant dry matter (DM) and then ground until a fine powder in a ball mill. Each material was divided in five sub-samples (n = 5) for the extraction procedure and considered as technical replicates. Extraction was performed using solvents of increasing polarity by simple sequential dynamic maceration in hexane, dichloromethane, ethyl acetate, methanol, and water (80 °C) in a ratio of 1 g DM in 30 mL of solvent. The maceration extraction in hexane, dichloromethane, ethyl acetate, and methanol was performed at room temperature and three-times for each solvent, changing the respective solvent every 24 h. Water extraction was carried out at 80 °C in a water bath and three-times for 3 h each. The supernatant from each solvent was filtered and pooled as a single extract sample, always keeping the individuality of each technical replicate. For each material, we obtained five crude extracts (with n = 5) in hexane, dichloromethane, ethyl acetate, methanol, and water. Organic extracts were concentrated by oven drying at 40 °C and then lyophilized. Aqueous extracts were directly lyophilized. Extract yield was calculated in percentage based on initial DM used for the extraction. As both methanolic and aqueous extracts exhibited higher yields in relation to other solvents, further quantification analysis was performed only with these extracts.

Antioxidant potential

Antioxidant activities were assessed based on simple spectrophotometric and colorimetric *in vitro* methods. Lyophilized methanolic and aqueous extracts were dissolved in DMSO 10% solution, antioxidant tested in 96-well microplates with final volume of 300 μL, and the respective absorbance of each assay read in a UV-Vis microplate spectrophotometer (Epoch Biotek, USA).

DPPH (1,1-diphenyl-2-picrylhydrazyl) free-radical scavenger activity is based on an redox reaction oxy-reduction mediated by the capture of the DPPH radical by antioxidants, producing a decrease in absorbance. The assay was based on modifications performed by Pires et al. (2017a) and Santos et al. (2019) for 96-well microplate, in which an aliquot of 20 μL of sample (or standard, or negative control) was added to 280 μL methanolic DPPH solution (Sigma-Aldrich, Brazil). The reaction was incubated at 25 °C for 20 min at room temperature and then the absorbance was measured at 517 nm.

Table 1 List of selected species of beach-cast marine algae harvested from the Brazilian coast at northeast Ceará (CE) and Paraíba (PB) and southeast Espírito Santo (ES)

Taxonomical group Species	Beach (state)	Localization	No. of voucher (herbarium)	Collection date
Ochrophyta/Phaeophyceae (brown algae)				
Dictyopteris jolyana E.C. Oliveira & R.P. Furtado	Pontal Beach (ES)	20° 58′ 22.5″ S; 40° 48′ 38.6″ W	SPF58249	06/09/2017
Dictyopteris jolyana	Coqueirinho Beach (PB)	07° 17′ 58″ S; 34°47′ 54″ W	SPF58249	02/25/2012
Dictyopteris polypodioides (A.P. De Candolle) J.V. Lamouroux	Ponta do Cabo Branco Beach (PB)	07° 08′ 43.6″ S; 34° 48′ 20.7″ W	SPF58249	07/18/2016
Zonaria tournefortii (J.V. Lamouroux) Montagne	Pontal Beach (ES)	20° 58′ 22.5″ S; 40° 48′ 38.6″ W	SPF58252	06/09/2017
Rhodophyta (red algae)				
Agardhiella ramosissima (Harvey) Kylin	Itaoca Beach (ES)	20° 54′ 18.0″ S; 40° 46′ 42.3″ W	SP470206	04/30/2018
Alsidium seaforthii (Turner) J. Agardh	Piúma Beach (ES)	20° 50′ 31.5″ S; 40° 43′ 46.0″ W	SPF58253	06/11/2018
Alsidium triquetrum (S.G. Gmelin) Trevisan	Emboaca Beach (CE)	3° 12′ 23.5″S; 39° 18′ 37.1"W	SPF58318	03/30/2018
Botryocladia occidentalis (Børgesen) Kylin	Emboaca Beach (CE)	3° 12′ 23.5″ S; 39° 18′ 37.1″ W	SPF58317	03/30/2018
Gracilaria domingensis (Kützing) Sonder ex Dickie	Emboaca Beach (CE)	3° 12′ 23.5″ S; 39° 18′ 37.1″W	SPF58316	03/30/2018
Osmundaria obtusiloba (C. Agardh) R.E. Norris	Piúma Beach (ES)	20° 50′ 31.5″ S; 40° 43′ 46.0″ W	SPF58344	06/11/2017
Osmundaria obtusiloba	Ponta do Cabo Branco Beach (PB)	7° 08′ 43.6″ S; 34° 48′ 20.7″ W	SPF58082	07/18/2016
Spyridia clavata Kützing	Pontal Beach (ES)	20° 58′ 22.5″ S; 40° 48′ 38.6″ W	SPF58251	06/09/2017
Chlorophyta (green algae)				
Codium isthmocladum Vickers	Itaoca Beach (ES)	20° 54′ 18.0″ S; 40° 46′ 42.3″ W	SP470207	04/30/2018
Mix 1	Emboaca Beach (CE)	3° 12′ 23.5″ S; 39° 18′ 37.1″ W	-	03/30/2018
Mix 2	Emboaca Beach (CE)	3° 12.443″ S; 39° 18.537″ W	-	03/31/2018

Capture of the ABTS (2,2′-azinobis-(3-ethylbenzthiazolin-6-sulfonic acid) radical is one of the most used in literature able to measure the antioxidant activity of hydrophilic and lipophilic substances (Rufino et al. 2007). The assay was based on modifications performed by Torres et al. (2017) and Santos et al. (2019) for 96-well microplate, in which the ABTS solution was prepared by mixing 1 mL of ABTS (7 mM) with 17.6 μL of potassium persulfate (140 mM) and waiting reaction time for 16 h at room temperature. The absorbance of the solution was adjusted to 0.8 at 734 nm by methanol dilution (1:60 v/v). The antioxidant reaction was performed adding aliquot of 20 μL of sample (or standard, or negative control) to 280 μL of ABTS solution (pH 6.7). Absorbance was measured at 734 nm after 20 min reaction at room temperature.

Metal chelator activity is an informative feature as antioxidant test, because metals are capable of generating free radicals. Ferrozine is one of the most common compounds frequently used in the determination of Fe^{2+}. When chelating agents are present, less Fe^{2+} ions will be available for complex formation with ferrozine, which will induce a decrease in absorbance. For the metal chelating assay, we followed the method described by Harb et al. (2016) and Santos et al. (2019) modified for 96-well microplate, in which an aliquot of 20 μL of sample (or standard, or negative control) was mixed to 250 μL ammonium acetate 10% and 15 μL ammonium sulfate 1 mM. After 5 min of reaction, 15 μL ferrozine 6.1 mM was added, and the reactive mixture was incubated for more 10 min at room temperature under stirring. The absorbance was measured at 562 nm.

Ferric-reducing antioxidant power (FRAP) method emerged as an alternative to determine the iron reduction in biological fluids and aqueous solutions of pure compounds. At acid pH, ferric tripyridyl hydrazine complex (Fe^{3+} TPTZ)

is reduced producing an intense blue color, which can be monitored by measuring the absorbance. The assay was based on modifications performed by Urrea-Victoria et al. (2016) and Santos et al. (2019) for 96-well microplate, in which the FRAP solution was prepared by mixing 25 mL acetate buffer (0.3 M; pH 3.6), 2.5 mL TPTZ (10 mM in 40 mM hydrochloric acid), and 2.5 mL ferric chloride 20 mM. The reaction assay was carried out by mixing an aliquot of 20 μL of sample (or standard, or negative control), 15 μL ultrapure water, and 265 μL FRAP solution, incubated at 37 °C for 30 min and the absorbance read at 595 nm.

Standard curves of gallic acid, Trolox, and phloroglucinol were assessed as there are different equivalent units in the literature, and conversion factors (CFs) were calculated according to gallic acid equivalent (GAE), and the CF results were obtained by the ratio of ax values between the curves of the different standards ($y = ax + b$) (Supplementary Materials, Table S1). GAE was used as reference substance, and the results were expressed in mg GAE g^{-1} of lyophilized algal extract. One concentration of crude extract (600 or 1000 μg mL^{-1}) was initially tested for each antioxidant assay and material, and depending on the response of antioxidant activity, other concentrations above or below were chosen in order to evaluate the EC_{50} (half maximal effective concentration). The values of EC_{50} were calculated from dose-response curves, in which the antioxidant activities were transformed in percentage (see Supplementary Materials, Fig. S2–S7) and calculated by using a sigmoidal dose-response fit model curves (Chen et al. 2013) and the software GraphPad Prism v6.01. The results were expressed as $1/EC_{50}$ (mg mL^{-1}) to facilitate the interpretation; it means, the major EC_{50} value represents higher antioxidant activity.

For an overview of the total antioxidant activities of methanolic and aqueous extracts, the Total Antioxidant Capacity (TAC) was assessed as described in Seeram et al. (2008) based on the results of $1/EC_{50}$ (mg mL^{-1}) for DPPH, ABTS, and chelator assays and antioxidant level (mg GAE g^{-1}) for FRAP assay. Zero values were attributed to species that was not able to calculate $1/EC_{50}$. The highest value of $1/EC_{50}$ or mg GAE g^{-1} per extract and assay was assigned as 100% and the following percentages calculated according to this ratio.

Total phenolic compound, total carbohydrate, and sulfur content

The determination of phenolic compounds was carried out by the colorimetric method of Folin-Ciocalteu (Pires et al. 2017b; Santos et al. 2019). Lyophilized methanolic and aqueous extracts were dissolved in DMSO 10% solution and tested at different concentrations in a 96-well microplates with final volume of 300 μL and the absorbance of 760 nm read in a UV-Vis microplate spectrophotometer. Standard curves of phloroglucinol, gallic acid, and Trolox were performed, and conversion factors were calculated according to phloroglucinol equivalent (PGE) (Supplementary Materials, Table S1). PGE was used as reference substance, and the results were expressed in mg PGE g^{-1} of lyophilized algal extract.

The quantification of total soluble carbohydrates was performed by the phenol-sulfuric acid colorimetric method according to Masuko et al. (2005). Lyophilized methanolic and aqueous extracts dissolved in DMSO 10% were tested in 96-well microplates at final volume of 230 μL and detected at 490 nm in a UV-Vis microplate spectrophotometer. Standard curves of galactose (GAL), glucose, and fucose were performed and conversion factors were calculated according to GAL equivalent (Supplementary Materials, Table S1). GAL was used as reference substance, and the results were expressed in μg equivalent of GAL per mass of lyophilized algal extract (μg GAL mg^{-1}).

The esterified sulfur content of the extracts was quantified by the turbidimetric method of barium chloride gelatin, according to Torres et al. (2018) adapted from Dodgson and Price (1962). This procedure was chosen as a method to estimate the sulfation of hydrolyzed (that confer the amount of total sulfur) and non-hydrolyzed (free sulfur content) samples that could be related to the content of sulfated polysaccharides. Lyophilized extracts were dissolved in HCl 0.5 N at the concentration of 10 mg mL^{-1} ($n = 5$) and placed in a dry bath at 100 °C for 2 h (hydrolyzed samples). Another part of the extracts was solubilized at the same concentration (10 mg mL^{-1}) in 958 μL of ultrapure water, without heating, and just before the analysis, 42 μL of HCl 0.5 N was added (non-hydrolyzed samples). All samples were centrifuged, and the supernatant was analyzed in 96-well microplates by mixing 125 μL of ultrapure water, 25 μL of the hydrolyzed or non-hydrolyzed sample, and 50 μL of the turbidimetric reagent (5 mg mL^{-1} animal gelatin, 10 mg mL^{-1} barium chloride, 10 mg mL^{-1} sodium chloride). The microplate was placed under agitation for 5 min at 200 rpm. After 15 min rest, the absorbances were read at 405 nm in a UV-Vis microplate spectrophotometer. Standard curve of sodium sulfate was performed in concentrations from 200 to 600 μg mL^{-1} ($y = 0.0052x + 0.0703$; $R^2 = 0.97$), and data were expressed in percentage of esterified sulfur, obtain by the difference between non-hydrolyzed sample and hydrolyzed sample.

Data analysis

Statistical analyses were carried out from the extraction of five subsamples, considered as technical replication with Statistica 10 software. Data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Bartlett's test) previous one-way analysis of variance (ANOVA) to compare the species ($p < 0.05$). When differences were detected, Newman-Keuls *post hoc* multiple comparison test was applied.

Additionally, a clustered correlation matrix (Pearson correlation coefficient, *r*) by Euclidean distance was associated to a heatmap graphic for assessing the multiple paired comparisons for species and dependent variables (antioxidant potential and chemical composition). The Pearson correlation coefficient values were categorized based on Callegari-Jacques (2003): $r = 0.10$ to 0.30, weak; $r = 0.40$ to 0.6, moderate; $r = 0.70$ to 0.90, strong; $r = 0.90$ to 1, very strong.

Results

The fifteen samples of beach-cast algae collected from the northeast and southeast of the Brazilian coast were identified as four representatives of brown macroalgae (Ochrophyta, Phaeophyceae), eight of red macroalgae (Rhodophyta), one of green macroalga (Chlorophyta), and two mix samples including a mixture of different species of macroalgae (see Supplementary Materials, Fig. S1).

Results of one-way ANOVA analysis for extract yield values from methanolic (MeOH) and aqueous extracts showed significant p values < 0.005 (Supplementary Materials, Table S2). The highest extract yields were registered in the MeOH extracts, which ranged from 2.55 ± 0.13 to 29.00 ± 1.22%, and in the aqueous extracts, which ranged from 9.51 ± 0.42 to 58.50 ± 6.11% (Supplementary Materials, Table S3). The percentage of crude extract yield with other solvents, such as hexane, dichloromethane, and ethyl acetate, was lower than 1.90%. Therefore, only MeOH and aqueous extracts were evaluated for antioxidant activity and chemical composition.

The antioxidant potential of MeOH and aqueous extracts by DPPH, ABTS, Chelator, and FRAP *in vitro* assays is shown in Table 2. Regarding MeOH extracts, *Osmundaria obtusiloba* (ES), *Spyridia clavata* (ES), *Dictyopteris jolyana* (ES and PB), *Zonaria tournefortii* (ES), and *Alsidium seaforthii* (ES) showed the best results for DPPH, which ranged from 3.17 ± 0.08 to 4.10 ± 0.02 mg GAEg^{-1}. In the ABTS assay, *Z. tournefortii* (ES), *D. jolyana* (ES and PB), *Dictyopteris polypodioides* (PB), and *O. obtusiloba* (ES and PB) had the best responses between 2.72 ± 0.07 and 3.16 ± 0.03 mg GAE g^{-1}. For the Chelator assay, the best activities were observed for *D. jolyana* (PB and ES), *O. obtusiloba* (ES), *Z. tournefortii* (ES), *O. obtusiloba* (PB), and *D. polypodioides* (PB), which ranged from 8.63 ± 0.25 to 10.80 ± 0.53 mg GAE g^{-1}. The brown alga *D. jolyana* (PB and ES) had better response for the FRAP assay, followed by *O. obtusiloba* (ES), *D. polypodioides* (PB), and *Z. tournefortii* (ES) with values from 3.85 ± 0.16 to 6.99 ± 0.10 mg GAE g^{-1}.

For the aqueous extracts (Table 2), *O. obtusiloba* (PB), *Z. tournefortii* (ES), *S. clavata* (ES), *O. obtusiloba* (ES), and *D. jolyana* (PB and ES) showed the best activities in DPPH assay, with range from 3.59 ± 0.12 to 4.09 ± 0.14 mg GAE g^{-1}. For ABTS, *D. jolyana* (PB) and *O. obtusiloba* (PB) were highlighted followed by *D. jolyana* (ES), *Z. tournefortii* (ES), *D. polypodioides* (PB), and *O. obtusiloba* (ES) with results from 3.25 ± 0.11 to 4.97 ± 0.04 mg GAE g^{-1}. Regarding Chelator assay, *O. obtusiloba* (PB), *D. jolyana* (PB), *Z. tournefortii* (ES), *D. jolyana* (ES), and *O. obtusiloba* (ES) showed the highest activities with range from 12.27 ± 0.49 to 16.79 ± 2.09 mg GAE g^{-1}. For FRAP assay, *O. obtusiloba* (PB), *D. jolyana* (PB), and *Z. tournefortii* (ES) showed the best responses which ranged from 4.63 ± 0.35 to 5.71 ± 0.19 mg GAE g^{-1}. All antioxidant activities showed significant differences among the species for the respective antioxidant assay for MeOH and aqueous extracts (Supplementary Materials, Table S2).

The results of $1/EC_{50}$ for the different antioxidant assays per extract and species are described in Table 3. For MeOH extracts, $1/EC_{50}$ values for the DPPH assay ranged from 1.18 ± 0.01 to 1.56 ± 0.02 mg mL^{-1}. For the ABTS test, the $1/EC_{50}$ results ranged from 1.08 ± 0.02 to 3.57 ± 0.02 mg mL^{-1}. Finally, for Chelator assay, the values ranged between 1.18 ± 0.01 and 1.86 ± 0.03 mg mL^{-1}. Regarding aqueous extract (Table 3), the $1/EC_{50}$ for the DPPH assay ranged from 1.13 ± 0.02 to 2.22 ± 0.02 mg mL^{-1}. For the ABTS test, the $1/EC_{50}$ results ranged from 1.16 ± 0.06 to 3.20 ± 0.03 mg mL^{-1}. Finally, for Chelator assay, the values ranged between 2.16 ± 0.03 and 4.31 ± 0.04 mg mL^{-1}. For FRAP assay and some species, the $1/EC_{50}$ could not be calculated because the antioxidant activity was lower than 50% for both extracts. All the estimated $1/EC_{50}$ showed significant differences among the species for the respective antioxidant assay for MeOH and aqueous extracts (Supplementary Materials, Table S2).

Total antioxidant capacity (TAC) represents the global antioxidant activity covering all antioxidant assays, which is shown in Fig. 1. For MeOH extracts (Fig. 1a), the top five most efficient beach-cast algae with antioxidant properties were *D. jolyana* (PB—62.82%), *D. jolyana* (ES—60.46%), *O. obtusiloba* (PB—53.39%), *Z. tournefortii* (ES—52.15%), and *O. obtusiloba* (ES—51.28%), whereas the lowest TAC index (< 5%) was registered for the red algae *Botryocladia occidentalis* (CE—1.14%) and *Agardhiella ramosissima* (ES—3.04%) and the green alga *Codium isthmocladum* (ES—3.51%) (Fig. 1a). For the aqueous extract (Fig. 1B), the top five best species with highest TAC index values (> 60%) were *Z. tournefortii* (ES—79.46%), *D. jolyana* (ES—78.48%), *O. obtusiloba* (PB—78.36%), *D. jolyana* (PB—74.81%), and *O. obtusiloba* (ES—63.70%), whereas the lowest index was for *Gracilaria domingensis* (CE—2.11%) (Fig. 1b).

The percentages of calculated TAC index for antioxidant assays in MeOH and aqueous extracts are shown in Supplementary Materials, Table S4. For methanolic extracts, the antioxidant activity, considering all species, was better evaluated by the ABTS (48.21%) and FRAP (42.67%) assays. Regarding aqueous extracts, the best activities were obtained

Table 2 Antioxidant activity (mg GAE g^{-1}) for DPPH, ABTS, Chelator, and FRAP *in vitro* assays of beach-cast marine algae from Ceará (CE), Espírito Santo (ES), and Paraíba (PB) states for MeOH and aqueous extracts (mean ± SD; $n = 5$). Lowercase letters indicate significant statistical differences ($p < 0.05$) among the species according to one-way ANOVA and Newman-Keuls post hoc test per extract and assay separately

Species/assay	DPPH	ABTS	Chelator	FRAP
MeOH extracts				
Ochrophyta (brown algae)				
Dictyopteris jolyana (ES)	3.61 ± 0.26^{bc}	3.08 ± 0.06^{ab}	9.33 ± 0.48^{b}	5.22 ± 0.16^{b}
Dictyopteris jolyana (PB)	3.45 ± 0.11^{cd}	2.93 ± 0.15^{bc}	10.80 ± 0.53^{a}	6.99 ± 0.10^{a}
Dictyopteris polypodioides (PB)	2.86 ± 0.15^{e}	2.92 ± 0.10^{bc}	8.63 ± 0.25^{b}	3.93 ± 0.20^{cd}
Zonaria tournefortii (ES)	3.37 ± 0.14^{cd}	3.16 ± 0.03^{a}	8.94 ± 0.51^{b}	3.85 ± 0.16^{d}
Rhodophyta (red algae)				
Agardhiella ramosissima (ES)	0.52 ± 0.02^{g}	0.37 ± 0.15^{g}	1.99 ± 0.34^{e}	0.85 ± 0.10^{k}
Alsidium seaforthii (ES)	3.17 ± 0.08^{d}	1.87 ± 0.14^{f}	7.01 ± 0.03^{c}	3.26 ± 0.11^{e}
Alsidium triquetrum (CE)	0.50 ± 0.04^{g}	1.88 ± 0.16^{f}	4.29 ± 0.04^{d}	2.30 ± 0.18^{i}
Botryocladia occidentalis (CE)	0.59 ± 0.08^{g}	0.53 ± 0.06^{g}	1.31 ± 0.04^{e}	0.32 ± 0.01^{l}
Gracilaria domingensis (CE)	0.58 ± 0.01^{g}	2.36 ± 0.05^{e}	1.21 ± 0.06^{e}	1.29 ± 0.17^{j}
Osmundaria obtusiloba (ES)	4.10 ± 0.02^{a}	2.83 ± 0.09^{c}	9.17 ± 0.45^{b}	4.09 ± 0.02^{c}
Osmundaria obtusiloba (PB)	2.82 ± 0.09^{e}	2.72 ± 0.07^{d}	8.80 ± 0.22^{b}	3.17 ± 0.05^{ef}
Spyridia clavata (ES)	3.86 ± 0.42^{ab}	1.98 ± 0.03^{f}	6.83 ± 0.53^{c}	2.69 ± 0.13^{h}
Chlorophyta (green algae)				
Codium isthmocladum (ES)	0.35 ± 0.02^{g}	0.40 ± 0.01^{g}	2.07 ± 0.34^{e}	0.98 ± 0.04^{k}
Mix 1 (CE)	1.09 ± 0.14^{f}	2.46 ± 0.15^{e}	1.37 ± 0.04^{e}	2.81 ± 0.03^{gh}
Mix 2 (CE)	1.22 ± 0.05^{f}	2.05 ± 0.07^{f}	1.80 ± 0.04^{e}	2.99 ± 0.01^{fg}
Aqueous extracts				
Ochrophyta (brown algae)				
Dictyopteris jolyana (ES)	3.59 ± 0.12^{b}	3.91 ± 0.11^{c}	13.33 ± 0.80^{bc}	3.94 ± 0.20^{cd}
Dictyopteris jolyana (PB)	3.71 ± 0.18^{b}	4.97 ± 0.04^{a}	16.69 ± 1.29^{a}	5.48 ± 0.06^{a}
Dictyopteris polypodioides (PB)	2.61 ± 0.15^{d}	3.40 ± 0.08^{d}	10.95 ± 0.20^{d}	3.49 ± 0.04^{d}
Zonaria tournefortii (ES)	3.82 ± 0.21^{b}	3.77 ± 0.19^{c}	14.24 ± 0.30^{b}	4.63 ± 0.35^{b}
Rhodophyta (red algae)				
Agardhiella ramosissima (ES)	1.09 ± 0.01^{e}	1.97 ± 0.19^{f}	4.09 ± 0.84^{g}	2.20 ± 0.45^{g}
Botryocladia occidentalis (CE)	0.06 ± 0.01^{f}	0.81 ± 0.14^{i}	2.79 ± 0.69^{ghi}	2.25 ± 0.09^{g}
Alsidium seaforthii (ES)	3.15 ± 0.16^{c}	3.05 ± 0.07^{e}	5.90 ± 0.29^{f}	2.59 ± 0.06^{f}
Alsidium triquetrum (CE)	0.07 ± 0.03^{f}	0.55 ± 0.06^{j}	1.15 ± 0.21^{i}	3.94 ± 0.29^{c}
Gracilaria domingensis (CE)	0.25 ± 0.09^{f}	2.95 ± 0.12^{e}	1.99 ± 0.03^{hi}	0.59 ± 0.03^{h}
Osmundaria obtusiloba (ES)	3.71 ± 0.07^{b}	3.25 ± 0.11^{d}	12.27 ± 0.49^{c}	3.94 ± 0.23^{c}
Osmundaria obtusiloba (PB)	4.09 ± 0.14^{a}	4.22 ± 0.11^{b}	16.79 ± 2.09^{a}	5.71 ± 0.19^{a}
Spyridia clavata (ES)	3.81 ± 0.11^{b}	2.91 ± 0.11^{e}	8.92 ± 1.02^{e}	2.75 ± 0.20^{f}
Chlorophyta (green algae)				
Codium isthmocladum (ES)	0.15 ± 0.06^{f}	0.14 ± 0.04^{k}	1.27 ± 0.05^{i}	2.19 ± 0.17^{g}
Mix 1 (CE)	0.07 ± 0.09^{f}	1.22 ± 0.05^{h}	2.12 ± 0.04^{hi}	3.03 ± 0.13^{e}
Mix 2 (CE)	0.21 ± 0.03^{f}	1.71 ± 0.07^{g}	3.64 ± 0.03^{gh}	3.88 ± 0.04^{cd}

by the methods FRAP (48.27%), DPPH (43.73%), and ABTS (41.26%). Considering the average of the total TAC of all species and assays, it was possible to observe that aqueous extracts were more efficient than MeOH, reaching 40.82% and 34.57% of TAC, respectively.

The contents of total phenolic compounds, soluble carbohydrates, and sulfation degree are shown in Table 4. The top five highest content of phenolic compounds for MeOH extracts was registered in the beach-cast seaweeds *D. jolyana* (PB), *O. obtusiloba* (PB), *Z. tournefortii* (ES), *O. obtusiloba* (ES), and *D. jolyana* (ES), ranging between 77.30 ± 0.99 and 141.55 ± 6.35 mg PGE g^{-1}. Regarding aqueous extract, the top five phenolic compound contents were higher for *D. jolyana* (PB) and *O. obtusiloba* (PB), followed by *Z. tournefortii* (ES), *D. polypodioides* (PB), and *D. jolyana* (ES), with values ranged between 69.51 ± 2.35 and 118.51 ± 1.20 mg PGE g^{-1}. All phenolic compound contents showed significant differences among the species for MeOH and aqueous extracts (Supplementary Materials, Table S2).

Table 3 Antioxidant activity expressed as $1/EC_{50}$ (mg mL^{-1}) of beach-cast seaweeds from Ceará (CE), Espírito Santo (ES), and Paraíba (PB) states for MeOH and aqueous extracts (mean ± SD; $n = 5$). Lowercase letters indicate significant statistical differences ($p < 0.05$) among the species according to one-way ANOVA and Newman-Keuls post hoc test. *ND* $1/EC_{50}$ not detected due activity lower than 50%

Species/assay	DPPH	ABTS	Chelator
MeOH extracts			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	1.56 ± 0.02a	1.90 ± 0.02c	1.85 ± 0.04a
Dictyopteris jolyana (PB)	1.32 ± 0.01b	1.76 ± 0.06c	1.78 ± 0.01a
Dictyopteris polypodioides (PB)	1.18 ± 0.01c	1.99 ± 0.01c	1.18 ± 0.01d
Zonaria tournefortii (ES)	1.35 ± 0.03b	1.94 ± 0.03c	1.61 ± 0.04b
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	ND	ND	ND
Alsidium seaforthii (ES)	1.51 ± 0.03a	1.08 ± 0.05d	ND
Alsidium triquetrum (CE)	ND	1.72 ± 0.03c	ND
Botryocladia occidentalis (CE)	ND	ND	ND
Gracilaria domingensis (CE)	ND	2.94 ± 0.04b	ND
Osmundaria obtusiloba (ES)	1.27 ± 0.01b	1.62 ± 0.01c	1.86 ± 0.03a
Osmundaria obtusiloba (PB)	1.29 ± 0.01b	2.79 ± 0.02b	1.37 ± 0.02c
Spyridia clavata (ES)	1.30 ± 0.01b	1.08 ± 0.02d	ND
Chlorophyta (green algae)			
Codium isthmocladum (ES)	ND	ND	ND
Mix 1 (CE)	ND	3.22 ± 0.02b	ND
Mix 2 (CE)	ND	3.57 ± 0.02a	ND
Aqueous extracts			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	2.07 ± 0.01ab	3.20 ± 0.03a	3.16 ± 0.01b
Dictyopteris jolyana (PB)	2.06 ± 0.03ab	2.41 ± 0.20b	2.57 ± 0.01bc
Dictyopteris polypodioides (PB)	1.13 ± 0.02e	2.21 ± 0.20b	2.16 ± 0.03c
Zonaria tournefortii (ES)	1.79 ± 0.02cd	2.50 ± 0.02b	4.31 ± 0.04a
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	ND	ND	ND
Botryocladia occidentalis (CE)	ND	ND	ND
Alsidium seaforthii (ES)	1.59 ± 0.04d	2.23 ± 0.30b	ND
Alsidium triquetrum (CE)	ND	ND	ND
Gracilaria domingensis (CE)	ND	ND	ND
Osmundaria obtusiloba (ES)	1.76 ± 0.02cd	2.20 ± 0.20b	2.43 ± 0.03bc
Osmundaria obtusiloba (PB)	2.22 ± 0.02a	2.54 ± 0.10b	2.57 ± 0.01bc
Spyridia clavata (ES)	1.92 ± 0.04bc	1.88 ± 0.03c	2.20 ± 0.06c
Chlorophyta (green algae)			
Codium isthmocladum (ES)	ND	ND	ND
Mix 1 (CE)	ND	1.16 ± 0.06e	ND
Mix 2 (CE)	ND	1.49 ± 0.11d	ND
Standard references			
Gallic acid[a]	460.38	611.11	179.52
Trolox[a]	119.15	264.92	
Phloroglucinol[a]	18.16		
Alfa-tocopherol[b]	3.22		
BHT[b]	6.25		
Ascorbic acid[b]	11.11		
BHA[b]	16.66		

[a] Present study

[b] Zubia et al. (2007)

Regarding carbohydrate content (Table 4), the species that showed better results in MeOH extracts were *D. jolyana* (ES and PB), *D. polypodioides* (PB), *O. obtusiloba* (PB), *A. seaforthii* (ES), *O. obtusiloba* (ES), and *Z. tournefortii* (ES), with values varying among 49.48 ± 4.06 to 95.19 ± 9.73 μg GAL mg^{-1}. For aqueous extracts, the species *D. jolyana* (ES), *Z. tournefortii* (ES), *D. jolyana* (PB), *S. clavata* (ES), and *O. obtusiloba* (ES) showed the highest carbohydrate content, with values of 123.89 ± 15.32 to 416.91 ± 27.07 μg GAL mg^{-1}. All carbohydrate contents showed significant differences among the species for MeOH and aqueous extracts (Supplementary Materials, Table S2).

The sulfation degree (Table 4) of MeOH extracts was highlighted for the red alga *O. obtusiloba* (ES and PB) and the brown algae *D. jolyana* (ES) with results among 2.64 ± 0.91 to 5.10 ± 0.08%. In respect of aqueous extract, sulfation degree showed high values for *S. clavata* (ES), *G. domingensis* (CE), *A. ramosissima* (ES), *O. obtusiloba* (ES), and *D. polypodioides* (PB), with data ranging from 4.39 ± 0.97 to 7.88 ± 1.00%. All sulfation degree values showed significant differences among the

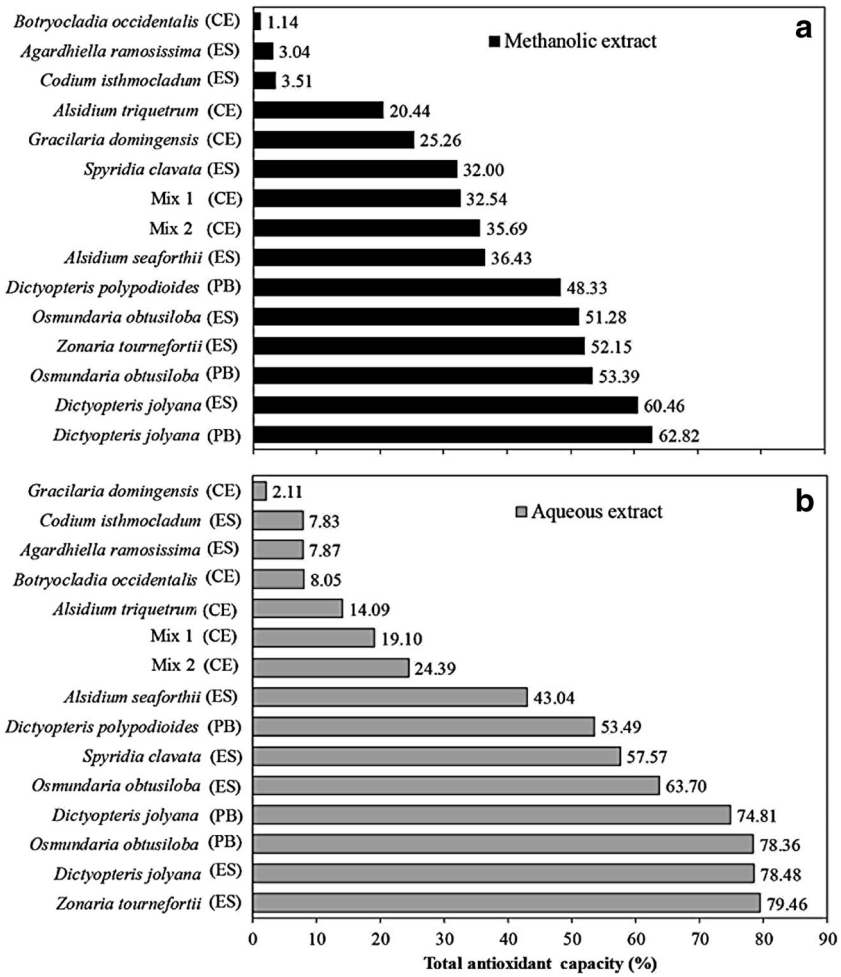

Fig. 1 Ranking of total antioxidant capacity (TAC) percent of the selected beach-cast marine algae for **a** MeOH and **b** aqueous extracts (mean; $n = 5$) from Ceará (CE), Espírito Santo (ES), and Paraíba (PB) states, considering DPPH, ABTS, Chelator in $1/EC_{50}$ (mg mL^{-1}), and FRAP (mg GAE g^{-1}) in vitro assays

species for MeOH and aqueous extracts (Supplementary Materials, Table S2).

Hierarchical clustering Euclidean distance was performed for similarity comparison of antioxidant potential and chemical composition for the MeOH and aqueous extracts (separately) (Fig. 2). The species with the best response for the parameters tested in MeOH extracts were grouped together by the brown beach-cast seaweeds and the red species *O. obtusiloba* (PB and ES), *A. seaforthii* (ES), and *S. clavata* (ES) (Fig. 2a). They were arranged according to the higher results in most of the parameters analyzed, except for *S. clavata* and *D. polypodioides* for sulfation degree and *S. clavata* and *A. seaforthii* for ABTS assay. Sulfation degree was the most distant descriptor.

According to the values of Pearson's correlation coefficient described in Supplementary Materials, Figure S8, for MeOH extracts, the data show a positive strong–very strong correlation (between r 0.78 and 0.96; $p < 0.05$) for most of the antioxidant assays, amount of carbohydrates, and phenolic compounds for the MeOH extracts, except for some pair-comparison for DPPH, ABTS, and Chelator assays and carbohydrates. Sulfation degree showed moderate correlation with the other parameter and r values from 0.35 to 0.55.

The hierarchical clustering Euclidean distance for the aqueous extracts (Fig. 2b) and the same species grouped for MeOH extracts were arranged together for aqueous extracts, and these species showed the best results in the analyzed parameters, except for some species in FRAP assay and sulfation degree. Sulfation degree was again the most distant descriptor.

Regarding aqueous extract and Pearson's correlation coefficients, a positive strong–very strong correlation (between r 0.70 and 0.93, $p < 0.05$) for most of the antioxidant assays (DPPH and Chelator), phenolic compounds, and carbohydrates (see Supplementary Materials, Fig. S9). Correlation from FRAP assay showed weak (r 0.24) and moderate correlations (r 0.42–0.57). Positive strong correlation was also observed between DPPH (r 0.74) or Chelator (r 0.72) or phenolic compounds (r 0.70) and carbohydrate content. Sulfation degree showed no correlation or weak correlation with the other parameters.

Table 4 Phenolic compounds (mg PGE g^{-1}), carbohydrates (μg GAL mg^{-1}), and sulfation degree (%) of the beach-cast seaweeds from Ceará (CE), Espírito Santo (ES), and Paraíba (PB) states for MeOH and aqueous extracts (mean ± SD; $n = 5$). Lowercase letters indicate significant statistical differences ($p < 0.05$) among the species according to one-way ANOVA and Newman-Keuls post hoc test per extract and assay separately

Species/parameter	Phenolic compounds	Carbohydrates	Sulfation degree
MeOH extracts			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	77.30 ± 0.99e	95.19 ± 9.73a	2.64 ± 0.91c
Dictyopteris jolyana (PB)	141.55 ± 6.35a	93.80 ± 13.38a	1.00 ± 0.25def
Dictyopteris polypodioides (PB)	58.59 ± 0.64f	74.36 ± 4.55b	0.05 ± 0.11h
Zonaria tournefortii (ES)	89.85 ± 1.66c	49.48 ± 4.06d	1.54 ± 0.25d
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	36.52 ± 3.49h	7.60 ± 0.86f	0.49 ± 0.08fgh
Alsidium seaforthii (ES)	46.69 ± 3.56g	57.86 ± 2.49cd	1.39 ± 0.27de
Alsidium triquetrum (CE)	20.49 ± 0.35j	11.27 ± 0.64f	0.87 ± 0.14efg
Botryocladia occidentalis (CE)	12.30 ± 0.29k	3.95 ± 0.37f	0.13 ± 0.02gh
Gracilaria domingensis (CE)	28.15 ± 4.39i	9.23 ± 0.88f	0.10 ± 0.05gh
Osmundaria obtusiloba (ES)	82.20 ± 3.33d	53.81 ± 1.88d	5.10 ± 0.08a
Osmundaria obtusiloba (PB)	117.56 ± 3.48b	63.75 ± 3.41c	3.34 ± 0.25b
Spyridia clavata (ES)	62.15 ± 2.15f	34.92 ± 2.29e	0.39 ± 0.19fgh
Chlorophyta (green algae)			
Codium isthmocladum (ES)	19.17 ± 1.60j	6.02 ± 1.20f	0.42 ± 0.05fgh
Mix 1 (CE)	22.97 ± 0.49ij	5.35 ± 1.24f	0.40 ± 0.10fgh
Mix 2 (CE)	25.53 ± 0.56ij	6.13 ± 0.44f	0.54 ± 0.16fgh
Aqueous extracts			
Ochrophyta (brown algae)			
Dictyopteris jolyana (ES)	69.51 ± 2.35e	416.91 ± 27.07a	3.33 ± 0.11d
Dictyopteris jolyana (PB)	118.51 ± 1.20a	258.70 ± 9.57c	1.21 ± 0.21f
Dictyopteris polypodioides (PB)	79.82 ± 2.95d	84.25 ± 13.72gf	4.39 ± 0.97bc
Zonaria tournefortii (ES)	98.42 ± 2.42c	354.66 ± 8.59b	1.15 ± 0.43f
Rhodophyta (red algae)			
Agardhiella ramosissima (ES)	8.82 ± 2.64m	99.27 ± 6.86f	4.85 ± 0.49b
Alsidium seaforthii (ES)	55.89 ± 3.01g	100.41 ± 12.32f	2.09 ± 0.32ef
Alsidium triquetrum (CE)	20.84 ± 0.79k	74.45 ± 10.69g	3.14 ± 0.97de
Botryocladia occidentalis (CE)	6.46 ± 0.83m	64.82 ± 7.27gh	1.66 ± 0.77f
Gracilaria domingensis (CE)	14.71 ± 1.35l	47.58 ± 3.30h	5.17 ± 1.03b
Osmundaria obtusiloba (ES)	59.60 ± 2.90f	123.89 ± 15.32e	4.51 ± 0.26bc
Osmundaria obtusiloba (PB)	105.90 ± 2.85b	81.99 ± 9.89gf	2.22 ± 0.12ef
Spyridia clavata (ES)	45.96 ± 2.79h	157.91 ± 14.08d	7.88 ± 1.00a
Chlorophyta (green algae)			
Codium isthmocladum (ES)	5.36 ± 1.15m	49.28 ± 5.75h	1.32 ± 0.22f
Mix 1 (CE)	35.39 ± 2.79j	18.81 ± 2.14i	3.68 ± 0.22cd
Mix 2 (CE)	39.67 ± 1.32i	70.23 ± 3.15hg	2.88 ± 0.35de

Discussion

Algal secondary metabolites of polar and nonpolar nature have great interest due to their bioactive properties. Among the prominent polar metabolites from methanolic extracts in marine algae, there are monosaccharides; heterosides; amino acids; sulfonic, dicarboxylic, and tricarboxylic acids; phenolic compounds; polar terpenoids; polyketides like acetogenins; and others (Esquivel-Hernández et al. 2017). Some compounds such as mycosporine-like amino acids (MAAs) are found mainly in red algae extracts, and phlorotannins are generally rich in brown macroalgae (Shibata et al. 2008). Aqueous crude extracts present as major components sulfated polysaccharides (Dobrinčić et al. 2020) that differ in the three groups of seaweeds (Phaeophyceae, Rhodophyta, and Chlorophyta).

Metabolites of nonpolar nature include fatty acids, glycolipids, terpenes, and steroids, among others. Although these metabolites have a wide bioactivity described as antiviral, antitumor, antifungal, and other activities (Mayer et al. 2013), there is a major limitation due to low yield, which makes prospection or more in-depth studies difficult.

The extract yields obtained in this study with hexane, dichloromethane, and ethyl acetate were low (lower than 1%) when compared with MeOH and aqueous extracts, which justifies our targeting with these last extracts. Similar results were obtained by Araújo et al. (2020) studying the red macroalga *Kappaphycus alvarezii*. Additionally, the highest extract yields obtained for MeOH and aqueous extracts for our selected species of beach-cast marine algae suggest that the main matrix of secondary metabolites is composed of polar components. These compounds may be involved in the defense process in macroalgae, suggesting that these metabolites, or at least some of them, may have an important role in the antioxidant results observed for the beach-cast seaweeds.

Sulfated polysaccharides, with polar nature, have been widely studied in macroalgae due to their bioactivity and also as a source of antioxidants; these compounds can be used as a functional ingredient in many applications to obtain functional foods (Porse and Rudolph 2017). Brown algae synthesize

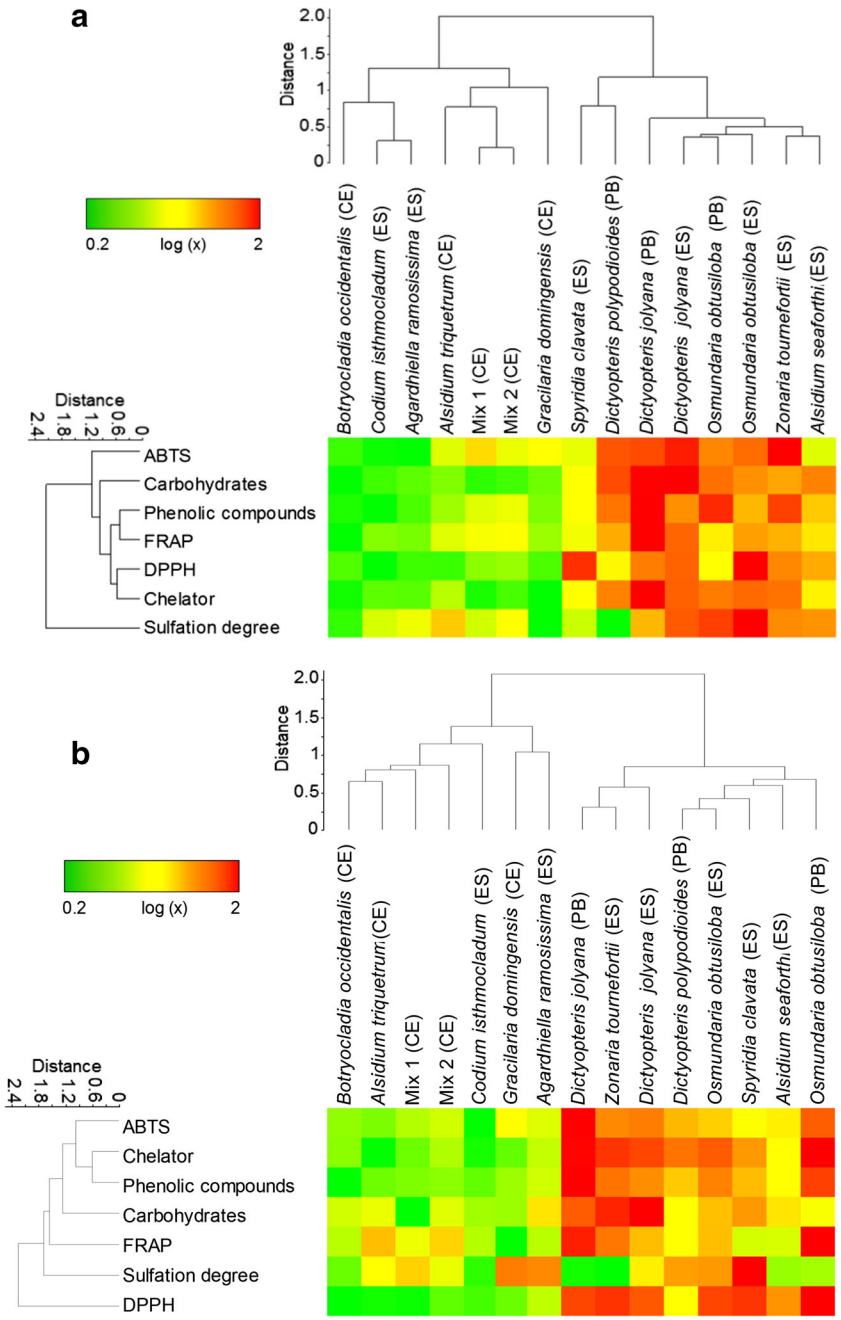

Fig. 2 Hierarchical analysis of cluster Euclidean distance associated to the heatmap representation with the responses of the **a** MeOH and **b** aqueous extracts of all parameters analyzed with beach-cast macroalgae species from Ceará (CE), Espírito Santo (ES), and Paraíba (PB) states of the Brazilian coast

fucans and fucoidans as sulfated polysaccharides, while red algae synthesize carrageenan and agar sulfated galactans, and green algae are characterized by high levels of heterofucans (de Jesus Raposo et al. 2015). The fact that most of these polysaccharides are water-soluble compounds and are present in the external matrix of the cell wall makes extraction with high temperature a necessary procedure. Besides, the increase of temperature in the extraction methods allows the development of faster and more efficient extraction of target compounds of interest.

Therefore, the highest yield percentages obtained from our aqueous beach-cast extracts compared with MeOH extracts can be explained by the cell wall polysaccharide composition, which can constitute at least 50% of dry weight in macroalgae; also, this behavior can be explained due to accelerated molecule diffusion and higher solubility of solutes at high temperature. It has been previously demonstrated that the use of water at high temperatures is a valuable tool for the extraction of metabolites in macroalgae, such as polysaccharides and phenolic compounds. Extraction yield, total phenols, total phlorotannins, and antioxidant activity have been optimized with increasing temperature in macroalgae extracts (del Pilar Sánchez-Camargo et al. 2016), in which polyphenols are attached to cell wall polysaccharides.

In the present study, all the beach-cast species showed content of phenolic compounds in both extracts; these results are in agreement with other studies, which confirms the presence of these metabolites in cold, warm, aqueous and hydroalcoholic extracts (Esquivel-Hernández et al. 2017). Phenolic compounds, such as polyphenols, phenolic acids, and bromophenols, are reported as excellent antioxidants for red algae (Mayer et al. 2013), and the antioxidant activity is attributed to these compounds because the –OH group of phenolic substances is capable of transferring an electron and also donating hydrogen (Fernando et al. 2016).

High antioxidant activities were corroborated for the extracts of the brown beach-cast *D. jolyana*, *D. polypodioides*, and *Z. tournefortii*. The high antioxidant activity in brown algae has been also attributed to the presence of high levels of phenolic compounds, especially phlorotannins (Shibata et al. 2008). In our results, the antioxidant activity reported for the beach-cast algae is related to the presence of phenolic compounds in the extracts analyzed, as positive Pearson's correlation was attributed. The presence of phenolic compounds suggests a great potential for functional foods. Nowadays, phenolic acid salts are used as industrial food preservatives (Cotas et al. 2020) and could be used in other forms in the preservation of food products, such as biofilms for fruits and vegetables, which are highly perishable or even in the composition of packaging to avoid product degradation. Therefore, as phlorotannins are active antioxidants in our studied brown beach-cast seaweeds, these compounds could be used in food products to limit or prevent the oxidation process as potential substitute of synthetic antioxidants in the food industry.

It is important to note that the material analyzed are crude extracts of beach-cast algae; therefore, substances such as carbohydrates, phenolic compounds, amino acids, or even the synergy among them can contribute to the reported antioxidant activity. The evaluation of EC_{50} is very useful because it is a typically employed parameter to express the antioxidant capacity and to compare the activity of different compounds in the literature (Chen et al. 2013), but the negative aspect is that it requires a greater amount of extract. A higher $1/EC_{50}$ indicates better antioxidant activity. The major antioxidant activities were registered for the aqueous extracts, indicating, once again, the great potential of the polysaccharides as an antioxidant. The beach-cast seaweeds are considered unusable materials; our results of $1/EC_{50}$ show that the extracts analyzed have similar or better antioxidant activity than reported by Zubia et al. (2007) and Bianco et al. (2015) for some attached macroalgae species. Also, the antioxidant activity from MeOH and aqueous extracts of the beach-cast seaweeds showed values of $1/EC_{50}$ close to those reported for the commercial standards alpha-tocopherol and BHT described by Zubia et al. (2007). The antioxidant tests performed present distinct mechanisms of action and reaction systems with different conditions, which can affect the reactivity of the substances present in the raw extracts. These reinforce the practice of evaluating more than one assay to better estimate the antioxidant activity in macroalgae.

All beach-cast species from this study showed antioxidant activity; however, some species exhibited better antioxidant levels than others by the global evaluation regarding the TAC index and the cluster analysis. The antioxidant test by ABTS method was the most sensitive to detect antioxidant activity, indicating that the transfer of electrons may be the main mechanism of antioxidant action of the substances present in the extracts analyzed. *D. jolyana* (PB and ES), *D. polypodioides* (PB), *Z. tournefortii* (ES), *O. obtusiloba* (PB and ES), and *A. seaforthii* (ES) were the species that showed the best results.

However, the values of extracts yield need to be considered for application. All these species proved to be more efficient with aqueous extraction and reinforce the differences in chemical composition between the extracts, which can be attributed to the large amount of sulfated polysaccharides, especially in the aqueous extract. In addition, the high yields of aqueous extracts associated with the low cost of the green solvent and simple chemical composition, mainly sulfated polysaccharides with promising biological activities, are characteristics that make it interesting for commercial application. Regarding all, *D. jolyana* (PB and ES) becomes more interesting for commercial application, due to the high yield and results showed with both extracts.

Particularly, we would like to comment about the beach-cast *G. domingensis*, because gracilarioid species have great commercial importance as source of agar, an exclusive phycocolloid produced for some red algal species (Porse and Rudolph 2017), and commonly used in salads and beverages with increasing commercial input as animal feed ingredients (Ferdouse et al. 2018). Despite the low antioxidant activity, *Gracilaria* species have shown high biological activity, such as antiviral and anti-inflammatory (Porse and Rudolph 2017). Beach-cast seaweeds with low antioxidant activity, such as *G. domingensis*, may have high biological activity. Polar metabolites are extremely promising in their bioactivity, which is why it is suggested that future studies should also invest in the evaluation of these fractions in beach-cast seaweeds.

Beach-cast seaweeds analyzed proved to be a good source of antioxidants, which makes it an excellent option to incorporate into a heart-healthy diet. The supplementation with beach-cast seaweeds to obtain functional low fat foods could be interest in countries with a high prevalence of cardiovascular disease, for instance.

The potential use of beach-cast seaweeds offers several economic and ecological benefits, whereas there is limited biomass to supply the industrial sectors. In Brazil, the northeast region account for the major volume of maricultured native seaweeds focused on gracilariod species (Marinho-

Soriano 2017), despite the marine algae, harvest from natural beds is still an important activity in the region. Most maricultured seaweed is sold as dry biomass without any added value processing (Andrade et al. 2020). Mariculture is an increasing activity in the region; however, the commercialization of beach-cast-derived bioproducts as complementary activity has the ability to promote employment and income generation improving quality of life of coastal communities.

The vast and large availability of underused biomass, usually discarded in landfills, makes overplus beach-cast algae biomass an attractive and sustainable alternative as raw material. The appropriate use of this biological material can meet growing social, economic, and public health requests in a sustainable way, being able to supply several social and commercial needs. With the expected population growth scenario, beach-cast seaweeds will be highlighted in the next years to answer the growing market and its high demand for foods and nutraceuticals. Food enrichment or natural nutraceutical stimulant is a technically and economically viable alternative that can be developed in a regulated manner on the Brazilian coast. The present research is the first report of the antioxidant potential and chemical composition for the beach-cast algae species collected in the Brazilian Coast, where the vast majority of studies in the country evaluate only taxonomy and abundance of these organisms.

Our results showed the potential of this unexplored and wasted biomass in Brazil. The beach-cast seaweeds studied may become valuable for the development of natural products and food supplementation, presenting potential as a rich source of antioxidant substances. Their use allows the development of new functional food products, fortifying their nutritional composition, quality, and health beneficial properties.

Conclusions

Action mechanisms of antioxidant substances differ depending on the chemical feature, as well as the *in vitro* antioxidant assays have different reactivity characteristics. Therefore, we strongly recommend testing different antioxidant assays to assess the total antioxidant capacity. Phenolic compounds and sulfated polysaccharides correlated positively with antioxidant capacity; therefore, it is possible to consider the contribution of these two chemical classes for the antioxidant efficiency in both extracts. Nevertheless, as crude extracts were obtained without specific fractionation or purification, the presence of other active substances is not discarded. The beach-cast seaweeds from the Brazilian coast are renewable, economically viable, and abundant potential resource of natural antioxidant with high valuable biomass for the prospection of novel functional foods and an eco-efficient production with social and environment benefits. Our study enhances the attractiveness of the use of the Brazilian beach-cast seaweeds for the development of new bioproducts, increasing employment opportunities.

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Author contribution Talissa Barroco Harb: conceptualization, methodology, formal analysis, project administration resources, writing—original draft, writing—review and editing. Mariana S. Pereira: formal analysis, writing—review and editing. Maria Irisvalda L.G. Cavalcanti: taxonomical analysis, review final version. Mutue T. Fujii: taxonomical analysis, review final version. Fungyi Chow: conceptualization, formal analysis, funding acquisition, resources, supervision, discussion and assessment all research steps, writing—review and editing.

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Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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