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Antioxidant Activity of Dictyotales from Tropical Reefs of Brazil

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Abstract
Macroalgae produce a large range of primary and secondary metabolites with ecological and economical importance. Studies on antioxidants from marine algae have increased notably, focusing on searching new sources of natural compounds for different applications, in which tropical species have been shown to have high potential, often improved by stressful environmental conditions during the tide cycle with periods of emersion and submersion. Therefore, in order to characterize the antioxidant activity and relate it to local environmental tide exposure, three species of brown marine algae, *Canistrocarpus cervicornis*, *Dictyopteris delicatula*, and *Lobophora variegata* from two beaches on the northeast coast of Brazil were studied. Dichloromethane:methanol (DCM:M) and aqueous extracts were tested for ferric reducing antioxidant power, 2,2-diphenyl-1-picrylhydrazyl, and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) antioxidant assays and total phenolic compounds. Aqueous extracts of *C. cervicornis* showed up to 10 times major antioxidant activity and phenolic compounds than DCM:M extracts. Different characteristic of antioxidant activity were identified among the beaches, species, and extracts, in which aqueous extracts from *C. cervicornis* and *L. variegata* showed the most promising matrices for future prospection of natural antioxidants.

Keywords Bioactivity · Bioprospecting · Brown algae · Coral reefs · Intertidal exposure · Marine algae · Natural products · Oxidative stress · Phenolic compounds

Introduction

Marine and freshwater macroalgae produce a large range of chemical compounds with ecological and economic importance by means of different metabolic pathways. Due to their high chemical

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diversity and relevant bioactivity of their primary and secondary metabolites, macroalgae become promising sources of bioactive molecules, among which those exhibiting antioxidant activity, have attracted greatest interest especially for human nutrition [1, 2]. Natural antioxidant researches from macroalgae have increased considerably, especially for incorporation into foods and drugs, mainly due to the toxicity of synthetic antioxidants used as additives. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butyl hydroquinone (TBHQ), and propyl-gallate (PG), have demonstrated harmful effect at high concentrations, including liver damage and neoplasms [3]. Among macroalgae, brown algae evidence great biological activity, including antioxidant activity [4–7].

Brazilian coast presents great biodiversity of macroalgae, among them are brown algae of Dictyotales order, which are rich in primary and secondary metabolites and are distributed from the north to the south coast, comprising over 6000 km of shoreline and also occurring on the oceanic islands. For the northeastern coast, Dictyotales represents the group with the greatest contribution in terms of biomass, overcoming other brown species and green and red algae [8]. Despite its great biomass in this region, there is lack of studies focused on bioactivity of tropical species of brown macroalgae, including antioxidant activity. At present, to our knowledge, only two studies have been published with tropical Brazilian northeastern species on the antioxidant activity approach of brown algae [6, 9].

Tropical reef species are interesting biological study models to understand responses to stressful conditions, as they are subject to great variations during tidal fluctuation and can be new raw material sources of natural products with antioxidant properties. Therefore, studies assessing antioxidant activity of tropical brown algae represent a huge global demand, especially in the northeastern coast of Brazil, because there is an evidence indicating that tropical macroalgae developed an effective antioxidant defense system, which may reflect an adaptation to a more aggressive solar radiation exposure [4]. In this sense, screening of antioxidant activity of Brazilian tropical macroalgae is an improved tool for searching potential species with prospection purpose for natural sources of antioxidants. Furthermore, research focused on species that have high contribution in biomass promotes a wide range of possibilities for future bioapplication and prospection for the region.

Natural products from brown algae of Dictyotales have been studied for their bioactivity and ecological role against herbivores. According to Oliveira Filho [8], *Dictyopteris delicatula* J.V. Lamouroux is considered one of the most common species along the Brazilian coast. Costa et al. [10] obtained extracts rich in polysaccharides from this species, which exhibited diverse biological activities, including anticoagulant, antiproliferative, and antioxidant potential. An extensive characterization of antioxidant potential of algae was performed by Zubia et al. [4], with 48 macroalgae from Mexico, and considering their major antioxidant potentials, one Dictyotales stood out, *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira. Moreover, this species, together with *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck, were the most active species of the eight brown algae tested.

The efficiency of biological activities depends not only on the composition characteristic of the species. In other words, it depends on the bioavailability of chemical compounds and is affected by extraction methods and differs between populations and the local environmental conditions [2, 5, 11]. Therefore, researches that prioritize the understanding of biological potential variation in macroalgae, as well as the general bioactivity screening, are essential to incentive the use of these organisms as natural sources for industrial application purpose [5]. This kind of study can improve the basic subsidies for mariculture and the inducing of selective production of determined bioactive potential [2, 11].

Thus, based on the background previously exposed, in order to characterize the antioxidant activity and relate it to local environmental tide exposure, our aim was to evaluate the variability of antioxidant potential of the most abundant Dictyotales species (*C. cervicornis*, *D. delicatula*, and *L. variegata*) from two beaches in the northeast coast of Brazil, highlighting the extract polarity and identifying the most promising species for natural antioxidant purposes.

Materials and Methods

Study Area

The coast of Pernambuco state, with 187 km of extension, is located in the tropical southwestern Atlantic ecoregion zone [12] and is considered one of the areas of greater phycological biodiversity of Brazil due to the large occurrence of consolidated subtracts**,** mainly formed by sandstone reefs [13]. The north coast of this state comprises a reef-lagoon complex, formed mainly by the Santa Cruz Channel, its estuaries, riverside areas, and a continental shelf [14]. On this region, there are two annual seasons: rainy season (March–August) with average monthly rainfall of 100 mm and dry season (September–February) with monthly rainfall below 100 mm [14].

Two beaches were selected on the northeastern coast of the state for this study, the Ponta de Pedras Beach in Goiana (07° 38′ 40″ S, 34° 47′ 58″ W) and the Jaguaribe Beach in Itamaracá Island (07° 44′ 30″ S, 34° 49′ 11″ W). Reef environments of both beaches are inserted in the intertidal zone. Ponta de Pedras reefs are much closer to coastal line, around 947 m, characterized by a greater diversity of subtracts, such as rock, sand, and algae [15]; they form a sandbed exposed intertidal environment of benthic organisms during low tide. Jaguaribe reefs consist of sand layers arranged horizontally, cemented by carbonates, approximately 40-cm thick, far from the coast 2601 m, and are examples of recent reef formations [16]. These reefs remain almost totally submerged during low tide.

Collection and Study Species

Based on greater coverage of species in Pernambuco beaches (verified in previous collections), three species of the brown algae Dictyotales order were selected, *C. cervicornis*, *D. delicatula*, and *L. variegata*. At Ponta de Pedras Beach, individuals were collected on an exposed intertidal zone during low tide in a sandbed area. At Jaguaribe Beach, the collected individuals were found submerged in the intertidal zone during low tide. Both expeditions occurred on the rainy season in 2017. Rainy season was chosen based on results of Costa Jr et al. [17], who found Dictyotales dominating the algal community in the rainy season at northeast of Brazilian coast. Afterwards, Santos et al. [7] confirmed that this season not only is better for biomass supply but also is the most promising season for algae in relation to yield and bioactivity of extracts.

Taxonomic identification was based on morphological, anatomical, and reproductive characteristics, according to specialized bibliography using Wynne [18]. The material was registered at Professor Vasconcelos Sobrinho Herbarium (PEUFR) of the Federal Rural University of Pernambuco (UFRPE) under the voucher numbers 54105 (*C. cervicornis* Ponta de Pedras), 54106 (*C. cervicornis* Jaguaribe), 54131 (*D. delicatula* Ponta de Pedras), 54107 (*D. delicatula* Jaguaribe), and 54289 (*L. variegata* Jaguaribe).

For comparisons between polarity of extracts, *C. cervicornis* and *D. delicatula* sampled from both beaches were used, whereas species comparisons were performed with three species collected only on Jaguaribe Beach, *C. cervicornis*, *D. delicatula*, and *L. variegata*.

Extraction Procedure and Extract Yield

Sampled material was washed successively in distilled water and cleaned of sand, associated fauna, epiphytes, calcareous inclusions, and salts. Then, samples were dried in open air for 7 days at shadow condition, grounded in a knife mill (Marconi, MA 630/1, Brazil), weighed, and stored at − 20 °C (Esmaltec, EFH350, Brazil).

Extraction was obtained by using the serial maceration method at the Research Support Center (CENAPESQ / UFRPE), using 150 mL of a mixture of dichloromethane and methanol (2:1 v/v; DCM:M) (PA, Modern Chemistry, Brazil) added to 15 g of powdered dry material (1 g/10 mL) for 24 h at room temperature. The supernatant was collected, and the residue was extracted three times again with DCM:M at the same ratio, obtaining four supernatants, which were pooled as a single extract and concentrated in a rotary evaporator under reduced pressure at 60 °C. This extract was identified as DCM:M.

Subsequently, in the Molecular Synthesis and Isolation Laboratory (SIM), the residue from the DCM:M extraction was again extracted by infusion for 1 h adding 200 mL of water at 60 °C; this procedure occurred only once. The obtained filtered aqueous extract was subsequently dried by lyophilization (SP Scientific, VirTis Sentry 2.0, USA). This extract was identified as H_2O.

DCM:M and H_2O extracts were kept in a desiccator and had their yields calculated taking into account the amount of algal sample used and the crude extract (CE) obtained, following this formula:

Yield (%) = (dry weight of crude extract × 100)/(dry weight of alga)

All extracts were kept at room temperature, dried condition environment, and light protected to avoid oxidation.

Antioxidant Activity and Quantification of Total Phenolic Compounds

Antioxidant assays were performed at the Laboratory of Marine Algae "Édison José de Paula" of the Institute of Biosciences at University of São Paulo; three in vitro assays were used: ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) scavenging activity. The total phenolic compounds were also assessed by using the Folin-Ciocalteu method. Gallic acid (Sigma-Aldrich, Brazil) was used as reference standard, and the results expressed in terms of gallic acid equivalent by mass of crude extract (mg GAE/g CE).

All dry algal extracts were previously solubilized in dimethyl sulfoxide (DMSO) 10% at a concentration of 3 mg/mL for DCM:M extracts and 15 mg/mL for H_2O extracts. Before performing the antioxidant assays, the diluted extracts were stored in a freezer at − 20 °C to avoid oxidation. The extracts were tested for antioxidant activity and phenolic compounds at concentration of 200 μg/mL extract as described below. The respective absorbances were read in a UV-visible microplate spectrophotometer (Epoch, Biotek, USA).

Ferric Reducing Antioxidant Power

FRAP assay was determined according to Urrea-Victoria et al. [19] modified from Benzie and Strain [20] with a reaction mixture of 20 μL of sample, 15 μL of ultrapure water, and 265 μL of FRAP reagent in a 96-well microplate. The reaction was incubated in the dark for 30 min at 37 °C, and the absorbance was read at 595 nm.

DPPH Scavenging Activity

DPPH assay was determined according to Pires et al. [21] modified from Brand-Williams et al. [22] by adding 20 μL of sample and 280 μL of DPPH solution in a 96-well microplate. The reaction was incubated in the dark for 30 min at room temperature, and then the absorbance was read at 517 nm.

ABTS•+ Scavenging Activity

ABTS assay was determined according to Torres et al. [23] modified from Rufino et al. [24] by mixing 20 μL of sample and 280 μL of ABTS•+ radical solution. The microplate with the reaction mixture was incubated in the dark for 20 min at room temperature, and then the absorbance was read at 734 nm.

Total Phenolic Compounds

The content of polyphenols was determined by using the Folin-Ciocalteu method according to Pires et al. [25] modified from Waterman and Mole [26] by adding 20 μL of sample, 200 μL of ultrapure water, 60 μL of Na_2CO_3 10%, and 20 μL of Folin-Ciocalteu reagent (2 N, Sigma-Aldrich®, Brazil). The microplate was incubated in the dark for 20 min at room temperature, and then the absorbance was read at 760 nm.

Antioxidant Activity Index

From the results obtained for antioxidant activity and polyphenol content, an index of antioxidant activity was constructed according to Seeram et al. [27], ranking the efficiency of the extracts relative to antioxidant capacity between the solvents and the species. Equal weights were assigned to all assays and also an index value of 100 for the highest activity of each assay. Subsequently, index value for the samples was calculated considering the following formula: AAI = [(sample activity/best assay activity) × 100]. For antioxidant activity index (AAI) determination, the mean of all assays for each sample was calculated. With this result, a rank of extracts was established.

Statistical Analysis

Procedural technical repetitions were performed for all parameters, in which were considered here as repetitions ($n = 3$) for statistical condition. Results were tested in normality by using the Kolmogorov-Smirnov test and homoscedasticity of variances by using the Bartlett test ($p < 0.05$). Statistical analysis was performed using analysis of three-factorial variance (three-way ANOVA) for determining differences between solvents, locations, and species, and one-

factorial variance (ANOVA) for determining differences between species from the same beach. A posteriori Newman-Keuls test was carried out for multiple comparison to detect differences among means. All statistical tests were performed with a significance level of 95% ($p < 0.05$). Results were expressed as mean ± standard deviation ($n = 3$). Analyses were performed in the Statistica v10 software (Stat Soft. Inc., USA).

Results and Discussion

Extract Yields

Yields of DCM:M and H_2O extracts can be seen in Table 1. DCM:M extracts resulted in higher yields than H_2O extracts for all studied species. It is considered that both polarity of solvent and solubility of compounds are crucial for extract yield but also the time of extraction [28]. In our case, despite the different polarities between the extractants, the differences in yields are attributed mainly to the time of extraction procedures. DCM:M extracts were obtained from four consecutive extractions for 24 h each, whereas H_2O extracts resulted from one single extraction for 1 h. As reported by Turkmen et al. [28], the time of extraction improves the extract yield, as the saturation of extractants is avoided.

Concerning yields of the three species from Jaguaribe, for both solvents, *C. cervicornis* had the highest yields followed by *D. delicatula* and *L. variegata*, evidencing an interspecific variation.

Antioxidant Potential of *C. cervicornis* and *D. delicatula* Extracts from Both Beaches

The antioxidant potentials by the FRAP assay for DCM:M and H_2O extracts are presented in Fig. 1a. Differences between solvents were evidenced; H_2O extracts exhibited higher antioxidant potential, except for *D. delicatula* from the Jaguaribe Beach, which did not present significant differences between solvents (DCM:M, 5.28 ± 0.12-mg GAE/g CE and H_2O, 4.33 ± 0.20-mg GAE/g CE; Fig. 1a). H_2O extracts of *C. cervicornis* presented the greatest antioxidant potential at both beaches (Ponta de Pedras 25.93 ± 1.60-mg GAE/g CE and Jaguaribe 20.48 ± 1.60-mg GAE/g CE; Fig. 1a).

Concerning the DPPH assay (Fig. 1b), differences between solvents were also evidenced; H_2O extracts exhibited higher DPPH scavenging potential for *C. cervicornis* extracts, while DCM:M extracts were more active for *D. delicatula* extracts (Ponta de Pedras 3.27 ± 0.05-mg GAE/g CE and Jaguaribe 4.99 ± 0.39-mg GAE/g CE; Fig. 1b). Once more, H_2O extracts of

Table 1 Yields of DCM:M and H_2O extracts of *Canistrocarpus cervicornis*, *Dictyopteris delicatula*, and *Lobophora variegata* from the Ponta de Pedras Beach and the Jaguaribe Beach. Results are expressed as percentage of dry mass of algae

Species	DCM:M extracts	H_2O extracts	Total yield
C. cervicornis Ponta de Pedras	10.96	1.97	12.93
D. delicatula Ponta de Pedras	7.74	1.50	9.24
C. cervicornis Jaguaribe	7.89	3.47	11.36
D. delicatula Jaguaribe	5.74	2.86	8.60
L. variegata Jaguaribe	2.29	2.16	4.45

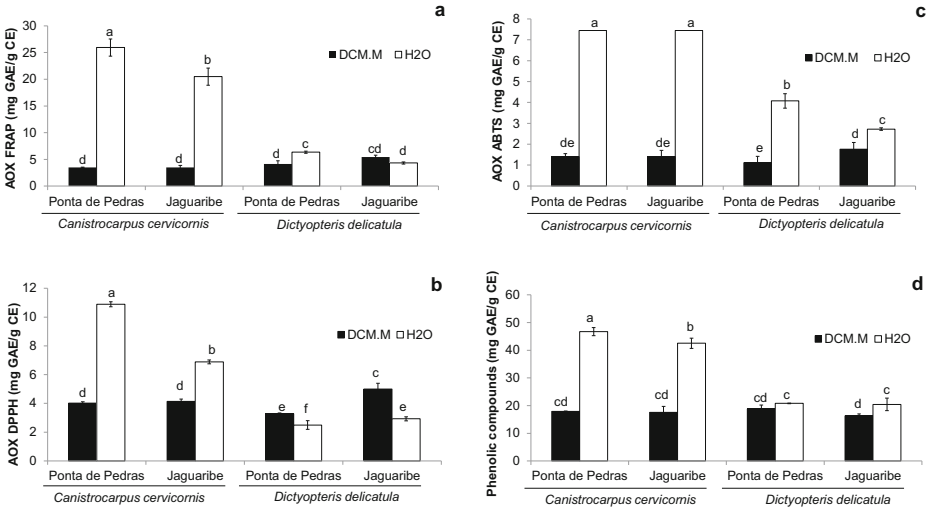

Fig. 1 Antioxidant potential (AOX) by the **a** FRAP, **b** DPPH, and **c** ABTS assays, and **d** total phenolic compounds of DCM:M and H_2O extracts of *Canistrocarpus cervicornis* and *Dictyopteris delicatula* collected on the beaches of Ponta de Pedras and Jaguaribe. Values are expressed as mean ± SD ($n = 3$). Different letters represent significant differences ($p < 0.05$)

C. cervicornis presented the greatest antioxidant potential at both beaches (Ponta de Pedras 10.89 ± 0.17-mg GAE/g CE and Jaguaribe 6.88 ± 0.13-mg GAE/g CE; Fig. 1b).

The $ABTS^{\bullet+}$ scavenging activity data are shown in Fig. 1c. As the last two antioxidant assays, differences between the solvents were also evidenced but, unlike them, H_2O extracts exhibited higher $ABTS^{\bullet+}$ scavenging potential for all species tested. Highlighting once more, H2O extracts of C.cervicornis with the greatest antioxidant potential on both beaches, which did not present differences between them. (7.45 ± 0-mg GAE/g CE; Fig. 1c).

All antioxidant assays from *C. cervicornis* showed higher activity for H_2O extracts than DCM:M, and the same trend was not majority for extracts of *D. delicatula*. In that case, it is clear that the chemical composition of DCM:M extracts is different from that of H_2O extracts. Even when the yield of H_2O extracts was half or 10 times smaller than DCM:M extracts, H_2O extracts were more efficient in terms of antioxidant capacity, reinforcing the interpretation of differences in chemical composition between the extracts. Divergence between the extracts can be due to the large amount of phenolic compound and sulfated polysaccharides found on mucilaginous matrix of brown algae that are usually water-soluble components and also extracted at high temperature, specially sulfated polysaccharides like mannitol, laminarin, fucoidan, and alginate [29]. These compounds have great antioxidant potential, which was demonstrated by Costa et al. [10] through assays of total antioxidant capacity, hydroxyl radical scavenging activity assay, superoxide radical scavenging activity assay, ferric chelating, and reducing power. The authors extracted sulfated polysaccharides from 11 species of tropical marine algae, including six brown algae, and emphasized the great antioxidant potential of *C. cervicornis*, *Sargassum filipendula* C. Agardh, and *D. delicatula*.

Despite the morphological similarity between *C. cervicornis* and *D. delicatula*, this last species showed no apparent trend between the solvents, presenting higher activities for H_2O

extracts in some assays (FRAP in Ponta de Pedras Beach and ABTS in both beaches) and for DCM:M extracts in others (DPPH in both beaches). According to this, we realize that polarity of solvent determines quantitatively the antioxidant compounds extracted [30]. However, concerning the beaches, for most H_2O extracts, the Ponta de Pedras Beach was the site that showed species with higher bioactivity, while for DCM:M extracts, the Jaguaribe Beach exhibited higher or equal antioxidant potential. Therefore, we consider the possibility of spatial variation influence in a distinct way on the antioxidant activity of these extracts, probably due to different reef environmental conditions of these beaches, as described before [31]. Thus, the differences in *D. delicatula* extracts could be associated with the inherent characteristic of the variable chemical composition and strategies of antioxidant defenses of the species. However, major interpretations and clear trends are difficult and could be preliminary speculations. Further studies are recommended.

Polyphenolic quantification of DCM:M and H_2O extracts from *C. cervicornis* and *D. delicatula* at Ponta de Pedras and Jaguaribe beaches is shown in Fig. 1d. The phenolic compounds for DCM:M extracts (Fig. 1d) ranged from 16.28 ± 0.70 to 18.78 ± 1.42-mg GAE/g CE, and non-differences were registered between species. For H_2O extracts, phenolic content ranged from 20.43 ± 2.27 to 46.72 ± 1.44-mg GAE/g CE (Fig. 1d), and differences were registered only between the samples of *C. cervicornis*, which had the greatest antioxidant potential at both beaches (Ponta de Pedras 46.72 ± 1.44-mg GAE/g CE and Jaguaribe 42.51 ± 1.86-mg GAE/g CE; Fig. 1d), similar to that described for all antioxidant activity. Differences between the solvents were also evidenced; H_2O extracts exhibited higher phenolic content, except for *D. delicatula* from Ponta de Pedras, which did not present significant differences between solvents (DCM.M 18.78 ± 1.42-mg GAE/g CE and H_2O 20.81 ± 0.10-mg GAE/g CE; Fig. 1d).

It is important to note that H_2O extracts exhibited a much higher concentration of phenolics than DCM:M extracts for *C. cervicornis*, evidencing that water-soluble polyphenols were efficiently extracted by aqueous extracts in this species, probably due to the greater amount of these components in the chemical composition of this species. At least for *C. cervicornis*, the antioxidant activity of H_2O extracts can be explained by the presence of phenolic compounds, once the same trend was observed for these parameters. The greater activity and phenolic content in H_2O extracts of *C. cervicornis* from Ponta de Pedras probably resulted from higher production and extraction of water-soluble compounds, such as phenolic compounds and polysaccharides, as a defense mechanism to protect against stressful abiotic factors. Since these compounds are commonly characterized as being produced as a result of stressful conditions. In this study, it was noted, on previous expeditions, higher exposure to radiation in macroalgae from the sandbank of this beach, and it is well established that exudation of these compounds into the surrounding seawater aid the photoprotection [2, 32].

When relating our results of extract's yields with phenolic content, it is clear that probably H_2O extracts of *C. cervicornis* from Jaguaribe (3.47% dry mass), compared with H_2O extracts of *C. cervicornis* from Ponta de Pedras (1.97% dry mass), extracted other water-soluble compounds more than phenolics from the cellular matrix of these algae, such as alginic acid and fucoidans (sulfated polysaccharides), since this extract, despite presenting higher yield, exhibited lower phenolic content [29].

Polyphenols are characterized as mainly responsible for antioxidant activity in extracts of brown algae [33]. This also was observed in our study, where the extracts with higher polyphenol content were responsible for the greatest antioxidant potential. In general, H_2O extracts obtained better bioactivity than DCM:M extracts in this study. Turkmen et al. [28],

when testing black tea extracts (*Camellia sinensis* L.), also observed that aqueous solvents obtained both higher antioxidant activity and higher content of phenolic compounds. According to this, phenolic content has a great variation with respect to polarity of solvent, and aqueous solvents or mixtures containing water are more efficient in extraction of these hydrophilic substances [28].

Similar results of greater antioxidant activity for aqueous extracts than for organic extracts were found by Santos et al. [7], who analyzed methanolic, aqueous, and hot aqueous (70 °C) extracts from *Sargassum vulgare* C. Agardh (brown alga), *Palisada flagellifera* (J. Agardh) K.W. Nam (red alga), and *Ulva fasciata* Delile (green alga). For *S. vulgare*, the authors found the highest phenolic content for hot aqueous extract (18.94 ± 1.16-mg GAE/g CE for dry season and 38.93 ± 1.88-mg GAE/g CE for rainy season), followed by aqueous extract (13.31 ± 0.88-mg GAE/g CE for dry season and 18.07 ± 1.39-mg GAE/g CE for rainy season). Chandini et al. [34] tested Indian brown algae and also found aqueous fractions of *Sargassum marginatum* (C. Agardh) J. Agardh and *Turbinaria conoides* (J. Agardh) Kützing with the highest phenolic contents (24.61-mg GAE/g CE and 49.16-mg GAE/g CE, respectively), compared with other fractions and methanolic extracts. They also observed that aqueous fraction of *Padina tetrastromatica* Hauck showed higher phenolic content (20.04-mg GAE/g CE) than other fractions.

Therefore, the algal extracts from this study, especially aqueous extracts, show similar or higher phenolic contents than algal extracts described in the literature, highlighting the potential of Dictyotales as a great source of natural antioxidant and phenolic compounds. Despite the high level of antioxidant capacity and phenolic compounds, general screening of antioxidant properties by several antioxidant assays is recommended, as our results showed differential capacities among the assays.

Antioxidant Potential of *C. cervicornis*, *D. delicatula*, and *L. variegata* Extracts from the Jaguaribe Beach

Inter- and intra-specific variations in chemical composition and bioactivity are natural features in macroalgae, as the environment and phylogenetic diversities impose wide versatility [31]. Then, the general screening of antioxidant capacity across the biological marine diversity is fundamental to identify potential species with biotechnological prospecting purposes. Comparisons of antioxidant activity and phenolic compound contents among *C. cervicornis*, *D. delicatula*, and *L. variegata* from the Jaguaribe Beach for DCM:M and H_2O extracts are presented in Figs. 2 and 3, respectively.

Antioxidant activity for DCM:M extracts showed significant differences among the species only in FRAP (Fig. 2a) and DPPH (Fig. 2b) assays. For FRAP assay (Fig. 2a), the activity of *L. variegata* (7.80 ± 0.66-mg GAE/g CE) was higher than *D. delicatula* (5.43 ± 0.35-mg GAE/g CE) and *C. cervicornis* (3.48 ± 0.38-mg GAE/g CE). For DPPH assay (Fig. 2b), *D. delicatula* (4.99 ± 0.40-mg GAE/g CE) displayed the highest antioxidant potential followed by *C. cervicornis* (4.14 ± 0.16-mg GAE/g CE) and *L. variegata* (3.42 ± 0.30-mg GAE/g CE). Non-differences among the species were registered for ABTS assay (Fig. 2c) and total phenolic compounds (Fig. 2d), ranging between 1.40 ± 0.29 to 1.76 ± 0.32-mg GAE/g CE for ABTS assay.

Differences among antioxidant assays usually happen probably due to the sensibility of substances extracted to the particular characteristic of the assay, such as mechanism (HAT and/or SET) of action, pH condition, reaction time, among other, causing algae extracts to react in

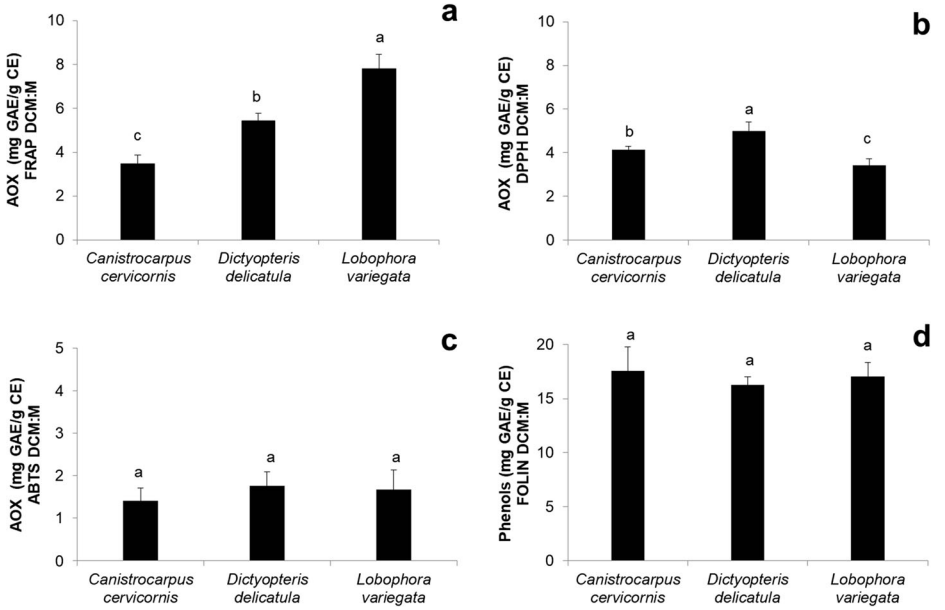

Fig. 2 Antioxidant potential and quantification of total phenolic compounds of DCM:M extracts of *Canistrocarpus cervicornis*, *Dictyopteris delicatula*, and *Lobophora variegata* collected from Jaguaribe Beach. **a** FRAP, **b** DPPH, and **c** ABTS antioxidant assays, and **d** phenolic compounds. Values are expressed as mean ± SD ($n = 3$). Different letters represent significant differences ($p < 0.05$)

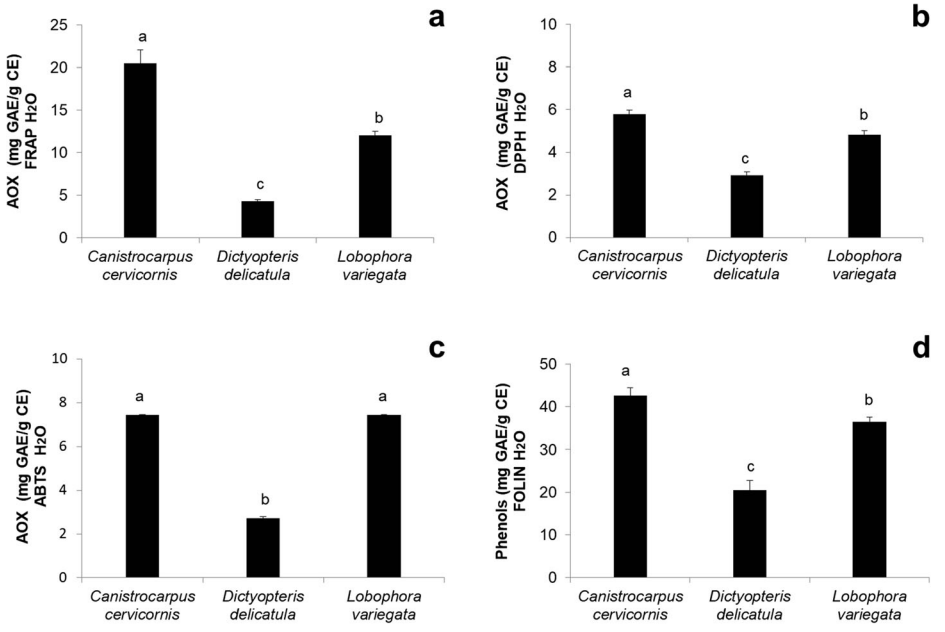

Fig. 3 Antioxidant potential and quantification of total phenolic compounds of H_2O extracts of *Canistrocarpus cervicornis*, *Dictyopteris delicatula*, and *Lobophora variegata* collected from the Jaguaribe Beach. **a** FRAP, **b** DPPH, and **c** ABTS antioxidant assays, and **d** phenolic compounds. Values are expressed as mean ± SD ($n = 3$). Different letters represent significant differences ($p < 0.05$)

different ways [35]. In this case, we can point out that *L. variegata* DCM:M extract has more active compounds to FRAP assay, probably due to fatty acids normally found in brown algae. Manilal et al. [36] characterized the presence of palmitic acid, lauric acid, stearic acid, α-linolenic acid, oleic acid, myristic acid, and hexadecatrienoic acid in DCM:M extracts from *L. variegata*, which could indicate the presence of these metabolites in our extracts. All of these non-polar metabolites identified in *L. variegata* have also showed effective bioactivity, and their complex synergy can be responsible for the higher activity of *L. variegata* in our study by the FRAP assay.

Concerning the quantification of total phenolic substances, the phenolic content ranged from 16.28 ± 0.70 to 17.54 ± 2.19-mg GAE/g CE (Fig. 2d), and no differences between the species were observed, but a higher concentration of phenolics (expressed as gallic acid equivalent) was noticed when compared with the same equivalency for antioxidant activity. These results could evidence that phenolic compounds are also responsible for the antioxidant potential of these extracts. Similar observations were made by Vasconcelos et al. [9], which suggested that the antioxidant activity of DCM:M extracts of the brown alga *Sargassum furcatum* Kützing also were related to the presence of phenolic compounds. In our study, we noticed that mixed nature of the DCM:M solvent allowed us to extract a partial amount of phenolic compounds. However, large amounts of phenolics were more successful when extracted with water (see the discussion below).

Regarding antioxidant activity for H_2O extracts a clear pattern was evidenced among the species for all antioxidant assays (Fig. 3a–c) and phenolic compounds (Fig. 3d). Major antioxidant activity and phenolics were registered for *C. cervicornis* followed by *L. variegata* and lower levels in *D. delicatula*. For FRAP assay (Fig. 3a), the activity of *C. cervicornis* was 20.48 ± 1.60-mg GAE/g CE, for *L. variegata* was 12.06 ± 0.42-mg GAE/g CE, and for *D. delicatula* was 4.33 ± 0.20-mg GAE/g CE. In ABTS assay (Fig. 3c), *C. cervicornis* and *L. variegata* displayed similar antioxidant potential (around 7.50-mg GAE/g CE) and the lowest activity for *D. delicatula* (2.72 ± 0.06-mg GAE/g CE).

Concerning the quantification of total phenolic substances for H_2O extracts, the content ranged from 20.43 ± 2.27 to 42.51 ± 1.86-mg GAE/g CE (Fig. 3d); the same pattern was noticed as antioxidant assays with *C. cervicornis* (42.51 ± 1.86-mg GAE/g CE) showing the greatest content of phenols followed by *L. variegata* (36.45 ± 1.09-mg GAE/g CE) and *D. delicatula* (20.43 ± 2.27-mg GAE/g CE). According to this, it was understood that phenolic compounds had a major influence on the antioxidant potential of these extracts. Thus, a clear correlation can be established between the responses of the antioxidant potential and quantification of phenolic compounds for these extracts. Vasconcelos et al. [9] also had observed this relation; the specie with higher phenolic content in their study, *Osmundaria obtusiloba* (C. Agardh) R.E. Norris, also had the highest antioxidant activity, establishing a positive correlation between phenol contents and antioxidant potential. This correlation was also observed in this study, with *C. cervicornis* achieving the greatest phenolic content and higher antioxidant activity, indicating that this macroalgae fits as a rich source of natural antioxidants on both beaches of this study.

Antioxidant Activity Index

From data of antioxidant assays and phenolic compound concentration, for both solvents and all species from both beaches, an AAI was calculated to assess the antioxidant ranking considering the overall responses, since AAI would represent a set of complex antioxidant

responses from the extract. The AAI values are shown in Table 2, in which the highest antioxidant potential for H_2O extracts is verified, exhibiting ranking from 1 to 4 and 6 among all extracts. H_2O extract of *C. cervicornis* from the Ponta das Pedras Beach and the Jaguaribe Beach registered the first and second AAI ranking, displaying almost the maximal antioxidant and phenolic compound level among the extracts. H_2O extract of *L. variegata* from the Jaguaribe Beach reached the 3rd ranking position. The ranking trend of DCM:M extracts was different than H_2O extracts, in which *D. delicatula* from Jaguaribe followed by *L. variegata* from Jaguaribe attained the best score among the DCM:M extracts.

The index summarizes and confirms the obtained results described separately before, with higher activity of H_2O extracts than DCM:M extracts, greatest bioactivity of *C. cervicornis* for both beaches, and different reactivity between assay and extracts, while other data was discovered, as the greater activity of H_2O extracts of *L. variegata* over H_2O extracts of *D. delicatula* of both beaches. Moreover, we could point out trends relating the best beach for bioactivity of these species, Ponta de Pedras for both *C. cervicornis* extracts and H_2O extract of *D. delicatula* and Jaguaribe for DCM:M extract of this species. Thus, through this rank, a clear pattern can be established from the set of data of this study, achieving what was proposed by the index, a single result of antioxidant activity through the combination of many antioxidant assays [27].

Therefore, this index proved to be an important tool in the evaluation of antioxidant potential and to confirm the patterns of this study. The index allowed to clarify which solvent and which species have the best antioxidant potential, besides indicating the best site to collect and to produce improved extracts. Thus, this set of data helps in the selection of the commercial harvest and the guarantee of stable and high-quality products [31].

Conclusion

The set of data from this study is vital not only to increase the knowledge of Brazilian biodiversity of bioactive compounds but also to incentive the possible application of natural

Table 2 Antioxidant activity index (AAI) calculated from the scores of antioxidant potential and phenolic compound concentration of H_2O extracts and DCM:M extracts of *Canistrocarpus cervicornis*, *Dictyopteris delicatula*, and *Lobophora variegata* from the beaches Ponta de Pedras and Jaguaribe

Species	FRAP	DPPH	ABTS	Phenolics	Antioxidant activity index (AAI)	Rank
H_2O extracts						
C. cervicornis Ponta de Pedras	100	100	99.86	100	99.96	1°
C. cervicornis Jaguaribe	78.98	63.17	100	91	83.28	2°
L. variegata Jaguaribe	46.5	44.16	100	78.03	67.17	3°
D. delicatula Ponta de Pedras	24.48	22.86	54.7	44.55	36.64	4°
D. delicatula Jaguaribe	16.69	26.9	36.55	43.73	30.96	6°
DCM:M extracts						
D. delicatula Jaguaribe	20.36	45.82	23.65	34.83	31.16	5°
L. variegata Jaguaribe	27.8	31.4	22.44	36.43	29.51	7°
C. cervicornis Ponta de Pedras	14.19	36.91	18.68	38.25	27	8°
C. cervicornis Jaguaribe	9.71	38.01	18.81	37.55	26.02	9°
D. delicatula Ponta de Pedras	18.81	30.02	14.91	40.2	25.98	10°

antioxidant from macroalgae in the diverse industrial sectors. According to these data, we can come to the conclusion that despite the lower antioxidant activity, DCM:M extracts showed higher extraction yield than H_2O extracts, indicating that a higher amount of polar and non-polar compounds was found in these macroalgae extracts. In general, all extracts of these three macroalgal species showed interesting antioxidant activity, mainly H_2O extracts which, even when presenting until 10 times lower extraction yield, exhibited higher antioxidant potential. H_2O extracts also were responsible for the highest amount of phenolic compounds, indicating a correlation between antioxidant potential and these compounds. From bioactivity of *C. cervicornis* extracts, clear variation was verified among the beaches, with local environmental characteristics influence species bioactivity and Ponta de Pedras exhibited higher antioxidant potential. Regarding *D. delicatula*, the data indicate that H_2O extracts had higher bioactivity in Ponta de Pedras, while DCM:M extracts were more active in Jaguaribe. Extracts exhibited variable antioxidant activity depending on the type of solvent, the collection site, and the assays performed. The screening of species from Jaguaribe highlight H_2O extract of *C. cervicornis* as the most active specie. In general, for better and more accurate results, it is suggested that during the rainy season, aqueous extraction on these species must be preferred, prioritizing macroalgae from Ponta de Pedras for future research and/or commercial farms. Besides, the most active extracts of this study, H_2O extracts of *C. cervicornis* from both beaches and H_2O extract of *L. variegata*, could be used as natural antioxidants in diverse industrial sectors. Future investigations about the chemical composition of these extracts are needed for a better understanding of the bioactivity behavior.

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Compliance with ethical standards

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

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