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# SCREENING FOR ANTIOXIDANT CAPACITY OF TROPICAL REEF SEAWEEDS: PROSPECTION FOR NEW NATURAL ANTIOXIDANTS

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**RESUMO.** O screening da capacidade antioxidante pode ser uma ferramenta útil, rápida e prática para identificar espécies de macroalgas marinhas potenciais para futuros estudos de prospecção. Apresentamos o primeiro screening da atividade antioxidante de 11 macroalgas marinhas representativas da região entre-marés de recifes de Pernambuco, Nordeste do Brasil, especificamente extraídas com diclorometano:metanol. Cinco diferentes ensaios in vitro (DPPH, ABTS, Quelante de metais, FRAP e Folin-Ciocalteu) foram escolhidos para avaliar a atividade antioxidante. Todas as espécies estudadas apresentaram um padrão antioxidante dose-dependente e elevada capacidade, mesmo em baixas concentrações de extrato. Ambientes recifais são ecossistemas estressantes, os quais impõem extremas condições abióticas, resultando em macroalgas marinhas adaptadas, com extrema competência para gerir o estresse oxidativo nesses ambientes. As macroalgas pardas estudadas foram as mais proeminentes junto com as verdes, reforçando a recomendação de estudos de prospecção destas espécies como fontes de antioxidantes naturais para aplicações funcionais.

**Palavras-Chave:** algas tropicais, antioxidante, espécies reativas de oxigênio, produtos funcionais, radicais livres.

**ABSTRACT.** The screening of antioxidant capacity may be a useful, rapid and feasible tool to identify potential seaweed species for future prospecting approaches. For intertidal reefs of Pernambuco, Northeastern of Brazil, we report the first antioxidant activity screening of 11 abundant seaweeds, specifically dichloromethane:methanol extracts. Five different in vitro antioxidant assays (DPPH, ABTS, Chelator, FRAP and Folin-Ciocalteu assays) were chosen for evaluating the antioxidant ability. All studied species showed a dose-dependence antioxidant pattern and elevated capacity even at low extract concentration. Reef environments are stressful ecosystems, which impose extreme abiotic conditions, resulting in adapted seaweeds that inhabit there with expressive competence to manage oxidative stress. The studied brown algae were the most prominent along with the green algae, enabling the possibility to reinforce the recommendation of further studies to prospect these species as sources of natural antioxidants for functional applications.

**Keywords:** antioxidant, free radicals, functional products, reactive oxygen species, tropical algae.

## INTRODUCTION

Among tropical marine ecosystems, the reef environments are characterized as extremely rich, holding great diversity of species (Sheppard *et al.,* 2009). The Pernambuco beach-rock reefs, located in the Northeastern Brazil, consist in marine outcrops parallel to the coastline (Kempf, 1970), with variable oceanographic conditions. In those reefs, seaweeds are often found in the intertidal zone, remaining emerged during low tides and immersed the rest of the time. As they are sessile, seaweeds are exposed to a combination of variable factors, such as desiccation, solar light, UV radiation, wave action, herbivory, among others, that may lead to the formation of free radicals and other agents that cause oxidative stress.

However, the lack of oxidative damage in seaweeds suggest that they may have diverse mechanism for protecting against oxidative damage, like production of antioxidant enzymes (superoxide dismutase, peroxidase, glutathione reductase, catalase) and antioxidant molecules (phlorotannins, ascorbic acid, tocopherols, carotenoids, phospholipids, chlorophyll related compounds, bromophenols, catechins, mycosporine-like amino acids, polysaccharides, etc.) (Fujimoto, 1990; Le Tutour *et al.*, 1998; Matsukawa *et al.*, 1997; Rupérez *et al*., 2002; Yuan *et al.,* 2005).

Seaweeds that inhabit harsh environments, such as the tropical reefs, which are abundant in Pernambuco, can overcome excessive UV radiation and temperature conditions that result from tropical geographic position (Zubia *et al.*, 2007). Several authors have demonstrated that tropical seaweeds show higher amount of antioxidant substances than other species from increasing latitudes (Kelman *et al*., 2012, Li *et al*., 2012, Martins *et al*., 2012, Silva *et al*., 2012; Zubia *et al*., 2007). Despite that, only a few studies on the antioxidant potential of tropical seaweeds have been performed, enabling an appropriate scenario for the search of new candidate species as potential sources of natural antioxidant and functional bioproducts.

The Brazilian littoral has an extensive coastline with great biodiversity of seaweeds that present several bioactivities against human pathologies (*e.g.*, chronic inflammation, atherosclerosis, cancer and cardiovascular disorders) and ageing processes (Kohen and Nyska, 2002). In view of that, our main goal was to evaluate the antioxidant capacity of the crude extracts of 11 seaweed species for prospecting purpose as natural antioxidant candidates.

## MATERIAL AND METHODS

### SAMPLING AND PREPARATION OF CRUDE EXTRACTS

A total of 11 seaweed species, belonging to Phaeophyceae, Chlorophyta and Rhodophyta (Tab. 1), were collected along the Pernambuco coastline in Northeastern Brazil, at the reefs of Enseada dos Corais (8°18'44.7"S; 34°56'49.8"W) and Boa Viagem (8°07'30.8"S 34°53'45.9"W).

The region is characterized by a tropical climate, with average temperatures ranging from 25°C to 30°C, and semidiurnal meso-tides (ranging between 2 and 4 m) dominated by waves (Amaral *et al*., 2016).

Approximately 500 g of each species were manually collected, transported to the laboratory and frozen at -20ºC. The material was washed with tap water to get rid of the excess of salt and sand, and it was also removed the associated fauna and epiphytes. Then, the material was air-dried at room temperature for about 72 h.

The dried seaweeds were ground to a fine powder and extracted for three days with a mixture of dichloromethane:methanol (2:1 v/v) with adequate solvent to cover and soak the material. The extraction procedure was repeated three times and all filtered extracts from the same species were gathered as a single extract and then evaporated in a rotavapor (R-215 Buchi, Switzerland) at 40ºC and pressures between 70 and 500 torr (Vacuubrand CVC 3000). The final dried extracts were removed from the rotary flask by acetone dilution and left to completely evaporate in an exhaustion hood. The extract yield was calculated according to the formula: yield (%) = (dried extract weight x 100) / dried seaweed weight.

## ANTIOXIDANT ASSAYS

The antioxidant capacity can act on the basis of the chemical reaction, therefore, the use of different antioxidant assays are valuable tools for a preliminary prospecting profile. The antioxidant analyses were performed in the crude extracts described above, dissolved in methanol, at different extract concentrations depending on the species. Results were expressed as percentage of antioxidant activity and $EC_{50}$ (concentration which induces a response halfway between the baseline and the maximum at defined time reaction). Gallic acid (Sigma-Aldrich, Brazil) was used as standard. All antioxidant assays were performed in triplicate and the respective absorbance was read with a UV-vis microplate spectrophotometer (Epoch Biotek, USA).

## DPPH RADICAL SCAVENGING ACTIVITY

The DPPH (2,2-diphenyl-1-picryhydrazyl) antioxidant assay was performed according to Brand-Williams *et al*. (1995), as modified by Pires *et al*. (2017a). The method is based on DPPH radical capture by antioxidants, producing an absorbance decrease at 517 nm. Aliquots of 20 µl of extracts, or standard, (dissolved in methanol) were added to 280 µl of DPPH (32 µg $ml^{-1}$) (Sigma-Aldrich, Brazil) solution. After standing for 30 minutes in the dark at room temperature, the absorbance was read at 517 nm. The DPPH radical scavenging percentage was calculated through the following equation: $[(Abs_{DPPH} - Abs_{sample}) / Abs_{DPPH}] \times 100$, where $Abs_{DPPH}$ is the control absorbance of DPPH, and $Abs_{sample}$ is the absorbance of sample in DPPH reactive solution. For

DPPH assay was also calculated the $EC_{50}$ index as the activity of the tested crude extracts that enabled their estimation.

## ABTS RADICAL SCAVENGING ACTIVITY

The antioxidant assay by ABTS (2,2–azinobis (3-ethylbenzthiazoline-6-sulfonic acid) free radical scavenging is one of the most used methods and measures the antioxidant potential of hydrophilic and lipophilic substances (Rufino *et al*., 2007). The method used was described by Rufino *et al*. (2007) and modified by Torres *et al.* (2017). Aliquots of 20 µl of extract, or standard, were added to 280 µl of ABTS radical. Absorbance was read at 734 nm after 20 minutes of incubation in the dark. ABTS radical scavenging percentage was calculated by the following formula: $[(Abs_{ABTS} - Abs_{sample})/Abs_{ABTS}] \times 100$, where $Abs_{ABTS}$ is the ABTS's control absorbance of ABTS, and $Abs_{sample}$ is the absorbance of the sample in ABTS reactive solution.

## METAL CHELATING ACTIVITY (CHELATOR)

The metal chelating activity (Chelator) determines the quantity of non-chelated iron ions in the reaction mixture remaining after chelation. The method was performed as described by Dinis *et al*. (1994) and modified by Harb *et al*. (2016). The Solutions were composed of 250 µl of acetate 10%, 15 µl of ammonium sulfate, and 20 µl of extract, with standard or methanol as blank. After 15 minutes, 15 µl of ferrozine solution were added. The microplates were homogenized and incubated in the dark for 10 minutes, followed by shaking at 100 rpm for further reading at 562 nm. The percentage of chelating potential was calculated using the following formula: $[(Abs_{Chelator} - Abs_{Chelator})/Abs_{Chelator}] \times 100$, where $Abs_{Chelator}$ is the control absorbance of the reaction mixture, and $Abs_{sample}$ is the absorbance in the Chelator reactive solution.

## FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

The ability of seaweed to act as a reducer of substances was tested through the FRAP assay, as described by Benzie and Strain (1996) and modified by Urrea-Victoria *et al*. (2016). Change of absorbance is directly related to the reducing power of the electron donors present in the reaction mixture (Ferreira *et al*., 2007). The microplates were assembled in the dark, mixing 15 µl of ultrapure water with 20 µl of sample, with standard or methanol as blank, and 265 µl of FRAP reagent (25 ml of buffer acetate 0.3 M, 2.5 ml of ferric tripyridyl hydrazine solution 10 mM and 2.5 ml of a ferric chloride aqueous solution 20 mM; Sigma-Aldrich, Brazil). Absorbance was read after 30 minutes of incubation at 595 nm. FRAP percentage was calculated as $(Abs_{sample} \times 100)/Abs_{FRAP}$, where $Abs_{sample}$ is the absorbance of the sample in the reaction mixture, and $Abs_{FRAP}$

is the maximum absorbance obtained by gallic acid standard, indicating the maximum antioxidant activity.

## FOLIN-CIOCALTEU ASSAY

This assay was described by Singleton and Rossi (1965) and Waterman and Mole (1994) and then modified by Pires *et al*. (2017b). It is based on the reductive property of total phenolic compounds that react with Folin-Ciocalteu reagent under alkaline conditions. The reaction mixture was composed of 200 µl of ultrapure water, 20 µl of sample, with standard or methanol as blank, 20 µl of Folin-Ciocalteu (1 N) reagent and 60 µl of $Na_2CO_3$ (10 mg $ml^{-1}$ of ultrapure water), followed by incubation in the dark for 30 minutes and subsequent reading at 760 nm. Folin-Ciocalteu percentage was calculated as follows: $(Abs_{sample} \times 100)/Abs_{Folin\text{-}Ciocalteu}$, where $Abs_{sample}$ is the absorbance of sample in the reaction mixture, and $Abs_{Folin\text{-}Ciocalteu}$ is the maximum absorbance obtained by gallic acid standard, indicating the maximum antioxidant activity.

## ENVIRONMENTAL PARAMETERS

Temperature (Temp), salinity (Sal), pH, dissolved oxygen (DO) and turbidity (Turb) were registered from each sampling site using a multiparameter probe (Horiba, USA) for correlating possible relationships between the antioxidant activity and abiotic parameters in a Redundancy Analysis.

## STATISTICAL ANALYSIS

The antioxidant activity percentages obtained for DPPH, ABTS, Chelator, FRAP and Folin-Ciocalteu assays with different extract concentrations were compared with one-way analysis of variance (ANOVA), prior transformation of the percentages achieve the statistical assumptions. Significant differences were determined by Tukey's post-hoc test at 95% significance level ($p < 0.05$), comparing the same extract concentration between species tested for each method. The results were expressed as mean ± standard deviation ($n = 3$). All analyses were performed with Statistica v 10. A Redundancy Analysis (RDA) was performed in Excel with XLSTAT (2014.5.03) to correlate the response of antioxidant activity with the environmental data (temperature, salinity, DO, pH and turbidity) as explanatory variables.

## RESULTS

Eleven representative species of seaweed were collected from the Pernambuco reefs: 3 Phaeophyceae, 3 Chlorophyta and 5 Rhodophyta (Tab. 1). The extract yield of each species was

variable, ranging between 0.39% for *Palisada perforata* and 10.63% for *Caulerpa cupressoides* (Tab. 1). All crude extract showed antioxidant activity for the different tests analyzed (Tab. 2), exhibiting a positive reactivity for the selected methodologies.

**Table 1**. Summary of the collected species, indicating the local and date, in Pernambuco State, Northeastern Brazil. The extract yield (%) for each species is also included.

| Species | Extract yield | Sampling site | Sampling date |
|---|---|---|---|
| PHAEOPHYCEAE | | | |
| *Padina tetrastomatica* Hauck | 3.76% | Enseada dos Corais | 05/17/2015 |
| *Padina gymnospora* (Kützing) Sonder | 6.29% | Boa Viagem | 05/18/2015 |
| *Dictyopteris delicatula* J.V. Lamouroux | 4.05% | Enseada dos Corais | 05/17/2015 |
| CHLOROPHYTA | | | |
| *Caulerpa racemosa* (Forsskål) J. Agardh | 4.79% | Enseada dos Corais | 05/17/2015 |
| *Caulerpa cupressoides* (Vahl) C. Agardh | 10.63% | Enseada dos Corais | 05/17/2015 |
| *Bryopsis pennata* J.V. Lamouroux | 7.12% | Boa Viagem | 05/18/2015 |
| RHODOPHYTA | | | |
| *Acanthophora spicifera* (M. Vahl) Børgesen | 3.28% | Boa Viagem | 05/18/2015 |
| *Chondracanthus acicularis* (Roth) Fredericq | 2.21% | Boa Viagem | 05/18/2015 |
| *Palisada perforata* (Bory) K.W. Nam | 0.39% | Enseada dos Corais | 04/27/2017 |
| *Gracilaria cearenses* (A.B. Joly & Pinheiro) A.B. Joly & Pinheiro | 5.69% | Boa Viagem | 05/18/2015 |
| *Gracilaria caudata* J. Agardh | 1.26% | Enseada dos Corais | 05/17/2015 |

All species at different assays presented a dose-dependence activity. Specifically, for DPPH assay, the $EC_{50}$ index was calculated and is shown in Fig. 1. A lower $EC_{50}$ indicates better antioxidant activity, and then the studied species of Phaeophyceae and Chlorophyta presented better antioxidant activity by the DPPH scavenging assay.

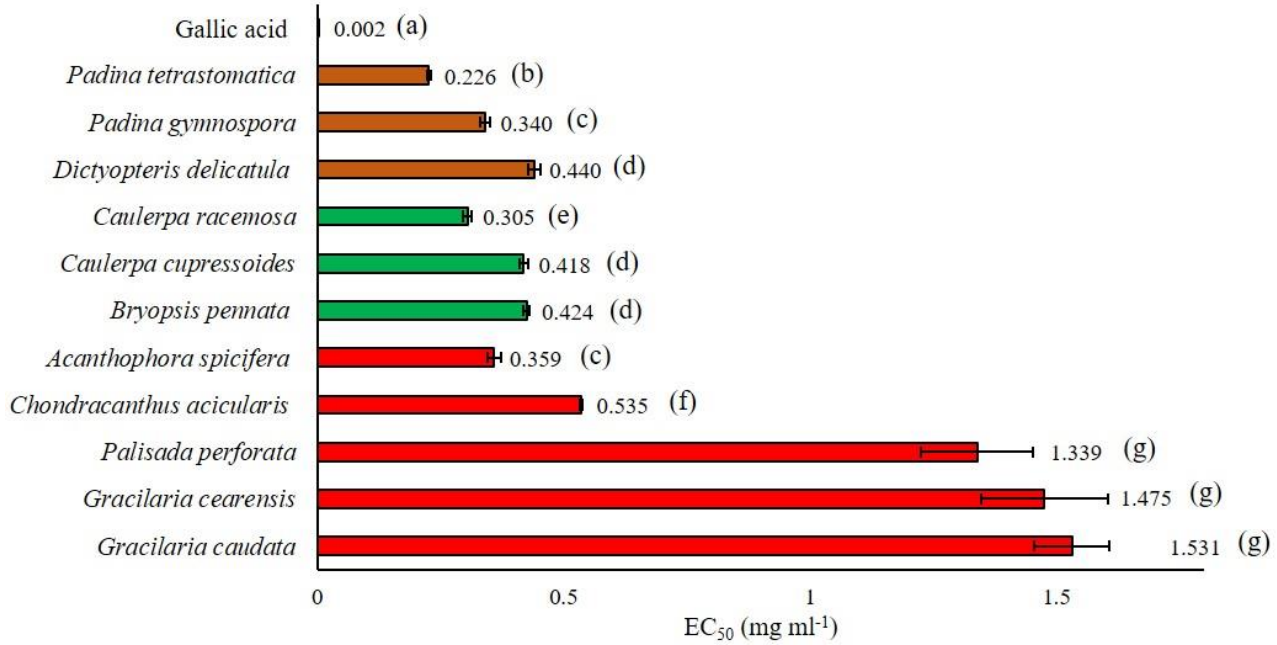


**Figure 1**. DPPH radical scavenging activity expressed in oxidation index $EC_{50}$ given in mg $ml^{-1}$ (mean ± SD, *n* = 3) for tropical seaweeds from Pernambuco reefs and standard gallic acid. Bars represent standard deviations. Brown bars indicate Phaeophyceae species, green bars indicate Chlorophyta species and red bars indicate Rhodophyta species. Significant differences are indicated by different letters as determined by Tukey HSD test ($p < 0.05$).

The species showed different levels of antioxidant activity for the DPPH assay, evidencing a potential characteristic of the species (Tab. 2). Nevertheless, brown algae showed the highest percentage of antioxidant activity among the studied seaweeds, with a mean value of 182.53% for DPPH assay at 1 mg $mL^{-1}$ of extract concentration, followed by the green algae, with 142.00%. Red algae had the lowest mean of antioxidant activity, with a value of 67.14% for DPPH.

Among the brown algae, *P. tetrastomatica* exhibited the highest activity at DPPH (251.51±3.56%) and ABTS (102.39±0.23%) assays (Tab. 2), with a very low $EC_{50}$ (Fig. 1), which was statistically different from all others ($p < 0.05$). The green algae *C. racemosa* exhibited the second highest antioxidant activity at DPPH (163.48±1.97%) and ABTS (87.54±0.09%) assays (Tab. 2), with a low $EC_{50}$, differing from the other species ($p < 0.05$). For the red algae, *A. spicifera* showed the best antioxidant results at DPPH (126.96±0.95%) and Chelator (74.90±5.84%) assays, with a low $EC_{50}$, similar to a brown species, *P. gymnospora* ($p > 0.05$) (Fig. 1).

The influence of environmental parameters (temperature, salinity, pH, DO and turbidity) on the antioxidant activities of seaweeds was assessed through a redundancy analysis (Fig. 2). For the species collected in Boa Viagem beach (Fig. 2A), pH, DO and turbidity seem to be the main parameters related to activities of the red species *A. spicifera* and *C. acicularis*, while *P. gymnospora* antioxidant potential may be mostly affected by salinity. In Enseada dos Corais beach

(Fig. 2B), pH, salinity and temperature had greater influence on the activities of both *Caulerpa* species, *G. caudata* and *D. delicatula*. This last one was more affected by increase of temperature.

**Table 2**. Antioxidant activity (%) of gallic acid, used as standard, and tropical seaweeds from Pernambuco reefs (Northeastern Brazil) at different standard or extract concentrations denoted in brackets. Data are expressed as mean ± SD (*n* = 3).

| | DPPH | ABTS | Chelator | FRAP | Folin-Ciocalteu |
|---|---|---|---|---|---|
| | [3 µg mL$^{-1}$] | [1.75 µg mL$^{-1}$] | [8 µg mL$^{-1}$] | [6 µg mL$^{-1}$] | [12 µg mL$^{-1}$] |
| Gallic acid | 72.69±2.07 | 75.52±3.14 | 59.75±1.80 | 100.00±6.44 | 100.00±2.60 |

| | DPPH | | | | ABTS | | | Chelators | FRAP | Folin-Ciocalteu | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | [1 mg mL$^{-1}$] | [0.6 mg mL$^{-1}$] | [0.4 mg mL$^{-1}$] | [0.2 mg mL$^{-1}$] | [0.6 mg mL$^{-1}$] | [0.4 mg mL$^{-1}$] | [0.2 mg mL$^{-1}$] | [0.6 mg mL$^{-1}$] | [0.2 mg mL$^{-1}$] | [0.2 mg mL$^{-1}$] | [0.15 mg mL$^{-1}$] |
| PHAEOPHYCEAE | | | | | | | | | | | |
| *P. tetrastomatica* | 251.51±3.56 | 114.84±1.51 | 118.05±14.83 | 22.91±1.40 | - | 102.39±0.23 | 57.83±0.14 | 8.90±6.33 | 26.35±0.74 | 31.80±1.76 | 24.31±3.57 |
| *P. gymnospora* | 153.87±0.59 | 89.99±1.26 | 59.81±0.10 | 15.38±3.09 | - | 77.70±0.19 | 57.33±3.03 | 41.39±11.80 | 69.42±2.40 | 32.70±3.53 | 18.14±11.22 |
| *D. delicatula* | 142.21±2.95 | 55.12±0.76 | 42.27±0.52 | 20.40±2.80 | 62.34±0.62 | 51.38±0.38 | - | 30.62±3.50 | 44.84±1.46 | 66.04±2.30 | 38.25±9.03 |
| CHLOROPHYTA | | | | | | | | | | | |
| *C. racemosa* | 163.48±1.97 | 65.04±3.40 | 71.41±0.33 | 21.19±2.61 | - | 87.54±0.09 | 40.64±0.78 | 37.94±7.89 | 30.69±0.65 | 86.12±6.50 | 65.61±3.76 |
| *C. cupressoides* | 133.51±2.20 | 50.04±0.61 | 49.95±0.92 | 17.03±0.59 | 74.02±2.37 | 44.00±1.96 | - | 28.52±9.55 | 20.73±0.82 | 37.28±1.64 | 52.78±6.42 |
| *B. pennata* | 129.01±5.45 | 86.77±1.97 | 40.80±1.62 | 11.75±1.64 | - | 65.96±1.01 | 53.87±0.80 | 21.29±7.26 | 53.89±3.44 | 60.10±6.99 | 30.80±11.04 |
| RHODOPHYTA | | | | | | | | | | | |
| *A. spicifera* | 126.96±0.95 | 75.72±0.52 | 56.65±1.38 | 14.58±3.31 | 62.59±2.63 | 52.94±0.44 | 44.25±3.20 | 74.90±5.84 | 53.35±1.62 | 33.48±2.47 | 20.62±8.18 |
| *C. acicularis* | 89.04±0.42 | 48.33±0.24 | 33.91±1.19 | 14.75±0.85 | 60.75±3.07 | 45.00±1.51 | 47.33±1.16 | 42.39±2.90 | 32.29±1.79 | 42.43±7.76 | 23.61±4.69 |
| *P. perforata* | 44.37±2.44 | 27.73±1.38 | 18.15±0.28 | 15.67±0.67 | 84.22±1.26 | 68.72±1.05 | - | 47.95±15.10 | 65.36±6.14* | 39.41±5.48* | |
| *G. cearensis* | 35.22±1.41 | 32.65±1.35 | 11.04±0.37 | 6.64±2.89 | 31.79±2.11 | 28.02±0.21 | 29.67±0.83 | 37.17±4.51 | 29.14±0.24 | 39.19±3.65 | 24.31±4.88 |
| *G. caudata* | 40.00±0.55 | 28.25±2.34 | 28.89±0.88 | 10.28±1.24 | 36.00±2.94 | 27.34±0.44 | - | 34.39±6.26 | 9.34±0.28 | 30.55±7.25 | 18.46±4.22 |

*Results for 0.4 mg mL$^{-1}$ extract concentration.

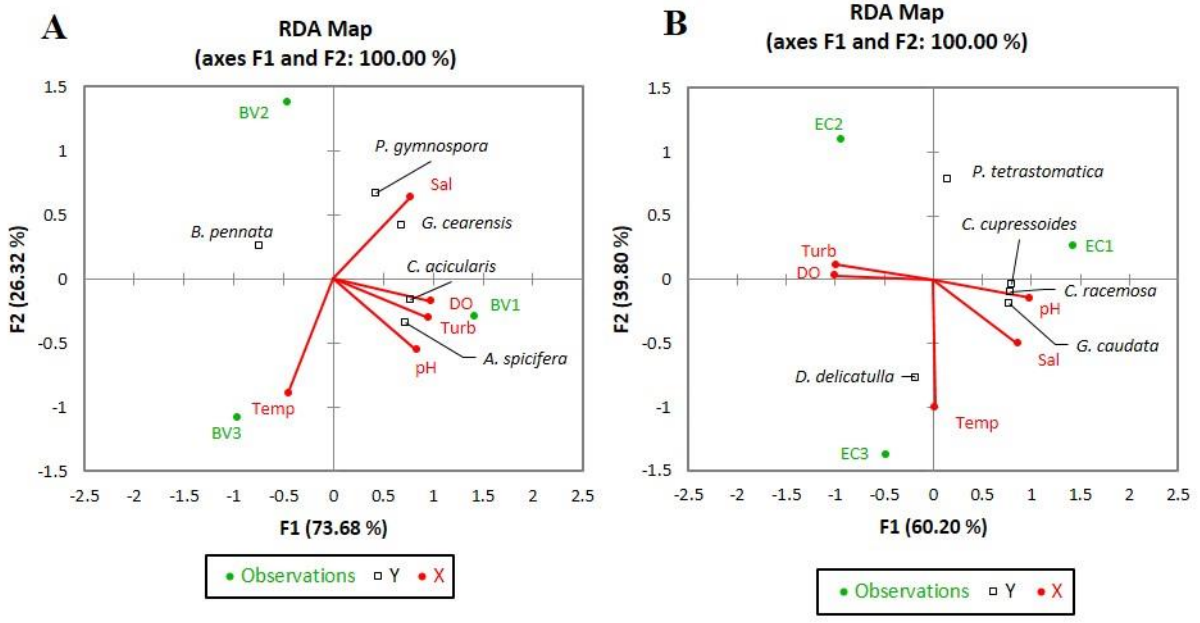


**Figure 2**. Redundancy analysis for the influence of abiotic parameters on the antioxidant activity of seaweed species collected from the reefs of (A) Boa Viagem beach and (B) Enseada dos Corais beach, in Pernambuco.

Observations: replicates ($n$ = 3) made in Boa Viagem (BV) and Enseada dos Corais (EC). Y: analyzed species. X: abiotic parameters [Temperature (Temp), salinity (Sal), pH, dissolved oxygen (DO) and turbidity (Turb)].

## DISCUSSION

All sampled seaweeds showed antioxidant activity through the analyzed assays. Those seaweeds inhabit harsh environments in tropical regions and, in Pernambuco, the reefs have extremely stressful conditions. When tide level lowers, seaweeds get exposed to air and direct UV radiation, leading to desiccation. When tide level rises, they must cope with osmotic variation, which leads to oxidative stress (Burritt *et al*., 2002; Collén and Davison, 1999a,b; Contreras *et al*., 2005; 2009; Kumar *et al*., 2010,2011). This condition of desiccation and osmotic variation may be the main abiotic factor regulating the production of antioxidants on seaweeds.

The analyzed seaweeds were sampled at the intertidal zone of the reefs during low spring tides, so, it´s possible that their high antioxidant activity is related to the desiccation stress. Among them, the brown species showed higher potential. Screening 30 Hawaiian seaweed species, Kelman *et al*. (2012) found *Turbinaria ornata* (Turner) J. Agardh, a brown alga, to be the most active, and identified fucoxanthin as the major bioactive antioxidant. Bioprospecting studies that test several seaweeds usually find that brown species tend to have higher antioxidant potential (Fujimoto and Kaneda, 1980; 1984; Kelman *et al*., 2012; Le Tutour, 1990; Matsukawa *et al*., 1997). Fujimoto and Kaneda (1984) studied the antioxidant potential of 36 seaweeds and confirmed that brown species had higher antioxidant activity. Le Tutour *et al*. (1990) analyzed the polar extracts of seven seaweeds from the French coast and obtained similar results.

Zubia *et al*. (2007) found antioxidant potential in 48 tropical seaweeds from Mexico, highlighting the brown species *Lobophora variegata* (J.V. Lamouroux) Womersley ex E.C. Oliveira as the most active, with very low oxidation index $EC_{50}$ (0.32±0.01 mg $mL^{-1}$). We found even lower oxidation index $EC_{50}$ (0.226 mg $mL^{-1}$) for *P. tetrastomatica*. Studying this same species, Vinayak *et al*. (2011) found high antioxidant activity and low cytotoxicity for its methanolic extracts, suggesting that *P. tetrastomatica* could be employed in functional food and cosmetic industries for preventing the oxidative stress.

The high activity of brown algae was positively correlated to phenolic contents (Connan *et al*., 2006; Pedersen, 1984). Jiménez-Escrig *et al.* (2001) showed that antioxidant activity measured by the DPPH assay was closely related to the phenolic content of brown algae. Studies also show that phenolic compounds may have anti-inflammatory, antitumor and antiviral activities, with positive effects on human health (Novoa *et al*. 2011; Thomas and Kim, 2011; Wijesingher and Jeon, 2012).

Green species analyzed also showed great antioxidant potential. *Caulerpa racemosa*, that had the higher activity, can be found at the top of the reefs, at extremely dry habitats, during

low tides. It´s high antioxidant activity found here, and *e.g*. by Cavas and Yurdakoc (2005) and Li *et al.* (2012), may explain its capacity to occupy such harsh environments. In fact, *C. racemosa* is an invasive species in the Mediterranean (Chisholm *et al*., 2000; Verlaque *et al*., 2003) due to its efficient chemical defense strategy, for the production of the phytotoxic caulerpenyne (Raniello *et al*., 2007), for instance.

The low antioxidant activity reported here by the red species it's not in accordance with the literature. Guaratini *et al*. (2012) found high antioxidant activity in carotenoids and fatty acids isolated from several *Gracilaria* species of Brazil. Great activity was also found for sulfated polysaccharides from *Gracilaria* spp. by Souza *et al*. (2012). The antioxidant activity registered for this genus is often related to water soluble compounds, like pigments and hydrocolloids. Nonetheless, the methodology we used favors finding relatively lipophilic compounds because of the very nonpolar solvent used. This could explain the low activity we found for red species, that are rich in those hydrophilic contents and that probably these components would be in low quantity in our extract due to the fact of being an extract with components mostly nonpolar.

This is the first study to comprehensively evaluate the antioxidant potential of tropical seaweeds from Pernambuco reefs. Even in low concentrations, we found that these seaweeds still showed antioxidant potential, featuring the brown alga *P. tetrastomatica* as a source of natural antioxidants that could be employed in cosmetic, nutraceutical, pharmacological and other industries.

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