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COMPARED ANTIOXIDANT ACTIVITY OF SIX TROPICAL MACROALGAL SPECIES FROM TRINDADE, A BRAZILIAN OCEANIC ISLAND

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Abstract Trindade is an oceanic and pristine island located ~1,100 km off the Brazilian coast. Macroalgal assemblages from remote islands are exposed to several abiotic and biotic stressors that may induce synthesis of chemical defense compounds, including antioxidants. Six species of seaweeds from Trindade were analyzed: *Palisada flagellifera* (Rhodophyta), *Chaetomorpha antennina* and *Cladophoropsis membranacea* (Chlorophyta), *Canistrocarpus cervicornis, Sargassum vulgare* and *Dictyopteris delicatula* (Phaeophyceae). Antioxidant activity (AA) was evaluated by (1) DPPH free radical sequestration, (2) Folin-Ciocalteu assay, and (3) carotenoid colorimetric assay. The higher AA using DPPH method was detected in filamentous Chlorophyta using both extracts (dichloromethane and methanolic), *C. antennina* 36.4 ± 3.7% and *C. membranacea* 39.1 ± 3.9%. Considering phenolic and carotenoids compounds, all seaweed groups showed higher AA using dichloromethane extracts, with maximum concentration in filamentous Chlorophyta (1418 ± 40 μg g^{-1} EAG). Carotenoids also were higher in Chlorophyta (3.5 to 2.9 ± 0.2 μg g^{-1}). Green filamentous seaweed from Trindade seem to be the most suitable and promising candidates for AA studies in this area. Despite seaweed AA from Trindade are still considered lower compared to species from higher latitudes, these screenings will be useful to fill gaps in comparative ecophysiology of seaweeds from tropical oceanic ecosystems.

Keywords: Benthic algae, remote ecosystems, free radicals, bioactivity

INTRODUCTION

Remote islands are of great ecological importance and scientific interest due to their unique biota, natural oligotrophy and lower pollution compared to coastal environments. Biogeographic isolation also may confer distinct abiotic features that favor development of a peculiar biodiversity. Thus, oceanic islands are natural models to elucidate changing biochemical and oceanographic patterns (Pellizzari *et al.* 2020a; Santos-Silva *et al.* 2018). Trindade is a volcanic island located *ca.*, 1,170 km off the Brazilian coast, and is the most isolated macroalgal community from Brazil, influenced by a complex ocean circulation pattern (Pellizzari *et al.* 2020b).

The oceans, a prolific source of biological and chemical diversity, are a promising reservoir of bioactive chemicals. According to Harizani *et al.* (2016), more than 27,000 metabolites from marine organisms have been isolated to develop potential new products for pharmaco-cosmetical industries. Macroalgae, the first marine organisms investigated globally as sources of bioactive metabolites, contain antioxidant compounds, including: fucoxanthin, carotenoids, pyrophaeophytin-a, meroditerpenes, phlorotannin, phospholipids, chlorophyll, tocopherols, among others. Water-soluble and low molecular weight sulfated polysaccharides (*e.g.,* alginates, agar, and carrageen), fat-soluble polysaccharides (chitosan) and phenolic acids (synopsis in Harizani *et al.* 2016) may also be present.

Macroalgae, as a survival strategy, may synthesize several bioactive compounds in response to the stressing environmental conditions, and biotic pressures. These biochemical and physiological processes mediated by metabolites determine their survival, increase their adaptation and consequently, the diversity in marine ecosystems. Macroalgae from extreme environments, such as desert and polar zones,

or from remote and oligotrophic areas, such as Trindade, usually receive an extra uptake in the synthesis of these compounds, due to higher exposition to several stressors (Gomez-Zavaglia *et al.* 2019).

Along Trindade beaches, the major stressing factors are herbivory (Tavares *et al.* 2021), higher temperatures and UV radiation throughout the year (Pellizzari *et al.* 2020b). One hypothesis posits that these environmental stressors may interact and engender synergistic or antagonistic effects on the physiological responses of seaweeds, thereby predisposing cell oxidation. Moreover, it is postulated that regulating the cellular concentrations of these chemical compounds is imperative for the survival of seaweeds amidst adverse conditions. (Gomes & Huovinen 2020). Macroalgae derived from secluded tropical islands comprise a distinctive assemblage of species referred to as "turf," which exerts dominance over the biomass by manifesting as intricate mats comprising delicate filamentous, foliaceous, calcareous, and fleshy thalli or morpho-functional clusters. (Afonso *et al.* 2021).

The biotic interactions, besides meteorological and oceanographic patterns will interfere in the macroalgal physiology and metabolism, that respond with the synthesis of several bioactive compounds and molecules, many of them with applied uses (synopsis in Bernardi *et al.* 2016). Considering these, many studies focusing on seaweed compounds have been published, including anti-tumor action, anti-microbial, anti-leishmaniosis, anti-HIV, and antioxidants (synopsis in Sanniyasi *et al.* 2019, Melo *et al.* 2021).

Antioxidants are molecules that are able to delay, prevent and remove the oxidative damage of a target molecule caused by the action of free radicals and reactive oxygen species (EROS). According to Briani *et al.* (2018), they are present in low concentrations, in relation to the oxidizing substrate. Antioxidants can be classified into enzymatic and non-enzymatic, in which enzymatic mechanisms are catalyzed through the action of specific enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GP), catalase (CAT), and ascorbate peroxidase (APX). The non-enzymatic systems include substances such as ascorbic acid, carotenoids, phenolic compounds and tannins. Within macroalgae and terrestrial plants, several antioxidants are described, among them phenolic compounds, phlorotannins, ascorbic acid, carotenoids, phospholipids, compounds related to chlorophyll, catechins, mycosporin-like amino acids (MAAs) and polysaccharides. Most of them probably improve human health (Briani *et al.* 2018, Torres *et al.* 2019, Leandro *et al.* 2020, Monteiro *et al.* 2020).

Phenolic compounds represent the most numerous group of secondary metabolites from plants and macroalgae, including bromophenol, phlorotannin, tocopherol, besides vitamin C and E (Hentati *et al.* 2018). In seaweed physiology, phenolics compounds are involved in protection against UV radiation, resistance to pathogens, defense against epiphyte and anti-herbivory (Pellizzari *et al.* 2020b). Carotenoids are essential to the conjugation and stability of some complex photosynthetic pigments-proteins and the photoprotection of these apparatus against oxidative damage (McDermid *et al.* 2019). The primordial interest in macroalgal antioxidant activity occurred in Japan during the research of new food additives to replace synthetic antioxidants and preservatives (*e.g.,* butylhydroxyanisol -BHA and butylhydroxytoluene -BHT). These substances are not stable at high temperatures and research has suggested carcinogenic effects and enzymatic changes in animals, stimulating this new research approach for natural antioxidants. Incorporation of antioxidants

derived from seaweeds in cosmetics and nutraceuticals has grown, following scientific evidence (Uribe *et al.* 2020). In addition, changes in food consumption behavior, and the search for natural products rich in antioxidants, improved seaweeds western market.

Notwithstanding the remarkable surge in studies on algal antioxidants, primarily centered around the quest for novel reservoirs of natural compounds with diverse applications, wherein tropical species have showcased significant potential, often augmented by taxing environmental circumstances during the tidal cycle involving intervals of emergence and submersion, nevertheless, investigations concerning AA in remote and pristine oceanic islands are poorly studied.

Rodrigues *et al*. (2020) in order to characterize the antioxidant activity and relate it to local environmental tidal exposure, studied three species of Dictyotales (Phaeophyceae), from the northeast coast of Brazil. They also compared different solvents such as dichloromethane:methanol (DCM:M) and aqueous extracts for ferric reducing antioxidant power, DPPH antioxidant assays and total phenolic compounds. Aqueous extracts of *Canistrocarpus cervicornis* showed up to 10 times more antioxidant activity and phenolic compounds than DCM:M extracts.

Recently Vasconcelos *et al.* (2021) studied the environmental stress tolerance and antioxidant response of *Palisada perforata* (Rhodophyta) from a Brazilian Tropical Reef. Theses author found that *P. perforata* has a low antioxidant activity throughout all reef microhabitats, suggesting that photoinhibition plays a more complex and vital role in protecting photosystem centers from ROS damage than the antioxidant activity.

Macroalgae produce a large range of primary and secondary metabolites with ecological and economical importance, constituting a unique and promising biotechnological raw material, based on its multiple benefits and high growth rates, which allows sustainability in production, supply, and uses in industrial scale. Although Brazilian studies along the coast have previously demonstrated seaweed high potential a natural source of antioxidants (Raymundo *et al.* 2004, Sousa *et al.* 2008, Vasconcelos *et al.* 2017), comparative eco-physiological and macroecological investigations in oceanic islands are still scarce. Thus, the objective of this study was to evaluate the antioxidant activity in different red, brown and green macroalgal species from a pristine and remote island, providing a baseline for further conservation and biotechnological studies.

MATERIALS AND METHODS

STUDY AREA

Trindade Island (20°29-32”S; 29°17-20”W) is the visible portion of Vitoria-Trindade Seamountain Chain (Fig. 1). The island is currently under the jurisdiction of Brazilian Navy. Trindade is in the middle of the Subtropical Gyre, bathed by the Brazilian Current. Mean sea surface temperature ranges from 22.0 ± 2.2 °C to 27.5 ± 2.0 °C during winter and summer, respectively. Salinity ranges from 36.5 ± 3.0 to 39.5 ± 1.5 during winter and summer, respectively. pH showed higher values in summer (8.3 ± 0.3) compared to the winter (8.2 ± 0.5). UV radiation rates ranged from 5 ± 1°C during winter, to 9 ± 2°C in summer (Pellizzari *et al.* 2020b).

SAMPLING SURVEY

Logistical support was provided by Brazilian Navy (SECIRM - PROTRINDADE), within a scientific research program in Trindade Island. Samplings occurred along beaches by hand scraping rocks in the intertidal and shallow subtidal zones. Higher biomass macroalgae were collected: Rhodophyta (one species): *Palisada flagellifera* (J. Agardh) K.W.Nam; Chlorophyta (two species): *Chaetomorpha antennina* (Bory) Kützing, and *Cladophoropsis membranacea* (Hofman Bang ex C. Agardh) Børgesen; Phaeophyceae (three species): *Dictyopteris delicatula* J.V. Lamouroux, *Canistrocarpus cervicornis* (Kützing) De Paula & De Clerck, and *Sargassum vulgare* C. Agardh. Samples were washed and sorted aiming to remove sediment and associated microorganisms. Fractions of the samples were preserved in 4% formaldehyde (diluted in local filtered sea water), for later taxonomic identification; and *ca.*, of 1 kg of fresh biomass, of each species, was frozen for further biochemical analysis.

PREPARATION OF CRUDE EXTRACTS

The samples were dehydrated in a freeze-dried Telstar LyoQuest (Telstar Technologies, S.L) and grounded until powdered. The extract preparation and the potential antioxidant analysis were performed according to methodology adapted from Bernardi *et al.* (2016). Extracts were obtained sequentially by maceration of 40 g of each material in dichloromethane and methanol. After the solvent evaporation, stock solutions of 20 mg mL^{-1} of each extract were prepared and kept at -20 ºC.

ANTIOXIDANT ASSAYS

Extracts antioxidant activity were characterized by DPPH and Folin-Ciocalteu essay. The antioxidant essay and extract concentrations (5.0, 2.5, 1.25 and 0.6 mg mL^{-1}) were analyzed in triplicate (n= 3) for each target species.

DPPH RADICAL SCAVENGING ACTIVITY

Tests were carried out using the inhibition of free radical DPPH (2,2-diphenyl-1-picryhydrazyl), adapted from Bernardi *et al.* (2016). An aliquot of 1,500 µL of DPPH solution (Sigma, 0.01 g eluted in ethanol PA) was added to 1,200 µL of Tris-HCl (15 g of Trisma Base in 100 mL of ultra-pure water, pH= 7) and 1,000 µL of sample. Control sample was prepared with 80% methanol. After homogenizing the solution, samples were left to stand for 20 min, protected from light, and analyzed by UV-visible spectrophotometer (Hiatachi U-1800), wavelength 517 nm. Percentage of AA was calculated by the following expression:

$$\% \text{ inhibition} = \left[\frac{\text{Mean Control Absorbance} - \text{Mean Sample Absorbance}}{\text{Mean Sample Absorbance}}\right] \times 100$$

FOLIN-CIOCALTEU ASSAY

AA was also tested by measuring phenolic compounds using a methodology adapted from Pires *et al.* (2017). To quantify phenolic compounds, solutions composed of 500 µL of sample, 2 mL of the Folin-Ciocalteu reagent and 2.5 mL of Na_2CO3 (10% in water), were stirred and left in the dark for 20 min, protected from light, and subsequently analyzed by UV spectrophotometer, wavelength 760 nm. Percentage of AA was calculated based on the standard curve of the gallic acid of R^2= 0.987, added to the formula:

$$\% \text{ Fenolic} = \frac{\text{Sample Absorbance} \times 100}{\text{Folin} - \text{Ciocalteu Absorbance}}$$

QUANTIFICATION OF TOTAL CAROTENOIDS AND CHLOROPHYLL-*a*

The quantification method was adapted from Torres *et al.* (2014). Freeze-dried samples were grounded separately using liquid nitrogen. Afterwards, 1.5 mL of methanol was added to 50 mg of seaweed sample. The mixture was vortexed and centrifuged (at 4 ºC, during 5 min). Extraction was repeated twice and analyzed in triplicates (n= 3). Afterwards, the extracts were immediately analyzed on a UV-visible spectrophotometer in wavelengths between 400 nm and 700 nm. Chl-*a* and total carotenoid concentrations were calculated in accordance to the equations:

$$Chl_a(\mu g.mL^{-1}) = 12.61 \times Absorbance_{666}$$

$$\text{Total Carotenoid}(\mu g.mL^{-1}) = \frac{1000 \times Absorbance_{470} - 1.63 \times Chl_a}{221}$$

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation (n= 3). Percentages of AA obtained by DPPH and gallic acid equivalent (EAG) essay were compared by unifactorial analysis of variance (ANOVA). Analyses and graphs were generated by R Statistics (R Core Team 2020), using Vegan and Sciplot packages (Morales 2020, Oksanen *et al.* 2020). A multifactorial analysis, non-metric multidimensional scaling, was performed within DPPH, total phenolics and carotenoids data. The graph was constructed using the stress index, which was calculated from a similarity matrix. In the ordering space, distinct tested factors are clustered, while similar ones are placed close together (Dexter et al., 2018). The distance matrix was obtained using the Bray-Curtis coefficient, which was computed using Software R (R Core Team, 2023) and the "vegan" package.

RESULTS

DPPH

The higher AA using DPPH method was detected in Chlorophyta for both extracts, dichloromethane at concentrations of 0.5 and 5 mg mL^{-1} (*C. membranacea* 36.0 ± 8.2 and 39.1 ± 3.9%, respectively), and methanol at 2.5 mg mL^{-1} (*C. antennina*: 36.4 ± 3.7%, *C. membranacea* 36.1 ± 5.8%) (Table S1).

AA concentrations using DPPH method were followed by Rhodophyta (*P. flagellifera)* 31.6 ± 7.2% with methanolic extracts at 0.5 mg mL^{-1}, and 31.0 ± 3.2% with dichloromethane at 2.5 mg mL^{-1} (Table S2). Considering Phaeophyceae, *C. cervicornis* showed the higher AA among species tested (31 ± 3.6 %), at concentration of 2.5 mg mL^{-1} of dichloromethane extract (Table S2).

In general, the higher extraction capacity of antioxidant compounds, among the studied species, was obtained using dichloromethane (39.1 ± 3.9%) in its higher concentration (5 mg mL^{-1}) for Chlorophyta species, mainly *C. membranacea*. Chlorophyta showed the higher concentration of antioxidant compounds, followed by Phaeophyceae, and then Rhodophyta (Fig. 2C), according to one-way analysis of variance (ANOVA) to compare samples ($p < 0.05$)

DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

The results obtained by the Folin-Ciocalteu method demonstrated higher concentrations of TPC using dichloromethane extracts, for both Chlorophyta species (Fig. 3), *C. antennina* and *C. membranacea,* showing maximum values of 1418 ± 40.0 μg g^{-1} EAG and 1417.9 ± 56.0 μg g^{-1} EAG, at 1.2 and 2.5 mg mL^{-1} concentrations, respectively (Table S2).

The following species detaining higher AA concentrations among these tropical seaweed

species were the brown algae *S. vulgare* and the red, *P. flagellifera*, both extracted using dichloromethane, and showing AA averages of 488.9 μg g^{-1} EAG and 326.6 μg g^{-1} EAG, at 2.5 and 0.6 mg mL^{-1}, respectively (Table S2). Considering *C. cervicornis* (Phaeophyceae), no significant differences were observed between mean values using the different solvents (Fig. 3).

DETERMINATION OF PIGMENT CONTENT

Average values found for the content of carotenoids and chlorophyll-*a,* among the different species are present in Figure 4. Total carotenoid concentrations ranged from the lower value (0.31 ± 0.06 μg g^{-1}) in Rhodophyta, to the higher concentration of 3.51 ± 0.35 μg g^{-1} in Chlorophyta (*C. membranacea*). The Phaeophycean *C. cervicornis* showed the second highest value of 1.99 ± 0.12 μg g^{-1} (Table S3). Values of chlorophyll-*a* were higher than those observed for carotenoids in all groups, showing higher concentrations in Chlorophyta species *C. anteninna* (6.95 ± 0.92 μg g^{-1}), Phaeophyceae *C. cervicornis* (6.12 ± 0.45 μg g^{-1}), and then Rhodophyta (1.07 ± 0.09 μg g^{-1}).

STATISTICAL ANALYSIS

Significant differences ($P < 0.01$) were observed between concentration mean values analyzed of chlorophyll-*a* and total carotenoids considering seaweed groups. Total Carotenoids in Chlorophyta were higher than Phaeophyceae, and Rhodophyta (Fig. 4). AA was tested among algal groups, in different solvents. The analysis showed a significant difference ($P < 0.01$) among DPPH percentages of inhibition for Chlorophyta (Fig. 5A). Using DPPH as antioxidant indicator there was no significant statistical difference using distinct concentrations of crude extract, or comparing solvents (dichloromethane and methanol) (Fig. 5C). Considering TPC, Chlorophyta was significantly different, showing higher concentrations when compared to the brown and red species tested. Besides, green seaweeds also showed differences among different concentrations of the extracts (Fig. 5B); and dichloromethane was more effective to extract TPC in all concentrations tested (Fig. 5D). In addition, nMDS (Fig. 6) suggests that the total Carotenoid and DPPH content probably induced the clusterization, with higher values and similarity between Chlorophyta and Phaeophyceae. Solvent plots are randomly distributed, and no significant difference was observed.

DISCUSSION

In this preliminary scanning, the higher AAs using both indicators (DPPH and TPC), were obtained from the target Chlorophyta species *C. membranacea* and *C. antennina*, the same pattern was observed for total carotenoids and chlorophyll-*a*, suggesting that this group is a potential candidate for further AA studies, and characterizations using HPLC method, in tropical oceanic islands.

Rodrigues *et al.* (2020) found differences in antioxidant activity between three species of brown algae from the NE Brazilian coast, comparing aqueous and DCM:M solvents, suggesting that aqueous extracts from *C. cervicornis* and *Lobophora variegate* are more promising. Studies that compare red, green and brown seaweeds simultaneously, are showing the most promising matrices for future prospection of natural antioxidants.

Ruiz-Medina *et al.* (2022) studying AA in 24 seaweed species from Tenerife (Canary Islands) found that, regardless of their phylogenetic group, the species exhibited a variable antioxidant activity. The authors, as well in our study, also found the highest potential scavenging activity in a filamentous Chlorophyte from this oceanic island,

Dasycladus vermicularis. In certain species, this activity decreased after desiccation, highlighting the importance of considering the role of processing and storage aiming to stabilize AA for practical applications in macroalgae.

Raymundo *et al.* (2004), studying green seaweeds from the costal Santa Catarina Island (SE Brazil), showed highest percentage of oxidation inhibition (75.75%), and TPC (610.31 mg EAG g^{-1}), from the filamentous chlorophyte *Ulva intestinalis*, similarly to what was observed in the present study. Although these extracts, at the tested concentrations, did not surpass the 92.45% efficacy of the commercial and standard synthetic antioxidants (eg. BHA). In general, natural antioxidants are not as efficient in deactivating free radicals as synthetic antioxidants (*e.g.,* hydroxybutylanisole HBA), according to *in vitro* essays. This reinforces the need for further research aiming to investigate more effective natural antioxidants for industrial purposes.

Also, Raymundo *et al.* (2004) suggested that methanolic extract is the most effective solvent for extractions in chlorophytes, showing higher ability to sequester hydrogen peroxide. The authors demonstrated that the most effective extracts expressed by percentage of inhibition of DPPH were from *C. antennina* (1.26 to 20.01%) and *U. intestinalis* (1.18 to 13.16%). In our study, the average values acquired from all tested concentrations for the green filamentous algae *C. antennina* were 20.1% when using dichloromethane as extractor, and 29.8% when extracted by methanol. Similarly for *C. membranacea*, the corresponding values were 33.2% and 30.4%, respectively, representing higher values compared to those previously reported.

These data also corroborate the results of Santos *et al.* (2019), where AA for the DPPH sequestration assay in enzymatic extracts showed a decreasing concentration starting from *Ulva fasciata* (42.19%), *Palisada flagellifera* (38.68%) and *Sargassum vulgare* (37.01%), sampled from Brazilian Northeast coast. Red algae *P. flagellifera,* tested in the present study, showed average AA of 23.7% using dichloromethane, and 28.5% using methanol, lower values compared to Santos *et al.* (2019).

Red algae from *Laurencia* complex, such as *Palisada*, are reported as promising sources of new secondary metabolites, *ca.*, of 1,047 secondary metabolites have been described (Harizani *et al.* 2016), and classified mainly into sesquiterpenes, diterpenes, triterpenes, acetogenins, indoles, aromatic compounds, steroids. According to Fujii *et al.* (2011), 47 sesquiterpenes and one triterpene were isolated from Brazilian species. Given the extensive research on bioactive compounds of *Laurencia* complex species (overview in Koishi et al., 2012; Harizani et al., 2016), our survey also focuses to obtain AA data from *Palisada* sp.

The Folin-Ciocalteu assay exhibits higher sensitivity to hydrophilic compounds, in contrast to DPPH, which, despite being a conventional test, lacks the same level of precision and is susceptible to interference from other reducing substances, such as ascorbic acid, proteins, and reducing sugars. Results of both tests will depend on the hydrophilicity/lipophilicity and its affinity with the different tests and reactants, besides of sample types and solvents. Despite DPPH radical sequestration being the most widely employed AA assay (Monteiro et al., 2020), it is advisable to test and adapt several extract concentrations aiming to scan macroalgae AA from different biogeographical regions (e.g., polar, temperate, and tropical species).

Higher total phenolic compounds (TPC) concentration, and lower AA obtained using DPPH were also observed by Ebrahimzadeh *et al.* (2018), testing *Gracilaria gracilis*

(Rhodophyta) extracts, where AA was 17.4 ± 1.73% in DPPH assay, and 29.39 ± 2.01% for TPC. Bernardi *et al.* (2016) observed a similar pattern when analyzing the green algae *Gayralia brasiliensis* from Paraná coast, where AA was 15.8 ± 5.2% using DPPH method, and 77.5 ± 1 μg g^{-1} EAG for TPC. Furthermore, the higher concentrations of TPC tested in Trindade seaweeds were consistent with the observed pattern of higher carotenoid concentrations, primarily in Chlorophyta. Fernando *et al.* (2018) highlighted the antioxidant and the broad range of anti-inflammatory functionality of the ethanol extract of *Caulerpa racemosa* (Chlorophyta) from the southwestern coast of Sri Lanka, which can be used as pharmaceutical functional ingredients in cosmeceutical formulations and as functional food.

Following Melo *et al.* (2021), TPC are thought to protect algae from ultraviolet radiation-induced photo-destruction by exhibiting free-radical scavenging properties. In this study, the brown alga (*Sargassum vulgare*) had higher TPC than green (*U. fasciata*) and red alga (*Crassiphycus corneus*, former as *Gracilaria cornea*) from Bahia reefs (NE Brazilian coast). The authors mentioned that phenolic content of *S. vulgare* from Bahia reefs was 10 times higher than Camariñas, Spain (Agregán *et al.* 2017), were TPC from *Ascophylum nodosum* was 0.96 mg (PGE) $100g^{-1}$, and *Fucus vesiculosus* was 1.15 (PGE) $100g^{-1}$.

Considering geographical locations, algal groups, and DPPH values (**Table 1**), comparing the brown species from Trindade (current study) and Tenerife Island (Ruiz-Medina et al., 2022), both belonging to Dictyotaceae, AA mean was ca. of 28% using the DPPH assay. Although in the present study the higher AA (34%) was measured in the filamentous green species (Chlorophyta) *C. membranaceae.*

Considering TPC, the highest concentration was also detected in a filamentous green alga (*Cladophora liebetruthii*) from Tenerife, followed by *Canistrocarpus cervicornis* from Trindade and *Dictyopteris polypodioides* from Tenerife, both belonging to Dictyotaceae. Finally, considering total carotenoids, the concentrations were similar among localities, with higher concentrations in brown species and lower in red. *Canistrocarpus cervicornis* from Trindade and *Sargassum vulgaris* from a coastal reef in Bahia (Melo *et al.* 2021) showed similar values (1.65 to 2 μg g^{-1}). Filamentous green macroalgae (which presented comparatively intermediate concentrations) both from Trindade and Tenerife Island, ranged from 0.3 to 0.62 μg g^{-1}.

Considering that environmental stress may increase the synthesis of AA (Pellizzari & Reis 2011, Pellizzari *et al.* 2020b), the higher concentration of TPC in green seaweeds from Trindade may also be related to the strong herbivory along the island. This stressful condition in the algal turfs may induce the production of phenolic substances, as a protection mechanism against grazing of fishes, sea urchins and green sea turtles (Pinheiro *et al.* 2015, Santos *et al.* 2020). Natural occurrence of phenols and polyphenols in macroalgae has been widely studied and it is associated with biotic pressures (Vasconcelos *et al.* 2017, Gómez & Huovinen 2020, Lalegerie *et al.* 2020). Besides that, Trindade is a tropical island where UV levels are high throughout the year, which may also stimulate the production of phenolic compunds.

Comparatively, TPC values found in the macroalgae from Trindade were similar to Antarctica, suggesting that besides in different abiotic parameters and intensities, both ecosystems (polar and remote tropical) suffer of environmental stresses, probably resulting in higher AA as ecophysiology adaptation and

defense response. Studies within this subject in the Southern Ocean islands are scarce. However, Bernardi *et al.* (2016) suggest that the seasonal high incidence of ultraviolet radiation during summer in Antarctica, which causes cellular oxidation, may be related to the higher concentrations of AA registered in polar marine organisms. In contrast, in Trindade, a tropical oceanic island, the stressors that seem to influence the secondary metabolism of macroalgae are herbivory pressure (Tavares *et al.* 2021), and the high and constant UV rates throughout the year (Pellizzari *et al.* 2020b), justifying the higher concentration of phenolic compounds in both environments, polar and oceanic tropical.

Oligotrophic waters, UV patterns, and biological interactions seem to have a central role in chemical defenses for macroalgae from tropical remote islands, opening interesting questions about the activation of anti-stress mechanisms based on chemical substances with multiple primary and secondary functions, vulnerable to climate and oceanographic changes.

Higher AA found in Chlorophyta may be associated to the protection mechanisms for the UV radiation daily cycle, since the analyzed filamentous species belong to the exposed intertidal turf habitats, and as consequence, are forced to develop an efficient biochemical arsenal against stress. Several antioxidant compounds such as phenolics, tannins and glycosides, as well as vitamins A and C, were found in high concentrations in *Chaetomorpha antennina* from South Pacific Islands (Haq *et al.* 2019). A similar pattern was observed in samples from Hawaii, where the authors also associated higher AA with abiotic and biotic factors, such as herbivory, shallow habitats, high irradiance and constant photoperiods, (McDermid *et al.* 2019), similar to observed in Trindade. Considering the wide distribution of Chlorophyta, further tropical remote insular studies are recommended to better understand induction mechanisms of antioxidant compounds synthesis, related to the macroecology of each ecosystem and species.

Raymundo *et al.* (2004) measured total carotenoids in *Ulva intestinalis* from 7.2 to 13 $\mu g\ g^{-1}$; the pattern observed in the present study was similar, showing chlorophyll values higher than carotenoids in green species. Le Tutour *et al.* (1996) studying synergistic effect of adding chlorophyll-*a* to vitamin E in a model system, suggested that chlorophyll-*a* acts synergistically, providing 24% increase in the inhibitory effect, concomitant with lutein, the main carotenoid in Chlorophyta, as well β-carotene (Mortensen *et al.* 1997, Sousa *et al.* 2008). Le Tutour *et al.* (1998), studying the ability of macroalgal species to sequester peroxyl radicals, reported that AA may not be only assigned to their tocopherol content (pro-vitamin E), but to many other fat-soluble compounds found in seaweeds (including alpha and β-carotenes, and chlorophyll), which in association would generate integrated bioactivity. For these reasons, the utilization of combined AA indicators is highly recommended for studies regarding antioxidant performance in seaweeds.

Some Ulvales may contain concentrations of β-carotene higher than many terrestrial plants, and that β-carotene reacts with lipid peroxides (Sousa *et al.* 2008). Pellizzari *et al.* (2020a) stated that this biochemical repertoire varies among macroalgae species across different global regions Following these authors, the biochemical arsenal may vary according to the biogeographic distribution of the taxa, being possible that the same genus and even a same species that broadly occurs in tropical and polar zones, may have chemical components in different concentrations, following the abiotic/biotic pressures, and even have a completely different chemical

characterization.

In addition to AA intra- and inter-specific differences, the distinct methods of analysis are not always comparable, and should be considered in data interpretation. Sousa *et al.* (2008) evaluated concentrations of α- and β-carotene using high-performance liquid chromatography (HPLC) in 32 seaweed species of Chlorophyta, Rhodophyta and Phaeophyceae, from Brazilian NE coast. Both β- and α-carotene were found in all species of green species analyzed. The authors detected higher concentrations of α-carotene in *Caulerpa*, and β-carotene in *Ulva fasciata*. Species of Phaeophyceae showed β- but no α-carotene, and in Rhodophyta only β-carotene was detected, in very low concentrations.

In contrast, carotenoids, and chlorophyll-*a* from green seaweed extracts from Trindade were higher than red, and lower than brown algae concentrations (synopsis in Table 1, Nauer *et al.* 2019, Amorim *et al.* 2020). Photossynthetic pigments concentrations in seaweeds are strongly influenced by sunlight wavelength, and time of exposure (Polo & Chow 2020). The constant high temperatures that impact seaweeds in Trindade, may also influence the concentration, considering photo-inhibition, and that any modification in irradiance (photoperiod, intensity and/or wavelength), as well as thermo-haline patterns, will affect algal ecophysiology, and should be better investigated.

AA from Trindade seaweeds contrasts with studies from polar and temperate zones (Sanniyasi *et al.* 2019, Bordoloi & Goosen 2020, Gómez & Huovinen 2020, Puspita *et al.* 2020), which suggests that brown algae, the most abundant group in Antarctica, are a promising candidate to the synthesis of antioxidant compounds.

Nevertheless, since Bernardi et al. (2016) investigation on the AA of monostromatic green seaweeds (Chlorophyta) distributed along a latitudinal gradient from Antarctica to Paraná (Southern Brazil), contrasting findings have emerged. These findings showed higher concentrations of AA mainly in specimens from polar zones, compared to lower latitudes.

The higher AA also found in green seaweeds from the tropical and remote Trindade Is., is possibly associated with the fact that Chlorophyta species from this isolated and warm ecosystem is also exposed to many stressors, such as, oligotrophy, constant heat and high UV, besides high herbivory pressure. Furthermore, in this area, a peculiar formation of branched calcareous red algae in the bottom, covered by delicate and filamentous green and red algae, called turf, is dominant. This intricate macroalgal cluster, by itself, may represent a stress that drives the synthesis of secondary metabolism compounds in chlorophytes, the most abundant group in these turfs.

Unlike this study, where dicloromethane and methanolic extracts were tested, Ebrahimi *et al.* (2021) studied polyphenols content, antioxidant and antibacterial activities of *Caulerpa sertularioides* (Chlorophyta); *Padina distromatica* and *Sargassum boveanum* (Phaeophyceae) from the Persian Gulf using different solvents, including aqueous. The highest extraction yield was found in *C. sertularioides* (also a green seaweed) from the aqueous extract. The highest phenolic content was found in *S. boveanum* (Phaeophyceae) from aqueous extract, more efficient in extracting phenolic compounds in comparison with n-hexane and ethyl acetate, and consistent with DPPH results. These authors also recommended incorporating the aqueous extract of *S. boveanum* as a natural additive with antioxidant potential for developing functional foods. Additionally, they noted that *n*-hexane extracts could exhibit antibacterial effects when included in edible products.

Ruiz-Medina *et al.* (2022) studied changes in antioxidant activity of phytoconstituents (total carotenoids, proline, phenols, flavonoids and condensed tannins content) in fresh and dried macroalgae from the Canary Islands. Results suggested that from fresh material, the filamentous green species *Cladophora liebetruthii*, and *Dasycladus vermicularis*, in addition to the brown species *Dictyopteris polypodioides* presented the highest scavenging activity, supported by a high correlation with phenolics and flavonoids content. In air-dry extracts, *Anadyomene saldanhae* and *D. polypodioides* showed the highest antioxidant potential, correlated with high phenolic compounds content. The study uncovered those fresh extracts of certain species, rather than dried extracts, exhibit promising characteristics as raw materials for deriving biologically active substances in the food and pharmaceutical industries. This finding emphasizes the importance of considering the selection of preservation methods, whether or not air-drying is employed.

According to Jacobsen *et al.* (2019), AA differences are also related to polarity, nature and position of the chemical constituent in the compound structure. Therefore, for the quantification, isolation and identification of the bioactive compounds in natural sources, extractions with solvents of different polarities are necessary. The use of distinct methods, extractors, and indicators of antioxidant, as performed in this pilot study, enables more refined responses. In addition to DPPH method, FRAP test (Ferric Reducing Antioxidant Power) can be used (Urrea-Victoria *et al.* 2016, Torres *et al.* 2015, Vasconcelos *et al.* 2017, Polo & Chow 2020).

Further studies also may associate several AA indicators, including (1) more refined tests using other markers (such as FRAP), (2) elucidation of bioactive molecules in High Performance Liquid Chromatography (HPLC), and (3) stability essays of potential molecules, the most challenging step to suggest the AA potential for industrial uses. In addition, this data enables to select potential species aiming to evaluate their performance *in vitro*, as well as their ability to produce and store higher concentrations of metabolites under controlled conditions (Gomez-Zavaglia *et al.* 2019).

Finally, further research is required on the chemo-diversity of macroalgae from tropical remote oceanic ecosystems, as a reservoir of active ingredients. Trindade, such as other isolated and pristine islands, are natural laboratories poorly investigated, where macroalgae have adapted and thrive in different habitats and conformations (*e.g.,* turf). However, the shifts in atmospheric and oceanographic features, and direct anthropogenic pressures are challenging the strategies of seaweeds adaptations in ways not yet well understood. Therefore, it is necessary to intensify studies that elucidate the action of defense mechanisms and physiological tolerance facing climate changes.

For the first time, a comprehensive investigation was conducted to assess the antioxidant activity of six marine macroalgae species from Trindade Island, a pristine oceanic island in Brazil. These algae, regarded as a natural reservoir of biochemical compounds for the nutraceutical, pharmaco-cosmetic, and food industries, exhibited significant antioxidant activity across the analyzed species. Tropical filamentous chlorophytes, including species with broad biogeographical distribution and physiological plasticity, seem to be the best candidates among seaweed groups for bioprospecting antioxidants in this oceanic island. Although the percentage of DPPH inhibition of methanol extracts was *ca*., of 37% in the tested concentrations, no higher efficiency than the commercial (synthetic) antioxidant was detected. However, this baseline of concentrations and ecophysiology

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approach may support further studies for natural antioxidants research.

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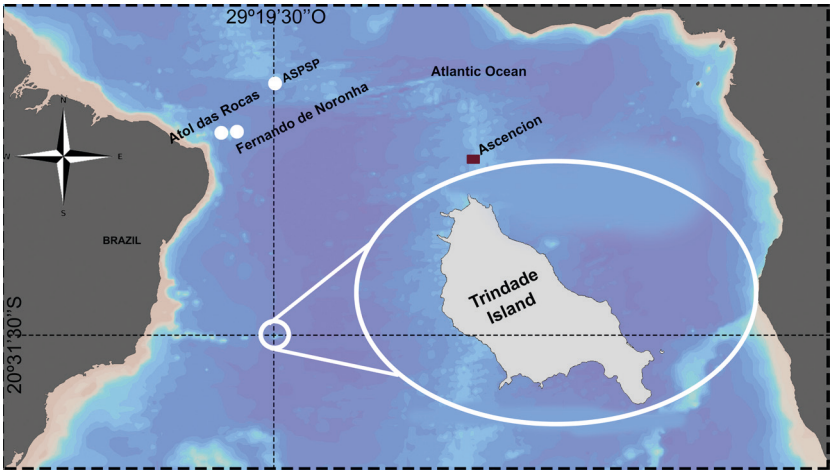
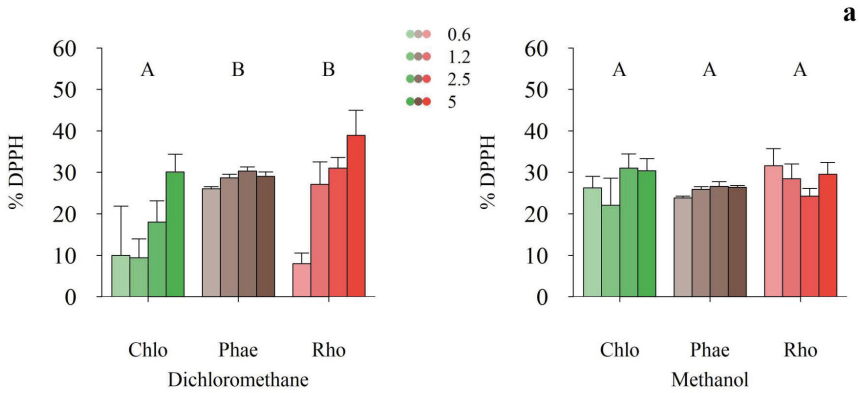


Figure 1. Trindade Island (in white detail), located off the Brazilian coast, Southwestern Atlantic Ocean. Other oceanic Islands (ASPSP= Saint Peter and Saint Paul Archipelago)



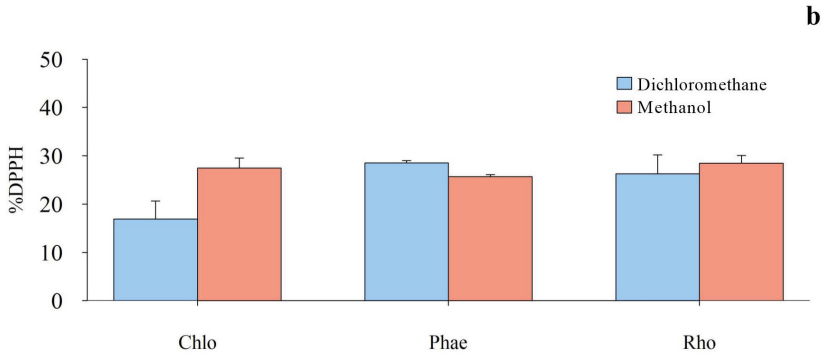


Figure 2. (a) Compared antioxidant activity (AA) of seaweed using dichloromethane and methanol extracts at different concentrations, comparing macroalgal groups: Chlo = Chlorophyta; Phae = Phaeophyceae and Rho = Rhodophyta. Capital letters (A and B) mean statistical differences among the groups. (b) Average values of antioxidant activity (AA) using DPPH method by seaweeds groups and solvents

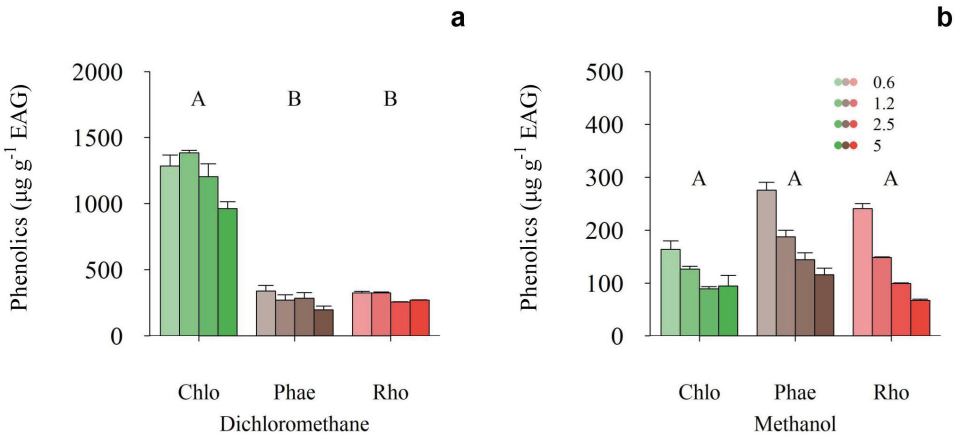


Figure 3. Compared concentrations of phenolic compounds (µg g^{-1} EAG) of macroalgal species from Trindade Island, using different concentrations of solvents, and extracted sequentially with (a) dichloromethane and (b) methanol. Chlorophyta (Chlo), Phaephyceae (Phae) and Rhodophyta (Rho). Different capital letters (A and B) mean statistical differences among seaweed groups.

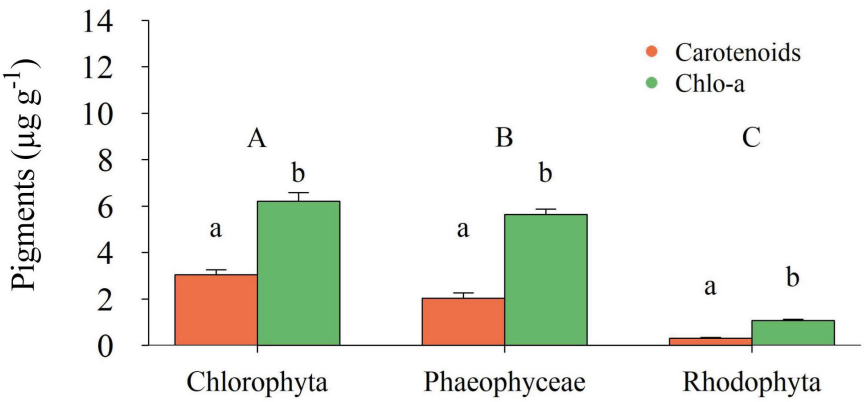


Figure 4. Pigments quantification (µg g^{-1}) of chlorophyll-a (Chlo-a) and total carotenoids of seaweed groups from Trindade Island. Letters mean statistically significant differences. Capital letter (A, B, C) = among seaweed groups; Lowercase letter (a and b)= between solvents (dichloromethane and methanol); $P < 0.01$

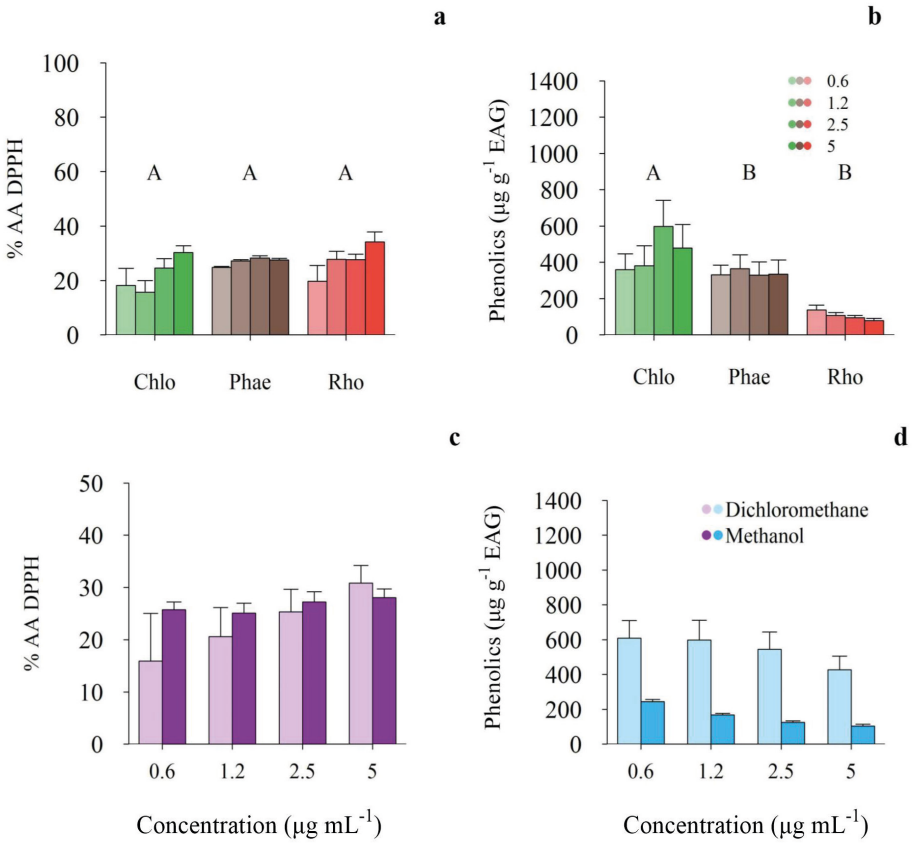


Figure 5. Comparative indicators of AA, DPPH (a and c) and total phenolics content (b and d), considering mean values of algal groups, and extract concentrations of seaweeds from Trindade Island. Letters. Capital letters (A and B) mean statisticall differences among seaweed groups.

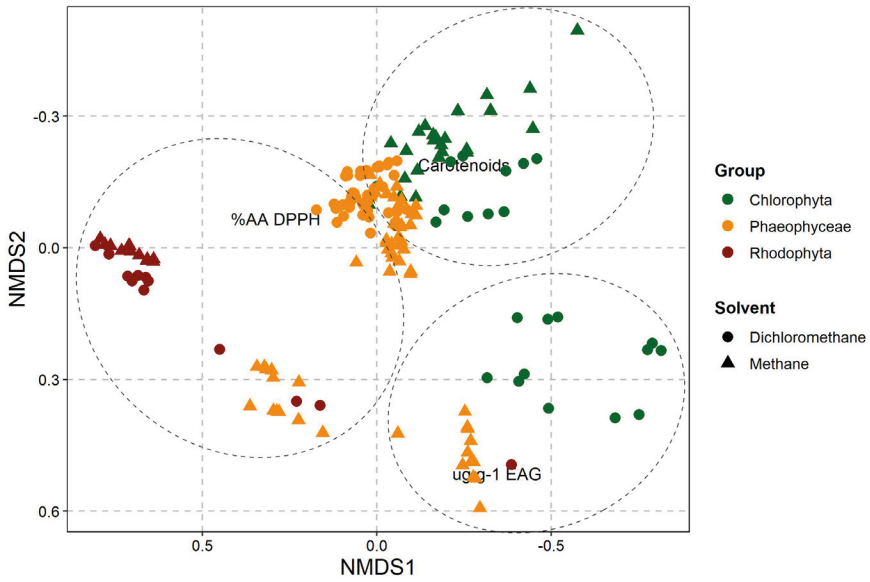


Figure 6. Multifactorial analysis of non-metric multi-dimensional scaling performed considering different groups of seaweeds and solvents, using the following AA indicators: DPPH, total phenolics and carotenoid data.

Seaweed species (Morphofuncional group)	Locality / Island	DPPH ($mg.g^{-1}$)	Total phenolic content (mg GAE.g^{-1})	Total carotenoids ($\mu g.g^{-1}$)	Reference
CHLOROPHYTA					
Chaetomorpha antennina (filamentous)	Trindade	29.5±4.1	10.9±2.8	3.0 ± 0.2	Present data
Cladophoropsis membranacea (filamentous)	Trindade	34.2 ± 3.5	13±2.7	3.5 ± 0.4	Present data
Cladophora liebetruthii (filamentous)	Canary Islands (Tenerife)	10.37 ± 0.87	33.59 ± 1.28	0.63 ± n.i.	Ruiz-Medina *et al.* 2022
Anadyomene saldanhae (filamentous)	Canary Islands (Tenerife)	23.12 ± 0.64	20.70 ± 0.99	0.42 ± n.i.	Ruiz-Medina *et al.* 2022
Ulva fasciata (foliaceous)	Bahia reef, NE Brazil	n.i.	1.9 ± n.i.	0.13 ± n.i.	Melo *et al.* 2021
PHAEOPHYCEAE					
Canistrocarpus cervicornis (foliose dicotomous)	Trindade	30 ± 2.7	23 ± 1.3	2.0 ± 0.1	Present data
Dictyopteris delicatula (foliose dicotomous)	Trindade	25± 2.2	12.1±0.4	0.6 ± 0.1	Present data
Sargassum vulgare (leathery)	Trindade	26.2± 2.3	14±1.6	1.7 ± 0.3	Present data
Canistrocarpus cervicornis (foliose dicotomous)	Canary Islands (Tenerife)	5.88 ± 0.76	5.65 ± 0.78	0.64 ± n.i.	Ruiz-Medina *et al.* 2022
Dictyopteris polypodioides (foliose dicotomous)	Canary Islands (Tenerife)	29.92 ± 0.55	31.43 ± 0.90	0.63 ± n.i.	Ruiz-Medina *et al.* 2022
Sargassum vulgare (leathery)	Bahia reef, NE Brazil	n.i.	12.1±n.i.	1.65 ± n.i.	Melo *et al.* 2021
RHODOPHYTA					
Palisada flagellifera (terete / fleshy)	Trindade	28.5± 0.7	2.9±0.73	0.3 ± 0.1	Present data
Palisada perforata	Canary Islands (Tenerife)	1.82 ± 0.04	7.81 ± 0.61	0.19±n.i.	Ruiz-Medina *et al.* 2022
Laurencia dendroidea	Canary Islands (Tenerife)	3.10 ± 0.27	7.05 ± 0.83	0.08 ± n.i.	Ruiz-Medina *et al.* 2022
Laurencia pyramidalis	Canary Islands (Tenerife)	3.52 ± 0.57	6.60 ± 0.61	0.12 ± n.i.	Ruiz-Medina *et al.* 2022
Crassiphycus corneus (terete / fleshy)	Bahia reef	n.i.	3.1 ± n.i.	0.05 ± n.i.	Melo *et al.* 2021

Table 1. Comparative data for different species, using methanolic extracts for distinct AA indicators (DPPH, TPC and total carotenoids) from Trindade Island, Canary Islands and NE Brazil (Bahia Coast). n.i.= not informed. Higher values for each AA indicator (columns) in bold.

SUPPLEMENTARY MATERIAL

Species sampled (Algal group)	Solvent	DPPH (%)			
		0.6 mg mL^{-1}	1.2 mg mL^{-1}	2.5 mg mL^{-1}	5 mg mL^{-1}
C. cervicornis (Phae)	Dichloromethane	30.2 ± 9.0	28.1 ± 3.2	31.0 ± 3.6	30.5 ± 2.8
	Methanol	24.5 ± 1.0	25.8 ± 0.7	28.1 ± 5.36	27.1 ± 1.1
D. delicatula (Phae)	Dichloromethane	NA	NA	NA	NA
	Methanol	22.6 ± 1.8	26.2 ± 5.1	25.1 ± 0.6	27.0 ± 0.3
S. vulgare (Phae)	Dichloromethane	25.4 ± 0.4	28.12 ± 0.71	28.41 ± 1.04	24.5 ± 3.3
	Methanol	23.23 ± 1.34	28.56 ± 2.71	24.18 ± 0.27	23.73 ± 0.79

P. flagellifera (Rho)	Dichloromethane	7 ± 3.4	27.1 ± 7.1	31.0 ± 3.2	28.9 ± 7.6
	Methanol	31.6 ± 7.2	28.5 ± 4.6	24.3 ± 2.5	29.6 ± 3.8
C. antennina (Chlo)	Dichloromethane	ND	ND	17.9±6.9	22.3 ± 4.2
	Methanol	29.1 ± 4.2	28.1 ± 2.3	36.4 ± 3.7	25.4 ± 0.4
C. membranacea (Chlo)	Dichloromethane	36.0 ± 8.2	29.4 ± 1.70	28.1 ± 4.3	39.1 ± 3.9
	Methanol	24.1 ± 5.9	26.0 ± 17.1	36.1 ± 5.8	35.4 ± 7.81

Table S1. Antioxidant activity (AA) by sequestering the DPPH radical (expressed in %), in different concentrations of extracts. Values are expressed as mean ± sd (mg mL^{-1}), where: Phae= Phaeophyceae, Rho= Rhodophyta and Chlo= Chlorophyta ($P < 0.01$).

NA= There was not enough extract yield for analysis in dichloromethane, ND= Antioxidant activity not detected.

Species sampled (Algal group)	Solvent	Concentrations - Phenolic compounds (**µg g^{-1} EAG**)			
		0.6 mg mL^{-1}	1.2 mg mL^{-1}	2.5 mg mL^{-1}	5 mg mL^{-1}
C. cervicornis (Phae)	Dichloromethane	303.1 ± 143.3	263.1 ± 155.3	218.3 ± 99.7	158.2 ± 76.2
	Methanol	310.0 ± 36.0	220.2 ± 33.2	177.2 ± 38.0	143.5 ± 42.0
D. delicatula (Phae)	Dichloromethane	ND*	ND*	ND*	ND*
	Methanol	191.6 ± 23.0	129.1 ± 19.0	94.8 ± 5.0	71.1 ± 2.1
S. vulgare (Phae)	Dichloromethane	306.6 ± 11.3	299.8 ± 4.1	488.9 ± 20.9	315.9 ± 14
	Methanol	255.9 ± 6.1	146.5 ± 13.4	95.9 ± 2.2	76.3 ± 3.0
P. flagellifera (Rho)	Dichloromethane	326.6 ± 14.3	325.5 ± 15.7	257.4 ± 4.0	271.6 ± 19.5
	Methanol	240.9 ± 16.4	148.6 ± 2.3	98.9 ± 3.1	67.5 ± 3.2
C. antennina (Chlo)	Dichloromethane	1182.0 ± 22.2	1418.0 ± 40.1	992.0 ± 23.0	849.4 ± 4.8
	Methanol	135.8 ± 26.3	115.6 ± 5.5	97.4 ± 3.8	138.7 ± 2.7
C. membranacea (Chlo)	Dichloromethane	1387.6 ± 271.7	1350.2 ± 33.5	1417.9 ± 56.3	1075.9 ± 56.6
	Methanol	191.6 ± 29.3	137.2 ± 9.2	80.9 ± 5.1	50.8 ± 5.4

Table S2. Concentration of phenolic compounds, in different concentrations of extracts, expressed as Gallic Acid Equivalent (EAG), where: Phae= Phaeophyceae, Rho= Rhodophyta and Chlo= Chlorophyta ($P < 0.01$).

* Concentration no detected.

Seaweed species	Pigment content (µg g^{-1})	
	Total carotenoids	Chlorophyll-*a*
C. cervicornis (Phae)	2.0 ± 0.1	6.1 ± 0.5
D. delicatula (Phae)	0.6 ± 0.1	5.8 ± 0.5
S. vulgare (Phae)	1.7 ± 0.3	4.7 ± 0.6
P. flagellifera (Rho)	0.3 ± 0.1	1.0 ± 0.1
C. antennina (Chlo)	2.9 ± 0.2	7.0 ± 0.9
C. membranacea (Chlo)	3.5 ± 0.4	4.3 ± 0.2

Table S3. Quantification (µg g^{-1}) of chlorophyll-***a*** and total carotenoids of seaweed extracts from Trindade Island. Phae= Phaeophyceae, Rho= Rhodophyta and Chlo= Chlorophyta.