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Chemical Compounds from Seaweeds on the Tropical Coast of Brazil

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Seaweeds have been explored by humans for thousands of years as a source of chemical compounds. This study describes the content of minerals, ash, carbohydrates, protein, lipids, and main metabolites of dichloromethane / methanol extracts of the seaweed *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata* and *Gelidiella acerosa* from sandstone reefs on the Brazilian tropical coast (Pernambuco, Northeastern of Brazil). The content (% dry weight) of carbohydrates ranged from 14.35-48.52, proteins 7.49-14.98, total lipids 0.40-8.92, and ash 18.51-37.02. The concentration (mg kg dry algae^{-1}) of Ca (900-3468), Mg (1655-4902), K (810-1707), Na (1062-4580), Mn (19-4462), and Cu (3.6-6.4) were maximum in *Palisada* and minimum in *Padina*. In turn, the lowest and highest contents (mg kg dry algae^{-1}) of Fe (100-2312), Zn (18-43), and Cr (0.08-0.93) were recorded in *Gelidiella* and *Ulva*, respectively. Neophytadiene was the major compound. Phytol and palmitic acid were found in all seaweeds, although in low quantities. *Palisada* had the highest contents (% dry weight) of metabolites (neophytadiene: 23.89, phytol: 8.29; palmitic acid: 8.32), while *Ulva* had the lowest, except phytone, which was present only in this species. Our findings highlight the potential of these macroalgae from the coastal reefs as a source of chemical compounds.

Keywords: benthic seaweeds, lipid metabolites, sandstone reefs, tropical coast

Introduction

Humans have been using seaweed for more than 14,000 years as food or medicine. Dillehay *et al.*[1] and Cornish *et al.*[2] hypothesized that modern humans evolved due to the consumption of seaweed. Almost 300 species of seaweed are exploited in some way by humans, and it is predicted that more than 500 million tons (dry weight) of seaweed will be consumed annually by 2050.[3] The global seaweed market was worth US$9.9 billion in 2021 with perspective growth at an annual rate of 2.3% from 2022 to 2030.[4] A large number of studies have demonstrated the potential of chemical compounds extracted from marine seaweed for the production of human food,[5,6] cosmetics,[7,8] fertilizers,[9,10] and medicinal drugs.[11-13] These compounds may also have a range of other industrial[14,15] and biotechnological uses.[16,17]

Seaweeds are a diverse group of photosynthetic organisms, which are classified as red (Rhodophyta), green (Chlorophyta), or brown algae (Ochrophyta, Phaeophyceae), according to their pigmentation, the composition of their cell walls, and their polysaccharide reserves.[18-20] Seaweeds are exceptionally resilient organisms, which arose between one and 1.6 billion years ago and have survived numerous mass extinction events.[21,22] Worldwide, nearly

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12,000 seaweed species are known to exist,[23] of which, 1,707 have been recorded in the tropical and subtropical waters of the western Atlantic.[24] Along the 194 km coastline of Pernambuco, a state in the Northeast Region of Brazil, sandstone reefs are very abundant, occurring in parallel to the coastline as patches or elongated bank reefs attached to the coast or at depths of 5-10 m.[25,26] These reefs are densely and mostly colonized by macroalgae typical of the tropical phytogeographic region.[27]

For maintenance in harsh natural conditions, seaweeds produce a variety of unique metabolites, which have a wide range of commercial and industrial applications.[28-31] Several bioactive compounds can be extracted from seaweed, such as minerals (calcium, magnesium, potassium, and iron), trace elements (zinc, manganese, and selenium), fiber, proteins, carbohydrates, lipids, and vitamins, including vitamins A, B, C, E, and K.[5,30,32] Seaweed extracts also tend to have high concentrations of secondary metabolites (phenolic and halogenated compounds, sterols, and terpenes) that have antioxidant, anti-inflammatory, anti-cancer, antiviral, bactericide, anti-fungal, and immune-modulating properties.[33,34] The specific chemical composition of seaweed will depend on a range of factors, including genetic variation, geographic distribution, and environmental conditions.[32,35,36]

The vastness and great biodiversity of the oceans have meant that every day more researchers have been moving away from terrestrial to the marine ecosystem looking for unique therapeutic molecules with high pharmaceutical and biotechnological potentials.[37,38] Despite the toxicity of some macroalgae[39,40] and the challenges of cultivating, producing, and processing,[41,42] several species have non-toxic, edible, inexpensive, and easy-to-grow properties, making them excellent candidates for a source of compounds for human use.[43] Nearly 427 seaweed species are known to produce marine natural products (MNPs), and from these, more than 3129 compounds have been discovered (Rhodophyta, 1658 MNPs or 53% of the total; Ochrophyta, 1213 MNPs; 39%; Chlorophyta, 258 MNPs; 8%).[44] Seaweed-derived compounds have been used as functional ingredients to boost the nutritional value of food,[5,31] and to produce high-value cosmetics,[45] and have shown active properties such as anticancer,[46] antidiabetic,[47] antifungal,[33] antibacterial,[48] antihypertensive,[49] antiviral,[50] immunomodulatory,[51] and thyroid-stimulating.[52]

Considering the potential of seaweed as a biocompound source in a world with a growing population and ever-increasing demand for products from natural origin, in particular, seaweed that could supply the protein needed by many populations while conserving natural resources,[53] identifying and quantifying nutrients and bioactive compounds in marine seaweed species can be extremely valuable. The present study investigated the chemical composition of the red algae *Gelidiella acerosa* and *Palisada perforata*, the brown alga *Padina gymnospora*, and the green alga *Ulva lactuca* from sandstone reefs on the tropical Brazilian coast, aiming to quantify their minerals and metabolites, and to discuss the potential of these algae for providing bioactive compounds for use by humans.

Experimental

Study area

The study was conducted in Enseada dos Corais Beach (Pernambuco, Northeastern of Brazil). The beach is approximately 3 km long and has offshore sandstone reefs running parallel to the coastline.[54] These reefs are colonized by *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa*, where they occur throughout the year.[26] The local climate is tropical humid, with a mean temperature of 28 ºC and two well-defined seasons. The dry season lasts from September to February, and the rainy, from March through August.[55] The tidal regime is of the mesotidal semi-diurnal type, with tide heights ranging from 0.7 m (neap tide) to 2.5 m, on the spring tide.[56]

Materials and methods

Sample collection and identification

Samples of red algae *Gelidiela acerosa* and *Palisada perforata*, the brown algae *Padina gymnospora,* and the green algae *Ulva lactuca* (approximately 1 kg of fresh alga *per* species) were collected randomly by hand from the intertidal zone of the sandstone reefs during the low spring tide in December 2018 (SISBIO (Research Authorization in Federal Conservation Units (FUCs)) license number: 66638-3). After collection, the samples were washed thoroughly in seawater to remove the attached fauna, epiphytes, and sand particles, and then stored on ice in a cooler for transportation to the laboratory. The macroalgae species were identified based on Joly and Pereira,[57] Littler and Littler,[58] Pedrini[59] and Guiry and Guiry.[23]

Preparation of the seaweed for chemical analysis

In the laboratory, the samples were washed under running water to remove the salt, dried at room temperature, and ground to a fine powder. The investigation of the metabolites (carbohydrates, protein and lipids) was carried out following the procedures described in "Determination of the ash"; "Determination of the minerals"; "Carbohydrates"; "Protein" and "Lipids" sub-sections. For major metabolites, samples of the seaweed powder were extracted using a 2:1 solution of dichloromethane (Neon, Suzano, Brazil) and methanol (Neon, Suzano, Brazil). After 72 h, the extracts were filtered, and the solvent was removed by evaporation under reduced pressure and a maximum temperature of 40 °C in a rotary evaporator. The dried extracts were subsequently analyzed as described in "Determination of the major metabolites" sub-section. The percentage yield of the extracts of each seaweed was calculated based on the algae dry weight.

Determination of the ash

The ash content was quantified as described by Robledo and Freile-Pelegrin,[60] with modifications. Samples of 2 g were calcined at 300 °C for ca. 1 h, and then, at 800 °C for 2 h. At the end of the process, the crucibles containing the ash were cooled in a desiccator, and the mass of the ash (g) was determined by the equation 1:

$$\text{Total ash} = \text{acm} - \text{crm} \qquad (1)$$

where, acm is the ash mass (g) plus the crucible mass (g), and crm is the crucible mass (g).

Determination of the minerals

The amount of Ca, Mg, Fe, Cu, Zn, Mn, and Cr was determined by dissolving 2.0 g of the dried seaweed biomass in 10 mL of 2% nitric acid (Neon, Suzano, Brazil), which was then quantified in a Shimadzu AA-6300 atomic absorption spectrophotometer (Shimadzu Scientific Instruments, Columbia, USA). The Na and K content was determined in a DM-61 Digimed Flame Photometer (Digimed, Vila Gea, Brazil).

Determination of the metabolites (carbohydrates, protein and lipids)

Carbohydrates

Soluble carbohydrates were extracted from the dry seaweed biomass using 5% trichloroacetic acid (Merck, Darmstadt, Germany) and the concentrations were determined by the phenolic sulphuric acid colorimetric method described by Dubois *et al.*[61] The percentage of soluble carbohydrates (% dry weight) was calculated based on the absorption at 490 nm in a UV-Vis spectrophotometer HP 8452 (Hewlett-Packard/Agilent technologies, Santa Clara, CA, United States), which was compared to a glycogen standard.

Protein

The protein content (% dry weight) of the dry seaweed biomass was determined using the method described by Kjedahl. The total nitrogen was multiplied by factor 6.25.[62]

Lipids

The lipid content (% dry weight) of the dry seaweed biomass was determined by extraction in a Soxhlet apparatus for 8 h, using petroleum ether (Sigma-Aldrich, Barueri, Brazil) as the solvent. The extracted material was dried in an oven at 105 ± 2 ºC until reaching a constant weight (determined gravimetrically).

Determination of the major metabolites

The samples were analyzed in a Shimadzu gas chromatograph (GC-2010) coupled to a Shimadzu mass spectrometer (GCMS-QP2010 Ultra) (Shimadzu, Kyoto, Japan) equipped with a 30 m long RTX-5MS capillary column, with an internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium 5.0 (purity: 99.9990%) with a flow rate of 1 mL min^{-1}. The starting oven temperature was 40 °C, with an initial heating ramp of 5 °C min^{-1} to 220 °C and 20 °C min^{-1} to 280 °C. The injection mode was spitless, with 1 µL being injected. The run lasted 25 min. The mass spectra were obtained by 70 eV electron impact ionization (EI), with the ion source being maintained at a temperature of 250 °C. The NIST08, NIST08+S, and FFNSC 1.3 databases were used for comparison, complying with a minimum similarity of 90%. Substances with a concentration over 5% were considered to be majority compounds. To express the data, the chromatographic peak areas were used to determine relative peak area (%).

Statistical analysis

A one-way analysis of variance (ANOVA) was applied to compare the amount of minerals, ash and metabolites in different seaweed species (fixed factor), using data log (x+1) transformed. When the ANOVA results were significant, a Tukey's *post hoc* test was applied for pairwise comparisons. These analyses were run in Statistica® 12,[63] and a significance level of 95% was considered in all cases.

Results

The yields of the crude extracts from the four algal species are shown in Table 1. *Palisada perforata* had the highest yield (1.9%), followed by *Gelidiella acerosa* (1.2%), *Padina gymnospora* (1.1%), and *Ulva lactuca* (0.7%).

Table 1. Percentage yield of the crude extracts of *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from coastal sandstone reefs on the tropical Brazilian coast (Pernambuco, Northeastern of Brazil)

Phylum	Species	Amount of dried seaweed collected / g	Dried extract / g	Yield / %
Chlorophyta	*Ulva lactuca*	140.5	1.1	0.7
Phaeophyta	*Padina gymnospora*	115.4	1.2	1.1
Rhodophyta	*Gelidiella acerosa*	90.6	1.1	1.2
	Palisada perforata	262.8	5.0	1.9

Metabolites (carbohydrates, protein, lipids) and ash

The content of carbohydrates (F = 1202.7, $p < 0.01$), proteins (F = 88.2, $p < 0.01$), lipids (F = 2408.4, $p < 0.01$), and ash (F = 385.5, $p < 0.01$) varied significantly among the four seaweed species (Figure 1). Significantly lower amounts of all these metabolites were found in the brown alga *P. gymnospora*, while the red algae *P. perforata* had significantly more carbohydrates and lipids than the other species. The highest protein content was recorded in *P. perforata*, and *U. lactuca*, and *P. perforata* also contained significantly more ash than the other species (Figure 1).

Minerals

Except for Cr (F = 1.34, p = 0.33), the concentrations of all the minerals varied significantly among seaweed species (Figure 2). The greatest variation was recorded in Ca (F = 2135, $p < 0.01$), Mg (F = 2512, $p < 0.01$), Fe (F = 60087, $p < 0.01$), Na (F = 3721, $p < 0.01$), and Mn (F = 290549, p < 0.01). Less pronounced but still significant variation was recorded in K (F = 1983, $p < 0.01$), Cu (F = 124.8, $p < 0.01$), and Zn (F = 37.33, $p < 0.01$). In general, maximum contents of most minerals (Ca, Mg, Na, K, Mn, Cu) occurred in *P. gimnospora*. *U. lactuca* had extremely high amounts of Fe and Zn (Figure 2).

Major metabolites

The major chemical groups were terpenes and fatty acids. Neophytadiene, phytol, and palmitic acid were recorded in all species, and neophytadiene was the major compound. *Palisada perforata* had the highest concentrations of major metabolites (neophytadiene: 23.89% dry weight, phytol: 8.29% dry weight; palmitic acid: 8.32% dry weight), while *U. lactuca* had the lowest concentrations, except for phytone, which was present only in this species (Figure 3 and Table 2).

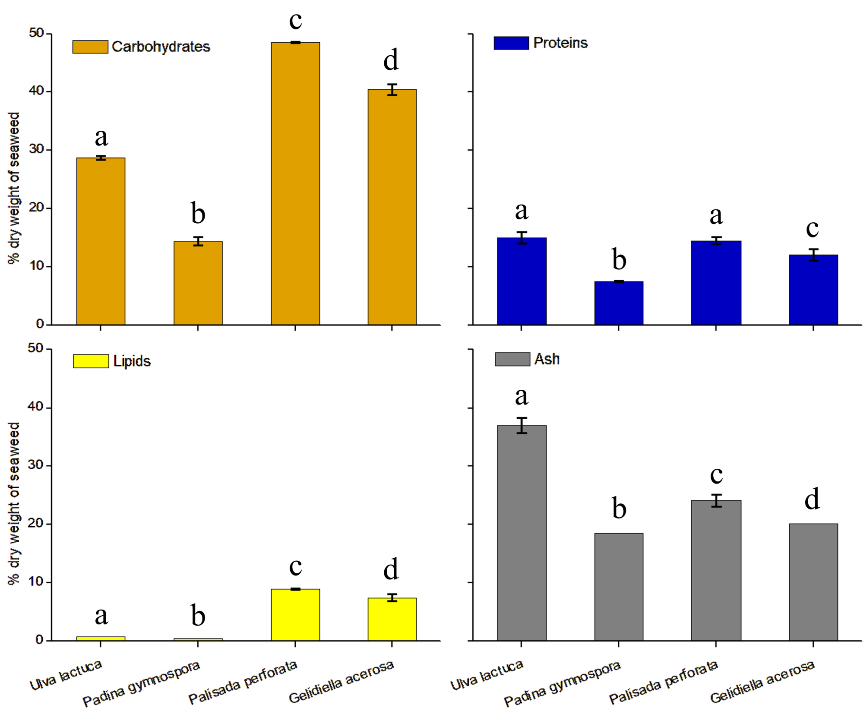

Figure 1. Amount (mean ± standard deviation) of metabolites in *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from coastal sandstone reefs on the tropical Brazilian coast (Pernambuco, Northeastern of Brazil). Distinct letters indicate significant differences.

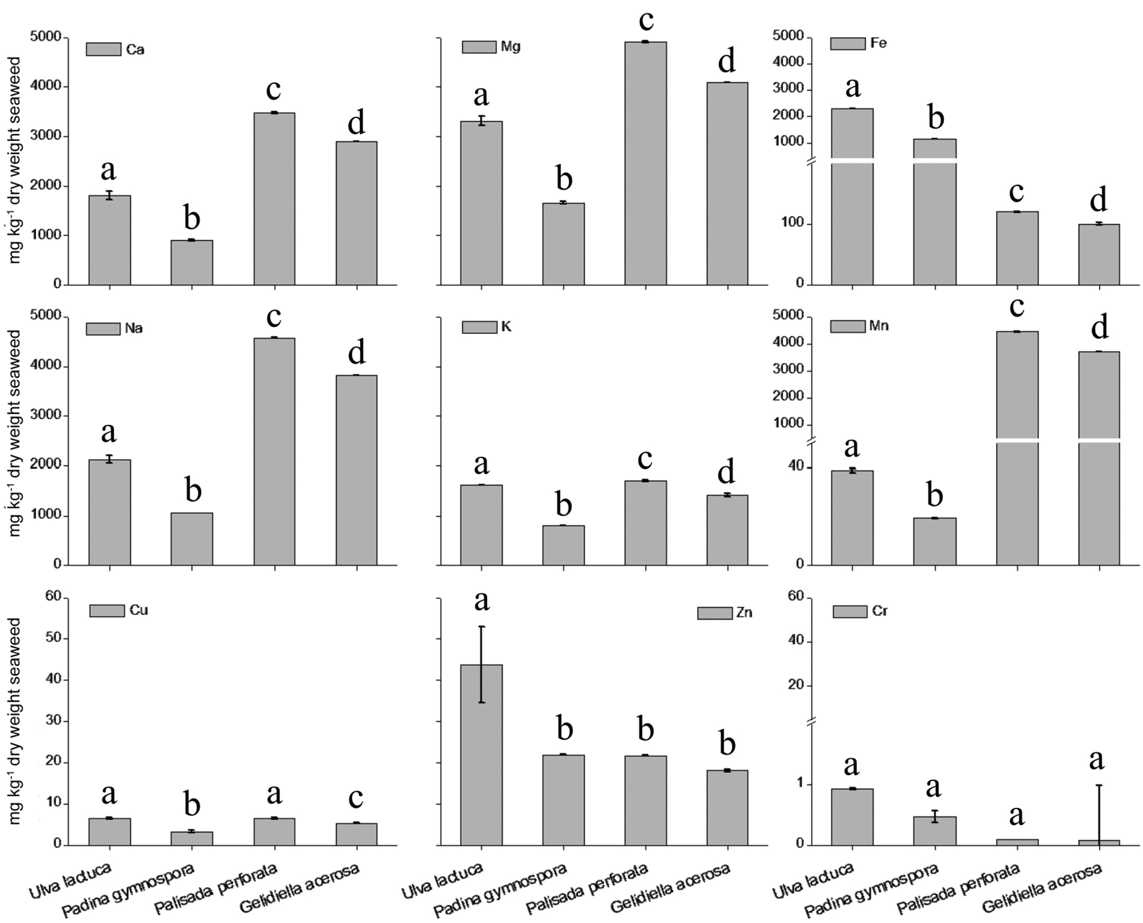

Figure 2. Amount (mean ± standard deviation) of minerals in *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from coastal sandstone reefs on the tropical Brazilian coast (Pernambuco, Northeastern of Brazil). Distinct letters indicate significant differences.

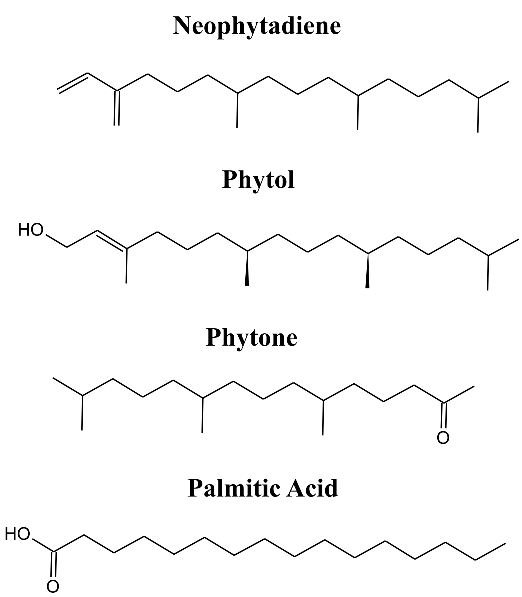

Figure 3. Chemical structures of the major metabolites in *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from coastal sandstone reefs on the tropical Brazilian coast (Pernambuco, Northeastern of Brazil).

Discussion

The four seaweed species, *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata,* and *Gelidiella acerosa*, from the sandstone reefs of Enseada dos Corais, on the Brazilian tropical coast, have very distinct chemical composition. Seaweeds produce metabolites in response to both abiotic and biotic factors,[29,35] and the chemical composition of these organisms is known to vary according to genetic variation, geographic distribution, and environmental conditions, such as salinity, temperature, luminosity, and growth habitats.[32,36,64] Despite occupying the same reefs, the studied species belong to different phyla, being probably the principal determinant of the differences observed in their chemical composition, as already observed in other studies comparing chemical compounds in brown, red, and green algae.[65-67]

Carbohydrates were the most abundant compound in all species, as is typical in seaweeds.[32,34,68] Carbohydrates derived from seaweeds are classified into different classes, namely fucoidan, alginate, carrageenan, ulvan, laminarin, and cellulose and hemicellulose, depending

Table 2. Major compounds, retention time (t_R), and concentration (% seaweed dry weight) of major metabolites in *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from coastal sandstone reefs on the tropical Brazilian coast (Pernambuco, Northeastern of Brazil)

Compound	Chemical group	*Ulva lactuca*		*Padina gymnospora*		*Palisada perforata*		*Gelidiella acerosa*	
		t_R / min	Concentration / %	t_R / min	Concentration / %	t_R / min	Concentration / %	t_R / min	Concentration / %
Neophytadiene	terpene	18.69	17.60	18.712	23.89	18.68	29.04	18.73	24.13
Phytol		19.14	8.47	19.164	8.29	19.13	11.29	19.18	09.69
Palmitic acid	fatty acid	20.01	6.20	20.035	8.32	19.99	08.10	20.05	07.75
Phytone	ketone	18.77	6.05	–	–	–	–	–	–

on their chemical composition.[65] Marine algae contain relatively large amounts of polysaccharides including mucopolysaccharides, and cell-wall and storage polysaccharides, which account for 4-76% of their total dry weight.[69] Many of these carbohydrates function as either a structural component of the cell wall or as storage molecules in the plastids, which provide the energy required for various metabolic processes.[70]

The red algae *P. perforata* had the highest carbohydrate content (49.7% dry weight). The taxonomic group is the principal determinant of the occurrence, composition, and structure of the carbohydrates found in marine seaweeds[69] and each class of macroalga produces its unique compounds.[71] Red algae have κ-carrageenan polysaccharides and agar composed of a variety of monomers.[5,72] Although few studies have compared the carbohydrate content of different types of seaweed, namely green, red, and brown algae,[73] one study recorded the highest carbohydrate content in the red alga *Gracillaria corticata* (41.72%) and the lowest in the brown alga *Colpomenia sinuosa* (11.3%). Ilhami *et al.*[74] obtained similar findings and concluded that red macroalgae typically have a higher carbohydrate content than either brown or green macroalgae.

The maximum protein content was recorded in the green alga *U. lactuca* (14.98% dry weight) and the minimum value in the brown alga *P. gimnospora* (1.49% dry weight). In algae, protein plays a crucial role in processes such as enzymatic catalysis, transport and storage, and mechanical sustentative control.[6] Protein content may vary considerably among species, seasons, and environmental conditions,[34,75] although marine macroalgae tend to contain high concentrations of essential amino acids, lectins, glycoproteins, and phycobiliproteins.[30] As observed in the present study, red (12.5-35.2% dry weight) and green algae (9.6-23.3% dry weight) tend to have a higher protein content than brown algae (4.5-16.8% dry weight).[30,34,76]

All studied species had a relatively low lipid content, with the highest concentrations in the red algae *P. perforata* (8.9% dry weight) and *G. acerosa* (7.43% dry weight) and the lowest in the brown alga *P. gymnospora* (0.4% dry weight). In general, seaweeds are not considered a rich source of lipids,[77] and tend to contain on average approximately 4% dry weight.[78,79] The results of the present study are in partial disagreement with previous studies, such as those of Rohani-Ghadikolae *et al.*[68] and Barot *et al.*,[80] which recorded higher lipid content in green algae when compared with red and brown species. However, it is important to note that these authors studied different species from ours and used analytical methods that are also different from those used in the present study, which recommends caution when comparing results.

The ash content varied widely among the seaweed species (18.51-37.02% dry weight). High ash contents are common in seaweed,[81] and values are generally around 20-25% dry weight,[82,83] although higher values have already been recorded (Rupérez:[84] 20.6-39.3% dry weight; Mohammadi *et al.*:[73] 15.84-33.68% dry weight; Jeliane *et al.*:[85] 31-36% dry weight). Most algae have a greater ash content than terrestrial plants, and some of the trace elements found in seaweeds are rare or absent in terrestrial plants.[24] The ash content of a plant tends to correlates with its mineral content,[65,86,87] as observed in the present study. The ash of edible seaweed is known to contain larger amounts of macrominerals (8.083-17.875 mg 100 g^{-1}; Na, K, Ca, Mg) and trace elements (5.1-15.2 mg 100 g^{-1}; Fe, Zn, Mn, Cu) than edible terrestrial plants.[28,84]

Except for Cr, the mineral content of the different elements (Ca, Mg, Fe, Na, K, Mn, Cu, and Zn) varied significantly among the study species. These minerals play a vital role in the growth, development, and protein synthesis of seaweed[88,89] and the availability of these nutrients can affect the production of metabolites by marine algae.[90] Seaweeds can absorb minerals selectively from the seawater and accumulate them in their thallus.[91] In general, the composition and concentrations of minerals found in seaweeds are species and location specific.[68] Minerals such as Ca, Mg, K, and Na are important for the development of the plant and are generally present in larger quantities in marine algae than in freshwater species.[92]

The metabolites groups in the present study were terpenes (neophytadiene and phytol) and fatty acids (palmitic acid), although both groups varied considerably in their abundance among the different seaweed species. The red algae *P. perforata* had a higher content of these metabolites in comparison with the green alga *U. lactuca*. Both chemical groups are typical of seaweeds[93-95] and neophytadiene, phytol, and palmitic acid often comprise the major compounds of terpenes and fatty acids in these organisms.[12,96] In general, this metabolites are excretory produced under stressful conditions, such as exposure to ultraviolet radiation, shifts in temperature and salinity, and pressures from competitors and herbivores,[90,97,98] and are predominantly phenolic and halogenated compounds, sterols, terpenes, and small peptides.[99,100]

Neophytadiene and phytol are the predominant terpenes in many types of seaweed, and their potential industrial applications have been the focus of several studies.[12,101] Phytol is an isoprenoid compound derived primarily from chlorophyll,[102] and is known to have antinociceptive, antioxidant,[103,104] antimicrobial,[33] and immunostimulatory activity in humans.[105] Like phytol, neophytadiene is a diterpene with known antibacterial[33] and antioxidant activity.[106] Bhardwaj *et al.*[11] also found that neophytadiene extracted from the brown macroalga *Turbinaria ornata* significantly inhibited the production of nitric oxide and inflammatory cytokines in *in vivo* and *in vitro* experiments.

Palmitic acid is the most abundant saturated fatty acid found in green, brown, and red algae.[68] This compound has known antioxidant, antifungal, and antibacterial activity,[33,48] and may protect the seaweed against physical, chemical, and biological stressors.[8] While phytone was found only in *U. lactuca* in the present study, it has been observed in other species of green, red, and brown macroalgae.[107,108] The presence of this compound may be the result of the hydrolysis of chlorophyll or bacteriochlorophyll-a photoproducts[109,110] or the biodegradation of phytol.[111,112]

Conclusions

The chemical compounds, including minerals, ash, and metabolites, of the seaweed species *Ulva lactuca*, *Padina gymnospora*, *Palisada perforata*, and *Gelidiella acerosa* from sandstone reefs of the tropical coast of Northeastern of Brazil, are described in this study. In general, these species are rich in proteins (all four), carbohydrates (primarily the red and green algae), and lipids (the red algae in particular). The red and green algae also have high concentrations of essential minerals, while the red and brown algae have the highest concentrations of major metabolites. These findings highlight that the four studied species have considerable potential as a source of chemical compounds for human use in a world with a continuously growing population, and ever-increasing demand for food and medicine. The study also emphasizes the need for further studies to better evaluate the potential of marine seaweeds for commercial, industrial, and pharmaceutical uses, and contribute to the prospection of products from marine origin.

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Author Contributions

Nykon Craveiro was responsible for conceptualization of the study, data analysis, figures (preparation and editing) formal analysis, methodology, and writing original draft and review and editing; Fausthon F. da Silva for formal analysis, and writing original draft review and editing; Marcia S. Nascimento for formal analysis, and writing original draft; Josean Fechine for formal analysis, and writing original draft; Yuri Mangueira for formal analysis, and writing original draft; José S. Rosa Filho for supervision, conceptualization, data analysis, and writing original draft and review and editing. All authors discussed the results and contributed to the final manuscript.

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