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CLINICAL MICROBIOLOGY - RESEARCH PAPER

Bioactivity and composition of lipophilic metabolites extracted from Antarctic macroalgae

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Abstract

Macroalgae comprise a vast group of aquatic organisms known for their richness in phytochemicals. In this sense, the lipophilic profile of five Antarctic seaweed species was characterized by chromatographic and spectroscopic analysis and their antioxidant and antimicrobial potential was evaluated. Results showed there were 31 lipophilic substances, mainly fatty acids (48.73 ± 0.77 to 331.91 ± 10.79 mg.Kg^{-1}), sterols (14.74 ± 0.74 to 321.25 ± 30.13 mg.Kg^{-1}), and alcohols (13.07 ± 0.04 to 91.87 ± 30.07 mg.Kg^{-1}). Moreover, *Desmarestia confervoides* had strong antioxidant activity, inhibiting 86.03 ± 1.47% of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical at 1 mg.mL^{-1}. Antimicrobial evaluation showed that extracts from *Ulva intestinalis*, *Curdiea racovitzae*, and *Adenocystis utricularis* inhibited the growth of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhimurium* (ATCC 14028) from concentrations of 1.5 to 6 mg.mL^{-1}. Therefore, the evaluated brown, red, and green macroalgae contained several phytochemicals with promising biological activities that could be applied in the pharmaceutical, biotechnological, and food industries.

Keywords Lipophilic extract · Bioactive compounds · Macroalgae · Antioxidant activity · Antimicrobial activity

Introduction

The marine environment is known for its biodiversity and for being a source of more than 25,000 natural products [1]. Among photosynthetic organisms that inhabit aquatic ecosystems are macroalgae, which can be divided into green, brown, and red algae depending on morphological and biochemical aspects [2, 3]. Given their biological, chemical, and breeding properties, seaweeds are becoming increasingly important marine resources. Indeed, their harvesting is growing approximately 15% per year, with capture or aquaculture reaching 25 million metric tons in 2014 [4, 5].

The high degree of adaptation of macroalgae allows these organisms to inhabit many environments, running the gamut of tropical, subtropical, temperate and polar regions [4, 6, 7]. In complex habitats that include the Antarctic Peninsula, seaweeds are subjected to several abiotic parameters, such as low water temperature, restricted nutrient availability, high exposure to ultraviolet radiation, limited photoperiod, and high water salinity [3, 8]. These extreme environmental conditions cause aquatic organisms to develop defense and survival strategies, including activation of biochemical processes related to the production of metabolites [9].

Secondary metabolites found in aquatic ecosystems have unique structural and chemical moieties that are not commonly found in natural products from terrestrial plants [1]. According to previous reports, approximately 15,000 secondary metabolites have been identified in macroalgae, including, for instance, fatty acids (FAs), sterols, polysaccharides, amino acids, flavonoids, and terpenoids [3, 8]. In this sense,

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seaweeds compose an important reserve of possible bioactive compounds that can be used for antimicrobial or antioxidant purposes [2].

The search for novel bioactive compounds is important to minimize antimicrobial resistance. Due to the increasing demand for novel therapeutic drugs, there is growing interest in metabolites found in marine organisms. Several algal species have been reported to produce bactericidal or bacteriostatic substances. In this sense, macroalgal extracts could provide important bioactive compounds for use in biotechnological and pharmaceutical areas, among others [1].

Indeed, macroalgae comprise an almost unlimited reserve of potential biochemical compounds that could be employed in industrial applications. However, although more than 10,000 species of seaweeds have been identified, only a few representatives have been chemically characterized to screen them for molecules with potential applications. In recent years, our research group has analyzed lipophilic components of sub-Antarctic and Antarctic macroalgae including FAs [4, 6] and sterols [10] and successfully indicated their biological applications [11]. The aims of this work were to evaluate fatty acids, sterols, and carboxylic, dicarboxylic, and tricarboxylic acids, among other lipophilic constituents, of five extracts of Antarctic macroalgae and to evaluate their antioxidant and antibacterial activities.

Materials and methods

Sampling

Approximately 5 to 10 individuals of each species of brown, red, and green Antarctic macroalgae were manually collected in the eulittoral or infralittoral zone in several locations of the Antarctic Peninsula between November and December 2015 (Table 1) as part of Brazil's Thirty-Fourth Antarctic Expedition. The samples were washed with seawater and further cleaned with distilled water to remove impurities, microorganisms, and salt. After morphological identification, the specimens were lyophilized, milled, and stored in hermetically sealed bags at −20°C before analyses.

Chemicals and materials

Pyridine, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide, 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH), methyl nonadecanoate, cholesterol, and 1-decanolwere were purchased from Sigma-Aldrich (St. Louis, USA), while *n*-hexane and methanol were obtained from J.T. Baker (Radnor, USA). All other solvents and reagents were analytical grade

Extraction

The lipophilic fraction of each macroalgal sample (5 g) was extracted using *n*-hexane by means of a Soxhlet apparatus for 6 h after the sample was soaked with solvent overnight. Subsequently, the lipophilic extract was dried under reduced pressure. The procedure was performed in triplicate (n=3) and followed the modified method of Santos et al. (2015), replacing dichloromethane with *n*-hexane.

Chemical composition

Hydrolysis and derivatization

Briefly, 10 mg of the lipophilic extract and 10 mL of a 0.5-M solution of sodium hydroxide in methanol:water (50:50, v/v) were constantly mixed and refluxed for 1 h. Afterwards, the system was cooled and acidified to pH 2 by the gradual addition of a 1-M solution of hydrochloric acid. Furthermore, samples were extracted three times with 5 mL of dichloromethane. Lipophilic layers were combined and dried under reduced pressure. The hydrolyzed extracts were reconstituted in 100 μL of chloroform and further derivatized with 100 μL of *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide and 100 μL of pyridine at 70°C for 30 min. Procedures were performed in triplicate (n=3) and followed the method of Santos et al. (2015).

Table 1 Sampling information of the brown, red, and green Antarctic macroalgae

Species	Collection site	Coordinates	Collection date
Ochrophyta			
Adenocystis utricularis	Greenwich Island	62° 29′ S × 59° 47′ W	December, 2015
Desmarestia confervoides	Hennequim Point	62° 7′ S × 58° 23′ W	November, 2015
A.1.1.1.1. Rhodophyta			
Curdiea racovitzae	Punta Plaza	62° 5′ S × 58° 24′ W	November, 2015
Myriogramme manginii	Snow Island	62° 46′ S × 61° 31′ W	December, 2015
A.1.1.1.2. Chlorophyta			
Ulva intestinalis	Robert Island	62° 22′ S × 59° 41′ W	December, 2015

Chromatographic analysis

Chromatographic analysis followed the method of Santos et al. (2015) and was performed in a GCMS-QP2010 system (Shimadzu, Kyoto, Japan) followed by the injection of 1 μL in split mode (1:33) of the derivatized material into an Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 μm; Restek, Bellefonte, USA) with helium gas flow of 1.50 mL.min^{-1}. The injection port and interface operated at 250 and 290°C, respectively. The initial oven temperature was set at 80°C, maintained for 5 min, and increased at 4°C.min^{-1} to 260°C and then at 2°C.min^{-1} until the final oven temperature of 285°C, which was maintained for 8 min.

The mass spectrometer operated using electron ionization at 70 eV with the ion source scanned from m/z 30 to m/z 550. Identification of compounds was performed using the NIST08s spectral library. Quantitation followed the method of Santos et al. (2015) and was performed using pure reference standards as representatives of the major lipophilic families (methyl nonadecanoate, cholesterol, and 1-decanol) in solutions of 1, 0.500, 0.250, 0.125, 0.625, and 0.312 mg.mL^{-1} in *n*-hexane, injected in triplicate (n=3).

Spectroscopic analysis

Approximately 10 μL of each lipophilic extract was analyzed using attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) with a Shimadzu Prestige 21 FTIR spectrometer (Kyoto, Japan) operating from 4000 to 600 cm^{-1} with resolution of 4 cm^{-1}.

Antioxidant activity

Concentrations of 1, 0.500, 0.250, and 0.125 mg.mL^{-1} of lipophilic extracts were evaluated for antioxidant activity by mixing them with 300 μL of DPPH radical methanolic solution (0.394 mg.mL^{-1}) and 3 mL of methanol. Samples were incubated at room temperature for 15 min in the dark and analyzed by spectrophotometry (UV-M51; Bel, Piracicaba, Brazil) at 517 nm. Positive controls were performed using ascorbic acid at the same concentrations of the tested samples. An analytical blank using 300 μL of DPPH radical mixed with 2.7 mL of methanol was also tested. All experiments were performed in triplicate (n=3) and followed the method of Pellati et al. [12]. Inhibition of the DPPH radical at different concentrations of the lipophilic extracts was measured by Eq. 1.

$$\%Inhibition = \left[\frac{A_{DPPH} - (A_{Extract} - A_{Blank})}{A_{DPPH}}\right] \times 100 \quad (1)$$

where A_{DPPH} is the absorbance of the DPPH radical without sample, $A_{Extract}$ is the absorbance of lipophilic extracts mixed with DPPH radical, and A_{Blank} is the absorbance of methanol.

Antibacterial activity

Test organisms

Antimicrobial activity was measured using the gram-positive standard strains *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 51299) as well as the gram-negative standard strains *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028). Microorganisms were provided by the Oswaldo Cruz Foundation (FIOCRUZ). The evaluated strains were maintained in Mueller-Hinton agar at 4°C and reactivated prior to antimicrobial evaluation.

Minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) were determined according to the broth microdilution method following the Clinical and Laboratory Standards Institute (CLSI [13]) guidelines. First, *n*-hexane extracts from Antarctic macroalgae were diluted in brain-heart infusion (BHI) broth at concentrations ranging from 6 to 0.0078 mg.mL^{-1} in 5% of ethanol. As negative control, 100 μL of BHI broth was used, while the positive control consisted of 50 μL of bacterial suspension and 50 μL of BHI broth.

Microorganisms were cultured in BHI broth and standardized to 0.5 on the McFarland scale, resulting in optical density between 0.08 and 0.1 at 630 nm. Afterwards, 50 μL of each cultured sample was diluted in 4950 μL of BHI broth, and 50 μL of each suspension was inserted in a well, resulting in a final concentration of microorganisms of 3.10^4 CFU.mL^{-1}. The MIC values were investigated in triplicate (n=3) and plates were incubated at 37°C for 24 h. Subsequently, 20 μL of *2,3,5*-triphenyl tetrazolium chloride (0.5%, *w/v*) was placed in each well and further incubated for 20 min at 37°C. Finally, bacterial growth was evaluated by color development.

Minimum microbicidal concentration

The minimum microbicidal concentration (MMC) was determined for all samples that had antimicrobial activity. Briefly, 5-μL aliquots were placed in Mueller-Hinton agar plates and incubated at 37°C for 24 h. Afterwards, the presence or absence of bacterial growth was evaluated for the determination of bacteriostatic or bactericidal activity. The experiments were performed in triplicate (n=3).

Statistical analysis

Two-way analysis of variance (ANOVA) followed by the Tukey test (p<0.05) was applied to determine significant differences between the constituents of the samples using GraphPad version 7 (La Jolla, USA). Principal component

analysis (PCA) was used to evaluate similarities in the lipophilic composition of the algal extracts, employing the Minitab software version 17 (State College, USA).

Results

Chemical evaluation

Lipophilic yields of the studied Antarctic macroalgae (Table 2) were generally in the range of 0.189 ± 0.005 to 0.356 ± 0.038% DW in brown seaweed; 0.134 ± 0.005 to 0.138 ± 0.003% DW in red seaweed; and 0.187 ± 0.021% DW in *U. intestinalis* (green seaweed). Generally, brown macroalgae had the highest amounts of extracted lipophilic compounds, reaching as much as 0.356 ± 0.038% DW in *D. confervoides*. On the other hand, the lowest lipophilic yield was observed in *M. manginii*, of 0.134 ± 0.005% DW.

Evaluation of chemical composition of the lipophilic extracts from Antarctic macroalgae (Table 3) showed that together the macroalgae had 31 distinct compounds, identified as FAs, alcohols, sterols, ketones, aldehydes, hydrocarbons, and other chemical classes. The highest number of compounds was found in Ochrophyta representatives such as *D. confervoides* and *A. utricularis*, which had 24 and 23 constituents, respectively, while *U. intestinalis* had the lowest variety, reaching 19 compounds.

As can be observed in Table 3, *D. confervoides* contained the most compounds found in higher concentrations compared to the other analyzed specimens. Generally, 2-ethylhexanoic acid (11.12 ± 5.48 to 49.65 ± 6.32 mg.kg^{-1}), hexadecanoic acid (7.92 ± 0.09 to 49.01 ± 6.18 mg.kg^{-1}), 2-butoxyethanol (7.48 ± 0.12 to 56.37 ± 26.20 mg.kg^{-1}), and fucosterol (nd—282.96 ± 29.02 mg.kg^{-1}) were the constituents found in the most relevant concentrations in the samples. The high presence of these compounds made the lipophilic extracts dominated by FAs (48.73 ± 0.77 to 331.91 ± 10.79 mg.kg^{-1}), sterols (14.74± 0.74 to 321.25 ± 30.13 mg.kg^{-1}), and alcohols (13.07 ± 0.04 to 51.87 ± 30.07 mg.kg^{-1}).

Table 2 Lipophilic extract yields in dry weight (% DW) of brown, red, and green Antarctic macroalgae

Sample	Extract yield (DW %)
U. intestinalis	0.187 ± 0.021^{abc}
D. confervoides	0.356 ± 0.038^{bc}
A. utricularis	0.189 ± 0.005^{c}
M. manginii	0.134 ± 0.005^{a}
C. racovitzae	0.138 ± 0.003^{a}

Results expressed as mean ± standard deviation of triplicates (n=3)

Different superscript letters indicate significant difference (p<0.05)

Spectroscopic analysis of the lipophilic extracts (Table 1S) showed that samples mainly showed vibrations that corresponded to aliphatic carbon-hydrogen bonds (2963 to 2855 cm^{-1}, stretching; 1457 to 1371 cm^{-1}, bending), carbonyl groups (1742 to 1715 cm^{-1}), and carbon-oxygen bonds (1230 to 1203 cm^{-1}). Moreover, extracts from *A. utricularis* and *D. confervoides* had unsaturation (3009 to 3004 cm^{-1}), while *M. manginii* and *U. intestinalis* had hydroxyl groups (3393 to 3357 cm^{-1}). IR spectra and GC-MS chromatograms of the lipophilic extracts can be seen in the Supplementary Information section.

Antioxidant activity

Evaluation of antioxidant activity of the lipophilic extracts (Fig. 1) showed that *D. confervoides* inhibited 86.03 ± 1.47% of the DPPH radical at 1 mg.mL^{-1} and maintained similar antioxidant potential at 0.500 and 0.250 mg.mL^{-1}, which inhibited 83.79 ± 2.12 and 80.40 ± 1.94%, respectively. The lowest antioxidant capacity of the lipophilic extract was observed at 0.125 mg.mL^{-1}, which had 63.39 ± 0.49% of inhibition of the DPPH radical. Moreover, comparison of the samples with the positive control revealed that all lipophilic extracts had lower antioxidant activity than ascorbic acid.

As can be seen in Fig. 1, lipophilic extracts from *A. utricularis*, *U. intestinalis*, *M. manginii*, and *C. racovitzae* did not have significant antioxidant activity compared to *D. confervoides*. Generally, samples inhibited the DPPH radical in major proportions at 1 mg.mL^{-1}, reaching 15.21 ± 1.01, 10.30± 1.26, and 8.70 ± 0.91% inhibition for *M. manginii*, *C. racovitzae*, and *A. utricularis*, respectively. However, at the maximum tested concentration, *U. intestinalis* had little antioxidant capacity, inhibiting 1.80 ± 0.36% of the DPPH radical. The lipophilic extracts mostly acted as antioxidants at 0.500 mg.mL^{-1}, but their potential decreased or could not be observed at concentrations lower than 0.250 mg.mL^{-1}.

Antimicrobial activity

Antimicrobial evaluation of *n*-hexane extracts from Antarctic macroalgae (Table 4) indicated the materials had activity against all the tested microorganisms except *Enterococcus faecalis*, which had bacterial growth in the evaluated concentrations (0.187 to 6 mg.mL^{-1}). *Desmarestia confervoides* and *M. manginii* extracts did not have antimicrobial activity in the experimental conditions. In general, the lipophilic extracts had MICs that ranged from 6 to 1.5 mg.mL^{-1}, varying according to the specimen and the microorganism. Furthermore, the MMC values of the samples indicated that concentrations that inhibited bacterial growth were bacteriostatic to the tested organisms.

Table 3 Chemical constitution of the lipophilic extracts of green, brown, and red Antarctic macroalgae expressed as mg.kg^{-1} of dry material

Compound	*C. racovitzae*	*D. confervoides*	*M. manginii*	*U. intestinalis*	*A. utricularis*
2-Ethylhexanoic acid	11.12 ± 5.48^{a}	49.65 ± 6.32^{b}	11.57 ± 0.48^{a}	22.12 ± 0.07^{c}	30.91 ± 0.30^{c}
5-Oxohexanoic acid	4.69 ± 0.01^{a}	12.42 ± 0.18^{a}	4.50 ± 0.03^{a}	6.29 ± 0.05^{a}	6.47 ± 0.03^{a}
Decanoic acid	nd^{a}	nd^{a}	nd^{a}	10.36 ± 0.03^{b}	nd^{a}
Dodecanoic acid	5.27 ± 0.13^{ab}	nd^{b}	nd^{b}	10.89 ± 0.18^{a}	10.42 ± 0.09^{a}
Tetradecanoic acid	5.44 ± 0.14^{ac}	28.93 ± 0.38^{b}	nd^{c}	8.24 ± 0.15^{ac}	14.00 ± 1.00^{a}
Hexadecenoic acid	nd^{a}	22.84± 0.62^{b}	5.08 ± 0.03^{a}	13.54 ± 0.00^{c}	8.69 ± 0.34^{c}
Hexadecanoic acid	10.10± 0.00^{ad}	49.01± 6.18^{b}	7.92 ± 0.09^{d}	18.92 ± 0.14^{ac}	24.73± 1.93^{c}
Octadecanoic acid	4.71 ± 0.00^{a}	20.97 ± 0.32^{b}	4.44 ± 0.01^{a}	8.27 ±0.25^{a}	7.82 ±0.60^{a}
Octadecenoic acid	5.19± 0.00^{a}	75.89± 1.55^{b}	5.86 ± 0.12^{a}	13.23 ± 0.19^{ac}	18.85 ± 0.38^{c}
trans-Octadecenoic acid	4.92± 0.11^{a}	nd^{a}	4.55 ± 0.02^{a}	nd^{a}	nd^{a}
Octadecadienoic acid	4.67 ± 0.13^{ac}	26.67 ± 0.01^{b}	nd^{c}	nd^{c}	10.92 ±1.60^{a}
Octadecatrienoic acid	nd^{a}	nd^{a}	nd^{a}	6.37 ± 0.02^{a}	nd^{a}
Eicosapentaenoic acid	4.96 ± 0.07^{ac}	18.49 ± 0.55^{b}	4.79 ± 0.02^{ac}	nd^{c}	9.69 ±0.35^{a}
Eicosatetraenoic acid	4.63± 0.01^{ac}	27.00± 11.41^{b}	nd^{ac}	nd^{ac}	7.51 ± 0.37^{a}
Fatty acids	66.75 ± 5.01^{a}	331.91 ± 10.79^{b}	48.73 ± 0.77^{c}	118.28 ± 0.16^{d}	150.05 ± 4.29^{e}
Hexanedioic acid	nd^{a}	nd^{a}	5.20 ± 0.03^{a}	nd^{a}	nd^{a}
Nonanedioic acid	nd^{a}	25.23± 0.38^{b}	nd^{a}	nd^{a}	nd^{a}
Benzoic acid	4.77 ± 0.04^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
Benzeneacetic acid	4.69 ± 0.00^{a}	nd^{a}	4.47 ± 0.03^{a}	nd^{a}	7.07 ± 0.05^{b}
Benzenedicarboxylic acid	nd^{a}	13.67 ± 0.25^{b}	4.88 ± 0.03^{ab}	nd^{a}	nd^{a}
Acetyltributylcitrate	4.79 ± 0.06^{a}	nd^{a}	4.93 ± 0.08^{a}	nd^{a}	nd^{a}
Carboxylic acids	14.26 ± 0.10^{a}	38.90 ± 0.64^{b}	19.50 ± 0.17^{a}	nd^{c}	7.07 ± 0.05^{d}
Cyclopentanol	5.17 ± 0.02^{ac}	13.29 ± 0.08^{ab}	nd^{c}	6.95 ± 0.00^{ac}	nd^{c}
2-Butoxyethanol	30.98 ± 0.11^{a}	56.37 ± 26.20^{b}	7.48 ± 0.12^{c}	12.92 ± 0.20^{c}	55.14 ± 9.64^{b}
Methylcyclohexenol	7.81 ± 0.11^{a}	22.20 ± 3.79^{b}	5.59 ± 0.08^{ac}	8.28 ± 0.01^{ac}	12.55 ± 0.45^{ac}
Alcohols	43.97 ± 0.20^{a}	91.87 ± 30.07^{b}	13.07 ± 0.04^{c}	28.16 ± 0.22^{d}	67.70 ± 10.09^{e}
Cholesterol	28.82 ± 0.40^{a}	18.14 ± 0.07^{a}	14.74 ± 0.74^{b}	9.42 ± 0.12^{b}	11.88 ± 0.75^{b}
Hydroxymethylcholesterol	nd^{a}	20.15 ± 1.02^{bc}	nd^{a}	nd^{a}	12.83 ± 0.25^{c}
Fucosterol	nd^{a}	282.96 ± 29.02^{b}	nd^{a}	12.02 ± 0.00^{c}	41.60 ± 3.32^{d}
Sterols	28.82 ± 0.40^{a}	321.25 ± 30.13^{b}	14.74 ± 0.74^{c}	21.45± 0.11^{d}	66.32 ± 4.33^{e}
α-Tocopherol	nd^{a}	14.80 ± 0.36^{b}	nd^{a}	nd^{a}	nd^{a}
Phytol	5.66 ± 0.08^{a}	27.40 ± 1.08^{b}	7.23 ± 0.09^{a}	13.14 ± 0.11^{a}	10.05 ± 0.42^{a}
L-cysteine	nd^{a}	4.99 ± 0.09^{b}	4.81 ± 0.00^{b}	nd^{a}	nd^{a}
Tetradecane	5.08 ± 0.00^{a}	4.66 ± 0.09^{a}	4.84 ± 0.03^{a}	7.92 ± 0.14^{a}	6.51 ± 0.00^{a}
Dihydroactinolide	nd^{a}	13.45 ± 0.11^{b}	nd^{a}	8.09 ± 0.42^{c}	7.89 ± 0.35^{c}
Benzaldehyde	nd^{a}	nd^{a}	4.68 ± 0.10^{a}	nd^{a}	nd^{a}
Hydroxymethylpentanone	5.21 ± 0.04^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
Trimethylbenzene	5.27 ± 0.06^{a}	5.01 ± 0.07^{a}	4.56 ± 0.02^{a}	4.49 ± 0.00^{a}	6.51 ± 0.37^{a}
Others	21.22 ± 0.18^{a}	70.32 ± 1.61^{b}	26.16 ± 0.01^{ac}	33.65 ± 0.69^{c}	30.97 ± 0.43^{c}

Results expressed as mean ± standard deviation of triplicates (n=3)

Results without a common superscript letter are significant different (p<0.05)

According to the results seen in Table 4, growth of *E. coli* was inhibited at concentrations above 1.5 mg.mL^{-1} by *A. utricularis* and *U. intestinalis* extracts, while *C. racovitzae* had MIC of 3 mg.mL^{-1}. In the case of *Staphylococcus aureus*, the MIC value of *A. utricularis* and *U. intestinalis* extracts was 3 mg.mL^{-1} while *C. racovitzae* inhibited bacterial growth at 6 mg.mL^{-1}. Similar results were observed for *Salmonella typhimurium*, for which lipophilic extracts from *C. racovitzae* and *A. utricularis* had MIC of 6 mg.mL^{-1}, while *U. intestinalis* had antimicrobial activity at concentrations above 3 mg.mL^{-1}.

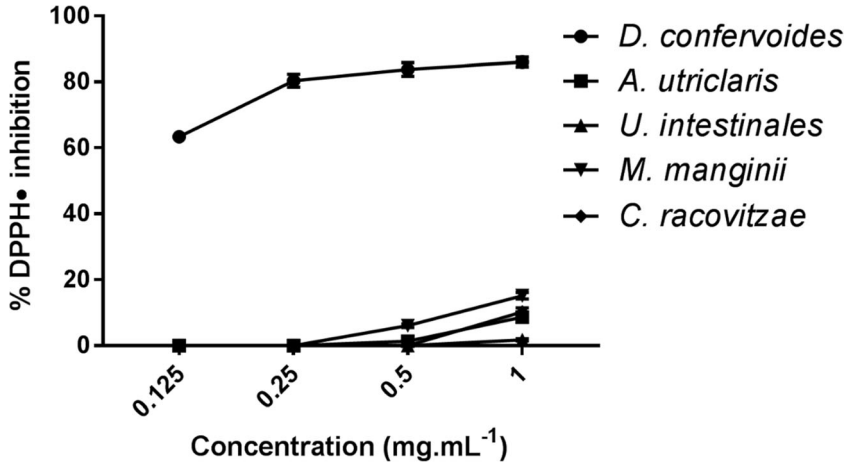

Fig. 1 Antioxidant evaluation of lipophilic extracts from Antarctic macroalgae

Multivariate analysis

PCA was applied to evaluate the chemical composition, antioxidant activity, and antimicrobial activity among the studied specimens (Fig. 2). For the multivariate analysis, substances that caused significant variations including hexadecanoic, eicosatetraenoic, and eicosapentaenoic acids as well as fucosterol and 2-butoxyethanol were chosen. The obtained loading plot (Fig. 2b) indicated that most substances clustered along the positive axis of the first principal component (PC1) while those in the second principal component (PC2) varied from the negative to positive axis. On the other hand, the antimicrobial activity of the tested organisms was found to be negative in PC1 and PC2.

The resulting score plot (Fig. 2a) showed that the studied Antarctic macroalgae could be differentiated based on the chosen variables. In general lines, Ochrophyta representatives were found along the positive axis of PC1, while *Rhodophyta* and *Chlorophyta* species were observed along the negative axis of PC1. The results showed that bioactive potential can be associated with the three phyla of seaweeds.

Table 4 Antimicrobial evaluation of *n*-hexane extracts from Antarctic macroalgae

Microorganism	MIC of macroalgae extract (mg.mL^{-1})		
	U. intestinalis	*C. racovitzae*	*A. utricularis*
Escherichia coli	1.5	3	1.5
Staphylococcus aureus	3	6	3
Salmonella Typhimurium	3	> 6	6
Enterococcus faecalis	> 6	1.5	> 6

Note: Minimum inhibitory concentration (MIC). Extracts from *D. confervoides* and *M. manginii* did not have antimicrobial activity in the tested microorganisms

Discussion

Chemical evaluation

Extractive yield and spectroscopic analysis

Previous reports in the literature indicate that lipophilic extracts of brown, green, and red macroalgae had values that ranged from 0.12 ± 0.01% DW to 1.74 ± 0.08% DW, which agree with the results found by us [4, 9]. Martins et al. (2018) analyzed Antarctic macroalgae and found small extractive yields in their specimens. Nonetheless, the extracts had antimicrobial and antifungal activities, indicating the presence of potential bioactive substances [11].

Spectroscopic evaluation of the lipophilic extracts indicated that the samples mainly consisted of lipid esters, since the main vibrations observed in the spectra corresponded to carbon-hydrogen bonds, carbon-oxygen bonds, and carbonyl groups [14]. Moreover, hydroxyl groups in the extracts of *M. manginii* and *U. intestinalis* were associated with non-esterified lipids and alcohols, as indicated in Table 3. Curiously, little information is available regarding the spectroscopic profiles of macroalgal extracts, despite the importance of fully characterizing these substances.

Fatty acids

FAs are among the vast lipid classes found in macroalgae, acting as membrane constituents (e.g., phosphoglycerides) or for energy storage (e.g., triacylglycerol). They generally have higher concentrations than other lipids, including sterols, hydrocarbons, and fatty alcohols, for instance [15–17]. In this sense, comparison between our results and those reported in the literature showed that the majority FAs found in *U. intestinalis* (hexadecanoic, hexadecanoic, and octadecenoic acids) were also identified by Martins et al. (2016), who analyzed the same species collected in the sub-Antarctic region [6].

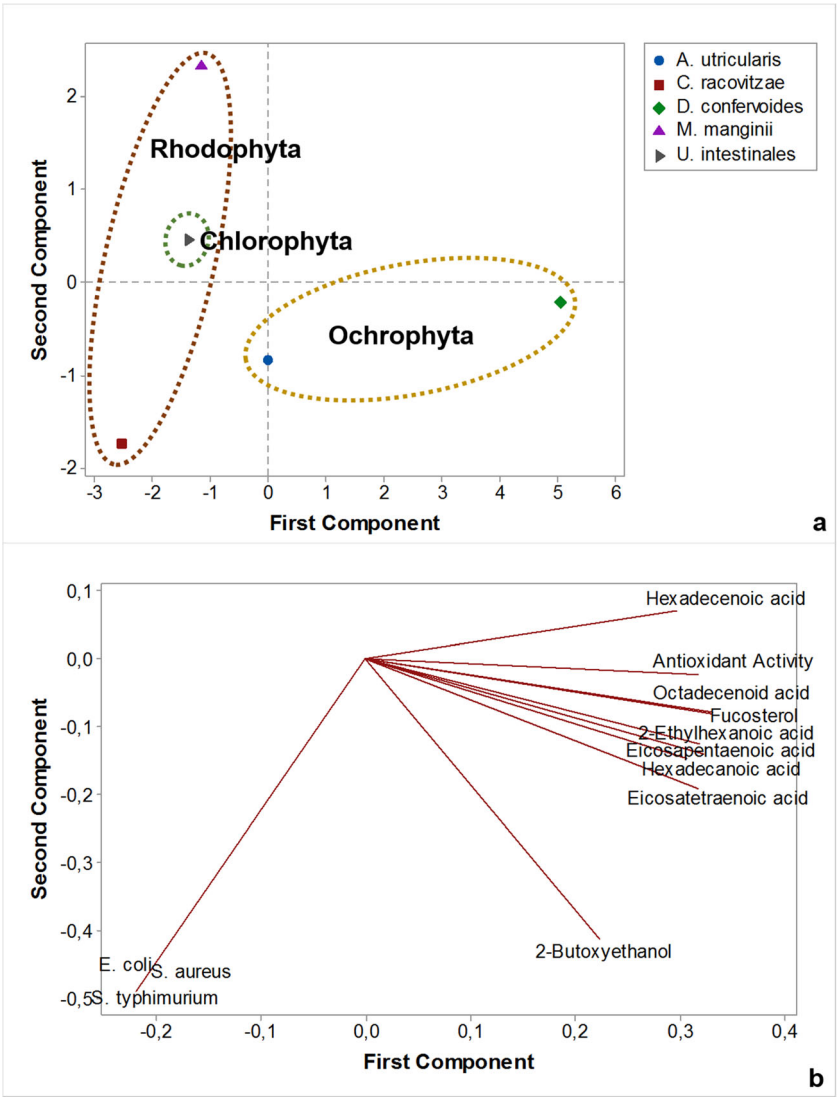

Fig. 2 Multivariate analysis of the chemical composition and bioactivity of lipophilic extracts from Antarctic macroalgae

Regarding *C. racovitzae* and *A. utricularis*, qualitative results were similar to the literature, but we found lower concentrations of PUFAs than reported by Pacheco et al. (2018). To the best of our knowledge, the FA profile of *M. manginii* and *D. confervoides* has not been previously reported in the literature. Nonetheless, representatives from the same order had similar patterns to those observed by us [7, 18].

Among the reasons explaining the variations between our results and those reported in the literature are the influences of the Antarctic environment, which include limited photoperiod and low water temperature, salinity, nutrient disposal, and pH [4, 19]. These abiotic parameters influence the production of FAs in seaweeds, primarily inducing the biosynthesis of PUFAs in order to maintain the integrity of membranes [18, 19]. Moreover, differences in the extraction approaches may also have influenced the overall results, since the extraction of FAs is generally performed using a solution of chloroform:methanol [20].

Carboxylic, dicarboxylic, and tricarboxylic acids

Carboxylic and dicarboxylic acids, including hexanedioic, octanedioic, nonanedioic, and undecanedioic acids, have been widely reported in the literature as constituents of seaweeds [3, 4]. These compounds have been associated with biological activities such as antimicrobial action, as well as for treatment of skin hyperpigmentation [21]. Previous research has identified the presence of carboxylic and dicarboxylic acids in macroalgae. Santos et al. (2016) detected octanedioic and 2-butenedioic acid as majority dicarboxylic acids in *Undaria pinnatifida* and *Cystoseira tamariscifolia*, with total amounts of 99.8 and 2.2 $mg.kg^{-1}$ DW, respectively [3]. In turn, [9] reported that nonanedioic acid was found in higher concentrations in green seaweed species compared to the other phyla, while red macroalgae mainly contained octanedioic acid.

Benzoic acid derivatives comprise a class of aromatic carboxylic acids that are formed by the shikimate pathway [22].

We found benzoic, benzeneacetic, and benzenedicarboxylic acids in the green, red, and brown Antarctic macroalgae, although other compounds have been detected in seaweeds, such as salicylic, gentisic, vanilic and gallic acids [23]. Furthermore, benzeneacetic acid can be categorized as an auxin, which is a plant hormone related to root development [24]. To the best of our knowledge, this article is the first to report detection of carboxylic and dicarboxylic acids in the studied Antarctic macroalgae.

Sterols

Sterols include a vast number of compounds that play roles as membrane constituents and hormonal precursors in aquatic and terrestrial organisms [10]. According to the literature [23], cholesterol and its derivatives are generally predominant in red and green algae, while fucosterol and its derivatives are more commonly found in brown seaweeds. Our findings corroborate these reports. Sterols are known for their biological activities, which include antioxidant, antitumor, antibacterial, antiviral, antifungal, and antiulcerative, among others [23, 25].

According to the literature [10], specimens of *A. utricularis* and *Desmarestia anceps* collected on King George Island (Antarctica) also contained fucosterol as the main sterol [10]. Moreover, in that study, other steroidal components were also detected in small amounts, including stigmasterol, cholesterol, and ergosterol, which were not observed in our samples. Differences in extraction and analytical approaches may have influenced the results, since the specimens were collected under similar environmental conditions. Sterols were also found in relevant concentrations in two other studies that evaluated *Ulva lactuta* and *Sargassum muticum* collected along the Portuguese coast [3, 9].

Previous reports indicate that the content of steroids in macroalgae varies considerably during the year, reaching maximum values in winter and minimum levels in summer, showing that seasonal variations play an important role in the steroid biosynthesis of seaweeds [2, 26]. As can be observed in Table 1, our samples were collected in the Antarctic summer, which could have lowered the concentration of steroids compared to what would be observed in other seasons [26]. Moreover, it is thought that other abiotic conditions, including water temperature and growth stage, may also affect the production of sterols by macroalgae [26, 27].

Other constituents

Several other constituents were found in the macroalgae and distributed in various chemical classes, including ketones, aldehydes, hydrocarbons, and amino acids. The presence of these constituents can be related to several biochemical mechanisms in seaweeds to survive in the Antarctic environment, since these compounds are linked to defense against oxidant agents and ultraviolet radiation, as well as to chemical signaling among individuals [28, 29].

Among the detected substances were α-tocopherol and phytol, which can be found in lipid membranes and storage structures. According to the literature, the content of tocopherols is higher in brown macroalgae than the other classes of seaweeds. Our results corroborate those findings, since we only observed α-tocopherol in Ochrophyta specimens. Moreover, concentrations previously reported for α-tocopherol ranged from 9.6 to 14 $\mu g.g^{-1}$ DW in *Undaria pinnatifida*, as also found by us [26]. It is worth noting that the consumption of α-tocopherol and phytol can have biological benefits due to the antioxidant and anticancer activities of these compounds [9, 26].

Evaluation of the lipophilic profile of Antarctic macroalgae showed the presence of trimethylbenzene, which is thought to derive from sugar or carotenoid degradation [30]. Other types of alkylbenzenes have also been detected in macroalgae, including, for instance, ethylbenzene, ethyltoluene, and tetramethylbenzene when analyzing volatile organic compounds of *Oscillatoria perornata* and *Palmaria palmata* [31, 32]. Tetradecane was also observed in small amounts in our specimens, and its presence can be associated with chemical signaling during the algal reproductive cycle [28].

Concerning other compounds found in the lipophilic profile, dihydroactinidiolide is a carotenoid derivative widely reported in the analysis of volatile compounds produced by macroalgae. It has been associated with the prevention of coronary diseases and tumors in humans [24]. Benzaldehyde was also detected in the specimens, and its presence can be associated to pathways of amino acid biosynthesis [33]. Finally, *L*-cysteine was observed in the samples, which can be related to defense mechanisms of seaweeds against antioxidant stress [34]. Studying the metabolites of macroalgae, Belghit et al. (2017) also detected relevant amounts of *L*-cysteine in brown and red seaweeds, corroborated by our results [29].

Antioxidant activity

Antioxidant evaluation showed that lipophilic extracts from Antarctic macroalgae have distinct levels of inhibiting DPPH radical scavenging. Generally, brown algae had greater antioxidant activity than red and green algae, in agreement with data reported in the literature [35]. In this sense, Paiva et al. (2016) indicated that extracts from *Ulva compressa*, *Gelidium microdon*, and *Pterocladiella capillacea* at concentrations of 2 $mg.mL^{-1}$ inhibited DPPH radical activity by 40.21 ± 2.84, 47.73 ± 3.01, and $26.14 \pm 1.90\%$ [36]. We found that *D. confervoides* had greater antioxidant capacity than indicated in the literature, while the other specimens had lower antioxidant activity.

The presence of antioxidants in seaweeds can be associated with mechanisms of defense and survival against oxidative stress and abiotic parameters [37, 38]. Generally, the antioxidant capacity of extracts is related to certain constituents, such as phenolic compounds, carbohydrates, FAs, and sterols [39, 40]. Among the components of lipophilic extracts, octadecenoic and octadecadienoic acids as well as phytosterols have been highlighted as compounds with antioxidant activity [39]. Indeed, extracts of *D. confervoides*, which had higher amounts of these lipid components compared to the other samples, had higher inhibition of DPPH radical activity than the other macroalgal extracts.

Antimicrobial activity

Previous studies of the lipophilic extracts of macroalgae have identified several biological activities, including antimicrobial, anti-inflammatory, antifungal, anticoagulant, antitumor, and antioxidant properties [11, 35, 41]. In this sense, metabolites from seaweeds have been receiving increased attention for use in various areas, including cosmetics, foods, and pharmaceuticals, due to their wide possible applications [2].

According to previous research works, the antibacterial activity of macroalgal extracts can be associated with the presence of distinct FAs [42, 43]. Based on our results, these biomolecules were present in greater amounts in the samples compared to other biochemical classes. Despite generally having weak bioactivities when isolated, several FAs acting together can promote bacterial inhibition, probably due to synergistic effects [43]. Among the reasons that can explain the antimicrobial activity of FAs are the amphipathic features of these molecules, allowing them to interact and penetrate membranes, inducing damage, and allowing the diffusion of other molecules that can further affect other biological processes [11, 42].

The antimicrobial activity found in the extracts from *U. intestinalis*, *C. racovitzae*, and *A. utricularis* agrees with the previous results of previous studies of macroalgal extracts. In this sense, Shanmughapriya et al. (2008) studied methanol:toluene extracts of *Sargassum wightii* and found inhibition of *E. faecalis*, *Staphylococcus epidermidis*, *E. coli*, *S. aureus*, and *Pseudomonas aeruginosa* [44]. Moreover, Cortés et al. 2014 used dichloromethane extracts from *Ceramium rubrum* against *Yersinia ruckeri* and *Syspastospora parasitica*, finding MICs of approximately 0.5 and 2 $mg.mL^{-1}$. Therefore, macroalgal extracts are potential antimicrobial agents with promising health applications.

Multivariate analysis

Evaluation of the PCA results showed that the Antarctic macroalgae could be distinguished according to their respective phyla according to parameters that involve biological activities and chemical composition. In this sense, *D. confervoides* was found along the positive axis of PC1, possibly due to its higher antioxidant capacity, while intermediate concentrations of bioactive compounds and antibacterial activity probably influenced the results of *A. utricularis*. Similarly, antibacterial activity was probably a key parameter for the presence of *C. racovitzae* and *U. intestinalis* along the negative axis of PC1. Finally, intermediate concentrations of FAs, sterols, and alcohols as well as little to no biological potential possibly influenced the results of *M. manginii*, with presence along the negative axis of PC1.

The results obtained in the multivariate analysis agree with those reported by Kumar et al. (2011), who evaluated more than 20 macroalgal species from Rhodophyta, Chlorophyta, and Ochrophyta phyla found along the Indian coast [45]. The study indicated that PCA was influenced by the lower antioxidant activity of red macroalgae compared to brown seaweeds, which we also observed. Moreover, the use of FAs and VOCs as variables in the multivariate analysis allowed the discrimination of phyla, as also accomplished by other studies in the area [45, 46].

Conclusion

The lipophilic profiles of *n*-hexane extracts from red, brown, and green Antarctic macroalgae were characterized, indicating the presence of various compounds, mainly sterols, fatty acids, and other carboxylic acids. Several constituents are biosynthesized within seaweeds as defense mechanisms against the extreme conditions of the Antarctic environment. PCA indicated that bioactive compounds and their biological activities were associated with the macroalgal phyla. Therefore, the analyzed species have noteworthy potential for use in the biotechnological, pharmaceutical, and food areas, since their lipophilic components are associated with beneficial biological activities including antibacterial, antifungal, and antioxidant.

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Declarations

Conflicts of interest The authors declare no competing interests.

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