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Comparative Bioactivity of Marine Macroalgae and Microalgae From the Brazilian Coast

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Abstract

Marine algae are recognized as important sources of bioactive compounds with diverse biological activities and biotechnological potential. This study investigated the antimicrobial, antioxidant and toxicity profile of extracts obtained from the red macroalgae *Amansia multifida* and *Enantiocladia duperreyi*, and the microalgae *Chaetoceros muelleri* and *Thalassiosira pseudonana*. Biomass was extracted using solvents of increasing polarity (hexane, ethyl acetate and ethanol), and the resulting extracts were evaluated by disk diffusion and broth microdilution assays against bacterial and fungal strains. Antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl method, while toxicity was assessed using the *Artemia salina* lethality assay. Several extracts exhibited antimicrobial properties, particularly hexane extracts, which exhibited a broader spectrum of inhibition. Minimum inhibitory concentration values ranged from 62.5 to 500 μg mL^{-1}. Five extracts demonstrated radical scavenging above 50%, with the ethyl acetate extract of *E. duperreyi* showing the highest performance. Toxicity assays indicated low to moderate effects, with median lethal concentration values ranging from 248 to 548.8 μg mL^{-1}. Differences in antimicrobial, antioxidant and toxicity profiles were observed between species and collection sites, as well as among extraction solvents. These findings reinforce the potential of marine macroalgae and microalgae as sources of bioactive compounds and contribute to the bioprospecting of marine biodiversity from the Brazilian coast. Further studies are warranted to characterize the compounds responsible for the observed biological activities.

Introduction

Marine biodiversity represents a vast source of bioactive compounds with significant applications in biotechnology and the sustainable use of marine biological resources. Among marine organisms, macro- and microalgae have gained increasing attention due to their ability to synthesize structurally diverse secondary metabolites, including phenolic compounds, pigments, polysaccharides and lipids, many of which exhibit antimicrobial and antioxidant properties. These characteristics make algae promising candidates for the development of natural products, particularly in response to increasing antimicrobial resistance and the demand for sustainable bioresources.

Recent studies demonstrated the capacity of algal extracts to inhibit a wide range of microorganisms, including bacteria and fungi, reinforcing their potential as sources of bioactive metabolites with antimicrobial activity (Hassan et al. 2022). In addition, the presence of antioxidant molecules such as carotenoids, phenols and flavonoids has been widely reported, highlighting their role in neutralizing free radicals and reducing oxidative stress (Afrin et al. 2023; Pradhan, Bhuyan and Ki 2023). These properties have stimulated interest in biotechnology, cosmetics and other bio-based applications.

Recent studies have further reinforced the antifungal potential of microalgal extracts, particularly against dermatophytes and phytopathogenic fungi, demonstrating their relevance as sources of bioactive compounds (Lage et al. 2023; Santos et al. 2024; Sacramento et al. 2025). In addition, previous studies have shown that cultivation and harvesting conditions may influence the antioxidant potential and toxicity of microalgal extracts (Ramos et al. 2020).

Despite these advances, the biological properties of many algal species remain insufficiently characterized, particularly when considering variations associated with species, environmental conditions and extraction methods. Furthermore, toxicity assessment is essential to ensure the safe application of these compounds, and studies have contributed to understanding the biological effects of algal extracts, including their potential toxicity and bioactivity (Aravinth et al. 2024). However, comparative investigations involving different algal groups and extraction solvents are still limited.

In this context, the present study aimed to comparatively evaluate the antimicrobial, antioxidant and toxicity profiles of extracts obtained from selected marine macroalgae and microalgae from the Brazilian coast using solvents of different polarities. By integrating biological activity and toxicity assessments, this study contributes to the bioprospecting of marine biodiversity and to the identification of promising algal resources for future biotechnological applications.

Methods

Macroalgae collection and processing

Samples of the red macroalgae *Amansia multifida* and *Enantiocladia duperreyi* were collected from the midlittoral zone during low spring tides along the coast of Bahia, Brazil, between 2015 and 2018, following standard marine phycology procedures. Tidal conditions were determined using the Tide Tables database of the Brazilian Navy. Collection was conducted under authorization from SISBIO/IBAMA (licenses no. 25423-8 and 25423-9), and the study complied with Brazilian regulations for access to genetic resources (SISGEN registration no. A2733A8). Specimens were washed with seawater to remove epiphytes and debris, transported to the laboratory, rinsed with distilled water and air-dried at room temperature under light-protected conditions. Voucher specimens were deposited in the Alexandre Leal Costa Herbarium (ALCB), a Brazilian university (Table 1).

Table 1
List of species collected to obtain seaweed extracts.

Species (Seaweed)	Collection site	Voucher
***Amansia multifida* J. V. Lamouroux (Ceramiales, Rhodophyta)**	**Vilas do Atlântico**	**132695**
***Amansia multifida* J. V. Lamouroux (Ceramiales, Rhodophyta)**	**Jauá**	**129949**
Enantiocladia duperreyi (C. Agardh) Falkenberg (Ceramiales, Rhodophyta)	Jauá	133179
Enantiocladia duperreyi (C. Agardh) Falkenberg (Ceramiales, Rhodophyta)	Saquaíra (Maraú)	129877

Microalgae cultivation and biomass production

The microalgae *Chaetoceros muelleri* and *Thalassiosira pseudonana* (SISGEN registration no. A653955; deposit numbers at a Brazilian herbarium, ALCB, IBL-C 003 and IBL-C 008, respectively) were obtained from the Marine Microorganism Bank (IO/USP) and incorporated into a microalgae collection maintained in a research laboratory (SISGEN accreditation no. C945879). Seawater used for cultivation was adjusted to a salinity of 28 PSU with distilled water, filtered and autoclaved. Culture conditions followed Nascimento et al. (2015), with a 12 h light:12 h dark photoperiod, irradiance of 35 µmol photons m^{-2} s^{-1}, temperature of 22°C, pH 7.0 and constant aeration. Strains were maintained in Conway medium (Walne 1966 apud Lourenço 2006), supplemented with sodium silicate ($Na_2SiO_3 \cdot 9H_2O$) at 0.32 M. Biomass was collected at the stationary growth phase by centrifugation at 5000 rpm for 20 min (Chu et al. 2004). The resulting pellet was stored in glass containers at − 25°C, while the supernatant was discarded. To obtain dry biomass, the frozen material was freeze-dried for 24 h under vacuum (137 µHg) at − 30°C using an Enterprise II freeze dryer (Terroni®). The lyophilized biomass was stored in a desiccator until extraction.

Extraction procedure

Extracts from macroalgae and microalgae were obtained by cold maceration through sequential extraction using organic solvents of increasing polarity: hexane, ethyl acetate and ethanol. For macroalgae, dried biomass was manually ground using a mortar and pestle, weighed, identified and stored in sealed glass containers. Organic solvents (Êxodo Científica®) were added sequentially to the biomass in order of increasing polarity: hexane (P.A., 98.5%), ethyl acetate (P.A., 99.5%) and ethanol (P.A.). For microalgae, biomass in contact with the same solvents was subjected to sonication (2 × 6 min, 10% power, on ice) to enhance cell disruption. After 72 h of extraction, the macerates were centrifuged at 4500 rpm for 10 min. The supernatants were collected, and the solvents were evaporated at room temperature until complete dryness. Extracts obtained from microalgae were subsequently processed under the same conditions as those from macroalgae.

Antimicrobial activity

The antimicrobial activity of the extracts was evaluated using two methods: agar disk diffusion and broth microdilution.

• Disk diffusion assay

Antimicrobial activity was evaluated using the agar disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI 2003), with adaptations (Rabanal et al. 2002; Karaman et al. 2003). Extracts were tested against bacterial and fungal strains (Table 2).

Table 2
Strains submitted to microbial susceptibility testing. ATCC = American Type Culture Collection

Microorganism	ATCC	Feature
Bacillus subtilis	6633	Gram-positive
Micrococcus luteus	10240	Gram-positive
Staphylococcus aureus	6538	Gram-positive
Escherichia coli	94863	Gram-negative
Pseudomonas aeruginosa	15442	Gram-negative
Salmonella cholerae	14028	Gram-negative
Aspergillus niger	16404	Filamentous fungi
Candida albicans	18804	Yeast
Candida glabrata		Yeast

Extract solutions were prepared in dimethyl sulfoxide (DMSO) at concentrations of 10 and 100 mg mL^{-1}. Sterile filter paper disks (6 mm diameter) were impregnated with 10 µL of each extract and placed on previously inoculated agar plates. Chloramphenicol (30 µg $disk^{-1}$) was used as a positive control for bacteria, and ciclopirox olamine (5.0 mg mL^{-1}) as a positive control for fungi. DMSO was used as a negative control. Plates were incubated at 37°C for 24 h for bacteria and 48 h for fungi. After incubation, inhibition zones were measured in millimeters. All assays were performed in triplicate (n = 3) under sterile conditions.

• Broth microdilution assay

Minimum inhibitory concentration (MIC) was determined using the broth microdilution method according to Balouiri et al. (2016), with adaptations. Extracts were prepared in DMSO and tested in 96-well microplates at concentrations ranging from 3.9 to 500 µg mL^{-1}.

Each well contained 100 µL of culture medium and 100 µL of extract solution, followed by serial dilution. Plates were incubated at 37°C for 24 h for bacteria and 48 h for fungi.

MIC was defined as the lowest concentration at which no visible microbial growth was observed. To determine bactericidal or fungicidal effects, aliquots (10 µL) from wells without visible growth were inoculated onto agar plates and incubated under the same conditions. The absence of growth was interpreted as bactericidal or fungicidal activity, whereas the presence of growth indicated bacteriostatic or fungistatic effects.

Toxicity assessment

Toxicity was evaluated using the Artemia salina lethality assay according to Meyer et al. (1982). Extracts were initially dissolved in ethanol to prepare a stock solution (11 mg mL^{-1}) and subsequently diluted to final concentrations of 10, 100 and 1000 µg mL^{-1}.

After hatching, 10 nauplii of Artemia salina were transferred using a micropipette to wells containing the test solutions, with a final volume of 3 mL completed with seawater. Ethanol (200 µL plus seawater) and seawater alone were used as controls. All assays were performed in triplicate, totaling 30 organisms per concentration.

After 24 and 48 h of exposure, mortality was recorded using a stereomicroscope (LABOMED Luxeo 4z). Individuals showing no movement for more than 10 s were considered dead (Lhullier et al. 2006). Mortality percentages were calculated, and median lethal concentration (LC_{50}) values were estimated using GraphPad Prism 5.0. Controls were used to confirm that mortality was associated with extract toxicity rather than starvation or handling effects (Carballo et al. 2002).

Antioxidant activity

The antioxidant activity of the extracts was determined by evaluating the scavenging capacity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, according to the methodology described by Duan et al. (2006) and Pires et al. (2017), with adaptations. An ethanolic solution of DPPH (Sigma-Aldrich) was prepared at a concentration of 120 µM, and the extracts were initially prepared at 6.0 mg mL^{-1}. Serial dilutions were performed to obtain concentrations ranging from 3.0 to 0.045 mg mL^{-1}. Ethanol was used as blank, and wells containing 100 µL of DPPH solution without extract were used as negative control.

After preparation, the microplate was kept in the dark for 30 min, and absorbance was measured at 492 nm using a microplate spectrophotometer (Sirio S, SEAC). All assays were performed in triplicate.

The antioxidant activity of the extracts was expressed as percentage of radical scavenging (%AA), calculated based on absorbance values according to Pires et al. (2017). For pigment-rich extracts, sample blanks were prepared, and their absorbance values were subtracted from the corresponding test readings.

$$\% \text{ AA} = \frac{[Abs\ DPPHControl\ - (Abs\ sample - Abs\ white\ sample)]}{Abs\ Control\ DPPH} \times 100$$

Determination of total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu method according to Slinkard and Singleton (1977), with adaptations. Extracts were diluted in ethanol to a concentration of 6.0 mg mL⁻¹. A 2% sodium carbonate solution (Vetec) and 10% Folin–Ciocalteu reagent (Cromoline) were prepared and used as reagents.

Serial dilutions of the extracts were performed to obtain concentrations ranging from 3.0 to 0.045 mg mL⁻¹. After reagent addition, the reaction mixtures were incubated for 40 min under light exposure. Absorbance was measured at 620 nm using a microplate spectrophotometer (Sirio S, SEAC).

Gallic acid was used as standard and prepared in ethanol at concentrations ranging from 0.15 to 1.0 mg mL⁻¹ (twofold serial dilution). Total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE g⁻¹), based on the calibration curve equation (y = 3.8883x + 0.1141), where y corresponds to the absorbance of the extract at 0.75 mg mL⁻¹ and x to the concentration of gallic acid (Fig. 1A).

Determination of total flavonoid content

Total flavonoid content was determined according to the methodology of the China Pharmacopoeia Committee (2005), with adaptations. Initially, a stock solution of the extracts was prepared in ethanol at a concentration of 6.0 mg mL⁻¹. From this solution, 100 µL were transferred to a microtube, followed by the addition of 400 µL of distilled water and 30 µL of 5% sodium nitrite. After 3 min, 30 µL of 10% aluminum chloride were added, and the mixture was allowed to stand for 1 min. Subsequently, 200 µL of 1.0 M sodium hydroxide and 240 µL of distilled water were added to complete a final volume of 1.0 mL.

Aliquots of 200 µL were transferred to a 96-well microplate, and absorbance was measured using a microplate spectrophotometer (Sirio S, SEAC) at 492 nm. Total flavonoid content was quantified using a quercetin standard curve (Fig. 1B) at concentrations of 0.01, 0.04, 0.08, 0.14, 0.24 and 0.36 mg mL⁻¹. Results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE g⁻¹), calculated based on the linear equation obtained from the calibration curve.

Statistical analysis

Statistical analyses were performed using the R statistical environment. Macroalgae and microalgae were initially analyzed as independent groups. Analysis of variance (ANOVA) was used to evaluate differences in total phenolic content (TPQ), total flavonoid content (FTQ), and antioxidant activity (%AA) among extracts. When significant differences were detected, Tukey's post hoc test was applied for pairwise comparisons. Additional analyses were conducted to compare macroalgae and microalgae groups and to assess differences among macroalgae collected from distinct locations. A significance level of 10% (α = 0.10) was adopted for all analyses.

Results

Antimicrobial activity

At a concentration of 10.0 mg mL⁻¹ (Table 3), the lowest tested in this assay, only extracts from the Rhodophyceae *Enantiocladia duperreyi* collected in Saquaíra and the diatom *Thalassiosira pseudonana* exhibited antimicrobial activity. These extracts were active against four bacterial strains, with inhibition zones ranging from 7.3 to 9.0 mm, and against one yeast, with inhibition zones ranging from 8.3 to 10.7 mm (Fig. 2).

The hexane extract of *Enantiocladia duperreyi* inhibited the growth of the three Gram-positive bacteria (*Bacillus subtilis, Micrococcus luteus and Staphylococcus aureus*), whereas the ethyl acetate and ethanolic extracts inhibited only *S. aureus*, with inhibition zones of 7.3 ± 0.6 and 7.7 ± 0.6 mm, respectively. For the microalga *Thalassiosira pseudonana*, hexane and ethyl acetate extracts showed activity against the Gram-negative bacterium Salmonella cholerae, with inhibition zones of 9.0 ± 0.0 and 7.3 ± 0.6 mm, respectively. The yeast *Candida albicans* was sensitive to all extracts, with the largest inhibition zone observed for the hexane extract (10.7 ± 0.6 mm).

Table 3. Mean halo values (mm ± standard deviation) of microbial growth inhibition at 10 mg mL⁻¹ (100 µg/disc).

Algae	Extract	Test-microorganisms								
		Gram-positive			Gram-negative				Fungi	
		Bacillus subtilis	*Micrococcus luteus*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella cholerae*	*Aspergillus niger*	*Candida albicans*	*Candida glabrata*
***Enantiocladia duperreyi* (SAQ)**	Hexane	7.7 ± 0.6	8.7 ± 0.6	9.0 ± 0.0	-	-	-	-	-	-
	EA	-	-	7.3 ± 0.6	-	-	-	-	-	-
	Ethanol	-	-	7.7 ± 0.6	-	-	-	-	-	-
Thalassiosira pseudonana	Hexane	-	-	-	-	-	9.0 ± 0.0	-	10.7 ± 0.6	-
	EA	-	-	-	-	-	7.3 ± 0.6	-	9.3 ± 0.6	-
	Ethanol	-	-	-	-	-	-	-	8.3 ± 0.6	-
Controls										
DMSO		-	-	-	-	-	-	-	-	-
CHL*		28.0	25.0	25.0	25.0	26.0	19.0	-	-	-
CPO**		-	-	-	-	-	-	23.0	25.0	25.0

SAQ = Saquaíra; EA = Ethyl acetate; – Absence of inhibition; * Chloramphenicol (30 µg/disc); ** Ciclopirox olamine (50 µg/disc).

At a concentration of 100 mg mL⁻¹, ten extracts inhibited the growth of six bacterial strains and one fungus, with inhibition zones ranging from 7.0 to 16.7 mm (Table 4). The three extracts of *Amansia multifida* collected in Vilas do Atlântico showed activity against the Gram-negative bacterium *Escherichia coli*. The hexane and ethyl acetate extracts inhibited *Bacillus subtilis*, whereas only the ethyl acetate extract inhibited *Candida albicans* (9.7 ± 0.6 mm). Only the ethanolic extract of *A. multifida* collected in Jauá showed activity against the Gram-negative bacterium *Pseudomonas aeruginosa* (9.7 ± 0.6 mm). Regarding the extracts *of Enantiocladia duperreyi* from Saquaíra, the hexane extract inhibited *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus* and *Candida albicans*. The ethyl acetate extract inhibited S. aureus, as well as the Gram-negative bacteria Escherichia coli and *Pseudomonas aeruginosa*, which were also sensitive to the ethanolic extract, although forming smaller inhibition zones. Increasing the extract concentration (Table 4) resulted in increased susceptibility of E. coli and *P. aeruginosa* to ethyl acetate and ethanolic extracts, and of *C. albicans* to hexane extracts. For *Bacillus subtilis*, no change in sensitivity was observed with increasing concentration. However, for Staphylococcus aureus, the ethyl acetate extract showed increased activity (9.0 ± 0.0 mm), whereas the ethanolic extract showed no inhibition and the hexane extract exhibited smaller inhibition zones compared to those observed at 10.0 mg mL⁻¹.

Table 4. Mean halo values (mm ± standard deviation) of microbial growth inhibition at 100 mg mL⁻¹ (1000 µg/disc).

Algae	Extract	Test-microorganisms								
		Gram-positive			Gram-negative				Fungi	
		Bacillus subtilis	*Micrococcus luteus*	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Salmonella cholerae*	*Aspergillus niger*	*Candida albicans*	*Candida glabrata*
***Amansia multifida* (VA)**	Hexane	7.0 ± 0.0	-	-	8.0 ± 1.0	-	-	-	-	-
	EA	8.0 ± 1.0	-	-	7.7 ± 0.6	-	-	-	9.7 ± 0.6	-
	Ethanol	-	-	-	9.0 ± 1.0	-	-	-	-	-
***Amansia multifida* (Jauá)**	Hexane	-	-	-	-	-	-	-	-	-
	EA	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	9.7 ± 0.6	-	-	-	-
***Enantiocladia duperreyi* (Jauá)**	Hexane	-	-	-	-	-	-	-	-	-
	EA	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-
***Enantiocladia duperreyi* (SAQ)**	Hexane	7.7 ± 0.6	9.0 ± 0.8	7.0 ± 0.0	-	-	-	-	12.3 ± 0.6	-
	EA	-	-	9.0 ± 0.0	7.0 ± 0.0	9.3 ± 0.5	-	-	-	-
	Ethanol	-	-	-	7.7 ± 1.2	7.7 ± 0.6	-	-	-	-
Chaetoceros muelleri	Hexane	-	-	-	-	-	-	-	-	-
	EA	-	-	-	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	-	-	-	-
Thalassiosira pseudonana	Hexane	-	16.7 ± 0.6	7.7 ± 0.6	-	-	9.0 ± 0.0	-	11.0 ± 0.0	-
	EA	-	13.3 ± 1.2	-	-	-	8.7 ± 0.6	-	10.3 ± 0.6	-
	Ethanol	-	-	-	-	-	7.3 ± 0.6	-	10.0 ± 0.0	-
Controls										
DMSO		-	-	-	-	-	-	-	-	-
CHL*		28.0	25.0	25.0	25.0	26.0	19.0	-	-	-
CPO**		-	-	-	-	-	-	23.0	25.0	25.0

VA = Vilas do Atlântico; SAQ = Saquaíra; – Absence of inhibition; EA = Ethyl acetate; – Absence of inhibition; * Chloramphenicol (30 µg/disc); ** Ciclopirox olamine (50 µg/disc).

For the diatom *Thalassiosira pseudonana*, increasing the extract concentration resulted in an expansion of the antimicrobial spectrum, from two to four susceptible microorganisms, accompanied by an increase in inhibition zone size. The hexane extract inhibited the growth of Micrococcus luteus (16.7 ± 0.6 mm, the largest inhibition zone observed), *Staphylococcus aureus*, the Gram-negative bacterium *Salmonella cholerae*, and the yeast *Candida albicans*. The ethyl acetate extract inhibited *M. luteus*, *S. cholerae* and *C. albicans*, while the latter two were also inhibited by the ethanolic extract.

In the current work, *Enantiocladia duperreyi* (Saquaíra) exhibited the broadest antimicrobial spectrum, whereas the diatom *Thalassiosira pseudonana* showed the largest inhibition zones.

In the broth microdilution assay, all algal species exhibited antimicrobial activity (Table 5). The 18 extracts tested inhibited the growth of the six bacterial strains, with minimum inhibitory concentration (MIC) values ranging from 62.5 to 500 µg mL^{-1}, and varying modes of action (bactericidal or bacteriostatic). Regarding fungi, 12 extracts inhibited *Aspergillus niger*, with only one showing fungicidal activity, while 17 extracts inhibited *Candida albicans* and *C. glabrata*, all with fungistatic effects. MIC values for fungi ranged from 250 to 500 µg mL^{-1}. Hexane, ethyl acetate and ethanolic extracts showed similar efficiency against both bacterial and fungal strains.

In the present investigation, *Micrococcus luteus* was the most sensitive microorganism, presenting the lowest MIC values (125 µg mL^{-1}) for all extracts of *Amansia multifida* collected in Vilas do Atlântico and Jauá, for the hexane extract of *Chaetoceros muelleri*, and for the ethyl acetate extract of *Thalassiosira pseudonana*. The ethanolic extract of *T. pseudonana* showed the highest activity, with a MIC value of 62.5 µg mL^{-1}.

Table 5. Values of Minimum Inhibitory Concentration (MIC) (µg mL⁻¹) and type of activity of seaweed extracts against bacteria and fungi. BC = Bactericidal; BT = Bacteriostatic; FC = Fungicidal; FT = Fungistatic

Table 5. Values of Minimum Inhibitory Concentration (µg/mL) and type of activity of seaweed extracts against bacteria and fungi. VA = Vilas do Atlântico; BC = Bactericide; BT= Bacteriostatic; FC= Fungicide; FT= Fungiostatic.

		Minimum Inhibitory Concentration (MIC)															
		Gram-positive						Gram-negative						Fungi			
Algae	Extracts	*Bacillus subtilis*		*Micrococcus luteus*		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Salmonella choleras*		*Aspergillus niger*		*Candida albicans*		*Candida glabrata*	
Amansia multifida VA	Hexane	500	BC	125	BT	500	BT	250	BC	500	BC	250	BT	250	FT	250	FT	500	FT
	Ethyl Acetate	250	BT	125	BT	500	BT	250	BC	250	BT	250	BT	250	FT	250	FT	250	FT
	Ethanol	250	BC	125	BT	250	BT	250	BC	250	BC	250	BT	250	FC	250	FT	250	FT
Amansia multifida Jauá	Hexane	500	BC	125	BT	500	BT	500	BC	500	BT	500	BT	500	FT	500	FT	500	FT
	Ethyl Acetate	250	BC	125	BT	250	BT	250	BC	500	BT	250	BT	500	FT	250	FT	250	FT
	Ethanol	250	BC	125	BT	250	BT	250	BC	250	BT	250	BT	500	FT	250	FT	250	FT
Enantiocladia duperreyi Jauá	Hexane	250	BC	250	BC	250	BT	250	BC	500	BT	500	BT	-	-	250	FT	250	FT
	Ethyl Acetate	250	BT	250	BT	250	BC	250	BT	125	BT	125	BT	-	-	250	FT	-	-
	Ethanol	250	BT	500	BT	250	BT	250	BC	500	BT	250	BT	-	-	250	FT	250	FT
Enantiocladia duperreyi Saquaíra	Hexane	250	BT	250	BC	500	BC	250	BC	500	BT	250	BT	500	FT	-	-	500	FT
	Ethyl Acetate	250	BC	250	BT	500	BC	250	BC	500	BT	125	BT	500	FT	250	FT	250	FT
	Ethanol	250	BC	250	BT	500	BT	250	BC	500	BT	125	BT	500	FT	250	FT	250	FT
Chaetoceros muelleri	Hexane	250	BC	125	BT	500	BT	250	BC	500	BT	250	BT	500	FT	500	FT	250	FT
	Ethyl Acetate	250	BT	125	BT	500	BT	250	BC	500	BT	250	BT	500	FT	250	FT	250	FT
	Ethanol	250	BC	125	BT	500	BT	250	BC	500	BT	250	BT	500	FT	250	FT	250	FT
Thalassiosira pseudonana	Hexane	250	BT	125	BC	500	BT	250	BC	500	BT	250	BT	-	-	250	FT	250	FT
	Ethyl acetate	250	BC	125	BC	500	BT	250	BT	500	BT	250	BT	-	-	250	FT	250	FT
	Ethanol	250	BT	62,5	BT	250	BT	250	BT	250	BC	125	BT	-	-	250	FT	250	FT

The 18 extracts showed a minimum inhibitory concentration (MIC) of 250 µg mL⁻¹ against *Escherichia coli*. Most extracts exhibited bactericidal activity, except for the ethyl acetate extracts of *Enantiocladia duperreyi* (Jauá) and *Thalassiosira pseudonana*, as well as the ethanolic extract of *T. pseudonana*, which showed bacteriostatic activity.

Regarding the fungi, not all extracts were active. No activity was observed for extracts of *E. duperreyi* (Jauá) and *T. pseudonana* against *Aspergillus niger*; for the ethyl acetate and ethanolic extracts of *E. duperreyi* (Jauá) against *Candida glabrata*; and for the hexane extract of *E. duperreyi* (Saquaíra) against *Candida albicans*.

Toxicity

Extracts that exhibited lethality above 50% included the hexane extract of *Enantiocladia duperreyi* (Jauá), as well as the hexane and ethyl acetate extracts of *Thalassiosira pseudonana*. For these extracts, the median lethal concentration (LC_{50}) was calculated using a sigmoidal fit of viability percentage as a function of the logarithm of crude extract concentration (Fig. 3).
In this study, LC_{50} values were 248 µg mL⁻¹ and 287.2 µg mL⁻¹ for the hexane and ethyl acetate extracts of *T. pseudonana*, respectively, and 548.8 µg mL⁻¹ for the hexane extract of the macroalga *E. duperreyi* (Jauá).

According to Meyer et al. (1982), plant extracts with LC_{50} values above 250 µg mL⁻¹ are considered to have low toxicity, those between 80 and 250 µg mL⁻¹ present moderate toxicity, and those below 80 µg mL⁻¹ are considered toxic. Nguta et al. (2011) further classify extracts with LC_{50} < 100 µg mL⁻¹ as highly toxic; those with LC_{50} between 100 and 500 µg mL⁻¹ as moderately toxic; those between 500 and 1000 µg mL⁻¹ as having low toxicity; and those with LC_{50} > 1000 µg mL⁻¹ as non-toxic. Based on these criteria, none of the active extracts evaluated in this study were classified as highly toxic (Table 6).

The toxicity observed for the hexane and ethyl acetate extracts of *Thalassiosira pseudonana* may be associated with their antimicrobial potential, as bioactive compounds are often toxic at concentrations required to inhibit the growth of pathogenic microorganisms (Guillén, 2012). To date, no studies have reported toxicity assessments using this method for the algae *Enantiocladia duperreyi*, *T. pseudonana* and *Chaetoceros muelleri*.

Table 6. Classification of the toxicity of algal extracts according to LC_{50} values. LC_{50}: median lethal concentration. * Other 15 extracts tested in this study. EDJ = *Enantiocladia duperreyi* (Jauá); TP = *Thalassiosira pseudonana*.

		Toxicity Classification	
Extract	**LC50 (µg/mL)**	**Meyer et al. (1982)**	**Nguta et al. (2011)**
TP - Hexane	248	Moderate	Moderate
TP – Ethyl acetate	287.2	Low toxicity	Moderate
EDJ - Hexane	548.8	Low toxicity	Low toxicity
Other Extracts*	⩾ 1000	-	Non-toxic

Antioxidant activity, total phenolic content and flavonoid content

Of the total extracts tested, only five exhibited antioxidant activity above 50% against the DPPH radical and were therefore considered to have good antioxidant potential. Antioxidant activity values ranged from 1.78 ± 0.8% to 81.32 ± 1.0% (Table 7).

Total phenolic content ranged from 0.0 to 192.80 ± 1.0 mg GAE g^{-1} of extract, while total flavonoid content ranged from 194 ± 1.7 to 1800 ± 0.0 mg QE g^{-1} of extract (Table 7). For most of the extracts evaluated in this study (n = 4), ethyl acetate proved to be the most effective solvent for extracting compounds with antioxidant activity, as also reported by Chakraborty, Joseph and Praveen (2015) for the alga *Hypnea musciformis* (Wulfen) J.V. Lamouroux.

However, these results differ from those reported in other studies. Saranya et al. (2014) observed that the methanolic extract of *Isochrysis galbana* Parke exhibited the highest antioxidant potential when compared to acetone and hexane extracts. Similarly, Foo et al. (2015) recommended methanol as the most suitable solvent for extracting antioxidant compounds from *Chaetoceros calcitrans* (Paulsen) H. Takano. These findings suggest that the selection of the most appropriate solvent depends on both the species and the types of compounds of interest.

The most effective extract in scavenging the DPPH radical was the ethyl acetate extract of *Enantiocladia duperreyi* (Saquaíra), with an activity of 81.32 ± 1.0%. Among the three extracts obtained from this alga, this extract also presented the highest levels of phenolic compounds (66.17 ± 1.5 mg GAE g^{-1}) and flavonoids (609.3 ± 2.3 mg QE g^{-1}).
Among all evaluated extracts, the highest phenolic content was observed for the ethyl acetate extract of *E. duperreyi* (Jauá), which also exhibited the highest flavonoid content. Janarthanan and Kumar (2013) quantified total phenolic compounds and flavonoids in two algal species and reported values higher than those commonly found in the literature, comparable to those observed in this study. For *Acanthophora spicifera* (M. Vahl) Børgesen, a species also belonging to the order Ceramiales, the authors reported values of 127.24 ± 6.541 and 190.12 ± 14.24 for phenolic compounds and flavonoids, expressed as caffeic acid and catechin equivalents, respectively.

The diversity of antioxidant compounds present in algae is considerable, and their synthesis may vary due to ecological factors as well as the composition of associated symbiotic microbial communities. For this reason, Almeida and Palermo (2017) emphasize that each extract should be considered as a unique sample, given the combination of factors influencing its chemical composition.

Based on the results obtained, it can be observed that, under the conditions of the method employed, the Rhodophyceae species evaluated in this study showed the highest antioxidant activity. Red algae are widely investigated, and according to Simões et al. (2017), they are characterized by the presence of halogenated structures belonging to several chemical classes, which explains the large number of bioactive compounds isolated from this group.

The microalgae *Chaetoceros muelleri* and *Thalassiosira pseudonana* showed relatively high flavonoid contents compared to the lowest values observed in this study; however, this was not reflected in their antioxidant potential. In contrast, these species exhibited the lowest total phenolic contents, as well as the lowest %AA values, suggesting that, for these microalgae, antioxidant capacity is more closely associated with phenolic compounds.

The microalgae used in this study were cultivated under controlled, non-stress conditions, which may have resulted in lower production of phenolic compounds. A similar pattern was reported by Goh, Yusoff and Loh (2010), who evaluated antioxidant properties and total phenolic content in extracts of *Chaetoceros* sp. and *Nannochloropsis* sp. In that study, nonpolar extracts of *Chaetoceros* sp. showed higher antioxidant activity than polar extracts, and no correlation was observed between total phenolic content and antioxidant activity ($p > 0.05$). Similarly, Maadane et al. (2015) reported a low correlation between antioxidant activity and phenolic content in nine microalgal species, as well as superior performance of ethanolic extracts in terms of antioxidant activity. Comparison between the literature and the results obtained here highlights divergences regarding the most suitable solvent for extraction. This same principle applies to the investigation of other biological properties, as both biotic and abiotic factors influence the synthesis and extraction of bioactive compounds.

Statistical comparison among extracts and algal groups

For macroalgae, ANOVA did not indicate significant differences in TPQ among the three extracts tested ($p = 0.143$). In contrast, for FTQ, ANOVA indicated significant differences between at least two groups ($p = 0.08$), and Tukey's test was applied to identify pairwise differences. This analysis revealed a significant difference between hexane and ethyl acetate extracts ($p = 0.07$). For %AA, ANOVA also indicated significant differences among the extracts ($p = 0.02$). Tukey's test showed a significant difference between hexane and ethanolic extracts ($p = 0.019$).

For microalgae, ANOVA did not reveal significant differences for any of the evaluated variables (p-values > 0.1). Subsequently, differences between macroalgae and microalgae were evaluated. ANOVA indicated significant differences for TPQ ($p = 0.076$) and %AA ($p = 0.017$), demonstrating that these variables differed between the two groups. No significant differences were observed for FTQ.

Finally, ANOVA was applied to assess whether macroalgae collected from different locations exhibited differences in TPQ, FTQ, and %AA. For *Amansia multifida* collected in Vilas do Atlântico and Jauá, significant differences were observed for TPQ ($p = 0.097$) and %AA ($p = 0.0342$), whereas no differences were observed for FTQ. For *Enantiocladia duperreyi* collected in Jauá and Saquaíra, significant differences were found only for %AA ($p = 0.0168$).

Table 7. In vitro antioxidant activity (%) and total phenolic and flavonoid contents of macroalgae and marine microalgae extracts (mean ± standard deviation).
Extracts: HE = hexane; EA = ethyl acetate; ET = ethanolic; ND = not detected.
%AA = percentage of antioxidant activity; GAE = gallic acid equivalents; QE = quercetin equivalents.

Species	Extract	%AA (0.75 mg mL^{-1})	Total phenolic content (mg GAE g^{-1})	Total flavonoids (mg QE g^{-1})
Amansia multifida (Vilas do Atlântico)	HE	23.26 ± 4.0	44 ± 0.6	1197.1 ± 0.2
	EA	53.33 ± 1.9	70 ± 1.7	1233.7 ± 1.7
	ET	20.78 ± 1.2	24 ± 1.7	293 ± 5.0
Amansia multifida (Jauá)	HE	18.37 ± 1.5	8.9 ± 0.8	1173 ± 3.5
	EA	40.62 ± 2.0	53.8 ± 2.5	1488 ± 0.0
	ET	24.03 ± 0.5	43.63 ± 0.8	574 ± 1.7
Enantiocladia duperreyi (Jauá)	HE	9.15 ± 0.7	43.50 ± 1.6	1050 ± 0.0
	EA	70.85 ± 2.1	192.80 ± 1.0	1800 ± 0.0
	ET	79.53 ± 0.4	78.48 ± 6.5	849 ± 0.6
Enantiocladia duperreyi (Saquaíra)	HE	9.65 ± 1.2	51.70 ± 0.5	194 ± 1.7
	EA	81.32 ± 1.0	66.17 ± 1.5	609.3 ± 2.3
	ET	50.78 ± 1.9	7.67 ± 0.4	282 ± 1.7
Chaetoceros muelleri	HE	10 ± 1.4	12.58 ± 1.3	905 ± 0.0
	EA	8.99 ± 0.5	7.23 ± 2.7	497.3 ± 0.6
	ET	4.26 ± 0.6	ND	673.7 ± 1.2
Thalassiosira pseudonana	HE	1.78 ± 0.8	19.03 ± 0.7	268 ± 0.6
	EA	6.05 ± 1.0	13.75 ± 1.2	440.7 ± 1.2
	ET	11.01 ± 1.1	22.7 ± 0.5	442.3 ± 4.0

Discussion

The findings of this study demonstrate that macroalgae and microalgae extracts exhibit relevant antimicrobial and antioxidant activities, with variations depending on species, extraction solvent and assay method. Antimicrobial activity showed a clear dependence on concentration and solvent polarity. At lower concentration (10 mg mL^{-1}), only extracts of *Enantiocladia duperreyi* (Saquaíra) and *Thalassiosira pseudonana* were active, whereas at 100 mg mL^{-1} a broader spectrum of inhibition was observed. The predominance of activity in hexane extracts suggests an important role of nonpolar compounds, in agreement with studies that associate antimicrobial effects in algae with lipophilic metabolites such as fatty acids (Amaro et al., 2011; Smith et al., 2010; Morais et al., 2015). Similarly, Qin et al. (2013) reported antimicrobial activity in extracts of *Thalassiosira rotula*, while Stein et al. (2011) described antifungal properties in nonpolar extracts of Laurencia species. In contrast, other studies have reported higher antimicrobial activity in polar extracts of algae (Osman et al., 2010; Lavanya and Veerappan, 2011; Dantas et al., 2019). These contrasting results reinforce the conclusion of Arulkumar et al. (2018) that extraction efficiency and antimicrobial activity depend on both species characteristics and extraction methodology. Among the species evaluated, *Enantiocladia duperreyi* (Saquaíra) exhibited the broadest antimicrobial spectrum, while *Thalassiosira pseudonana* showed the largest inhibition zones. The activity observed against clinically relevant microorganisms such as *Escherichia coli* and *Staphylococcus aureus* reinforces the relevance of these algae as sources of bioactive compounds with antimicrobial activity. *Escherichia coli* is frequently associated with healthcare-associated infections and exhibits increasing resistance to several classes of antibiotics, including quinolones, beta-lactams and aminoglycosides (ANVISA 2004; ANVISA 2007; Ribeiro and Cortina 2016). Likewise, *Staphylococcus aureus* remains one of the most important human pathogens due to its multiple antimicrobial resistance mechanisms and its involvement in both community- and hospital-acquired infections (Bassetti et al. 2009; Hinrichsen 2013). In the microdilution assay, all extracts showed activity, with MIC values ranging from 62.5 to 500 µg mL^{-1}, confirming the higher sensitivity of this method compared to disk diffusion (Ramirez and Castaño 2009).

The greater sensitivity of the microdilution assay may be related to the direct contact between extracts and microorganisms, allowing a more accurate assessment of antimicrobial activity. Similar observations have been reported by Bona et al. (2014), who compared methodologies for evaluating plant extracts and highlighted the influence of assay design on the detection of antimicrobial effects.

Toxicity analysis indicated that most extracts presented low to moderate toxicity according to Meyer et al. (1982) and Nguta et al. (2011). The moderate toxicity observed for some extracts, particularly from *Thalassiosira pseudonana*, may be associated with the presence of bioactive compounds responsible for antimicrobial activity (Guillén, 2012). In contrast, extracts of *Enantiocladia duperreyi* combined relevant antioxidant activity with low toxicity, suggesting a favorable profile for potential applications.

Antioxidant activity varied considerably among extracts, with ethyl acetate generally showing better performance. However, no consistent relationship was observed between antioxidant activity and phenolic or flavonoid content, indicating that antioxidant capacity depends on the chemical nature of the

compounds present. Similar patterns have been reported in microalgae studies (Goh et al. 2010; Maadane et al., 2015). The higher antioxidant activity observed in Rhodophyceae is consistent with their known diversity of secondary metabolites (Simões et al., 2017), while the lower activity in microalgae may be related to cultivation under non-stress conditions.

Finally, the variability observed among extracts from the same species collected at distinct locations reinforces the influence of environmental and methodological factors on metabolite production (Almeida and Palermo 2017). Overall, the evaluated marine macroalgae and microalgae exhibited distinct antimicrobial, antioxidant and toxicity profiles influenced by species, collection site and extraction solvent. The results highlight the value of these organisms as sources of bioactive compounds and contribute to the bioprospecting of marine biodiversity from the Brazilian coast. Further studies aimed at chemical characterization and identification of the compounds responsible for the observed biological activities are warranted.

Declarations

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Conflicts of interest

The authors declare no conflict of interest.

Availability of data and material

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Arielle Araujo Santos: Investigation, Methodology, Data curation, Formal analysis, Writing – original draft.

Edson de Jesus Marques: Methodology, Supervision, Writing – review & editing.

Gabriel do Nascimento Santos: Resources, Taxonomic identification, Writing – review & editing.

José Marcos de Castro Nunes: Resources, Taxonomic identification, Writing – review & editing.

Edson Delgado Rodrigues: Formal analysis, Methodology, Writing – review & editing.

Suzana Telles da Cunha Lima: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

All authors have read and approved the final manuscript and consent to its publication.

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Figures

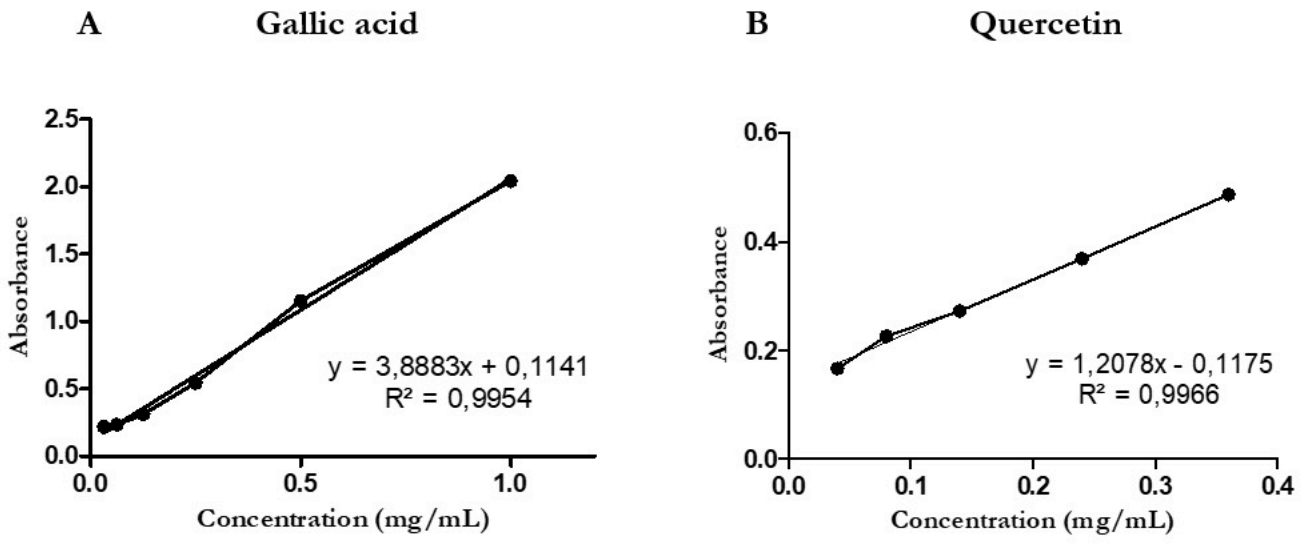


Figure 1

Calibration curves of gallic acid (A) and quercetin (B) used for the quantification of total phenolic compounds and flavonoids.

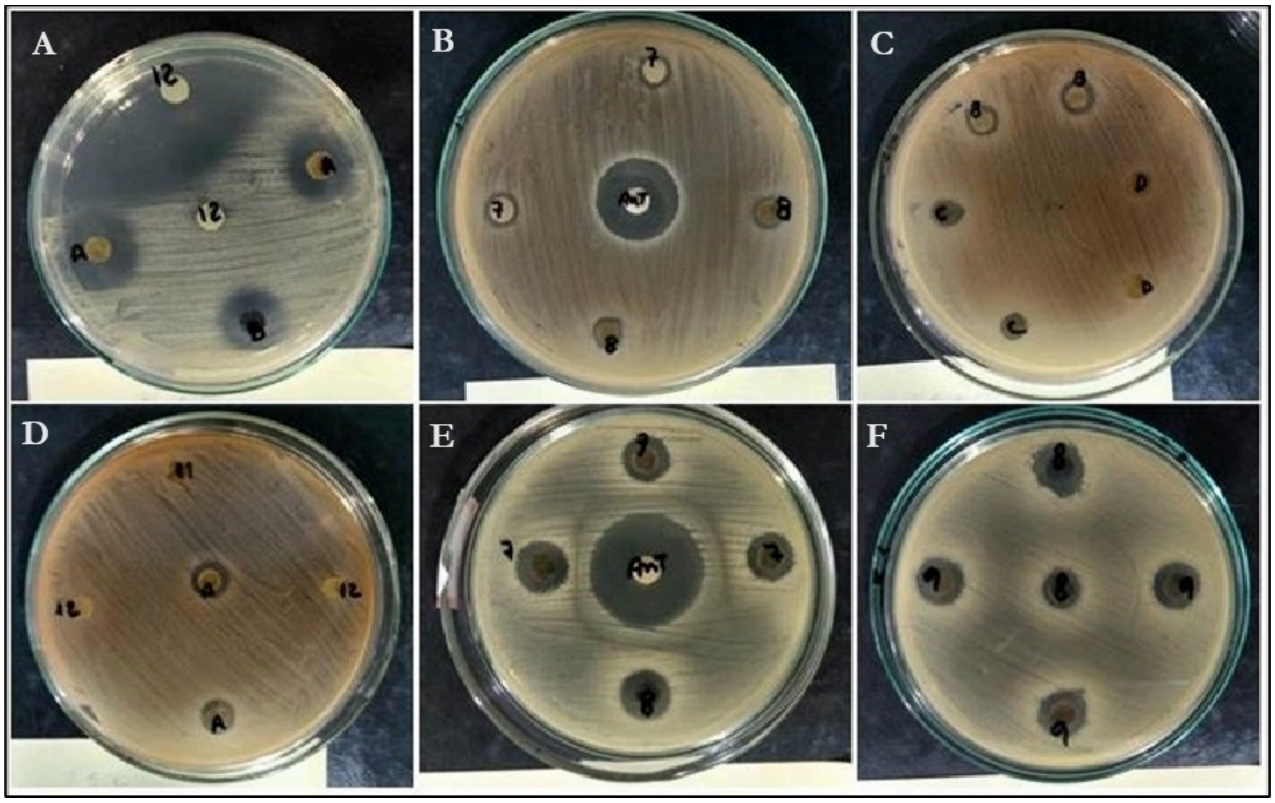


Figure 2

Antimicrobial activity of *Thalassiosira pseudonana* extracts evaluated by the agar disk diffusion method.

(A) *Micrococcus luteus*; (B–D) *Salmonella choleraesuis*; (E–F) *Candida albicans*.
Discs: (1) hexane extract of *Thalassiosira pseudonana* (100 mg mL⁻¹); (2) ethyl acetate extract of *T. pseudonana* (100 mg mL⁻¹); (3) ethanolic extract of *T. pseudonana* (100 mg mL⁻¹); (4) hexane extract of *Chaetoceros muelleri* (100 mg mL⁻¹); (5) hexane extract of *T. pseudonana* (10 mg mL⁻¹); (6) ethyl acetate extract of *T. pseudonana* (10 mg mL⁻¹); (7) ethanolic extract of *T. pseudonana* (10 mg mL⁻¹); (8–9) hexane and ethyl acetate extracts of *C. muelleri* (10 mg mL⁻¹).

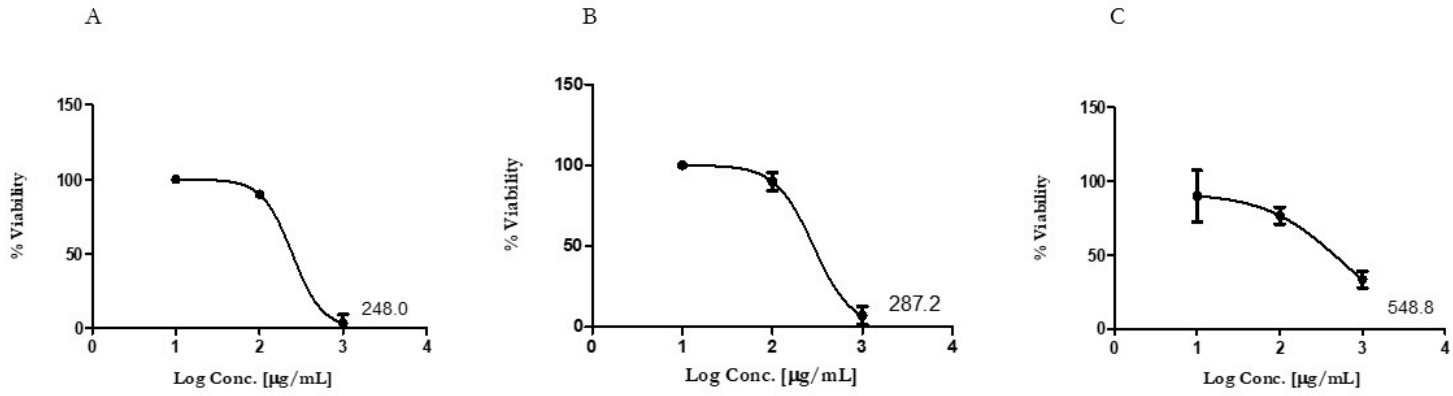


Figure 3

Relationship between the percentage of viability of *Artemia salina*nauplii and the concentration of seaweed extracts (µg/mL).

A: hexane extract of *Thalassiosira pseudonana*; B: ethyl acetate extract of *T. pseudonana*; C: hexane

extract of *Enantiocladia duperreyi* (Jauá).