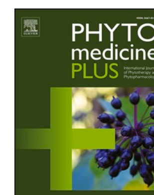
Phytomedicine Plus 2 (2022) 100246

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus



Bioprospecting of new Antarctic seaweed selective antitumor molecules: chemical characterization and in vitro analysis

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ARTICLE INFO

Keywords:
Antitumor activity
Cytotoxicity
Glioma
Lung adenocarcinoma
Antarctic seaweeds

ABSTRACT

Background: The Antarctic continent exposes seaweeds to extreme environmental conditions, which may facilitate the production of novel metabolites. Algae represent a highly diverse group of little explored organisms. Thus, marine biomass has emerged as a possible source of new biologically active molecules for the treatment of diseases requiring novel therapeutic options.
Purpose: The ultrasonic and Soxhlet extractions were used to evaluate chemical profiles and antitumor activities of Desmarestia anceps, Iridaea cordata, and Pyropia endiviifolia extracts using solvents of different polarity.
Methods: Chemical characterization of the extracts was performed using gas chromatography. The anticancer activities of the extracts were evaluated by exposing rat glioma (C6), human glioblastoma (U87), and human lung adenocarcinoma epithelial (A549) cell lines to increasing extract concentrations (10–1000 μg mL^{-1}) for 24 and 48 h. In parallel, rat astrocyte and human lung fibroblast (MRC-5) were treated under the same conditions to represent non-transformed cell controls, and permit tumor selectivity evaluations of the extracts.
Results: An MTT assay was used to measure cell cytotoxicity. Hexane, chloroform, and ethanol extracts reduced glioma and lung cancer cell viability. The hexane and ethanol extracts of *P. endiviifolia* showed the best antiproliferative effect, decreasing glioma cell viability by 40% at 10 μg mL^{-1}, and the chloroform extract reduced lung adenocarcinoma cell viability by 50% at 46 μg mL^{-1} after 24 h of treatment. None of the extracts affected the viability of non-transformed cells, as was observed in tumor cells. Fatty acids, fatty alcohols, and phthalate esters were the main compounds identified in the seaweed extracts.
Conclusions: Antarctic seaweeds displayed selective antitumor activity against glioma and lung cancer cells, particularly the endemic red alga *P. endiviifolia*. Therefore, these algae could play an important role as novel prototype molecule source for oncology drug development.

Introduction

Polar regions such as Antarctica have severe environmental conditions, including desiccation, salinity, radiation, and extreme temperatures, that induce protective secondary metabolite production in seaweeds, which may have potential biological activities (Núñez-Pons et al., 2020). Seaweeds are usually used as nutritious food resources and have proved valuable against some diseases, thus contributing to good health. In particular, in the red edible seaweed genus *Porphyra*, redefined as the genus *Pyropia, Porphyra haitanensis* polysaccharide antitumor effect can inhibit colon cancer cell growth but not normal keratinocyte growth (Yao et al., 2020). Furthermore, a novel porphyrin extracted from *Pyropia yezoensis* shows antitumor activity against cervical carcinoma cell line (HeLa) higher than that of 5-fluorouracil, a chemotherapeutic drug, blocking the cell cycle at the G2/M phase (He

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https://doi.org/10.1016/j.phyplu.2022.100246
Received 16 December 2021; Received in revised form 11 February 2022; Accepted 15 February 2022
Available online 17 February 2022
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Abbreviations

ANOVA analysis of variance
BF_3 Boron Trifluoride
CNS central nervous system
DMEM Dulbecco's modified Eagle's medium
DMSO dimethyl sulfoxide
DHA docosahexaenoic acid
EPA cis-5,8,11,14,17-eicosapentaenoic
FAME fatty acid methyl esters
GC-FID gas chromatography with flame ionization detector
IC_{50} half maximal inhibitory concentration
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
ω-3 omega-3 polyunsaturated fatty acids
PI propidium iodide
TMZ temozolomide

et al., 2019). Some studies have reported anticancer, antileishmanial, and antimicrobial activities of *I. cordata* (Martins et al., 2018; Rangel et al., 2019). Because *Desmarestia anceps, Iridaea cordata*, and *Pyropia endiviifolia* are endemic to a poorly studied region, little is known about their pharmacological properties.

Glioblastoma, a grade IV glioma, is the most aggressive type of primary central nervous system (CNS) cancer due to its invasive phenotype. Prognosis for patients with malignant glioma is poor, with a low survival time of approximately 15 months (Ramezani et al., 2019) regardless of the recent progress in brain tumor therapy and conventional treatments such as maximal resection, radiotherapy, and chemotherapy with temozolomide (TMZ). Glioma rapid proliferation, high aggressiveness, vascular hyperplasia, and immunosuppressant effects have become an increasing concern for researchers. Another cancer with extremely high mortality rates is lung cancer, which has a survival rate of 21% (Siegel et al., 2021). Controlling this cancer with conventional therapies and surgery has proven difficult. Therefore, the search for effective and safe drugs to prevent, inhibit, or reverse tumor development is a priority.

In addition, undesirable side effects of cancer treatment drugs emphasize the need to discover better and more specific anticancer compounds. Approximately 60% of anticancer drugs are derived or synthesized from natural sources, such as plants, animals, and microorganisms. Marine algae synthesize a large amount of secondary metabolites; therefore, they are the target of interest in the medical field. This variety of compounds, especially in polar environments, allows to target distinct hallmarks of cancer and improve treatment (Calcabrini et al., 2017). Thus, ultrasonic and Soxhlet extractions were used to investigate the chemical profile and antitumor activity of different polarity extracts of Desmarestia anceps, Iridaea cordata, and Pyropia endiviifolia collected in Antarctica.

Material and methods

Algal sample

The Rhodophyta *Pyropia endiviifolia* (A. Gepp & E. Gepp) H. G. Choi & M. S. Hwang (Bangiaceae), *Iridaea cordata* (Turner) Bory de Saint-Vincent (Gigartinaceae), and Phaeophyta *Desmarestia anceps* Montagne (Desmarestiaceae) were collected at the Demay site in Admiralty Bay, King George Island (62°13′13.93"S, 58°26′17.85"W) in Antarctica on January 27, 2012. The algae fixed to the substrate were collected during low tide periods. A portion of the seaweed was preserved in 4% formaldehyde in seawater solution for identification and herbarium specimen preparation, and the other part was washed with seawater to remove epiphytes, frozen at -20°C, and lyophilized for biochemical analysis. The botanist Mutue T. Fujii performed species identification according to the proposed morphology and classification system (Guiry and Guiry, 2020; Wiencke, C.; Clayton, 2002). The species were included in the book Marine Macroalgae of Antarctica with full characterization (Fujii et al., 2014).

Algal extract preparation

Conventional extraction of bioactive compounds from seaweeds

The ultrasonic and Soxhlet extractions were performed with four solvents: hexane, chloroform, ethanol, and distilled water. The aqueous extraction was performed under reflux in an oil bath at 100°C, after which the extracts were frozen at -80°C in an ultra-freezer, and lyophilized (Liotop L101 with vacuum pump; power, 15.4 W). For Soxhlet extraction, 1 g of seaweed was added to each solvent (100 mL), incubated for 4 h, and the macroalgal biomass cartridge remained submerged in the solvent overnight, as per the adapted method (Punín Crespo and Lage Yusty, 2006).

Ultrasound assisted extraction of bioactive compounds from seaweeds

Ultrasonic probe extraction was performed using an ultrasonic processor (VCX-500) and a probe horn of 20 kHz frequency and 500 W power (Sonics & Materials Inc., Newton, USA). A 13 mm-diameter horn tip was used, the power was set at 25% amplitude, total irradiation period was 30 min with the solvents mentioned previously (40 mL), and the same depletion technique was used in three cycles of solvent renewal and extract evaporation. The sample bottle was immersed in ice to avoid large temperature fluctuations, and the maximum temperature was below 20°C throughout the extraction period, as per the modified method (Stein et al., 2011). The extracts were evaporated (Büchi rotary evaporator RII) to remove the solvents.

Lipid extraction and fatty acid methyl ester preparation

Lipid extraction was performed using 1 g of algae following the conventional method (Bligh and Dyer, 1959). This method is based on the extraction of fatty acids using chloroform and methanol at room temperature. The samples were shaken for 30 min with a magnetic stirrer bar, and the chemical extractor contained 30 mL of chloroform/methanol (1:2 v/v) and 10 mL of 1.5% sodium sulfate (w/v). After stirring, 10 mL of chloroform and 10 mL of 1.5% sodium sulfate (w/v) were added. The extracts were centrifuged at 3000 rpm for 30 min, after which the organic phase was collected and dried.

Lipids extracted from algal biomass were methylated and converted to their respective fatty acid methyl esters (FAMEs), according to the modified methodology (Moss et al., 1974). Further, 2 mL of 0.5 M NaOH solution in 2% (w/v) methanol was added to a 100 mL flask containing the lipid with stirring and heating to 100°C for 5 min under reflux. BF_3 (3 mL) was added, followed by stirring for 2 min for acid catalysis to occur, after which 3 mL of NaCl solution was added at 20% (w/v). The sample was transferred to a separator funnel with hexane (20 mL). The organic phase was separated and dried using anhydrous sodium sulfate (2 g). The solvent was evaporated with N_2, and the samples were weighed for further analysis.

Gas chromatography of the extracts

Gas chromatography/mass spectrometer (QP 2010SE, Shimadzu, Kyoto, Japan) was used for the qualitative analysis of the extracts. Separation was carried out on a capillary column (RTX-5MS, 30 m × 0.25 mm I.D. × 0.25 μm film thickness). Automated injections were made with a 1:10 split ratio using an autosampler AOC-20i (Shimadzu, Kyoto, Japan). Helium was used as a carrier gas at a constant flow rate of 1.2 mL min^{-1}, and the initial injection temperature was 40°C, which increased at 5°C/min to 280°C for 10 min. In the mass spectrometer, impacts with ionization mass spectra were recorded at 70 eV ionization energy in full scan mode (40–700 m/z) (Tang and Row, 2013).

Moreover, gas chromatography with a flame ionization detector (GC-FID) was used to identify and quantify FAMEs, and analytical curve from the FAME MIX-37 standard and internal standardization to qualitatively and quantitatively analyze them. A gas chromatograph GC-FID with an autosampler AOC-20i equipped with a fused-silica capillary column (RTX-WAX, dimensions of 30 m × 0.25 mm I.D. × 0.25 μm film thickness) was used for quantitative analysis as previously described (Tang and Row, 2013). An internal standard solution containing non-adecanoate (C19:0 ≥ 99.0%, Sigma-Aldrich) was prepared at a concentration of 2 mg mL^{-1}.

Cell Culture

The rat (C6, CCL-107) and human (U87-MG, HTB-14) glioma, human lung adenocarcinoma epithelial (A549, CCL-185), and human lung fibroblast (MRC-5, CCL-171) cell lines were obtained from American Type Culture Collection. The cells were cultured in low glucose DMEM with 0.1% fungizone and 100 U/L penicillin/streptomycin supplemented with 10% fetal bovine serum and maintained at 37°C and 5% CO_2. The cells were kept in cell culture flasks (25 cm^2). After cell growth, the cells were trypsinized, seeded (5×10^3 cells per well) in 96-well plates, and cell viability analysis was performed when they reached 70–80% confluence. The cells were exposed to increasing extract concentrations of 10–1000 μg mL^{-1} for 24 and 48 h (glioma cells) and 10–250 μg mL^{-1} for 24 h (lung adenocarcinoma and lung fibroblast cells).

Cortical astrocyte primary cultures were prepared from neonatal Wistar rats (1–2 days old) by mechanical tissue dissociation using calcium-magnesium-free phosphate-buffered saline, seeded in 96-well plates, and maintained under standard culture conditions for 20 days (Bona et al., 2020). All procedures were approved by the Ethics Committee of Animal Experimentation (protocol number 10321, approved on November 25, 2011).

MTT assay

Cell viability was assessed using an MTT assay (Bona et al., 2020). The cells were incubated for 2 h with 0.5 mg mL^{-1} of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dissolved in DMEM at 37°C in a humidified 5% CO_2 atmosphere. The medium was then removed, and DMSO was added to dilute the formazan crystals formed by MTT reduction by viable cells. Optical density was measured at 570 nm using a spectrophotometer. The results are expressed as the percentage of cell inhibition against the control.

Propidium iodide (PI) assay

C6 cells were cultured in 24-well plates (1×10^4 cells per well) until they reached 70–80% confluence. The cells were treated with extracts (1, 10, 50, 100, 250, and 1000 μg mL^{-1}) for 48 h. Further, the cells were incubated with 7.5 mM PI for 1 h. PI fluorescence was excited at 515–560 nm using an inverted microscope (Olympus IX71, Tokyo, Japan) fitted with a standard rhodamine filter. Images were captured using a digital camera connected to the microscope. This cell viability indicator monitors plasma integrity through nuclear staining with membrane-impermeant dyes (Nieminen et al., 1992).

Statistical analysis

The results are expressed as the mean ± standard deviation (SD) of at least three independent experiments. All experiments were performed in quadruplicates. Statistical significance of the parameters investigated was determined by one-way analysis of variance (ANOVA) with Tukey's post hoc multiple comparisons test to assess statistical differences in normal distribution. Differences were considered statistically significant at $P < 0.05$ or $P < 0.001$. Analyses were performed in GraphPad Prism v.8.

Results and Discussion

Antiglioma activity of the extracts

To assess the antiglioma activity of the extracts of *D. anceps, I. cordata*, and *P. endiviifolia*, the rat glioma (C6) cell line was exposed to different extracts obtained by Soxhlet and ultrasonic extractions for 24 and 48 h. The percentage of cell viability inhibition was evaluated using the MTT assay (Fig. 1).

Cell viability results showed very similar responses to extracts of *D. anceps* and *I. cordata*. The hexane and chloroform extracts of *D. anceps* showed better antiproliferative activity in C6 glioma than the ethanol extract did, inhibiting C6 cell growth by approximately 25% at 100 μg mL^{-1} after 24 h of exposure and 50% at 250 μg mL^{-1} after 48 h. The ethanol extract inhibited C6 cell growth by 50% at 500 μg mL^{-1} after 48 h (Fig. 1A, B). The best extract of *I. cordata* was the chloroform extract after 48 h of exposure, which inhibited C6 cell growth by more than 30% at 100 μg mL^{-1} and more than 45% at 1000 μg mL^{-1}; however, it was less effective than the other extracts (Fig. 1A, B).

The hexane extract of *P. endiviifolia*, obtained by ultrasonic extraction, showed significant results at lower concentrations after 24 h (37% inhibition at 10 μg mL^{-1}, $P < 0.001$; Fig. 1B) compared with that obtained by Soxhlet extraction (8% inhibition at 10 μg mL^{-1}, Fig. 1A). Similar conditions were observed in the ethanol extract obtained by ultrasonic (40% inhibition at 10 μg mL^{-1}, $P < 0.001$; Fig. 1B) and Soxhlet (29% inhibition at 10 μg mL^{-1}, Fig. 1A) extractions. Marked contrast was observed in the hexane extract cell growth inhibition after 48 h of treatment, which decreased from 37% to 12%, and in that of the ethanol extract, which decreased from 40% to 17%, suggesting better extract activity after 24 h. The chloroform extract exhibited a lower antitumor activity than the other tested extracts. The aqueous extract did not show significant antitumor activity even at the highest concentration tested (1000 μg mL^{-1}, data not shown). Therefore, the best anti-C6-glioma activity was exhibited by the hexane and ethanol extracts of *P. endiviifolia*, and ultrasonic extraction was the best extraction method for this alga. Heatmap analysis showed that the *D. anceps* extracts inhibited glioma growth more than those of *I. cordata* did, followed by those of *P. endiviifolia* (Fig. 1C).

Fatty acids represented a major class of molecules in apolar fractions, as observed in the hexane extract. Previously, our research group showed that a combination of fatty acids extracted from macroalgae has cytotoxic activity against human breast cancer cells (Pacheco et al., 2018). In this study, the viability assay showed that fatty acids contributed to glioma growth inhibition after 48 h of exposure (Fig. 1D) as compared to the antitumor activity of TMZ, the standard chemotherapy drug for glioblastoma (Fig. 1E). However, upon evaluating the inhibition percentage, it was noted that such activity was highest in the hexane extract, possibly due to the synergistic effect of other extracted molecules. These data show that 100 μM of TMZ (19.4 μg mL^{-1}) inhibited C6 cell growth by 45% after 48 h (Fig. 1E), demonstrating the potential of the hexane extract at 10 μg mL^{-1}, which inhibited C6 cell viability by approximately 40% after 24 h.

Rat C6 glioma cell viability was evaluated by PI staining. The hexane extract of *D. anceps* at 50 μg mL^{-1} induced morphological changes and cell death after 24 h (in red, Supplementary material 1). The chloroform and ethanol extracts of *D. anceps* changed the density and cell morphology at 50 μg mL^{-1} after 48 h of treatment and remarkable cell death was observed at 100 μg mL^{-1} after 24 h (Supplementary material 2, 3), as well as the chloroform extract of *I. cordata* (Supplementary material 4). These data emphasize the antitumor effect of apolar extracts of *D. anceps* and *I. cordata* at low concentrations (below 100 μg mL^{-1}).

The antiglioma activity of the extracts was also evaluated in a human glioblastoma cell line (U87, Fig. 2). The U87 cells showed a distinct response to the treatments when compared to C6 cells. Indeed, the

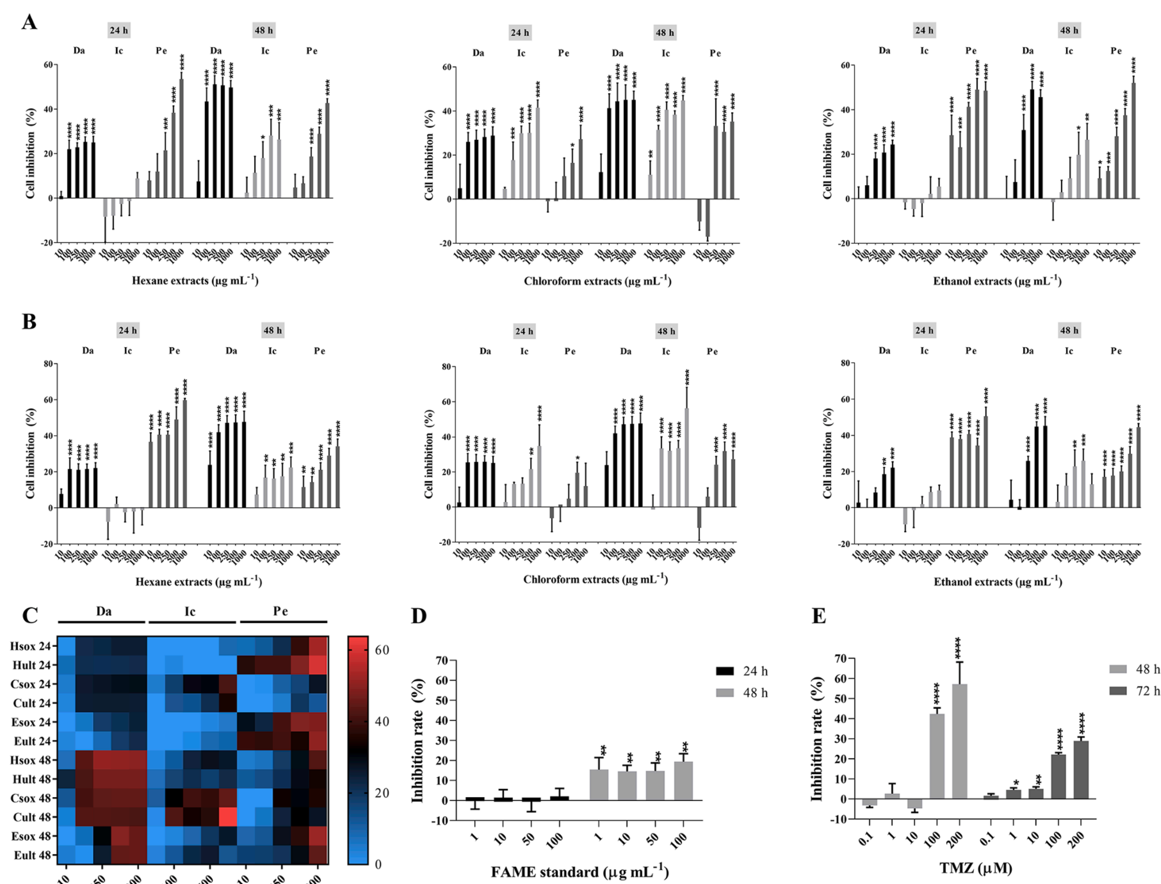

Fig. 1. Rat glioma viability was determined by MTT assay after 24 and 48 h of treatment. The hexane, chloroform, and ethanol extracts of *Desmarestia anceps, Iridaea cordata,* and *Pyropia endiviifolia* obtained by Soxhlet **(A)** or ultrasonic **(B)** extraction. Heatmap analysis of treatment effect on rat glioma cells, correlations between concentrations and different extracts of *D. anceps, I. cordata,* and *P. endiviifolia* **(C)**. Red and blue colors indicate high and low antiglioma activity levels, respectively. FAME (fatty acid methyl ester) analytical standard **(D)**, and TMZ (temozolomide) treatment **(E)**. Results are expressed as cell viability inhibition rate percentage and represent the media ± SD of at least three independent experiments (one-way ANOVA, post-hoc Tukey; $P < 0.001$). Extract concentrations are expressed in µg mL^{-1}. Da, *D. anceps*; Ic, *I. cordata*; Pe, *P. endiviifolia*; H, hexane; C, chloroform; E, ethanol; sox, Soxhlet extraction; ult, ultrasonic extraction.

hexane, chloroform, and ethanol extracts of *D. anceps* inhibited U87 cell growth by 20%, 11%, and 16%, respectively, at 250 µg mL^{-1} after 24 h (Fig. 2A-C); whereas the chloroform extract of *I. cordata* inhibited U87 glioma cell growth by 16% (Fig. 2D). A previous study with the control drug-treated U87 cells has shown that 200 µM (38.8 µg mL^{-1}) of TMZ can inhibit U87 cell growth by 50% after 72 h of treatment (Souza et al., 2018), demonstrating a greater treatment resistance of the human glioma lineage. Heatmap analysis showed that the chloroform extract had the highest U87 cell growth inhibition activity (66%) compared with that of the hexane and ethanol extracts (Fig. 2E).

To assess whether the best extracts for glioma treatment were also cytotoxic to healthy cells, rat astrocytes were exposed to the hexane and ethanol extracts of *P. endiviifolia* obtained using ultrasonic extraction, and all the extracts of *D. anceps* and the chloroform extract of *I. cordata* obtained using Soxhlet extraction (Fig. 3). As shown in Figure 3, neither the hexane nor ethanol extract of *P. endiviifolia* (Fig. 3A, C) was significantly cytotoxic to astrocytes, which served as a healthy CNS cell model. Although *D. anceps* (Fig. 3) and *I. cordata* (Fig. 3B) extracts showed some cytotoxic activity against astrocytes, the growth inhibition was lower than that caused by the same extract concentrations in C6 glioma cells. Astrocyte viability was not significantly affected by the treatment; therefore, seaweed extracts showed a selective antiglioma effect, as noted in the heatmap analysis (Fig. 3D).

Antitumor effects of the extracts on lung adenocarcinoma

Further, the effect of different extracts on the viability of human lung adenocarcinoma (A549) cells was determined after 24 h (Fig. 4). The MTT assay was used to compare the antitumor activities of the extracts obtained through the two extraction systems. Similar to the results obtained using glioma cells, the highest cytotoxic activity was observed in *D. anceps* and *I. cordata* extracts prepared using the Soxhlet method and in *P. endiviifolia* extracts prepared using the ultrasonic method. It is important to discuss the differences in responses between the extraction methods and, consequently, the types of compounds extracted depending on extraction temperature, as Soxhlet extraction occurs at high temperatures, and ultrasonic extraction is controlled in an ice bath. The extraction temperature is important to keep the compounds active. The mechanism of action of molecules may vary depending on the temperature at which they are extracted due to differences in molecular weight (Guerra-Rivas et al., 2011). According to the same authors, hot water extracts of seaweeds are expected to have high molecular weights, while cold extracts are expected to have lower molecular weights. It has also been reported that ultrasound increases the extraction rate by disrupting plant cell walls, leading to increased diffusion of cell contents into a small amount of solvent (Al Jitan et al., 2018).

The hexane and chloroform extracts of *D. anceps* inhibited A549 cell growth by 50% at low concentrations (70 and 61.2 µg mL^{-1}; Fig. 4A, B). The same extracts of *I. cordata* reduced A549 cell viability by 50% at 157.0 and 67.5 µg mL^{-1}, while the ethanol extract inhibited A549 cell

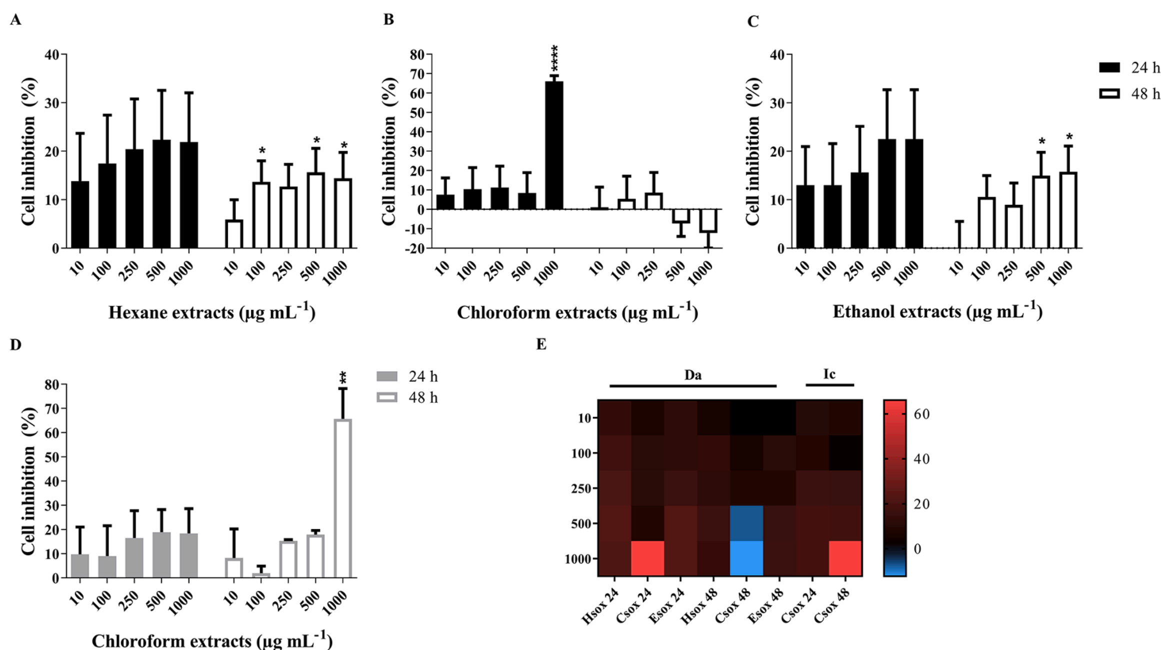

Fig. 2. Human glioblastoma cell growth inhibition after 24 and 48 h of treatment with the hexane **(A)**, chloroform **(B),** and ethanol **(C)** extracts of *Desmarestia anceps* and the chloroform extract of *Iridaea cordata* **(D)** obtained by Soxhlet extraction. Heatmap analysis of treatment effect, correlations between concentrations and different extracts of *D. anceps* and *I. cordata* **(E)**. Red and blue colors indicate high and low antiglioma activity levels, respectively. Extract concentrations are expressed in µg mL^{-1}. Da, *D. anceps*; Ic, *I. cordata*; H, hexane; C, chloroform; E, ethanol; sox, Soxhlet extraction.

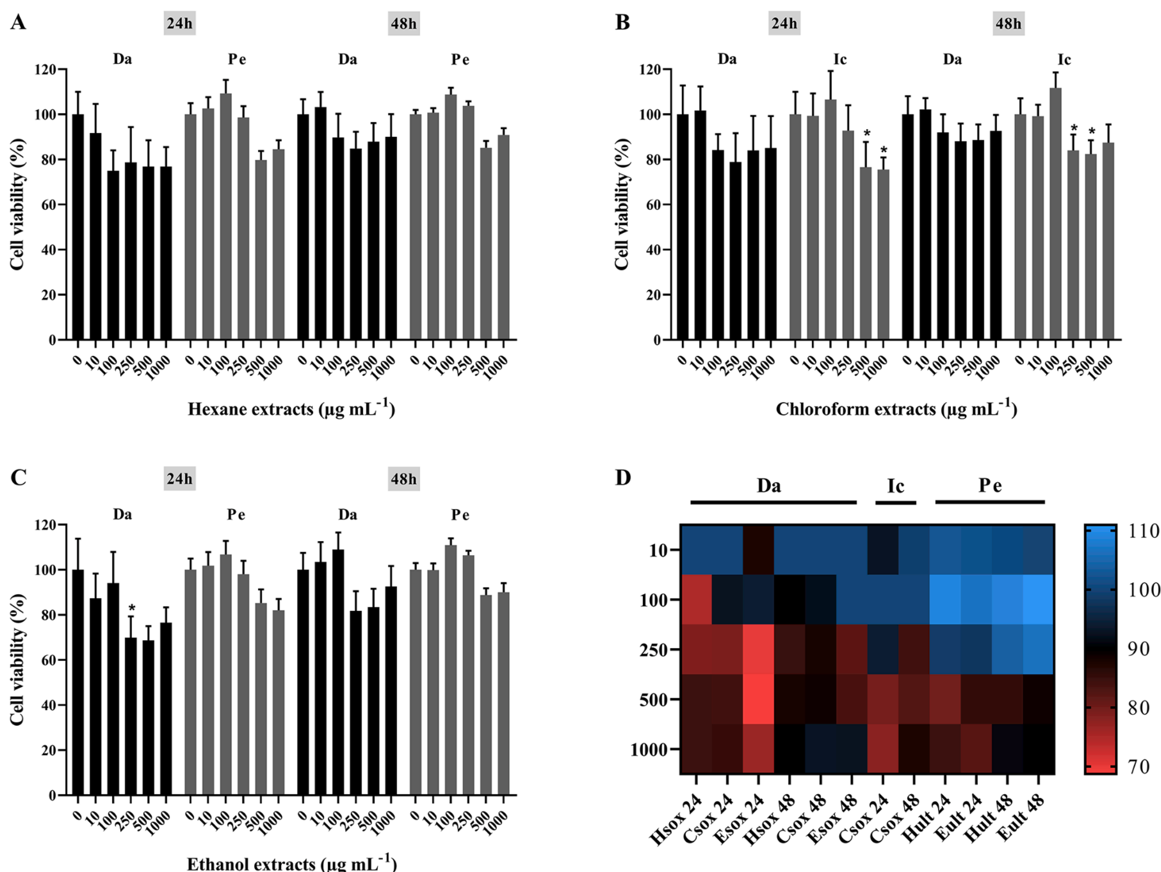

Fig. 3. Primary rat astrocyte cell viability after 24 and 48 h of treatment with the hexane **(A)**, chloroform **(B),** and ethanol **(C)** extracts of *Desmarestia anceps* and *Iridaea cordata* obtained by Soxhlet extraction and of *Pyropia endiviifolia* obtained by ultrasonic extraction. Heatmap analysis of treatment effect on rat astrocytes, correlations between concentrations and different extracts of *P. endiviifolia* **(D)**. Blue indicates high cell viability levels. Extract concentrations are expressed in µg mL^{-1} (one-way ANOVA, post-hoc Tukey; $P < 0.05$). Da, *D. anceps*; Ic, *I. cordata*; Pe, *P. endiviifolia*; H, hexane; C, chloroform; E, ethanol; sox, Soxhlet extraction; ult, ultrasonic extraction.

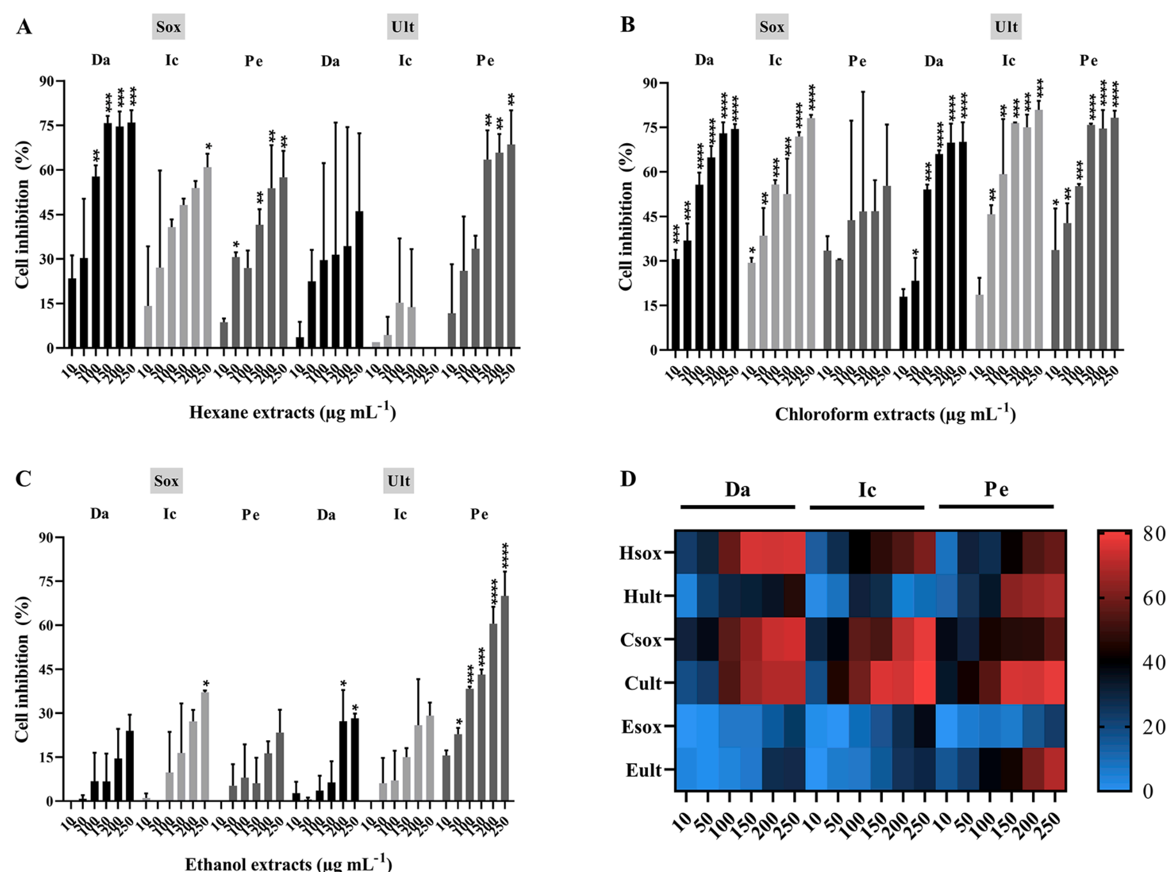

Fig. 4. Human lung adenocarcinoma cell viability was determined by MTT assay after 24 h of treatment. The hexane **(A)**, chloroform **(B)**, and ethanol **(C)** extracts of *Desmarestia anceps, Iridaea cordata,* and *Pyropia endiviifolia* obtained by Soxhlet and ultrasonic extractions. Heatmap analysis of treatment effect on A549 cells, correlations between concentrations and different extracts **(D)**. Red and blue colors indicate high and low antitumor activity levels, respectively. Results are expressed as cell viability inhibition rate percentage and represent the media ± SD of at least three independent experiments (one-way ANOVA, post-hoc Tukey; *P < 0.001*). Da, *D. anceps*; Ic, *I. cordata*; Pe, *P. endiviifolia*; H, hexane; C, chloroform; E, ethanol; sox, Soxhlet extraction; ult, ultrasonic extraction.

growth by 37% at 250 μg mL^{-1} (Fig. 4A-C). The most promising result was that of the chloroform extract of *P. endiviifolia* which reduced A549 cell viability from 66% to 22% at 10–250 μg mL^{-1}, exhibiting a concentration-dependent profile and a half maximal inhibitory concentration (IC_{50}) of 46 μg mL^{-1} (Fig. 4B). The hexane and ethanol extracts obtained using ultrasonic extraction also reduced cell viability, but at higher concentrations than those of the chloroform extract, inhibiting A549 cell growth by 50% at 124 and 147 μg mL^{-1}, respectively (Fig. 4A, C). Heatmap analysis showed that the hexane and chloroform extracts of *D. anceps, I. cordata*, and *P. endiviifolia* exhibited higher antitumor activity than the ethanol extract did (Fig. 4D).

All the extracts were evaluated in human lung fibroblast (MRC-5) cells to verify their selectivity (Fig. 5). Extracts obtained by the two extraction systems had very similar effects on cell viability. The hexane and chloroform extracts of *D. anceps* exhibited cytotoxic activity in MRC-5 cells, reducing cell growth by 50% at 90.1 and 123.3 μg mL^{-1} (Fig. 5A, B). The chloroform extract of *I. cordata* also reduced MRC-5 cell viability by 50% at a low concentration of 95.4 μg mL^{-1} (Fig. 5B). However, these cytotoxic activities occurred at higher concentrations than those that caused cytotoxic activities in A549 cells. The chloroform extract of *P. endiviifolia* (Fig. 5B) also reduced MRC-5 cell viability by 50% at a concentration twice as high as that in A549 cells (87 μg mL^{-1}). These data are promising because cisplatin, a drug generally used in the clinical treatment of lung cancer, has an IC_{50} of 17.3 μg mL^{-1}in A549 cells, being more lethal in healthy cells such as MRC-5 at a dosage of 13.2 μg mL^{-1} (Thiagarajan et al., 2020). The ethanol extracts of *D. anceps* and *I. cordata* did not show significant lung fibroblast inhibition, as evidenced by heatmap analysis of the extracts (Fig. 5C, D). Taken together, these results suggest that the endemic Antarctic macroalga *P. endiviifolia* has a greater potential antitumor activity to be explored in glioma and lung cancer treatment.

Chromatographic analysis

Gas chromatography-mass spectrometry was used for the chemical characterization of the hexane, chloroform, and ethanol extracts of *D. anceps* and *P. endiviifolia* and the chloroform extract of *I. cordata* (Supplementary material 5, 6). The identified compounds are shown for the first time for these Antarctic algae (Supplementary material 7-10). The main compound classes were fatty acids, fatty alcohols, and phthalate esters. Considering the remarkable activity of the hexane extract, an apolar solvent, an analysis of the chromatographic profile of the major fatty acids of *P. endiviifolia* was performed (Supplementary material 6D). The concentration of each fatty acid is shown in Supplementary material 11.

Gas chromatography analysis of *P. endiviifolia* fatty acid composition showed that the main compounds with high proportions were palmitic acid, C16:0 (24%), and cis-5,8,11,14,17-eicosapentaenoic (EPA), C20:5n3 (60%), also found in the chloroform extract. In this context, omega-3 polyunsaturated fatty acids (ω-3) have certain biological properties, including anti-angiogenic, pro-apoptotic, antioxidant, anti-inflammatory, and anti-proliferative properties (Keene et al., 2019; Layé et al., 2018; Pal et al., 2019). EPA antitumor activity has been shown in breast cancer in vitro and in vivo; it can reduce cell growth and enhance apoptosis (Torres-Adorno et al., 2019).

Lipophilic molecules, which can passively enter the CNS by diffusion

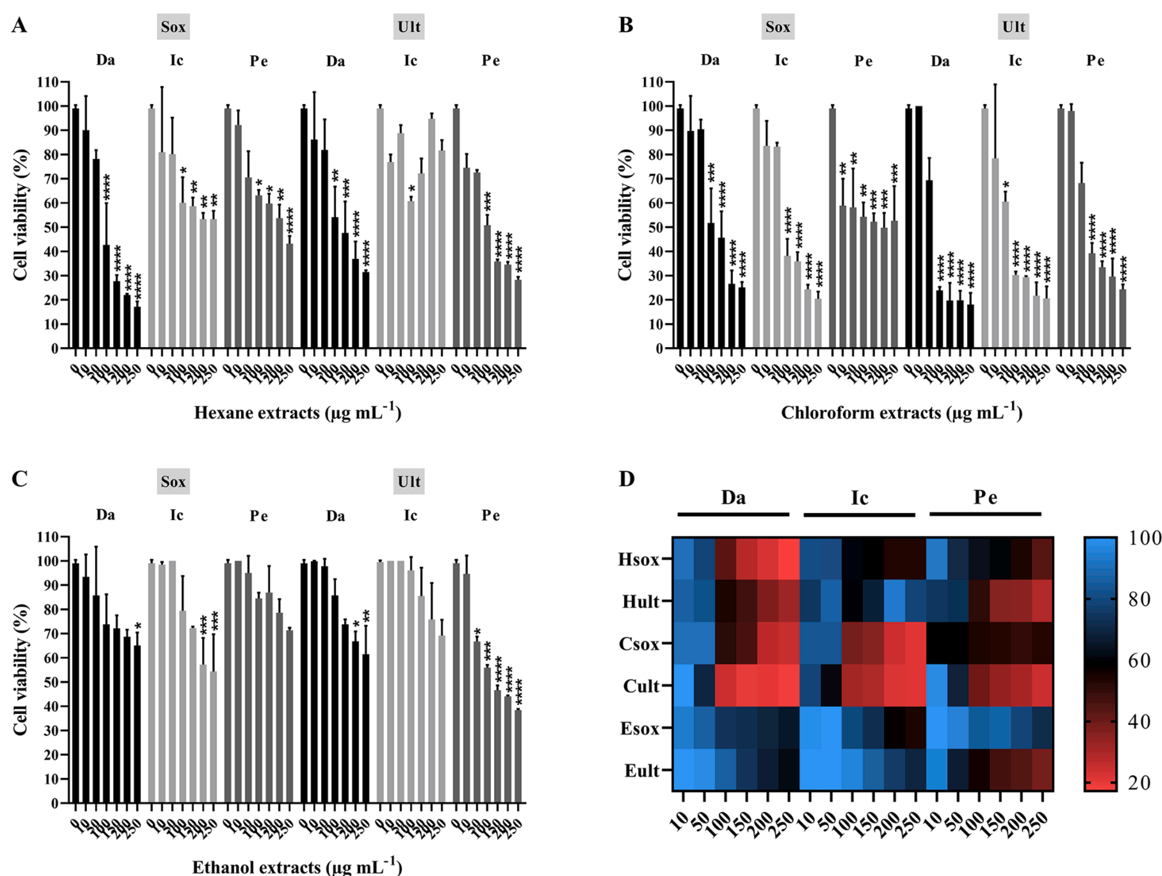

Fig. 5. Human lung fibroblast viability analysis after 24 h of treatment with the hexane **(A)**, chloroform **(B)**, and ethanol **(C)** extracts of *Desmarestia anceps, Iridaea cordata*, and *Pyropia endiviifolia* obtained by Soxhlet and ultrasonic extractions. Heatmap of treatment effect, correlations between concentrations and different extracts **(D)**. Blue and red colors indicate high and low cell viability levels, respectively. Extract concentrations are expressed in μg mL^{-1} (one-way ANOVA, post-hoc Tukey; $P < 0.001$). Da, *D. anceps*; Ic, *I. cordata*; Pe, *P. endiviifolia*; H, hexane; C, chloroform; E, ethanol; sox, Soxhlet extraction; ult, ultrasonic extraction.

once the blood-brain barrier exhibits low permeability to hydrophilic molecules, are relevant in the therapeutic area (Laurens et al., 2019). Chemical analysis of the hexane extract showed docosapentaenoic acid methyl ester and squalene, which have shown some biological activities. Interestingly, ω-3 docosahexaenoic acid (DHA) can induce glioblastoma cell death by apoptosis and autophagy in vitro and in vivo (Kim et al., 2017). Myristic acid, found in the chloroform extract of *D. anceps*, is a fatty acid that has shown antioxidant activity, which can be associated with the binding of fatty acid alkyl chain to serine/threonine protein kinase (Prasath et al., 2019). Squalene, a triterpenoid and intermediate metabolite in cholesterol synthesis, can reduce inflammation by regulating prostaglandin E2 production during anticancer drug doxorubicin treatment, indicating its potential as an adjuvant treatment during chemotherapy in tumor-bearing mice (Narayan Bhilwade et al., 2019).

Moreover, a study with 21 species of algae has shown that *Porphyra crispata* had the highest lipid content (28.34 ± 2.98 mg g^{-1} dry basis), including palmitic acid and EPA, as observed in the present study of *P. endiviifolia*, and the highest ω-3/ω-6 ratio (4.11) (Tsai and Sun Pan, 2012). These data can be correlated with health benefits of omega fatty acids, since epidemiological and molecular studies have shown the importance of diet with an optimum ω-3/ω-6 essential fatty acid ratio close to 4:1 (Candela et al., 2011). These data corroborate the relationship established in this study, as they confirm the high quantity of omega fatty acids present in the studied algal genus. Considering the potential antitumor activity of ω-3 reported in the literature, the glioma and lung adenocarcinoma cell growth inhibition by the hexane extract of *P. endiviifolia* at low concentrations can be associated with high levels of EPA and DHA found in this alga.

The ethanol extracts contained high amounts of fatty acids, fatty alcohols, and phthalate esters. Fatty alcohol compounds can inhibit DNA synthesis and tumor cell proliferation through efficient transmembrane permeation (Takada et al., 2001). Additionally, n-heptadecanol-1 is a fatty alcohol that has shown antibacterial activity against *Staphylococcus gallinarum* at a minimum inhibitory concentration of 15.08 μg mL^{-1} (Chatterjee et al., 2018). Docosanol, a highly lipophilic fatty alcohol, is an antiviral agent approved by the Food and Drug Administration for recurrent herpes labialis, which makes cells resistant to viral fusion and entry (Sadowski et al., 2021).

Moreover, 2-methylpropyl ester is a phthalate derivative that shows potential antioxidant activity (Druzian et al., 2020). Further, n-hexadecanoic acid is a fatty acid with an antitumor effect associated with its immunomodulatory activity (Boubaker et al., 2018). No description of the biological activity of the other identified molecules has been found in the literature. These results suggest that fatty acids induce changes in tumor cell lipid composition, resulting in cell damage and treatment effectiveness. These data highlight the complexity of the extract composition of *D. anceps, I. cordata*, and *P. endiviifolia,* and the need for further analysis of these molecules present in endemic Antarctic algae to investigate their application in cancer therapy.

Conclusions

All the extracts of brown (*D. anceps*) and red (*P. endiviifolia*) endemic seaweeds, and the chloroform extract of *I. cordata* exhibited potential antitumor activity against glioma and lung adenocarcinoma cells. Apolar extracts had high amounts of fatty acids, which are more able to penetrate biological membranes. These compounds are easily dispersed in the cell membrane, making them potential candidates to cross the

blood-brain barrier, which is relevant in the aspect of developing new therapeutic approaches for cancer treatment. Therefore, the molecules identified in these unique Antarctic algae might be interesting as potential therapeutics in preclinical cancer models.

Author Contributions

Priscila O. de Souza: conceptualization, methodology, formal analysis, investigation, writing, original draft, and visualization. **Felipe A. Silva:** investigation. **Caroline O. da Silva Frozza:** methodology and investigation. **Rafaele Frassini:** formal analysis. **Mariana Roesch-Ely:** methodology and resources. **Marco A. Z. dos Santos:** formal analysis; investigation; original draft writing. **Rogério A. Freitag:** methodology and resources. **Pio Colepicolo:** resources and funding acquisition. **Claudio M.P. Pereira:** conceptualization; methodology, resources, original draft writing, supervision, and funding acquisition. **Elizandra Braganhol:** conceptualization, methodology, resources, original draft writing, visualization, and supervision. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of the work, ensuring integrity and accuracy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was financially and logistically supported by the Brazilian Antarctic Program (CNPq/MCTI/PROANTAR 407588/2013-2). The authors are grateful for the financial and fellowship support from the Brazilian research funding agencies FAPERGS, FAPESP (2010/50193-1), and Capes (General Program of International Cooperation-99999.002378/2015-9). We would like to thank Editage (www.editage.com) for English language editing and the botanist Mutue T. Fujii for algae identification.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phyplu.2022.100246.

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