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Antarctic Macroalgae *Palmaria decipiens* (Rhodophyta, Palmariales) Extracts Present Antioxidant and Antitumor Activity against Colorectal Cancer

Marina Della Giustina[1], Rafaele Frassini[1], Sidnei Moura[2], Fernando Joel Scariot[3], Sérgio Echeverrigaray[3], Vanessa Sayuri Osaki[4], Franciane M. Pellizari[4], Pio Colepicolo[5], João Antonio Pêgas Henriques[1], Janaina da Silva Crespo[6], Mariana Roesch-Ely[1]*

[1]Laboratory of Applied Toxicology and Bioproducts, University of Caxias do Sul, Rio Grande do Sul, RS, Brazil
[2]Laboratory of Biosynthetic and Natural Products, University of Caxias do Sul, Rio Grande do Sul, RS, Brazil
[3]Laboratory of Applied Microbiology, Biotechnology Institute, University of Caxias do Sul, Rio Grande do Sul, RS, Brazil
[4]Laboratory of Phytology and Quality of Marine Water, Paraná State University, Paranaguá Campus, PR, Brazil
[5]Laboratory of Biochemistry and Molecular Biology of Algae, University of São Paulo, São Paulo, SP, Brazil
[6]Exact Sciences and Engineering Area, University of Caxias do Sul, Rio Grande do Sul, RS, Brazil
Email: *mrely@ucs.br

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Abstract

Despite therapeutic advances in recent decades, colorectal cancer is still the third most frequent neoplasm worldwide, with significant morbidity and mortality in young and middle-aged adults. Therefore, advance in treatment options for patients who are afflicted with tumor subtypes without effective therapies is needed. Antarctica macroalgae are substances-producing organisms with important biological activities, in which antitumor properties are investigated, showing promising cytotoxic results. There are no reports so far showing antitumor activity of macroalgae *Palmaria decipiens* extracts against colorectal tumors. This study aims to evaluate the effect of macroalgae *P. decipiens* extract from Antarctic on tumor cell HCT-116 and non-tumor cell HaCaT lines. The phenolic compounds present were identified by high performance liquid chromatography. The antioxidant activity of the extracts was determined by the DPPH radical inhibition method and cytotoxicity was evaluated through MTT assay. Cell death events were identified using dual staining with acridine orange/ethidium bromide and flow cytometry. The quantification of phenolic compounds present in the extracts identified the presence of three main compounds among them is kaempferol. The metanolic extract showed inhibition within 72 h of treatment in HCT-116 and potential antioxidant activity. The results presented in this study point out an im-

balance in the redox metabolism and also a loss of mitochondrial membrane potential integrity, most likely inducing cell death mechanisms after 72 h exposure to treatment with metanolic extract. These events could be observed by penetration of propidium iodide through membrane damage. The results indicate that the extract of the Antarctic macroalgae *P. decipiens* interferes in the mechanisms of action of colorectal cancer tumor cells, acting as a potential antitumor and antioxidant agent.

Keywords

Antarctic Seaweeds, Antitumor Activity, Colorectal Cancer, Antioxidant Activity

1. Introduction

Among all types of cancers, colorectal cancer stands out for its epidemiological relevance and association with the population's lifestyle and dietary factors. Despite therapeutic advances in recent decades, colorectal cancer is the third most frequent neoplasm worldwide and mortality rates remain around 40% in developed countries and 55% in developing countries [1] [2]. In Brazil, positive cases are distributed predominantly in the South and Southeast regions [3].

Antarctic macroalgae are organisms that produce substances with important biological activities, such as antiviral, anti-inflammatory, anti-phytophagic, antimicrobial and cytotoxic actions, in addition to antifreeze and algaecide [4]-[10]. From the bioactive compounds identified in marine algae, studies point out a variety of phytochemicals with anticancer activity, these compounds can be used as agents for chemoprevention or chemotherapy of cancer, producing anticancer effects through various mechanisms of action, including growth inhibition, invasion and metastasis, and through the induction of apoptosis [11] [12].

The red macroalgae *Palmaria decipiens* stands out for its abundance in the Antarctic continent, its particularities have been the subject of research to understand the survival mechanisms developed by the species. The antitumor activity has already been investigated in other macroalgae, such as *I. cordata*, *C. jacquinotii* and *D. anceps*, showing very promising cytotoxic results against colorectal cancer tumor lineage HCT-116 [13]. So far there are no reports of antitumor activity of extracts of the macroalgae *Palmaria decipiens* against colorectal tumors. This study aims to evaluate the effects of macroalgae Antarctica *Palmaria decipiens* extracts (hexane, chloroform and methanol) against colorectal cancer HCT-116 and non-tumor HaCaT lines.

2. Materials and Methods

2.1. Reagents

Dulbecco's modified eagle's medium–high glucose (DMEM), trypsin-EDTA, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), dime-

thylsulfoxide (DMSO), inactivated by heat fetal bovine serum (FBS), Annexin V-FITC Apoptosis Detection Kit, standard phenolic compounds and DPPH (2,2-diphenyl-picrylhydrazyl), were purchased from Sigma-Aldrich (St. Louis, MO, USA). DPPH reagent (2,2-diphenyl-picrylhydrazyl) was prepared daily. Hexane, chloroform and methanol ultrapure were obtained from Merck (Brazil). All other chemicals were ultra-pure grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Seaweed Sample and Preparation of Extracts

The red alga *Palmaria decipiens* (Reinsch) R.W.Ricker 1987 was collected in Whalers Bay (62˚59"S and 60˚34"W) during the Brazilian Antarctic Operation XXXIII in September 2016. Seaweeds fixed to the substrate were taken from the intertidal region during periods of low tide. The biomass was first washed with seawater to remove epiphytes, frozen at −20˚C and posteriorly lyophilized for biochemical analyzes. Species identification was performed according to its morphology and the classification system proposed by Wynne (2017), and specimens were deposited in the Herbarium of Botanical Institute of São Paulo (SP), Brazil.

The extracts were obtained by macerating the dry powder of *P. decipiens* immersed in hexane, chloroform and methanol, individually, in the proportion of 1:10, remaining in contact with the solvent for 24 hours, through the exhaustion process. Extracts from three consecutive baths were collected and filtered (Whatmann n˚1). After filtration, the extracts were combined and concentrated to dryness under vacuum at 40˚C. Crude extracts were kept in the dark and dried at −20˚C until biological testing.

2.3. Phenolic Compounds by High Performance Liquid Chromatography (HPLC)

The analysis was performed on HPLC equipment, HP model 1100, column Lichrospher RP18 (5 μm), equipped with 210 nm UV detector and quaternary pump system. The reverse phase analysis consisted of: solvent A-Milli-Q water with 1% phosphoric acid and solvent B-Acetonitrile. The pumping system of the mobile phase was prepared in agradient, with 90% of the solvent A from 0 to 5 min, 60% from A from 5 to 40 min and 90% from A from 45 to 50 min. The standard flow was maintained at 0.5 mL/min according to Morelli (2010) [14]. The samples were solubilized in water Milli-Q (5 g/L) and filtered through Nylon membranes of 0.45 μm pore diameter. The phenolic compounds were identified according to their order of elution and by comparison of their time of retention with those of your pure standards. Quantification was performed using external standardization, correlating the area (mAU * s) of the compound peak to the standard curve performed. The use of standards was as described in the literature (gallic acid, epigallocatechin, catechin, epicatechin, epigallocatechin gallate, rutin, ferulic acid, naringin, hesperidin, myricetin, resveratrol, quercetin, apigenin and kampferol). The result was expressed in μg/ mL of extract.

2.4. Radical DPPH Scavenging Activity

The antioxidant properties of the macroalgae were performed using the inhibition of the free radical DPPH (2,2-diphenyl-picrylhydrazyl), described by Kim *et al.* (2002) [15] and adapted from Bernardi *et al.* (2016) [16]. 1.5 mL aliquot of the DPPH solution (0.01 g in ethanol PA) was added to 1.2 mL of Tris-HCl (15% of Trisma Base in ultrapure water, pH = 7) and 1 mL of sample. The control blank was prepared with 80% methanol. After homogenizing the solution, the samples were left to stand for 20 minutes, protected from light, for later reading on a UV-visible spectrophotometer (Hiatachi U-1800) at a wavelength of 517 nm. The percentage of Antioxidant Activity (AA) was calculated by the algebraic expression: % inhibition = {[abs. final mean of the control − abs. final sample mean]/abs. final control mean} × 100.

2.5. Cell Culture and Cytotoxic Assay

HCT-116 (human colorectal cancer cell line) and HaCaT (human keratinocyte cell line) was purchased from American Type Culture Collection (ATCC - CCL247), Manassas, VA, US. Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS. Cells were maintained in a humidified atmosphere at 37˚C, in 5% CO_2, and 95% air. The cytotoxicity was performed as cells reached 70% - 80% confluence.

Cytotoxic analysis was assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method. Cells were seeded in 96-well plates at a density of 5×10^4 cells/cm^3. After 24 h incubation, the cells were treated with increasing concentrations of extracts for 72 h. Ethanol 3.5% (v/v) and DMSO 0.5% (v/v) was used as a negative control. MTT solution (0.4 mg/mL) was added after treatment and incubated for 2 h, followed by dimethyl sulfoxide [17]. The optical density (OD) was measured at 570 nm. IC_{50} was calculated by GaphPad Prism version 5.0.

2.6. Cellular Morphology Test by Giemsa Staining

HCT-116 cells were seeded over round coverslips in 24-well plates at 5×10^4 cells/cm^3. After cell binding, the medium was removed, and three concentrations of the selected extracts were added to the wells for 72 h: IC_{25}, IC_{50} and IC_{75}. The treatment medium was removed and cells were washed gently with PBS and fixed with methanol for 5 minutes. Subsequently, they received staining with 10% Giemsa for 10 minutes. The coverslips were washed with distilled water and samples were analyzed under light inverted microscopy (Nikon Eclipse Ts2) staining protocol [18].

2.7. Dual Acridine Orange/Ethydium Bromide Staining

HCT-116 cells were seeded in a 6-well plate and treated with concentrations of IC_{50} from the selected extracts. After 72 h, the cells were washed with PBS, detached from the wells with trypsin and resuspended in 50 µL of PBS. The cells

were stained with 100 μg/mL of acridine orange and 100 μg/mL of ethidium bromide and visualized on an Olympus BX43 microscope. Acridine orange penetrates in normal cells and early apoptosis events with intact cell membranes and shows green fluorescence. Ethidium bromide only entered cells with membrane damage, such as late apoptotic and necrotic cells, emitting orange-red fluorescence. Negative controls were treated with ethanol and DMSO [19].

2.8. *In Vitro* Migration Assay

HCT-116 cells were seeded in round coverslips on 24-well plates at 5×10^4 cells/cm^3. After 24 h of incubation at 37˚C, the monolayers were scratched, creating a cell-free slit, using a sterile micropipette tip at an angle of about 90 degrees to keep the slit width limited. To determine the proper slot size, three different sizes of micropipette tips (1000, 200 and 10 μl) were used. Wells were washed twice to remove cells in the supernatant. After this process, the selected extract at the concentration of IC_{50} was added to the wells and incubated for 72 h.

The groove area was photographed using an inverted microscope (Nikon Eclipse Ts2) before adding the extracts (time 0), as well as after 24, 48 and 72 h of treatment, using an objective that allows visualization of both edges of the wound with 40× magnification. Reference points were made by drawing a straight line, with an ultra-fine tipped marker pen, on the outer bottom of the plate wells. The reference mark was placed outside the capture imaging field but within the microscope field of view. Two areas of the cleft, above and below the guideline, were measured in each well between 0 and 72 h of treatment using Image J software (Software 1.48q, Rayne Rasband National Institutes of Health, US; http://rsb.info.nih.gov/ij/). Slot closure (%) was quantified using the percent change in the normalized measurement area divided by the original open area according to: % slot closure= $[A(0) - A(t)/A(0)] \times 100$ where the area at time zero (0) and the area after the incubation time (t) were used to calculate the percentage of slot closure [20].

2.9. Apoptosis, ROS and Cell Membrane Integrity Analysis by Flow Cytometry

Apoptosis events were assayed by the Human Annexin V-FITC/PI apoptosis Kit (Sigma-Aldrich, MO, USA), according to the manufacturer's instructions. The fraction of the cell population in different quadrants was measured using quadrant statistics with the FlowJo 10.0 software (LLC, Ashland, Ore). Generation of Reactive Oxygen Species (ROS) was analyzed by flow cytometry using DCFH-DA. Cells were treated with PSC-hex for 72 h, suspended in PBS and incubated with 10 μM DCFH-DA at 37˚C for 30 min. Fluorescence generation due to the hydrolysis of DCFH-DA to dichlorodihydrofluorescein (DCFH) by non-specific cellular esterases, and the subsequent oxidation of DCFH by peroxides was measured by means of flow cytometry (BD FACScalibur, San Jose, California). The uptake of the cationic fluorescent dye 3,3'-dihexyloxacarbocyanine iodide

(DiOC6(3)) (2 μL of 2 μmol/L stock solution in dimethyl sulfoxide [DMSO]) was used for the evaluation of mitochondrial membrane potential [21]. Cell treatment was performed as in ROS experiments. Untreated controls and treated cells were harvested and washed twice with PBS. The cell pellets were then re-suspended in 2 mL of fresh incubation medium containing DiOC6 and incubated at 37˚C in a thermostatic bath for 30 min. HTC-116 cells were separated by centrifugation, washed twice with PBS, and analyzed by flow cytometry using FL1 channel (488/533 nm) (BD FACScalibur, San Jose, Califórnia). Three independent experiments were performed in duplicate and the results were expressed as mean values ± standard deviation (SD). P values < 0.05 were considered as statistically significant.

2.10. Statistical Analysis

The results were expressed as means ± SD. Statistical analysis was performed using the Statistical Package for the Social Sciences - SPSS version 20.0. Normality tests (Shapiro-Wilk) and one-way ANOVA analysis of variance were performed, with Tukey's multiple post-hoc comparisons test. The significance of the statistical difference was considered for values of $p < 0.05$.

3. Results

3.1. Chemical Characterization of EXTRACTs through HPLC

Three extracts were obtained from the macroalgae *Palmaria decipiens*: MET (Methanol), CLO (Chloroform) and HEX (Hexane). The chemical quantification of phenolic compounds presents in *P. decipiens* extracts identified the presence of three main compounds: gallic acid, quercetin and kaempferol (**Table 1**).

3.2. Antitumor Activity

The cytotoxic activity of the extracts was tested in human colorectal cancer cells (HCT-116 cell line) using the MTT assay. The concentrations used in this assay ranged from 100 to 750 μg/mL, determined from the initial concentration of the extract, and exposed for periods of 24, 48 and 72 h, respectively. The results obtained for IC_{50} were more promising for MET (130.30 $\mu g \cdot mL^{-1}$) extracts after exposition of 72 h, as shown in **Table 2** and **Figure 1**.

Table 1. Major phenolic compound presents in the *P. decipiens* extracts by HPLC.

Seaweeds	Extract	RT (min)*	Concentration (μg/mL)	Component Name	Molecular Formula
P. decipiens	Hexane	42.36	56.56	Kaempferol	$C_{15}H_{10}O_6$
	Chloroform	42.36	2580.92	Kaempferol	$C_{15}H_{10}O_6$
		36.55	102.47	Quercetin	$C_{15}H_{10}O_7$
	Methanol	5.44	261.64	Gallic Acid	$C_7H_6O_5$
		42.36	95.52	Kaempferol	$C_{15}H_{10}O_6$

All extracts used in the study reduced cell viability after 24, 48 and 72 h exposition. However, the MET extract showed a decrease in cell survival, with the lowest IC_{50}, observed after 72 h treatment.

The cytotoxic activity of *P. decipiens* extracts was also tested on non-tumor cells (HaCaT cell line) through the MTT assay. The concentrations used in this assay ranged from 1000 to 5000 μg/mL, determined by the maximum concentration of the initial solution of the macroalgae extract *P. decipiens*, exposed for periods of 24, 48 and 72 h. The IC_{50} of the extracts presented in the non-tumor cell line are higher than for HCT-116, indicating selectivity. The IC_{50} of the MET extract in the HaCaT strain is almost 12 fold greater than in the HCT-116 strain as shown in Table 3 and Figure 2.

3.3. DPPH Analysis

The mean values of the antioxidant activity of the MET extract are shown in Table 4. The percentages of DPPH radical reduction varied from 28.66% ± 1.34%

Table 2. IC_{50} (μg·mL^{-1}) of *P. decipiens* extracts against HCT-116 cell line.

Extract	IC_{50} (Mean ± SD)		
	24 h	48 h	72 h
Hexane - HEX	1119.0 ± 3.05	383.6 ± 2.58	259.6 ± 2.41
Chloroform - CLO	576.6 ± 2.76	238.5 ± 2.38	141.4 ± 2.015
Methanol - MET	521.2 ± 2.71	242.5 ± 2.38	130.3 ± 2.11

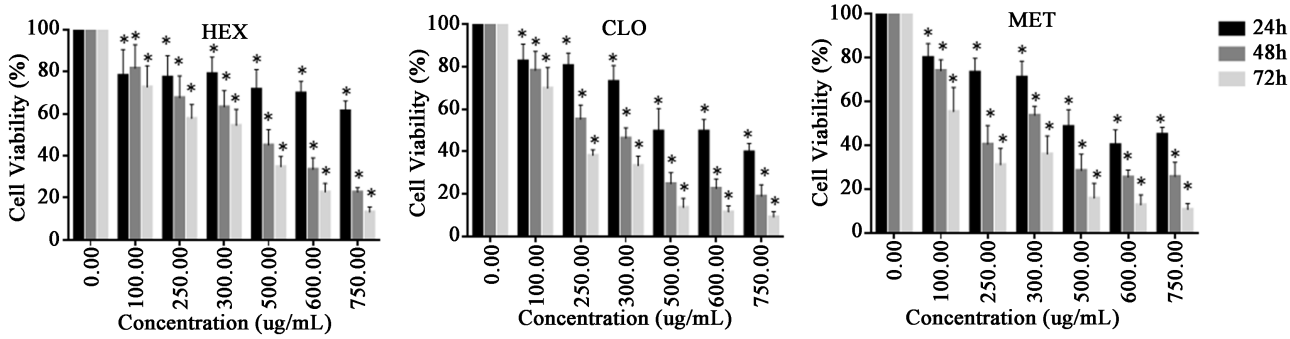

Figure 1. Cell viability (%) of the HCT-116 colorectal cancer tumor line, exposed to three different extracts (μg·mL^{-1}) of the alga *P. decipiens* (HEX, CLO and MET). Anova followed by Tukey's test expresses values as means ± SD and statistical analyzes (*p < 0.05 compared to control in the corresponding analysis of time).

Table 3. IC_{50} (μg·mL^{-1}) extracts of *P.decipiens* seaweeds against HaCaT cell line.

Extract	IC_{50} (Mean ± SD)		
	24 h	48 h	72 h
Hexane - HEX	2177.0 ± 3.34	1592.0 ± 3.20	1348.0 ± 3.13
Chloroform - CLO	1798.0 ± 3.26	1224.0 ± 3.09	1339.0 ± 3.13
Methanol - MET	3075.0 ± 3.49	1764.0 ± 3.25	1457.0 ± 3.18

in the macroalgae *P. decipiens*. The dilution of the extract at a concentration of 1.25 mg·mL^{-1} showed the most satisfactory results.

3.4. Cellular Morphology and Apoptotic Cell Identification

To assess whether the cells undergoes morphological changes after 72 h extract exposition, cells were stained with Giemsa solution and observed under an inverted microscope. As shown in Figure 3, treatment with MET extract changed the morphology and number of adhered cells. Cells clustered into colonies after exposure to the extract at the IC_{50} concentration. Cells in the control group maintained regular appearance and intense growth, characteristic of the morphology of the cell line.

The results evidenced by Giemsa staining suggest changes in the morphology of cells exposed to the MET extract. To confirm whether cells were induced to cell death events, a dual AO/EB staining was processed, followed by tests of cell

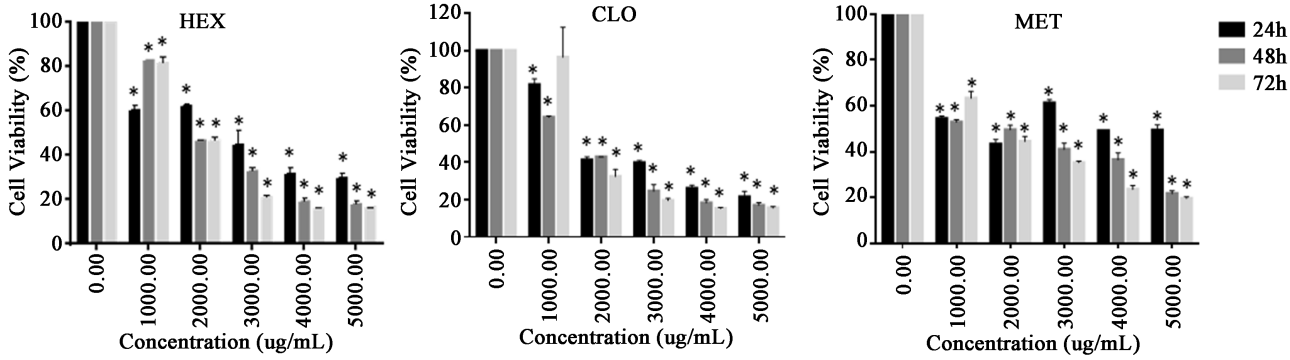

Figure 2. Cell viability (%) of the HaCaT non-tumor cell line, exposed to three different extracts (μg·mL^{-1}) of the alga *P. decipiens* (HEX, CLO and MET). Anova followed by Tukey's test expresses values as means ± SD and statistical analyzes (*p < 0.05 compared to control in the corresponding analysis of time).

Table 4. Antioxidant Activity (%) of MET extract.

Sample	Concentration (mg.mL-1)/Mean ± SD		
	0.6	1.25	2.5
P. decipiens (MET)	28.66 ± 0.57	31 ± 0	29.66 ± 1.15

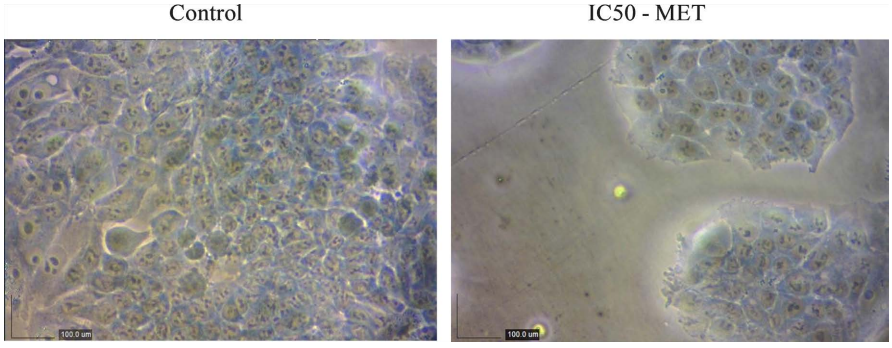

Figure 3. Morphological changes of the HCT-116 cell line after 72 hours of treatment with the *P. decipiens* MET extract.

migration, Annexin-PI, ROS and cell membrane integrity.

Changes in HCT-116 cell death pattern after metanolic extract exposition is observed with dual AO/EB staining (Figure 4), in which acridine orange (AO) penetrates only in normal cells and early apoptosis events, emitting green fluorescence. Ethidium bromide targets cells with severe membrane damage, such as cells undergoing late apoptosis and necrosis, emitting reddish-orange fluorescence.

3.5. Migration Test

For the evaluation of the migration test, cells were treated for 72 h with metanolic extract and photographed every 24 h. As seen in Figure 5, treatment with IC_{50} MET extract of the alga *P. decipiens* influenced cell proliferation and migration.

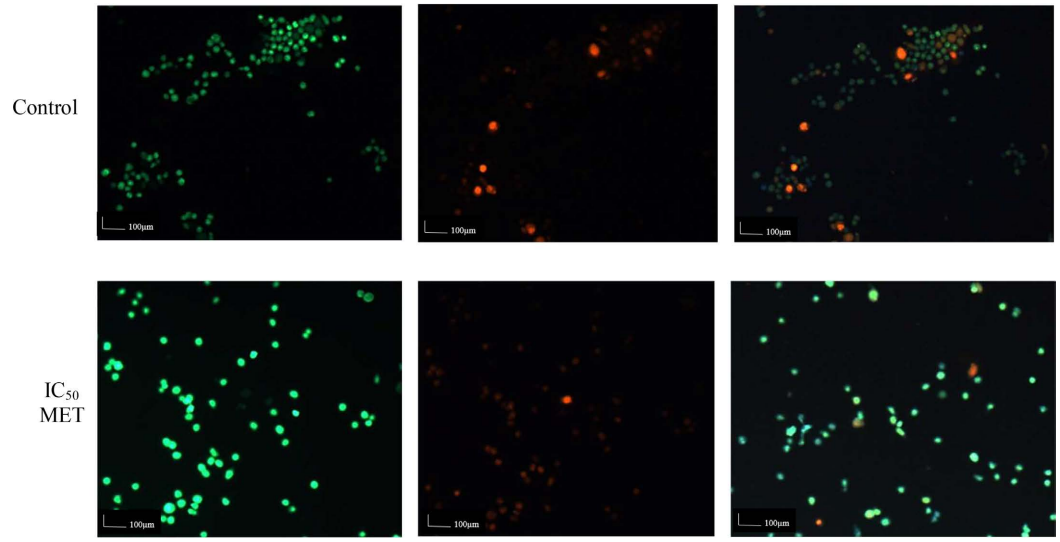

Figure 4. Morphological changes and cell death events after seaweeds extracts treatment stained with AO/EB in HCT-116. The images were taken under fluorescence microscopy at 100×. The third column in the right side refers to the merge images from acridine orange (green fluorescence in the left column) and ethidium bromide (reddish-orange fluorescence in the middle column).

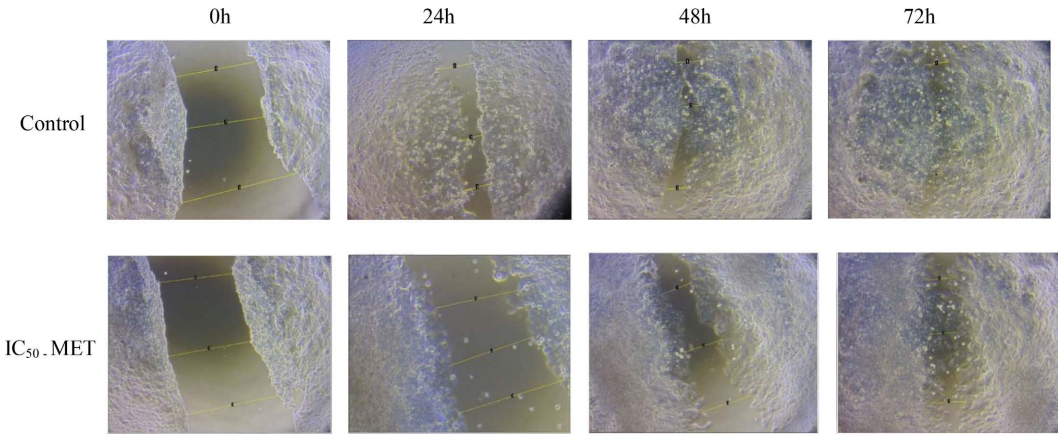

Figure 5. *In vitro* migration assay of HCT-116 cell line after treatment with the *P. decipiens* MET extract.

Length averages between the migration test margins are shown in Figure 5. Groove closure was decelerated in HCT-116 cells exposed to MET extract compared to control (Figure 6). The percentage of closure of the groove corroborates other information, being 89.69% of closure in the control and only 58.77% of closure in cells exposed to the MET extract of *P. decipiens*, indicating inhibition of proliferation and delay in cell migration.

3.6. ROS and Mitochondrial Membrane Potential Integrity Analysis by Flow Cytometry

Flow cytometry results indicated that at the IC_{50} concentration of the MET extract, the cells had already undergone a process of necrosis in more than 40% of the cells exposed to the treatment (Figure 7). The percentage of cells stained only with Annexin-V and double stained with Annexin-V and PI, which indicates necrosis events, as expressed in each quadrant. The penetration of propidium iodide stained events shows damage to the membrane and its ability to intercalate with DNA. The exposure time of HCT-116 cells for 72 h to the MET extract may be associated with the result obtained.

The analysis of intracellular Reactive Oxygen Species (ROS) and Cell Membrane Integrity was evaluated using the flow cytometry technique (Figure 8). ROS was performed with HCT 116 cells incubated for 72 h in the presence of MET extract of *P. decipiens* at the concentration of IC_{50}, and showed that there was increased fluorescence, which indicates accumulation of ROS. This result indicates a direct relationship between mitochondrial dysfunction and necrotic events observed in previous analyses.

Loss of mitochondrial membrane potential is a signal of stress inside the cell and may result in the release of apoptotic factors leading to cell death. The integrity of mitochondrial membrane potential was assessed using the flow cytometry technique, where cells were treated with MET extract of *P. decipiens* at IC_{50} concentration and incubated for 72 h (Figure 8). The observed results indicate that there was a loss of mitochondrial membrane potential integrity, inducing

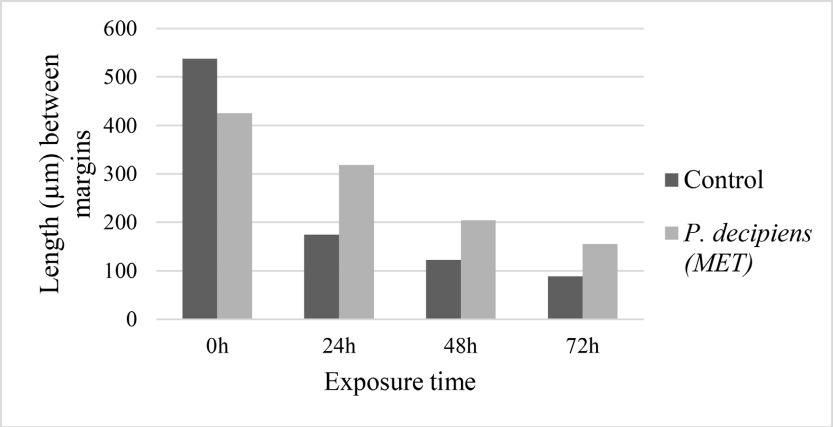

Figure 6. Migration (µm) of HCT-116 cells after different time exposure to MET of *P. decipiens*.

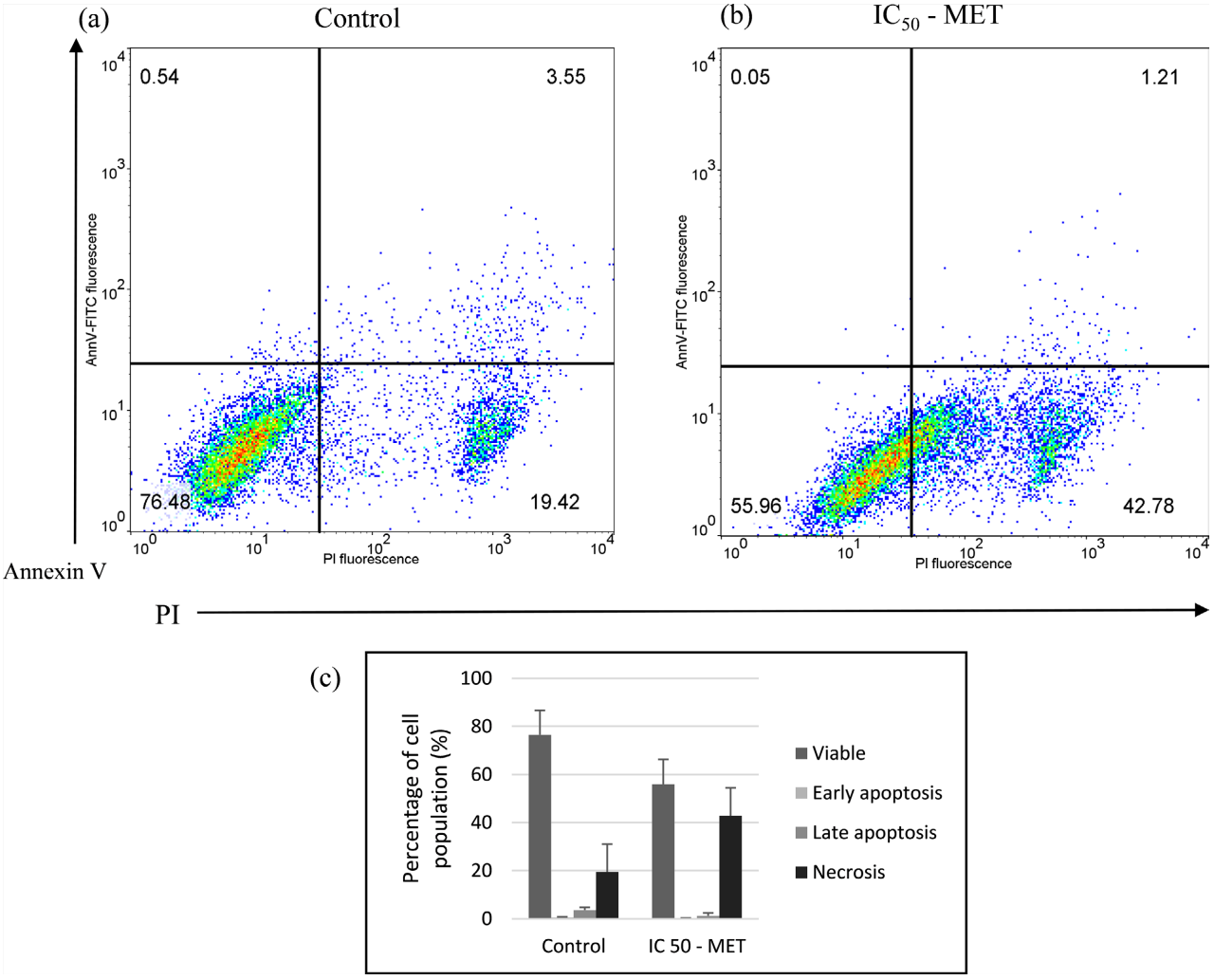

Figure 7. Annexin V/Propidium Iodide stain. (a) Control, HTC-116 cells without *P. decipiens* extract, incubated for 72 h; (b) IC_{50} MET, HCT-116 cells exposed to MET extract of *P. decipiens*, incubated for 72 h; (c) Percentage of cell population per sample. Results expressed as mean ± SD of three to six independent experiments performed in duplicate. * Statistically significant values ($p < 0.05$) different when compared to white.

cell death. These events may be associated with the time of exposure of cells to the MET extract of the macroalgae *P. decipiens*.

4. Discussion

4.1. Chemical Characterization of Extracts through HPLC

Red macroalgae are rich in phenolic compounds and their composition and applicability have been studied due to the reaction of these components with various proteins [22] [23]. Phenolic compounds is special are widely used in pharmaceuticals and other consumer products, as they have several biological properties in combating free radicals given their antioxidant activity, acting also as metal chelators, and modulating the activity of some enzymes [24] [25]. Some of their antimicrobial activity are related to the weak organic acids, and also due to their partially lipophilic nature that allows them to pass through the lipid bilayer

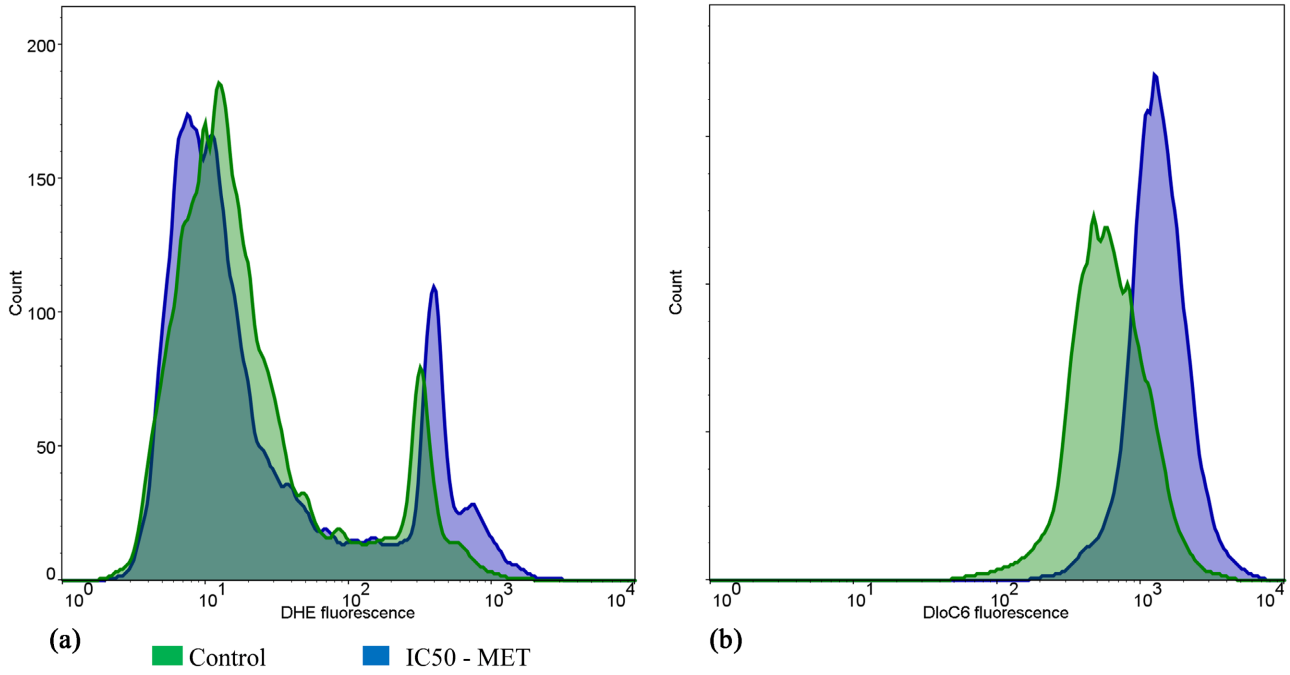

Figure 8. Flow cytometry evaluation. (a) Intracellular ROS in HCT-116 cells after exposure of *P. decipiens* MET extract at IC_{50} concentration. (b) Mitochondrial membrane potential of HCT-116 cells after treatment with MET extract of *P. decipiens* at IC_{50} concentration, indicating a loos of potential. Results are mean ± SD from three to six independent experiments performed in duplicate.

of the cell membrane, disrupting the cell structure and acidifying the cytoplasm [26]. Bernardi *et al.* (2016) reported a high content of phenolic compounds in two species of Antarctic macroalgae *P. rosulatum* and *U. hookeriana*. The authors have associated with the antioxidant potential found in extracts, probably as a metabolite related to the environmental adversities to which these species are exposed [16].

Studies have shown that phenolic compounds such as gallic acid has significant biological properties, directly affecting the proliferation and replication of tumor cells, acting as antimutagenic and antioxidant agents, being considered effective against cancer cells [26] [27] [28]. Another phenolic derivate is quercetin, considered an inhibitor of the development of different types of cancer, since this flavonoid can affect cell proliferation, cell cycle regulation and usually participate in multiple signaling pathways [26] [29] [30]. A third flavonoid identified in this study is kaempferol, that has also been reported to participate in biological properties such as anti-inflammatory, antioxidant and antimutagenic, reducing the risk of cancer and other diseases [25] [31] [32].

Cardozo *et al.* (2011) quantified phenolic compounds in three species of red macroalgae, *G. birdiae*, *G. domingensis* and *G. tenuistipitata*, and reported that there was greater detection efficiency of the compounds in the use of methanol solvent, when compared to other solvents at different concentrations [33]. Phenolic compounds was reported to decrease the amount of cellular protein and the mitotic index, while some flavonoids can modify hormone production and

inhibit aromatase to contain the progress of malignant cells [23] [34].

4.2. Antitumor Activity

Due to the extreme climatic conditions of the original habitat of Antarctic macroalgae, the organisms develop unique defense mechanisms to survive in these environments. Alves *et al.* (2016) [35] indicates that marine organisms develop secondary metabolites with therapeutic potential, as a form of protection against environmental adversities. The author tested methanolic extracts of the red algae *Asparagopsis armata* and *Sphaerococcus* and showed high cytotoxicity in HepG-2 cells, inducing a decrease in cell viability by more than 80%. Among all the algae tested in the study, *Asparagopsis armata*, *Fucus spiralis*, *Plocamium cartilagineum* and *Sphaerococcus coronopifolius* showed the highest cytotoxic potential and antiproliferative activities. The authors also report that the use of the dose-response assay is essential to define the effects and evaluate the cytotoxicity of macroalgae extracts on human cancer cells**.** Other studies with red macroalgae *I. cordata* and ethyl acetate extract proved to be cytotoxic against the tested cancer cell line. In this same study Martins *et al* (2018) [36] pointed that the tested algae extracts were not cytotoxic against the non-cancerous cell line. *I. cordata* extracts were evaluated in a study of Frassini *et al.* (2019) [13], showing cytotoxic potential of macroalgae in the HCT-116 lineage, with hexane (IC_{50} = 61.06 ± 1.79 μg/ml) and chloroform (IC_{50} = 115.9 ± 2.1 μg/ml) extracts.

4.3. DPPH Analysis

In agreement with the results observed in the study, it is suggested that the potential for antioxidant activity may be associated with the geographic location of greater occurrence of the *P. decipens* species analyzed and not necessarily with the order or taxonomic group to which the species belongs. Studies indicate that the antioxidant potential may be a strategy for the body's adaptation to the high ultraviolet radiation to which they are exposed in the algae habitat [16]. Mamani *et al.* (2020) [37] evaluated the antioxidant activity of the macroalgae *Solieria filiformis*, indicating that the methanol extract presented antioxidant potential (84.65% ± 1.56%) through the DPPH method. The study reports that antioxidant activity of macroalgae is related to their content of phenolic compounds.

4.4. Cellular Morphology and Apoptotic Cell Identification

Algae have established themselves as a potential source of bioactive compounds with nutritional value and potential therapeutic activities [11] [38]. The versatility of biological activities expressed by Antarctic macroalgae has already been proven through scientific studies, including its antitumor activity [36].

For decades, researchers from different parts of the world have studied the potential for antitumor activity in marine algae. Due to the extreme climatic conditions of these macroalgae's original habitat, organisms develop unique defense mechanisms to survive in these environments. A study indicates that ma-

rine organisms develop secondary metabolites with therapeutic potential, as a form of protection against environmental adversities [35].

The biochemical pathways of metabolism produce reactive oxygen species (ROS) as intermediaries between free radicals. Due to their abundance in the body these species are no longer able to neutralize or detoxify, impacting oxidative stress [39]. Deficiency in cellular oxidative repairs such as protein denaturation, lipid peroxidation and/or DNA conjugation is related to a series of diseases such as cancer, diabetes, cardiovascular diseases, neurodegenerative diseases, among others. Antioxidants are compounds that interrupt the cellular oxidation process by eliminating radicals and, therefore, preventing diseases [23] [40]. Al Monla *et al.* (2020) [41] identified a significant increase in the level of ROS in a concentration-dependent manner used in the treatment, the fluorescence intensity in treated cells was significantly increased compared to the respective untreated HCT-116 cells. The effects of ROS on mitochondria induce a sequence of events that include hyperpolarization of the mitochondrial membrane, oxidative explosion, followed by potential membrane breakdown and mitochondrial fragmentation [42]. The results presented in this study point out an imbalance in the redox metabolism and also a loss of mitochondrial membrane potential integrity, most likely inducing cell death mechanisms. These events could be observed by penetration of propidium iodide in the AO/BE assay, revealing membrane damage and its ability to intercalate with DNA. This technique allows the quantification of viable cells in the sample, as well as cells in apoptosis and necrosis [43].

Characterized by a variety of morphological changes, apoptosis is a sequential order of cell death that occurs regularly to ensure a homeostatic balance between the rate of cell formation and cell death [44]. One of the first events is the alteration of the plasma membrane, with translocation of phosphatidylserine (PS) found inside the cytoplasmic membrane to the extracellular surface of the membrane, promoting signaling to macrophages to phagocytose the injured cell [45].

Cell migration assay was performed and showed that groove closure was decelerated in HCT-116 cells exposed to MET extract compared to control. It is known that aberrant regulation of this process drives the progression of many diseases, including cancer invasion and metastasis [20]. Al Monla *et al* (2020) observed reduced migration of HCT-116 cells exposed to methanolic extract obtained from *C. sinuosa*, indicating a significant reduction in groove closure rates in a dose- and time-dependent manner [41].

5. Conclusion

As observed in the results, three extracts were prepared using different solvents from the Antarctic macroalgae *P. decipiens*. The evaluation of phenolic compounds identified the presence of gallic acid, quercetin and kaempferol present in the analyzed extracts. The cytotoxicity of the extracts was analyzed against HCT-116 colorectal cancer tumor cells, with the MET extract showing the most

significant results, inhibiting the growth and migration of cells, and inducing death mechanisms. These results are in agreement with other findings in the literature, indicating that the Antarctic macroalgae *P. decipiens* may have potential antioxidant and antiproliferative properties on the colorectal cancer cell line HCT-116. However, more studies are needed to determine the chemical composition by complementary methods, in addition to evaluating the antimicrobial and antitumor activity in order to determine the applicability of the extract.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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