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Chemical Characterization and Cytotoxic Activity of Antarctic Macroalgae Extracts against Colorectal Cancer

Rafaele Frassini[1], Yeslei Paulino da Silva[1], Sidnei Moura[2], Leonardo Zambotti Villela[3], Aline Paternostro Martins[3], Pio Colepicolo[3], Mutue Toyota Fujii[4], Nair S. Yokoya[4], Cláudio Martin Pereira de Pereira[5], Vagner Reinaldo Zingalli Bueno Pereira[6], João Antonio Pêgas Henriques[1], Mariana Roesch-Ely[1*]

[1]Laboratory of Genomics, Proteomics and DNA Repair, University of Caxias do Sul, Caxias do Sul, RS, Brazil
[2]Laboratory of Biosynthetic and Natural Products of Caxias do Sul, Caxias do Sul, RS, Brazil
[3]Laboratory of Biochemistry and Molecular Biology of Algae, University of São Paulo, São Paulo, SP, Brazil
[4]Phycologycal Research, Institute of Botany, São Paulo, SP, Brazil
[5]Laboratory of Lipidomics and Biorganic—LLipidomicaBio, Federal University of Pelotas, Pelotas, RS, Brazil
[6]General Hospital, University of Caxias do Sul, Caxias do Sul, RS, Brazil
Email: *mrely@ucs.br

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Abstract

Background/Aim: Antarctic seaweeds are considered a promising source of compounds with anticancer activity. Colorectal cancer (CRC) is one of the most incident cancers with high mortality rates worldwide. This work aimed to characterize chemically extracts of the Antarctic macroalgae *Iridaea cordata*, *Cystosphaera jacquinotii* and *Desmarestia anceps* and to evaluate the cytotoxic effects against human colon cancer HCT 116 cell line. Materials and Methods: The extracts were obtained by depletion using an ultrasound probe and were identified by High-Performance Liquid Chromatography (HPLC) and Gas Chromatography coupled with Mass Spectrometry (GC-MS). Cell viability was determined by MTT assay. Results: Hexanic and chloroform extracts of the *I. cordata* and the hexanic, chloroform and methanolic extracts of *D. anceps* were able to inhibit growth of colorectal cancer cells in the three different incubation times (24, 48 and 72 h). Through GC analysis, 01 compounds were identified in the hexane extract and 02 compounds in the chloroform extract of the algae *I. cordata*. The hexane extract of *D. anceps* macroalgae presented 5 compounds, chloroform extract 10 and methanolic extract 3 respectively, with special highlight to fucosterol. Carotenoid analysis by HPLC identified *β*-carotene in all species, while zeaxanthin was present in the spectrum of *I. cordata* and *C. jacquinotii*. Fucoxanthin and violaxanthin were confirmed in the brown seaweeds C. *jacquinotii* and *D. anceps*. Conclu-

sion: Extracts of macroalgae *I. Cordata* and *D. anceps* may be a source of therapeutic agents against CRC.

Keywords

Antarctic Seaweeds, Antitumor Activity, Colorectal Cancer

1. Introduction

Colorectal cancer (CRC) prevalence is one of the major public health problems worldwide due to its high incidence and mortality. CRC is the third most common cancer among men, and the second among women, accounting for about 1.4 million new cases and almost 700 thousand deaths in 2012 [1]. The most recent estimate of Brazilian National Cancer Institute [2] for 2018/2019 is that CRC will affect 17,380 men and 18,890 women, consisting of the third and second causes of cancer deaths respectively. CRC incidence distribution varies across Brazil's regions. A high incidence is reported in the most developed economically and industrialized regions, such as Southeast and South. These may be correlated with a western way of life as obesity, smoking, sedentary lifestyle, high consumption of red meat and processed foods, low vegetable intake, and aging of the population, similar to developed countries [2] [3]. Regardless the advances in therapeutic interventions over the last decades, mortality rates caused by CRC remain around 40%, mainly due to liver metastases in advanced stages of the disease [4]. Therefore, research on new natural compounds with anticancer properties and reduced side effects have become important in providing alternatives for the CRC treatment [5].

The oceans cover more than 70% of the earth's surface and contain an extraordinary diversity of life, with an enormous resource for potential therapeutic agents. Algae are adapted to a hostile environment, developing secondary metabolites as a defense and adaptation strategies. The anticancer potential activity of many compounds extracted from macroalgae, which provides important chemical scaffolds for the discovery of new drugs for the management of CRC has been recently reported [6] [7]. Previous studies have shown that algae-extracted compounds, such as fucoxanthin and sulfated polysaccharides (fucoidan), have antitumor activity against colorectal cancer cell lines. In addition, fucoidan had a protective effect against chemotherapy toxicity when used concomitantly with colorectal cancer therapy [8] [9]. Fucoidan reduced fatigue, allowing patients to have extended treatment time, which is crucial for chemotherapy effectiveness [10].

Among the thousands of macroalgae species, Antarctic seaweeds survive in one of the most hostile environment in the planet, being exposed to solar radiation in the summer, low luminosity and photosynthetic activity in the winter, ice melting and herbivory. Because they have adapted to the polar environment, these algae may be considered a promising source of new bioactive compounds,

including those with anticancer activity [7]. Therefore, the objective of this study was to chemically characterize different extracts obtained from Antarctic seaweeds and to evaluate the antitumor activity of these extracts in order to isolate new molecules with potential anticancer activity in the future.

2. Materials and Methods

Seaweed Sample and Preparation of Extracts: The red seaweed *Iridaea cordata* (Turner) Bory de Saint-Vincent and brown seaweeds *Cystosphaera jacquinotii* (Montagne) Skottsberg and *Desmaretia anceps* Montagne were collected during the Brazilian Antarctic Operation XXXIII (**Table 1**). Macroalgae fixed to the substrate were collected from the intertidal region during periods of low tide. The seaweeds were washed with seawater to remove epiphytes and frozen at −20˚C. Species identification and the classification were performed according to specimen morphology [11] and deposited in the Herbarium of Botanical Institute of São Paulo (SP), Brazil. The frash seaweeds were lyophilized and tritured with liquid nitrogen to obtain a uniform sample. The extracts were obtained by depletion, in which 10 g of each algal sample was sequentially soaked with 50 mL of hexane, chloroform and methanol, using ultrasound probe (VC 750, Sonics and Materials Inc., Newtown, USA) at a constant frequency of 20 kHz and 20% amplitude for 30 minutes. The temperature was monitored and did not exceed 18˚C. The extration was repeated three times. After, the extracts were vacuum filtered through 0.45 μm membrane and the solvent was rotoevaporated (Büchi R-300 Labortechnik AG, Flawil, Switzerland). Three extracts (hexane, choroform and methanol) were obtained for each algae.

Cell Culture and Cytotoxic Assay: HCT 116 (colorectal cancer cell line) was purchased from American Type Culture Collection (ATCC-CCL 247), Manassas, VA, USA. 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. Cytotoxic analysis was assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method according to established protocols [12].

Chemical Analysis of Extracts by Gas Chromatography Coupled with Mass (GC-MS): The extracts were analyzed by GC-MS (QP2010, Shimadzu, Kyoto, Japan) with a 30 m fused silica capillary column (HP-5MS with 0.25 μm film, Agilent). A sample (1 μL) was injected at temperature of 220˚C and with split of 1:10. Helium was used as the carrier gas at a flow rate of 1 $mL{\cdot}min^{-1}$ with the following temperature ramp: initial temperature of 60˚C with a of 5˚C per min ramp up to 260˚C, which was maintained for 10 min. Peak identification of crude algal extracts was performed based on comparing the obtained mass spectra with those available in NIST 08 and Wiley 9 libraries.

Carotenoids Analysis: The analysis of the carotenoids profile of the macroalgae was determined as described in literature [13]. Briefly, chromatographic separations were carried out by High-Performance Liquid Chromatography (HPLC, Shimadzu system) equipped with two LC-10AD pumps, a SIL-10AD *VP* automatic

Table 1. Collection data from Antarctic Macroalgae.

Seaweed	Date	Collecttion Site	Collection Data
I. cordata	13/01/2015	Greenwich Island	62˚26'40"S e 59˚43'29.8"W
D. anceps	10/01/2015	Snow Island	62˚44'07"S e 61˚13'45"W
C. Jacquinotti	08/01/2015	King George Island (Demay)	62˚12.1'15.4"S e 58˚26'28.6"W

sample injector, a DGU-14A degasser, and a SPD-M10A *VP* photodiode-array detector). Chromatographic separations were carried out on a C30 column (Ultracarb, 250 × 4.6 mm, 5 mm, Phenomenex) at 1.0 mL·min^{-1} at room temperature, using as mobile phase: (A) MeOH:H_2O:NH4Ac buffer 1 mol·L^{-1} (pH 4.6) (90:8:2) and (B) MeOH:MTBE:NH4Ac buffer 1 mol·L^{-1} (pH 4.6) (30:68:2). The gradient elution was performed as follows: a linear increase from 5% to 10% of solvent B (0 - 15 min); maintaining 10% B for 10 min; a linear gradient (10 min) to 15% B followed by another linear gradient (5 min) to 40% B and then an increase to 45% of solvent B in 2 min, an isocratic elution for 20 min and an increase to 100% B in 1 min and maintaining 100% B for 5 min, for a total run time of 68 min. The injection volume of standards and samples was 50 µL and all ultraviolet-visible spectra were recorded from 200 to 800 nm. Fourteen standards were used. The lyophilized powder of seaweeds were weighed, dissolved in methanol: acetone (1:1, v/v) and sonicated for 15 min. The extracts were then centrifuged and filtered through a 0.45 µm membrane (Millex HN nylon, 13 mm, Millipore). Aliquots (50 µL) of each extract (3 mg·mL^{-1}) were injected into the HPLC system.

Statistical Analysis: The results were expressed as the means ± SD. Statistical analysis was performed using SPSS version 20.0 and GaphPad Prism version 5.0. Normality tests (Shapiro-Wilk), Student's t-test for independent samples, and one-way analysis of variance ANOVA, with Tukey's post hoc multiple comparisons test were performed. The significance of difference was considered to include values of $p < 0.05$.

3. Results

Antitumor Activity: Nine extracts were obtained: ICH (*I. cordata*—hexane), ICC (*I. cordata*—chloroform), ICM (*I. cordata*—methanol); CJH (*C. jacquinotii*—hexane), CJC *(C. jacquinotti*—chloroform), CJM (*C. jacquinotti*—methanol); DAH (*D. anceps*—hexane), DAC *(D. anceps*—chloroform), DAM (*D. anceps*—methanol). The cytotoxic activity of extracts was tested against human cancer colon carcinoma (HCT 116 cell line) by MTT assay. The maximum concentration tested varied according to the solubility of each extract. The concentration that inhibited 50% of cell growth (IC_{50}) is shown in Table 2.

The extracts obtained from *C. jacquinotii* (CJH, CJC and CJM) and methanolic extract of *I. cordata* (ICM) were not cytotoxic against HCT 116 within the conditions tested. The hexane (ICH) and chlroformic (ICC) extracts of *I. cordata* and all extracts of *D. anceps* (DAH, DAC and DAM) inhibited the growth of the

colorectal cancer cell line with dose-dependent manner ($p < 0.05$). Figure 1 and Figure 2 show the cytotoxic profile of Antarctic seaweeds extracts.

GC-MS Analysis: Analysis of the extracts by gas chromatography was performed only for those who inhibited the growth of the HCT 116 cell line. One major compound was identified in the hexane extract and two in the chloroform extract. Both bioactive extracts of *I. coradata* (ICH and ICC) showed the compound cholest-5-en-3ß-ol (ephicolesterol). The chloroform extract presented two peaks of another major compound, known as octadecanoic acid. In the extracts of *D. anceps*, a greater number of compounds were identified (Table 3). Among them, hexadecanoic acid, identified in all extracts, fucosterol present in two extracts (DAH and DAC), and phytol (DAH) had biological activities reported.

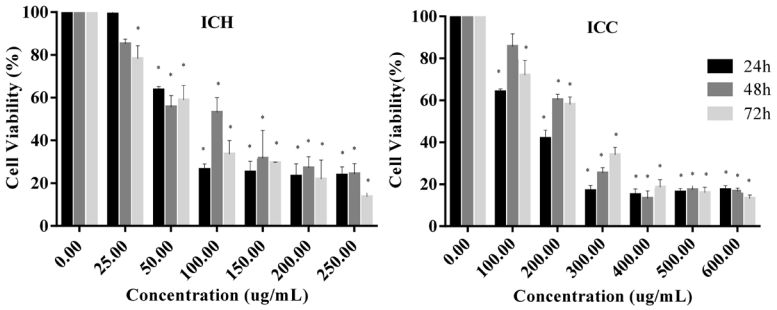

Figure 1. Cytotoxic profile of antartic seaweed *I. cordata* (ICH and ICC) extracts against HCT 116 cell line after 24, 48 and 72 h of exposure (*$p < 0.05$ compared to control in time corresponding analysis).

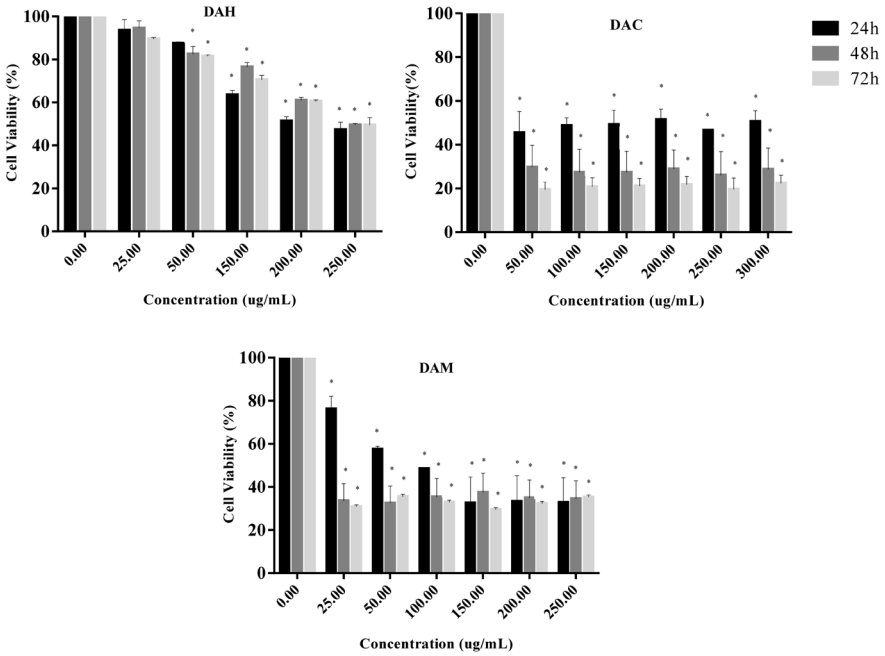

Figure 2. Cytotoxic profile of antartic seaweed *D. anceps* (DAH, DAC and DAM) extracts against HCT 116 cell line after 24, 48 and 72 h of exposure (*$p < 0.05$ compared to control in time corresponding analysis).

Table 2. IC50 (μg·mL^{-1}) extracts of antartic seaweeds against HCT 116 cancer cell line.

		IC_{50} (Mean ± SD)		
Seaweed	Extract	24 h	48 h	72 h
C. Jacquinotii	Hexane	>200	>200	>200
	Chloroform	>140	>140	>140
	Methanol	>240	>240	>240
I. cordata	Hexane	70.46 ± 1.84[a]	82.5 ± 1.91[a]	61.06 ± 1.79[a]
	Chloroform	115.9 ± 2.1[a]	172.4 ± 2.4[b]	164.5 ± 2.2[b]
	Methanol	>600	>600	>600
D. anceps	Hexane	242.6 ± 2.4[a]	315.2 ± 2.5[b]	284 ± 2.45[a]
	Chloroform	125.9 ± 2.1[a]	45.83 ± 1.7[b]	27.12 ± 1.43[c]
	Methanol	87.73 ± 1.94[a]	38.69 ± 1.58[b]	34.03 ± 1.53[b]

*Different letters in each treatment correspond to statistical difference ($p < 0.05$).

Table 3. Major compound presents in the hexanic, chloroformic and methanol extracts of I. Cordata and D. anceps by GC-MS.

Seaweed	Extract	Peak n°	RT (min)*	Component Name	Molecular Formula	SI*
I. cordata	Hexane	1	78.408	Cholest-5-en-3ß-ol	$C_{27}H_{46}O$	90
	Chloroform	1	60.783	Cholest-5-en-3ß-ol	$C_{27}H_{46}O$	90
		2	66.000	Octadecanoic acid	$C_{18}H_{36}O_2$	91
		3	78.358	Cholest-5-en-3ß-ol	$C_{27}H_{46}O$	90
D. anceps	Hexane	1	44.958	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	94
		2	49.617	Phytol	$C_{20}H_{40}O$	89
		3	60.775	Hexadecanoic acid	$C_{16}H_{32}O_2$	90
		4	79.817	Alfa-tocopherol	$C_{29}H_{50}O_2$	91
		5	89.575	Fucosterol	$C_{29}H_{48}O$	92
	Chloroform	1	12.175	Phosphoric acid	$C_6H1_5PO_4$	96
		2	38.133	9-Octadecenoic acid	$C_{18}H_{34}O_2$	90
		3	45.000	n-Hexadecanoic acid	$C_{16}H3_2O_2$	95
		4	50.417	Oleic acid	$C_{18}H_{34}O_2$	91
		5	51.192	Octadecanoic acid	$C_{18}H_{36}O_2$	93
		7	60.775	Hexadecanoic acid	$C_{16}H_{32}O_2$	88
		8	65.992	Octadecanoic acid	$C_{18}H_{36}O_2$	91
		9	89.475	Fucosterol	$C_{29}H_{48}O$	90
		10	93.125	(3β)-9,19-Cyclolanost-24-en-3-yl acetate	$C_{32}H_{52}O_2$	78
	Methanol	1	16.308	2-Phenoxyethanol	$C_8H_{10}O_2$	92
		2	44.917	Heptadecanoic acid	$C_{17}H_{34}O_2$	89
		3	60.767	Hexadecanoic acid	$C_{17}H_{34}O_2$	91

*SI = Similarity Index; *RT = Retention Time.

Carotenoids Profile: Two peaks were identified in the chromatogram of the red algae *I. cordata*: zeaxanthin and *β*-carotene. For brown seaweed *D. anceps*, four peaks were indetified: fucoxanthin, violaxanthin, zeaxanthin and *β*-carotene fucoxanthin. For brown algae *C. jacquinotii*, three peaks were identified: fucoxanthin, violaxanthin and *β*-carotene.

4. Discussion

Colorectal carcinoma has become a worldwide public health problem due to its high incidence and mortality. This type of cancer is the third most prevalent and represents the largest cause of non-smoking cancer deaths. The increase in incidence in recent years in developing countries such as Brazil has been observed [14].

Despite advances in diagnosis and treatment, mortality rates remain high due to the lack of government investments to create programs for the early detection of disease in developing countries, such as Brazil, and intrinsic characteristics such as tumor resistance to conventional chemotherapy and side effects. Due to these factors, the search for new compounds with anticancer activity is of extreme importance [15].

Natural compounds play a very important role in the discovery of new drugs with antitumor activity. In the last three decades, the discovery of secondary metabolites with biological activity from macroalgae has grown substantially, however, of approximately 25,000 species of algae described, only 15 are used biotechnologically on a large scale [16]. Antarctic algae are adapted to one of the most adverse conditions on the planet, such as the polar environment, may be a promising source of compounds with anticancer activity.

The present study analyzed the cytotoxicity of extacts of Antarctic macroalgae *I. cordata*, *D. anceps* and C. *jacquinotti* and found that hexanic and chloroform extracts of *I. cordata* and hexane, chloroform and methanolic extracts of *D. anceps* seaweeds were cytotoxic against the HCT 116 cell line after 24, 48 and 72 h exposition in a dose-dependent manner ($p < 0.05$). All extracts of *C. jacquinotti* and methanolic extract of *I. cordata* were not cytotoxic under the experimental conditions tested. Studies with antartic algae, *C. jacquinotii*, *I. cordata*, *H. grandifolius* and *P. endiviifolia* showed the antitumor activity. *C. jacquinotii* extracts (hexane, chloroform, acethyl acetate and ethanolic) had low cytotoxicity against the A-431 cell line, with an inhibitory rate lower than 50% (500 $\mu g \cdot mL^{-1}$). The acethyl acetate extract of *I. cordata* had cytotoxic effect against the human epidermoid carcinoma cell line (A-431), inhibiting cell growth in 91.1% and 95.6%, respectively, after 24 and 48 h of exposure in the concentration of 500 $\mu g \cdot mL^{-1}$ [17]. Cytotoxicity of sulfated polysaccharides, SP1 and SP2, purified of *I. cordata* displayed significant antitumor activity at 1000 μg/mL against HeLa cells (68.4% and 30.1%) and PC-3 cells (59.8% and 56.4%) [18]. The chemical composition of the extracts varies according to the extraction methodology and the intrinsic characteristics of each organism, as well as the seasonality. Evaluating the cyto-

toxicity of extracts is important as it is the initial step for the selection of new compounds with anticancer activity [18] [19]. The present study was the first to evaluate the cytotoxicity of *D. anceps* extracts.

GC-MS analysis shows the presence of cholest-5-en-3ß-ol (epicholesterol) and octadecanoic acid in *I. cordata* extracts. Epicholeterol is an epimeric form of cholesterol present in menbranes cell and is related to cell permeability and membrane movement [20]. Octadecanoic acid or stearic acid is a fatty acid, a unique long-chain satured fatty acid (SFA), the only one that is not correlated with the risk of cardiovascular diseases, being a candidate for use in the food industry [21]. In the spectrum of *D. anceps*, peaks corresponding to compounds with described biological activity were identified, such as hexadecanoic acid, phytol and fucosterol. Hexadecanoic acid is a saturated fatty acid with anti-inflammatory activity [22]. Phytol, open chain diterpene, is with antimycobacterium activity [23]. Fucosterol, a sterol typically found in brown algae and has been attributed to it several biological activities, such as anti-inflammatory, anti-oxidant, hepatoprotective and anticancer (against HL 60 cell line) [24].

HPLC—carotenoids analysis allowed to identify the presence of four compunds β-carotene, fucoxanthin, zeaxanthin and violaxanthin. Carotenoids have many functions in the human body, including antioxidant activity (β-carotene), retinal protective effect against solar radiation damage (zeaxanthin) and reduction of serum triglyceride levels (astaxanthin) [25]. Fucoxanthin is a colored marine carotenoid present in microalgae and brown algae. Many studies have reported the anticancer activity of fucoxanthin and its derivative fucoxanthinol against several types of tumor cell lines, including colorectal carcinoma [8] [26]. Also, fucoxanthin is related to decrease of risk factors for cardiac diseases, such as obesity, diabetes, high blood pressure, chronic inflammation, plasma and hepatic triglyceride, and cholesterol concentrations and antioxidant activity. Fucoxanthin, violaxanthin and β-carotene had a photoprotective effect [26] [27].

Many studies have reported on the anticancer activity of extracts and compounds isolated from macroalgae against colorectal cancer strains, but the biological structure/activity relationship needs to be elucidated [8] [9] [10]. The present study suggests that the hexanic and chloroform extracts of *I. cordata* and the hexanic, chloroform and methanolic extracts of *D. anceps* are promising candidates for investigations, including the development of drugs to combat colorectal cancer.

5. Conclusion

The hexanic and chloroform extracts of the red seaweed *I. cordata* and the hexanic, chloroform and methanolic extracts of brown seaweed *D. anceps* inhibited the growth of the HCT 116 cell line, suggesting that these algae are a promising source of bioactive compounds with anticancer properties. Further studies to isolate and elucidate the bioactive compounds present in these extracts are required.

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Conflicts of Interest

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

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