Scientific Research Publishing

Advances in Biological Chemistry, 2022, 12, 228-245
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

Desmarestia anceps Montagne (Phaeophyceae) against Colorectal Cancer Cells: Cytotoxic Activity and Proapoptotic Effects

Rafaele Frassini[1], Daniela Steffens[1], Sidnei Moura[2], Cesar Aguzzoli[3], Aline Paternostro Martins[4], Pio Colepicolo[4], Mutue Toyota Fujii[5], Nair S. Yokoya[5], Cláudio Martin Pereira De Pereira[6], Ana Cláudia Phillipus[7], Miriam De Barcellos Falkenberg[7], João Antonio Pêgas Henriques[1], Mariana Roesch-Ely[1]*

[1]Laboratory of Applied Toxicology and Bioproducts, University of Caxias do Sul, RS, Brazil
[2]Laboratory of Biosynthetic and Natural Products of Caxias do Sul, RS, Brazil
[3]Area of Exact Sciences and Engineering, University of Caxias do Sul, RS, Brazil
[4]Laboratory of Biochemistry and Molecular Biology of Algae, University of São Paulo, São Paulo, SP, Brazil
[5]Nucleus Research Phycology, Institute of Botany, São Paulo, SP, Brazil
[6]Laboratory of Lipidomics and Biorganic-LipidomicaBio, Federal University of Pelotas, Pelotas, RS, Brazil
[7]Study Group on Natural and Synthetic Products (GEPRONAS), Federal University of Santa Catarina, SC, Brazil
Email: *mrely@ucs.br

How to cite this paper: Frassini, R., Steffens, D., Moura, S., Aguzzoli, C., Martins, A.P., Colepicolo, P., Fujii, M.T., Yokoya, N.S., De Pereira, C.M.P., Phillipus, A.C., De Barcellos Falkenberg, M., Henriques, J.A.P. and Roesch-Ely, M. (2022) *Desmarestia anceps* Montagne (Phaeophyceae) against Colorectal Cancer Cells: Cytotoxic Activity and Proapoptotic Effects. *Advances in Biological Chemistry*, **12**, 228-245.
https://doi.org/10.4236/abc.2022.126019

Received: October 5, 2022
Accepted: December 24, 2022
Published: December 27, 2022

Abstract

Impairment of apoptosis promotes abnormal cellular proliferation and accumulation of genetic alterations. Identifying bioactive compounds extracted from seaweeds that induce apoptosis in cancer cells may be explored as new agents for cancer chemoprevention and/or chemotherapy. The present study aimed to determine the chemical composition and biological activity of the chloroform crude extract and fractions from Antarctic seaweed *Desmarestia anceps.* The chloroform extract was obtained by three consecutive macerations and the fractionation by vacuum liquid chromatography. The chemical characterization of the extract and fractions was performed through Gas Chromatography. The cytotoxicity of the crude extract and fractions was evaluated by the MTT assay. Cell death and cell cycle evaluation after 24 hours of exposure to chloroform extract were performed by flow cytometry. A total of 48 compounds were identified. The results indicate that chloroform extract and its fractions presented cytotoxic activity against HCT 116 cell line in a dose dependent-manner. Proapoptotic events were observed after chloroform extract exposition, which promoted an increase of multinucleated cells and reduced cell viability. This study was the first to explore cytotoxic potential of seaweed *D. anceps* fractions against HCT colorectal cancer cell line, suggesting that these macroalgae may be a promising candidate against

anticancer activity.

Keywords

Antarctic Seaweeds, Antitumor Activity, Apoptosis, Proapoptotic Effect, Colorectal Cancer

1. Introduction

Macroalgae have been used for millennia as food sources in different countries. Its nutritional interest is based on high levels of dietary fibers, polyunsaturated fatty acids, vitamins, and minerals. Due to the great biodiversity in the oceans, marine organisms, including macroalgae, are a valuable source of minerals, as well as a reservoir of new biologically active compounds [1] [2]. Antarctic macroalgae are adapted to an extreme environment, presenting a diversity of metabolites and, therefore, being considered promising sources of compounds with anticancer activity [3] [4].

Due to side effects promoted by many cancer therapies and also tumor cell resistance to conventional drugs, the search for compounds of natural origin is still being investigated [5] [6]. Anticancer chemotherapeutic drugs are currently developed to inhibit the growth of tumor cells, but chemotherapy may fail due to the development of drug resistance promoted by apoptosis inhibition mechanisms. Apoptosis is a regulated cell death program that plays an essential role in the development and maintenance of tissue homeostasis by eliminating unnecessary or mutated cells. As regulation of apoptosis relies on multiple cell signaling mechanisms, cancer cells use number of different strategies to suppress a protective apoptotic response. Phosphatidylserine exposure on the cellular surface occurring at the initial or early apoptosis stages promotes membrane blebbing, nuclear fragmentation, decreased cellular volume and a formation of apoptotic bodies [7] [8] [9]. It can be further defined as cell death accompanied by the activation of caspase proteases [10]. Impairment of this mechanism generates abnormal cellular proliferation and the accumulation of genetic alterations that may contribute to drug resistance [11].

Identifying the type of cell death after treatment may contribute to selecting the best therapy modality. Therefore, a better understanding of the cell death signaling pathways can improve the efficacy of cancer therapy and bypass resistance [11] [12] [13]. Bioactive compounds that induce apoptosis in cancer cells can be used as agents for cancer chemoprevention and/or chemotherapy [14]. Accumulating evidence suggests that bioactive compounds extracted from algae produce anticancer effects through multiple mechanisms of action, including inhibition of cancer cell growth, invasion, and metastasis, and through the induction of apoptosis [15] [16].

Until present, there is no literature demonstrating reduction of cellular viability of colorectal cancer cells after treatment exposition to extract fractions of

macroalgae *D. anceps* and the mechanism of cell death after de exposition of crude extract. However, some isolated compounds taken from brown Antarctic macroalgae such as fucoidan and fucosterol have been investigated with promising antitumor activity [17] [18].

In this context, the aim of this study was to evaluate mechanisms of cell death induced after treatment of colorectal cancer cell line HCT 116 with chloroform crude extract of *D. anceps.* In this study, the extract was fractioned and the chemical characterization of the crude extract and its fractions were further investigated.

2. Methods

2.1. Reagents

Dulbecco's modified eagle's medium-high glucose (DMEM), trypsin-EDTA, 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheyl-tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), heat inactivated fetal bovine serum (FBS), Annexin V-FITC Apoptosis Detection Kit were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hexane, chloroform, acetone, and methanol were obtained from Synth (Brazil). All other chemicals were of ultrapure grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Seaweed Sample

The brown seaweed *Desmarestia anceps* Montagne (Phaeophyceae) was collected during the Brazilian Antarctic Operation XXXIII in the Snow Land (62˚44'07"S and 61˚13'45"W in January 2015). Macroalgae fixed to the substrate were collected from the intertidal region during periods of low tide. The seaweeds was washed with seawater to remove epiphytes, frozen at −20˚C and posteriorly lyophilized for biochemical analyzes. Species identification was performed according to specimen morphology and the classification system proposed by Wynne [19] and herbarium specimens were deposited in the Herbarium of Botanical Institute of São Paulo, SP, Brazil.

2.3. Preparation of Extract

The extract was obtained by maceration using 200 g of dry powder of *D. anceps* that was mixed with 2 L of chloroform for 24 h. The extracts from three consecutive extractions were pooled and filtered using filter paper (Whatmann No. 1). After filtration, *D. anceps* chloroform extract (DACExt) was concentrated to dryness under vacuum at 40˚C (Büchi R-300 Labortechnik AG, Flawil, Switzerland). The crude extract was kept in dark and dry conditions at −20˚C until biological testing.

2.4. Liquid Vacuum Chromatography (LVC)

DACExt was fractionated by Liquid Vacuum Chromatography (LVC) with gradients of increasing polarity using the solvents n-hexane, ethyl acetate and me-

thanol. Each fraction (100 mL) was collected in a separate flask and concentrated in a rotatory evaporator. The extracts were verified by 1H NMR (Bruker-Fourier 300) and were obtained for comparison of the chemical profile.

2.5. Gas Chromatography (GC)

The extracts and fractions were analyzed by GC (QP2010, Shimadzu, Kyoto, Japan) with a 30 cm 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 an increase of 5˚C per min up to 260˚C, which was maintained for 10 min. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

2.6. Cell Culture and Cytotoxic Assay

HCT 116 (colorectal cancer cell line) was purchased from American Type Culture Collection (ATCC - CCL247), Manassas, VA, USA. Cells were cultured in DMEM supplemented with 10% heat inactivated FBS and maintained in a humidified atmosphere at 37˚C, in 5% CO_2 and 95% air. The cytotoxicity study was performed when the cells reached 70% - 80% confluence. Cytotoxicity 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 DACExt extract for 24, 48 and 72 h. The fractions were treated for 24 h. DMSO 0.5% was added to medium as a negative control. MTT solution (0.4 mg/mL) was added after treatment and incubated for 2 h, followed by dimethyl sulfoxide. The optical density (OD) was measured at 570 nm and IC_{50} was calculated by GaphPad Prism version 5.0.

2.7. Morphological Analysis through SEM

For morphological evaluation, HCT 116 cell line was plated at a density of 1×10^6 cells/mL in 24-well plates over a glass coverslip. After 24 h, the cells were treated with DACext at the IC_{50} concentration and values correspondent to 2 times the concentration of IC_{50} for 24 h**.** The cells were fixed with a 3% glutaraldehyde solution in PBS (v/v) for 15 minutes and with 30%, 50%, 70%, 90% and 100% (v/v) ethanol for 10 minutes for each concentration. The samples were kept in a desiccator until the analysis. The coverslips were removed from the wells, coated with gold and examined in a SHIMADZU Superscan SS-500 scanning electron microscope (SEM).

2.8. Cell Cycle Assays and Apoptosis Detection through Annexin V and Propidium Iodine by Flow Cytometry

HCT 116 cell line (5×10^5/well) was seeded into 6 well plates culture flasks with

DMEM supplemented culture medium. CRC cells were treated with IC_{50} concentration (37.77 μg·mL^{-1}) and double (75.44 μg·mL^{-1}) of DACExt extract for 24 h. The negative control was treated with DMSO 0.5%. For Annexin V assay, the Annexin V-FITC Apoptosis Detection Kit (APOAF, Sigma, USA) was used, according to manufacturer's instruction. For cell cycle analysis, cells went through trypsinization and collected for centrifugation (1800 rpm for 5 min) in PBS. Cell pellets were fixed in ice-cold 70% ethanol and stored overnight at −20˚C. After, cells were resuspended in Triton X-100 (1% in PBS) and incubated with RNAse and Propidium Iodide in the dark for 30 min at room temperature. Cells were analyzed by flow cytometry using a BD FACSAria III flow cytometer equipped with lasers 488 and 633 nm (Becton & Dickinson LTDA). Data were collected by FACSDiva software (BD Biosciences) and analyzed using FlowJo (TreeStar, Inc.).

2.9. 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 (Kolmogorov-Smirnov and Shapiro-Wilk), 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

3.1. Yield and Analysis of Fractions by 1H NMR

DACExt extract was obtained and fractionated by VLC as mentioned in item 2.4. In total 13 fractions were obtained, and their yield is shown in Table 1. Fractions

Table 1. Yield of fractions of DACExt.

Fractions	Solvents	Yield (mg)	Yield (%)
1	Hexane	39	10.8
2	Hexane:Ethyl acetate (9:1)	78.6	21.77
3	Hexane:Ethyl acetate (8:2)	106,2	29.42
4	Hexane:Ethyl acetate (7:3)	114.9	31.83
5	Hexane:Ethyl acetate (6:4)	68.4	18.95
6	Hexane:Ethyl acetate (1:1)	81	22.44
7	Hexane:Ethyl acetate (4:6)	86	23.82
8	Hexane:Ethyl acetate (3:7)	125.3	34.72
9	Hexane:Ethyl acetate (2:8)	73.8	20.44
10	Hexane:Ethyl acetate (1:9)	34.5	9.55
11	Ethyl acetate	22.2	6.15
12	Ethyl acetate:Methanol (1:1)	209.3	57.99
13	Methanol	89.7	24.85

3 and 4, 9 and 10, 11 and 12 were grouped by similarity of spectra, totaling 10 fractions.

3.2. Gas Chromatography

To examine the chemical profile of DACExt, the analysis of chloroform extract and fraction was carried out using GC analysis of crude chloroform extract and fractions shown in Table 2 and Table 3, respectively. The major compounds were expressed as peak area percentage. The Fraction 1 was not chemically characterized.

Table 2. Major compounds expressed as a peak area (%) of chloroform extract of *D. anceps*.

Entry	RT (min)	Identified compound	Molecular Formula	% Peak Area	SI
1	34.680	Bicyclo [3.1.1] heptane, 2,6,6-trime this-, (1*α*, 2*β*, 5*α*)	$C_{10}H_{18}$	0.34	60
2	37.087	Fucosterol	$C_{29}H_{48}O$	2.40	95
3	37.612	6,10-methano-19-norandrost-4-ene-3,17-dione, 6-methoxy-	$C_{20}H_{26}O_3$	5.93	96
4	37.732	Androst-5,15-dien-3ol acetate	$C_{21}H_{30}O_2$	7.48	83
5	42.933	Quinoline, 3-(methylthio)	$C_{10}H_9NS$	1.07	58
6	41.996	Stigmasta 4,24 (28)-dien-3-one	$C_{29}H_{46}O$	1.47	91

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

Table 3. GC Major compounds expressed as a peak area (%) of fractions of chloroform extract fractions of *D. anceps*.

Fraction	Entry	RT (min)	Identified compound	Molecular Formula	Peak area %	SI
2	1	34.673	Bicyclo [3.1.1]heptane, 2,6,6-trimethyl	$C_{10}H_{18}$	14.45	60
	2	37.445	Phytol	$C_{20}H_{40}O$	2.08	90
	3	39.354	Hexanedioic acid, ethyl ester	$C_{18}H_{36}O_2$	8.32	95
	4	46.287	Vitamin E	$C_{29}H_{50}O_2$	16.46	98
3/4	1	36.117	n-hexadecanoic acid	$C16H_{32}O_2$	34.10	98
	2	37.643	Oleic acid	$C_{18}H_{36}O_2$	13.30	95
5	1	36.849	Fucosterol	$C_{29}H48O$	13.79	93
	2	36.901	5-androsten-17.alpha.-ethynyl-3.beta.,17.beta.-diol	$C_{25}H_{36}O_4$	22.81	94
6	1	8.112	2-pentene, 5-bromo-2,3-dimethyl-	$C_7H_{13}Br$	4.91	64
	2	11.839	2-cyclopenten-1-one, 3-methyl-	C_6H_8O	6.39	94
	3	21.858	Hexadecane	$C_{16}H_{34}$	2.10	83
	4	27.453	Pentacosane	$C_{25}H_{52}$	2.60	90
	5	32.363	Octadecane, 1-iodo-	$C_{18}H_{37}I$	2.69	90
	6	35.712	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	4.19	95
7	1	32.362	Heneicosane	$C_{21}H_{44}$	4.52	83
	2	34.672	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl	$C1_0H1_8$	20.06	46

Continued

	3	35.003	1,2-dihexylcyclopropene	$C_{15}H_{28}$	4.31	53
	4	35.226	Phytol	$C_{20}H_{40}O$	7.81	53
	5	35.712	Heptacosane	$C_{27}H_{56}$	8.66	53
8	1	34.672	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	$C_{10}H_{18}$	4.21	60
	2	35.341	6-methyl-6-(5-methyl-furan-2-yl)-hept-3-en-2-one	$C_{13}H1_8O2$	22.86	43
	3	35.712	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	6.74	95
	4	39.930	7-pentadecyne	$C_{15}H2_8$	6.77	64
	5	40.022	Hexadecanoic acid	$C1_6H_{32}O_2$	14.08	72
9/10	1	35.226	Phytol	$C_{20}H_{40}O$	2.09	64
	2	37.626	Heptacosane	$C_{27}H_{56}$	1.76	83
	3	37.935	Hexacosane	$C_{26}H_{54}$	1.45	83
	4	39.038	Octacosane	$C_{28}H5_8$	3.41	72
	5	40.535	Eicosane	$C_{20}H_{42}$	3.62	68
11/12	1	27.453	2-bromo dodecane	$C1_2H_{25}Br$	1.49	78
	2	32.363	Heptacosane	$C_{27}H_{56}$	1.86	86
	3	36.100	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	1.63	62
	4	37.449	Phytol	$C_{20}H_{40}O$	1.18	72
	5	37.632	Octadecane	$C_{18}H_{38}$	2.44	91
	6	39.078	Octacosane	$C_{28}H_{58}$	0.38	83
	7	40.027	Hexadecanoic acid	$C_{16}H_{32}O_2$	8.05	76
	8	40.273	9-(2',2'-dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorene	$C_{30}H_{42}Cl_2N_4O_3$	2.24	91
13	1	27.454	Heptacosane	C_27H_{56}	2.40	83
	2	32.364	Pentadecane, 2,6,10-trimethyl-	$C_{18}H_{38}$	3.56	86
	3	35.713	7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	11.10	99
	4	39.040	Pentacosane	$C_{25}H5_2$	1.17	72
	5	40.028	Hexadecanoic acid	$C_{16}H_{32}O_2$	19.84	58

*RT: Retention Time *SI = Similarity Index.

Altogether, considering DACExt extract and fractions, 48 compounds were identified. The literature reports the biological activity of some of these compounds. The DACExt spectra reveled seven major compounds; among them is the fucosterol, in which seven peaks were identified. In F5, two peaks corresponded to fucosterol. In F3/4, two peaks were identified as n-hexadecanoic acid. This compound was also found in the fractions F2, F8, F11/12 and F13.

3.3. Cytotoxic Assay

The DACExt crude extract and its fractions were tested against HCT 116 cell line

and were able to inhibit the growth of cells in different times of incubation (24, 48 and 72 h) (Figure 1).

Results indicate that early incubation times (24 h) are already presenting cytotoxic activity and further experiments reveled no difference in cell proliferation rates. IC_{50} varied according incubation time, 37.77 ± 1.57 μg·mL^{-1} for 24 h, 25.99 ± 1.41 μg·mL^{-1} for 48 h and 30.48 ± 1.48 μg·mL^{-1} for 72 h. The cell viability show to be concentration dependent ($p < 0.05$), but not time dependent ($p > 0.05$). All fractions were cytotoxic against colorectal cancer cell line in a dose-dependent manner ($p < 0.05$), with the exception of Fraction 1 ($IC_{50} > 350$ μg·mL^{-1}). The general IC_{50} values were higher in the fractions with higher polarity (F11/12 and F13), decreased in fractions with intermediate polarity (F5 to F10) (Table 4).

3.4. Evaluation of Cell Morphology by SEM

The morphological analysis of the cells by SEM, after 24 hours of exposure to the concentration of the IC_{50} (37.77 μg·mL^{-1}) and twice this value (75.54 μg·mL^{-1}) of

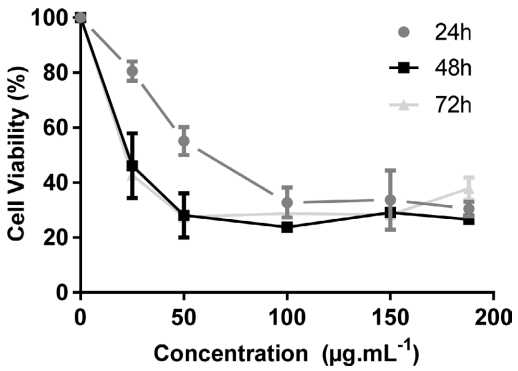

Figure 1. Cytotoxic effects of DACExt extract against HCT 116 cell line in 24, 48 and 72 h. Decrease in cell viability was reduced in lower concentrations (<50 μg·mL^{-1}) for longer incubation time (48 and 72 h).

Table 4. IC_{50} (μg·mL^{-1}) of Fractions of Chloroform Extract of *D. anceps* against HCT 116 cancer cell line.

Fractions	IC_{50}
1	>350
2	43.85 ± 1.64
3/4	39.86 ± 1.60
5	24.52 ± 1.38
6	18.82 ± 1.27
7	22.63 ± 1.35
8	14.62 ± 1.16
9/10	22.63 ± 1.35
11/12	308.8 ± 2.49
13	218.3 ± 2.33

DACExt showed morphological profiles indicating a reduction in the number of cells per area. Also retraction of the cytoplasm and altered morphology of the treated cells in comparison to the negative control was observed. Furthermore, in the highest concentration tested, sparse colonies were seen (Figure 2).

The morphological evaluation is a qualitative analysis and allows evidence of cellular changes after exposure of the cells to the extract, corroborating with the data obtained by the MTT assay. Untreated cells exhibited characteristics typical of adherent cells with cytoplasmic extensions and expected confluence according to the number of cells plated.

3.5. Flow Cytometry: Annexin-V and Cell Cycle Assays

The effect on cell cycle of HCT 116 of the DACExt in early incubation time (24 h) was evaluated in two concentrations, the IC_{50} (37.77 μg·mL^{-1}) and twice the

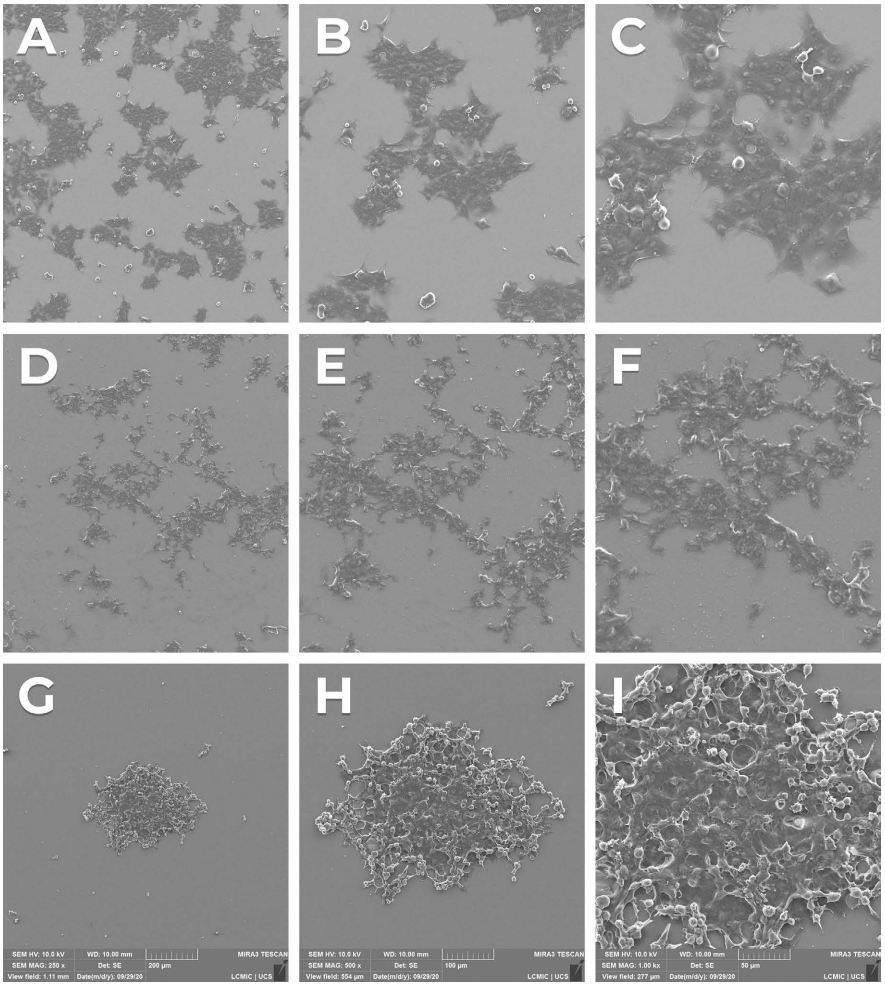

Figure 2. HCT 116 cell line images obtained by Scanning Electron Microscopy after 24 hours of exposure to the chloroform extract of *D. anceps* at the IC_{50} 37.77 ± 1.57 μg·mL^{-1} and 75.54 μg·mL^{-1}. (A)-(C) correspond to the control group, (D)-(F), the treatment in the concentration of the IC_{50} and G, H and I, to the treatment with concentration corresponding to twice the IC_{50}. The images on the left are 250× magnification, those in the center are 500× and on the right 1000×.

value (75.54 μg·mL^{-1}) in comparison with the control (DMSO 0.5%) (Figure 3).

There was a significant decrease in G1 cells with increased extract concentration ($p < 0.05$), a small but not significant increase in G2/M cells ($p > 0.05$). Moreover, multinucleated cells were increased with enhanced extract concentration ($p < 0.05$).

In the treatment with the IC_{50} concentration, the percentage of cells in early apoptosis presents a significant difference in relation to the control ($p < 0.05$). The percentage of cells in initial apoptosis increases after exposure to the lowest concentration of the extract and the number of cells is reduced using a higher concentration. With increasing concentration, the number of cells in late apoptosis increased ($p < 0.05$). Late apoptosis results increased after high extract concentration exposure. There was a reduction in the number of cells in necrosis ($p < 0.05$) and the percentage of viable cells decreased considerably ($p < 0.05$). Therefore, the DACExt extract inhibits the growth of colorectal cancer in a dose-dependent manner through the induction of apoptosis. Figure 4 and Figure 5 represents the results of triplicate after flow cytometry.

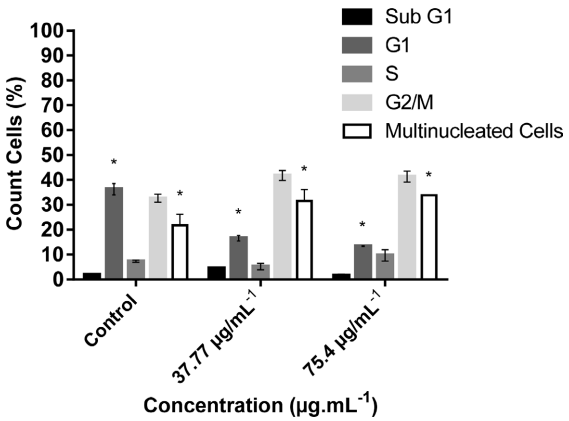

Figure 3. Flow cytometry analysis of Cell Cycle parameters: HCT 116 cells were treated for 24 h with DMSO 0.5% (control negative) and with the DACExt extract at the IC_{50} (37.7.7 μg·mL^{-1}) concentration and twice the IC_{50} (75.4 μg·mL^{-1}) (*$p < 0.05$ compared to control).

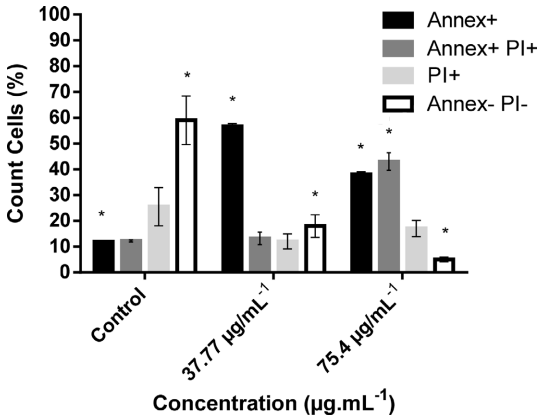

Figure 4. Annexin-V/PI staining expressed in mean and standard deviation after 24 h treatment with the DACExt at the concentration of IC_{50} (37.77 μg·mL^{-1}) and twice (75.4 μg·mL^{-1}) (*$p < 0.05$ compared to control).

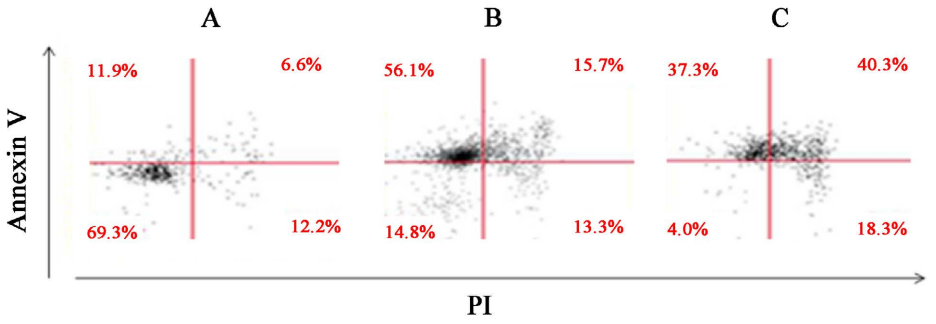

Figure 5. Representative analysis of flow cytometry data after Annexin V/Propidium Iodide staining. HCT 116 cells were treated for 24 h with DMSO 0.5% ((A) Control) and with the DACExt at the IC_{50} ((B) 37.77 μg·mL^{-1}) concentration and twice the IC_{50} ((C) 75.4 μg·mL^{-1}). Annexin-V only staining early apoptotic events (Q1-UL quadrant), Annexin V and PI staining indicated the late phase of apoptosis (Q1-UR quadrant), only PI staining indicated necrosis (Q1-LR) and Annexin-V and PI not stained (Q1-LL) indicated viable cells. Quadrant figures were representative of at least three independent experiments.

The mechanism of cell death, including initial apoptosis, late apoptosis or necrosis, was analyzed after exposure HCT 116 cell line to DACExt extract for 24 h.

4. Discussion

Seaweeds are used as functional food and medicinal herbs, and have a long history of applications in Asian countries. Since, crude extracts or partially purified or isolated compounds from many species of brown, green, and red algae have been tested for their antitumor activities. These studies have indicated that marine algae constitute a promising source of novel compounds with potential as human therapeutic agents. In particular, macroalgae have been considered as a source of new bioactive compounds [16].

In this study, GC identified many compounds in the chloroform extract and fractions of the Antarctic macroalgae *D. anceps*. Fucosterol is a sterol commonly seen in algae and has been attributed to several biological activities such as anti-inflammatory, antioxidant, hepatoprotective and anticancer [18] [20]. Khanavi *et al.* [21] isolated fucosterol from brown alga *Sargassum angustifolium* and showed an anticancer activity against HT29 colorectal and T47D breast cancer lines. Among them, fucosterol has been investigated for its anticancer properties [22]. Therefore, other unidentified compounds that may act in isolate or through synergism mechanism may be involved in the anticancer potential of *D. anceps*. Recently, in our studies, we identified a series of steroids in Antarctic macroalgae using liquid chromatography coupled with tandem mass spectrometry [22]. The chemical composition of extract may vary according to extraction technique and methodology.

Bicyclo [3.1.1] heptane, 2,6,6-trimethyl (pinane) is a compound from the terpene class and was identified by Santana *et al.* [23] as a constituent of the essential oil of *S. terebinthifolius*, showing cytotoxic properties against human melanoma (A2058), breast adenocarcinoma (MCF7), leukemia (HL-60) and cervical carcinoma (HeLa) cell lines. Boyom *et al.* [24] identified the presence of al-

pha-pinene in the essential oil composition of *Cleistopholis patens* and *Uvariastrum pierreanum*, both showing antimalarial effect. Maruthupandian & Mohan [25] identified the presence of acid n-hexadecanoic with biological activities such as antioxidant, nematicide, antiinflammatory, hypocholesterolemic, cannabinoid. Members of the vitamin E family are hydrophobic fat-soluble compounds found in a variety of food sources such as corn oil, peanuts, vegetable oils, fruits and vegetables consumed through diet. The health benefit of consuming vitamin E, present in present in F2, through diet or supplementation is believed to be for its antioxidant properties. Vitamin E protects cells from cell damage caused by free radicals that damage cell membranes through lipid oxidation, leading to DNA damage and cancer development [26].

The compound 3-methyl-2-cyclopenten-1-one and oleic acid observed in fractions 3/4 and 6, respectively, were found in plant extracts of *Foeniculum vulgare* and showed antimicrobial activity [27]. Phytol, the open chain diterpene, presented antimycobacterium activity [28] was identified in F2, F7, F9/10, F11/12.

Based on popular knowledge, algae have been investigated for their biological activities. However, few studies have reported the chemical composition and biological activity of Antarctic phytoflora [29] [30]. Clinical tests, both *in vivo* and *in vitro*, are performed to confirm the effectiveness of natural compounds [31].

Several studies have reported that compounds extracted from seaweed may be effective anticancer agents [16] [32] [33]. The DACExt and its fractions presented cytotoxicity against the colorectal cancer cell line HCT 116 at the analyzed times. The effect of the treatment on IC_{50} concentration (37.7.7 $\mu g \cdot mL^{-1}$) of the cell line after 24 hours of exposure to the extract was also analyzed by scanning electron microscopy and, in addition to the reduction in the number of cells, loss of cell adhesion, cytoplasm retraction was found. As the concentration of the extract increased (75.4 $\mu g \cdot mL^{-1}$), sparse colonic formation was observed. Cytotoxic activity of extracts of different Antarctic seaweeds, *C. jacquinotii*, *I. cordata*, *H. grandifolius*, and *P. endiviifolia* were observed from our research group [4] [34] [35]. The anticancer potential of several macroalgae species has been demonstrated against colorectal cancer cells lines such as HT 29 e HCT 116 [14] [30] [31], Caco-2, WiDr, HCT116, and DLD-1 [36] [37] [38] [39]. This was the first study to demonstrate the anticancer activity extract fractions of macroalgae *D. anceps* against colorectal cancer HCT 116 cell line. In this way, *D. anceps* was shown to be a promising source of compounds with anticancer activity and target for new studies.

The elucidation of the molecular mechanisms involved in the pathogenesis of colorectal cancer may reduce incidence, as well as the morbidity and mortality. Thus, the identification of markers and the mechanisms that inhibit apoptosis and stimulate cell proliferation of transformed cells is of fundamental importance to reduce the prevalence of this disease.

In this study we evaluated by flow cytometry the effects on cell cycle of exposing colorectal cancer cells to different concentrations of DACExt and the

mechanism of cell death. We verified an increase in the number of multinucleated cells and phase M progression, acquiring extra copies of the genome without making cytokinesis, perhaps by checkpoint failure in phase M. Increased ploidy may be causing genetic instability, which may be responsible for the cell being referred for apoptosis.

Tumors are complex systems that include heterogeneous cancer cells with markedly different sizes and genomic contents [40]. The bulk of cancer cells within the majority of solid tumors (~90%) are aneuploidy. Such cells have an alteration (often gain) of chromosome number that is not a multiple of the diploid (2n) component. Cancer stem cells are often much smaller than the bulk of cells, whereas polyploid cells are larger than bulk cells by virtue of their increased ploidy (>4n). Not surprisingly, cultures of established solid tumor-derived cell lines are also heterogeneous [41]. Similar results were found and described by Lu *et al.* [42]. In this study, reversine, a small molecule that was originally identified to induce dedifferentiation of murine myoblasts into multipotent progenitor cells, demonstrated anticancer activity and generated an increase in the number of multinucleated cells, suggesting that tumor cells can escape from mitosis without cell division.

Giant multinucleated cell formation is a cell phenotype due to verification failure at cell cycle checkpoints, mainly in DNA structure verification and mitotic spindle assembly. Failure to interrupt the cycle to phase M leads to an attempt at aberrant chromosomal segregation, which triggers the activation of the apoptotic mechanism. Due to damage to the mitotic spindle, cells are able to escape the M-phase without segregation of sister chromatids and cytokinesis, a process known as "mitotic slippage". Cells stop at G1 because they activate a p53-mediated checkpoint. In the presence of p53, p21 activates polyploidy, which generates cell death to prevent late mitosis and aneuploidy errors [41].

The mechanism of cell death identified was initial apoptosis. Cell death was evaluated by several studies using different compounds and extracts from algae in a wide variety of cell models. Laminarin from brown seaweed *Laminaria digitata* induced apoptosis in HT-29 colon cancer cells (5 $mg{\cdot}mL^{-1}$) in a dose-dependent manner [43]. The ethanolic extract of algae *Undaria pinnatifida* induced the reduction of cell viability of the HCT 116 cell line (2% of cell extract reduces in 80% cell viability) by apoptotic mechanism [44]. Fucoindan, sulfated polysaccharides purified from the brown algae *Fucus vesiculosus*, induced apoptosis in the HT 29 adenocarcinoma line (5 - 20 $\mu g{\cdot}mL^{-1}$) [14]. Fucoxanthin, a carotenoid present in brown seaweeds, has shown to suppress the level of Bcl-2 protein and induce apoptosis in human colon cancer cells Caco-2, HT-29 and DLD-1. After exposure at concentration 15.2 μM, fucoxoxantin inhibited, respectively, the viability of the cells in 14.8%, 50.8% and 29.4%. In addition, fucoxanthinol, fucoxanthin metabolite, also had a stronger anti-proliferative effect than fucoxanthin on Caco-2 human colon cancer cells [45] [46].

Most reports studying cytotoxic effects of macroalgae present a range of IC_{50} from 5 μg/mL to 869 $\mu g{\cdot}mL^{-1}$. Various therapeutic compounds from seaweed are

able to induce apoptosis through different pathways and molecular mechanisms. Recent cancer preventive studies have been focused on designing anticancer drugs from natural sources because of fewer side effects as compared to synthetic anticancer drugs [16] [32] [17] [47].

The inhibition of apoptosis in colorectal cancer cells enhances tumor growth, promotes neoplastic progression, and confers resistance to cytotoxic anticancer agents. Thus, bioactive compounds that induce apoptosis in cancer cells can be usefull as agents for cancer chemoprevention and/or chemotherapy [17].

5. Conclusion

This study was the first to demonstrate the reduction of the cellular viability of colorectal cancer cells through the induction of apoptosis pathway after treatment with chloroform extract of the brown Antarctic macroalgae *D. anceps*, suggesting that this macroalgae is a promising candidate for the isolation of molecules with anticancer activity. Fractionation by bioassay-guided is an interesting tool to separate, isolate and purify natural compounds with anticancer activity. Some isolated compounds such as fucoidan and fucosterol have been investigated with promising antitumor activity. However, more studies are necessary in order to elucidate the mechanism of action of macroalgae and the pathways of induction of apoptosis.

Acknowledgements

The authors thank Brazilian Research Funding Program (CAPES), University of Caxias do Sul (UCS), Brazilian Algae Research Group (RedeAlgas), Antarctic Brazilian Program (PROANTAR) for financial support for the development of this work.

Author Contribution Statement

The authors Rafaele Frassini, Daniela Steffens, Sidnei Moura, Aline Paternostro Martins, Cesar Aguzzoli, Pio Colepicolo, Mutue Toyota Fujii, Nair S. Yokoya, Cláudio Martin Pereira de Pereira, Ana Cláudia Phillipus, Miriam de Barcellos Flakenberg, João Antonio Pegas Henriques and Mariana Roesch-Ely, declare to be responsible for drafting the manuscript entitled "Desmaretia anceps Montagne Against Colorectal Cancer Cells: Cytotoxic Activity and Proapopitotic Effects."

The authors Rafaele Frassini, João Antonio Pegas Henriques and Mariana Roesch-Ely participated in project design, in the execution of all experiments and data collection. Daniela Steffens assisted in the cytometry, cell cycle assay and statistical analysis. Aline Paternostro, Mutue Toyota Fujii, Nair S. Yokoya and Pio Colepicolo were responsible for the collection, storage and botanical identification of seaweed *Desmarestia anceps*. Cesar Aguzzoli contributed to the SEM analysis. Cláudio Martin Pereira de Pereira, Ana Cláudia Phillipus, Miriam de Barcellos Flakenberg helped in chemical characterization, vacuum liquid

chromatography and nuclear magnetic resonance.

The authors participated in data analysis, discussion of results and writing of the manuscript. The manuscript was written, read and approved by all the authors. The material has not been published, in whole or in part, and it also is not under consideration for publication elsewhere.

Conflicts of Interest

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

References

[1] El-Beltagi, H.S., Mohamed, A.A., Mohamed, H.I., Ramadan, K.M.A., Barqawi, A.A. and Mansour, A.T. (2022) Phytochemical and Potential Properties of Seaweeds and Their Recent Applications: A Review. *Marine Drugs*, **20**, Article 342. https://doi.org/10.3390/md20060342

[2] Pal, A., Kamthania, M.C. and Kumar, A. (2014) Bioactive Compounds and Properties of Seaweeds—A Review. *Open Access Library Journal*, **1**, e752. https://doi.org/10.4236/oalib.1100752

[3] Wiencke, C., Clayton, M.N., Gómez, I., Iken, K., Lüder, U.H., Amsler C.D., Kastern, U., Hanelt, D., Bischof, K. and Dunton, J. (2006) Life Strategy, Ecophysiology, and Ecology of Seaweeds in Polar Waters. *Reviews in Environmental Science and Bio/Technology*, **6**, 95-126. https://doi.org/10.1007/s11157-006-9106-z

[4] Martins, R.M., Nedel, F., Guimarães, V.B.S., Da Silva, A.F., Colepicolo, P., De Pereira, C.M.P. and Lund, R.G. (2018) Macroalgae Extracts from Antarctica have Antimicrobial and Anticancer Potential. *Frontiers in Microbiology*, **9**, Article 412. https://doi.org/10.3389/fmicb.2018.00412

[5] Dagogo-Jack, I. and Shaw, T.A. (2018) Tumour Heterogeneity and Resistance to Cancer Therapies. *Nature Reviews Clinical Oncology*, **15**, 81-94. https://doi.org/10.1038/nrclinonc.2017.166

[6] Kumar, A. and Jaitak, V. (2019) Natural Products as Multidrug Resistance Modulators in Cancer. *European Journal of Medicinal Chemistry*, **176**, 268-291. https://doi.org/10.1016/j.ejmech.2019.05.027

[7] Xu, X., Lai, Y. and Hua, Z.C. (2019) Apoptosis and Apoptotic Body: Disease Message and Therapeutic Target Potentials. *Bioscience Reports*, **39**, BSR20180992. https://doi.org/10.1042/BSR20180992

[8] Chang, W., Fa, H., Xiao, D. and Wang, J. (2020) Targeting Phosphatidylserine for Cancer Therapy: Prospects and Challenges. *Theranostics*, **10**, 9214-9229. https://doi.org/10.7150/thno.45125

[9] Pietkiewicz, S., Schmidt, J.H. and Lavrik, I.N. (2015) Quantification of Apoptosis and Necroptosis at the Single Cell Level by a Combination of Imaging Flow Cytometry with Classical Annexin V/Propidium Iodide Staining. *Journal of Immunological Methods*, **423**, 99-103. https://doi.org/10.1016/j.jim.2015.04.025

[10] Van Opdenbosch, N. and Lamkanfi, M. (2019) Caspases in Cell Death, Inflammation and Disease. *Immunity*, **50**, 1352-1364. https://doi.org/10.1016/j.immuni.2019.05.020

[11] Pistritto, G., Trisciuoglio, D., Ceci, C., Garufi, A. and D'Orazi, G. (2016) Apoptosis as Anticancer Mechanism: Function and Dysfunction of Its Modulators and Tar-

geted Therapeutic Strategies. *Aging*, **8**, 603-619.
https://doi.org/10.18632/aging.100934

[12] Carneiro, B.A. and El-Deiry, W. (2021) Targeting Apoptosis in Cancer Therapy. *Nature Reviews Clinical Oncology*, **17**, 395-417.
https://doi.org/10.1038/s41571-020-0341-y

[13] Plati, J., Bucura, O. and Khosravi-Fara, R. (2011) Apoptotic Cell Signaling in Cancer Progression and Therapy. *Integrative Biology*, **3**, 279-296.
https://doi.org/10.1039/c0ib00144a

[14] Kim, E.J., Park, S.Y., Lee, S.Y. and Park J.H. (2010) Fucoidan Present in Brown Algae Induces Apoptosis of Human Colon Cancer Cells. *BMC Gastroenterology*, **10**, Article No. 96. https://doi.org/10.1186/1471-230X-10-96

[15] Pradhan, B., Nayak, R., Patra, S., Jit, B.P., Ragusa, A. and Jena, M. (2021) Bioactive Metabolites from Marine Algae as Potent Pharmacophores against Oxidative Stress-Associated Human Diseases: A Comprehensive Review. *Molecules*, **26**, Article 37. https://doi.org/10.3390/molecules26010037

[16] Moussavou, G., Kwak, D.H., Obiang-Obonou, B.W., Maranguy, C.A.O, Dinzouna-Boutamba, S., Lee, D.H., Pissibanganga, O.G.M., Ko, K., Seo, J.I. and Choo, W.K. (2014) Anticancer Effects of Different Seaweeds on Human Colon and Breast Cancers. *Marine Drugs*, **12**, 4898-4911. https://doi.org/10.3390/md12094898

[17] Narayani, S.S., Saravanan, S., Ravindran, J., Ramasamy, M.S. and Chitra, J. (2019) *In Vitro* Anticancer Activity of Fucoidan Extracted from *Sargassum cinereum* against Caco-2 Cells. *International Journal of Biological Macromolecules*, **138**, 618-628.
https://doi.org/10.1016/j.ijbiomac.2019.07.127

[18] Bae, H., Lee, J.Y., Song, G. and Lim, W. (2020) Fucosterol Suppresses the Progression of Human Ovarian Cancer by Inducing Mitochondrial Dysfunction and Endoplasmic Reticulum Stress. *Marine Drugs*, **18**, Article 261.
https://doi.org/10.3390/md18050261

[19] Wynne, M.J. (2017) A Checklist of Benthic Marine Algae of the Tropical and Subtropical Western Atlantic: Fourth Revision. Nova Hedwigia Beihefte, Beih.

[20] Meinita, M.D.N., Harwanto, D., Tirtawijaya, G., Negara, B.F.S.P., Sohn, J.H., Kim, J.S. and Choi, J.S. (2021) Fucosterol of Marine Macroalgae: Bioactivity, Safety and Toxicity on Organism. *Marine Drugs*, **19**, Article 545.
https://doi.org/10.3390/md19100545

[21] Khanavi, M., Gheidarloo, R., Sadati, N., Ardekani, M.R.S., Nabavi, S.M.B., Tavajohi, S. and Ostad, S.N. (2021) Cytotoxicity of Fucosterol Containing Fraction of Marine Algae against Breast and Colon Carcinoma Cell Line. *Pharmacognosy Magazine*, **8**, 60-64. https://doi.org/10.4103/0973-1296.93327

[22] Jiang, H., Li, J., Chen, A., Li, Y., Xia, M., Guo, P., Yao, S. and Chen, S. (2018) Fucosterol Exhibits Selective Antitumor Anticancer Activity against HeLa Human Cervical Cell Line by Inducing Mitochondrial Mediated Apoptosis, Cell Cycle Migration Inhibition and Downregulation of m-TOR/PI3K/Akt Signaling Pathway. *Oncology Letters*, **15**, 3458-3463. https://doi.org/10.3892/ol.2018.7769

[23] Santana, J.S., Sartorelli, P., Guadagnin, R.C., Matsuo, A.L., Figueiredo, C.R., Soares, M. G., Da Silva, A.M. and Lago, J.H.G. Essential Oils from *Schinus terebinthifolius* Leaves—Chemical Composition and *in Vitro Cytotoxicity Ev*aluation. *Pharmaceutical Biology*, **50**, 1248-1253. https://doi.org/10.3109/13880209.2012.666880

[24] Boyom, F.F., Ngouana, V. and Kemgne, E.A.M. (2011) Antiplasmodial Volatile Extracts from *Cleistopholis patens* Engler & Diels and *Uvariastrum pierreanum* Engl. (Engl. & Diels) (Annonaceae) Growing in Cameroon. *Parasitololy Research*, **108**,

1211-1217. https://doi.org/10.1007/s00436-010-2165-7

[25] Maruthupandian, A. and Mohan, V.R. (2011) GC-MS Analysis of Some Bioactive Constituents of *Pterocarpus marsupium* Roxb. *International Journal of Chemtech Research*, **3**, 1652-1657.

[26] Cardenas, E. and Gosh, R. (2013) Vitamin E: A Dark Horse at the Crossroad of Cancer Management. *Biochemical Pharmacology*, **86**, 845-852. https://doi.org/10.1016/j.bcp.2013.07.018

[27] Esquivel-Ferriño, P.C., Favela-Hernández, J.M.J., Garza-González, E., Waksman, N., Ríos, M.Y. and Camacho-Corona, M.R. (2012) Antimycobacterial Activity of Constituents from *Foeniculum vulgare* var. Dulce Grown in Mexico. *Molecules*, **17**, 8471-8482. https://doi.org/10.3390/molecules17078471

[28] Cantrell, C.L., Franzblau, S.G. and Fisher, N.H. (2001) Antimycobacterial Plant Terpenoids. *Planta Medica*, **67**, 685-695. https://doi.org/10.1055/s-2001-18365

[29] Ankisetty, S., Nandiraju, S., Win, H., Park, Y.C., Amsler, C.D., McClintock, J.M., Baker, J.A., Diyabalanage, T.K., Pasaribu, A., Singh, M.P., Maiese, W.M., Walsh, R.D., Zaworotko, M.J. and Baker, B.J. (2004) Chemical Investigation of Predator-Deterred Macroalgae from the Antarctic Peninsula. *Journal of Natural Products*, **67**, 1295-1302. https://doi.org/10.1021/np049965c

[30] Dembittsky, V.M. and Tolstikov, G.A. (2003) Natural Halogenated Alkanes, Cycloalkanes and Their Derivatives. *Chemistry for Sustainable Development*, **11**, 803-810.

[31] Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. and Latha, L.Y. (2011) Extraction Isolation and Characterization of Bioactive Compounds from Plnast's Extracts. *African Journal of Traditional, Complementary, and Alternative Medicine*, **8**, 1-10. https://doi.org/10.4314/ajtcam.v8i1.60483

[32] Sanjeewa, K.K., Lee, A., Suck, J., Won-Suck, K. and You-Jin, J. (2017) The Potential of Brown-Algae Polysaccharides for the Development of Anticancer Agents: An Update on Anticancer Effects Reported for Fucoidan and Laminaran. *Carbohydrate Polymers*, **177**, 451-459.

[33] Jin, Y., Qiu, S., Shao, N. and Zheng, J. (2018) Fucoxanthin and Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) Synergistically Promotes Apoptosis of Human Cervical Cancer Cells by Targeting PI3K/Akt/NF-κB Signaling Pathway. *Medical Science Monitor*, **24**, 11-18.https://doi.org/10.12659/MSM.905360

[34] Gambato, G., Baroni, E.G., Garcia, C.S.G., Frassini, R., Frozza, C.O.S., Frozza, M.S., De Pereira, C.M.P., Fujii, M.T., Cplepicolo, P., Lambert, A.P.F., Henriques, J.A.P. and Roesch-Ely, M. (2014) Brown Algae *Himantothallus grandifolius* (Desmarestiales, Phaeophyceae) Suppresses Proliferation and Promotes Apoptosis-Mediated Cell Death in Tumor Cells. *Advances in Biological Chemistry*, **4**, 98-108. https://doi.org/10.4236/abc.2014.42014

[35] Pacheco, B. S., Dos Santos, M.A.Z., Schultze, E., Martins, R.M., Lund, R.G., Seixas, F.K., Colepicolo, P., Collares, T., Paula, F.R. and De Pereira, C.M.P. (2018) Cytotoxic Activity of Fatty Acids from Antarctic Macroalgae on the Growth of Human Breast Cancer Cells. *Frontiers in Bioengeenering and Biotechnology*, **6**, Article 185. https://doi.org/10.3389/fbioe.2018.00185

[36] Park, H.K., Kim, J. and Nam, T.J. (2013) Induction of Apoptosis and Regulation of ErbB Signaling by Laminarin in HT-29 Human Colon Cancer Cells. *International Journal of Molecular Medicine*, **32**, 291-295.https://doi.org/10.3892/ijmm.2013.1409

[37] Lopes-Costa, E., Abreu, M., Gargiulo, D., Rocha, E. and Ramos, A.A. (2017) Anticancer Effects of Seaweed Compounds Fucoxanthin and Phloroglucinol, Alone and

in Combination with 5-Fluorouracil in Colon Cells. *Journal of Toxicology and Environmental Health*, **80**, 776-787. https://doi.org/10.1080/15287394.2017.1357297

[38] Nwosu, F., Morris, J., Lund, V.A., Stewart, D., Ross, H.A. and McDougall, G.J. (2011) Anti-Proliferative and Potential Anti-Diabetic Effects of Phenolic-Rich Extracts from Edible Marine Algae. *Food Chemistry*, **126**, 1006-1012. https://doi.org/10.1016/j.foodchem.2010.11.111

[39] Takahashi, K., Hosokawa, M., Kasajima, H., Hatanaka, K., Kudo, K., Shimoyama, N. and Miyashita, K. (2015) Anticancer Effects of Fucoxanthin and Fucoxanthinol on Colorectal Cancer Cell Lines and Colorectal Cancer Tissues. *Oncology Letters*, **10**, 1463-1467. https://doi.org/10.3892/ol.2015.3380

[40] Li, Q., Rycaja, K., Chena, X. and Tanga, D.G. (2015) Cancer Stem Cells and Cell Size: A causal link? *Seminars in Cancer Biology*, **35**, 91-199. https://doi.org/10.1016/j.semcancer.2015.07.002

[41] Mirzayans, R., Andrais, B. and Murray, D. (2018) Roles of Polyploid/Multinucleated Giant Cancer Cells in Metastasis and Disease Relapse Following Anticancer Treatment. *Cancers* (*Basel*), **10**, Article 118. https://doi.org/10.3390/cancers10040118

[42] Lu, Y.C., Lee, Y.R., Liao, J. D., Lin, C., Chen, Y., Chen, P. and Tseng, Y. (2016) Reversine Induced Multinucleated Cells, Cell Apoptosis and Autophagy in Human Non-Small Cell Lung Cancer Cells. *PLOS ONE*, **11**, e0158587. https://doi.org/10.1371/journal.pone.0158587

[43] Park, H.K., Kim, I.H., Kim, J. and Nam T.J. (2012) Induction of Apoptosis by Laminarin, Regulating the Insulin-Like Growth Factor-IR Signaling Pathways in HT-29 Human Colon Cells. *Internatianal Journal of Molecular Medicine*, **30**, 734-738.https://doi.org/10.3892/ijmm.2012.1084

[44] Nishibori, N., Itoh, M., Kashiwagi, M., Arimochi, H. and Morita, K. (2012) *In Vitro* Cytotoxic Effect of Ethanol Extract Prepared from *Sporophyll* of *Undaria Pinnatifida* on Human Colorectal Cancer Cells. *Phytotherapy Research*, **26**, 191-196. https://doi.org/10.1002/ptr.3527

[45] Hosokawa, M., Kudo, M., Maeda, H., Kohno, H., Tanaka, T. and Miyashita, K. (2004) Fucoxanthin Induces Apoptosis and Enhances the Antiproliferative Effect of the PPARγ Ligand, Troglitazone, on Colon Cancer Cells. *Biochimica et Biophysica Acta*, **1675**, 113-119. https://doi.org/10.1016/j.bbagen.2004.08.012

[46] Konishi, I., Hosokawa, M., Sashima, T., Kobayashi, H. and Miyashita, K. (2006) Halocynthiaxanthin and fucoxanthinol Isolated from *Halocynthia Roretzi* Induce Apoptosis in Human Leukemia, Breast and Colon Cancer Cells. *Comparative Biochemistry and Physiology*, **142**, 53-59. https://doi.org/10.1016/j.cbpc.2005.10.005

[47] Alghazeer, R., Howell, N.K., El-Naili, M.B. and Awayn, N. (2018) Anticancer and Antioxidant Activities of Some Algae from Western Libyan Coast. *Natural Science*, **10**, 232-246. https://doi.org/10.4236/ns.2018.107025