

# Algae from Portuguese Coast Presented High Cytotoxicity and Antiproliferative Effects on an *In vitro* Model of Human Colorectal Cancer

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## ABSTRACT

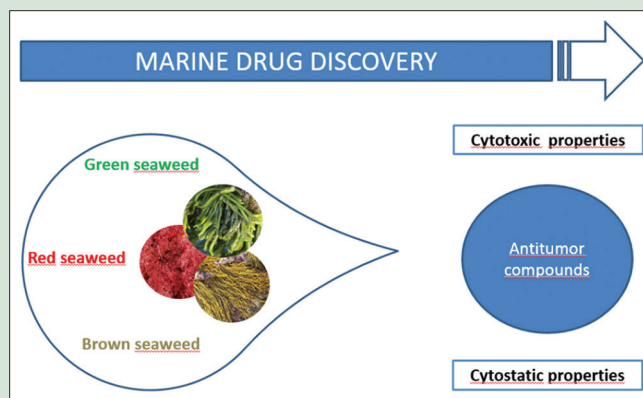
**Background:** The marine environment has shown to be an interesting source of new antitumor agents, representing an important tool in cancer research. **Objective:** The aim of this study was to evaluate the antitumor activities of 12 algae from Peniche coast (Portugal) on an *in vitro* model of human colorectal cancer (Caco-2 cells). **Materials and Methods:** The antitumor potential was accessed by evaluating Caco-2 cell's viability and proliferation through the 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide and calcein-AM methods. **Results:** The dichloromethane extracts of *Asparagopsis armata* and *Sphaerococcus coronopifolius* induced the highest decrease on cell's viability (1 mg/mL; 24 h), 98.96% ± 0.39% and 98.08% ± 0.89%, respectively, followed by the methanolic extracts of *S. coronopifolius* (96.47% ± 1.26%) and *A. armata* (92.68% ± 1.17%). Regarding cell proliferation, the highest decrease of Caco-2 cell's proliferation (1 mg/mL; 24 h) was induced by the dichloromethane extract of *A. armata* (100% ± 0.48%), *S. coronopifolius* (99.04 ± 0.51%), and *Plocamium cartilagineum* (95.05% ± 1.19%). The highest potency was shown by the dichloromethane extract of *S. coronopifolius* in both, cytotoxicity and antiproliferative tests, with an IC<sub>50</sub> of 21.3 and 36.5 µg/mL, respectively. **Conclusion:** The extracts of *A. armata* and *S. coronopifolius* are promising sources of new bioactive molecules with application in cancer therapeutics. **Key words:** Bioactive compounds, Caco-2 cells, marine natural products, red macroalgae, seaweed

## SUMMARY

- Algae from Peniche coast (Portugal) revealed to be a promising source of new bioactive compounds with potential application on cancer therapeutics.

**Abbreviations Used:** MTT: 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide; DMSO: Dimethyl sulfoxide; FBS: Fetal bovine serum;

MEM: Minimum Essential Medium; SEM: Standard error of the mean; SP : Sulfated polysaccharides



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## INTRODUCTION

The world's population is affected by many diseases, such as cancer, which have high social and economic impacts in all societies. This disease is one of the major public health problems worldwide with millions of new cancer patients diagnosed every year.<sup>[1]</sup> Among the different types of cancer, liver, breast, lung, stomach, and colorectal cancer present the highest mortality rates.<sup>[2]</sup> In fact, colorectal cancer is the third most frequently diagnosed, both in men and woman, and has been estimated in the US that in 2017 will occur around 135,430 of new cases and 50,260 deaths.<sup>[3]</sup> Along years, natural products have been an important source of new bioactive molecules with application in cancer therapy, and recent survey indicates that 40% of all anticancer drugs developed before 2002 are of natural products while another 20% are synthetic compounds based on natural products.<sup>[4]</sup> In the last years, as a consequence of an increasing demand for new therapeutic drugs from natural products, there is a great interest toward marine organisms. Marine organisms produce unique bioactive molecules synthesized mainly by their secondary metabolism in response to ecological pressures, such as competition for space, maintenance of fouled surfaces, deterrence of predation, and ability to successfully reproduce.<sup>[5]</sup>

The ability of these organisms live in harsh environments makes them an interesting source of novel or preexisting compounds with high value, which may provide sustainable economic and human benefits.<sup>[6,7]</sup> In fact, the marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features which are not found in the terrestrial environment.<sup>[8]</sup> Among marine organisms, algae, which can be classified into green algae (Phylum Chlorophyta), red algae (Phylum Rhodophyta), and brown algae (Phylum Ochrophyta or Heterokontophyta), have triggered interest in the biomedical area, mainly due to their content on bioactive substances with great potential,

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including antioxidant, antimicrobial, antiviral, anthelmintic, antialgal, antiprotozoan, anti-macroufouling, anti-inflammatory, and antitumor properties.<sup>[9-12]</sup>

Numerous algae extracts and metabolites have shown high cytotoxic activities; accordingly, some scientists suggest algae consumption as a chemopreventive routine against several cancers.<sup>[13-15]</sup> Different groups of bioactive molecules with antitumor activity have been isolated from algae, such as sulfated polysaccharides (SP), carotenoids, and terpenoids. Moreover, some of these compounds have shown interesting cytotoxic activities also in *in vivo* experimental models.<sup>[16-19]</sup> Portugal has an enormous and rich marine coast. However, it continues to be mainly unexplored. The high heterogeneity along the coast provides a rich flora biodiversity. The intertidal algae flora in northern Portugal is similar to that of the coast of Central Europe (Brittany, South of British Isles), and in southern, flora is very different suffering a marked influence of Mediterranean and African species.<sup>[20,21]</sup> Therefore, Portugal reveals excellent conditions for the discovery of novel products with high biotechnological potential, including antitumor potential. In line with this, the aim of this study was to evaluate the antitumor activities of 12 algae from Peniche coast (Portugal) on Caco-2 cells, an *in vitro* model of human colorectal cancer.

## MATERIALS AND METHODS

### Sampling, identification, and preparation of algae extracts

Algae were collected freshly from Peniche coast (Portugal) and immediately transported to our laboratory and identified as described in Table 1. Then, they were washed first in sea water followed by fresh water to remove epiphytes, sand, and other extraneous matter and stored at -80°C (Thermo, Electron Corporation). Frozen algae were mechanically reduced into a powder with a mixer grinder. To obtain algae extracts, the resulting powder was sequentially extracted in 1:4 biomass: solvent ratio with methanol and dichloromethane (Fisher Scientific, United Kingdom) at constant stirring for 12 h. Liquid-liquid extraction was then performed on the methanolic extract, using *n*-hexane (Lab-Scan Analytical Sciences, Poland). Solvents were evaporated in a rotary evaporator (Laborota 4000, Heidolph) at 40°C and 70 rpm, and dried extracts were solubilized in dimethyl sulfoxide (Sigma-Aldrich, Germany) at 100 mg/mL. Samples were then stored at -20°C until further use.

### Antitumor activities

#### Cell culture conditions

Human colorectal cancer model (Caco-2 cells) was obtained from the Leibniz Institute DSMZ – German Collection of Microorganisms and

Cell Cultures and cultured in minimum essential medium (MEM) (Sigma-Aldrich, USA) supplemented with 20% of fetal bovine serum (FBS) (Hyclone, UK) and 1% of antimycotic solution (100 U/mL penicillin G, 0.25 µg/mL amphotericin B, and 100 µg/mL streptomycin) (Hyclone, USA). Medium was changed every 3 days and cells reached total confluence after 5–6 days of initial seeding. For subculture, cells were dissociated with trypsin-ethylenediaminetetraacetic acid (Sigma-Aldrich, USA), split 1:3 and subcultured in petri dishes with 25 cm<sup>2</sup> of growth area. Caco-2 cells were maintained in 5% of CO<sub>2</sub>, 95% of humidified atmosphere at 37°C.

### Evaluation of cytotoxic activities

The cytotoxicity studies were adapted from the work developed by Pinteus *et al.*<sup>[11]</sup> Cells were seeded in 96-well plates and assays were done after cells reached the total confluence. Algae extracts were dissolved in culture medium without FBS and sterile filtered (0.2 µm Whatman, UK). Then, the cells were incubated 24 h with the extracts (1 mg/mL). For extracts that showed the highest activities, the effects were evaluated after 12 h of incubation, and they were also subjected to dose-response analysis (10–1000 µg/mL; 24 h).

### Evaluation of antiproliferative activities

The antiproliferative studies were adapted from the work developed by Yuan and Walsh.<sup>[22]</sup> Cells were seeded in 96-well plates, and after 36 h of initial seeding, they were incubated 24 h with algae extracts (1 mg/mL), previously dissolved in MEM supplemented with FBS and sterile filtered (0.2 µm Whatman, UK). Extracts that produced effects at 24 h were also evaluated after 12 and 48 h of incubation and were subjected to dose-response analysis (10–1000 µg/mL; 24 h).

### Evaluation of cytotoxic and antiproliferative effects of algae extracts on Caco-2 cells by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide method

Cytotoxicity and cell proliferation tests were evaluated through the 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) method. This assay is based on cleavage of tetrazolium salt MTT by mitochondrial dehydrogenase of viable cells to form a blue formazan product. The formazan produced is proportional to the number of living cells present and can be measured colorimetrically.<sup>[22]</sup> After the treatment with the methanolic and dichloromethane extracts, the medium was removed and cells were washed with Hank's buffer (composition, in mM: NaCl 137, KCl 5, MgSO<sub>4</sub> 0.8, Na<sub>2</sub>HPO<sub>4</sub> 0.33, KH<sub>2</sub>PO<sub>4</sub> 0.44, CaCl<sub>2</sub> 0.25; MgCl<sub>2</sub> 1.0, Tris HCl 0.15, and sodium butyrate 1.0, pH = 7.4). Cells were then incubated 4 h at 37°C with 1.2 mM MTT (Sigma-Aldrich, Germany) which was previously dissolved in Hank's buffer. The formazan products were dissolved in isopropanol (Panreac, Spain) containing 0.04 N HCl and quantified spectrophotometrically at 570 nm.

### Evaluation of algae extracts effects on Caco-2 cell's viability and proliferation by the calcein-AM method

For the extracts that exhibited high activity through the MTT assay, it was used a fluorometric method to cross results, the calcein-AM (Invitrogen, USA) method. The membrane-permeant calcein-AM, a nonfluorescent dye, is taken up and converted by intracellular esterases to calcein, which in turn is not permeable and emits green fluorescence that can be quantified.<sup>[23]</sup> After treatment with algae extracts, cells were washed twice with Hank's buffer and loaded with 2 µM calcein-AM, previously prepared in the same buffer, and incubated at room temperature for 30 min. The natural fluorescence of cells was determined on previously

**Table 1:** Algae collected from Peniche coast (Portugal) for evaluation of antitumor potential

Division	Algae
Rhodophyta	<i>Asparagopsis armata</i>
	<i>Ceramium ciliatum</i>
	<i>Plocamium cartilagineum</i>
	<i>Sphaerococcus coronopifolius</i>
Heterokontophyta	<i>Fucus spiralis</i>
	<i>Halopteris filicina</i>
	<i>Sacchorhiza polyschides</i>
	<i>Stypocaulon scoparium</i>
Chlorophyta	<i>Codium adhaerens</i>
	<i>Codium tomentosum</i>
	<i>Codium vermiculara</i>
	<i>Ulva compressa</i>

defined wells, incubated with Hank's buffer without calcein-AM. Fluorescence was measured at 485 nm excitation and 530 nm emission wavelengths in a multiplate reader (Spectramax Gemini, Molecular Devices).

### Data analysis

The results of both studies were expressed in percentage in relation to the control, calculated as:

$$(\% \text{ of control}) = (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100$$

One-way analysis of variance was used followed by Dunnett test to discriminate significant differences between algae extracts and controls (or vehicle). These analyzes were performed with GraphPad InStat for Windows (GraphPad Software, La Jolla, CA, USA). Results are presented as mean  $\pm$  standard error of the mean. The significance level was inferred at  $P < 0.05$  or  $P < 0.01$  for all statistical tests.

## RESULTS

### Cytotoxicity induced by algae extracts on Caco-2 cells

The methanolic and dichloromethane extracts of 12 algae from Peniche coast, belonging to the Heterokontophyta, Rhodophyta, and Chlorophyta divisions, were tested on Caco-2 cells. Among the methanolic extracts (1 mg/mL; 24 h), the highest capacity to reduce Caco-2 cell's viability was exhibited by *Asparagopsis armata* (7.32%  $\pm$  1.17% of viable cells), *Sphaerococcus coronopifolius* (3.53%  $\pm$  1.26% of viable cells), and *Fucus spiralis* (23.68%  $\pm$  2.93% of viable cells). The methanolic extracts of algae belonging to the Chlorophyta division (green algae) did not show any activity on Caco-2 cell's viability [Figure 1a].

Regarding results of dichloromethane extracts, it is possible to observe in Figure 1b that algae belonging to Rhodophyta division demonstrated the highest cytotoxic potential, namely, *A. armata* (1.04%  $\pm$  0.39% of viable cells), *Plocamium cartilagineum* (9.77%  $\pm$  2.28% of viable cells), and *S. coronopifolius* (1.92%  $\pm$  0.89% of viable cells). The green alga *Codium adhaerens* also showed cytotoxic activity (83.97%  $\pm$  1.40% of viable cells). For other side, dichloromethane extract of Heterokontophyta division did not exhibit any activity on Caco-2 cell's viability. For the extracts that exhibited capacity to reduce the cell's viability in at least 50%, we decided to corroborate the results using a fluorescence method (calcein-AM). The extracts were incubated with Caco-2 cells on the same conditions of the MTT method (1 mg/mL; 24 h). It is possible to verify that both extracts of *A. armata* (1.04%  $\pm$  0.39% of viable cells)

and *S. coronopifolius* presented the highest activity on Caco-2 cell's viability reduction [Figure 2], attesting therefore the previous results. For algae with major potential, we decided to understand if the effects were dependent of the concentration (10–1000  $\mu\text{g/mL}$ ; 24 h). The dichloromethane extract of *S. coronopifolius* displayed the smallest  $\text{IC}_{50}$  with 21.3  $\mu\text{g/mL}$  (12.9–35.2). The results were summarized in Table 2. The next step was to comprehend if the effects induced on Caco-2 cell's viability were time dependent (12 and 24 h at 1 mg/mL). All extracts showed dependence of the time, excepting the dichloromethane extract of *S. coronopifolius* (0.33%  $\pm$  0.18% of viable cells) that displayed a total reduction of cell viability after 12 h of incubation. This alga exhibited the highest potential in both extracts after 12 h [Figure 3].

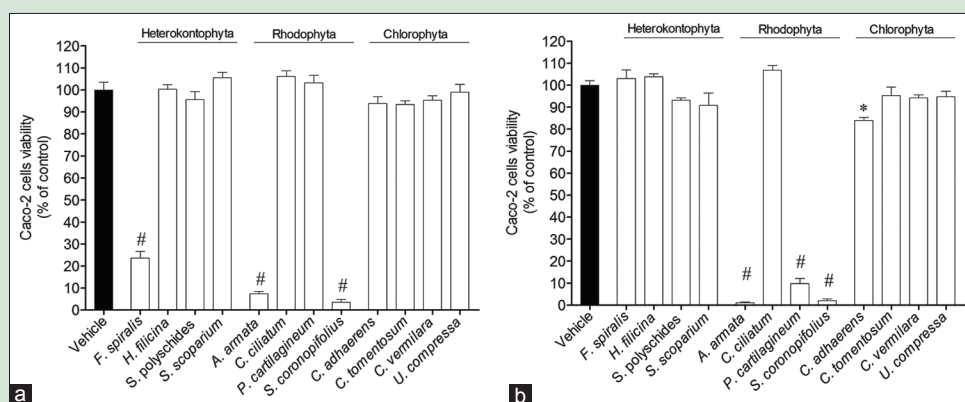
### Antiproliferative activity induced by algae extracts on Caco-2 cells

The antiproliferative activity of the different extracts obtained from 12 algae collected in Peniche coast (Portugal) was tested on Caco-2 cells. The cells were incubated with extracts at 1 mg/mL (24 h) after 36 h of the initial seeding. The results are shown in Figure 4. For both, extracts are possible to see that only the algae belonging to Rhodophyta and Heterokontophyta showed capacity to inhibit Caco-2 cell's proliferation. However, the major potential was exhibited by red algae (Rhodophyta) *A. armata* and *S. coronopifolius* methanolic extracts (24.32%  $\pm$  7.56% of viable cells; 9.27%  $\pm$  3.26% of viable cells, respectively) [Figure 4a] and dichloromethane extracts (0%  $\pm$  0.48% of viable cells; 0.96%  $\pm$  0.51% of viable cells, respectively) [Figure 4b]. The extracts that demonstrated capacity to inhibit the cell's proliferation in more than 50% were also screened through the calcein-AM method. The results showed a more marked effect on the inhibition of Caco-2 cell proliferation comparing with the results obtained

**Table 2:**  $\text{IC}_{50}$  values for the methanolic and dichloromethane extracts that showed the highest reduction of Caco-2 cell's viability (>50%) on maximum concentration (1000  $\mu\text{g/mL}$ ; 24 h)

Alga	Extract	
	Methanol $\text{IC}_{50}$ ( $\mu\text{g/mL}$ )	Dichloromethane $\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
<i>Fucus spiralis</i>	935.2 (573.3-1526.0)	-
<i>Asparagopsis armata</i>	823.0 (421.8-1606.0)	531.6 (384.6-734.7)
<i>Plocamium cartilagineum</i>	-	995.4 (756.0-1311.0)
<i>Sphaerococcus coronopifolius</i>	559.5 (374.2-836.4)	21.3 (12.9-35.2)

Values are expressed as means with 95% CI. Cisplatin was used as positive control with an  $\text{IC}_{50}$  of 80.11  $\mu\text{g/mL}$  (62.47-102.53). CI: Confidence interval



**Figure 1:** The effects on Caco-2 cell's viability induced by methanolic (a) and dichloromethane, (b) extracts of algae (1 mg/mL) (% of control) after 24 h obtained by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide method. Each column represents the mean of eight experiments per group; vertical lines show standard error of the mean. # $P < 0.01$  and \* $P < 0.05$  compared with control

by the MTT method. The dichloromethane extract of *P. cartilagineum* did not showed antiproliferative potential by this method [Figure 5]. For the extracts that exhibited high inhibition of Caco-2 cells, proliferation was

**Table 3:** IC<sub>50</sub> values for the methanolic and dichloromethane extracts that showed highest inhibition of Caco-2 cell's proliferation (>50%) on maximum concentration (1000 µg/mL; 24 h)

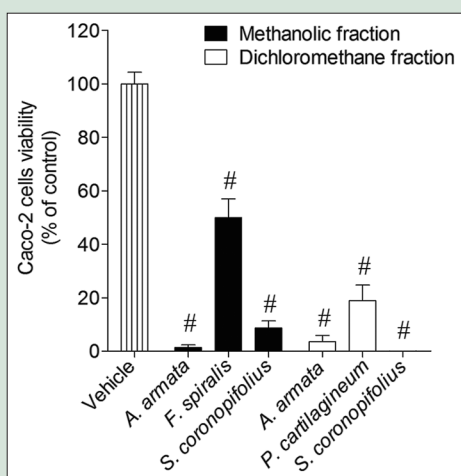
Alga	Extract	
	Methanol IC <sub>50</sub> (µg/mL)	Dichloromethane IC <sub>50</sub> (µg/mL)
<i>Asparagopsis armata</i>	508.1 (312.9-825.1)	271.5 (165.5-445.4)
<i>Plocamium cartilagineum</i>	-	343.4 (197.1-598.3)
<i>Sphaerococcus coronopifolius</i>	227.1 (161.0-320.3)	36.5 (22.5-59.2)

Values are expressed as means with 95% CI. Cisplatin was used as positive control with an IC<sub>50</sub> of 92.00 µg/mL (61.18-135.5). CI: Confidence interval

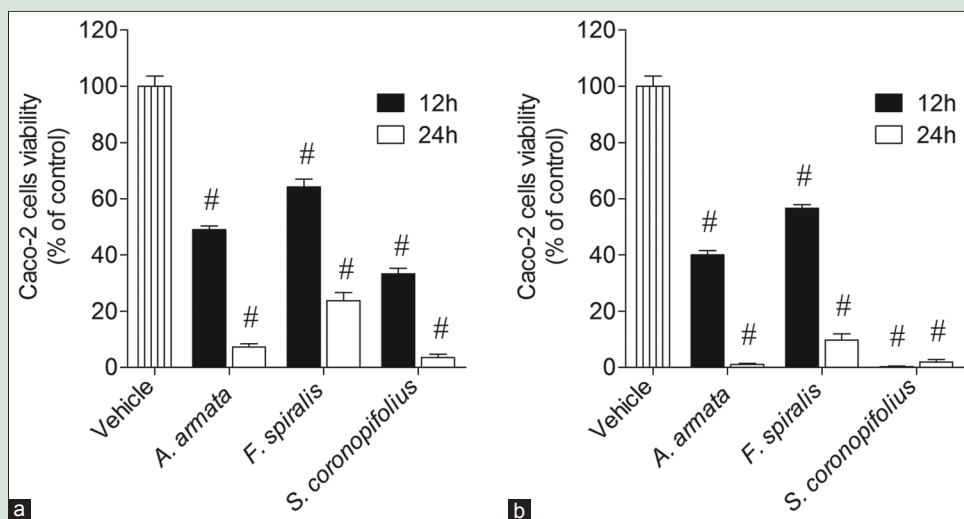
evaluated the potency of the effects. The IC<sub>50</sub> was defined testing different extract concentrations (10–1000 µg/mL) during 24 h. The results are represented in Table 3 and showed that *S. coronopifolius* had the smallest IC<sub>50</sub> both in the methanolic and dichloromethane extract, with 227.1 µg/mL (161.0–320.3) and 36.5 µg/mL (22.5–59.2), respectively. *A. armata* also demonstrated an interesting IC<sub>50</sub> on the dichloromethane extract with an value of 271.5 µg/mL (165.5–445.4). The time-course effects were assessed for the extracts that displayed the highest inhibition of Caco-2 cell's proliferation (1 mg/mL; 24 h). In this assay, the extracts were tested at 12, 24 and 48 h at 1 mg/mL. The methanolic extract of *A. armata* showed dependence of the time between 12 and 24 h, maintaining the effects after 48 h. By other side, methanolic extract of *S. coronopifolius* displayed the same inhibition in all incubation times as well as the dichloromethane extract of *A. armata*. Dichloromethane fraction of *S. coronopifolius* showed similar inhibition at 12 h and 24 h but lost activity after 48 h. *P. cartilagineum* showed dependence between 12 and 24 h and maintained approximately the activity after 48 h [Figure 6a and b].

### DISCUSSION

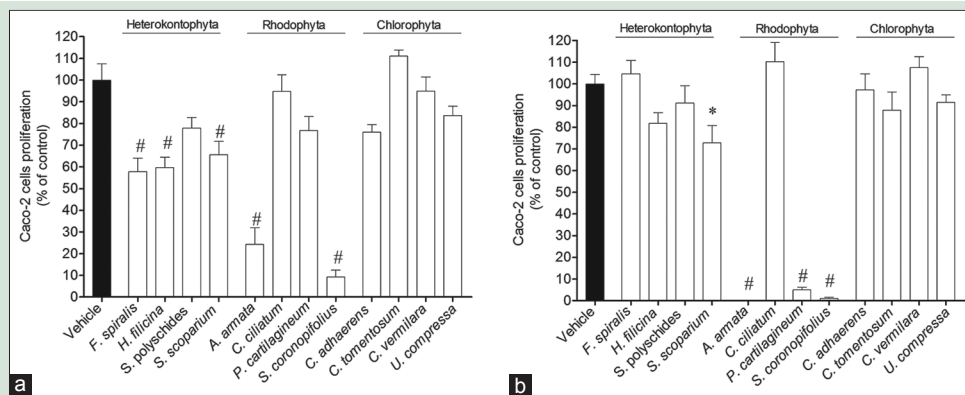
The present study was designed to evaluate the antitumor activities of different algae collected from the Portugal coast (Peniche) on an *in vitro* model of human colorectal cancer. The data gathered here indicated that four algae (*F. spiralis*, *A. armata*, *P. cartilagineum*, and *S. coronopifolius*) have interesting potential. Cancer represents a major threat against human health and one of the major causes of death in humans. Therefore, there is an increasing interest on the search and development of new anticancer drugs from marine resources.<sup>[2,24]</sup> The results obtained in this work are not surprising since algae are described as a rich source of structurally diverse secondary metabolites which includes terpenes, acetogenins, alkaloids, and polyphenolics, with many of these compounds being halogenated, which already reveal different biological activities.<sup>[25,26]</sup> It was possible to verify a high decrease of Caco-2 cell's viability and proliferation induced by algae belonging to Heterokontophyta (brown algae) and Rhodophyta (red algae) phylum. These effects can be associated with the presence of SP, which in previous works showed antiproliferative activities. SP can be found in variable amounts in the three major divisions of marine algae, Rhodophyta, Heterokontophyta, and Chlorophyta.<sup>[27-29]</sup> Fucans, for example, were



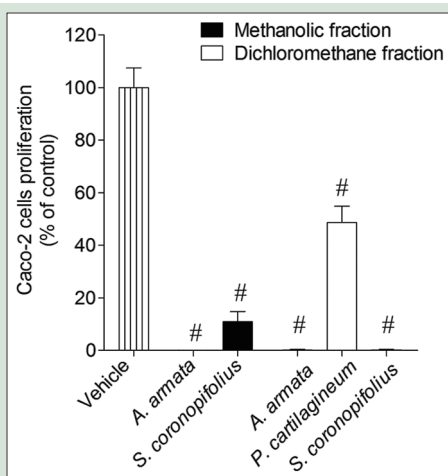
**Figure 2:** The effects on Caco-2 cell's viability induced by methanolic and dichloromethane extracts of algae (1 mg/mL) (% of control) with highest activity (>50%) after 24 h obtained by calcein-AM method. Each column represents the mean of eight experiments per group; vertical lines show standard error of the mean. *P* < 0.01 compared with control



**Figure 3:** The effects on Caco-2 cell's viability induced by methanolic (a) and dichloromethane (b) extracts of algae (1 mg/mL) (% of control) with highest activity (>50%) after 12 h and 24 h obtained by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide method. Each column represents the mean of eight experiments per group; vertical lines show standard error of the mean. *#P* < 0.01 compared with control



**Figure 4:** The effects induced on Caco-2 cell's proliferation by methanolic (a) and dichloromethane (b) extracts of algae (1 mg/mL) (% of control) after 24 h obtained by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide method. Each column represents the mean of eight experiments per group; vertical lines show standard error of the mean. #*P* < 0.01 and \**P* < 0.05 compared with control



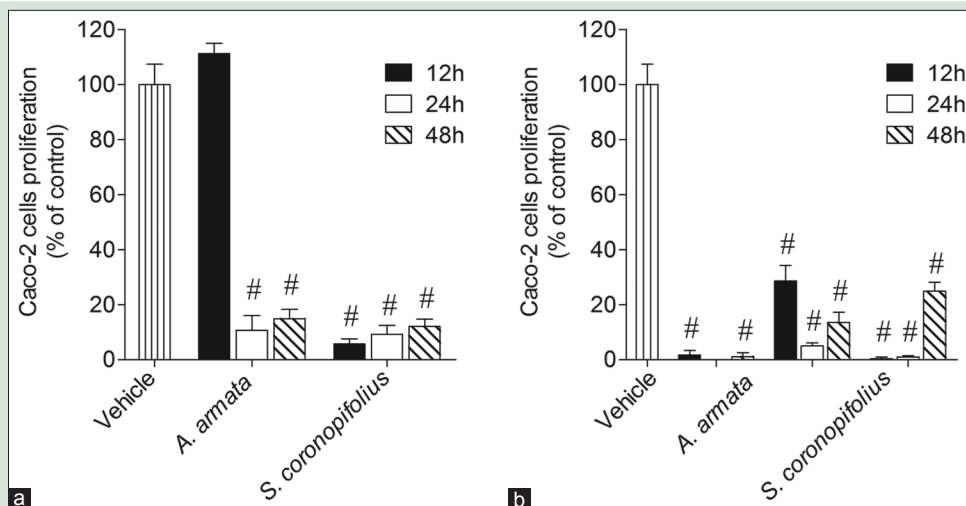
**Figure 5:** The effects induced on Caco-2 cell's proliferation by methanolic and dichloromethane extracts of algae (1 mg/mL) (% of control) with highest activity (>50%) after 24 h obtained by calcein-AM method. Each column represents the mean of eight experiments per group; vertical lines show standard error of the mean. #*P* < 0.01 compared with control

effective on several tumor cells, such as HL60 cells, sarcoma 180, murine colon cancer cell line (CT-26), mouse melanoma cell line (B-16), and human leukemia cell line (U-937).<sup>[30]</sup> As regards to *F. spiralis*, the activity exhibited can be mediated by the presence of fucoidan since this molecule was previously isolated from *Fucus vesiculosus* and strongly inhibited the growth of HCT-15 cells (human colon carcinoma cells).<sup>[31]</sup> In fact, polysaccharides are strong candidates to mediate the cytotoxic effects associated with *F. spiralis* since these molecules can be obtained with a methanolic extraction. Moreover, previous studies suggest that mechanisms mediated by fucoidan indicate induction of apoptotic processes in intestine tumor lines.<sup>[31-33]</sup> In line with these findings, this study also shows a possible cytotoxicity mechanism independent of the cell cycle inhibition since the cytotoxic effects were significantly more potent than the antiproliferative effects. However, more studies will be necessary to understand these mechanisms. Red algae (Rhodophyta division) produce a variety of secondary metabolites with considerable interest, such as mono and halogenated diterpenes, sterols, alkaloids, polyphenols, and sulfated sugars.<sup>[34]</sup> Some of these molecules have showed interesting antitumor activities on different cell lines, as for example the monoterpene-pentahalogenated halomon, which it was isolated from

the red alga *Portieria hornemannii* and shown to have cytotoxic activity on several cancer cell lines, including colon cancer cell lines.<sup>[25,35,36]</sup> These potential could also be observed by the extracts of *A. armata* and *S. coronopifolius*. Several studies have reported the antimicrobial activity of *A. armata* on different types of microorganisms; however, as concerns to cytotoxicity screenings, there are few studies.<sup>[36,37]</sup> Nevertheless, diterpenes with great interest were isolated from *S. coronopifolius* collected in the Mediterranean Sea. These molecules showed interesting results on different cellular lines and were able to overcome the natural resistance of certain tumor cells to apoptosis. Although the species studied in this work were collected from the Atlantic Coast, these molecules can also be responsible for the potent effect exhibited by the dichloromethane extract of *S. coronopifolius*.<sup>[38-40]</sup> The algae belonging to Chlorophyta did not showed activity on Caco-2 cells; however, previous studies reported antiproliferative activity by these kind of algae, namely, by SP isolated from *Ulva lactuca* that inhibit human epithelial colorectal adenocarcinoma cell's (Caco-2) proliferation.<sup>[30,41]</sup> For other side, the *Codium fragile* specimen studied by Villarreal-Gómez *et al.* (2010) did not show activity on HCT-116 cells (colorectal cancer). This situation may seem divergent; nevertheless, diverse authors have described that the bioactivities are strongly related with temporal-space variations. Moreover, the toxicity varies even intraspecies and interspecies, depending on the community or the season, pointing to the important role of biotic and abiotic factors in the production of chemical defenses by these organisms.<sup>[14,42,43]</sup> These facts can explain the data obtained for green species in this study. Regarding the time-dependent proliferation studies, all extracts showed time-dependent effects between 12 and 24 h. Nevertheless, the dichloromethane extract of *P. cartilagineum* and *S. coronopifolius* lost activity after 48 h of incubation. This fact can be explained by the existence of tumor cells that have capacity to recover from the toxicity induced by the dichloromethane extract. Analyzing the potency of the effects, the smallest IC<sub>50</sub> was exhibited by the dichloromethane extract of *S. coronopifolius* in cytotoxicity and antiproliferative tests, with 21.3 and 36.5 µg/mL, respectively. These results are particularly interesting when comparing with the IC<sub>50</sub> obtained for cisplatin (commercial drug) in cytotoxicity and antiproliferative tests (80.02 and 91.08 µg/mL, respectively), in the same test conditions of the algae extracts. It is possible to verify that the values obtained by dichloromethane extract of these algae are substantially lower.

## CONCLUSION

Our results indicate that the extracts of *A. armata*, *F. spiralis*, *P. cartilagineum*, and *S. coronopifolius* may contain compounds with great



**Figure 6:** The effects induced on Caco-2 cell's proliferation by methanolic (a) and dichloromethane (b) extracts of algae (1 mg/mL) (% of control) with highest activity (>50%) after 12 h, 24 h, and 48 h obtained by 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide method. Each column represents the mean of eight experiments per group; vertical lines show standard error of the mean. \* $P < 0.01$  compared with control

antitumor potential. Therefore, these algae reveal to be promising sources for new molecules with application on cancer therapeutics. However, it is very important to isolate and purify the molecules responsible for these activities and define their mechanisms of action, for instance through analysis of cell cycle impacts, the ability to activate caspases, and through the analysis of mitochondrial and DNA damages.

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### Conflicts of interest

There are no conflicts of interest.

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