Anticancer Effect of *Sargassum oligocystom* Hydroalcoholic Extract Against SW742, HT-29, WiDr, and CT-26 Colorectal Cancer Cell Lines and Expression of P53 and APC Genes

Ibrahem Rahem Jassim Al-Aadily¹ · Suzan Ibrahim Bajilan² · Dhafer A. F. Al-Koofee³ · Ali H. Al-Marzoqi⁴

Accepted: 12 November 2021 / Published online: 9 January 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

**Purpose** Colorectal cancer (CRC) is the third most common cancer in the world, with enhancing morbidity and mortality each year. Due to the drug resistance against CRC, the use of novel compounds besides chemotherapy is required. Natural seafood contains large amounts of biologically active substances with new chemical structures and new medicinal activities. The aim of this study was to evaluate the effects of hydroalcoholic extract of *Sargassum oligocystom* algae on SW742, HT-29, WiDr, and CT-26 CRC cell lines, and to evaluate the expression of P53 and APC genes using quantitative real-time PCR (RT-qPCR).

**Methods** The cytotoxicity of *S. oligocystom* hydroalcoholic extract was determined by MTT and trypan blue methods in six different concentrations including 0.1, 0.2, 0.5, 1, 2, and 4 mg/mL on various CRC cell lines and a control group. The expression of P53 and APC genes in exposure to 2 mg/mL of the extract was also evaluated using RT-qPCR.

**Results** The LD50 and LD90 of *S. oligocystom* included 0.5–1 and > 2 mg/mL, respectively mostly affecting SW742 and CT-26 cells. In the trypan blue test, 90% viability and death of cells were observed at 0.1 and 4 mg/mL of extract, respectively. The 2 mg/mL was a safe cytotoxic concentration. A significant viability decrease was observed at concentrations ≥ 1 mg/mL (p < 0.001). *Sargassum oligocystom* extract at 2 mg/mL significantly increased the expression of APC ranging 1.98–2.2-fold (p < 0.001) but not P53 gene which ranged 0.5–0.68-fold (p = 0.323) after 24 h.

**Conclusion** These results indicated that the brown algae *S. oligocystom* extract had significant antitumor effects against the SW742, HT-29, WiDr, and CT-26 CRC cell lines and especially CT-26, suggesting that it may be a potential candidate for further studies and therefore designing drugs of natural anticancer origin. The *S. oligocystom* had an anticancer effect via an increase in the APC gene expression.

Keywords Colorectal cancer · Cell lines · *Sargassum oligocystom* · Apoptosis

Introduction

One of the most common problems in the medical world has been the resistance of cancer cells to antitumor drugs; hence, finding novel anticancer compounds with minimal side effects seems necessary in this regard. Colorectal cancer (CRC) mainly originates from adenomatous polyps, some of which are pre-malignant and develop into cancer [1, 2]. CRC generally occurs among people > 50 years of age and older when abnormal cells divide in the large intestinal epithelium. The mortality rate from CRC is about 40%. Genetic factors and inflammation of colon epithelial cells, epigenetics, and individual behavior and nutrition are important in the onset and progression of CRC [3, 4]. Approximately 30% of cases are inherited and people who

---

1 Department of Biochemistry, Faculty of Medicine, University of Kufa, Najaf, Iraq
2 Basic Sciences Department, College of Nursing, University of Baghdad, Baghdad, Iraq
3 Department of Clinical Lab. Science, Faculty of Pharmacy, University of Kufa, Najaf, Iraq
4 Department of Biology, College of Science for Women, University of Babylon, Hillah, Iraq
consume more calories, protein, and fat are at greater risk. The apoptosis in cancer cells inhibits cancer progression [5, 6]. The development of anticancer drug resistance is also a dilemma. Anticancer drugs should act exclusively on cancer cells, while some of the chemotherapy drugs currently used in cancer patients have many side effects on the human body [7, 8]. These effects include bleeding, hair loss, diarrhea, and device suppression, so research is needed to find a compound with special targeting. Antitumor properties that have the ability to prevent the spread and growth of cancer cells have made significant progress in recent years due to the vital biological role of seaweed in the safety and improvement of life of cancer patients [7, 8]. Extensive studies in medical-industrial applications of these products have been developed and their antitumor effects have led to the pursuit of wider studies by researchers [9]. The cytotoxic effects of extracts of some green and brown algae in a dose-dependent response against leukemia in mice were subsequently studied, assessing the effect of Spirulina blue algae polysaccharide inhibitory effects against several tumors [10, 11]. The cytotoxic effects of red seaweed *Sargassum crispum* and *Sargassum oligocystom* have revealed promising results when determined with IC50 in vitro [12–14]. The apoptotic effects of brown algae have also been confirmed by microscopic observations and analytical methods by MTT assay and enzyme-linked immunosorbent assay (ELISA). Bioactive compounds that induce apoptosis in cancer cells can be considered as effective therapeutic agent. Aqueous algae compounds mostly include polysaccharides, flortanins, carotenoids, minerals, peptides, and sulfo-peptides [10, 11]. Among brown algae, *Sargassum* spp. contains a glycoprotein with anticancer effects against the human CRC [13, 14]. The anticancer effects of several algal genera, particularly *Sargassum* spp. against human leukemia cells (MOLT), K562, mouse lymphocytic leukemia cells (p.388), and 180-sarcoma cells have been revealed. Our aim was the assessment of the anticancer effect of *S. oligocystom* hydroalcoholic extract against SW742, HT-29, WiDr, and CT-26 CRC cell lines and expression of P53 and APC genes.

### Materials and Methods

#### Algae Collection and Extract Preparation

*Sargassum oligocystom* was collected from the Persian Gulf. After washing the algae, it was dried at room temperature for 2 weeks, and after cleaning the obtained fine powder, it was combined with 300 mL of deionized water and the obtained suspension was boiled for 3 h, and then, the suspension was passed through paper. The filtered hydroalcoholic extract was lyophilized into the powder and stored at 4 °C until use [15].

#### Preparation and Culture of Cell Lines

Various CRC cell lines including SW742, HT-29, WiDr, and CT-26 cells were purchased from Pasteur Institute of Iran. The cell lines were placed in DMEM medium with 10% fetal bovine serum (FBS) and 100 μg/mL of penicillin and streptomycin and incubated at 37 °C containing 5% CO2 and 90% of humidity.

#### Preparation of the Algae Extract

Firstly, 100 mg of lyophilized *S. oligocystom* powder was weighed and 1 mL of phosphate-buffered saline (PBS) was added to the powder, and after vortex, 9 mL of medium was added and the extract was reached to a volume of 10 mL and then filtered using a 0.2 μL filter and completely purified and homogenized. The prepared extract was stored at – 20 °C until use [15, 16].

#### The MTT Assay

In this method, cells (100 μL) were cultured in 96-well plates. Then, concentrations 0.1, 0.2, 0.5, 1, 2, and 4 mg/mL of the extract were prepared and each concentration was exposed to each 96-well plate containing each cell line as an independent group. The plates were incubated in 5% CO2 and 90% humidity for 24 and 48 h separately. Then, each supernatant was taken and MTT dye was added to wells and the plates were wrapped in aluminum foil and incubated for 4 h. Then, the MTT dye was taken and DMSO was added to each well and placed in the shaker for 20 min to make it completely uniform, and then, the light absorbance rate of each well was measured at 570 nm wavelength [16, 17].

#### Cells Viability Using Trypan Blue Dye

Briefly, the cells were cultured in 96-well plates containing the DMEM medium and various concentrations of *S. oligocystom* were added and incubated for 72 h at 37 °C and supplementation of 5% CO2. Next, 20 μL of trypan blue was mixed with 20μL of culture cell and the number of cells was counted using hemocytometer neobar lamella. The percentage of living cells was measured using the following formula:

\[
\text{Cells viability percentage} = 1 - \left( \frac{\text{living cells}}{\text{total cells}} \right) \times 100
\]

#### Expression of APC and P53 Genes

The cell lines were exposed to 2 mg/mL of *S. oligocystom* for 24 h. Next, RNA extraction from each cell line was
conducted using a Gen-All kit according to the protocol of the manufacturer. The real-time PCR reaction was performed at a final volume of 20λ and repeated twice for each group. The concentration of primers was 150 nM. The quantification and analysis of gene expression were performed using the semi-quantitative method considering ΔΔCT formula and Real Time PCR ABI software. The expression of each gene was repeated in triplicate and the mean level was reported for each cell line.

Data Analysis

Results analysis was performed using SPSS software version 21 and a one-way ANOVA statistical test. Quantity difference was defined at a level of 0.05.

Results

MTT Assay

The 50% cell cytotoxicity (LD50) of *S. oligocystom* against SW742, HT-29, WiDr, and CT-26 cell lines after 24 h included 0.5, 1, 1, and 0.5 mg/mL, respectively (Fig. 1). After 48 h, the LD50 of this algae included 0.2, 0.5, 0.5, and 0.2 mg/mL, respectively. Moreover, LD90 of this algae was > 2 mg/mL after 24 h and > 1 mg/mL after 48 h for all cell lines. The results exhibited that a concentration of ≥ 0.5 mg/mL of *S. oligocystom* can be considered for anticancer therapies.

According to the MTT assay, *S. oligocystom* exerted a substantial anticancer effect at 4 mg/mL; however, this concentration was also toxic against normal cells. Therefore, the concentration of 2 mg/mL was efficient against all cell lines. Moreover, these effects were time dependent.

Trypan Blue Test

In the concentration of 4 mg/mL of *S. oligocystom*, 96% of cells were killed, and at concentrations 2, 1, 0.5, 0.2, and 0.1 mg/mL, 91%, 81%, 56%, 31%, and 11% of them were killed, respectively (Table 1). There was no significant difference among various cell lines, but a significant viability decrease was observed at concentrations ≥ 1 mg/mL.

Gene Expression

The effect of 2 mg/mL of *S. oligocystom* on the expression of APC and P53 genes after 24 h ranged 1.98–2.2-fold increase (*p* < 0.0001) in the former ranged 0.5–0.68-fold (*p* = 0.323) increase in the latter genes (Table 2). Therefore,
the *S. oligocystom* had an anticancer effect via an increase in the APC gene.

**Discussion**

Cancer has an increasing trend around the world. Physicians and researchers have been trying to improve the general condition of cancer patients using different methods of chemotherapy, radiation therapy, and surgery [1–3]. However, despite the development of therapeutic interventions, the development of novel chemotherapeutics and the mortality rate of patients with CRC are still high [18]. Therefore, the application of novel alternative compounds in various extracts will be promising for inducing cell death (apoptosis) in cancer cells. It has been shown that hydroalcoholic extract of some algal species had significantly higher anticancer effects than other extracts against cancer cells [19–21]. In another study, alcoholic and chloroform extracts of *Poly-siphonia lanosa* were significantly more effective against DLD-1 and HTC-116 CRC cell lines [22]. Moreover, *Graciilaria edulis* methanolic extract had a significantly higher effect against HT-29 CRC cells [23].

In this study, the effect of hydroalcoholic extract of *S. oligocystom* was evaluated against several CRC cell lines. The 50% cell cytotoxicity (LD50) of *S. oligocystom* against SW742, HT-29, WiDr, and CT-26 cell lines after 24 h included 0.5, 1, 1, and 0.5 mg/mL, respectively (Fig. 1). After 48 h, the LD50 of this algae included 0.2, 0.5, 0.5, and 0.2 mg/mL, respectively. Moreover, LD90 of this algae was > 2 mg/mL after 24 h and > 1 mg/mL after 48 h for all cell lines. The results exhibited that a concentration of ≥ 0.5 mg/mL of *S. oligocystom* can be considered for anticancer therapies. It has been verified that *Sargassum* spp. has antioxidant and anticancer effects against some cancer cell lines such as HepG2, Hela, MDA-MB-231, MCF-7, HT-29, and LNCap in vitro. We also did not assess the in vivo results. In previous studies, *Sargassum* spp. has conferred anticancer effects at higher concentrations [21–26].

In the trypan blue test, in the concentration of 4 mg/mL of *S. oligocystom*, a mean of 96% of cells was killed, and at concentrations 2, 1, 0.5, 0.2, and 0.1 mg/mL, 91%, 81%, 56%, 31, and 11% of them were killed, respectively. There was no significant difference among various cell lines, but a significant viability decrease was observed at concentrations ≥ 1 mg/mL. It is crucial to determine a special dose for anticancer treatment using more exact verification of anticancer effects of *S. oligocystom* because of potential effects in this study.

We also observed that *S. oligocystom* significantly increased the expression of the APC gene, a regulatory gene necessary for the control of cell division. One of the most common mutations in CRC development includes the inactivation of the APC gene which results in uncontrolled cells proliferation and polypl development. However, patients with APC mutations have the risk of developing CRC approximately at the age of 40 [27]. The APC protein from mutation is truncated, abnormal, and dysfunctional. This short protein cannot prevent cell overgrowth, thus leading to the formation of polyps that can become cancerous. APC is also involved in the demonstration of microtubules by binding to the PD2 domain. APC inactivation can be initiated after specific chain reactions in the cytoplasm [28]. Mutations in the APC gene mostly occur early in cancers, such as CRC. Humans develop the CRC due to mutations in the APC gene.

In addition, P53 acts as a guardian of the genome to maintain genome stability by preventing the incidence of mutations. This suggests that the TP53 gene plays an important role in preventing cancer formation, with proteins encoded by TP53 binding to DNA and regulating gene expression to prevent genome mutation (in normal cells P53 binds to its negative regulator, MDM2 complex). Following DNA damage or other stresses, different pathways lead to the dissociation of P53 and the MDM2 complex. P53 activation causes the cell cycle arrest and allows cell repair or apoptosis [29, 30].

**Conclusion**

Herein, *S. oligocystom* was firstly studied to effect on SW742, HT-29, WiDr, and CT-26 CRC cell lines and it was confirmed that the algae hydroalcoholic extract conferred toxic activity and growth inhibition against CRC cells. Gene expression analysis exhibited cell death inducing by the extract of the *S. oligocystom* through activating and increasing the expression of the APC gene, which is a tumor suppressor gene, especially in CRC cells. According to our results, *S. oligocystom* exerted a substantial anticancer effect at 4 mg/mL; however, this concentration was also toxic against normal cells. Therefore, the concentration of 2 mg/mL was safe and efficient against all cell lines. Moreover, these effects were time dependent.

**Funding** This study was supported by Baghdad University.
Data Availability  Not applicable.

Code Availability  Not applicable.

Declarations

Ethics Approval  This study was supported by Baghdad University.

Consent to Participate  Not applicable.

Consent for Publication  The authors have the consent to submit and publish the manuscript in the journal.

Competing Interests  The authors declare no competing interests.

References

1. Kim EJ, Park SY, Lee JY, Park JH. Fucoidan present in brown algae induces apoptosis of human colon cancer cells. BMC Gastroenterol. 2010;10:96.

2. Siegel R, Naishadham D, Jemal A. Cancer statistics. CA. Cancer J Clin. 2012;62:10–29.

3. Perez EA. Impact, mechanisms, and novel chemotherapy strategies for overcoming resistance to anthracyclines and taxanes in metastatic breast cancer. Breast Cancer Res Treat. 2009;114(2):195–201.

4. Kranz D, Dobbelstein M. A killer promoting survival: p53 as a selective means to avoid side effects of chemotherapy. Cell Cycle. 2012;11(11):2053–4.

5. Athukorala Y, Kim KN, Jeon YJ. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga. Ecklonia cava Food Chem Toxicol. 2006;44(7):1065–74.

6. Turner JP, Shakhb S, Singhal N, Hogan-Doran J, Prowse R, Johns S, et al. Prevalence and factors associated with polypharmacy in older people with cancer. Support Care Cancer. 2014;22(7):1727–34.

7. Ghosh S. Cisplatin: The first metal based anticancer drug. Bioorgan Chem. 2019;88:102925.

8. Martin AC, Tomasin R, Luna-Dulcey L, Graminha AE, Naves MA, Teles RH, et al. Gingerol improves doxorubicin anticancer activity and decreases its side effects in triple negative breast cancer models. Cell Oncol. 2020;43(5):915–9.

9. Rocha DH, Seca AM, Pinto DC. Seaweed secondary metabolites: p53 as a selective means to avoid side effects of chemotherapy. Cell Cycle. 2012;11(11):2053–4.

10. Aghabozorgi AS, Bahreyni A, Soleimani A, Bahrami A, Khazaei M, Al-Rashed S, et al. Antioxidant, anticancer activity and phytochemical analysis of green algae, Chaetomorpha collected from the Arabian Gulf. Internat J Biologic Macromolecul. 2018;102:405–12.

11. Lefran C, Koutsaviti A, Ioannou E, Kornienko A, Roussis V, Kiss R, et al. Algae metabolites: from in vitro growth inhibitory effects to promising anticancer activity. Natural Product Rep. 2019;36(5):810–41.

12. Haq SH, Al-Ruwaished G, Al-Mutlaq MA, Naji SA, Al-Mogren M, Al-Rashed S, et al. Antioxidant, anticancer activity and phytochemical analysis of green algae, Chaetomorpha collected from the Arabian Gulf. Scient Rep. 2019;9(1):1–7.

13. Banoon SR, Ghasemian A. The characters of graphene oxide nanoparticles and doxorubicin against HCT-116 colorectal cancer cells in vitro. J Gastrointestinal Cancer. 2021;1–5.

14. Surits VV, Usoilteva RV, Shevchenko NM, Thinh PD, Ermakova SP. Structural characteristics and anticancer activity in vitro of fucoidans from brown seaweeds Sargassum miyabei and S. oligocystum. Chem Natural Compounds. 2020;56(1):34–8.

15. Ranabheva TH, Premarathna AD, Wijesundara RM, Wijewardana V, Jayasooriya AP, Rajapakse RP. Biochemical composition and anticancer effect of different seaweed species (in-vitro and in-vivo studies). Sustain MAr Struct. 2019;2.

16. Lim S, Cheung P, Ooi V, Ang P. Evaluation of antioxidative activity of extracts from a brown seaweed Sargassum siliquastrum. J Agric Food Chem. 2002;50(13):3862–6.

17. Li Y, Huang W, Huang S, Du J, Huang C. Screening of anti-cancer agent using zebralfish: Comparison with the MTT assay. Biochem Biophys Res Communicat. 2012;422(1):85–90.

18. Nath M, Vats M, Roy P. Tri- and diorganotin (IV) complexes of biologically important orotic acid synthesis, spectroscopic studies, in vitro anticancer, DNA fragmentation, enzyme assays and in vivo anti-inflammatory activities. Europ J Medical Chem. 2012;59:310–21.

19. Grasselli J, Eler E, Carati G, Maito J, Santos C, Macarulla T, et al. Concordance of blood- and tumor-based detection of RAS mutations to guide anti-EGFR therapy in metastatic colorectal cancer. Ann Oncol. 2017;28(6):1294–301.

20. Kiadaliri M, Firoozbakht F, Deldar H. Effects of feeding with red algae (Laurencia caspica) hydroalcoholic extract on antioxidant defense, immune responses, and immune gene expression of kidney in rainbow trout (Oncorhynchus mykiss) infected with Aeromonas hydrophila. Aquaculture. 2020;526:735361.

21. Movahed A, Ghaderi M, Daneshi A, Nabipour I, Keshavarz M. Hydroalcoholic extract of Sargassum oligocystum attenuates pentyleneetetrazole-induced seizures by potentiating antioxidant activity in mice. Internat J Epilep. 2017;4(2):159–66.

22. Moshfegh A, Jalali A, Salehzadeh A, Jozani AS. Biological synthesis of silver nanoparticles by cell-free extract of Polysiphonia alga and their anticancer activity against breast cancer MCF-7 cell lines. Micro & Nano Lett. 2019;14(5):581–4.

23. Jesus A, Corretia-da-Silva M, Alfonso C, Pinto M, Cidade H. Isolation and potential biological applications of haloaryl secondary metabolites from macroalgae. Mar Drugs. 2019;17(2):73.

24. Karimzadeh K, Zahratkeshe A. Phytochemical screening, antioxidant potential, and cytotoxic effects of different extracts of red algae (Laurencia ruderitae) on HT29 cells. Res Pharmaceutical Sci. 2021;16(4):400.

25. Pugazhendhi A, Prabhu R, Muruganantham K, Shanmuganathan N, Natarajan S. Anticancer, antimicrobial and photocatalytic activities of green synthesized magnesium oxide nanoparticles (MgONPs) using aqueous extract of Sargassum wittighii. J Photochemist Photo-biol B: Biol. 2019;190:86–97.

26. Palanisamy S, Vinosha M, Manikandakrishnan M, Anjali R, Rajasekar P, Marudhupandi T, Manikandan R, et al. Investigation of antioxidant and anticancer potential of fucoidan from Sargassum polycystum. Internat J Biologic Macromolec. 2018;116:151–61.

27. Palanisamy S, Vinosha M, Marudhupandi T, Rajasekar P, Prabhu NM. Isolation of fucoidan from Sargassum polycystum brown algae: Structural characterization, in vitro antioxidant and anticancer activity. Internat J Biologic Macromolec. 2017;102:405–12.

28. Aghabozorgi AS, Bahreyni A, Soleimani A, Bahrami A, Khazaei M, Ferns GA, et al. Role of adenomatous polyposis coli (APC) gene mutations in the pathogenesis of colorectal cancer; current status and perspectives. Biochimie. 2019;157:64–71.

29. Zhou X, Jiao D, Dou M, Zhang W, Hua H, Chen J, et al. Association of APC gene promoter methylation and the risk of gastric cancer: a meta-analysis and bioinformatics study. Medicine. 2020;99(16).

30. Gupta A, Shah K, Oza MJ, Behl T. Reactivation of p53 gene by MDM2 inhibitors: a novel therapy for cancer treatment. Biomed Res Int. 2012;59:310–21.