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Survey of Antioxidant and Cytotoxic Activities of *Gracilaria corticata* (a Red Seaweed), Against RKO, AGS and HepG2 Human Cancer Cell Lines

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ABSTRACT

One of the problems in cancer pharmaceutical treatment is development of drugs that not only have minimum complication, but are also prepared from natural compounds. Marine algae with antibacterial, antiviral, antifungal, antioxidative and anticancer potentials are one of the natural resources in this field. The source of *Gracilaria corticata* which a red alga is different sea coasts in the world including coasts in countries of India, China, Persian Gulf countries, etc. In this research, antioxidant activity and anticancer potential of aqueous extract from *Gracilaria corticata* against three kinds of human cancer cell lines, human gastric adenocarcinoma (AGS), human colon cancer (RKO) and human hepatocellular carcinoma (HepG2) were studied. Various concentration of algal extract were employed for cell treatment. In addition, extract cytotoxicity was measured using Methyl thiazolyl tetrazolium (MTT) assay in 540nm. The results showed that 9µg μl^{-1} of algal extract had the highest effectiveness against HepG2, RKO and AGS with 74%, 69% and 68% cytotoxicity effects respectively. 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) antioxidant assay was used to determine the probable antioxidant activity of this algae. Samples were prepared according to kit instruction and treated by different concentration of algal extract. Next, free radical scavenging activity of extracts was compared with measuring of the absorbency of the samples at 405nm using spectrophotometer. Descending order of the cell lines which had affected antioxidant activity of upper concentration of algal extract was as follows: HepG2, RKO and AGS respectively and the most effective concentration of algal extract that had the highest antioxidant efficacy on selected cancer cell lines was 9 µg μl^{-1}. The water crude extract of *Gracilaria corticata* had significant antioxidant and anticancer effect and it could be a good option for more studies for creating a compound with natural origin as an anticancer agent that can be employed for the development of potential anticancer medicine and new pharmaceutical endeavors.

Key words: Gracilaria corticata, RKO, AGS, HepG2, MTT assay, ABTS antioxidant assay.

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1. INTRODUCTION

The biological impact of some newly created drugs has been explored using the testing of different bioactive compounds from marine organisms (1). The results of exploring the bioactivities of marine algae have indicated different positive health-related effects, such as anti-oxidative, anti-inflammatory, antimicrobial, and anti-cancer impacts (2-6). Antioxidants have a significant role in inhibiting and neutralizing free radicals in human, providing protection against cellular damage related to cancer initiation (7). Cancer is one of the most significant serious threats to human health round the world and chemotherapy is one of the main treatment methods. Most drugs used for anticancer effects currently in chemotherapy have cytotoxic effects on normal cells and lead to immunotoxicity which not only negatively affects tumor development, but also deteriorates patient's status in some respects. Consequently, a major objective of researchers in the domain of immunopharmacology has been developing new antitumor drugs with low negative effects on immune system (8, 9). Marine algae are natural substances found in the marine ecosystem. Currently over 2400 marine natural products are developed from seaweeds of subtropical and tropical populations around the world (10). Water soluble antitumor active materials from

different marine algae have been highly explored. Most anticancer drugs have not been used clinically due to their negative effects on normal human cells (11). According to existing literature, more than ten new experimental antitumor agents developed from marine sources by researchers have been used in clinical trials, including bryostatin-1, aplidine, ecteinascidin-743 (ET-743), Kahalalide F, and some dolastatin derivatives like TZT-1027 and LU 103793 (12). *Gracilaria corticata* is a red alga which can be obtained from many sea coasts throughout the world like China, India, Persian Gulf, and the like (9). Here, the *in vitro* antioxidant and antitumor activities of aqueous extract of *Gracilaria corticata* on three human cancer cell lines was evaluated.

2. MATERIALS AND METHODS

2.1. Algal extract preparation

Gracilaria coticata which is a red alga, was obtained along the Bushehr coast of Persian Gulf. Afterwards, it was rinsed with distilled water. Around 10 g of fresh alga, being equal to 1 g of dried material, was completely homogenized in 100 ml of cold double distilled water. Algal mixture clarification was done using filtration through Whatman paper No.1 filter paper. At the end, algal clarified crude extract sterilization was done using millipore filter with 0.22 µm pore size and the extract was stored at -80°C until the date of use (9).

2.2. Cell lines

Human gastric adenocarcinoma (AGS), human colon cancer (RKO) and human hepatocellular carcinoma (HepG2) cell lines were purchased from American Type Culture Collection (ATCC), Manassas, USA.

2.3. Cell culture

The cells culturing was done in 50 ml cell culture flasks or 96 wells cell culture microplates by using Eagle's Minimum Essential Medium (EMEM), Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute (RPMI 1640) (Gibco) containing 10% fetal bovine serum or FBS (Gibco). Then, cell incubation was done at the temperature of 37°C in the presence of 5% CO_2 (13).

2.4. MTT assay test

For evaluation of the cytotoxicity of algal extract against the explored cancer cell lines, MTT assay test was employed as a quantitative and authentic method. In the aforementioned method, 10 µl of MTT stock solution (5 mg ml^{-1} in phosphate buffered saline or PBS) was added to 90 µl medium of wells which were treated using various algal extract concentrations (3, 6 and 9 µg $µl^{-1}$) for 72 h. The incubation of microplate was then done at 37°C for 4 h and afterwards, the optical density of each well was determined using microplate reader (Epoch – Biotek) at 540nm (14).

2.5. ABTS antioxidant assay test

For determining the overall antioxidant capacity of algal extract on the studied cancer cell lines, Zen-Bio ABTS Antioxidant Assay Kit was employed. The assay measures $ABTS^{+}$ radical cation creation resulted by metmyoglobin and hydrogen peroxide. For this assay, 10 µl of samples or Trolox standards were added to each well of the assay plate provided. 10µl of assay buffer was added to individual wells as a negative control. Next, 20 µl of the myoglobin working solution was added to each individual wells that contained standards and samples from previous step. To begin the assay, 100 µl of the ABTS solution was added to each well and placed on plate shaker at ambient room temperature. The reaction was allowed to proceed for 5 minutes and, in order to stop the reaction, 50 µl of stop solution was added per well. Absorbance at a wavelength of 405nm was read using plate reader and results were compared based on protocols (15-17).

3. RESULTS AND DISCUSSION

3.1. Cytotoxic activity of Gracilaria corticata against three cancer cell lines

We designed the following models of test for this assay and cells seeded in 6 wells per group.

Model Ⅰ: the group with cells only without algal extract(control group).

Model Ⅱ: the group with cells treated by 3 µg $µl^{-1}$ algal extract

Model Ⅲ: the group with cells treated by 6 µg $µl^{-1}$ algal extract

Model Ⅳ: the group with cells treated by 9 µg $µl^{-1}$ algal extract

The result of cytotoxic effect of algal extract on RKO, AGS and HepG2 cell lines which were monitored through MTT assay and related data was provided in Table 1.

Table 1. Average absorbance of MTT assay results in 4 models of designed test at three cancer cell lines, RKO, AGS and HepG2

Models/cell lines	Average absorbance at RKO cell line	Average absorbance at AGS cell line	Average absorbance at HepG2 cell line
Model Ⅰ (group with cells only without algal extract)	0.905 ±0.0054	0.906 ±0.0121	0.905 ±0.0084
Model Ⅱ (group with cells treated by 3 µg µl^{-1} algal extract)	0.420 ±0.0167	0.413 ±0.0150	0.395 ±0.0104
Model Ⅲ (group with cells treated by 6 µg µl^{-1} algal extract)	0.301 ±0.0147	0.295 ±0.0176	0.293 ±0.0121
Model Ⅳ (group with cells treated by 9 µg µl-1 algal extract)	0.276 ±0.0121	0.289 ±0.0949	0.238 ±0.0090

According to these data, it was shown that average absorbance for all cell line was decreased from model 1 to model 4. As shown in Table 1, by increasing concentrations of algal extract, the average absorbance and consequently viable cells were reduced which indicated the cytostatic activity of the algal extract against cancer cell lines. The most effective concentration in which the number of viable cells was significantly reduced was 9 µg µl^{-1} of algal extract. Dead cells were observed in every microplate well. The greatest number of dead cells has been seen in well whose cells were treated using 9 µg µl^{-1} of algal extract. Percentage of cytotoxicity of algal extract against RKO, AGS and HepG2 cell lines was calculated by statistical analysis that summarized in Table 2.

Percentage of cytotoxicity of Algal extract (%) was equal to (optical density (OD) of control-OD of treatment/OD of control)*100. According to the obtained data, it was concluded that 9 µg µl^{-1} of *Gracilaria corticata* extract showed potent cytostatic effect against HepG2, RKO and AGS respectively.

Table 2. Percentage of cytotoxic effect of different concentration of algal extract against RKO, AGS and HepG2 cell lines

Percentage in different Cell lines/ concentration of algal extract	*RKO Cell line*	*AGS Cell line*	*Hep G2 Cell line*
3 µg µl^{-1}	54%	55%	60%
6 µg µl^{-1}	67%	67%	67%
9 µg µl^{-1}	69%	68%	74%

3.2. Antioxidant activity of Gracilaria corticata against three cancer cell lines

The formation of ferryl myoglobin radical is originated in metmyoglobin and hydrogen peroxide. The ferryl myoglobin radical cause oxidization of ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), forming a radical cation, $ABTS^{\cdot+}$, that has a green color and is measurable using absorbance at 405nm. Antioxidants inhibit this reaction through electron donation radical scavenging and prevent colored ABTS radical development. Antioxidant concentration in the test sample is inversely proportional to the ABTS radical creation and 405nm absorbance. Results of average OD of ABTS radical cation at 405 nm in three cancer cell lines with treatment of different concentration of algal extract were compared in Table 3. As shown in this Table, by treatment of higher concentration of algal extract, average OD in all cancer cell lines was reduced more. Because antioxidants prevent the oxidation of ABTS through electron transfer radical scavenging and so decrease OD of samples at specific wavelength, it can be said that algal extract had the antioxidant activity against selected cancer cell lines. According to obtained data and statistical analysis, the most effective concentration of algal extract that had the highest antioxidant efficacy on selected cancer cell lines was 9 µg µl^{-1}. Descending order of the cell lines which had affected with antioxidant activity of upper concentration of algal extract was as follows: HepG2, RKO and AGS.

Table 3. Comparison of Average OD of ABTS radical cation in three cancer cell lines, RKO, AGS and HepG2 with treatment of different concentration of algal extract

OD in different Cell lines/ Concentration of algal extract	*RKO*	*AGS*	*HepG2*	*P value*
0 µg µl^{-1}	0.712± 0.0166	0.623± 0.013	0.761± 0.043	RKO vs AGS P<0.0001
3 µg µl^{-1}	0.707± 0.024	0.601± 0.0124	0.761± 0.0195	RKO vs HepG2 P<0.01
6 µg µl^{-1}	0.620± 0.020	0.565± 0.009	0.547± 0.010	AGS vs HepG2 P<0.0001
9 µg µl^{-1}	0.460± 0.007	0.425± 0.020	0.403± 0.032	RKO vs AGS P<0.0001

Currently more than 2400 marine natural substances have been isolated from seaweeds of subtropical and tropical

populations (9). Some algae have been employed in traditional Chinese herbal medicine for the treatment of cancer for many years (18). For exploring the bioactive compounds produced by marine algae, many studies have been conducted (19). The effect on cancer cell lines is one of the main significant characteristics of marine algae, and many algae have exhibited cytotoxic and antioxidative activities. Various metabolites including bromophenols, carotene and steroids were isolated and purified in some algae and their effects on some cancer cell lines were verified (20). In addition, in another study conducted, it was indicated that the sulfated compounds like fucoidans extracted from Sargassum polycystum and some other brown algae showed significant effects against some cancer cell lines in human body (21). In this study, the water crude extract of *Gracilaria corticata* was explored in order to determine its possible antitumor and antioxidant activities against RKO, AGS and HepG2 cell lines which are three types of human carcinoma cell lines. According to previous experience, filtration method is the optimal option for algal extract sterilization (22). The heat sensitivity of some biological constituents of algal extract is the main reason behind not employing autoclave for extract sterilization (23). In this study, the cold water extract of *Gracilaria corticata* indicated moderate activity against tumor cells replication. The results indicated that 9 µg μl^{-1} of algal extract had the greatest effect against HepG2, RKO and AGS with 74%, 69% and 68% cytotoxicity effect respectively. The result of MTT assay for all cell lines is consistent with the data of conducted viability tests for same cell lines. According to obtained data and statistical analysis, the most effective concentration of algal extract that has the highest antioxidant efficacy on selected cancer cell lines was 9 µg μl^{-1}. Descending order of the cell lines which had affected with antioxidant activity of upper concentration of algal extract was as follows: HepG2, RKO and AGS. Extracts that showed a good antioxidant property were proved to have a significant anticancer activities. In this study, the effective concentration is higher than those in similar studies that employed purified biological active compounds instead of crude extract. Therefore, future studies are recommended to do fractionation and purification of *Gracilaria corticata* extract. In addition, in relation to the significant findings of this study, further studies such as in vivo anticancer activity of *Gracilaria corticata* evaluation is suggested and it could result in discovering new natural compounds effective against tumors. The red marine alga, *Gracilaria corticata* is a worthy alga as it has anticancer effects against human leukemic cell lines as well as antioxidant effects. As a result, final application of this alga can be explored more and could be a significant effort in the field of natural antitumor studies.

4. CONCLUSION

In the present research, the authors have indicated that the aqueous extract of *Gracilaria corticata* as a type of marine algal natural products is rich source of antioxidants which demonstrated significant cytotoxic activity against selected human cancer cell lines. The authors found that water crude extract of this alga like other algal extracts and their bioactive components had significant modulating effects against oxidative stress and oxidative stress-related diseases like cancer. In the future, these marine algae-derived materials will be employed more frequently in pre-clinical investigations for developing drugs.

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CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this paper.

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