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INVESTIGATING ANTICANCER POTENTIAL OF RED ALGAE (RHODOPHYCEAE) FROM GWADAR SEA SHORE

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Abstract

Cancer is one of the most dangerous diseases in the world due to its poor prognosis, negative treatment outcomes, and medication resistance. Nowadays, many chemical therapies and harsh techniques are used to cure cancer, which can affect the body's other healthy cells. For this reason, red algae are a potential treatment option. The red algae have a low calorie content and are high in antioxidants, polyunsaturated fatty acids, proteins, minerals, vitamins, and soluble dietary fibers. However, just like other plants, their nutrient contents are affected by outside variables like the season, environment, geographic location, and sampling conditions. The purpose of this study was to check the anticancer activity of red algae (Rhodophyceae) which were collected from the sea shore of Gwadar, Balochistan, Pakistan. Five grams of the powder algae was macerated by immersing it in fifty milliliters of solvent (methanol 100%, chloroform/methanol (3:2), and dichloromethane 100%) for seventy-two hours. Then the samples were filtered, and a rotary evaporator operating at low pressure at 30 to 35°C was used to dry out the resulting algae extracts. The Cancerous cells were used to check the anticancer potential of the extract obtained from red algae. MTT assay was used to check the anticancer activity. Results were calculated with the help of IC_{50} calculation, and from IC_{50} calculation, the percent viability of cells was obtained.

Keywords: *Anticancer, Breast cancer, Cell viability, Gwadar, MTT assay, Red algae*

INTRODUCTION

Cancer is among the life threatening dieses with global prevalence having higher rates in developing countries. The drugs being used for the treatment of cancer impose harmful effects on healthy cells besides cancerous cells (1). Among the deadliest diseases in the world is cancer. Over the past ten years, there have been consistent advancements in the fields of therapeutic and chemopreventive medicine; yet, the hunt for new anticancer treatments has not stopped (2). The red algae (*Rhodophyceae*) has been reported to contain many biological compounds like poly saccharide which helps fight with cancer (1). Exploring that are particularly compound found in nature that can be used as chemotherapeutic agents, slow down the cancer cell growth or even reverse human carcinogenesis is the need of time. There is an urgent need to produce additional anti-cancer alternative and supplemental preventative or therapeutic drugs due to the rising incidence of cancer. A technique to stop, halt, or even reverse human carcinogenesis is called chemoprevention, using compounds occurring naturally in nature (3).There is very less work regarding anticancer activity of algae in Pakistan, particularly Balochistan. To check how much local red algae will be effective to cancer cells that's why red algae from the sea shore of Gwadar were tested.

The red algae have a low calorie content and are high antioxidants, polyunsaturated fatty acids, proteins, minerals, vitamins, and soluble dietary fibers. However, just like other plants, their nutrient contents are affected by outside variables like the season, environment, geographic location, and sampling conditions (4). The mechanisms behind the possible anticarcinogenic benefits of seaweed elements are being considered to explore their anticancer potential through cell culture and cell-free investigations. These

investigations include the assessment of antimutagenicity of kelp and red algal extracts against aflatoxin B1, N-methyl-N′-nitro-N-nitroguanidine, and breast and colon cancer inducers (5). With its wide range of species, the marine environment is a relatively unexplored source of new chemicals with strong anticancer potential in this field. Although there have been numerous reviews of marine anticancer compounds published, Red algae sustained angiogenesis, resistance to anti-growth signals, apoptosis avoidance, growth signal self-sufficiency, unrestricted reproduction, tissue invasion, and metastasis are the main topics discussion among researcher (2).

Numerous red algal polysaccharides, including agarans, carrageenan, alginate, fucan, laminaran, and naviculan, have antiviral properties. Depending on the chemistry of various secondary metabolites and cell line metabolism, the soluble fibers found in red algae's sulfated polysaccharides and carraginans may have anticancer properties (6). It has been noted that red algae have a variety of carotenoid compositions that are frequently under consideration (7). Given the paucity of knowledge regarding metabolites in seaweeds in comparison to terrestrial plants, results of this study will serve as a resource and guide for nutraceutical research projects (8). In some studies the scientists offer the first comprehensive, systematic metabolomics analysis of seaweed species (9).

One of the most significant characteristics of algae is their cytotoxic activity in cancer and tumor cell lines; in fact, numerous species have demonstrated these bioactive effects (10). Due to exposure to chemicals and pollutants, the body may have an imbalance between free radicals and antioxidants, which could result in an excess of free radicals and lower the biological defense system's capacity, causing irreversible oxidative damage (11).

METHODOLOGY

STUDY AREA AND SAMPLE COLLECTION

Samples of red algae (*Rhodophyceae*) were collected from seashore of Gwadar. The samples were shade dried and then were transported to CASVAB, University of Balochistan, Quetta for further processing.

IDENTIFICATION OF THE SAMPLE

The thallus of red algae is bushy 15 cm long. It is branched with smooth and proliferous margins. The cortex is rounded and medullary cells are flattened. The color of this alga is brownish red. The samples were washed with tape water so the samples should be cleaned from exotic and debris materials. After that Slides were made by mounting the crushed thalli of bigger seaweeds in 1% methylene blue in 5% HCl to observe the transverse section of the thalli. After that Acetic acid, distilled water, and glycerin were added on the slide. Cutex nail polish was then used to seal each slide. The slides were observed under the light microscope in order to identify the species (12).

EXTRACTION METHOD

The shade dried samples were chopped into tiny bites, and with the help of a mixer grinder the samples were ground and converted into powder. The 5g of algae powder was soaked in 50ml solvent which contain methanol 100% 12.5ml, chloroform/methanol (3:2) 25ml and dichloromethane 100% 12.5ml for 72 hours. This process was repeated for three times. After that the soaked samples were filter with whatman filter paper. After filtration the extract was dried (13).

CELLS INOCULATION WITH RED ALGAE DILUTIONS IN 96 WELLS

In order to inoculate cancerous cells with extract of red algae the stock solution 30mg/3ml of extract was prepared. To prepare stock solution 30mg extract was dissolved in 95 micro liter DMSO and 2905 micro liter cell culture media. Using this stock solution, five dilutions of red algae which were prepared in order 10µl/1000µl, 20µl/1000µl, 40µl/1000µl, 80µl/1000µl and 160µl/1000µl. These dilutions were used to inoculate human breast cancer cell line, MDA-MB-231 which was taken from the cell bank of CASVAB already grown in 96 well plates having confluency of around 70–80%.

CELL VIABILITY USING MTT ASSAY

After inoculation, MDA-MB-231cells which were grown in 96 well plates cell viability was assessed using quick colorimetric test utilizing the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) MTT assay. The incubation post inoculation was complete, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution prepared by dissolved it in PBS at a concentration of 5mg/ml was added to the plate and again incubated at 37°C for four hours (13). After that the Formazine was converted into formazan by adding dimethyl sulfoxide (DMSO), which changed the color. Than microplate reader was used to note absorbance at 630 nm. Blank and negative controls were also added to the plate besides inoculated wells in order to calculate the percentage viability using the formula below (14).

$$\%\text{viability} = \frac{(\text{ODSample} - \text{OD} - \text{Blank})}{(\text{OD Control} - \text{OD} - \text{Blank})} * 100$$

RESULTS

A standard curve was constructed to examine the results, and a percentage viability test was performed against rising extract concentrations. The concentration needed to achieve 50% inhibition (IC_{50}) was also calculated. The dose-response record was fitted using linear regression in order to get the IC_{50} value. The Hill coefficient formula was utilized to determine the IC_{50} value. The extract was found to be effective against MDA-MB-231 breast cancer cells by showing decreased viability with the increasing concentration of the extract. These findings also point to a possible dose-dependent impact of red algae extract on breast cancer cells. The IC_{50} value for MDA-MB-231 breast cancer was 26.9625. The result was calculated with the help of internet tool https://www.aatbio.com/tools/ic50-calculator .

Table I. The decreasing % viability of the cells by increasing the concentration of red algae extracts dosage

Cons.	% viability
10	92.3566879
20	87.4469214
40	76.0350318
80	72.2001062
160	68.3519108

$$Y = \text{Min} + \frac{\text{Max} - \text{Min}}{1 + (\frac{x}{ic50})}\ \text{Hill coefficient}$$

$$Y = 0.1405 + \frac{1.0273 - 0.1405}{1 + (\frac{X}{26.9625})^{3.476}}$$

$$IC_{50} = 26.9625$$

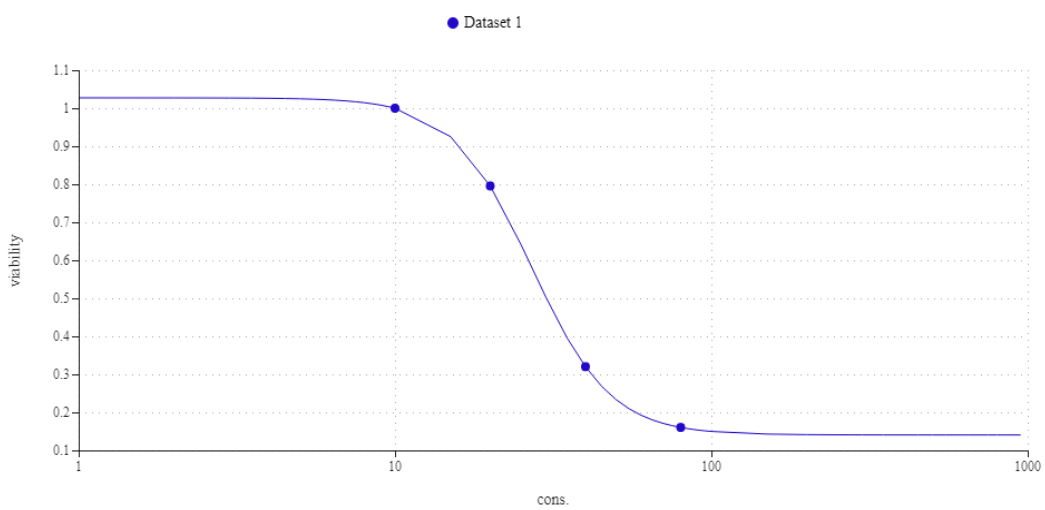

Fig. 1. The graph showing decreasing viability of cancerous cells with increasing concentration of red algae extract

DISCUSSION

This study focused on the anticancer activity of red algae from the seashore of Gwadar. There were different studies on the anticancer activity of red algae but data regarding red algae found in waters of Gwadar is not available. Marine macro-algae may generate a wide range of secondary metabolites that are beneficial to human health and contain bioactive. Omar, H. et.al (2018) reported the use of extract of algae to study DPPH radical scavenging activity and the MTT to check the cell viability using HCT-116 colon cancer. They checked the phytochemical composition of *L. papillosa*. The result was checked by performing FT-IR and GC-MS analysis for the most effective algal extract. Two components were shown by the result (1) dichloromethane and (2) methanol. Among the algal extracts these two components dichloromethane and methanol contain the highest antioxidant and anticancer activities with a very low IC_{50} which is 110.8 and 196 µg mL–1, respectively (15). In this study the chemicals used are same methanol and dichloromethane by which show that above work is supported by this work. Another research show that the red sea alga contain polysaccharides which can be a viable source of natural chemicals for anticancer and antioxidant treatments by Gheda, S. et.al (2018). The study showed that Lactate dehydrogenase enzyme activity was measured by using it as a biomarker of cell membrane integrity, and sea algal polysaccharides was used as an anticancer drug in vitro against colon cancer cell line and breast cancer cell line cell lines was assessed. The result showed that the red seaweed exhibited the greatest 2, 2 diphenyl-1-picrylhydrazyl free radical scavenging activity, according to an in vitro test of the antioxidant activity of eight marine seaweed species. To check the result the MTT assay was used to investigate the cytotoxic effects and anticancer potential of the isolated polysaccharides from red sea algae at doses ranging from 0.1 to 40.0 mg/mL against colon cancer cell lines. The red sea algal polysaccharide extract's inhibitory concentration at 50 (IC_{50}) value was 0.312, 5 mg/mL for MCF7, and 20 mg/mL for colon cancer cell line as Compared to the untreated cells (1). Results of these studies support the results obtained in present study in which IC_{50} value is 26 this show that the red algae which was collected from sea shore of Gwadar has the potential of anticancer activity.

Haq . *et al.,* (2019) reported that seaweeds are a type of marine multicellular algae that are rich in polysaccharides, minerals, and vitamins. They are thought to be a possible source of bioactive compounds with strong anticancer, antioxidant, and antibacterial qualities, including proteins, lipids, and polyphenols. The presence of antioxidant phytochemical elements in extracts from seaweeds a genus of marine multicellular algae, has drawn interest due to its potential to protect human health cure. The assessment of macro-algae extracts' antioxidant phytochemical components has drawn interest due to its significant contribution to the prevention of human illnesses. It was believed that the antioxidant compounds found in algae, including alkaloids, flavonoids, phenols, tannins, phlorotannin, terpenoids, pigments, glycosides, and steroids, served as a defensive mechanism, shielding the organisms from reactive oxygen species (ROS) brought on by severe environmental circumstances which can cause cancer. The study was applied on the breast cancer. The findings showed that the chaetomorpha sp. species under investigation has extremely special and innovative components as well as strong antitumor chemical elements. It can also function as a potential antioxidant and anticancer agent for use in pharmaceutical businesses in the future (16). These finding also support the results of this study which showed potential anticancer activity against MDA-MB-231 breast cancer cell line.

Data regarding anticancer activity of algae found in Pakistan, particularly Balochistan is scantly. This study could serve as a milestone in exploring anticancer potential of local algae. In Pakistan a work was done by Mustafa *et al.,* (2025) which is about marine algae but not specifically for red algae our work is specifically for red algae. Their study was about how marine red algae worked, which included controlling the immune response, causing cell death, and inhibiting angiogenesis. Their study showed the effectiveness of certain compounds, such as polysaccharides, against various types of cancer was discussed, along with the techniques employed to separate them. They discussed that the Natural compounds made from algae provide a promising new route, even though advancements in a number of areas show promise. They discussed that Algae's varied chemical makeup and potential benefits, such as expedited action and less toxicity, make them a viable alternative to traditional chemotherapy. They said that further study in this

area might yield a wealth of knowledge and pave the way for safer and more effective cancer therapies, even if the use of compounds produced from algae in medicine development is still in its infancy. The potential of different types of algae in the battle against cancer (17). So by combining the results of different research, it can be concluded that the red algae shows a very good potential of anticancer activity.

CONCLUSION

In the conclusion the study discovered anticancer effects in an extract of red algae (*Rhodophyceae*) from the seashore of Gwadar. Experiment performed in this study shows the evidence of dose dependent effect of red algae extract on MDA-MB-231 breast cancer cell line. Decrease in cell viability was considerably evident with the increasing concentration of extract obtained from red algae. This opens wide array of avenues to further investigate and specifically identify and evaluate the bioactive compounds found in red algae as anticancer treatment not only against breast cancer but also for the treatment of other types of cancer. In future there should be in vivo testing of red algae against different cancer cells.

Conflict of Interest:

The authors report no conflicts of interest

Author's Contributions:

MMR Conducted the experimental work and data collection; AU Supervised the study and reviewed the manuscript; ZT Performed data analysis and interpretation; HAA Assisted in laboratory assays; PG Contributed to sample collection and processing; SAB Participated in manuscript drafting and editing.

Declaration of generative AI-Assisted Tools:

No AI-assisted tools were used.

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