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Anticancer Activity of *Sargassum Sp.* Seaweed Crude Extracts against the Breast Cancer Cell Line

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Abstract

Introduction: Breast cancer is a cancer that develops from breast tissue. Signs of breast cancer include lumps in the breast tissue, change in shape, dimpling of the skin, and fluid coming from the nipple or a red scaly patch of skin. People with distant spreads of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin. Seaweed is a marine macro alga comprising many geniuses and species. *Sargassum* is a marine sulfated polysaccharide, extracted from brown seaweed that has a wide range of bio activities including anticancer properties. Marine organisms generally produce a variety of compounds with pharmacological activities.

Aim: The aim of this study is to estimate the anticancer activity of *Sargassum sp*. against breast cancer cell line.

Materials and method: The effect of *Sargassum Sp*. on breast cancer cell viability was measured by MTT assay following the method by Mossman. Briefly, MCF-7 cells were plated in 48 well plates at a concentration of 2 × 104 cells/well with replications. Treatment was conducted for 24 h with different concentrations of *Sargassum sp*. The IC50 was calculated as the concentration of sample needed to reduce 50% of the absorbance in comparison to the DMSO-treated control. Cell morphological changes were observed in phase contrast microscopy.

Results: The concentration of *Sargassum Sp*. extract and the viability of cancer cells. From this, it is seen that with increase in concentration of the extract, the viability of the cell decreases. So, it is clearly understood that this extract possesses significant anticancer activity against the breast cancer cell line. The cell viability was decreased in a time and dose dependent manner.

Conclusion: Our study indicates that the brown marine algae *Sargassum sp*. Collected from Thondi, Coastal areas have promising anticancer activity. In future, this can be developed into a new anticancer drug to be used, which has lesser side effects when compared to the commercially available synthetic drugs.

Keywords: *Sargassum sp*; Seaweed; Anticancer; Breast cancer; Cytotoxicity; Eco friendly

Introduction

Breast cancer is a cancer that develops from breast tissue. Signs of breast cancer include lumps in the breast tissue, change in shape, dimpling of the skin, and fluid coming from the nipple or a red scaly patch of skin [1]. People with distant spreads of the disease may have bone pain, swollen lymph nodes, shortness of breath, or yellow skin [2]. Risk factors may be obesity, lack of exercise, alcohol, hormone replacement therapy during menopause or Klinefelter syndrome [3]. Genetics is believed to be the primary cause of all cases. Common cause is hereditary breast-ovarian cancer syndrome. Diagnostic methods used for breast cancer are tissue biopsy and mammography [4]. Treatments done are surgery, radiation therapy, chemotherapy, hormonal and targeted therapy. Fucoidans are also valuable biologically active polysaccharides of brown algae. The native and DE acetylated Fucoidans inhibit the colony formation of human colon cancer cells. DE sulfated Fucoidans possessed weak anti-cancer activity. Sequence analysis has revealed that Fucoidans belongs to the GH107 family [5]. Recombinant Fucoidans was used to produce fuco oligosaccharides. Neutral and acidic polysaccharides and their protein complexes were fractionated and purified from the brown seaweed umitoranoo (*Sargassum thunbergii*) by fractional extraction, ion exchange chromatography, and gel filtration [6]. In ancient days, *Sargassum sp*., brown seaweed, has been used in the Traditional Chinese Medicine (TCM) to treat a variety of diseases including thyroid disease (eg. goiter). Seaweed is a marine macro alga comprising many geniuses and species. Sargassum is a marine sulfated polysaccharide, extracted from brown seaweed that has a wide range of bio activities including anticancer properties [7,8]. Marine organisms generally produce a variety of compounds with pharmacological activities. Discovery of anticancer drugs that kill or disable tumour cells in the presence of normal cells without undue toxicity is a potential challenge [9]. The ethanol fraction of *Sargassum sp*. induced cell shrinkages, cell membrane blabbing and formation of apoptotic bodies with evidence of bioactive components as profound influencing factors for antitumor effects. Some metabolites isolated from the algae, influence proliferation, apoptosis and cell cycle arrest with different mechanisms. Some mechanisms which are suggested included increasing natural killer cells, activation of nonspecific immune systems, inhibition of cell growth, angiogenesis and induction of apoptosis. The bioactive compounds in *Sargassum* species appear to play a role as immunomodulatory and could be useful in the treatment of thyroid related diseases such as Hashimoto's thyroiditis. Some studies have found both the preventative and therapeutic role of Sargassum species in thyroid health [10,11]. Our team has extensive knowledge and research experience that has translate into high quality

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publications [12-18]. The main objective of this study is to estimate the anticancer activity of *Sargassum* species against the breast cancer cell line.

Materials and Methods

Chemicals

DMEM medium, 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, Bovine Serum Albumin(BSA), low melting agarose, MTT from Sigma Chemicals Co. St. Louis, USA Fetal Bovine Serum(FBS) and antibiotic/antimitotic solution, DMSO were from HIMedia, Sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid and methanol were purchased from Sysco Research Laboratories (SRL), India.

Cell line maintenance

Human breast cancer cell line MCF-7 was procured from the National Centre for Cell Science (NCCS, Pune), India. The cells were grown in T25 culture flasks containing DMEM medium supplemented with 10% FBS. Upon reaching confluence, the cells were detached using Trypsin-EDTA solution.

Cell proliferation (MTT) assay

The proliferation of MCF-7 cells was assessed by MTT assay Safadi. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in color. Then the cells are lysed using a 20% SDS solution, which releases the formazan crystal. These crystals are solubilized by DMF present in the solubilize. The color developed is then determined in an ELISA reader at 620 nm. MCF-7 cells were plated in 48 well plates at a concentration of 2×10^4 cells/well 24 hours after plating; cells were washed twice with 500 µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37°C. After starvation, cells were treated with *Sargassum spp*. different concentrations for 24 hours. At the end of treatment, the medium from control and *Sargassum spp*. treated cells were discarded and 200 µl of MTT containing DMEM (0.5 mg/ml) was added to each well. The cells were then incubated for 4 h at 37°C in the CO_2 incubator.

The MTT containing medium was then discarded and the cells were washed with 1 × PBS. The crystals were then dissolved by adding 200 µl of solubilization solution and this was mixed properly by pipetting up and down. Then the formed crystals were dissolved in dimethyl sulfoxide (200 µl) and incubated in dark for an hour. Then the intensity of the color developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability=(A570 nm of treated cells/A570 nm of control cells) × 100.

Morphology study

Based on MTT assay we selected the optimal doses (150 µg/ml) for further studies. Analysis of cell morphology changes by a phase contrast microscope. 3×10^4 cells were seeded in 6 well plates and treated with *Sargassum spp*. (150 µg/ml for MCF-7 cells) for 24 h. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase contrast microscope.

Results and Discussion

Breast cancer is one of the most exciting recent advances in cancer research that has largely passed by breast cancer [4,5] (Figures 1 and 2). Immunotherapy has had tremendous success in melanoma, kidney cancer, lung cancer and prostate cancer. But no immunotherapy drugs have been approved for breast cancer. The chance that a woman will die from breast cancer is about 1 in 39 [2]. The first line treatment for this cancer is advanced hormone receptor positive (oestrogen receptor-positive or progesterone receptor positive), usually hormone therapy. Postmenopausal women with breast cancer may forgo chemotherapy. Having a heart attack may make breast cancer grow faster. Recent studies show tests that analyse tumour DNA in blood, called liquid biopsies, may help detect cancer early, guide precision cancer treatment

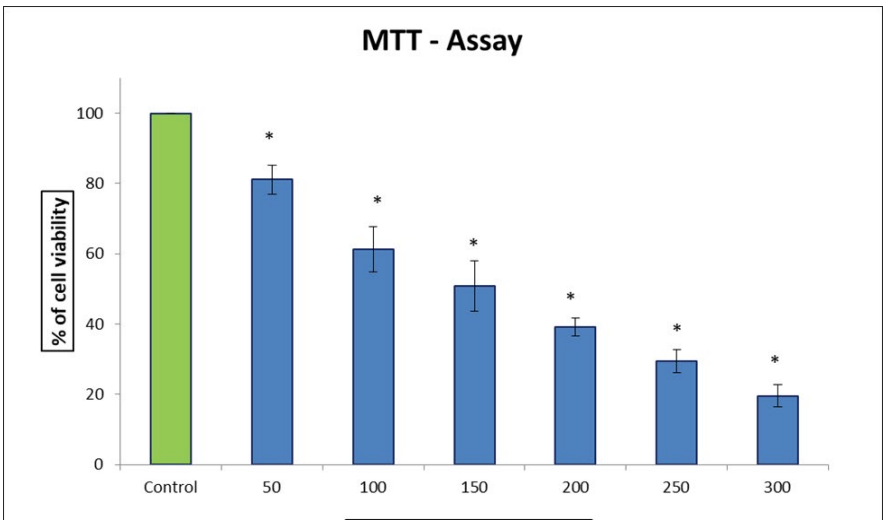

Figure 1: This figure represents about the percentage of cell viability of MCF-7 cells performed in MTT assay. The X-axis depicts concentration of seaweed extract and the Y-axis depicts the percentage of the viability of MCF-7 cells after 24 hours of incubation. **Note:** * Represents statistical significance between control versus treatment group was p<0.03 at 150 µg/ml, with p<0.05 level using Student's-Newman-Keuls test, statistically significant.

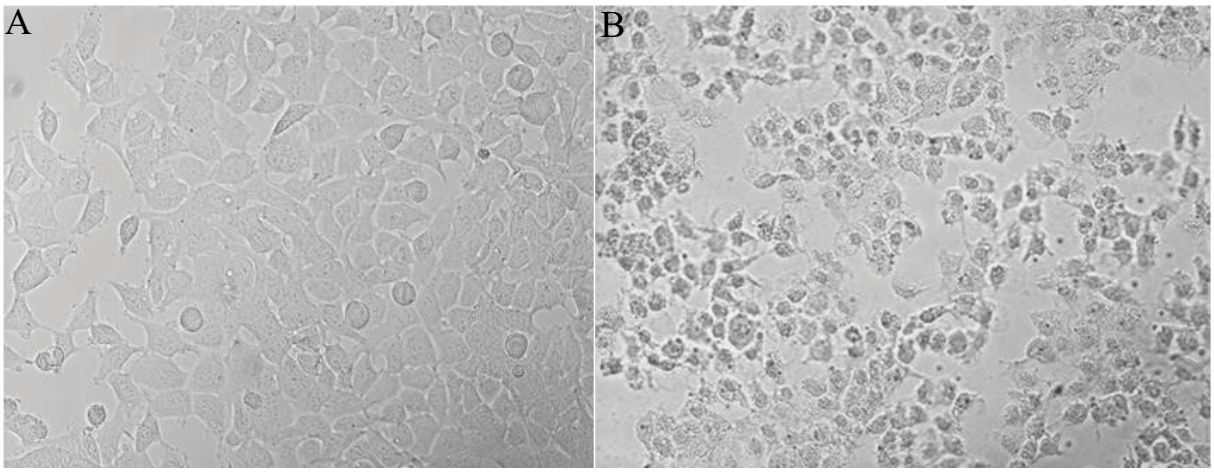

Figure 2: This figure represents the control group (A) and (B) represents MCF-7 cells. *ulva sp.,* extract (150 µg/ml). In which control showed no cytotoxicity as expected and tested seaweed extract showed strong selective cell proliferation inhibition of human breast cancer cells.

response [19]. Checkpoint inhibitors were ineffective against breast cancer. Mucinous ductal carcinoma has a better prognosis than more types of Invasive ductal carcinoma. Globally, utilization of plants as therapeutic agents is increasing. At the same time, when a plant is located as a valuable drug, its population becomes prone to wild crafting and unsustainability. Moreover, seaweed-derived compounds target important molecules that regulate cancer processes.

The concentration of *Sargassum Sp*. extract and the viability of cancer cells (Y-axis) (Figure 1). From this graph, it is seen that with increase in concentration of the extract, the viability of the cell decreases. So, it is clearly understood that this extract possesses significant anticancer activity against the breast cancer cell line. The cell viability was decreased in a time and dose dependent manner [20,21]. Our preliminary results show the cytotoxic activity of crude extracts of marine brown algae. Some literature highlights the potential implications of marine algae which exhibit antitumor activity. Similar results were also observed by other studies who emphasize a strong cytotoxicity of the crude extracts on Jurkat cell line. Jurkat cell viability decreased significantly after a treatment of 24 hrs. with extracts prepared from marine red algae. Similarly, the results obtained by show that the aqueous extract of *Sargassum Vulgare* was significantly cytotoxic against Hep-2 [22-24]. Many other studies have evaluated the cytotoxic effect of various algae species of the genus *Sargassum* as *Sargassum swartzii*. The methanolic extract prepared from *Sargassum* muticum inhibits the growth of cell lines in a dose and time dependent manner [25]. While the methanolic extract of *Sargassum* ilicifolium inhibits the proliferation of five lines of human cancer cells [1,8].

Further studies can be done by performing more assays and with a larger sample size. Since the extract of *Sargassum* possess less cytotoxic to humans, it can be used as a commercial product for treating breast cancer.

Conclusion

Our study indicates that the brown marine algae *Sargassum sp*. Collected from Thondi, Coastal areas have promising anticancer activity. Using the extract, a formulation was made to act on human breast cell cancer line. In future, this can be developed into a new anticancer drug to be used, which has lesser side effects when compared to the commercially available synthetic drugs. Various plant extracts can be combined with this preparation to elicit its capacity of killing breast cancer cells.

Conflict of Interest

None declared.

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