In vitro Cytotoxicity Screening of Brown and Red Seaweed, against MDA-MB-231 Cancer Cell Lines

Hingane. N. N.[1], Ambavade S. D.[2], Gajbhiye M. H.[1], Agnihotri S. N.[1]

[1]Tuljaram Chaturchand College of Arts, Science and Commerce College, Baramati-413102, Pune, Maharashtra, India.

[2]Institute of Chemical Technology. Matunga East-400019, Mumbai, Maharashtra.

**Corresponding author email: nnh.jscopr@gmail.com*

Abstract

Currently the challenges faced in medicine are investigating an anticancer drug that destroys tumor cells in the presence of normal cells without undue toxicity. Marine components such as seaweeds which exhibit antitumor activity are enlisted in the literature which has emphasized its potential implications. This Study is conducted to screen the antitumor effect of Brown and Red seaweed namely *Sargassum sp*, and *Corallina sp.* Isolated from Sindhudurg west coast from Maharashtra against MDA-MB-231 (Triple negative Breast Cancer) cell line. Ethanolic extract of both the marine seaweed was concentrated using a Soxhlet apparatus and its aqueous form is used for further evaluation.MTT assay (3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide, a yellow tetrazole) was performed for In vitro assessment of cytotoxic activity of Sargassum sp. at various concentrations (25 µg/ml-400 µg/ml) against the chosen cell lines. Investigation documented cell viability percentage, have been reduced with increased concentration, as evidenced by cell death. The marine extract showed significant potential cytotoxic activity ($P<0.001$) and ($P<0.05$) with IC50 of 28.01 µg/ml and 27.43 µg/ml respectively, against MDA-MB 231cell line. Anti- tumor effect of the ethanolic extract of Sargassum sp induced cell apoptosis with evidence of bioactive components. The successful application of Sargassum sp as a potent therapeutic tool against cancer need to be explore through further research.

Keywords: Marine seaweed, MTT Assay, Sargassum sp, *Corallina sp*, apoptosis, MDA-MB-231

Introduction

It's a period of reemerging natural product discovery and their potential social impact as a source of therapeutic drugs. In twenty-third century Cancer is one of the major fatal diseases to humans, responsible for millions of deaths annually. Seaweed derived compounds, such as polysaccharides, polyphenols, pigments, and sterols, possess various bioactivities, including anti-inflammatory, antitumor, antihypertension, antioxidant, antiobesity, and antidiabetics' activities [1–3]. The Marine seaweed *Sargassum sp.* and *Corallina sp.* isolated from the west coast of Sindhudurga Maharashtra are an edible Red and Brown alga, which is consumed as food,

herb medicine, and food additive in Asian countries, including China, Korea, and Japan, for a long time [4] Espoused by Hippocrates "Let food be thy medicine and medicine be thy food", around 2500 years ago, is now gaining momentum due to increased interest in health-promoting foods. Macroalgae an excellent source of health- promoting bioactive compounds which are rich in polyphenols. They are said to be superior in preventing oxidative stress compared with other edible plants due to the higher stability of macroalgae polyphenols than the terrestrial plants. [6, 7].Traditionally algae have been part of the cuisine in East Asia, especially Japan, Korea, the Philippines, Vietnam, Taiwan & China. Macroalga have attracted attention being natural reservoir of pharmaceutically active molecule. Marine floras have been used for medicinal purpose in India, China, and Near East & Europe since ancient times. [9,10] Having rich in antioxidants alga has gained so much attention and intended towards the development of ethno medicines due to having constituents such as phenols, flavonoids, alkaloids, tannins, vitamins, terpenoids and many more phytochemicals responsible for different pharmacological activities which have attracted researchers for drug discovery and development in the treatment of various human diseases. According to National Cancer Institute (NCI) of the United States of America (USA) has screened about 1, 14,000 extracts from an estimated 35,000 marine samples against a number of tumor systems [12]. Since from late 1960s the extensive efforts to extract drugs from the sea started. Since in the mid-1970s the systematic investigation began. Around 1977 to 1987, about 2500 new metabolites were reported from a variety of marine organisms. This discovery has come up with conclusion that the marine environment is an excellent source of novel chemicals, not found in terrestrial sources. [14]

Breast cancer is one of the most challenging conditions across the globe with high mortality. In many cases rural region left unattended due to the socio economic status .High morbidity is due to unavailability of cost effective treatment and side effects associated with current therapeutics hence the natural origin treatment with the marine biota found to have a great role. The studies undertaken using a triple negative MDA-MB-231 cell line. The cell line is a highly aggressive,invasive and poorly differentiated triple-negative breast cancer (TNBC) cell line as it lacks oestrogen receptor (ER) and progesterone receptor (PR) expression, as well as HER2 (human epidermal growth factor receptor 2).Extensive work need to be done as an urgent therapeutic aspect with a goal of eradicating cancer without destructing neighboring cell and without side effects. Marine macroalgae owing to their anticancer effect have been attracted in deep search of the marine potential. Present work is intended to check the effect of marine extract on the triple negative cell line MDA-MB-231.

Materials & Method

Seaweed material

Red and Brown seaweeds used in this study were *Corallina* sp. (Linnaeus) (Family: *Corallinaceae*, Order: Corallinales), *Sargassum* sp. (Turner) (Family: Sargassaceae, Order: Fucales respectively. Algae were freshly collected from the Sindhudurga coastal area, Maharashtra India. Samples collected were washed in running water for 10 min, transported to the laboratory and shade dried (35 ± 3 °C) for 72 h. The shade dried seaweeds were powdered

and used for further experiments.

Corallina sp

Sargassum sp

Figure 1. Images of plants species selected for study

Preparation of seaweed extracts

The powdered seaweed samples of both the seaweeds (100 g) were extracted with ethanol using Soxhlet extraction. The extract was concentrated using Rotary evaporator ABC/INC Biomedica BMI776, India and then aqueous form of it was subjected for MTT Assay. [13]

Cell lines and culture condition

MDA-MB-231 cell line ATCC culture was purchased from USA .Cells were cultured in DMEM medium purchased from Thermo Fisher Scientific and supplemented with 10% of fetal bovine serum (FBS) then the culture flasks were incubated for 3-4 days at 37˚C in 5% CO2 incubator.

MTT Assay

The cell viability was determined by MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) dye uptake assay. Briefly, MDA-MB-231 (triple negative breast cancer) cells were seeded at a density of 1 × 107 cells/ml density in 96-wellplate. An untreated group was kept as a negative control. The drug was added at different concentrations in 96 wells in triplicates. Next day, MTT Solution (5 mg/ml) was added to each well, and the cells were incubated for 4h at 37°C in 5% CO_2 incubator. The formazan crystals formed were dissolved by addition of 100µl of DMSO. The amount of colored formazan derivative was determined by measuring optical density (OD) using the ELISA microplate reader at 570 nm (OD570-630 nm). The percentage viability was calculated for both the extract as:

$$\% \text{ Viability} = [\text{OD of treated cells}/\text{OD of control cells}] \times 100$$

Statistical Analysis

Data was expressed as independent measurement. It was expressed as Means±S.D. Data was analyzed by an analysis of variance by Two way ANOVA using Graph Pad Prism version 9.5.1 ($P<0.001$) and ($P<0.05$) found to be significant.

Table 1. Statistical Analysis using Graph Pad Prism version 9.5.1

Source of variation MDA-MB-231 Concentration of *Sargassum sp.* & *Corallina sp.*	**SS**	**DF**	**MS**	**F (DFn, DFd)**	**P value**
Treatment (between row)	16622	5	3324	F(5,5) = 4789	P<0.001
Treatment (within columns)	0.4840	1	0.4840	F(1,5)=6.870	P<0.05
Residual	0.3522	5	0.07045		

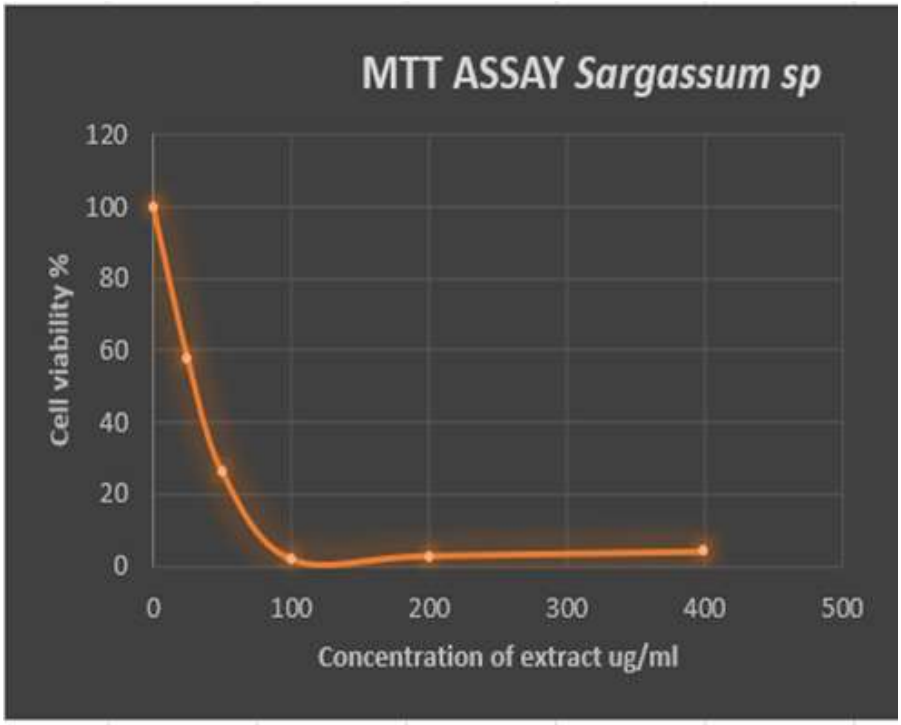

Figure 2. Cytotoxic Effect of *Sargassum sp* Extract at Various Concentration (25µg/ml-400 µg/ml) against MDA-MB-231 Cancer Cell Lines

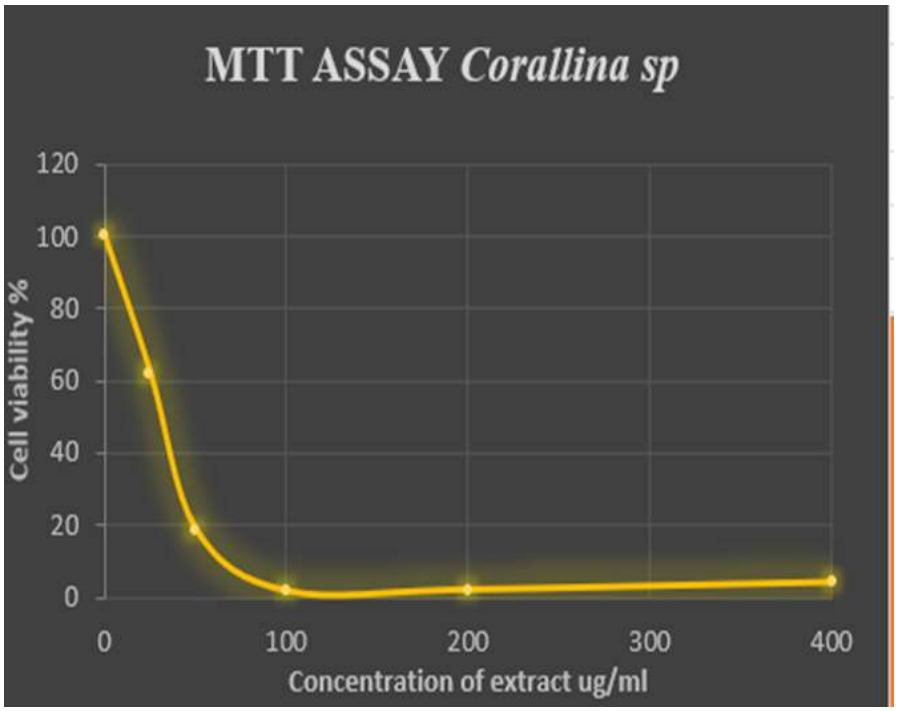

Figure 3. Cytotoxic Effect of *Corallina sp* Extract at Various Concentration (25µg/ml-400 µg/ml)against MDA-MB-231 Cancer Cell Lines

Result

The current work focused on In vitro cytotoxic activity of two marine seaweeds namely *Sargassum sp* and *Corallina sp.* using triple negative breast cancer cell line MDA-MB-231.The results of the cytotoxicity activity assessed using MTT Assay which are represented in the above Table. It shows that with increase in the concentrations (25-400ug/ml) of the extracts of the marine seaweed reduction in the cell viability has been recorded.IC50 value using cell viability percentage was calculated using GraphPad prism 9.5.1(733) software it was found to be 28.01ug/ml and 27.74 ug/ml for brown and red seaweed respectively. Statistical analysis using Two way ANOVA showed ($P<0.001$) and ($P<0.05$) are significant. From the depicted result the extract visualize Promising cytotoxic activity against triple negative MDA-MB-231 cell line with low IC_{50} vale.

Discussion

Currently across the globe one of the major cause of death in humans and have high impact in industrialized countries are tumor malignancies.[5] At present the challenges with therapeutic aids are to invent drug which potentially toxic against cancer cell keeping normal cells being unaffected [15].Literature has revealed the potential implications of marine seaweeds which manifest antitumor activity [16].The present work demonstrates that, *Sargassum Sp.* may be cytotoxic against triple negative breast cancer cell line and with increased concentration their exhibit decrease in cell viability. This surmise the existence of dose dependent cytotoxicity of *Sargassum sp* extract against cancer cell lines which was found effective and the IC50 value of it is 28.01µg/ml. Various studies have been developed in order to evaluate the bioactive

compounds produced by marine algae [17]Researchers have revealed that the sulfated compounds such as fucoidans extracts of brown seaweed disclose important roles against some human cancer cell lines[18] Researches have also come up with the findings that bioactive compounds including fucoidans, terpenes, sterols, polyunsaturated fatty acids and phenolic compounds have anticancer and cytotoxic activity[19]Our findings with Two way analysis shows significant ($p<0.001$) difference for the various concentration of Sargassum sp. extract against MDA-MB-231 cell lines. Present study suggests that the *Sargassum sp* and *Corallina sp*. extract exhibit effective antitumor activity. The brown algae are the potential source of sulfated polysaccharides fucoidans which are produce only by brown seaweed and has vast array of pharmacological activities. Red marine seaweed has sulfated polysaccharides as a primary component additional bioactivity of these marine seaweeds attributed to presence of carrageenan, laminarin as a bioactive molecules.[20] These marine therapies if implicated in future it could be cost effective, easy in production and purification. However, the best activity in both studies was manifested by the ethanolic extract of marine seaweeds that exhibited the lowest IC50 on cell viability and cell proliferation studies. It could be recommended in future to the patients as a effective therapeutic tool as a nutraceuticals. Further extensive research need to be carry out for the successful implication of this extract as a potent therapeutic tool against cancer.

Conclusion

Concisely, this study revealed interesting outcome which has showed the seaweeds used in this study has potential to exhibit anticancer agent due to vast array of molecules within them. In order to obtain therapeutic active molecule out of this work this study is one of the many tests. Further it need to isolate and purify the molecules exhibiting antitumor property associated with exact cell death mechanism ,However to our knowledge this is the first antitumor screening of the marine extract from the Sindhudurga west coast of Maharashtra, revealing anticancer potential with broad opportunities of anticancer research

Acknowledgement

The author extends sincere thanks to my principal &colleagues of JSPM's Jayawantrao Sawant College of Pharmacy & Research, Pune who constantly supported and motivated me during the research work.

Conflict of interest declaration

The authors declare that they have no conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or be perceived to influence, the present work.

Submission declaration

The authors vouch that the work has not been published elsewhere, either completely, in part, or in any other form and that the manuscript has not been submitted to another journal, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere without the written consent of the copyright-holder.

Reference

1. Wang L, Park Y-J, Jeon Y-J, Ryu B. Bioactivities of the edible brown seaweed, Undaria pinnatifida: A review. Aquaculture [Internet]. 2018;495:873–80. Available from: http://dx.doi.org/10.1016/j.aquaculture.2018.06.079**.**
2. Wang L, Lee W, Oh J, Cui Y, Ryu B, Jeon Y-J. Protective effect of sulfated polysaccharides from Celluclast-assisted extract of Hizikia fusiforme against ultraviolet B- induced skin damage by regulating NF-κB, AP-1, and MAPKs signaling pathways in vitro in human dermal fibroblasts. Mar Drugs [Internet]. 2018;16(7):239. Available from: http://dx.doi.org/10.3390/md16070239**.**
3. Fernando IPS, Jayawardena TU, Sanjeewa KKA, Wang L, Jeon Y-J, Lee WW. Anti-inflammatory potential of alginic acid from Sargassum horneri against urban aerosol-induced inflammatory responses in keratinocytes and macrophages. Ecotoxicol Environ Saf[Internet]. 2018;160:24–31. Available from:http://dx.doi.org/10.1016/j.ecoenv.2018.05.024**.**
4. Fernando IPS, Sanjeewa KKA, Kim H-S, Wang L, Lee WW, Jeon Y-J. Apoptotic and antiproliferative properties of 3β-hydroxy-Δ5-steroidal congeners from a partially purified column fraction of *Dendronephthya gigantea* against HL-60 and MCF-7 cancer cells: Anticancer activity of steroids from *Dendronephthya gigantea*. J Appl Toxicol [Internet]. 2018;38(4):527–36. Available from: http://dx.doi.org/10.1002/jat.3559**.**
5. Fukahori S, Yano H, Akiba J et al (2008) Fucoidan, a major component of brown seaweed, prohibits the growth of human cancer cell lines in vitro. Mol Med Rep 1:537–542.
6. Kumar S, Sahoo D, Levine I. Assessment of nutritional value in a brown seaweed Sargassum wightii and their seasonal variations. Algal Res [Internet]. 2015; 9:117–25. Available from: http://dx.doi.org/10.1016/j.algal.2015.02.024.
7. Santos F, Monteiro JP, Duarte D, Melo T, Lopes D, da Costa E, et al. Unraveling the lipidome and antioxidant activity of native Bifurcaria bifurcate and invasive Sargassum muticum seaweeds: A lipid perspective on how systemic intrusion may present an opportunity. Antioxidants (Basel)[Internet]. 2020;9(7):642. Available from: http://dx.doi.org/10.3390/antiox9070642.
8. Hwang JY, Park JH, Kim MJ, Kim WJ, Ha K-T, Choi BT, et al. Isolinderalactone regulates the BCL-2/caspase-3/PARP pathway and suppresses tumor growth in a human glioblastoma multiforme xenograft mouse model. Cancer Lett [Internet]. 2019;443:25–33. Available from: http://dx.doi.org/10.1016/j.canlet.2018.11.027.
9. *Sargassum ilicifolium* (turner) *C. agardh*: Algae Base [Internet]. Algaebase.org .Available from:https://www.algaebase.org/search/species/detail/?species_id=j6355e2efef8de401.
10. Malve H. Exploring the ocean for new drug developments: Marine pharmacology. J Pharm Bioallied Sci [Internet]. 2016;8(2):83. Available from: http://dx.doi.org/10.4103/0975-7406.171700.
11. Albertus J. University of Cape Town, Rondebosch, 7700, South Africa; Current address: School of Biology, Faculty of Science, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban, 4000, South AfriMedicinal and pharmaceutical uses of

seaweed natural products: A review. Private Bag X54001.

12. Yamamoto T. Animal models of systemic sclerosis. In: Animal Models for the Study of Human Disease. Elsevier; 2013. p. 1021–35.
13. Tung J, Zhou X, Alberts SC, Stephens M, Gilad Y. The genetic architecture of gene expression levels in wild baboons. Elife [Internet]. 2015; 4. Available from: http://dx.doi.or0.7554/eLife.04729.
14. Welsh J. Animal models for studying prevention and treatment of breast cancer. In: Animal Models for the Study of Human Disease. Elsevier; 2013. p. 997–1018.
15. Shagufta H, Viqar S, Jehan A, Syed E-H, Mohammad A. Toxicity of Fusarium solani Strains on Brine Shrimp (Artemia salina): Toxicity of <I>Fusarium solani</I> Strains on Brine Shrimp (<I>Artemia salina</I>). Dongwuxue Yanjiu [Internet]. 2009;30(4):468–72.
16. Mohammed F, Rashid-Doubell F, Taha S, Cassidy S, Fredericks S. Effects of curcumin complexes on MDA‐MB‐231 breast cancer cell proliferation. Int J Oncol [Internet]. 2020;57(2):445–55. Available from: http://dx.doi.org/10.3892/ijo.2020.5065.
17. Albano RM, Pavão MS, Mourão PA, Mulloy B. Structural studies of a sulfated L-galactan from Styela plicata (Tunicate): analysis of the Smith-degraded polysaccharide. Carbohydrate 1990; 208:163–74. Availablefrom:http://dx.doi.org/10.1016/0008-6215(90)80096-l.
18. Minh Ly B, Quoc Buu N, Duy Nhut N, Duc Thinh P, Thi Thanh Van T. Studies on fucoidan and its production from Vietnamese brown seaweeds. ASEAN J Sci Technol Dev [Internet]. 2017;22(4):371. Available from: http://dx.doi.org/10.29037/ajstd.173.
19. Sheikh H, El-Naggar A, Al-Sobahi D. Evaluation of antimycotic activity of extracts of marine algae collected from red sea coast, Jeddah, Saudi Arabia. J Biosci Med (Irvine) [Internet]. 2018;06(04):51–68. Available from: http://dx.doi.org/10.4236/jbm.2018.64004.
20. Khotimchenko M, Tiasto V, Kalitnik A, Begun M, Khotimchenko R, Leonteva E, et al. Antitumor potential of carrageenans from marine red algae. Carbohydr Polym [Internet]. 2020;246(116568):116568. Available from: http://dx.doi.org/10.1016/j.carbpol.2020.116568.