CYTOTOXIC EFFECTS OF *ACANTHOPHORA SPICIFERA* (M. VAHL) ON MCF-7 BREAST CANCER CELL LINE

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Abstract

Cancer remains one of the most pressing health concerns of the modern era. Seaweeds, known for their rich composition of bioactive compounds, present a valuable opportunity for exploring their therapeutic potential. This study examines the anticancer effects of Acanthophora spicifera (M. Vahl) at varying concentrations against human breast cancer cells (MCF-7) using the MTT assay. The results demonstrate a dose-dependent inhibition of cell growth, indicating the extract's potential as a natural anticancer agent with minimal adverse effects. Moreover, the study emphasizes the significance of A. spicifera as a promising natural alternative to synthetic cancer treatments. The bioactive compounds found in this seaweed may serve as the foundation for innovative therapeutic strategies. Given their low toxicity, these natural compounds offer a safer approach to cancer treatment. This research highlights the critical role of marine-derived resources in advancing future cancer therapies.

Keywords: *MCF-7, MTT assay, anti-cancer, acanthophora spicifera, therapies*

Introduction

Algae are aquatic, photosynthetic organisms that contain a nucleus but lack the true roots, stems, leaves, and specialized reproductive structures characteristic of plants. Among them, seaweeds—a diverse group of macroalgae—have garnered considerable interest due to their nutritional and medicinal properties (Carolin Joe Rosario & Eluvathingal, 2023). Marine organisms serve as abundant sources of bioactive secondary metabolites, offering significant potential for pharmaceutical advancements. Over the past four decades, researchers have identified numerous unique compounds from these organisms, many of which exhibit impressive biological activities (El Gamal, 2010). Seaweeds are particularly rich in beneficial compounds such as polysaccharides, phenolics, vitamins, and minerals. Their antioxidant, anti-inflammatory, and anticancer properties make them valuable for drug discovery and functional food development. Additionally, their high nutrient content combined with a low-calorie profile makes them a sustainable dietary option, promoting health and contributing to global food security (K Anitha & K Athulya, 2018).

Cancer is one of the most fatal and life-threatening diseases, marked by the uncontrolled proliferation of abnormal cells, commonly referred to as cancer or tumor cells (Pereira & Valado, 2023). Tumors are classified into two main types: benign and malignant. Malignant tumors pose a greater risk as they can spread to different parts of the body through a process known as metastasis. Cancer can develop in various organs, leading to different forms of the disease, such as leukemia, melanoma, colorectal cancer, breast cancer, and endometrial cancer (K Athulya & T Anitha, 2019).

The development of cancer is influenced by multiple factors rather than a single cause. These factors include genetic predisposition, exposure to ultraviolet radiation, lifestyle choices, environmental conditions, age, gender, and dietary habits (Sha-sha Guo & Zhen-guo Wang, 2022).. Various treatment options are available, including chemotherapy, hormone therapy, immunotherapy, radiation therapy, and surgery (Hannan et al., 2020). Depending on the cancer type and stage, doctors often recommend a combination of these treatments. However, conventional treatments such as surgery, radiation, and synthetic drugs

can lead to adverse side effects, impacting the patient's overall health (Guo & Wang, 2022).

The search for safer and more effective cancer therapies remains a significant challenge, as cancer continues to be a leading cause of death worldwide. Many chemotherapy drugs originate from plant-based compounds or their synthetic derivatives (Khairinisa et al., 2023) (Pereira & Valado, 2023). In recent years, marine algae have gained attention for their potential in drug development due to their diverse biological properties (Nova et al., 2024). Natural medicines, particularly those derived from seaweeds, are increasingly in demand. Seaweeds are rich in bioactive secondary metabolites, which offer protection against various disease-causing pathogens (Eluvathingal et al., 2024). Some species have shown promise as sources of potent anticancer compounds, making them valuable for the development of novel cancer treatments (Guo & Wang, 2022).

Materials and Methods

Collection of sample

The samples were collected from Dharmadam beach, Kannur, Kerala.

Preparation of the Sample

The collected seaweed samples were initially washed with seawater to eliminate impurities and sand particles. They were then rinsed three times with sterile distilled water to ensure the removal of any remaining adhered sand and dust. Afterward, the samples were placed on filter paper for a few hours to absorb excess moisture. Once dried, the seaweeds were cut into small pieces and shade-dried. The dried samples were then ground into a coarse powder using an electric mixer grinder and stored in airtight containers for further analysis.

Test for Anticancerous Activity

The tests for the selected samples' anti-cancerous activity were performed using the MTT assay technique.

Cell Culture Condition

The MCF-7 human breast cancer cell line was obtained from the National Center for Cell Sciences (NCCS) in Pune, India. These cells were cultured in Dulbecco's modified medium, enriched with fetal calf serum (FCS) and 2 mM L-glutamine. The medium was further supplemented with a balanced salt solution (BSS) containing 1.5 g/L sodium bicarbonate (Na_2CO_3), 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g/L glucose, and 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). Additionally, 10% fetal bovine serum (GIBCO, USA) was added, along with penicillin and streptomycin (100 IU/100 µg) at a final concentration of 1 mL/L. The cells were incubated at 37°C in a controlled atmosphere with 5% CO_2.

Evaluation of Cytotoxicity (MTT)

The half-maximal inhibitory concentration (IC_{50}) was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. MCF-7 cells were seeded at a density of 1×10^4 cells per well in a 96-well plate and cultured for 48 hours until they reached approximately 80% confluence. The existing medium was then replaced with fresh medium containing serially diluted pyrogalol or extract, followed by an additional 48-hour incubation.

After incubation, the culture medium was discarded, and 100 µL of MTT solution (Hi-Media) was added to each well. The plate was incubated at 37°C for 4 hours to allow the formation of formazan crystals. Subsequently, the supernatant was removed, and 50 µL of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The plate was incubated for 10 minutes, after which the optical density (OD) was measured at 620 nm using an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD values were then used to determine the percentage of cell viability using the appropriate formula.

Percentage of viability

$$=\frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}}\times100$$

IC_{50} Value

The half-maximal inhibitory concentration (IC_{50}) is a key parameter used to assess the potency of a compound in inhibiting a biological or biochemical function. This quantitative measurement determines the concentration of a drug or inhibitor required to reduce a specific biological process by 50%.

To determine the IC_{50} of a drug, a dose-response curve is generated by evaluating the impact of various concentrations of an antagonist on counteracting the activity of an agonist. The IC_{50} value is then derived by identifying the concentration at which half of the maximum biological response is inhibited. This measurement is widely used in pharmacology and drug development to compare the effectiveness of different therapeutic agents. (Aleena B *et al*., 2023).

Morphological Study

MCF-7 and A-549 cells were cultured on coverslips at a density of 1×10^5 cells per coverslip and treated with varying concentrations of the test compounds for 6 to 24 hours. Following incubation, the cells were fixed using a 3:1 ethanol and acetic acid solution. The coverslips were carefully mounted onto glass slides for morphological examination. Photomicrographs were captured for three monolayers from each experimental group. Cellular morphological changes were observed and analyzed using a Nikon (Japan) bright-field inverted light microscope at 40× magnification.

Statistics

All in vitro experiments were conducted in triplicate and repeated a minimum of three times to ensure reliability. Data analysis was performed using SPSS software, version 17.0. A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

This study investigated the anticancer potential of the selected algal species, *Acanthophora spicifera* (M. Vahl). The cytotoxic activity of the extract was evaluated against human breast cancer cell lines (MCF-7) at concentrations ranging from 10 to 100 µg/mL using the MTT assay. The results demonstrated a dose-dependent inhibition of cell proliferation. A graph was generated to illustrate the relationship between sample concentration and cell viability, and the IC_{50} value for *A. spicifera* against MCF-7 cells was determined to be 29 ± 1.5 µg/mL.

Furthermore, cytotoxicity in treated cells was significantly higher compared to the untreated control group. Since the extract exhibited a strong inhibitory effect on cell proliferation, its cytotoxic properties were further explored at different concentrations. The findings suggest that the extract may exert cytotoxic effects on cancer cells even at low concentrations while displaying minimal toxicity toward normal cells. The observed cytotoxicity may be associated with membrane-mediated apoptosis, highlighting its potential as a promising natural anticancer agent.

Cell Morphology Analysis

Plate 1 illustrates the morphological alterations observed in MCF-7 cells following treatment with varying concentrations of the extract for 24 hours. The treated cells exhibited increased shrinkage, membrane blebbing, and the formation of floating cells in a dose-dependent manner when compared to the untreated control group. Cytological analysis revealed that the extract exerted its antiproliferative effects by inducing membrane blebbing, destabilizing the cell membrane, and disrupting the cytoskeletal structure.In Plate 1, image (a) represents the condition of cells in the control group, while images (b), (c), and (d) depict the membrane blebbing and floating cell formation observed in treated cancer cells. These findings suggest that the isolated bioactive compounds demonstrate significant cytotoxic and antiproliferative effects on the selected cancer cell lines.

Over the past decade, there has been a substantial rise in the number of preclinical anticancer compounds derived from marine organisms advancing to human clinical trials. The therapeutic potential of algae is largely attributed to the presence of bioactive secondary metabolites, which contribute to their pharmacological significance. (Nagai *et al*., 2003). The anticancer potential of *Gracilaria corticata* was evaluated using a crude methanol extract at different concentrations against human breast cancer cell lines. The extract exhibited a maximum cell viability of 95.67% and a minimum of 39.9%. These findings highlight the need for further clinical trials and molecular characterization of algal bioactive compounds to validate their therapeutic potential (Jayashree *el al*., 2018).

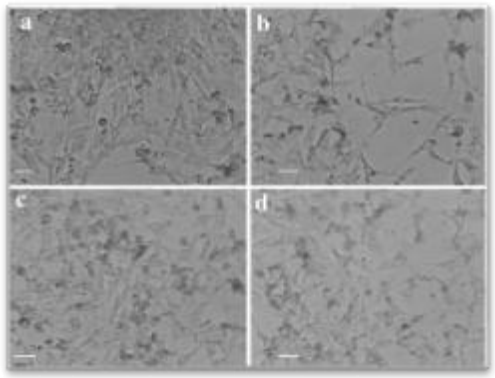
Additionally, the cytotoxic evaluation of selected algal extracts against human breast and lung cancer cell lines suggests that these marine-derived compounds could serve as promising alternatives to conventional anticancer drugs.

Table 1 *In vitro* Anti-Cancerous Activity of *Acanthophora Spicifera* Representing the Percentage of Cell Viability in MCF-7 Cell Line

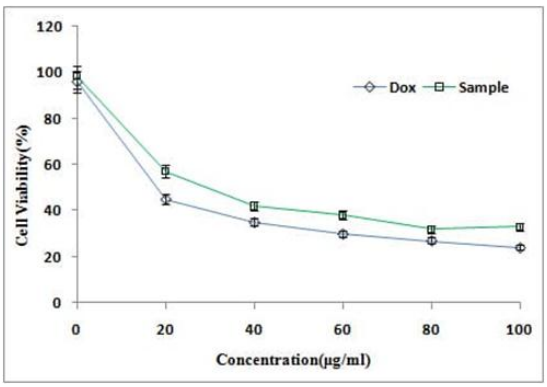
Sample Name	MCF-7
Acanthophora spicifera	29 ±1.5
Std (Doxorubicin)	0.8

IC_{50} – Values of the respective sample (at 24 hrs)
Inhibitory concentration expressed in µg/ml

Plate 1. Morphological Changes in MCF-7 Cells



(a) Control, (b) 25µg/ml, (c) 50µg/ml, (d) 100µg/ml



Conclusion

The impact of the extract on the viability of human breast cancer cells (MCF-7) was assessed using the MTT assay. The findings revealed that the extract effectively inhibited cell proliferation in a dose-dependent manner. As a natural bioresource, *Acanthophora spicifera* holds promise for breast cancer treatment, potentially reducing the adverse effects associated with conventional cancer therapies. While marine plants have long been explored for their nutritional and medicinal benefits, this study highlights the tumor-suppressing potential of *A. spicifera*, introducing it as a promising anticancer agent with minimal side effects.

References

1. Aleena B, E., Amitha, T. V, Carolin Joe Rosario, J., & Vimal Priya, S. (2023). An in vitro anticancerous and antioxidant potentials of the brown seaweed Sargassum polycystum C. Agardh. *Kongunadu Research Journal*, *10*(2), 31–34. https://doi.org/10.26524/krj.2023.13
2. Aleena B, E., Amitha, T. V, Rosario, C. J., & Priya, V. (2024). A comparative phytochemical investigation on selected Macroalgae in Thikkodi Coast, Kerala. *Kongunadu Research Journal*, *11*(1), 46–50. https://doi.org/10.26524/krj.2024.7
3. Carolin Joe Rosario, J., & Aleena B, E. (2023). International Journal of Pharmaceutical and Bio-Medical Science A Review on Potentiality of Marine Algae in Environmental Sustainability. *International Journal of Pharmaceutical and Bio-Medical Science*, *3*(9), 501–505. https://doi.org/10.47191/ijpbms/v3
4. El Gamal, A. A. (2010). Biological importance of marine algae. In *Saudi Pharmaceutical Journal* (Vol. 18, Issue 1, pp. 1–25). https://doi.org/10.1016/j.jsps.2009.12.001
5. Guo, S. S., & Wang, Z. G. (2022). Glyceroglycolipids in marine algae: A review of their pharmacological activity. *Frontiers in Pharmacology*, *13*, 1–12. https://doi.org/10.3389/fphar.2022.1008797
6. Hannan, M. A., Dash, R., Haque, M. N., Mohibbullah, M., Sohag, A. A. M., Rahman, M. A., Uddin, M. J., Alam, M., & Moon, I. S. (2020). Neuroprotective Potentials of Marine Algae and Their Bioactive Metabolites: Pharmacological Insights and Therapeutic Advances. In *Marine Drugs* (Vol. 18, Issue 7, pp. 1–50). MDPI AG. https://doi.org/10.3390/md18070347
7. Jayasree P, Thiruchelvi R, Balashanmugam P, 2018, Evaluation Of Antibacterial, Antioxidant, and Anticancer Potentials From Marine Red Algae GRACILARIA CORTICATA, Asian Journal of pharmacetical and clinical research, Vol 11(07), Pg: 347-350, ISSN: 2455-3891.

8. K Anitha, & K Athulya. (2018). Marine Green Algae As An Anti Breast Cancer Agent- Cytotoxic Test of Chaetomorpha compressa (Bory) Kutzing. *International Journal for Research Trends and Innovation*, *3*(8), 62–66. www.ijrti.org
9. K Athulya, & T Anitha. (2019). Algal Biodiversity Along Southern Coasts of India: A Review. Algal Biodiversity Along Southernn Coasts of Indi:: A Review. *Indian J Biology*, *6*(2), 93–101. https://doi.org/10.21088/ijb.2394.1391.6219.5
10. Khairinisa, M. A., Latarissa, I. R., Athaya, N. S., Charlie, V., Musyaffa, H. A., Prasedya, E. S., & Puspitasari, I. M. (2023). Potential Application of Marine Algae and Their Bioactive Metabolites in Brain Disease Treatment: Pharmacognosy and Pharmacology Insights for Therapeutic Advances. *Brain Sciences*, *13*(12), 1–18. https://doi.org/10.3390/brainsci13121686
11. Nova, P., Gomes, A. M., & Costa-Pinto, A. R. (2024). It comes from the sea: macroalgae-derived bioactive compounds with anti-cancer potential. In *Critical Reviews in Biotechnology* (Vol. 44, Issue 3, pp. 462–476). Taylor and Francis Ltd. https://doi.org/10.1080/07388551.2023.2174068
12. Pereira, L., & Valado, A. (2023). Harnessing the power of seaweed: unveiling the potential of marine algae in drug discovery. *Exploration of Drug Science*, 475–496. https://doi.org/10.37349/eds.2023.00032
13. Remya, R. R., Julius, A., Ramadoss, R., Parthiban, S., Bharath, N., Pavana, B., Samrot, A. V., Kanwal, S., Vinayagam, M., & Gemeda, F. W. (2022). Pharmacological Activities of Natural Products from Marine Seaweed Turbinaria ornata: A Review. *Journal of Nanomaterials*, *2022*. https://doi.org/10.1155/2022/4784608