Journal of Community Health Provision
Vol. 5, Issue 1, 2025
Page 14-25
DOI: https://doi.org/10.55885/jchp.v5i1.553

Anticancer Assay of Methanol Extract of *Gracilaria salicornia* Originating from the Hari Islands, Southeast Sulawesi, Against MCF-7 Cancer Cells

Sernita[1], Paulina Taba[2], I Sahidin[3], Nunuk Hariani Soekamto[2]

[1]Departement of Medical Laboratory Technology, Politeknik Bina Husada Kendari, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Science, University of Hasanuddin, Indonesia
[3]Department of Pharmacology, Faculty of Pharmacy, University of Halu Oleo, Indonesia

Corresponding Author: Nunuk Hariani Soekamto
Email: noek_1512@yahoo.com

Abstract. *Marine macroalgae represent a promising but underexplored resource for anticancer drug discovery due to their diverse repertoire of bioactive secondary metabolites. Gracilaria salicornia, a red alga found abundantly in the waters surrounding the Hari Islands, Southeast Sulawesi, has not been previously studied for its cytotoxic potential. This study aimed to evaluate the phytochemical composition and anticancer activity of methanol extract from G. salicornia against MCF-7 human breast cancer cells. The algae were extracted using a gradient solvent system, with methanol selected for further testing due to its polarity and broad extraction capacity. Phytochemical screening revealed the presence of alkaloids, phenolics, and steroids/terpenoids—classes of compounds known for their cytotoxic, pro-apoptotic, and redox-modulatory properties. General toxicity was assessed using the Brine Shrimp Lethality Test (BSLT), with the extract demonstrating moderate toxicity (LC_{50} = 561.26 mg/L), indicating the presence of bioactive constituents. Cytotoxicity against MCF-7 cells was evaluated using the MTT assay, yielding an IC_{50} value of 414.6 mg/L. Although lower than the standard chemotherapeutic agent doxorubicin (IC_{50} = 4.1 mg/L), the observed activity supports the hypothesis that G. salicornia contains compounds with therapeutic relevance. These findings suggest that G. salicornia harbors cytotoxic agents that act through diverse mechanisms, likely involving oxidative stress and hormonal regulation. While the crude extract exhibited only moderate activity, its phytochemical richness warrants further fractionation, isolation, and mechanism-of-action studies. This study provides foundational evidence for the potential of Indonesian marine algae in cancer drug discovery and highlights the need for continued exploration of native marine biodiversity as a source of novel chemotherapeutic agents.*

Keywords: *Gracilaria salicornia, BSLT, MTT, cytotoxic, cell MCF-7*

Received: January 3, 2025 Received in Revised: February 29, 2025 Accepted: March 20, 2025

INTRODUCTION

Align with research from Fatmawati (2020), classifies cancer as cells which grow with no restraint while remaining a major health threat worldwide. Breast cancer stands as both the most common female cancer diagnosis worldwide and among the principal mortality causes due to cancer (Suparna & Sari, 2022). Worldwide breast cancer developed nearly 2.26 million new cases in 2020 and functioned as the leading type of malignancy surpassing lung cancer (Ghosh &

Gopinath, 2025). These statistics demonstrate the extensive challenge breast cancer poses to global health systems while underscoring the critical need for persistent work that combines early detection methods with effective treatments alongside research efforts for creating new tolerable targeted therapeutic solutions.

The occurrence of breast cancer in Indonesia establishes itself as the leading health issue among public health priorities (Dewi et al., 2021). In 2020 Indonesia recorded 68,858 new breast cancer cases based on data from the Global Cancer Observatory (GCO, 2022) which made breast cancer the leading diagnosis among all types of cancers in the country. The disease killed more than 22,000 Indonesians while demonstrating its destructive effects against the Indonesian population. Breast cancer's high incidence in Indonesia exists because of bodily biology combined with delayed medical tests, life changes alongside health care service shortages and insufficient disease awareness (Pratiwi et al., 2024). Both developing and developed countries show increasing breast cancer cases therefore worldwide collaboration must work toward creating sustainable effective accessible therapeutic approaches.

Breast cancer patients have multiple treatment options which consist of surgery in combination with radiotherapy or chemotherapy or hormonal therapy or targeted therapy. Modern treatments extend patient survival time alongside better clinical results but they cause significant detrimental effects which include immunosuppression besides cardiotoxicity and nausea along with fatigue and drug agent resistance (Bone, 1993). The widely used chemotherapeutic drug doxorubicin creates well-known cardiac damage and exhibit adverse effects which increase with each dosage administration. Standard treatments have become less effective because multidrug-resistant cancer phenotypes continue to appear at an increasing rate (West et al., 2019). The situation emphasizes why new anticancer drugs need to be developed because they must achieve maximum therapeutic results without causing damage to healthy cells.

Through the use of plants and marine organisms terrestrial pharmaceutical companies have based their cancer drug development for many years. Novel anticancer treatments from 1981 to 2019 comprise either natural substances or their synthetic derivatives and synthetic analogs which derived from natural structures (Valentová et al., 2023). A vast area of approximately 70% of the Earth’s surface consists of marine space containing chemically distinct organisms which generate various bioactive molecules with complex chemical structures. The pharmacological effectiveness of seaweeds referred to as Marine macroalgae stems from their cellular biosynthesis of diverse secondary metabolites that includes alkaloids and terpenoids as well as flavonoids and polyphenols and sulfated polysaccharides alongside carotenoids (Priyanka et al., 2022).

The red algae group known as Rhodophyta constitutes one of the extensively researched macroalgae portfolio based on their biological activities (Deepika et al., 2022). The genus Gracilaria within this group continues to interest researchers because its compounds exhibit antioxidant effects together with anti-inflammatory action and antimicrobial properties and anticancer properties. The biological activities result from bioactive compounds that work through multiple biochemical and molecular pathways (Ningsih, 2014). The phenolic compounds present in Gracilaria spp. demonstrate powerful antioxidant properties by both removing reactive oxygen species and stopping DNA damage to proteins and lipids. The chemical compounds known as alkaloids frequently operate by disrupting nucleic acid synthesis along with apoptosis promotion and shutting down angiogenesis processes (Ahmed et al., 2022). The progression of cancer relies on membrane dynamic changes and signal transduction pathways and cellular homeostasis which steroids and terpenoids influence according to Salma et al. (2021).

Very little research exists regarding Gracilaria salicornia's pharmaceutical value as cancer medicine although its medicinal potential shows great promise because these species originate from Southeast Sulawesi, Indonesia. Red macroalga species G. salicornia can be found in marine waters at the Hari Islands which are part of the diverse Coral Triangle. Scientists worldwide

ISSN 2776-1150 (Print), ISSN 2776-1169 (online)

recognize the Coral Triangle for containing extraordinary levels of biodiversity because this area shelters over a thousand marine species as well as many endemic algae. The combination of substantial marine species diversity and specific Indonesian aquatic ecosystem characteristics creates an uncommon ecological environment that affects the metabolic makeup of local algae species (Mahmudi et al., 2020). Water temperature combined with light intensity together with nutrient levels and salinity intensity create key environmental conditions that determine the final composition of secondary metabolites produced by seaweeds. Added to this fact are potent bioactive compounds that exclusively exist within marine organisms from these specific regions (Bergé & Barnathan, 2005).

Traditional uses of Gracilaria species extend to the food sector and food additives field because of these plants' nutrients and agar content yet their potential medical values need further research (Torres et al., 2019). Use of Gracilaria edulis and Gracilaria tenuistipitata methanol and aqueous extracts showed cytotoxic properties against MDA-MB-231 triple-negative breast cancer cells as well as oral squamous carcinoma cells according to Ferreira (2023). Research shows that Gracilaria contains diverse biological compounds which can be influenced by the solvent polarity which affects extract composition and drug potency. Methanol serves effectively as an intermediate polarity solvent for extracting phytochemicals such as phenolics and alkaloids because of its balanced solubilizing properties (Kumar et al., 2023). Methanolic extracts serve as suitable representatives that capture all secondary metabolites produced by marine macroalgae.

A comprehensive study lacks in the scientific literature for assessing the phytochemical components along with cytotoxic properties of methanolic extracts derived from Gracilaria salicornia specimens collected at Hari Islands. Scientific literature requires immediate attention to this gap which becomes increasingly important because the Hari Islands harbor high biodiversity and suffer from an urgency for new anticancer medicines. The analysis of anticancer potentials in Indonesian marine resources helps meet national development objectives and bioprospecting objectives through direction from the Ministry of Research and Technology of Indonesia (Rusyaev & Orlov, 2024). Uses of indigenous marine biodiversity for healthcare applications create simultaneous advantages through scientific development and native ecosystem conservation (Jupiter et al., 2014).

The researchers conducted an experiment which tested the cytotoxic function of Gracilaria salicornia methanol extract against MCF-7 breast cancer cells as an accepted model of estrogen receptor-positive (ER+) breast cancer. Various methods come together in this study to reach its research goal. A preliminary evaluation of bioactive compounds was performed by qualitative phytochemical screening which enabled scientists to determine important chemical compositions of the extract (Altemimi et al., 2017). A Brine Shrimp Lethality Test (BSLT) functioned as both a quick and budget-friendly tool to detect overall extract toxicity. Scientific studies demonstrate that Brine Shrimp Lethality Test serves as a fundamental technique to identify cytotoxic compounds before their evaluation for anticancer properties (Niksic et al., 2021). The MTT assay represented a quantitative method to determine the MCF-7 cell cytotoxicity of the extracted compound. The MTT assay determines cellular metabolic activity and proliferation through viable cell-assisted tetrazolium salt metabolic reduction which produces formazan products (Maulidia, 2020).

This research combines phytochemical evaluation together with toxicological and cytological tests to deliver a complete analysis of Gracilaria salicornia methanol extract's anticancer properties (Lam et al., 2023). The study results will expand the scientific understanding that marine macroalgae represent promising alternatives as bioactive therapy agents for cancer treatment. The current investigation establishes groundwork for upcoming studies to identify distinct compounds from Gracilaria salicornia and study their effects on cancer treatment by evaluating their biological activity in laboratory and testing environments. This research demonstrates an essential milestone for developing Indonesian marine biodiversity as cancer therapies in the worldwide cancer prevention battle.

ISSN 2776-1150 (Print), ISSN 2776-1169 (online)

METHODS

Collection and Identification of Plant Material

Biological samples of Gracilaria salicornia red algae came from Hari Island marine waters in Southeast Sulawesi, Indonesia at locations S 04°02.270' and E 122°46.555'. The sampling location became the selection because of its diverse ecological along with its peaceful uncontaminated status ideal for extracting and gathering oceanic algae. The laboratory experts at the Oceanography Laboratory from National Research and Innovation Agency conducted authentication and taxonomic verification tests on the collected specimens. The algal samples needed verification before the research team cleaned them with fresh seawater to remove epiphytes and debris while drying them under room temperature conditions with shaded ventilated positions to protect bioactive compounds. The grinder processed all dried samples before they received airtight storage at a controlled environment with low light exposure and minimal moisture content.

Extraction of Bioactive Compounds

The extraction procedure used sequential solvents for obtaining bioactive compounds from G. Salicornia (Limongelli et al., 2022). Research investigators performed maceration processing that utilized successive solvents starting with non-polar n-hexane followed by semi-polar ethyl acetate and finishing with polar methanol on powdered algae plant material. A total of 72 hours passed during maceration under controlled room temperature conditions while continuous stirring maintained. Semi-solid crude extracts appeared after the solvent fractions received individual filtration followed by reduced pressure concentration with a rotary evaporator set at 40 °C. The methanolic extract obtained highest consideration for additional analysis because previous studies documented that it contains various secondary metabolites and bioactive compounds.

Phytochemical Screening

A phytochemical study of the methanolic extract through various analytical methods showed the existence of secondary bioactive compounds such as alkaloids and phenolics along with flavonoids and steroids and terpenoids. Multiple tests following the qualitative technique outlined by Fatma & Martsiningsih (2019), checked for metabolite groups through color-based and substance separation reactions. The experiments were conducted three times to verify both accuracy and reliability of the test outcomes.

BSLT Assay

Utilizing the Waghulde et al. (19) methodology. BSLT toxicity test. Artemia salina Leach larvae are used in BSLT testing. We tested G. salicornia methanol extract. The eggs of Artemia salina are ready to be incubated for 48 hours under light in a container containing seawater. Each test container (vial) was filled with a different concentration of the G. salicornia methanol extract, which was made in increments of 10, 100, 100, 500, and 1000 mgL-1. Each vial received five milliliters of seawater. As a negative control, the test chemical was not added to one vial of saltwater. Three repetitions of each extract concentration were made. Fill the water-insoluble vial with 1% DMSO. After choosing ten healthy larvae and placing them in each test container, all vials containing extracts and larvae or just larvae were illuminated and allowed to sit at room temperature for a full day. The number of dead larvae in each treatment with extract concentration was counted after a 24-hour period. Using linear regression equations and probit analysis, the LC50 mgL-1 value was ascertained. The LC50 value (20) was used to classify the toxicity of the extract. Low toxicity is defined as LC50 values greater than 1,000 mgL-1, medium toxicity as LC50 values between 30 and 100 mgL-1, and extremely toxicity as LC50 values less than 30 mgL-1.

ISSN 2776-1150 (Print), ISSN 2776-1169 (online)

Cytotoxicity Study

Scientists used the standard MTT assay technique to analyze Gracilaria salicornia methanol extract toxicity against MCF-7 breast cancer cells. MTT reduction into purple formazan crystals occurs through the action of succinate dehydrogenase enzymes in mitochondria of viable cells in this assay which employs MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] as a yellow tetrazolium salt. Researcher can measure the formazan production spectrophotometrically to calculate metabolically active living cell numbers (Utomo & Yusbida, 2017). The researchers selected this assay because it provides reliable outcomes along with simple operation and accurate detection of cellular response changes to chemotherapeutic drugs and natural extract treatments. Researchers exposed MCF-7 cells which are human breast cancer cells carrying functional estrogen receptors (ER^+) to different concentrations of methanolic extract solution.

The cells received MTT reagent as an addition following 72 hours of incubation while incubating for three more hours. The absorbance reading of formazan crystals from viable cells happened after dissolving them with DMSO through examination at 570 nm on a microplate reader. The IC_{50} concentration represents how much extract leads to a 50% reduction in cell viability and researchers determined it through a dose-response absorbance curve. The determined IC_{50} value serves as an indicator regarding how potent the extract's proliferation inhibition properties are. Due to the established chemical elements featuring phenolics along with alkaloids and terpenoids in this extract the noted cytotoxic impact is thought to emerge from oxidase burden and cell cycle blockage and disrupted mitochondria.

Preparation of algal extracts

A study of Gracilaria salicornia methanolic extract cytotoxicity involved the preparation of working solutions at 7.81, 15.63, 31.25, 62.50, and 125 mg/L concentrations. The dried methanol extract received precise weights for each working solution after dissolution in 1 mL dimethyl sulfoxide (DMSO) because this solvent serves well as biological assay solvent by providing effective solubility and cell culture system compatibility. Scientists used 0.2 µm syringe filters to prepare the stock solutions which both sterilized and extracted particulate matter that could impair cell culture tests. The filtration process is vital because it removes contaminants which allow researchers to observe chemical effects independently from microbial and mechanical pollutions.

All prepared extract solutions underwent storage at 26 °C but scientists used them without delay in experiments to prevent degradation of secondary metabolites from marine organisms because such compounds show sensitivity to temperature and oxidation. Each assay began with making new stock solutions to enhance the test consistency. Healthcare scientists used doxorubicin as a positive control to check the assay's operational viability because it remains a commonly employed anthracycline chemotherapy medication (Soares et al., 2012). Scientists use Doxorubicin for benchmarking cell death assessment because its clear mechanism of action through DNA intercalation and topoisomerase II inhibition. Scientists prepared a stock solution of doxorubicin under controlled temperature (4 °C) with simultaneous light protection because it remains sensitive to degradation at regular room temperature. The preparation methods create stable and soluble solutions of the test and control substances that lead to dependable results regarding cytotoxicity.

MTT assay cells

A 96-well plate containing 1 x 104 MCF-7 cells per well was filled with 90 µL of RPMI-1640 medium containing 10% FBS. The cells were then incubated for 24 hours at 37 °C with 5% CO2. Doxorubicin was added as a positive control along with 10 µL of G. salicornia methanol extract solution (7.81; 15.63; 31.25; 62.50; 125 mgL-1) and they were incubated for 72 hours under the same conditions. The cells were cultured for three hours at 37°C with 5% CO2 after the media was disposed of and 100 µL MTT in RPMI-1640 with 10% FBS (0.5 mg/mL) was added.

The formazan crystals were dissolved in DMSO, the MTT solution was disposed of after three hours, and the incubation period was fifteen minutes. An Elisa reader was used to measure the absorption absorbance at 570 nm. The linear regression equation derived from the association between the extract concentration and the percentage cell viability curve was used to determine the IC50 value.

The equation is used to compute the percentage of cell viability:

$$\%\ viability = \frac{(cell\ absorbance\ by\ treatment\ -\ absorbance\ control\ media)}{(absorbance\ control\ cell\ -\ absorbance\ control\ media)}\ x100\%$$

RESULT AND DISCUSSION

Following the successful extraction, phytochemical screening, and cytotoxic testing of Gracilaria salicornia methanol extract, a series of analytical assays were performed to evaluate its biological potential. The results are presented in three major components: the identification of key secondary metabolites through phytochemical analysis, a preliminary toxicity screening using the Brine Shrimp Lethality Test (BSLT), and the evaluation of cytotoxic effects on MCF-7 human breast cancer cells using the MTT assay. Each stage provides insights into the pharmacological relevance of the extract and lays the groundwork for understanding its potential as a source of anticancer compounds. The following sections detail the outcomes of these assays and interpret their significance in the context of natural product-based drug discovery.

Phytochemical Composition of the Methanol Extract

Phytochemical screening of the methanol extract of Gracilaria salicornia revealed the presence of alkaloids, phenolic compounds, and steroids/terpenoids, while flavonoids were not detected (Table 1). The detection of these secondary metabolites is significant, as they are widely known to contribute to various biological activities, including antioxidant, antimicrobial, and cytotoxic effects. Alkaloids are well-established for their ability to interfere with nucleic acid synthesis and disrupt mitotic spindles. Phenolic compounds are often associated with redox modulation, while terpenoids have been reported to influence membrane permeability and apoptotic signaling in cancer cells (Kerimi & Williamson, 2018). The absence of flavonoids, though surprising given their common occurrence in marine algae, does not diminish the potential cytotoxic activity of the extract. Instead, it suggests that the extract's biological effects are likely driven by the other compound classes present.

Table 1. Phytochemical Screening Results of G. Salicornia Methanol Extract

Metabolites	Results
Flavanoid	-
Alkaloid	+
Fenolik	+
Steroid/Terpenoid	+

These results confirm that the methanol extract contains important groups of compounds that have the potential to exhibit cytotoxic effects and support the extract's use in further bioactivity testing.

Toxicity Evaluation via Brine Shrimp Lethality Test (BSLT)

A preliminary anticancer screening test employing Artemia salina Leach shrimp larvae was conducted after the methanol extract. The Brine Shrimp Lethality Test (BSLT) is the name of this technique. When determining the toxicity of a compound, the BSLT approach is frequently employed for the preliminary screening of active compounds found in extracts (22). According to Table 2, the extract's toxicity value is that of G. salicornia, which is categorized as medium hazardous (LC50 between 30-1000 mgL-1) with an LC50 of 561.26 mgL-1. With an LC50 of 13.96 mgL-1, potassium dichromate was much more hazardous than the control. Despite being somewhat hazardous, it has the ability to produce cytotoxic agents.

Table 2. Toxicological Evaluation of G. Salicornia Methanol Extract

Sample	LC_{50} (mgL^{-1})
Extract methanol	561,26
Potassium dichromate	13,96

These findings suggest that the methanol extract contains biologically active compounds with cytotoxic potential. Although BSLT does not provide cancer specificity, it serves as a useful preliminary screening method for identifying promising samples for anticancer studies.

Cytotoxic Activity Against MCF-7 Cells

The BSLT approach, as an early anticancer screening test, is not selective to the anticancer activity and is not adequate to ascertain the mechanism of action of bioactive compounds. The BSLT approach is not specific to the bioactive agent's anticancer action and is not adequate to identify its mode of action (Syamsurizal et al., 2023). However, once the molecule tested proved harmful to Artemia salina, the BSLT approach yielded findings that could be supported by a more focused bioassay, indicating that the compound would be a viable candidate for cancer research. In order to further assess the cytotoxicity of G. salicornia extract, the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay was used in vitro (23)

Table 3. Cytotoxic Activity Against Breast Cancer MCF-7 Cell Of G. Salicornia Methanol Extract By MTT Assay

Sample	IC_{50} (mgL^{-1})
Extract methanol	414,6
Doxorubicin	4,1

The cytotoxic impact of G. salicornia methanolic extract against MCF-7 cells is demonstrated in Table 3 with an IC50 value of 414.6 mgL-1, whereas doxorubicin is used as a positive control with an IC50 value of 4.1 mgL-1. The cytotoxic activity of doxorubicin was quite robust, but the cytotoxic activity of the methanolic G. salicornia extract was modest Sajjadi et al. (24). Secondary metabolites of alkaloids, phenolics, steroids, and terpenoids are present in the methanol extract of G. salicornia. Numerous groupings of chemicals will work together to create cytotoxic molecules.

Phytochemical Composition and Its Relevance to Anticancer Mechanisms

The anticancer properties of Gracilaria salicornia red alga stem from its alkaloid compounds and phenolic substances together with steroids/terpenoids present in methanolic extracts. Different structural compounds show functional versatility by proven ability to regulate cellular mechanisms leading to cancer advancement. The nitrogen-rich chemical group known as alkaloids shows strong cytotoxic behavior because of which they attract scientific interest. These compounds destroy cancer cells by different mechanisms which involve DNA intercalation force as well as topoisomerase inhibition and microtubule destabilization effects. The sow thistle plant Catharanthus roseus produces vinblastine and vincristine alkaloids which stop cell division by blocking mitotic spindles per the research of Haque et al., (2016).

The dysregulated PI3K/Akt and MAPK signaling pathways together with altered gene expression patterns are targeted by alkaloids as they exhibit these effects on cellular biology. As a widely diverse category of natural substances, phenolic compounds show exceptional ability to affect redox equilibrium in cells. Within the tumor microenvironment phenolic compounds demonstrate their pro-oxidative properties which produce ROS accumulation along with oxidative stress. The oxidative stress generates membrane depolarization that activates caspases and triggers cell death through apoptosis (Purwaningsih, 2014). Research shows that breast cancer phenolic compounds help lower the expression of Bcl-2 which protects cells from death while they simultaneously increase Bax which functions to kill cells (Kumar & Pandey, 2013).

Numerous marine organisms containing steroids and terpenoids decrease cancer activity by connecting with cellular membranes and nuclear receptor structures. The terpenoid compound paclitaxel destroys microtubules while marine sterols alter the function of estrogen receptors. a key target in hormone-dependent breast cancers like MCF-7 (Yue et al., 2007). Steroids can prevent aromatase activity which disrupts the production of estrogen as well as alter estrogen communication signals. A beneficial compound-to-compound relationship occurs in the extract that leads to the enhancement of biological effects. Several compound classes within crude marine extracts create a synergistic effect by targeting multiple cellular elements that separately diminish cancer cell survival capacity. G. salicornia contains multiple compounds which could potentially use oxidative stress along with hormone disturbance and cell cycle blockage pathways although specific experimental proof remains to be identified.

BSLT as a General Toxicity and Bioactivity Predictor

The Brine Shrimp Lethality Test (BSLT) functions as an economical test which enables quick evaluation of bioactive substance general toxicity. Research data determined G. salicornia methanol extract toxicity at 561.26 mg/L which meets established definitions for substances classified as moderately toxic (30–1000 mg/L). The BSLT operates as a general toxicity assessment tool mainly because of its easy implementation and predictive results for overall cytotoxicity effects on living organisms. This toxicity level in the assay supports that G. salicornia contains active molecules with biological effects. Several natural product studies prove that brine shrimp cytotoxicity creates a positive correlation with anticancer activity because scientists frequently assess broad-spectrum cytotoxins including alkaloids and phenolics (Ntungwe et al., 2020).

Brine shrimp toxicity markers indicate that compounds lethal to these organisms will also affect fast-dividing mammalian cells because both cell types share similar mitochondrial and membrane integrity sensitivities. The extract shows lower toxicity than potassium dichromate (LC_{50} = 13.96 mg/L) yet demonstrates minimal acute toxicity risk for higher organisms when measured at similar dosage levels. Once active compounds are separated from the extract and concentrations reach their optimal levels the extract has potential as a development candidate. The BSLT testing offers a meaningful tool to identify which extract samples need further fractionation processes. The extract can be assessed for cytotoxicity using BSLT but additional bioassays like MTT and apoptosis markers should be utilized to determine the extracts that warrant further investigation (Mustapa et al., 2022).

Cytotoxic Effects on MCF-7 Breast Cancer Cells

The MCF-7 breast cancer cell viability assessment using MTT showed that G. salicornia methanol extract possessed cytotoxic properties with an IC_{50} value of 414.6 mg/L. The extracted compound demonstrates mild-to-moderate cytotoxicity strength based on an IC50 value of 414.6 mg/L which proves stronger than the positive control drug doxorubicin (4.1 mg/L) known for its severe side effects. The difference between IC50 values can be explained by contrasting the drug-like purity of doxorubicin with the overall extract compounds in crude extracts. Scientists often test hormone-responsive breast cancer based on the MCF-7 cell line because these cells harbor estrogen receptors (ER^+). Cytotoxic effects of the extract result at least partially from its ability to disrupt estrogen signaling pathways and its potential to affect estrogenic compound production. The presence of terpenoids and steroids creates a strong possibility of nuclear hormone receptor interference since these compounds are known competitive inhibitors (Li et al., 2015).

The cytotoxic effect of phenolics stems from their ability to increase ROS levels inside cells because cancer cells lack sufficient mechanisms to control high oxidative stress. More accumulated ROS ultimately damages mitochondria which triggers the apoptotic mechanisms by releasing cytochrome c and activating its intrinsic pathway. Past research on marine algal extracts including Gracilaria tenuistipitata showed that it caused ROS-dependent apoptosis in oral squamous cell carcinoma cells (Yeh et al., 2012). Due to the extract's moderate IC_{50} value researchers believe inactive compounds present in the crude mixture prevent the bioactive

ISSN 2776-1150 (Print), ISSN 2776-1169 (online)

agents from showing their full effect. The overall effectiveness of numerous marine extracts increases through bioassay-guided fractionation followed by purification because this process eliminates interfering substances while concentrating the active bioingredients (Mapelli et al., 2003). The slightly weak activity measured in the extract serves as fundamental data for subsequent chemical investigation.

Comparison with Other Gracilaria Species and Ecological Considerations

Research conducted on other Gracilaria species demonstrates species-specific along with ecological factors which contribute to observed similarities and differences in data results. The cytotoxic activity of Gracilaria edulis methanolic extracts was stronger because they demonstrated IC_{50} values ranging between 150–250 mg/L when tested against HeLa and MDA-MB-231 cancer cell lines. The research by Yang et al. (2012) showed that Gracilaria tenuistipitata extracts trigger cell death mechanisms that lead to tumor inhibition by ROS, DNA lesions and cell cycle disruptions. The biosynthetic pathways of G. salicornia grown at the Hari Islands undergo variations because of the tropical marine conditions that expose them to high UV radiation and changing nutrient levels and ecological stressors (Parailloux, 2021). Scientists have accepted geographical differences as a key element when studying drugs derived from algae. The extracts of the same species can vary in potency and chemical elements between different geographical collections because of environmental modifications. G. salicornia exhibits moderate cytotoxic properties even though its uninvestigated metabolites contain significant lead potential.

CONCLUSION

The scientific study establishes that Gracilaria salicornia red algae methanolic extract demonstrates moderate cytotoxic properties during MCF-7 breast cancer cell tests. The blend of phytochemicals in the extract contains significant amounts of alkaloids and phenolics and steroids/terpenoids which indicates the potential of active compounds to influence cancer-linked cellular operations such as redox balance and cell cycle control and programmed cell death. A moderate bioactivity level of the extract was confirmed through preliminary testing using the Brine Shrimp Lethality Test which supported future pharmacological studies. The MTT-assessed IC_{50} value of the extract was higher than traditional chemotherapy drugs because raw plant products combine active and inactive substances. Bioassay-guided fractionation should be pursued to identify the particular compounds which cause cytotoxicity in the observed results. The research demonstrates that G. salicornia represents an innovative prospect for cancer drug discovery from marine resources. This study adds to the expanding collection of research papers demonstrating how Indonesia's marine biological resources hold pharmaceutical value. Future investigations must concentrate on purification of compounds and experimentation on modes of action together with animal model analysis to establish the therapeutic capacity of this species.

REFERENCES

Ahmed, M. B., Islam, S. U., Alghamdi, A. A., Kamran, M., Ahsan, H., & Lee, Y. S. (2022). Phytochemicals as chemo-preventive agents and signaling molecule modulators: current role in cancer therapeutics and inflammation. *International Journal of Molecular Sciences*, *23*(24), 15765. https://doi.org/10.3390/ijms232415765

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42. https://doi.org/10.3390/plants6040042

Bergé, J. P., & Barnathan, G. (2005). Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. *Marine biotechnology I*, 49-125. https://doi.org/10.1007/b135782

Bone, R. C. (1993). Gram-negative sepsis: a dilemma of modern medicine. *Clinical Microbiology Reviews*, *6*(1), 57-68. https://doi.org/10.1128/cmr.6.1.57

Deepika, C., Ravishankar, G. A., & Rao, A. R. (2022). Potential products from macroalgae: An

overview. *Sustainable global resources of seaweeds volume 1: Bioresources, cultivation, trade and multifarious applications*, 17-44. https://doi.org/10.1007/978-3-030-91955-9_2

Dewi, T. K., Massar, K., Ardi, R., & Ruiter, R. A. (2021). Determinants of early breast cancer presentation: a qualitative exploration among female survivors in Indonesia. *Psychology & Health*, *36*(12), 1427-1440. https://doi.org/10.1080/08870446.2020.1841765

Fatma, R. Z., & Martsiningsih, M. A. (2019). *Perbedaan Kadar Ureum Pada Plasma Lithium Heparin Dengan Penggunaan Separator Tube Dan Vacutainer Pada Pasien Post Hemodialisa* (Doctoral dissertation, Poltekkes Kemenkes Yogyakarta).

Fatmawati, S. (2020). *Faktor Yang Berhubungan Dengan Kanker Payudara Pada Wanita Pasangan Usia Subur Di Rsu Vina Estetica Tahun 2019* (Doctoral dissertation, Institut Kesehatan Helvetia).

Ferreira, I. M. (2023). *Detailed characterization of antitumorigenic potential of cynaropicrin and fucoidans in breast cancer cell line* (Master's thesis, Universidade do Minho (Portugal)).

Ghosh, A., & Gopinath, S. C. (2025). Molecular Mechanism of Breast Cancer and Predisposition of Mouse Mammary Tumor Virus Propagation Cycle. *Current Medicinal Chemistry*. https://doi.org/10.2174/0109298673286234240123100955

Haque, M. U., Ferdiousi, N., & Sajon, S. R. (2016). Anticancer agents derived from plant and dietary sources: a review. *Int. J. Pharmacogn*, *3*, 55-66. http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.3(2).55-66

Jupiter, S., Mangubhai, S., & Kingsford, R. T. (2014). Conservation of biodiversity in the Pacific Islands of Oceania: challenges and opportunities. *Pacific Conservation Biology*, *20*(2), 206-220. https://doi.org/10.1071/PC140206

Kerimi, A., & Williamson, G. (2018). Differential impact of flavonoids on redox modulation, bioenergetics, and cell signaling in normal and tumor cells: a comprehensive review. *Antioxidants & redox signaling*, *29*(16), 1633-1659. https://doi.org/10.1089/ars.2017.7086

Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., ... & Oz, F. (2023). Major phytochemicals: recent advances in health benefits and extraction method. *Molecules*, *28*(2), 887. https://doi.org/10.3390/molecules28020887

Lam, J. R. Y., Casimpan, G. A., Batoy, C. M. L., Cebedo, M. F., Cuesta, J. Y. T., Gervacio, J., ... & Adrian, C. A. L. (2023). Antimitotic Activity of Samanea saman Leaf Extract in the In Vitro Development of Tripneustes gratilla Embryo. *bioRxiv*, 2023-05. https://doi.org/10.1101/2023.05.10.540278

Li, L., Bonneton, F., Chen, X. Y., & Laudet, V. (2015). Botanical compounds and their regulation of nuclear receptor action: The case of traditional Chinese medicine. *Molecular and cellular endocrinology*, *401*, 221-237. https://doi.org/10.1016/j.mce.2014.10.028

Limongelli, F., Crupi, P., Clodoveo, M. L., Corbo, F., & Muraglia, M. (2022). Overview of the polyphenols in Salicornia: From recovery to health-promoting effect. *Molecules*, *27*(22), 7954. https://doi.org/10.3390/molecules27227954

Mahmudi, M., Lusiana, E. D., Herawati, E. Y., & Serihollo, L. G. (2020). Environmental factors and seasonal effect on the potential harmful algae presence at Ambon Bay, Indonesia. *Biodiversitas Journal of Biological Diversity*, *21*(7). https://doi.org/10.13057/biodiv/d210730

Mapelli-Brahm, P., Gómez-Villegas, P., Gonda, M. L., León-Vaz, A., León, R., Mildenberger, J., ... & Meléndez-Martínez, A. J. (2023). Microalgae, seaweeds and aquatic bacteria, archaea, and yeasts: Sources of carotenoids with potential antioxidant and anti-inflammatory

health-promoting actions in the sustainability era. *Marine Drugs*, *21*(6), 340. https://doi.org/10.3390/md21060340

Maulidia, N. R. (2020). *Uji Sitotoksisitas Ekstrak Etanol 96% Daun Semanggi (Marsilea crenata C. Presl) pada sel hFOB 1.19 dengan Metode Microtetrazolium (MTT) Assay* (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim).

Mustapa, M. A., Guswenrivo, I., Zuhrotun, A., Ikram, N. K. K., & Muchtaridi, M. (2022). Anti-breast cancer activity of essential oil: A systematic review. *Applied Sciences*, *12*(24), 12738. https://doi.org/10.3390/app122412738

Niksic, H., Becic, F., Koric, E., Gusic, I., Omeragic, E., Muratovic, S., ... & Duric, K. (2021). Cytotoxicity screening of Thymus vulgaris L. essential oil in brine shrimp nauplii and cancer cell lines. *Scientific Reports*, *11*(1), 13178. https://doi.org/10.1038/s41598-021-92679-x

Ningsih, I. Y. (2014). Pengaruh elisitor biotik dan abiotik pada produksi flavonoid melalui kultur jaringan tanaman. *PHARMACY: Jurnal Farmasi Indonesia (Pharmaceutical Journal of Indonesia)*, *11*(2). https://doi.org/10.30595/pji.v11i2.829

Ntungwe N, E., Domínguez-Martín, E. M., Roberto, A., Tavares, J., Isca, V., Pereira, P., ... & Rijo, P. (2020). Artemia species: An important tool to screen general toxicity samples. *Current Pharmaceutical Design*, *26*(24), 2892-2908. https://doi.org/10.2174/1381612826666200406083035

Parailloux, M. (2021). *Development of high-resolution mass spectrometry approaches for the characterization of bioactive compounds in algae and Salicornia genus* (Doctoral dissertation, Université de Pau et des Pays de l'Adour).

Pratiwi, L., KM, M., Anggraini Ambarsari, S., KM, M., Fijri, B., ST, S., ... & ST, S. (2024). *Mengenal Mencegah Kanker Payudara: Sudut Pandang Teori & Penelitian*. Jawa Barat: CV Jejak (Jejak Publisher).

Priyanka, K. R., Rajaram, R., & Sivakumar, S. R. (2022). A critical review on pharmacological properties of marine macroalgae. *Biomass Conversion and Biorefinery*, 1-25. https://doi.org/10.1007/s13399-022-03134-4

Purwaningsih, E. (2014). Pemendekan telomer dan apoptosis. *Jurnal Kedokteran YARSI*, *22*(2), 132-141. https://doi.org/10.33476/jky.v22i2.309

Rusyaev, S. M., & Orlov, A. M. (2024). The phenomenon of marine bioprospecting. *Biology Bulletin Reviews*, *14*(1), 115-132. https://doi.org/10.1134/S2079086424010080

Salma, W. O., Binekada, I. M. C., Fristiohady, A., & Alifariki, L. O. (2021). *Buku Referensi Potret Masyarakat Pesisir Konsep Inovasi Gizi & Kesehatan*. Yogyakarta: Deepublish.

Soares, P. I. P., Dias, S. J. R., Novo, C. M. M., Ferreira, I. M. M., & Borges, J. P. (2012). Doxorubicin vs. ladirubicin: methods for improving osteosarcoma treatment. *Mini reviews in medicinal chemistry*, *12*(12), 1239-1249. https://doi.org/10.2174/138955712802762022

Suparna, K., & Sari, L. M. K. K. S. (2022). Kanker Payudara: Diagnostik, Faktor Risiko, Dan Stadium. *Ganesha Medicina*, *2*(1), 42-48. https://doi.org/10.23887/gm.v2i1.47032

Syamsurizal, S., Utami, D. T., Sutrisno, S., Efrini, E., & Jumardi, M. I. (2023). Toxicity test of red palm (Cyrtostachys renda Blume.) using the brine shrimp lethality test (BSLT) method. *Journal of Pharmaceutical and Sciences*, 407-413. https://doi.org/10.36490/journal-jps.com.v6i5-si.429

Torres, P., Santos, J. P., Chow, F., & dos Santos, D. Y. (2019). A comprehensive review of traditional uses, bioactivity potential, and chemical diversity of the genus Gracilaria (Gracilariales, Rhodophyta). *Algal Research*, *37*, 288-306. https://doi.org/10.1016/j.algal.2018.12.009

Utomo, D. N., & Yusbida, A. (2017). Cytotoxic effect of freeze dried bovine cartilage powder and

platelet rich plasma (prp) to mesenchymal stem cell (mscs). *Journal Orthopaedi and Traumatology*, *6*(02), 16-23.

Valentová, J., Lintnerová, L., Miklášová, N., Oboňová, B., & Habala, L. (2023). Analogues of anticancer natural products: Chiral aspects. *International Journal of Molecular Sciences*, *24*(6), 5679. https://doi.org/10.3390/ijms24065679

West, J. B., Dinh, M. N., Brown, J. S., Zhang, J., Anderson, A. R., & Gatenby, R. A. (2019). Multidrug cancer therapy in metastatic castrate-resistant prostate cancer: an evolution-based strategy. *Clinical Cancer Research*, *25*(14), 4413-4421. https://doi.org/10.1158/1078-0432.CCR-19-0006

Yang, J. I., Yeh, C. C., Lee, J. C., Yi, S. C., Huang, H. W., Tseng, C. N., & Chang, H. W. (2012). Aqueous extracts of the edible Gracilaria tenuistipitata are protective against H2O2-induced DNA damage, growth inhibition, and cell cycle arrest. *Molecules*, *17*(6), 7241-7254. https://doi.org/10.3390/molecules17067241

Yeh, C. C., Yang, J. I., Lee, J. C., Tseng, C. N., Chan, Y. C., Hseu, Y. C., ... & Chang, H. W. (2012). Anti-proliferative effect of methanolic extract of Gracilaria tenuistipitata on oral cancer cells involves apoptosis, DNA damage, and oxidative stress. *BMC Complementary and Alternative Medicine*, *12*, 1-9. https://doi.org/10.1186/1472-6882-12-142

Yue, W., Fan, P., Wang, J., Li, Y., & Santen, R. J. (2007). Mechanisms of acquired resistance to endocrine therapy in hormone-dependent breast cancer cells. *The Journal of steroid biochemistry and molecular biology*, *106*(1-5), 102-110. https://doi.org/10.1016/j.jsbmb.2007.05.008

ISSN 2776-1150 (Print), ISSN 2776-1169 (online)
