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Phytochemical Content and Toxicity Test of *Kappaphycus Alvarezii* Hot Water and Methanol Using BSLT Method

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Abstract

Investigating both the beneficial bioactive compounds and the potential toxicity of seaweed extracts provides crucial information to guide their development for nutritional, antioxidant activity, and even cytotoxicity against cancer cells. This study investigated the toxicity and phytochemical composition of hot water and methanol extracts of *Kappaphycus alvarezii* using a brine shrimp lethality test (BSLT) with *Artemia salina* larvae. *K. alvarezii* samples were extracted using hot water and methanol, and the extracts were subjected to phytochemical screening and toxicity testing. The hot water extract yielded 8.4%, comprising alkaloids, saponins, and flavonoids, whereas the methanol extract yielded 2.4%, consisting of alkaloids and saponins. The BSLT results showed that larval mortality increased with higher extract concentrations, indicating a dose-dependent toxicity. Probit analysis revealed LC_{50} values of 180.90 µg/mL for the hot water extract and 961.97 µg/mL for the methanol extract, classifying both as toxic according to Meyer's toxicity categories. The hot water extract demonstrated higher toxicity, potentially owing to its higher yield and presence of flavonoids. The bioactive compounds in *K. alvarezii* extracts, such as alkaloids, saponins, and flavonoids, may contribute to their toxic effects on *A. salina* larvae. These findings suggest that *K. alvarezii* extracts have potential applications as antitumor and antibacterial agents. Antibacterials should be further explored for their immunostimulatory properties in aquaculture.

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1. Introduction

Seaweed is a marine resource with considerable market value and long-term potential and its demand continues to increase. It is extensively used in various sectors and is the primary raw material used in the food, cosmetic, and pharmaceutical industries. The widespread distribution and relatively straightforward cultivation of seaweed have made its farming a prevalent practice in Indonesia (Mambai *et al*., 2020). One such seaweed is *Kappaphycus alvarezii*. *K alvarezii* cultivation has rapidly expanded in Indonesia, particularly among coastal communities, owing to its simple farming process, low capital costs, and short production cycles (Nurdin *et al*., 2023).

K. alvarezii, a commercially important red seaweed, is primarily cultivated because of its carrageenan content, a key primary metabolite. Carrageenan, a hydrocolloid, is widely used as a gelling and stabilizing agent in food and cosmetic industries (Kumar *et al*., 2020). The carrageenan content in dried Kappaphycus is approximately 34.6% (Wanyonyi *et al*., 2017). *K. alvarezii* can synthesize secondary metabolites containing a diverse range of phytochemicals with potential bioactive properties. Quantitative phytochemical analysis revealed the presence of flavonoids, tannins, phenolic compounds, saponins, glycosides, steroids, carbohydrates, and alkaloids in *K. alvarezii* (Das *et al*., 2023; Prasetiyo *et al*., 2023). The presence of these phytochemical constituents in *K. alvarezii* has a significant potential for further investigation.

Solvent maceration has been effectively used to extract metabolites from the red seaweed, *K. alvarezii*. The methanol extraction of *K. alvarezii* revealed 20 valuable bioactive compounds, including 3-hydroxybenzoic acid, gallic acid, chlorogenic acid, cinnamic acid, artemiseole, and hydrazine carbothioamide (Baskararaj *et al*., 2020). *K. alvarezii* has shown promising potential in various applications, particularly in the form of aqueous extracts. Aqueous extracts from *K. alvarezii* have versatile applications ranging from prebiotic ingredients to natural preservatives. It is rich in phytochemicals, including flavonoids, tannins, and phenolic compounds (Bajury *et al*., 2017; Das *et al*., 2023), and different extraction methods and solvents can significantly affect the yield and composition of the extracted metabolites. *K alvarezii* is a promising functional food ingredient and feed supplement, with potential benefits for metabolic health. Its applications extend beyond whole seaweed use as different fractions exhibit distinct bioactivities. Further research is needed to optimize the extraction methods and examine their toxicity in living organisms using animal testing.

The Brine Shrimp Lethality Test (BSLT) is a widely used method for the preliminary cytotoxicity screening of plant extracts, including seaweed. This assay involves exposing *Artemia salina* (brine shrimp) nauplii to different concentrations of the extract for 24 h and calculating the number of motile nauplii to determine the effectiveness of the extract (Sarah et al., 2017). BSLT is valued for its simplicity, low cost, and reproducibility in toxicity detection (Ntungwe *et al*., 2020; Salay *et al*., 2024). The Lethal Concentration 50% (LC_{50}) value is the concentration that kills 50% of the test organisms obtained from the BSLT, and correlates with the toxicity of the tested substances. Generally, lower LC_{50} values indicate a higher toxicity. Interestingly, the interpretation of BSLT results can vary. While some studies indicate general toxicity, others correlate it with specific bioactivities, such as cytotoxicity or anticancer potential (Ogbole *et al*., 2017; Olmedo *et al*., 2024). There are three categories of toxicity, based on the magnitude of the LC_{50} value: highly toxic (<30 µg/mL), toxic (30-1000 µg/mL), and non-toxic (>1000 µg/mL) (Meyer *et al*., 1982).

Utilizing both hot water and methanol as solvents is essential for a comprehensive examination of the diverse compounds present in seaweeds. These solvents enable efficient extraction of active metabolites from *K. alvarezii*, thereby facilitating further analysis of compounds that may serve as foundational materials for product development. This methodological approach is particularly significant before evaluating the toxicity of *K. alvarezii* extract. Currently, there is insufficient scientific research regarding the influence of different solvents on the toxicity of extracts and the effect of varying concentrations on the mortality rate of *Artemia salina*. This study aimed to investigate the impact of extract type and concentration on *A. salina* mortality using BSLT to assess toxicity. Furthermore, the potential for application of the extract was analyzed based on its LC_{50} value.

2. Material and methods

2.1. Material

The material used in this study was *K. alvarezii* grown under wet conditions and sourced from the Brackish Water Aquaculture Development Center (BBPBAP) in Jepara, Central Java. This sample served as the primary material in the extraction process for the toxicity assessment. Newly hatched *A. salina* larvae were used as test organisms. *A. salina* cysts were procured online from Supreme Plus. Seawater with a salinity of 30 ppt was used to maintain the *A. salina* larvae during the test. Two polar solvents, water and methanol, were used in the extraction process and obtained from a chemical supply store.

2.2. Sample Preparation

Test samples, weighing 500 g in a wet state, were obtained by cultivation. These samples were securely sealed and stored in an icebox for transportation to the Biology Laboratory at Diponegoro University for subsequent analysis. Upon arrival, the samples were meticulously rinsed under running water to remove residual dirt. They were then allowed to dry for four days, ensuring that they were not exposed to direct sunlight.

2.3. Extraction

The maceration technique was used in the extraction process to obtain polysaccharides through the dissolution of the cell walls. This extraction was conducted over three consecutive 24-hour periods, employing a methanol solution to treat samples with both hot water and methanol solvents. Initially, the samples were ground using a blender to prepare 50 g of sample powder. Subsequently, one 50 g batch was extracted with 500 mL of methanol, whereas another 50 g batch was extracted using 500 mL of hot water at 80°C (Lalopua 2020).

2.4. Evaporation

Evaporation was performed using a rotary evaporator to separate the extract from solvent. For the samples utilizing methanol as the solvent, the temperature was maintained at 40°C at a rotation speed of 65 rpm. In contrast, for the sample prepared using hot water as the solvent, the temperature was set at 50°C at a rotation speed of 60 rpm (Panjaitan & Meze, 2023). The samples, which were in the paste form after rotary evaporation, were subsequently placed in an oven at 40°C until their weight was reduced to 10% of the initial weight.

2.5. Phytochemical Screening

The alkaloid assay was performed by dissolving each sample in methanol (10 mL) in a beaker. Subsequently, 2 mL of Dragendorff's reagent was added and the solution was agitated and observed. A positive outcome was indicated by the formation of a white or orange-yellow precipitate. For the saponin assay, 2 mL of the extract was combined with 2 mL of distilled water, shaken for 1 min, and 2 drops of HCl were added. Foam stability was monitored for 7 min. The tannin assay involved the addition of 1 mL of the extract to 10 drops of $FeCl_3$, and the color changes were recorded after 5 min. For the flavonoid assay, 2 mL of the sample was mixed with 2 mL of n-hexane to create two layers, followed by the addition of 1 mL of methanol, magnesium powder (0.5 g), and HCl (5 drops). Steroid assays included Mayer's, Wagner's, and Liebermann's tests. For this, 2 mL of methanol was mixed with the sample, and then 2 mL of the Liebermann-Burchard reagent was added to observe changes. For Mayer's and Wagner's tests, 2 mL of the extract was mixed with 2 mL of n-hexane, and the n-hexane layer was treated with the respective reagents (Panjaitan and Meze, 2023).

2.6. Hatching of *Artemia salina*

The procedure was initiated by measuring 1 g of dry *A. salina* cysts, which were subsequently immersed in freshwater for an hour. Then, 1 L of sterilized seawater was prepared. *A. salina* was then transferred to a container containing seawater, where it was aerated and incubated at room temperature for 24 h (Yudiati *et al*., 2023).

2.7. Toxicity Testing

The toxicity assessment was conducted over 24 h, commencing with the preparation of the stock solutions. A stock solution with a concentration of 1,000 µg/mL was prepared in a volume of 50 mL for each test solution type. Specifically, 0.5 g of the sample with methanol solvent and 0.5 g of the sample with hot water solvent were weighed and subsequently dissolved in 50 mL of seawater, ensuring thorough mixing. Subsequently, five series of extract solvent concentrations were prepared, with test concentrations of 500, 250, 125, 62.5, and 31.3 µg/mL; Each concentration was replicated five times. Twenty *A. salina* individuals were introduced into each vial, and the initial time (t_0) was recorded as the reference point for mortality calculations. The mortality assessment commenced immediately upon the introduction of the samples and *A. salina* into the vials. Observations were conducted over 24 h, and mortality was recorded at the 1st, 2nd, 4th, 8th, and 24th hour (Damayanti *et al*. 2025).

2.8. Data Analysis

The probit analysis method was used to model the biological response to the concentration of *the K. alvarezii* extract and provided an accurate estimate of the LC_{50} value based on the probit distribution of the response. The data were processed using Microsoft Excel with a linear graph of the relationship between the probit value and log concentration. The graph provides data for the equation y = a + bx, where y is the probit value, and x is the log concentration. The LC_{50} value is the specific concentration that produces a 50% mortality response in the test biota.

3. Results

3.1 Yield of *Kappaphycus alvarezii* Extract

Based on the data presented in Table 1, it can be observed that the initial weight of the samples before extraction for each sample was 50 g. After evaporation via a rotary evaporator, the samples exhibited different final weights: the extract obtained using hot water as a solvent weighed 4.2 g, whereas that obtained using methanol weighed 1.2 g. The percentage yield of the extract obtained using hot water was 8.4%, whereas that obtained using methanol was 2.4%.

Table 1. Yield percentage of hot water and methanol extract

Type of Extract	Weight of Sample (g)	Weight of Extract (g)	Yield Percentage (%)
Hot Water	50	4.2	8.4
Methanol	50	1.2	2.4

3.2. Results of Phytochemical Screening

Based on the data presented in Table 2, it can be observed that several groups of secondary metabolite compounds were successfully identified through the various tests conducted earlier. The groups of compounds identified included alkaloids, tannins, and saponins in the hot water extract and alkaloids and saponins in the methanol extract.

Table 2. Phytochemical test on hot water and methanol extract

Type of Phytochemical Test	Hot Water Extract	Methanol Extract
Alkaloids	+	+
Tanins	-	-
Flavonoids	+	-
Saponins	+	+
Steroids	-	-

Note: + present, - absent

3.3. Probit Regression Analysis for Toxicity Testing

Tables 3 and 4 present the mortality data of Artemia larvae following treatment with the extracts at specified concentrations. The regression equation derived from probit analysis is illustrated in Figures 1 and 2. As shown in Figure 1, the regression equation derived for the hot water extract was y = 1.8579x + 0.8059 with an R^2 value of 0.9066. Similarly, as shown in Figure 2, the equation for the methanol extract was y = 1.3362x + 1.0139 with an R^2 value of 0.9348. The LC_{50} value was determined using a regression equation, where y = 5. Subsequently, the value of x was calculated using the equation y = ax + b, and this value was employed to determine LC_{50} using the antilogarithmic function, where LC_{50} = antilog(x). The resulting LC_{50} values are presented in Table 5.

Table 3. Mortality rates of hot water extracts against *Artemia* larvae.

Extracts Concentration (μg/mL)	Log Concentration	Number of Larvae	Average Number of Dead Larvae	Mortality (%)	Probit Value
500	2.70	20	15	75	5.68
250	2.40	20	13	65	5.39
125	2.10	20	10	50	5.00
62.5	1.80	20	2	10	3.72
31.5	1.50	20	2	10	3.72

Tabel 4. Mortality rates of methanol extracts against *Artemia* larvae.

Extracts Concentration (μg/mL)	Log Concentration	Number of Larvae	Average Number of Dead Larvae	Mortality (%)	Probit Value
500	2.70	20	8	40	4.76
250	2.40	20	4	20	4.16
125	2.10	20	2	10	3.72
62.5	1.80	20	1	5	3.22
31.5	1.50	20	1	5	3.22

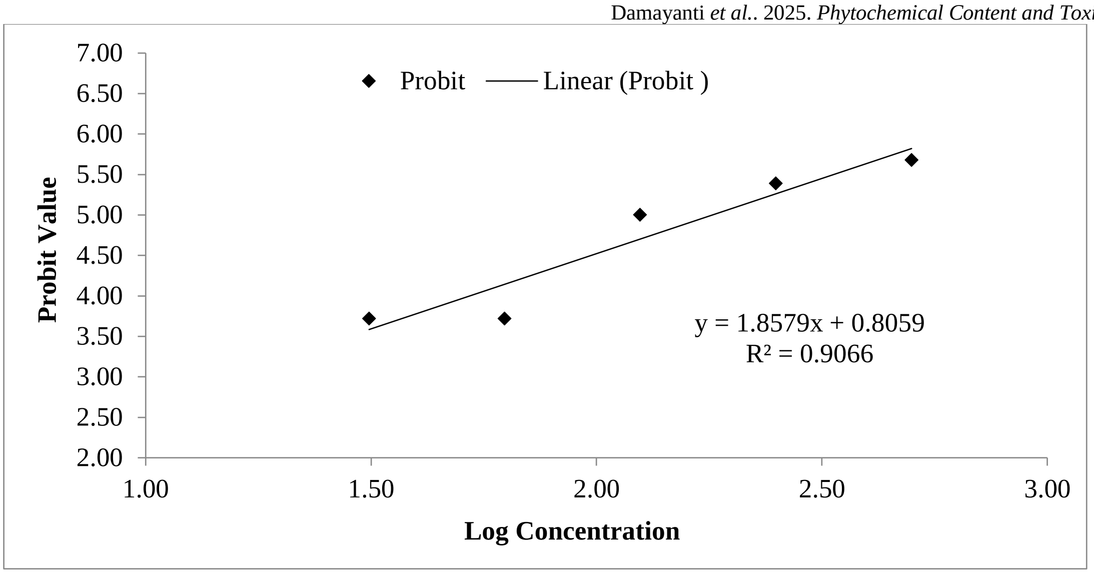

Figure 1. Probit analysis of LC_{50} for hot water extract

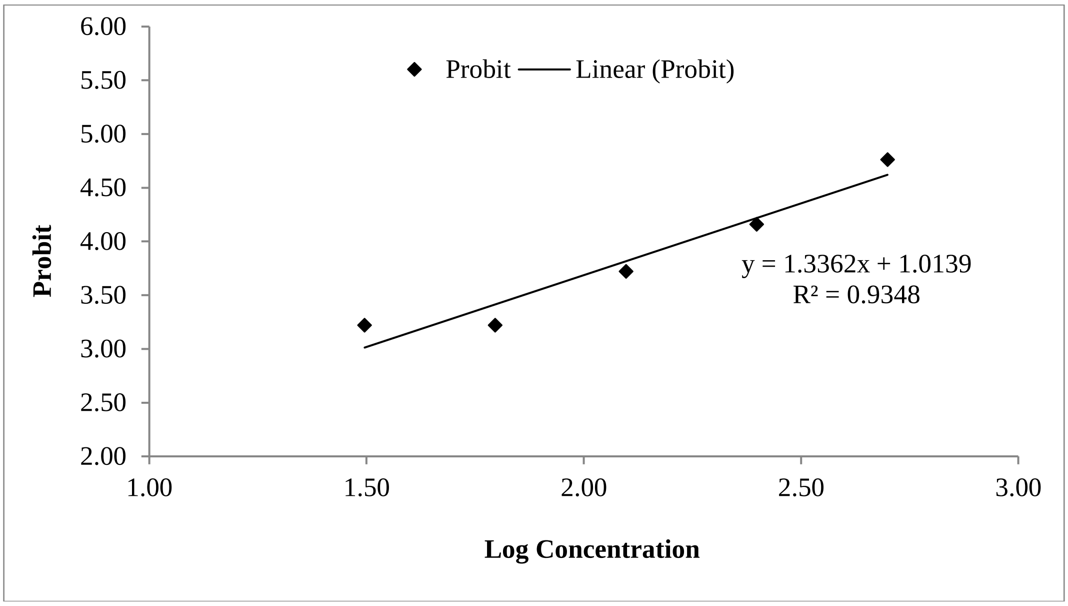

Figure 2. Probit Analysis of LC_{50} for methanol extract

4. Discussion

The yield of the extract obtained using hot water was 8.4%, while that obtained using methanol was 2.4%. The high yield from the hot water extract can be attributed to the presence of phycocolloids, particularly carrageenan, which dissolve in larger quantities than in the methanol extraction. This resulted in the characteristic gel-like form of the hot water extract. Although carrageenan can still be extracted using plain water, extraction using hot water yielded significantly higher amounts. The high temperature used during hot water extraction helps break down cell structures, thereby increasing the solubility of carrageenan. This was supported by Chin *et al*. (2019), who reported that *K. alvarezii* contains a significant amount of carrageenan, a water-soluble hydrocolloid. Wanyonyi *et al*. (2017) reported that dried Kappaphycus contains approximately 34.6% carrageenan as a soluble fiber.

The hot water extract of *K. alvarezii* was positive for alkaloids, saponins, and flavonoids, whereas the methanol extract was positive for alkaloids and saponins. Several studies have identified specific phytochemical groups that are potentially toxic to artemia. Various phytochemical groups found in seaweed extracts, such as flavonoids, tannins, phenolic compounds, and terpenoids, have demonstrated potential toxicity in *Artemia salina*. The brine shrimp lethality assay serves as a valuable tool for preliminary screening of seaweed extracts for bioactive compounds with potential therapeutic applications, including anticancer and antimicrobial properties (Das *et al*., 2023; N *et al*., 2020).

The phytochemical content of the samples also influenced the toxicity levels, as bioactive compounds in the test samples interacted with *A. salina* and affected the mortality rates. Based on these research findings, extraction using a hot water solvent was able to extract three groups of secondary metabolites: alkaloids, saponins, and flavonoids, whereas extraction with methanol yielded only two groups: alkaloids and saponins. This phytochemical screening aligns with the solvent characteristics used. Hot water, which is highly polar at elevated temperatures, enhances the diffusion of compounds from plant tissues and softens the cell wall structures, thereby facilitating the release of secondary metabolites. Additionally, polar flavonoids such as glycoside flavonoids have a high affinity for hot water because of their numerous hydroxyl groups (Sagala *et al*., 2025). This explains why flavonoids were detected in the hot water extract but not in the methanol extract. Although methanol is a polar solvent, its semi-organic nature, owing to the presence of methyl groups, can reduce the solubility of highly hydrophilic compounds such as polar flavonoids. Thus, the differences in the detected compounds between the two solvents indicate that the choice of solvent type and properties significantly affect the effectiveness of secondary metabolite extraction from natural materials.

The results presented in Figures 1 and 2 indicate that larval mortality increased at higher extract concentrations. The increase in mortality rates at higher concentrations indicates that the active compounds in *the K. alvarezii* extract possess dose-dependent toxic properties. As the concentration of the extract increased, the amount of bioactive compounds available to interact with the biological systems of the larvae also increased, thereby increasing the likelihood of mortality. This phenomenon is known as the dose-response relationship, where biological effects (mortality) increase proportionally with increased exposure to active substances (concentration). In acute toxicity testing, such relationships are often used as the basis for determining the toxicity potential of a compound or a natural extract.

Figures 1 and 2 show that the concentration of the extract was directly proportional to the percentage of mortality, with higher concentrations resulting in a higher probability of mortality. The toxicity of the test compounds was assessed by examining the LC_{50} value over 24 h. Based on the data presented in Table 4.3, both the hot water and methanol extracts of *K. alvarezii* fall into the toxic category, with an LC_{50} value of 180.90 µg/mL for the hot water extract, while the methanol extract had an LC_{50} value of 961.97 µg/mL. The LC_{50} values for the extracts of *K. alvarezii* using both hot water and methanol are 30-1000 µg/mL, indicating that the extracts are toxic. A relevant application in the marine field is antibacterial or antitumor activity for human health.

Previous studies have demonstrated the cytotoxic and antitumor potential of various seaweed extracts prepared using BSLT. Interestingly, BSLT results correlated with the specific mechanisms of antitumor activity (Olmedo *et al*., 2024). *K. alvarezii* extract has shown promising antitumor potential in several studies. The methanol extract of *K. alvarezii* contains 20 valuable bioactive compounds, including phenolic acids and other phytochemicals, which demonstrate anticancer activity. A lower LC_{50} value suggests a higher potency of the extract in inhibiting cancer cell growth (Baskararaj *et al*., 2020).

Das *et al*. (2023) reported that seaweeds can demonstrate antibacterial properties against several pathogenic bacteria. These findings suggest its potential use in promoting gut health and as a natural antimicrobial agent, and can be applied for feed supplementation and immunostimulants for aquaculture biota, such as shrimp and fish. According to Chowdhury *et al.* (2021), the alkaloids, saponins, and flavonoids found in seaweeds are bioactive compounds that exhibit significant antibacterial activity and can function as immunostimulants. Alkaloids inhibit bacterial growth by disrupting cellular metabolism and inhibiting essential enzymes, thereby reducing the bacterial viability. In contrast, saponins damage bacterial cell membranes, leading to the leakage of cellular components, ultimately resulting in cell death. Flavonoids also contribute to the inhibition of protein synthesis and bacterial enzyme activity and possess anti-inflammatory effects that support immune responses. Furthermore, these three compounds can enhance the activity of immune cells, such as macrophages and lymphocytes, and increase the production of cytokines, which play a crucial role in strengthening the immune response to infections.

Based on the results of this study, hot water extract-rich carrageenan has potential as an effective and safe natural immunostimulant for the cultivation of various organisms. These findings align with those of Dhewang *et al*. (2023), who reported that the supplementation of *K. alvarezii* carrageenan in shrimp feed significantly enhances various innate immune parameters, and the mechanism of immunostimulation is believed to be closely related to the bioactive compound content of carrageenan, including alkaloids, saponins, and flavonoids, which are known to possess antioxidant, antimicrobial, and immunomodulatory activities.

5. Conclusions

K. alvarezii extract contained phytochemical compounds. Based on the phytochemical screening results, the hot water extract contained alkaloids, saponins, and flavonoids, whereas the methanol extract contained alkaloids and saponins. These bioactive compounds have the potential to act as toxic agents in *A. salina* larvae. There was a correlation between the concentration of *the K. alvarezii* extract and the mortality rate of *A. salina*. The hot water extract is potentially more toxic than the methanol extract, with LC_{50} values of 180.90 and 961.97 µg/mL, respectively. Based on the toxicity category, both extracts could be further tested for their potential as antibacterial or antitumor agents.

Ethics approval

No permits were required.

Data availability statement

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Author contributions

SOW is responsible for data curation, conceptualization, project administration, funding acquisition, writing – review & editing. FA and DNR are contributed to investigation, resource acquisition, methodology, formal analysis and writing the original draft.

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Declaration of competing Interest

None

References

Baskararaj, S., Panneerselvam, T., Sankaranarayanan, M., Pandian, S. R. K., Ravishankar, V., Kunjiappan, S., Govindaraj, S., Mohan, U. P., Palanisamy, P., Parasuraman, P., & Arunachalam, S. 2020. Formulation and characterization of folate receptor-targeted PEGylated liposome encapsulating bioactive compounds from *Kappaphycus alvarezii* for cancer therapy. *Biotech*, 10(3). https://doi.org/10.1007/s13205-020-2132-7

Chin, Y. X., Cao, W. X., Mi, Y., Xue, C. H., Lim, P. E., & Tang, Q. J. 2019. A Pilot Study on Anti-Obesity Mechanisms of *Kappaphycus alvarezii*: The Role of Native κ-Carrageenan and the Leftover Sans-

Carrageenan Fraction. *Nutrients*, 11(5): 1133. https://doi.org/10.3390/nu11051133

Chowdhury, A., Ara, J. & Islam, M. S. 2021. Green Synthesis of Phytochemical Nanoparticles and Their Antimicrobial Activity, A Review Study. *Biomed J Scientific Techn Res*, 34(4): 26929-26935. https://doi.org/10.26717/BJSTR.2021.34.005580

Damayanti, M., Salma, U., Susetya, I. E., & Ighwerb, M. I. 2025. Evaluation of Carrageenan Yield and Toxicity from *Kappaphycus alvarezii*: Implications for Aquaculture and Environmental Impact. *Journal of Marine Biotechnology and Immunology*. 3(1): 1-7. https://doi.org/10.61741/5r66rh88

Das, D., Lopez-Santamarina, A., Paramasivam, S., Miranda Lopez, J. M., Del Carmen Mondragon, A., & Arulkumar, A. 2023. Phytochemical Constituents, Antimicrobial Properties and Bioactivity of Marine Red Seaweed (*Kappaphycus alvarezii*) and Seagrass (*Cymodocea serrulata*). *Foods*, 12(14): 2811. https://doi.org/10.3390/foods12142811

Dhewang, I. B., Yudiati, E., Subagyo, S., & Alghazeer, R. 2023. Supplementation of Carrageenan (*Kappaphycus alvarezii*) for Shrimp Diet to Improve Immune Response and Gene Expression of White Shrimp (*Litopenaeus vannamei*). *ILMU KELAUTAN: Indonesian Journal of Marine Sciences*, 28(2): 46-53. https://doi.org/10.14710/ik.ijms.28.2.161-172

Kumar, Y. N., Sade, A., Lim, P.-E., Brodie, J., Poong, S.-W., & Gachon, C. 2020. Impact of elevated temperature on the physiological and biochemical responses of *Kappaphycus alvarezii* (Rhodophyta). *PLOS ONE*, 15(9): e0239097. https://doi.org/10.1371/journal.pone.0239097

Lalopua, V. M. 2020. Rendemen ekstrak kasar dan fraksi pelarutan alga merah (*Kappaphycus alvarezii* Doty). *Majalah Biam* , 16 (1), 1-5

Lara, A.D., Elisma, E. & Kasmadi, M. S. 2021. Uji Aktivitas Analgesik Infusa Daun Jeruju (*Acanthus ilicifolius* L.) pada Mencit Putih Jantan (*Mus musculus*). *Indonesian Journal of Pharma Science*, 3(2): 71-80. https://doi.org/10.22437/ijps.v3i2.15383

Mambai, R. Y., Salam, S., & Indrawati, E. 2020. Analisis Pengembangan Budidaya Rumput Laut (*Euchema cottoni*) di Perairan Kosiwo Kabupaten Yapen. *Urban and Regional Studies Journal*, 2(2): 66-70. https://doi.org/10.35965/ursj.v2i2.568

Meyer, B., Ferrigni, N., Jacobsen, L., Mclaughlin, J., Nichols, D., & Putnam, J. 1982. Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, 45(05): 31–34. https://doi.org/10.1055/s-2007-971236

Nurdin, N., Aris, A., Asis, H., Komatsu, T., Oiry, S., Barillé, L., Brunier, G., Syamsuddin, R., Alevizos, E., & Zainuddin, E. N. 2023. Precision Aquaculture Drone Mapping of the Spatial Distribution of Kappaphycus alvarezii Biomass and Carrageenan. *Remote Sensing*, 15(14): 3674. https://doi.org/10.3390/rs15143674

Ntungwe N, E., Pereira, P., Domínguez-Martín, E. M., Roberto, A., Isca, V. M. S., Cebola, M.-J., Rijo, P., & Tavares, J. 2020. Artemia species: An Important Tool to Screen General Toxicity Samples. *Current Pharmaceutical Design*, 26(24): 2892–2908. https://doi.org/10.2174/1381612826666200406083035

Ogbole, O. O., Adeniji, A. J., & Segun, P. A. 2017. In vitro cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. *BMC Complementary and Alternative Medicine*, 17(1): https://doi.org/10.1186/s12906-017-2005-8

Olmedo, D. A., Solís, P. N., De León, E. G., Vasquez, Y., Morán, J. A., & Caballero-George, C. 2024. Understanding the *Artemia salina* (Brine Shrimp) Test: Pharmacological Significance and Global Impact. *Combinatorial Chemistry & High Throughput Screening*, 27(4): 545–554. https://doi.org/10.2174/1386207326666230703095928

Panjaitan, R. S. & Meze, M. F. 2023. Variasi Metode Ekstraksi, Skrining Fitokimia dan Uji Toksisitas Ekstrak Metanol *Eucheuma cottonii*. *Indonesian Journal of Pharmaceutical Research*, 3(2): 8-19. https://doi.org/10.31869/ijpr.v3i2.5045

Prasetiyo, H., Sasongko, A. S., Fahira, D. D. & Ayuningsih, T. 2023. Skrining Fitokimia dan Uji Aktivitas Antioksidan secara Kualitatif pada Ekstrak Rumput Laut Eucheumma cottonii. *Jurnal Kemaritiman: Indonesian Journal of Maritime*, 4(1): 25-34. https://doi.org/10.17509/ ijom.v4i1.60288

Sagala, V. Z., Ridwanto, R., Daulay, A. S. & Pulungan, A. F. 2025. Uji Toksisitas menggunakan Uetode BSLT dan uji Antibakteri *Staphylococcus aureus* dan *Escherichia coli* Ekstrak dan Fraksi Daun karamunting (*Rhodomyrtus tomentosa* (Aiton) Hassk.). *Journal of Pharmaceutical and Sciences*, 603-625. https://doi.org/10.36490/journal-jps.com.v8i1.823

Salay, G., Fonseca, F. L. A., Carvalho, S. S. D., Reis, B. D. C. A. A., Gascón, T. M., Veiga, G. R. L. D., & Lucarelli, N. 2024. Acute Toxicity Assays with the *Artemia salina* Model: Assessment of Variables. *Alternatives to Laboratory Animals*, 52(3): 142–148. https://doi.org/10.1177/02611929241242443

Sarah, Q. S., Anny, F. C., & Misbahuddin, M. 2017. Brine shrimp lethality assay. *Bangladesh Journal of Pharmacology*, 12(2). https://doi.org/10.3329/bjp.v12i2.32796

Wanyonyi, S., Brown, L., Du Preez, R., Panchal, S., & Paul, N. 2017. *Kappaphycus alvarezii* as a Food Supplement Prevents Diet-Induced Metabolic Syndrome in Rats. *Nutrients*, 9(11): 1261. https://doi.org/10.3390/nu9111261

Yudiati, E., Arifin, Z., Santoso, A., Hidayati, J. R., Alghazeer, R., & Azhar, N. 2023. *Artemia* with synbiotics enrichment improves resistance against Vibrio parahaemolyticus AHPND of *Litopenaeus vannamei* larvae. *Buletin Oseanografi Marina*. 12(3): 357-364. https://doi.org/10.14710/buloma.v12i3.52523