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Evaluation of Carrageenan Yield and Toxicity from *Kappaphycus alvarezii*: Implications for Aquaculture and Environmental Impact

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Abstract

Carrageenan, a polysaccharide derived from red seaweeds, is widely used across industries due to its gelling, thickening, and stabilizing properties. Among the species of red algae, *Kappaphycus alvarezii* is a key source for carrageenan production, especially in tropical mariculture systems. The extraction process involves several steps, including grinding, hot water extraction, ethanol precipitation, and drying, each critical for optimizing both yield and quality of the final product. Despite its industrial applications, concerns about carrageenan's toxicity have been raised, particularly its potential to induce inflammation and immunosuppression, which may affect aquatic organisms in mariculture systems. This study aimed to optimize the extraction of carrageenan from *K. alvarezii* and assess its toxicity on *Artemia salina* larvae as a bioassay model. Extraction was conducted at varying concentrations (6% to 10% of the seaweed weight), with the highest yield obtained at 6%. The carrageenan extract was then tested for toxicity on *A. salina* larvae at concentrations of 10,000 ppm, 5,000 ppm, 2,500 ppm, 1,250 ppm, and 625 ppm. Mortality rates increased with higher concentrations, with the highest concentration causing near-total mortality within 24 hours (94.67%). The LC50 value for carrageenan extract was calculated to be 4.24 µg/mL, indicating a concentration-dependent toxicity. The regression analysis showed a strong linear relationship between concentration and mortality, supporting the use of *A. salina* as an effective model for toxicity testing in aquaculture. These findings highlight the need for further investigation into the safety of carrageenan use in aquatic environments and contribute to the development of more sustainable practices in marine-based.

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1. Introduction

Carrageenan, a water-soluble polysaccharide extracted from red seaweeds, is widely utilized in the food, pharmaceutical, and aquaculture industries due to its excellent gelling, thickening, and stabilizing properties (Prajapati *et al.*, 2014); Zia *et al.* (2017); (Shafie *et al.*, 2022). *Kappaphycus alvarezii*, a species of red seaweed, stands out as a significant source of carrageenan, particularly in tropical regions where it is cultivated in large-scale mariculture operations (Rupert *et al.*, 2022). This seaweed is renowned for its high carrageenan content, which is crucial for the extraction process that yields a valuable product for diverse industrial applications. The extraction of carrageenan from *K. alvarezii* typically involves a multi-step process, including initial grinding of the seaweed, hot water extraction, ethanol precipitation, and drying. Each of these steps is essential to maximize both the quantity and quality of carrageenan obtained from the biomass, influencing its final functional properties and suitability for various applications (Martín-del-Campo *et al.*, 2021; Premarathna *et al.*, 2024).

Despite its widespread use in the food and pharmaceutical industries, concerns have been raised regarding the potential toxicity of carrageenan, particularly in relation to its use in food products, supplements, and immunostimulants for aquaculture (Cohen and Ito, 2002; Dhewang *et al.*, 2023b; Komisarska *et al.*, 2024). Some studies suggest that carrageenan may pose health risks, including inflammation and immunosuppression, when consumed or administered in large quantities, especially over prolonged periods (McKim, 2014; Dhewang *et al.*, 2023b;

Wagner *et al.*, 2024). These concerns are compounded by the widespread use of carrageenan in immunostimulants for shrimp and fish (Cheng *et al.*, 2007; Cheng *et al.*, 2008; Mariot *et al.*, 2021), where its safety profile has been questioned, particularly with regard to its effects on the immune response and overall health of aquatic organisms.

The potential for carrageenan to cause adverse effects has led to calls for more comprehensive safety assessments to better understand its impact on both human health and aquatic ecosystems. One of the primary concerns is carrageenan's possible toxicity in aquatic species, such as shrimp and fish, where it may interfere with immune function or contribute to adverse health outcomes. *Artemia salina*, a small brine shrimp species, is frequently used in bioassays to evaluate the toxicity of various substances, as its larvae are highly sensitive to environmental changes and contaminants. Toxicity testing with *A. salina* is a reliable method for screening the safety of substances intended for use in aquaculture, particularly in assessing their potential effects on aquatic organisms' survival, immunity, and overall health (Salay *et al.*, 2024; Sunaryo *et al.*, 2024; Suryono *et al.*, 2024).

The primary objective of this study was twofold: to optimize the extraction process of carrageenan from *K. alvarezii* and to assess the toxicity of the resulting carrageenan extract on *A. salina* larvae. The study aimed to determine the optimal conditions for carrageenan extraction to achieve high yield and quality, while also evaluating the potential toxicological effects at various concentrations of the extract. Furthermore, the study sought to provide a deeper understanding of how carrageenan concentrations affect *A. salina* mortality, contributing to the development of more sustainable practices in marine-based industries.

2. Material and methods

2.1. Materials

The dried *Kappaphycus alvarezii* seaweed used for carrageenan extraction was collected from Ngrehan Beach, Gunungkidul, Yogyakarta, Indonesia. Hot water (80°C), 96% ethanol, and distilled water were utilized during the extraction process. The equipment used included a blender, graduated cylinders, centrifuge tubes, petri dishes, an oven set at 60°C, a mortar, and aerators. *Artemia salina* cysts were purchased for toxicity testing, with 1 gram of dry *Artemia* cysts used per test.

2.2. Extraction Process

Carrageenan extraction from *Kappaphycus alvarezii* was carried out in two stages, with three replicates per stage, to isolate carrageenan. Approximately 500 grams of seaweed was ground using a blender, and 6%, 7%, 8%, 9%, 10% of the total seaweed weight was weighed and subjected to extraction with hot water (80°C) for 24 hours per stage. After extraction, the solution was filtered to remove solid residues, and the liquid extract was processed for centrifugation (Heriyanto *et al.*, 2018; Rudke *et al.*, 2022; Dhewang *et al.*, 2023a).

2.3. Centrifugation of Extracts

Following extraction, the combined samples were measured using a graduated cylinder. Ethanol (96%) was added in a 1:1 ratio to the extracted solution, and the mixture was allowed to cool to room temperature. The sample was centrifuged at 3,000 rpm for 1-2 minutes. The precipitate was separated from the supernatant, and the solid carrageenan was transferred to petri dishes for drying. This process was repeated for all 15 samples (Dhewang *et al.*, 2023a; Sunaryo *et al.*, 2024).

2.4. Drying Process

The dried carrageenan samples were placed in an oven at 60°C for 24 hours or until they formed paper-like sheets. After drying, the samples were finely ground using a mortar to obtain carrageenan powder, which was then used for subsequent toxicity testing (Yudiati *et al.*, 2018; Sunaryo *et al.*, 2024).

2.5. *Artemia* Hatching

Artemia cysts were weighed (1 gram of dry cysts), soaked in freshwater for 1 hour to activate them, and then transferred to 1 liter of sterilized seawater with aeration. The hatching process was carried out in a light-proof room for 24 hours (Yudiati *et al.*, 2021a; Yudiati *et al.*, 2021b; Yudiati *et al.*, 2023).

2.6. Toxicity Testing

Toxicity testing was conducted using five concentrations of carrageenan extract: 10,000 ppm, 5,000 ppm, 2,500 ppm, 1,250 ppm, and 625 ppm, with five replicates for each concentration. The carrageenan extract was added to vial bottles, with sterilized seawater to make a total volume of 10 mL. A total of 25 *Artemia* were placed in each vial, and initial mortality (t0) was recorded. Mortality was monitored at 1, 2, 4, 8, 16, and 24 hours after the introduction of *Artemia* into the vials (Banti and Hadjikakou, 2021; Istiqomah *et al.*, 2024; Pramudyo *et al.*, 2024; Sunaryo *et al.*, 2024).

3. Results

3.1 Extraction Results

The extraction results are presented in Figure 1. The carrageenan extraction from *Kappaphycus alvarezii* was conducted at varying concentrations of 6%, 7%, 8%, 9%, and 10%. The highest yield of carrageenan was obtained at 6%,

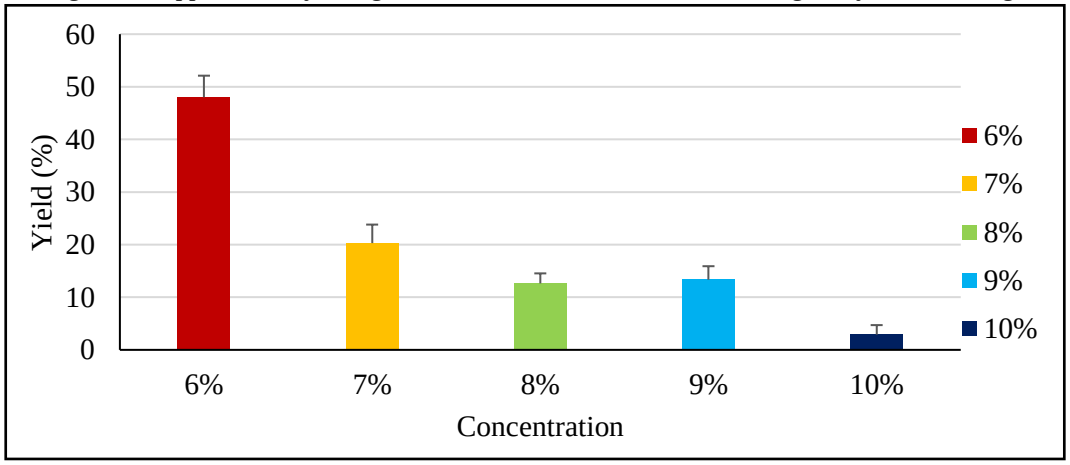

Figure 1. Extraction results of carrageenan from *Kappaphycus alvarezii*.

with a value of 20.29, which represents the most efficient extraction. At 7%, the yield decreased slightly to 12.63, while at 8%, it further declined to 13.33. The yields at 9% and 10% were significantly lower, at 2.90, indicating that higher concentrations reduce extraction efficiency. These results suggest that the optimal extraction concentration lies between 6% and 7%, where efficiency is highest.

3.2 Mortality Rate of *Artemia* larvae

The mortality rates of *Artemia* larvae exposed to different concentrations of *Kappaphycus alvarezii* extract (10,000 ppm, 5,000 ppm, 2,500 ppm, 1,250 ppm, and 625 ppm) were observed at various time intervals (0, 1, 2, 4, 8, 16, and 24 hours) and are presented in Figure 2. At the highest concentration (10,000 ppm), mortality was first observed at 10.67% after 1 hour, and it increased significantly over time, reaching 94.67% by 24 hours. Similarly, at 5,000 ppm, the mortality rate began at 8% and rose to 96% after 24 hours. The 2,500 ppm treatment showed a gradual increase in mortality, reaching 94% at 24 hours. At the lower concentrations of 1,250 ppm and 625 ppm, the mortality rates were slower, with the 1,250 ppm concentration showing 41.33% mortality at 24 hours, and the 625 ppm concentration demonstrating a final mortality rate of 36%. These findings indicate a concentration-dependent increase in mortality, with higher extract concentrations causing more rapid and higher lethality across all time intervals.

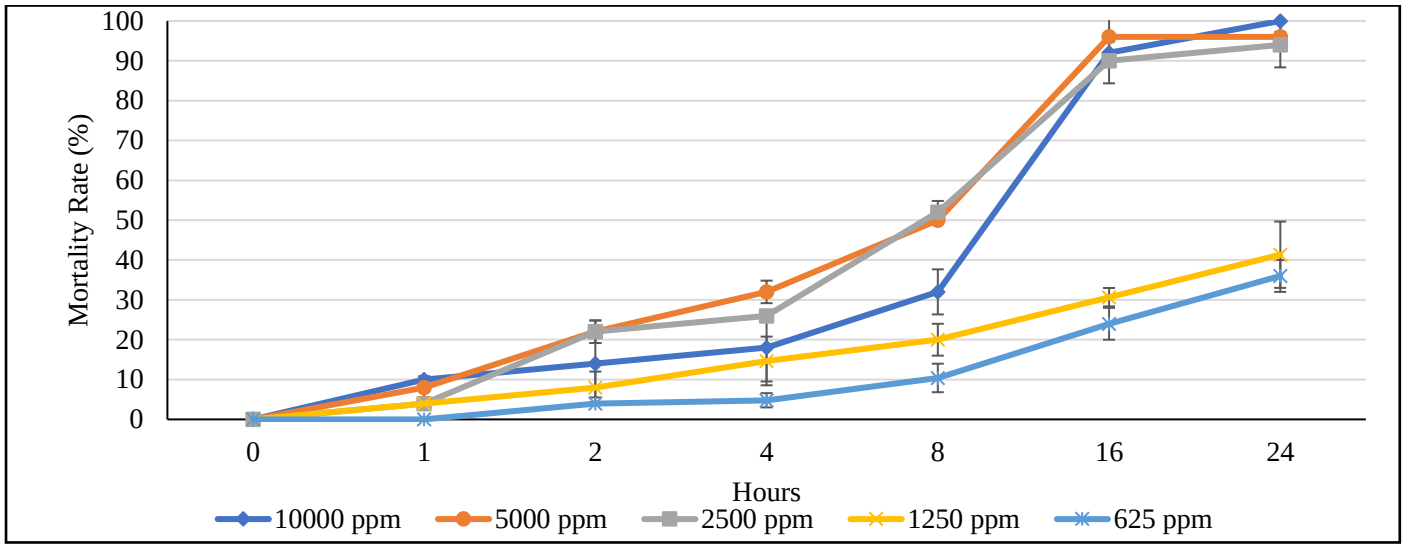

Figure 2. The mortality rates of *Atermia* larvae exposed to different concentrations of *Kappaphycus alvarezii* extract (10,000 ppm, 5,000 ppm, 2,500 ppm, 1,250 ppm, and 625 ppm) at various time intervals (0, 1, 2, 4, 8, 16, and 24 hours).

3.3 Mortality Test Results

The mortality rates of *Artemia* larvae exposed to different concentrations of *Kappaphycus alvarezii* carrageenan extract are presented in Table 1. The test was conducted over 24 hours, with the larvae exposed to carrageenan concentrations of 10,000 ppm, 5,000 ppm, 2,500 ppm, 1,250 ppm, and 625 ppm. At the highest concentration of 10,000 ppm, 100% mortality was observed across all five larvae samples (U1–U5), with mortality increasing steadily over time, reaching 94.67% by 24 hours. At 5,000 ppm, the mortality rate was slightly lower, at 96.8%, and the pattern of increasing mortality over time was similar. At 2,500 ppm, the mortality rate reached 94.4%, while at 1,250 ppm, the mortality was significantly reduced to 40%. At 625 ppm, the mortality rate was the lowest, at 35.2%, with notable variation among the different samples. The LC50 value was calculated to be 4.24 µg/mL, which represents the concentration at which 50% of the *Artemia* larvae are expected to die within the 24-hour exposure period. These results demonstrate that higher concentrations of *Kappaphycus alvarezii* carrageenan extract significantly increase the toxicity to *Artemia* larvae, with lower concentrations showing milder effects. These findings suggest a direct relationship between carrageenan concentration and mortality rate, which can serve as an indicator for evaluating the toxicity of *Kappaphycus alvarezii* carrageenan in aquatic environments.

Table 1. Mortality rates and probit values for *Kappaphycus alvarezii* carrageenan extract concentrations tested on *Artemia* larvae.

Test Concentration (ppm)	Log Concentration	Number of Test Larvae	U1	U2	U3	U4	U5	Average	Mortality (%)	Probit Value
10,000	4	25	25	25	25	25	25	25	100	8.95
5,000	3.70	25	23	25	25	25	23	24.2	96.8	6.75
2,500	3.40	25	23	24	24	24	23	23.6	94.4	6.55
1,250	3.10	25	8	11	12	8	11	10	40	4.76
625	2.80	25	9	8	8	10	9	8.8	35.2	4.61
LC_{50} = 4.24 µg/mL										

The regression curve is presented in Figure 3. The regression analysis of log concentration versus probit value revealed a strong linear correlation between the two variables. The obtained regression equation was y = −5.7687 + 3.5567x, where y represents the probit value and x denotes the log concentration. The R-squared value of 0.90990.90990.9099 indicates that approximately 91% of the variation in the probit value can be explained by the log concentration. This suggests a robust relationship between the concentration of the extract and the mortality rate, supporting the reliability of the toxicity test results.

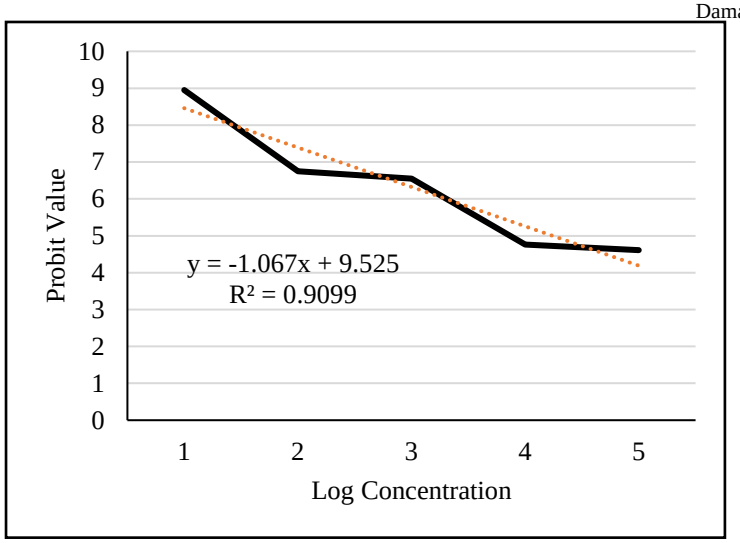

Figure 3. The regression curve

4. Discussion

The extraction of carrageenan from *Kappaphycus alvarezii* using a 6–7% concentration of hot water solution proved to be the most effective for maximizing polysaccharide yield while ensuring manageable extraction conditions. This concentration range is consistent with findings from similar studies on carrageenan extraction from *Kappaphycus alvarezii*. The results of extraction of *K.alvarezii* seaweed by Mahyati and Azis (2019) with a time variation of 40 minutes and temperature variation is 70 °C, with the optimum value of temperature and extraction time is 70 °C for 30 minutes which produces carrageenan as much as 44.46%. Another research by Webber *et al.* (2012) demonstrated Optimal extraction conditions were 74 ºC and 4 hours. In these conditions, the carrageenan extract properties determined by the polynomial model were 31.17%. Another seaweed for carrageenan resource for Chondrus crispus by Bahari *et al.* (2021) shows In the mentioned range of temperature, the maximum yield was obtained after extraction at 90 °C for 8 h (39.2 ± 0.0 wt% of dry seaweed mass). This suggests that *K. alvarezii* is a promising source for carrageenan production, a polysaccharide widely used in various industries such as food, pharmaceuticals, and cosmetics (Rupert *et al.*, 2022; Hans *et al.*, 2023; Nurani *et al.*, 2024)

The toxicity test using *Artemia salina* (brine shrimp) revealed a concentration-dependent increase in mortality, with the LC50 calculated at 4.24 µg/mL. This result indicates that carrageenan, despite its beneficial applications in many sectors, can be toxic to marine organisms at higher concentrations. The toxicity observed in *Artemia salina* is in line with previous research that suggests carrageenan and other polysaccharides extracted from marine algae can exhibit varying levels of toxicity depending on the molecular weight and chemical structure. High molecular weight carrageenan, which is typically found in *K. alvarezii*, has been shown to have more pronounced toxic effects, possibly due to its ability to disrupt cellular functions and ion balance in marine organisms (Nair et al., 2020; García et al., 2017) (Lichtfouse *et al.*, 2024).

The variability in mortality at different carrageenan concentrations highlights the need to carefully consider the dose used in potential applications, particularly in marine environments. While carrageenan is non-toxic at lower concentrations, its accumulation could have detrimental effects on aquatic life, especially in enclosed or low-exchange water systems such as aquaculture ponds. This suggests that while carrageenan can be safely used in products for human consumption (Cohen and Ito, 2002), its use in marine ecosystems especially in aquaculture or biofouling control equires further scrutiny (Kim and Kang, 2020; Dhewang *et al.*, 2023b).

Moreover, these findings point to the need for further studies on the environmental impact of carrageenan, particularly in terms of its long-term presence in marine ecosystems. Key factors to consider include the persistence of carrageenan in aquatic environments, its bioaccumulation in marine organisms, and its potential to alter ecosystem dynamics. It is also important to investigate how carrageenan interacts with other pollutants or chemicals in the water, as this could exacerbate its toxic effects or lead to unforeseen ecological consequences (Yasmin et al., 2021).

In addition to the toxicity assessment, the potential for carrageenan to impact non-target marine organisms and the broader aquatic food chain should be addressed. Research into the bioavailability of carrageenan and its breakdown products could provide insights into its persistence and ecological footprint. For example, while carrageenan is often considered biodegradable, the rate at which it breaks down in marine environments, particularly under varying temperature and salinity conditions, is still not fully understood. The long-term effects on marine species, particularly those at lower trophic levels, remain an area requiring further investigation. Overall, while carrageenan remains a valuable resource for industrial applications, its use in marine ecosystems, particularly in aquaculture and biofouling management, must be balanced with environmental sustainability considerations. A more detailed understanding of its toxicity, environmental behavior, and long-term effects on marine biodiversity is crucial to ensure safe and responsible use of this bioactive polysaccharide. Further research should explore the mechanisms behind carrageenan toxicity and the potential for developing safer, environmentally-friendly alternatives.

5. Conclusions

This study optimized the extraction of carrageenan from *Kappaphycus alvarezii*, achieving maximum yield at 6–7% concentration. Toxicity testing on *Artemia salina* revealed a concentration-dependent increase in mortality, with an LC50 value of 4.24 µg/mL. These results emphasize the need for caution in carrageenan use in aquaculture and suggest further studies on its environmental impact.

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

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

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Declaration of competing Interest

None

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