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A Comparative Assessment on the Cytotoxicity of *Kappaphycus alvarezii* and *Kappaphycus striatus* Seaweeds using Brine Shrimp Lethality Assay

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ABSTRACT

Seaweed farming plays a crucial role in the Philippine economy, particularly in the Caraga region, where the cultivation of *Kappaphycus* spp. is highly prevalent and abundant. This study provided a preliminary assessment on its toxicology with a comprehensive comparison between *K. alvarezii* and *K. striatus* collected from Barobo, Surigao del Sur. Brine shrimp lethality assay (BSLA) was employed to assess the cytotoxic activity of both species. Results revealed that both methanolic crude extracts of two species exhibited bioactivity (% mortality) towards the test samples with highly significant Spearman's rho correlation among all concentrations ($p<0.001$). Comparison of extracts per concentration also revealed that *K. alvarezii* significantly exhibited higher mortality over *K. striatus* at concentrations of 1000 µg/mL ($p<0.001$) and 10 µg/mL ($p<0.01$). Median Lethal Concentration (LC_{50}) values showed that *K. alvarezii* (38.9 ppm) had higher lethality compared to *K. striatus* (2187.76 ppm) with a highly significant statistical difference value of 45.54 ($p<0.0001$). This study showed potential pharmaceutical implications for both species of *Kappaphycus*. Moreover, the significantly higher lethality of *K. alvarezii* indicates it as a more promising species for further studies on bioactivity potential and medicinal research. Studies on these fields would mean an additional potential market for the thriving seaweed industry in Caraga, Philippines.

Keywords: *Seaweeds, Kappaphycus, Median Lethal Concentration, Cytotoxicity, Brine Shrimp Lethality Assay*

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1 Introduction

The cultivation of *Kappaphycus* spp. in marine coastal environments has resulted in substantial socioeconomic benefits for communities, especially those in coastal areas. Additionally, local coastal households' economic reliance on seaweed farming is growing, making farmers crucial partners in the prudent management of coastal and marine resources (Samonte 2017). Through this, seaweed farming is regarded as a significant direct-use value for coastal and marine ecosystems that contributes to positive net present values, along with tourism and fishing (Mateo et al. 2021).

For the Caraga Region in the Philippines, its aquaculture subsector contributes mainly to the fisheries production in the country, with a total production value reaching Php 1.14 billion in 2019 and a 16.30% share of the total fisheries value of production. Specifically in its seaweed industry, Caraga has produced 8,468.17 metric tons of seaweeds belonging to the genus *Kappaphycus*, and the majority of it came from the province of Surigao del Sur, accounting for 8,367.62 metric tons (Philippine Statistics Authority, 2020). Consequently, the consumption of seaweeds, especially *Kappaphycus* spp., is known to be a healthy source of high fibers and minerals with low calorie content.

Seaweeds generally provide significant amounts of protein, vitamins, trace elements, and a wide range of secondary metabolites not identified in other organisms (Ferraces-Casais et al. 2012, Senthil et al. 2011). Some species of edible seaweed contain significant amounts of vitamins, proteins, fibers, carbohydrates, macronutrients (N, P, K, Ca, Mg, and S), and micronutrients (Zn, Cu, and Mn), are low in fat (about 5%) and calorific value (i.e. 827.6 kcal kg-^{1} dry alga), and also possess important biological compounds (e.g. terpenoids, alkaloids, photosynthetic pigments, and polyamines) that are known to help combat disease (Gullón et al. 2021, Oucif et al. 2020, Pati et al. 2016, Rengasamy et al. 2020, Smith et al. 2010).

The Philippines' seaweed sector is likewise threatened by several interconnected hazards. Farmers are mostly confronted with environmental dangers (such as disease and pest infestations), which, if not controlled well, might lead to the failure of seaweed production. The fluctuation of the seaweed supply and the poor quality of the raw materials are viewed by dealers and processors as predecessors to other problems, such as greater competition among regional traders and processors (Suyo et al. 2021).

To highlight, one of the environmental-related risks that can cause potential issues on health are the heavy metals and metalloids (cadmium accumulation) from anthropogenic activities, including mining, milling, petrochemicals processing, printing, the electronics industry, and municipal waste, be it directly discharged into the marine environment or transported into the greater aquatic system via estuaries (Filippini et al. 2021, Wang et al. 2013). Accordingly, once toxic metals are introduced into the aquatic systems where seaweeds are cultivated or grow naturally, they easily accumulate in the multicellular marine macroalgae. With this, it is important to recognize that metal accumulation in seaweed is influenced not only by anthropogenic sources but also by various natural factors impacting the marine ecosystem, including volcanic activity and tsunamis (Jarvis & Bielmyer-Fraser 2015, Malain et al. 2012, Santawamaitre et al. 2011).

Given the significant economic value and vulnerability of *Kappaphycus* species to anthropogenic pressures, this study sought to enhance their commercial potential by evaluating their medical applications. Specifically, it aimed to conduct a preliminary cytotoxicity assessment of *Kappaphycus alvarezii* and *Kappaphycus striatus* harvested from Barobo, Surigao del Sur. Furthermore, the research compared the median lethal concentrations (LC_{50}) and overall mortality rates of both species to identify potential pharmaceutical advantages.

2 Materials and Methods

2.1 Sampling Area

The study was conducted in the coastal waters of Barobo, Surigao del Sur. Three sampling stations were set up within the study area (Figure 1). Station 1, located at 8.56389° latitude and 126.12972° longitude, cultivated *Kappaphycus alvarezii*. Stations 2 and 3, located at 8.56389° latitude, 126.13583° longitude, and 8.56389° latitude, 126.13611° longitude, respectively, cultivated *Kappaphycus* striatus. Barobo Lianga Bay is a vital coastal zone in the Caraga region, contributing significantly to the country's fisheries sector (Rasonable et al. 2023). This highlights the bay's importance as a key source of livelihood and economic activity for the local communities involved in milkfish farming.

Samples shown in Fig. 2 were harvested, placed in ziploc bags, and refrigerated under 4 °C for proper storage.

2.2 Preparation of Samples for Methanolic Extraction

The crude extraction process for *K. alvarezii* and *K. striatus* was carried out following the method outlined by Guevara (2005). The procedure was performed and closely supervised by the Regional Standards and Testing Laboratory of the Department of Science and Technology in Caraga. Stored seaweed samples were washed 3 times with distilled water. The samples were then air-dried for 86 hours and then freeze-dried for 14 hours to achieve the texture ideal for pulverization.

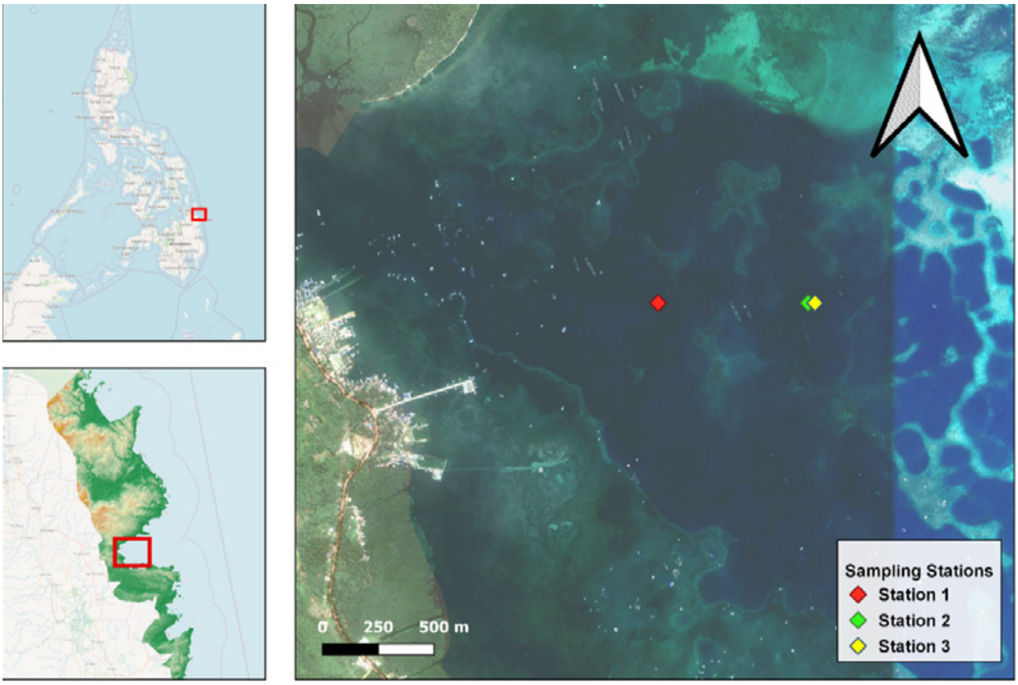

Figure 1. Sampling stations within the coastal waters of Barobo, Surigao del Sur. Aerial view of all sampling sites of *Kappaphycus* spp. farms and the established permanent sites using QGIS version 3.18.2.

Figure 2. Actual samples of *K. alvarezii* (A) and *K. striatus* (B) harvested from Barobo, Surigao del Sur.

Pulverized 250 grams of *K. alvarezii* and 183 grams of K. striatus were soaked separately in Erlenmeyer flasks with 160 ml 95% methanol solution. The flasks were covered with rubber stoppers, and the samples were soaked for 48 hours. After the soaking process, the samples were filtered through a Buchner funnel with gentle suction. The flask and seaweed material were rinsed with fresh portions of methanol. The washings and seaweed material were transferred to the funnel, combining the washings with the first filtrate. Gentle suction was applied to complete the collection of the seaweed extract. The residue was then discarded. The filtrate was concentrated under vacuo at a temperature below 50 degrees Celsius to the resulting crude extract of 20 mL *K. alvarezii* and 14 mL *K. striatus*. A 95% solution of Methanol was used as a solvent for the extraction process since methanol was recorded as the best solvent for extracting bioactive compounds, yielding a high content of plant secondary metabolites (Truong et al. 2019).

2.3 Preparation of Reagents

Serial dilution of extracts. Clean test tubes were taken and labeled. Stock solutions were then prepared for each *Kappaphycus* spp. crude extracts at 100 mg in 10 mL artificial seawater. Concentrations of 1000 µg/mL, 100 µg/mL, 10 µg/mL, and 1 µg/mL were prepared by serial dilution from the stock solution. Nine (9) replicates were prepared for each of the concentrations.

2.4 Cytotoxicity Test

For the determination of potential cytotoxicity of the *Kappaphycus* spp. crude extracts, brine shrimp lethality assay (BSLA) was performed. The method was adapted from Sarah et al. (2017) and initially developed by Meyer et al. (1982), with minor modifications to suffice the needed number of replicates and effectively detect brine shrimp nauplii.

Three (3) liters of distilled water were prepared in a rectangular container with 27 grams of rock salt to serve as artificial seawater during the hatching of brine shrimp eggs. An electric air pump was activated into the bottom of the container for proper and continuous aeration. Five (5) grams of brine shrimp eggs were then placed in the aerated seawater with proper illumination for optimal conditions of hatching (Fig. 3A). Hatched nauplii were observed after 24 hours of the hatching process. Ten nauplii were then carefully transferred to the prepared concentrations via a glass dropper (Fig. 3B). The number of surviving nauplii was then observed after 24 hours of exposure in each replicate of serially diluted concentrations (Fig. 3C). Artificial seawater served as the control variable.

2.5 Data Analysis

The nauplii mortality percentage for each concentration and the control was calculated, with the data presented as the mean along with the corresponding standard error. For each test tube, the numbers of dead and live nauplii were recorded, and the % death was determined as:

$$\%\ \text{Death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \text{ x } 100$$

Probit Analysis for the percent mortalities was done initially to calculate the median lethal concentrations (LC_{50}). Transformation of % mortality to probit values was done using Finney's Table (Finney 1952) adapted by Randhawa (2009). The 0% and 100% mortality corresponding to a probit value of 1.0334 and 8.9538, respectively, were followed according to Bliss (1935), which was also adopted by (Gomaa et al. 2021). Regression analysis was then performed to the probit values (y range) with their corresponding concentrations at log base 10 (x range). The resulting ***x variable 1*** and ***intercept*** values were then used to calculate the LC_{50} with the following formula:

$$Y = ax + b$$
$$x = \frac{(y-b)}{a}$$
$$LC_{50} = \text{antiLog } x$$

Wherein:

a = x variable 1 (slope); y = intercept value.

Analysis of the ***T-test*** was also done to determine the significant difference between the same concentrations of *K. alvarezii* and *K. striatus*. Spearman's rho correlation coefficient was also computed to determine the correlation of the concentrations to the percentage of mortalities for both extracts. Correlation analysis was computed through the JASP software version 0.14.1.0. Calculation of the descriptive statistics, probit analysis, LC_{50}, ***T-test,*** and formulation of graphs were done using the Microsoft Excel 2016 edition and Google Colab.

3 Results and Discussion

3.1 Effects of K. alvarezii and K. striatus on Brine Shrimp Larvae Mortality

The cytotoxic effect of bioactive compounds is frequently assessed using the brine shrimp lethality assay (BSLA). It serves as an initial investigation of toxicity among tested plant extracts (Ghosh et al. 2015, Kibiti & Afolayan 2016, Sarah et al. 2017, Sufian & Haque 2015, Syahmi et al. 2010), cytotoxicity testing of dental materials, heavy metals, cyanobacteria toxins, pesticides, fungus toxins, and among others (Sarah et al. 2017). Based on the results, data on both *K. alvarezii* and *K. striatus* showed potential bioactivity towards the tested nauplii. Mortalities were observed in each concentration after 24 hours of exposure for most replicates. Also, the control group had a very low mortality rate, which only accounts for 3% of its population, indicating the acceptability of the

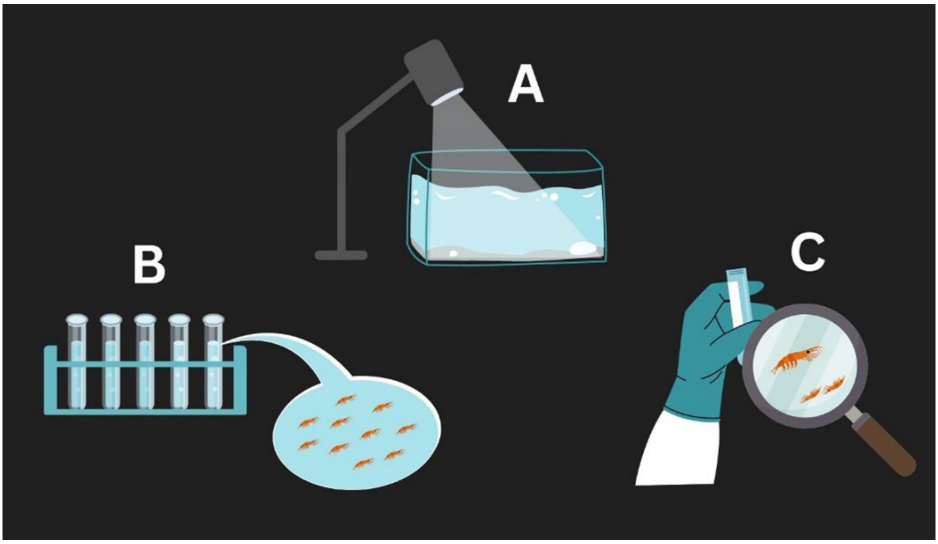

Figure 3. Illustration of the Brine Shrimp Lethality Assay Set-up. A) Brine shrimp hatching stage using a rectangular container with continuous aeration and proper illumination, B) Transfer of nauplii to the respective concentrations (10 nauplii per concentration), and C) Manual counting of nauplii after 24-hour exposure to treatments.

Table 1. Data on percent (%) mortality described as Means with Standard Error Mean (SEM) for both *Kappaphycus* spp extracts.

Concentrations	Mortality (%)									
	K. alvarezii					*K. striatus*				
	Mean	**SEM**	**Min.**	**Max.**	**LC50 (µg/mL)**	**Mean**	**SEM**	**Min.**	**Max.**	**LC50 (µg/mL)**
1000 µg/mL	88.9	6.8	40	100	38.9	32.2	4.6	10	50	2187.76
100 µg/mL	30	4.4	0	40		26.7	3.3	20	50	
10 µg/mL	20	2.9	10	30		6.7	1.7	0	10	
1 µg/mL	14.4	3.8	0	30		5.6	2.4	0	20	

experiment (Sadat Sadeghi 2018).

The BSLA revealed distinct toxicity profiles between *K. alvarezii* and *K. striatus. K. alvarezii* demonstrated a significantly higher mortality rate than *K. striatus* across all tested concentrations. At the highest concentration (1000 µg/mL), *K. alvarezii* induced an average mortality of 88.9%, indicating a substantial toxic effect. While mortality decreased with lower concentrations, it remained notably higher than that of *K. striatus. K. striatus,* on the other hand, consistently exhibited lower mortality rates. Even at the highest concentration, mortality was only 32.2%, suggesting a significantly lower toxic potential than *K. alvarezii.* This trend persisted across all concentration levels.

The observed disparity in toxicity between *K. alvarezii* and *K. striatus* (Fig. 4) has significant implications for various applications. If these seaweeds are considered for use in food, pharmaceuticals, or cosmetics, the higher toxicity of *K. alvarezii* necessitates rigorous safety evaluations. Identifying the specific toxic compounds and their mechanisms of action is crucial for developing mitigation strategies or determining safe usage limits (Desideri et al. 2016). However, this also means that the higher toxicity exhibited by *K. alvarezii* compared to *K. striatus* suggests the presence of potentially bioactive compounds within *K. alvarezii* that could have pharmacological applications. Many drugs are derived from natural products that exhibit some toxicity, often refined through chemical modification or isolation of the active compound (Khan 2018 Thomford et al. 2018). This being said, the toxicity differences between *K. alvarezii* and *K. striatus* highlight the importance of species-specific toxicity assessments (Peng et al. 2022). Further research is warranted to characterize the toxic profiles of these seaweeds fully, identify

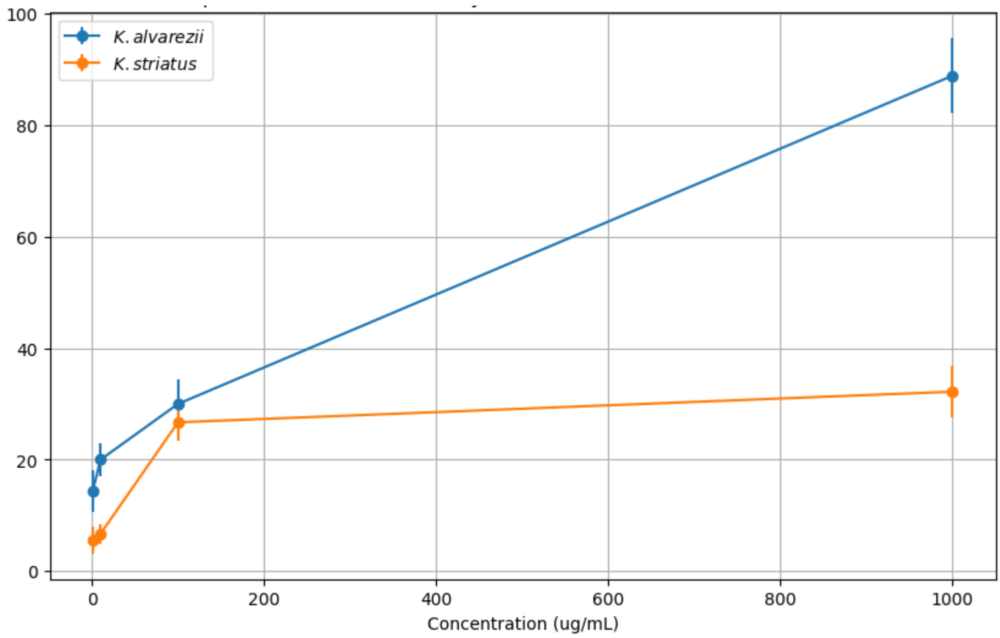

Figure 4. Graph showing the concentration-dependent toxicity of *Kappaphycus* species.

potential risks, and explore their safe and sustainable utilization. Studies employing a wider range of concentrations, additional toxicity assays, and in vivo models are essential to evaluate the safety profile of these seaweeds comprehensively.

3.2 Median Lethal Concentration (LC_{50}) and correlation of treatments to mortalities.

For the analysis of the correlation between the level of concentrations and the percentage of mortalities, Spearman's rho correlation coefficients were employed (Table 2). The established level of concentrations and the percentage of mortalities showed a positive correlation with highly significant *p* values for both crude extracts of *K. alvarezii* and *K. striatus.* For *K. alvarezii*, the Spearman's rho value is 0.797 ($p<0.001$), while for K. striatus, the Spearman's rho value is 0.781 ($p<0.001$). The positive correlation between concentration and mortality rates for both *K. alvarezii* and *K. striatus*, as indicated by the highly significant Spearman's rho values, provides strong evidence for a concentration-dependent toxic effect of both seaweed extracts on brine shrimp larvae. This finding is consistent with the general toxicological principle that increasing exposure to a toxic or bioactive substance typically results in increased adverse effects (Vilas-Boas et al., 2021). Furthermore, the similar correlation coefficients for both species suggest that while there is a difference in the overall toxicity, they exhibit a comparable pattern of increasing toxicity with increasing concentration. This information is crucial for understanding the potential hazards of these seaweeds and determining safe exposure levels.

The cytotoxicity of both species, as indicated by their concentration-dependent toxicity on brine shrimp larvae, warrants further investigation into their potential bioactivity. While cytotoxicity is often associated with negative effects, it can also be a precursor to identifying valuable pharmacological compounds (Majolo et al. 2019). Many natural products with therapeutic properties exhibit cytotoxicity at certain concentrations. These compounds often serve as lead compounds for drug discovery and development (Chopra & Dhingra 2021). The concentration-dependent toxicity observed in both seaweed species suggests the presence of bioactive compounds that could potentially be harnessed for pharmaceutical applications.

The higher cytotoxicity of *K. alvarezii* compared to *K. striatus* is particularly intriguing. This could indicate a greater abundance or potency of bioactive compounds in *K. alvarezii.* However, further studies are required to isolate and characterize these compounds to determine their specific biological activities and potential therapeutic applications. It is essential to approach this potential with caution. While cytotoxicity is a preliminary indicator of bioactivity, it does not guarantee the presence of safe and effective drug candidates (Aware et al. 2022). Rigorous toxicity testing, pharmacological evaluation, and drug development processes are essential to ensure the safety and efficacy of any potential drug derived from these seaweeds. Hence, the observed cytotoxicity of *K. alvarezii* and *K. striatus* provides a foundation for exploring

Table 2. Data showing the correlation coefficient of percent mortalities from each extract in response to the established level of concentrations along with their respective LC_{50}

Species	Spearman's rho	*p* - value	LC_{50} (ppm)
K. alvarezii	0.797***	<0.001	38.9
K. striatus	0.781***	<0.001	2187.6

Note: **p<0.01, ***p<0.001

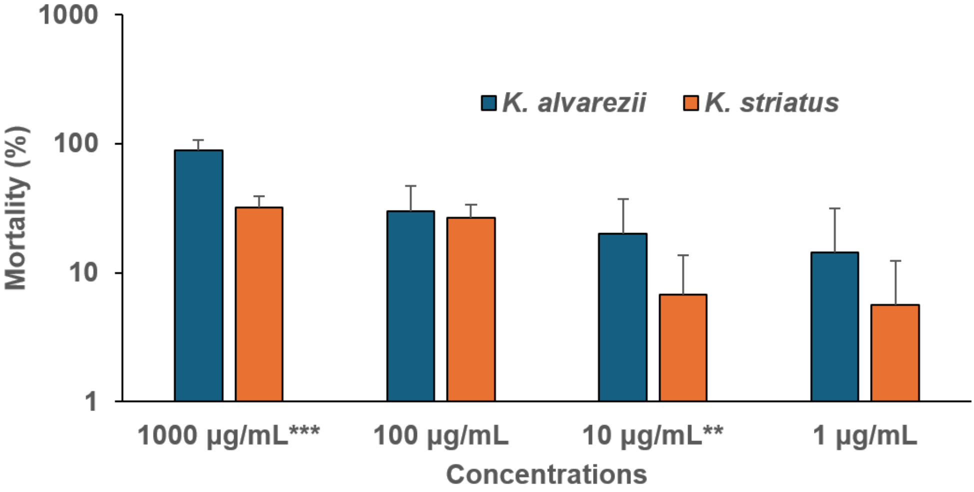

Figure 5. Graph showing the comparison of means (%mortality) for both *Kappaphycus* extracts. Concentrations with asterisks have *p* values <0.01** and <0.001***.

their potential as sources of bioactive compounds with pharmaceutical applications. However, comprehensive studies are necessary to unlock these species' full potential and develop safe and effective drug candidates.

The comparative analysis of mean mortality rates between *Kappaphycus alvarezii* and *Kappaphycus striatus* at varying concentrations provides further insights into their toxicity profiles. A consistent trend emerges, with *K. alvarezii* consistently demonstrating significantly higher mortality rates than *K. striatus* across all concentration levels. This observation reinforces the previous findings regarding the greater toxic potential of *K. alvarezii.* The most pronounced difference in mean mortality was observed at the highest concentration (1000 µg/mL), followed by the 10 µg/mL concentration (Fig. 5). These findings suggest a potential concentration-dependent increase in toxicity for both species, but with a more pronounced effect in *K. alvarezii.* The highly significant differences in mean mortality between the two species at the 1000 µg/mL and 10 µg/mL concentrations, as determined by the ***t***-test ($p < 0.001$ and $p < 0.01$, respectively), provide strong statistical support for the observed differences. This statistical significance strengthens the conclusion that the observed differences in mortality rates are not due to chance but are likely attributable to intrinsic differences in the toxicity of the two seaweed species. This strong statistical support further solidifies the notion that *K. alvarezii* possesses a significantly higher toxic potential than *K. striatus.* The concentration-dependent nature of the toxicity, particularly evident at higher concentrations, warrants further investigation to elucidate the underlying mechanisms and to assess the potential implications for various applications.

Median lethal concentration (LC_{50}) is defined as the concentration level of a particular substance killing half or 50% of the population of test samples, the Brine Shrimp nauplii, as used in the previous study (Orsine et al. 2012). LC_{50} is being considered in the study since the values vary less than LD_1 and LD_{99}, defined as the dosage needed to kill 1% or 99% of a particular test population, respectively (Gupta 2020). For this study, the differences in bioactivity were further supported by the evaluation of their respective LC_{50} (Table 2). The LC_{50} values provide quantitative support for the qualitative observations made in the previous analyses. The significantly lower LC_{50} value for *K. alvarezii* (38.9 µg/mL) compared to *K. striatus* (2187.76 µg/mL) unequivocally demonstrates the superior toxicity of *K. alvarezii.* This implies that a substantially lower concentration of *K. alvarezii* extract is required to induce mortality in 50% of the brine shrimp population compared to *K. striatus.*

The substantial statistical difference (45.54,

$p<0.0001$) between the LC50 values reinforces the conclusion that the observed difference in toxicity between the two seaweed species is highly unlikely to be due to chance (Pocock 2006). These findings further emphasize the potential of *K. alvarezii* as a source of bioactive compounds with cytotoxic properties. However, as previously mentioned, further investigation is necessary to isolate and characterize these compounds to determine their specific biological activities and potential therapeutic applications. It is crucial to note that while the LC_{50} values provide a quantitative measure of acute toxicity, they do not necessarily reflect the chronic toxicity or other potential adverse effects of these seaweed extracts (Pillai et al. 2021).

3.3 Biochemical composition from previous studies

Cytotoxicity assessments are pivotal in the preliminary evaluation of potential drug candidates. As Aslantürk (2018) noted, these assays are indispensable tools in in vitro studies. Their efficacy in identifying compounds that inhibit or halt cell proliferation makes them cornerstone methods in cancer research. The present study's findings align with this perspective. The markedly lower LC_{50} value of *K. alvarezii* compared to *K. striatus* strongly suggests a higher cytotoxic potential in the former species. This differential cytotoxicity profile indicates that *K. alvarezii* may harbor compounds with more potent biological activities, warranting deeper exploration.

Previous research has consistently demonstrated that *K. alvarezii* possesses a richer biochemical composition than *K. striatus,* particularly in polysaccharides and fat-soluble antioxidants (Ariano et al. 2021, Bhuyar et al. 2021). This disparity in chemical constituents likely plays a pivotal role in the observed differences in bioactivity between the two species.

The presence of these compounds in *K. alvarezii* may be correlated with its enhanced cytotoxic properties. By delving deeper into the specific compounds responsible for the observed cytotoxicity in *K. alvarezii,* researchers can identify potential lead molecules for therapeutic interventions. For example, studies have shown that certain polysaccharides derived from marine algae can exhibit anti-cancer properties by inhibiting cell proliferation and inducing apoptosis (Yao et al. 2022). Similarly, antioxidant compounds from marine algae have been investigated for their potential to protect against neurodegenerative diseases and cardiovascular disorders (Barbalace et al. 2019, Murray et al. 2018). Therefore, the richer biochemical composition of *K. alvarezii*, particularly its higher content of polysaccharides and fat-soluble antioxidants, provides a strong foundation for exploring its potential as a source of bioactive compounds with therapeutic applications. However, further research is necessary to isolate and characterize these compounds to fully understand their biological activities and safety profiles.

4. CONCLUSION

This study evaluated the cytotoxic effects of *K. alvarezii* and *K. striatus* on brine shrimp larvae. The results demonstrated a significant toxicity disparity between the two seaweed species. *K. alvarezii* consistently exhibited a markedly higher mortality rate across all tested concentrations, indicating a substantially greater toxic potential. A positive correlation between concentration and mortality was observed for both species, suggesting a concentration-dependent toxic effect. This finding is further supported by the LC_{50} values, with *K. alvarezii* exhibiting a significantly lower LC_{50}, indicating a higher acute toxicity. The higher cytotoxicity of *K. alvarezii* is potentially attributed to its richer biochemical composition, particularly in terms of polysaccharides and fat-soluble antioxidants from recent literature. This suggests the presence of bioactive compounds within *K. alvarezii* that could potentially have pharmacological applications. However, rigorous safety evaluations and further research are necessary to isolate and characterize these compounds, ensuring their safety and efficacy for therapeutic use. In conclusion, this study underscores the importance of species-specific toxicity assessments and highlights the potential of *K. alvarezii* as a source of bioactive compounds. While the higher toxicity necessitates careful handling and application, further research is warranted to explore its potential benefits and develop strategies for safe and sustainable utilization. Research in these areas could expand the market potential for the thriving seaweed industry in Caraga, Philippines.

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6. STATEMENT OF CONFLICT OF INTEREST

The authors declared that they have no conflict of interest associated with this research and the publication of this manuscript.

7. LITERATURE CITED

Ariano, A., Musco, N., Severino, L., De Maio, A., Tramice, A., Tommonaro, G., Damiano, S., Genovese, A., Olanrewaju, O. S., Bovera, F., & Guerriero, G. (2021). Chemistry of Tropical Eucheumatoids: Potential for Food and Feed

Applications. *Biomolecules,* **11**(6), 804. https://doi.org/10.3390/biom11060804

Aslantürk, Ö. S. (2018). In Vitro Cytotoxicity and Cell Viability Assays: Principles, Advantages, and Disadvantages. In M. L. Larramendy & S. Soloneski (Eds.),*Genotoxicity—APredictableRisk to Our Actual World.*InTech.https://dorg/10.5772intechopen.71923

Aware, C. B., Patil, D. N., Suryawanshi, S. S., Mali, P. R., Rane, M. R., Gurav, R. G., & Jadhav, J. P. (2022). Natural bioactive products as promising therapeutics: A review of natural product-based drug development. S*outh African Journal of Botany,* **151**,512–528.https://doi.org/10.1016/j.sajb.2022.05.028

Barbalace, M. C., Malaguti, M., Giusti, L., Lucacchini, A.,Hrelia,S.,&Angeloni,C.(2019).Anti-Inflammatory Activities of Marine Algae in Neurodegenerative Diseases. I*nternational Journal of Molecular Sciences*,**20**(12),3061.https:doiorg10.3390ijms20123061

Bhuyar, P., Sundararaju, S., Rahim, M. H. Ab., Unpaprom, Y., Maniam, G. P., & Govindan, N. (2021). Antioxidative study of polysaccharides extracted from red (Kappaphycus alvarezii), green (Kappaphycus striatus) and brown (Padina gymnospora)marinemacroalgae/seaweed.*SNApplie Sciences,* **3**(4), 485. https://doi.org/10.1007/s42452-021-04477-9

Chopra, B., & Dhingra,A. K.(2021).Natural products: A lead for drug discovery and development. *Phytotherapy Research*, **35**(9),4660–4702.https://doi.org/10.1002/ptr.7099

Desideri, D., Cantaluppi, C., Ceccotto, F., Meli, M. A., Roselli, C., & Feduzi, L. (2016). Essential and toxic elements in seaweeds for human consumption. Journal of Toxicology and Environmental Health, Part A, 79(3), 112–122. https://doi.org/10.1080/15287394.2015.1113598

Ferraces-Casais,P.,Lage-Yusty,M.A.,Rodríguez-Bernaldo De Quirós, A., & López-Hernández, J. (2012). Evaluation of Bioactive Compounds in Fresh Edible Seaweeds. *Food Analytical Methods*, **5**(4), 828–834. https://doi.org/10.1007/s12161-011-9321-2

Filippini, M., Baldisserotto, A., Menotta, S., Fedrizzi, G., Rubini, S., Gigliotti, D., Valpiani, G., Buzzi, R., Manfredini, S., & Vertuani, S. (2021). Heavy metals and potential risks in edible seaweed on the market in Italy. *Chemosphere*, **263**, 127983. https://doi.org/10.1016/j.chemosphere.2020.127983

Ghosh, A., Banik, S., & Islam, Md. A. (2015). In vitro thrombolytic, anthelmintic, anti-oxidant and cytotoxic activity with phytochemical screening of methanolic extract of Xanthium indicum leaves. *Bangladesh Journal of Pharmacology,***10**(4), 854.https://doi.org/10.3329/bjp.v10i4.23829

Gomaa, S. A. S., Barakat, E. M. S., Salama, M. S., & El-Gohary, E. E. (2021). Effect of the Bacterium Paenibacillus larvae larvae on Vitellogenin Gene Expression of the Queen Honey Bee Apis mellifera L. *African Entomology,* **29**(1). https://doi.org/10.4001/003.029.0096

Guevara, B. Q. (2005). *A guidebook to plant screening: Phytochemical and biological* (Rev. ed). University of Santo Tomas Publishing House.

Gullón, P., Astray, G., Gullón, B., Franco, D., Campagnol, P. C. B., & Lorenzo, J. M. (2021). Inclusion of sea weeds as healthy approach to formulate new low-salt meatproducts. *Current Opinion in Food Science,* **40**, 20–25. https://doi.org/10.1016/j.cofs.2020.05.005

Gupta, P. K. (2020). Principles of Toxicology. In P. K. Gupta, *Problem Solving Questions in Toxicology:* (pp. 27–45). Springer International Publishing. https://doi.org/10.1007/978-3-030-50409-0 _3

Jarvis,T.A.,&Bielmyer-Fraser,G.K.(2015).*Accumulation and effects of metal mixtures in two seaweed species.Comparative Biochemistry and Physiology PartC:Toxicology&Pharmacology,***171**,28–33.https://doi.org/10.1016/j.cbpc.2015.03.005

Khan, R. A. (2018). Natural products chemistry: *The emerging trends and prospective goals. Saudi Pharmaceutical Journal,* **26**(5), 739–753. https://doi.org/10.1016/j.jsps.2018.02.015

Kibiti, C. M., & Afolayan, A. J. (2016). Antifungal activity and brine shrimp toxicity assessment of Bulbine abyssinica used in the folk medicine in the Eastern Cape Province, South Africa. *Bangladesh Journal of Pharmacology,* **11**(2), 469. https://doi.org/10.3329/bjp.v11i2.24405

Majolo, F., De Oliveira Becker Delwing, L. K., Marmitt, D. J., Bustamante-Filho, I. C., & Goettert, M. I. (2019). Medicinal plants and bioactive natural compounds for cancer treatment: Important advances for drug discovery. *Phytochemistry Letters,* **31,** 196–207.https://doi.org/10.1016/j.phytol.2019.04.003

Malain, D., Regan, P. H., Bradley, D. A., Matthews, M., Al-Sulaiti, H. A., & Santawamaitre, T. (2012). An evaluation of the natural radioactivity in Andaman beach sand samples of Thailand after the 2004 tsunami. *Applied Radiation and Isotopes,* **70**(8), 1467–1474.https://doi.org/10.1016/j.apradiso.2012.04.017

Mateo, J. P., Campbell, I., Cottier-Cook, E. J., Luhan, M. R. J., Ferriols, V. M. E. N., & Hurtado, A. Q. (2021). Understanding biosecurity: Knowledge, attitudes and practices of seaweed farmers in the Philippines. *Journal of Applied Phycology,* **33**(2), 997–1010. https://doi.org/10.1007/s10811-020-02352-5

Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D., & McLaughlin, 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

Murray, M., Dordevic, A. L., Ryan, L., & Bonham, M. P. (2018). An emerging trend in functional foods for the prevention of cardiovascular disease and diabetes: Marine algal polyphenols. *Critical Reviews in Food Science and Nutrition,* **58**(8), 1342–1358. https://doi.org/10.1080/10408398.2016.1259209

Orsine, J. V. C. (n.d.). *The acute cytotoxicity and lethal concentration (LC50) of Agaricus sylvaticus through hemolytic activity on human erythrocyte.*

Oucif, H., Benaissa, M., Ali Mehidi, S., Prego, R., Aubourg, S. P., & Abi-Ayad, S.-M. E.-A. (2020). Chemical Composition and Nutritional Value of Different Seaweeds from the West Algerian Coast. *Journal of Aquatic Food Product Technology,* **29**(1), 90–104. https://doi.org/10.1080/10498850.2019.1695305

Pati, M. P., Sharma, S. D., Nayak, L., & Panda, C. R. (2016). Uses of Seaweed and its Application to Human Welfare: *A Review. International Journal of Pharmacy and Pharmaceutical Sciences,* **8**(10),12. https://doi.org/10.22159/ijpps.2016v8i10.12740

Peng, Z., Guo, Z., Wang, Z., Zhang, R., Wu, Q., Gao, H., Wang, Y., Shen, Z., Lek, S., & Xiao, J. (2022). Species-specific bioaccumulation and health risk assessment of heavy metal in seaweeds in tropic coasts of South China Sea. *Science of The Total Environment,* **832**,155031.https://doi.org/10.1016/j.scitotenv.2022.155031

Pillai, S. K., Kobayashi, K., Mathai, T., & Michael, M. (2021). Use of LC50 in aquatic regulatory toxicology-Disharmony in global harmonization of hazard classification of chemicals. *Ecotoxicology and Environmental Contamination,* **16**(1), 91–96. https://doi.org/10.5132/eec.2021.01.12

Pocock, S. J. (2006). The simplest statistical test: How to check for a difference between treatments. *BMJ,* **332**(7552),1256.https://doi.org/10.1136/bmj.332.7552.1256

Randhawa, M. A. (2009). Calculation of LD50 values from the method of Miller and Tainter, 1944. J Ayub Med Coll Abbottabad, 21(3), 184-185.Rasonable, G., Seronay, R., & Asufre, G. (2023). Effects of Fish Farming on Sediment, Water Quality and Plankton Communities in Barobo Coastal Waters in Lianga Bay, Surigao Del Sur, Philippines. *International Conference on Fisheries and Aquaculture,* 1–22. https://doi.org/10.17501/23861282.2023.8101

Rengasamy, K. Rr., Mahomoodally, M. F., Aumeeruddy, M. Z., Zengin, G., Xiao, J., & Kim, D. H. (2020). Bioactive compounds in seaweeds: An overview of their biological properties and safety. *Food and Chemical Toxicology,* **135,** 111013. https://doi.org/10.1016/j.fct.2019.111013

Sadat Sadeghi, M. (2018). Evaluation of toxicity and lethal concentration (LC50) of silver and selenium nanoparticle in different life stages of the fish Tenualosa ilish (Hamilton 1822). *Oceanography & Fisheries Open Access Journal,* **7**(5). https:/doi.org/10.19080/OFOAJ.2018.07.555722

Samonte, G. P. B. (2017). Economics of *Kappaphycus* spp. Seaweed Farming with Special Reference to the Central Philippines. In A. Q. Hurtado, A. T. Critchley, & I. C. Neish (Eds.), *Tropical Seaweed Farming Trends, Problems and Opportunities* (pp. 147–154). Springer InternationalPublishing.https://doi.org/10.1007/978-3-319-63498-2_9

Santawamaitre, T., Malain, D., Al-Sulaiti, H. A., Matthews, M., Bradley, D. A., & Regan, P. H. (2011). Study of natural radioactivity in riverbank soils along the Chao Phraya river basin in Thailand. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators,Spectrometers,Detectors and Associated Equipment,* **652**(1),920–924.https://doi.org/10.1016/j.nima.2010.10.057

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

Senthil, A., Mamatha, B. S., Vishwanath, P., Bhat, K. K., & Ravishankar, G. A. (2011). Studies on development and storage stability of instant spice adjunct mix from seaweed (Eucheuma). *Journal of Food Science and Technology,* **48**(6), 712–717. https://doi.org/10.1007/s13197-010-0165-3

Smith, J., Summers, G., & Wong, R. (2010). Nutrient and heavy metal content of edible seaweeds in New Zealand. *New Zealand Journal of Crop and Horticultural Science,* **38**(1), 19–28. https://doi.org/10.1080/01140671003619290

Sufian, Md. A., & Haque, M. R. (2015). Cytotoxic, thrombolytic, membrane stabilizing and anti-oxidant activities of *Hygrophila schulli. Bangladesh Journal of Pharmacology,* **10**(3), 692. https://doi.org/10.3329/bjp.v10i3.23718

Suyo, J. G. B., Le Masson, V., Shaxson, L., Luhan, M. R. J., & Hurtado, A. Q. (2021). Navigating risks and uncertainties: Risk perceptions and risk management strategies in the Philippine seaweed industry. *Marine Policy,* **126,** 104408. https://doi.org/10.1016/j.marpol.2021.104408

Syahmi, A. R. M., Vijayarathna, S., Sasidharan, S., Latha, L.Y., Kwan, Y.P., Lau, Y.L., Shin, L.N., & Chen, Y. (2010). Acute Oral Toxicity and Brine Shrimp Lethality of Elaeis guineensis Jacq., (Oil Palm Leaf) Methanol Extract. *Molecules,* **15**(11), 8111–8121. https://doi.org/10.3390/molecules15118111

Thomford, N. E., Senthebane, D. A., Rowe, A., Munro, D., Seele, P., Maroyi, A., & Dzobo, K. (2018). Natural Products for Drug Discovery in the 21st Century: Innovations for Novel Drug Discovery. *International Journal of Molecular Sciences,* **19**(6), 1578. https://doi.org/10.3390/ijms19061578

Truong, D.-H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., & Nguyen, H. C. (2019). Evaluation of the Use of Different Solvents for Phytochemical Constituen ts, Antioxidants, and *In Vitro Anti-Inflammatory Activities of Severinia buxifolia. Journal of Food Quality,* **2019**, 1–9. https://doi.org/10.1155/2019/8178294

Vilas-Boas, A. A., Pintado, M., & Oliveira, A. L. S. (2021). Natural Bioactive Compounds from Food Waste: Toxicity and Safety Concerns. *Foods,* **10**(7), 1564. https://doi.org/10.3390/foods10071564

Wang, S., Wang, Q., Jiang, X., Han, X., & Ji, H. (2013). Compositional analysis of bio-oil derived from pyrolysis of seaweed. *Energy Conversion and Management,* **68,** 273–280. https://doi.org/10.1016/j.enconman.2013.01.014

Yao, W., Qiu, H.-M., Cheong, K.-L., & Zhong, S. (2022). Advances in anti-cancer effects and underlying mechanisms of marine algae polysaccharides. International Journal of Biological Macromolecules, 221, 472–485.https://doi.org/10.1016/j.ijbiomac.2022.09.055

Book:

Guevara, B. Q. (2005). A guidebook to plant screening: Phytochemical and biological (Rev. ed). University of Santo Tomas Publishing House.

Website:

Philippine Statistics Authority (2020). Fisheries Statistics of the Philippines. https://psa.gov.ph/