J. Mar. Biotechnol. Immunol
Vol. 2 No. 3 (2024)

ISSN 3026-1457 (Online)
ISSN 3026-5274 (Print)

Journal of Marine Biotechnology and Immunology

Journal homepage : https://ejournal.immunolmarbiotech.com

Extraction of Sulfated Polysaccharides from *Ulva* sp. Using Acid and Toxicity Testing with the Brine Shrimp Lethality Test (BSLT)

Sunaryo Sunaryo[1]. Alief Oktavian Saputra[1]. Jelita Rahma Hidayati[2*]. Ipanna Enggar Susetya[3]

[1] Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro, Jl Prof. Sudharto, Tembalang, Semarang, Indonesia.
[2] Department of Marine Science, Faculty of Marine and Fisheries Sciences, Raja Ali Haji Maritime University Jl. Politeknik Senggarang Tanjung Pinang, Riau Islands, Indonesia.
[3] Aquatic Resource Management Study Program, Faculty of Agriculture, Universitas Sumatera Utara, Jl. Dr. A. Sofian No.3, Padang Bulan, Kec. Medan Baru, Kota Medan, Sumatera Utara 20155, Indonesia.

OPEN ACCESS

Article Info
Received: August 15, 2024
Accepted: August 31, 2024
Published: September 30, 2024
Available online: October 6, 2024

Keywords:
Sulfated Polysaccharides
Ulva sp.
Artemia salina
Brine Shrimp Lethality Test

*Corresponding Author email: jelitarahmahidayati@umrah.ac.id

Abstract

This study investigates the extraction of sulfated polysaccharides from *Ulva sp.* at pH 3 and evaluates their toxicity using the Brine Shrimp Lethality Test (BSLT) with *Artemia salina*. *Artemia salina* is utilized for its rapid life cycle and sensitivity to various chemical compounds, making it an effective bioassay organism for assessing the toxicity of natural extracts. The extraction process was conducted at both hot (80°C) and cold (room temperature) conditions, with the hot extraction yielding higher amounts of polysaccharides. The results indicated a wet extraction yield of 890 mL at 80°C and 800 mL at room temperature, while the highest dry weight yield was achieved at 80°C (0.57 g). The toxicity assessment revealed an LC50 value of 15,815.85 ppm, classifying the sulfated polysaccharides as non-toxic to *Artemia salina*. Maintaining optimal water quality parameters, including temperature, salinity, pH, and dissolved oxygen levels, is essential for the successful cultivation of *Artemia salina*. Furthermore, the acidic extraction pH significantly influences the structure and chemical properties of the resulting polysaccharides, emphasizing the importance of this parameter in future applications. These findings support the potential use of sulfated polysaccharides from *Ulva sp.* as a safe and effective feed ingredient in aquaculture.

1. Introduction

Artemia salina is widely used in bioassays to evaluate the toxicity of various chemical compounds, including polysaccharide extracts from marine sources like *Ulva* sp. This organism is often chosen due to its simple maintenance, rapid life cycle, and sensitivity to a broad range of chemical agents (Rajabi *et al.*, 2015). Toxicity assays using *Artemia salina* provide an efficient means of assessing the LC50 (lethal concentration for 50% of test organisms), which reflects the relative toxicity of compounds in natural environments (Salay *et al.*, 2024).

Sulfated polysaccharides derived from *Ulva* sp., a green seaweed, have garnered attention due to their wide range of bioactive properties, including antioxidant, anti-inflammatory, anticoagulant, and antitumor activities (Kidgell *et al.*, 2019; Cindana Mo'o *et al.*, 2020; Li *et al.*, 2023). These polysaccharides show promising potential in pharmaceutical and nutraceutical applications, particularly as natural bioactive compounds that could serve as immunostimulants and prebiotics (Azhar *et al.*, 2024a; Azhar *et al.*, 2024b). The demand for alternative and sustainable shrimp feed ingredients is increasing globally, and sulfated polysaccharides have been shown to enhance shrimp health and growth by acting as natural immunomodulators (Vijayaram *et al.*, 2023; Vijayaram *et al.*, 2024). This highlights the importance of evaluating their toxicity and bioactivity before commercial use in aquaculture or pharmaceuticals.

The extraction of sulfated polysaccharides from *Ulva* sp. at an acidic pH, particularly acid pH, has been reported to be effective in optimizing both yield and biological activity (Kidgell *et al.*, 2019). Different acidic

condition have been applied to extract from seaweed, resulting in shorter and lower molecular weight due to hydrolysis (Kadam *et al.*, 2017). The acidic condition facilitated the release of protein bound to the cell wall polysaccharide but did not promote protein solubilization (Sari *et al.*, 2015). Therefore, in this study, the extraction of sulfated polysaccharides at acidic pH was employed to ensure optimal extraction efficiency and bioactivity retention.

Toxicity testing using the Brine Shrimp Lethality Test (BSLT) is a widely accepted method for evaluating the toxicological profile of natural products, as it offers a quick, low-cost, and reliable means of assessing the safety of bioactive compounds (Banti and Hadjikakou, 2021). Combining the extraction of sulfated polysaccharides from *Ulva* sp. with the BSLT provides critical insights into both the efficacy and potential toxic effects of these compounds (Tran *et al.*, 2023). The objective of this research is to optimize the extraction of sulfated polysaccharides from *Ulva* sp. at pH 3, determine their toxicity using the Brine Shrimp Lethality Test (BSLT) with *Artemia salina*, and contribute to understanding their potential as natural, health-enhancing agents in aquaculture and related industries.

2. Material and methods

2.1 Material

The material studied and analyzed in this research is *Ulva* sp., which was sourced from the southern coast of Java, specifically in Yogyakarta. After collection, the seaweed was meticulously cleaned to remove any epiphytes, sand, and other debris to ensure the purity of the sample. This cleaning process was carried out by washing the *Ulva* thoroughly with seawater, followed by freshwater rinses to eliminate all foreign substances that could potentially interfere with the extraction and testing processes. Afterward, the cleaned seaweed was dried and prepared for further extraction of sulfated polysaccharides.

2.2 Methods

2.2.1. Extraction of *Ulva* sp.

The Extraction of Ulva sp. was conducted twice with five repetitions, utilizing two different temperatures: hot (80 ^{o}C) and cold (room temperature) at pH 3. The objective of this extraction process was to obtain sulfated polysaccharide compounds from the *Ulva* sp. sample by disrupting the cell walls. The extraction was performed over 24 hours using seawater solution at pH 3. The initial stage of the extraction involved grinding the sample into small pieces using a blender, followed by weighing out 5 grams of the sample for each extraction. Subsequently, the samples were extracted using hot and cold water at pH 3 as solvents (Yaich *et al.*, 2013; Yaich *et al.*, 2014).

2.2.2. Sample Centrifugation and Sample Drying

Before the centrifugation process, empty vials were weighed using an analytical balance. The centrifugation of samples was carried out in stages for a total of 20 samples (10 at room temperature and 10 at elevated temperature). The samples, which had undergone extraction twice, were combined and measured using a graduated cylinder. The measured sample volume was then mixed with alcohol at a 1:1 ratio (Azhar *et al.*, 2024a; Azhar *et al.*, 2024b). The mixture was subsequently placed into a centrifuge and centrifuged for 2 minutes at a speed of 30 rpm. After centrifugation, the precipitate was separated from the supernatant and transferred into the previously weighed vials. This process was repeated until all 20 samples were processed. Centrifugation is a common technique used to separate solid components from liquids, allowing for the concentration of precipitates. The speed and duration of centrifugation can significantly affect the efficiency of separation, making it essential to optimize these parameters for specific applications (Rickwood and Birnie, 1978).

2.2.3. Hatching of *Artemia* sp.

The hatching process of *Artemia* was conducted using 0.5 grams of dried *Artemia* cysts, which were weighed and soaked in freshwater for 1 hour. Subsequently, 400 mL of sterilized seawater was prepared. The soaked *Artemia* cysts were then introduced into the container containing the sterilized seawater, aerated, and allowed to hatch for 24 hours. This method ensures optimal hatching conditions, promoting the viability and health of the *Artemia* larvae for subsequent bioassay applications. Proper aeration and sterilization are critical factors in maximizing hatching success and minimizing contamination (Briski *et al.*, 2008; Arun *et al.*, 2017; Dey *et al.*, 2023).

2.2.4. Toxicity Test

The toxicity test was conducted over a 24-hour period. The procedure began with the preparation of five concentrations of sulfated polysaccharide extract solutions: 5,000 ppm, 2,500 ppm, 1,250 ppm, 625 ppm, and 312.5 ppm, with each concentration prepared in triplicate. Each solution was placed into vials according to standard protocol, and sterilized seawater was added to reach a final volume of 10 mL per vial. A total of 25 *Artemia* were introduced into each test vial, and the initial time for mortality observation (t0) was recorded. Mortality observations were made immediately after the *Artemia* were introduced into the vials, with further observations conducted at 1, 2, 4, 8, 16, and 24 hours. This method ensures a systematic and reliable assessment of the lethal effects of the sulfated polysaccharide extract on *Artemia* over time, following established toxicity testing protocols (Meyer *et al.*, 1982; Ara *et al.*, 1999)

3. Results

3.1 Extraction of *Ulva* sp.

The following data represent the wet extraction yields, comparing the results between cold and hot temperature conditions. Table 1 shows the dry extraction yield of sulfated polysaccharide samples after undergoing two stages of extraction, centrifugation, and drying, comparing cold (room temperature) and hot (80°C) conditions. The wet extraction yield produced 800 mL at room temperature and 890 mL at hot temperature (80°C). The highest dry weight yield was obtained at hot temperature (80°C), with 0.57 ± 0.11 g, compared to the room temperature yield of 0.17 ± 0.04 g.

Table 1. Extraction of *Ulva* sp.

	Yield	
Treatment	Extraction (mL)	dry weight (gram)
room temperature	800	0.17±0.04
hot temperature (80 ^{o}C)	890	0.57±0.11

3.2 Mortality rate and LC_{50}

Based on Figure 1, the mortality rate ranges from 9.33% to 41.33%. According to Table 2, the LC50 value of the sulfated polysaccharides is 15,815.85 ppm with an R^2 value of 0.8197 (y = 0.9036x + 1.2057) as shown in Figure 2. This means that the concentration of 15,815.85 ppm is estimated to cause 50% mortality of Artemia within 24 hours. The equation y=0.9036x+1.2057 represents the linear regression used to calculate the LC_{50} value, while the R^2 value of 0.8197 indicates a strong correlation between the logarithm of the concentration and mortality, reflecting the reliability of the model.

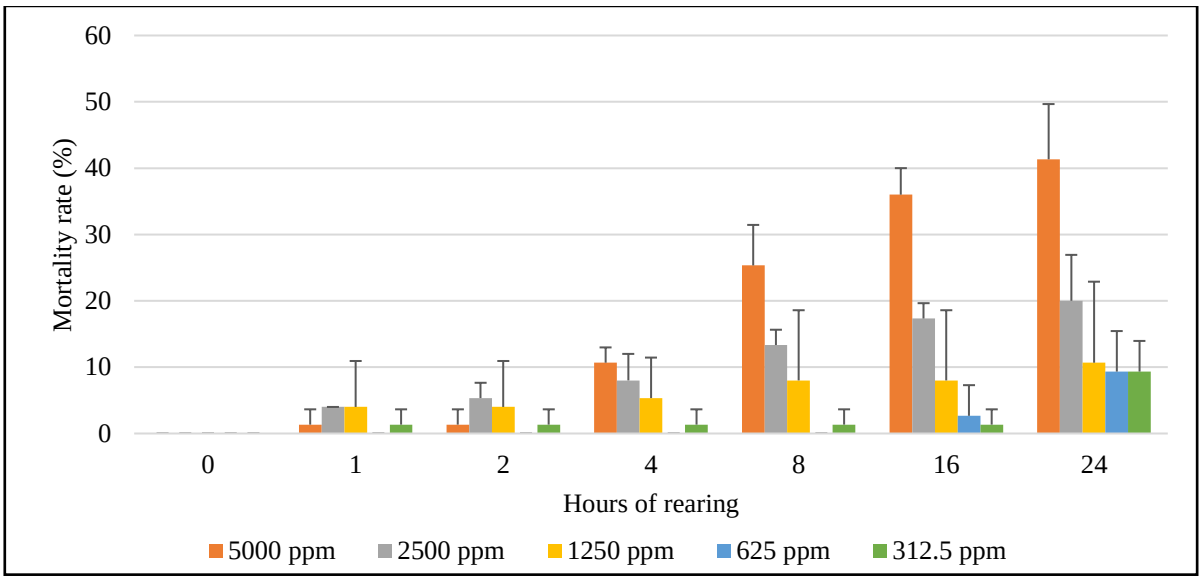

Figure 1. Artemia Mortality with Sulfated Polysaccharides over 24 Hours

Table 2. LC_{50} (Lethal Concentration 50%)

Test Concentration (ppm)	Log Concentration	No. of Test Larvae	U1	U2	U3	Average	% Mortality	Probit Value
5000	3,698970004	25	11	8	12	10,33	41,33	4,77
2500	3,397940009	25	4	7	4	5	20	4,16
1250	3,096910013	25	6	0	2	2,66	10,66	3,77
625	2,795880017	25	1	4	2	2,33	9,33	3,66
312,5	2,494850022	25	3	3	1	2,33	9,33	3,66

LC_{50}= 15.815,85

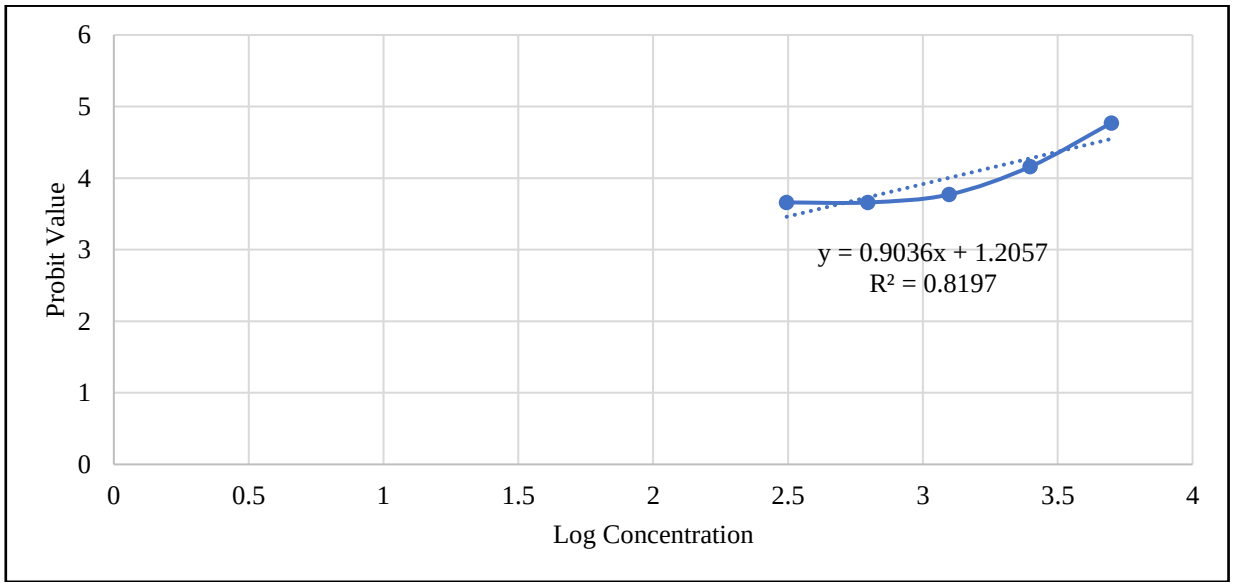

Figure 2. Log Concentration Graph

4. Discussion

Optimal water quality parameters are crucial for the successful cultivation of *Artemia salina* and toxicity testing. Key parameters include temperature, salinity, pH, and dissolved oxygen levels. The optimal temperature for the growth and hatching of *Artemia* cysts ranges from 25 to 30°C. Temperatures that are too low or too high can hinder the hatching process and the growth of nauplii. Additionally, sudden fluctuations in temperature can induce stress in *Artemia*, potentially decreasing their survival rates.

Salinity also plays a significant role, with optimal levels around 30-35 ppt. Moreover, the ideal pH for *Artemia*

salina falls between 7.5 and 8.5. Extreme pH levels, whether too low or too high, can stress *Artemia* and adversely affect their survival. Unsuitable pH conditions can disrupt ionic balance and cellular metabolism, which can be fatal for *Artemia* (Thirunavukkarasu and Munuswamy, 2019; Dey *et al.*, 2023).

Dissolved oxygen levels must also be maintained adequately, typically above 5 mg/L, to ensure efficient respiration. The use of aerators can help maintain optimal dissolved oxygen levels, particularly in closed systems or high population densities. Maintaining suitable incubation parameters for *Artemia* cysts can prevent external mortality due to unsuitable conditions during the testing process (Gajardo and Beardmore, 2012; Pacheco-Vega *et al.*, 2015).

The use of pH 3 in the extraction of sulfated polysaccharides from *Ulva sp.* has a significant impact on the structure and chemical properties of the resulting compounds. Acidic pH can aid in breaking down the algal cell walls, facilitating the release of sulfated polysaccharides into the extract solution. Phomkaivon *et al.* (2024) and He *et al.* (2016) reinforce that during acid extraction, cellular materials from algae can also be co-extracted along with the necessary components. A pH of 3 can also prevent the degradation of bioactive compounds that are sensitive to alkaline conditions, thereby preserving the integrity and biological activity of the polysaccharides. However, extreme acidic conditions can also affect the stability of other components in the extract, such as proteins or lipids, which may degrade during the process. Therefore, determining the appropriate pH is critical for maximizing extraction yields and minimizing damage to active compounds.

Both hot and cold extraction methods (room temperature) are commonly employed to obtain bioactive compounds from natural materials, such as sulfated polysaccharides from *Ulva sp.* Hot extraction involves heating the extraction solution to a specific temperature to enhance the speed and efficiency of extraction. The results of this study demonstrate that hot extraction yields a greater quantity of extract compared to extraction at room temperature. Increased temperatures can improve the solubility of polysaccharides in the solvent, accelerate the diffusion rate of compounds from the cellular matrix, and more effectively break down cell walls. Additionally, heat can denature proteins and other components that may interact with polysaccharides, thereby facilitating their release into the solution (Zhou *et al.*, 2020; Lomartire and Gonçalves, 2022; Antonisamy and Rajendran, 2024).

The results of the Brine Shrimp Lethality Test (BSLT) using *Artemia salina* against sulfated polysaccharides indicate an LC50 value of 15,815.849 ppm. LC50 represents the concentration of a substance that causes death in 50% of the test organism population, in this case, *Artemia salina*. Such a high LC50 value suggests that the sulfated polysaccharides from this source exhibit low toxicity towards *Artemia salina*. Setianingsih *et al.* (2023) supports this by categorizing toxicity levels: <30 ppm as highly toxic, 30-1000 ppm as toxic, and >1000 ppm as non-toxic. *Artemia salina* serves as a sensitive bioassay model for assessing the relative toxicity potential of chemical compounds or natural extracts. This finding indicates that sulfated polysaccharides can be considered relatively safe at the concentrations used in the study concerning *Artemia salina*. Thus, this high LC50 value may also encourage further research into the pharmacological potential or biomedical applications of sulfated polysaccharides from *Ulva sp.*, as the results demonstrate low toxicity.

5. Conclusions

The extraction of sulfated polysaccharides from *Ulva sp.* at pH 3 yields adequate rendement and exhibits good biological activity as a potential feed for *Artemia salina*. Toxicity testing using the Brine Shrimp Lethality Test (BSLT) indicates that the compounds are classified as non-toxic. Important water parameters, such as temperature, salinity, pH, and dissolved oxygen levels, must be carefully managed to support the survival and growth of *Artemia salina*. Additionally, the use of pH 3 in the extraction process affects the structure and chemical properties of the compounds, which should be taken into account for future development.

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

AOS : sample image collection, water quality sampling collection. S.S, J.R.H and IES : research ideas, supervising and writing

Funding

No funding available

Acknowledgments

We would like to state my special thanks of gratefulness to Diponegoro University.

Declaration of competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Referrences

Antonisamy, A. J., & Rajendran, K. 2024. Comparative study on the extraction methods, characterization, and bioactivity of crude fucoidan, a polysaccharide derived from *Sargassum ilicifolium*. *Biochemical Engineering Journal.* 209: 109398. https://doi.org/10.1016/j.bej.2024.109398

Ara, J., Sultana, V., Ehteshamul-Haque, S., Qasim, R., & Ahmad, V. U. 1999. Cytotoxic activity of marine macro-algae on *Artemia salina* (Brine shrimp). *Phytotherapy Research.* 13(4): 304-307. https://doi.org/10.1002/(SICI)1099-1573(199906)13:4<304::AID-PTR439>3.0.CO;2-9

Arun, V. V., Saharan, N., Ramasubramanian, V., Babitha Rani, A. M., Salin, K. R., Sontakke, R., Haridas, H., & Pazhayamadom, D. G. 2017. Multi-response optimization of Artemia hatching process using split-split-plot design based response surface methodology. *Sci Rep.* 7: 40394. https://doi.org/10.1038/srep40394

Azhar, N., Yudiati, E., Ambariyanto, & Trianto, A. 2024a. The Effect of Enhancing Ulvan's Antioxidant Properties in Supplemented Diets on Accelerating The Phenoloxidase Immune Response in White

Shrimp. *HAYATI Journal of Biosciences.* 31(6): 1116-1129. https://doi.org/10.4308/hjb.31.6.1116-1129

Azhar, N., Yudiati, E., Ambariyanto, A., & Trianto, A. 2024b. Immunostimulatory Effects of Ulvan on Trypsin-Mediated Protein Digestion in The Gut of Pacific Whiteleg Shrimp (*Litopenaeus vannamei*). *Jurnal Riset Akuakultur.* 19(1): 45-56. http://dx.doi.org/10.15578/jra.19.1.2024.45-56

Banti, C. N., & Hadjikakou, S. K. 2021. Evaluation of Toxicity with Brine Shrimp Assay. *Bio Protoc.* 11(2): e3895. https://doi.org/10.21769/BioProtoc.3895

Briski, E., Van Stappen, G., Bossier, P., & Sorgeloos, P. 2008. Laboratory production of early hatching *Artemia* sp. cysts by selection. *Aquaculture.* 282(1): 19-25. https://doi.org/10.1016/j.aquaculture.2008.06.034

Cindana Mo'o, F. R., Wilar, G., Devkota, H. P., & Wathoni, N. 2020. Ulvan, a Polysaccharide from Macroalga *Ulva* sp.: A Review of Chemistry, Biological Activities and Potential for Food and Biomedical Applications. *Appl Sci.* 10(16): 5488. https://doi.org/10.3390/app10165488

Dey, P., Bradley, T. M., & Boymelgreen, A. 2023. The impact of selected abiotic factors on Artemia hatching process through real-time observation of oxygen changes in a microfluidic platform. *Sci Rep.* 13(1): 6370. https://doi.org/10.1038/s41598-023-32873-1

Gajardo, G. M., & Beardmore, J. A. 2012. The brine shrimp artemia: adapted to critical life conditions. *Front Physiol.* 3: 185. https://doi.org/10.3389/fphys.2012.00185

He, J., Xu, Y., Chen, H., & Sun, P. 2016. Extraction, Structural Characterization, and Potential Antioxidant Activity of the Polysaccharides from Four Seaweeds. *Int J Mol Sci.* 17(12). https://doi.org/10.3390/ijms17121988

Kadam, S. U., Álvarez, C., Tiwari, B. K., & O'Donnell, C. P. 2017. Extraction and characterization of protein from Irish brown seaweed *Ascophyllum nodosum*. *Food Research International.* 99: 1021-1027. https://doi.org/10.1016/j.foodres.2016.07.018

Kidgell, J. T., Magnusson, M., de Nys, R., & Glasson, C. R. K. 2019. Ulvan: A systematic review of extraction, composition and function. *Algal Research.* 39: 101422. https://doi.org/10.1016/j.algal.2019.101422

Li, C., Tang, T., Du, Y., Jiang, L., Yao, Z., Ning, L., & Zhu, B. 2023. Ulvan and *Ulva* oligosaccharides: a systematic review of structure, preparation, biological activities and applications. *Bioresour Bioprocess.* 10(1): 66. https://doi.org/10.1186/s40643-023-00690-z

Lomartire, S., & Gonçalves, A. M. M. 2022. Novel Technologies for Seaweed Polysaccharides Extraction and Their Use in Food with Therapeutically Applications-A Review. *Foods.* 11(17). https://doi.org/10.3390/foods11172654

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E., & McLaughlin, J. L. 1982. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med.* 45(5): 31-34. https://doi.org/10.1055/s-2007-971236

Pacheco-Vega, J., Cadena, M., Ascencio, F., Rangel-Dávalos, C., & Rojas, M. 2015. Assessment of endemic microalgae as potential food for *Artemia franciscana* culture. *Latin american journal of aquatic research.* 43: 23-32. https://doi.org/10.3856/vol43-issue1-fulltext-3

Phomkaivon, N., Pongponpai, P., Kosawatpat, P., Thongdang, B., & Pan-Utai, W. 2024. Extraction, Characterisation and Evaluation of Antioxidant and Probiotic Growth Potential of Water-Soluble Polysaccharides from *Ulva rigida* Macroalgae. *Foods.* 13(11). https://doi.org/10.3390/foods13111630

Rajabi, S., Ramazani, A., Hamidi, M., & Naji, T. 2015. *Artemia salina* as a model organism in toxicity assessment of nanoparticles. *Daru.* 23(1): 20. https://doi.org/10.1186/s40199-015-0105-x

Rickwood, D., & Birnie, G. D. (1978). 1 - Introduction: Principles and Practices of Centrifugation. In G. D. Birnie & D. Rickwood (Eds.), *Centrifugal Separations in Molecular and Cell Biology* (pp. 1-6). Butterworth-Heinemann. https://www.sciencedirect.com/science/article/pii/B9780408708036500054 https://doi.org/https://doi.org/10.1016/B978-0-408-70803-6.50005-4

Salay, G., Lucarelli, N., Gascón, T. M., Carvalho, S. S., Veiga, G., Reis, B., & Fonseca, F. L. A. 2024. Acute Toxicity Assays with the Artemia salina Model: Assessment of Variables. *Altern Lab Anim.* 52(3): 142-148. https://doi.org/10.1177/02611929241242443

Sari, Y. W., Mulder, W. J., Sanders, J. P. M., & Bruins, M. E. 2015. Towards plant protein refinery: Review on protein extraction using alkali and potential enzymatic assistance. *Biotechnology Journal.* 10(8): 1138-1157. https://doi.org/10.1002/biot.201400569

Setianingsih, N. L. P. P., Sudiarta, I. W., Andriani, A. A. S. P. R., Jiwantara, G. N. O., Djelantik, S. A. M. A. P., & Darmawan, K. D. R. 2023. Toxicity Test of Moringa (Moringa Oleifera Lam) Essential Oil with the Brine Shrimp Lethality Test (BSLT) Method. *International Journal of Scientific Multidisciplinary Research.* 1(6): 627-640. https://doi.org/10.55927/ijsmr.v1i6.5255

Thirunavukkarasu, S., & Munuswamy, N. 2019. Do environmental factors influence the morphotypes in *Artemia franciscana* Kellogg, 1906 (Crustacea: Anostraca)? *SN Applied Sciences.* 1(4): 282. https://doi.org/10.1007/s42452-019-0291-0

Tran, V. H. N., Mikkelsen, M. D., Truong, H. B., Vo, H. N. M., Pham, T. D., Cao, H. T. T., Nguyen, T. T., Meyer, A. S., Thanh, T. T. T., & Van, T. T. T. 2023. Structural Characterization and Cytotoxic Activity Evaluation of Ulvan Polysaccharides Extracted from the Green Algae *Ulva papenfussii*. *Mar Drugs.* 21(11). https://doi.org/10.3390/md21110556

Vijayaram, S., Ringø, E., Ghafarifarsani, H., Hoseinifar, S. H., Ahani, S., & Chou, C.-C. 2024. Use of Algae in Aquaculture: A Review. *Fishes.* 9(2): 63. https://doi.org/10.3390/fishes9020063

Vijayaram, S., Ringø, E., Zuorro, A., van Doan, H., & Sun, Y. 2023. Beneficial roles of nutrients as immunostimulants in aquaculture: A review. *Aquaculture and Fisheries*. https://doi.org/10.1016/j.aaf.2023.02.001

Yaich, H., Garna, H., Besbes, S., Barthélemy, J.-P., Paquot, M., Blecker, C., & Attia, H. 2014. Impact of extraction procedures on the chemical, rheological and textural properties of ulvan from *Ulva lactuca* of Tunisia coast. *Food Hydrocoll.* 40: 53-63. https://doi.org/10.1016/j.foodhyd.2014.02.002

Yaich, H., Garna, H., Besbes, S., Paquot, M., Blecker, C., & Attia, H. 2013. Effect of extraction conditions on the yield and purity of ulvan extracted from *Ulva lactuca*. *Food Hydrocoll.* 31(2): 375-382. https://doi.org/10.1016/j.foodhyd.2012.11.013

Zhou, P., Eid, M., Xiong, W., Ren, C., Ai, T., Deng, Z., Li, J., & Li, B. 2020. Comparative study between cold and hot water extracted polysaccharides from *Plantago ovata* seed husk by using rheological methods. *Food Hydrocolloids.* 101: 105465. https://doi.org/10.1016/j.foodhyd.2019.105465