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Effect of Alginate Oligosaccharides (AOS) from *Sargassum* sp. on Cutaneous Wound Healing in Zebrafish (*Danio rerio*)

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Abstract

Wound healing is a regenerative process that restores damaged connective tissue, but inadequate management can delay repair. Sodium alginate may promote wound healing by modulating oxidative stress through its antioxidant, antimicrobial, and immunomodulatory properties. This study aimed to evaluate the effectiveness of alginate in supporting wound healing using zebrafish as an experimental model. Alginate oligosaccharides (AOS) were produced by oxidative depolymerization of crude alginate using hydrogen peroxide and ascorbic acid. Zebrafish with induced cutaneous wounds were immersed in AOS solutions at concentrations of 350, 500, 650, and 800 ppm. Each treatment consisted of 20 randomly assigned fish and was conducted in triplicate. Immersion treatments were administered at three time points: 12 h before wound induction, after wound induction, and on the fourth day post-injury. Each immersion exposure lasted for 1 h. Biological responses were evaluated using a zebrafish cutaneous wound model. Alginate oligosaccharide (AOS) treatments (350-800 ppm) accelerated early wound healing, with higher wound closure on day 4 (6.67 ± 2.89-8.33 ± 2.89%) compared to the control (0%). The highest closure was observed at 500 and 800 ppm, although no clear dose-dependent or statistically significant differences were detected. Complete wound closure occurred in all groups by day 21. Overall, the findings indicate that alginate oligosaccharide treatment was well tolerated and compatible with the wound-healing process, supporting its further evaluation as a marine-derived biomaterial.

1. Introduction

Wound healing remains a major challenge in regenerative medicine and biomedical science, particularly in the search for natural, biocompatible, and cost-effective materials that can support tissue repair while minimizing adverse effects (Deng *et al.*, 2022). In this context, marine-derived natural polymers have attracted increasing attention due to their sustainability and favorable biological properties. Alginate, a polysaccharide abundantly found in brown algae, is one of the most widely studied marine biopolymers and is well known for its biocompatibility, biodegradability, and suitability for wound-dressing applications and gel formulations with high exudate absorption capacity (Kuznetsova *et al.*, 2020).

Alginate oligosaccharides (AOS), which are produced through the depolymerization of alginate, have attracted interest as bioactive derivatives with improved solubility and biological functionality. Several studies have reported that AOS possess antioxidant and immunomodulatory activities, suggesting their potential role in supporting tissue repair (Xing *et al.*, 2020). These effects are commonly associated with the ability of AOS to reduce oxidative stress and modulate inflammatory responses, both of which are key factors influencing the early stages of wound healing.

Excessive production of reactive oxygen species (ROS) is known to impair wound healing by disrupting cellular migration and proliferation and by prolonging inflammation. For this reason, materials with antioxidant activity have been proposed as supportive agents to improve the wound microenvironment and facilitate tissue regeneration (Ukaegbu *et al.*, 2025; Fadilah *et al.*, 2023). Although the antioxidant properties of AOS derived from brown seaweeds, including *Sargassum* sp., have been well documented through in vitro assays, evidence of their biological effects in in vivo wound-healing models remains

limited. In particular, it is still unclear whether the antioxidant capacity of AOS directly contributes to observable wound-closure responses.

The zebrafish (*Danio rerio*) has emerged as a well-established in vivo model for cutaneous wound-healing studies due to its rapid regenerative capacity, ease of maintenance, and suitability for non-invasive morphological observation (Utami, 2018; Seo *et al*., 2017; Ali *et al*., 2021). A previous study by Seo *et al*. (2017) using zebrafish model to evaluate the effects of silver nanoparticles (AgNPs) on cutaneous wound healing using both immersion (50 mg/L) and direct application (2 mg/wound) treatments over a 20-day post-wounding period. The study reported accelerated wound closure in AgNP-treated groups compared to the control, with the immersion treatment showing the highest wound healing percentage at 5 days post-wounding (36.6%), followed by direct application (23.7%) and the control (18.2%), findings that were supported by histological observations. Importantly, immersion-based exposure provides a practical and biologically relevant approach for evaluating water-soluble bioactive compounds such as alginate oligosaccharides (AOS) in aquatic models. In adult zebrafish, cutaneous wound healing involves conserved processes, including re-epithelialization and granulation tissue formation, which closely resemble those observed in mammalian wound repair (Richardson *et al.,* 2013). However, studies examining the effects of immersion-administered AOS on wound healing in zebrafish are still scarce, particularly those linking antioxidant-related properties to morphological wound outcomes.

Therefore, this study investigates the effects of marine-derived AOS applied through immersion on cutaneous wound healing in zebrafish. By extending previous antioxidant characterization studies to an in vivo wound model, this work aims to provide preliminary insight into the biological compatibility and potential wound-healing relevance of AOS, which may support further mechanistic and applied research on marine-based biomaterials.

2. Material and methods

2.1 Preparation of alginate oligosaccharides (AOS)

Alginate oligosaccharides (AOS) used in this study were prepared from *Sargassum* sp. following previously published procedures, including alginate extraction (Yudiati *et al.*, 2018) and oxidative depolymerization (Chen *et al*., 2016), as reported in Rizfa *et al.* (2020). No modifications were applied, and AOS was used directly for the wound-healing experiments.

2.2 Fourier-Transform IR (FTIR) and UV-visible spectroscopic analysis

FTIR and UV-visible analyses were used to confirm the chemical characteristics of standard alginate and AOS, following the methods described in Rizfa *et al.* (2020). Detailed analytical conditions were not repeated, as no methodological changes were introduced.

2.3 Antiradical activity assay

The antiradical activity of AOS was evaluated using the DPPH assay exactly as described in Rizfa *et al.* (2020), based on the method of Chen *et al.* (2016). AOS samples (10, 20, 30, 40, 50, and 100 ppm) were reacted with 0.1 mM DPPH, and percentage inhibition was calculated from absorbance values. IC_{50} values were obtained by linear regression of inhibition percentage versus concentration, representing the concentration required to scavenge 50% of DPPH radicals.

2.4 Skin injury and AOS immersion

Adult zebrafish (*Danio rerio*), approximately four months old, were obtained from a local supplier and acclimated for five days in glass aquaria (50 × 40 × 35 cm) with continuous aeration. Fish were maintained under standard freshwater laboratory conditions at 28±1 °C, and pH 7.0–7.5, with a 12 h light/12 h dark photoperiod. During acclimation and throughout the 21-day experimental period, fish were fed a commercial diet three times daily at 4% of body weight (Seo *et al*., 2017).

The experiment used a randomized design with a single factor, namely AOS concentration. Four AOS concentrations (350, 500, 650, and 800 ppm) and a control were tested using a single immersion duration of 1 h. Each treatment group consisted of 20 fish. Fish were anesthetized using hypothermal shock (±10 °C) (Collymore *et al.*, 2014), and a standardized cutaneous wound (~0.5 cm diameter) was induced on the posterior skin near the lateral line. AOS immersion was applied 12 h prior to wounding, immediately after wounding, and on day 4 post-wounding. Fish were then maintained under standard conditions and observed for up to 21 days. Morphological wound closure was assessed at 2 h, day 4, and day 21 post-wounding using digital caliper measurements.

3. Results

3.1 Characterization of AOS derived from H_2O_2-ascorbic acid treatment

Alginate was extracted from *Sargassum* sp. collected from Awur Bay, Jepara. The extracted alginate appeared as a dark brown solid. Alginate extraction yielded an average recovery of 40.82 ± 2.166%, based on three independent extractions (Table 1).

To obtain alginate oligosaccharides (AOS), the extracted raw alginate was subjected to oxidative depolymerization using a combination of hydrogen peroxide and ascorbic acid. This treatment was applied to reduce the molecular size of alginate without altering its primary functional groups. The structural characteristics of the AOS were subsequently evaluated using Fourier Transform Infrared (FTIR) spectroscopy.

FTIR analysis revealed no additional peaks or changes in functional group composition between AOS and standard alginate. The characteristic alginate bands were observed at approximately 3400 cm^{-1} (–OH) and 1600–1400 cm^{-1} ($–COO^-$). (Figure 1 and Table 2).

Table 1. Percentage alginate yield

No.	Dried *Sargassum* sp. (g)	Alginate (g)	Mean (g)	Yield (%)	Mean (%)
1	20	7.82		39.10	
2	20	8.19	8.22±0.415	40.10	40.82±2.166
3	20	8.65		43.25	

Table 2. FTIR spectral features of standard alginate and AOS

Wavenumber (cm^{-1})	Functional group assignment	Standard alginate	AOS	Observed difference
~3431	O–H stretching (hydroxyl groups)	√	-	Characteristic hydroxyl band
~3419	O–H stretching vibration	-	√	Slight shift within O–H region
~1656	Asymmetric stretching of $–COO^–$	√	√	No change in functional group
~1437-1436	Symmetric stretching of $–COO^–$	√	√	Comparable peak position
~1315	C–OH stretching / C–C deformation	√	√	Typical alginate band
~1021	C–O–C stretching vibration	√	√	No additional peak detected
~953	O–H bending vibration	√	√	Minor variations in intensity
750-950	Fingerprint region of sodium alginate	Typical pattern	Typical pattern	Within sodium alginate range
~3400 (T%)	O–H band transmittance	~35%	~10%	Reduced transmittance after treatment

Note: FTIR data presented in this table are taken from the published work of Rizfa *et al.* (2020). No new FTIR characterization was performed in the present study.

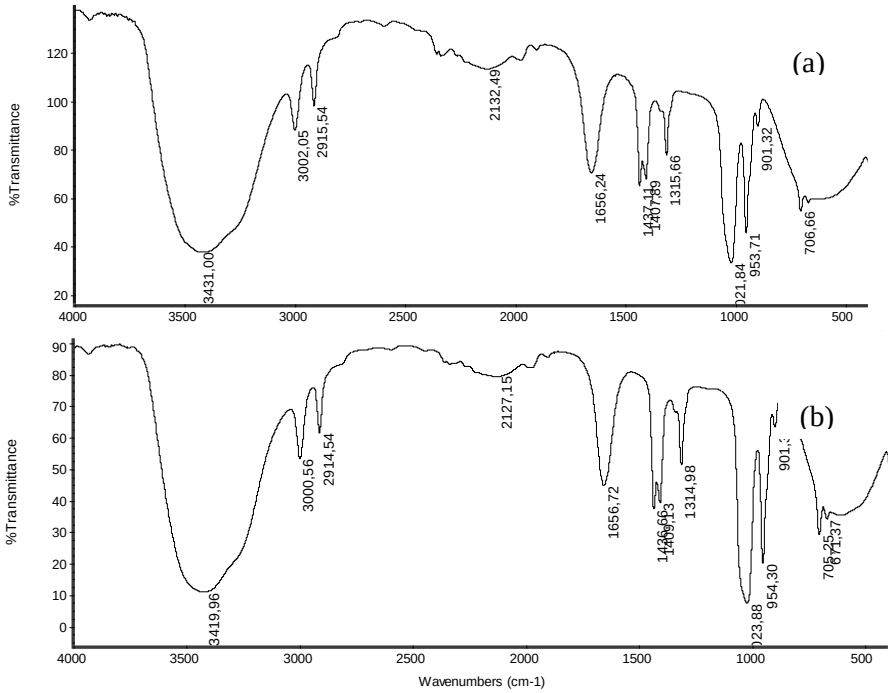

Figure 1. FTIR spectra of (a) standard alginate; and (b) alginate oligosaccahrides (AOS)

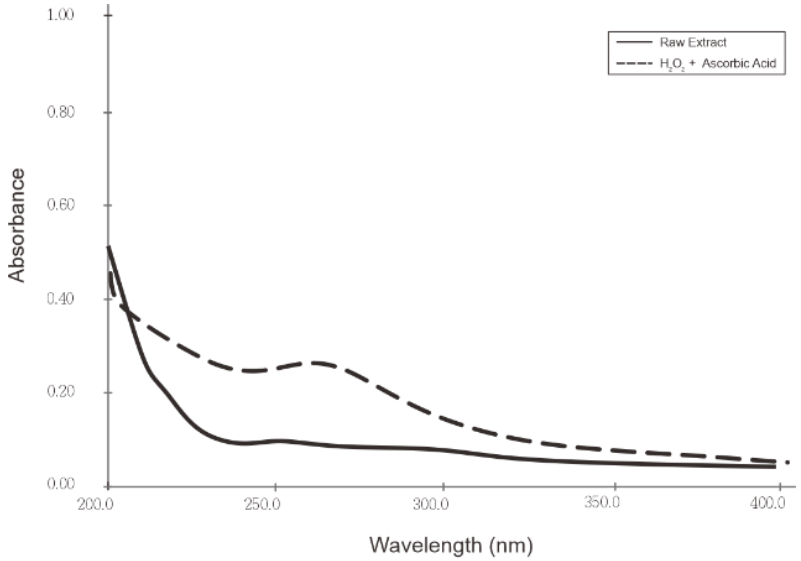

Figure 2. UV-Vis Spectrophotometric. (⟶) raw extract and (---) AOS.

Figure 1 shows the FTIR spectra of standard alginate and AOS. Both spectra exhibited broad O–H stretching bands at 3431 and 3419 cm^{-1}. Characteristic carboxylate ($–COO^-$) bands were observed at 1656 cm^{-1} (asymmetric) and 1437–1436 cm^{-1} (symmetric). Additional peaks at approximately 1315, 1021, and 953 cm^{-1} were detected, corresponding to C–OH stretching, C–O–C stretching, and O–H bending vibrations, respectively.

UV–Vis spectrophotometric analysis was performed following FTIR characterization to examine differences in the absorption characteristics of the alginate samples. Crude alginate exhibited a smooth absorption profile, whereas AOS showed a distinct absorption peak at approximately 250-260 nm, which was not observed in the crude extract.

3.2 DPPH radical scavenging activity

DPPH radical scavenging activity of AOS extracts was evaluated at concentrations of 10–100 ppm, with maximum absorbance observed at 516 nm. Antioxidant activity, expressed as IC_{50}, was determined by linear regression (y = 0.1489x + 52.447), with 53.71% inhibition observed at 10 ppm, followed by a gradual increase in antioxidant activity at higher concentrations up to 100 ppm. The antioxidant data were adopted from Rizfa *et al.* (2020).

3.3 Cutaneous wound healing

Wound healing in zebrafish was evaluated based on the percentage of wound closure and macroscopic morphology (Table 3). No wound closure was observed at 2 h post-injury in either the control or AOS groups. By day 4, partial wound closure was detected in AOS, with closure percentages of 5–10%, while no closure was observed in the control group. Complete wound closure (100%) was observed in all groups by day 21. Morphological observations showed open wounds at 2 h, early epithelialization at day 4, and complete closure with scar formation at day 21 in both control and treated groups. No signs of wound infection were observed throughout the 21-day observation period.

Table 3. Percentage of zebrafish wound closure (%)

Observation Time	Control (a)	Concentration (ppm)			
		350 (b)	500 (c)	650 (d)	800 (e)
2h post-injury	0	0	0	0	0
Day 4	0	6,67±2,89	8,33±2,89	6,67±2,89	8,33±2,89
Day 21	100	100	100	100	100

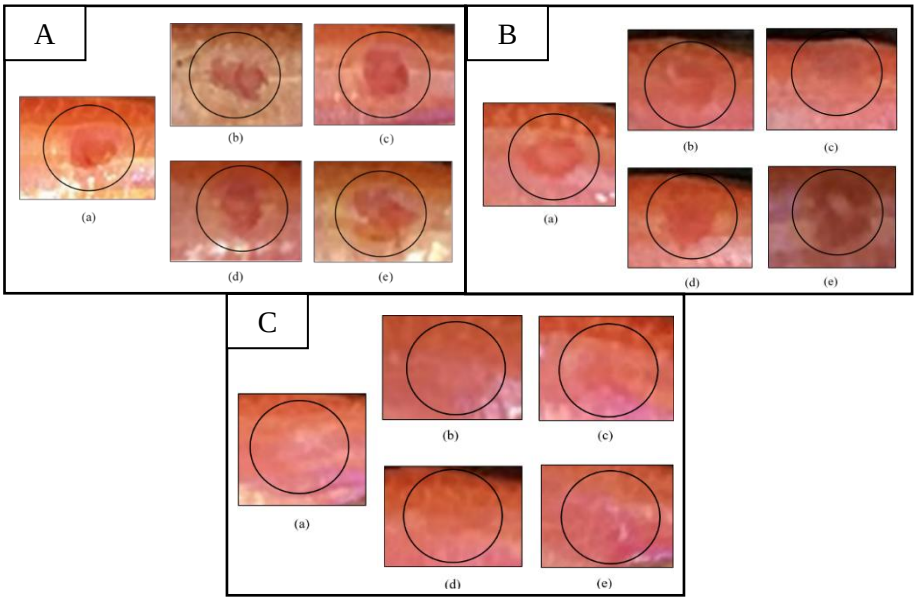

Figure 3. Morphological appearance of zebrafish cutaneous wounds (A) at 2 h, (B) 4 days, and (C) 21 days post-injury in the control group and AOS groups at different immersion concentrations: (a) control, (b) 350 ppm, (c) 500 ppm, (d) 650 ppm, and (e) 800 ppm.

4. Discussion

The alginate yield obtained from *Sargassum* sp. collected in Awur Bay reached 40.82 ± 2.166%, indicating an efficient extraction process. This yield is within the upper range reported for brown seaweeds, where alginate content generally varies depending on species, environmental conditions, and extraction methods. Recent studies have reported alginate yields ranging from approximately 20% to 40% using optimized alkaline or environmentally friendly extraction approaches, supporting the comparability of the present results (Rahardjo and Prasetyaningsih, 2018).

Oxidative depolymerization using a combination of hydrogen peroxide and ascorbic acid successfully produced alginate oligosaccharides while preserving the main functional groups of alginate, as indicated by FTIR analysis adopted from Rizfa *et al.* (2020). The retention of characteristic –OH and $–COO^-$ bands suggests that the treatment reduced molecular size without altering the primary chemical structure (Liu *et al*., 2019; Yao *et al*., 2023), a feature commonly reported for controlled oxidative depolymerization methods aimed at producing bioactive alginate derivatives.

Similar spectral features have been reported in recent studies on depolymerized polysaccharides and are generally associated with reduced polymer chain length and modified electronic transitions (Camacho-González *et al*., 2025).

Oxidative depolymerization of alginate has been reported to enhance antioxidant properties by increasing the availability of hydroxyl and carboxyl functional groups, which contribute to radical scavenging activity. Alginate oligosaccharides with reduced molecular weight commonly exhibit higher antioxidant capacity than native alginate due to greater chain flexibility and exposure of active sites (Liu *et*

al., 2019; Yao *et al.*, 2023). In this study, antioxidant data are referenced from earlier work and are provided for contextual comparison only.

In the zebrafish model, AOS immersion treatments resulted in earlier partial wound closure at day 4 compared to the control, although complete wound closure was observed in all groups by day 21. At 2 h post-injury, no wound closure was observed in either the control or AOS groups, indicating that the initial injury response was comparable across all treatments and that AOS immersion did not interfere with the acute phase of wound healing. The absence of infection and the comparable final closure suggest that AOS immersion did not adversely affect the wound healing process. Previous studies have shown that alginate-based materials can support wound environments by maintaining moisture balance and potentially mitigating oxidative stress (Bi *et al*., 2023); however, the present study focuses on descriptive morphological outcomes, and further investigations involving histological or molecular analyses would be required to clarify the underlying biological mechanisms.

5. Conclusions

This study presents preliminary descriptive evidence that alginate extracted from *Sargassum* sp. can be depolymerized into alginate oligosaccharides without altering its main functional groups. FTIR and UV-Vis analyses indicated polymer chain degradation following treatment, while zebrafish wound observations showed that AOS immersion was biocompatible and did not affect normal wound healing. Although earlier wound closure was observed in treated groups, complete healing occurred in all groups, indicating that the findings demonstrate compatibility rather than therapeutic efficacy. Further studies involving histological and molecular analyses are required to clarify the biological mechanisms and therapeutic potential.

Ethics approval

No permits were required.

Data availability statement

The findings of this study are based on a combination of primary and secondary data. The secondary data were previously published and are available in The data supporting the findings of this study have been published previously and are available in the Jurnal Kelautan Tropis under the following DOI: https://doi.org/10.14710/jkt.v23i3.8946

Author contributions

MSR contributed to the conceptualization of the study, data collection and curation, conducted the investigation, and prepared the original draft of the manuscript. EY contributed to funding acquisition, provision of research resources, and supervised the overall research process. DPW contributed to data validation, visualization, and provided scientific supervision. All authors contributed to manuscript revision and approved the final version.

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Declaration of competing Interest

None

References

Ali, M.S., Anuradha, V. and Yogananth, N., 2021. *Zebrafish-A model organism for regeneration studies* (Vol. 1). Darshan publishers.

Bi, D., Yang, X., Lu, J. and Xu, X., 2023. Preparation and potential applications of alginate oligosaccharides. *Critical reviews in food science and nutrition*, *63*(29): 10130-10147. https://doi.org/10.1080/10408398.2022.2067832.

Camacho-González, C.E., Cardona-Félix, C.S., Pérez-Larios, A., Sáyago-Ayerdi, S.G., Zamora-Gasga, V.M., López-García, U.M. and Sánchez-Burgos, J.A., 2025. Development and characterization of a novel biocomposite from alginate oligosaccharides loaded with endolysin LysPA26: Study of its physico-chemical properties and antibacterial activity. *Reactive and Functional Polymers*, 106386. https://doi.org/10.1016/j.reactfunctpolym.2025.106386.

Chen, B.J., Shi, M.J., Cui, S., Hao, S.X., Hider, R.C. and Zhou, T., 2016. Improved antioxidant and anti-tyrosinase activity of polysaccharide from *Sargassum fusiforme* by degradation. *International journal of biological macromolecules*, 92: 715-722. https://doi.org/10.1016/j.ijbiomac.2016.07.082.

Collymore, C., Tolwani, A., Lieggi, C. and Rasmussen, S., 2014. Efficacy and safety of 5 anesthetics in adult zebrafish (*Danio rerio*). *Journal of the American Association for Laboratory Animal Science*, 53(2): 198-203.

Deng, X., Gould, M. and Ali, M.A., 2022. A review of current advancements for wound healing: Biomaterial applications and medical devices. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 110(11): 2542-2573. https://doi.org/10.1002/jbm.b.35086.

Fadilah, N.I.M., Phang, S.J., Kamaruzaman, N., Salleh, A., Zawani, M., Sanyal, A., Maarof, M. and Fauzi, M.B., 2023. Antioxidant biomaterials in cutaneous wound healing and tissue regeneration: A critical review. *Antioxidants*, 12(4): 787. https://doi.org/10.3390/antiox12040787.

Liu, J., Yang, S., Li, X., Yan, Q., Reaney, M.J. and Jiang, Z., 2019. Alginate oligosaccharides: Production, biological activities, and potential applications. *Comprehensive Reviews in Food Science and Food Safety*, 18(6): 1859-1881. https://doi.org/10.1111/1541-4337.12494.

Hidayati, J.R., Yudiati, E., Pringgenies, D., Mansur, Z.M.B. and Idris, F., 2021. Antioxidant activity of Alginate Oligosaccharides (AOS) from *Sargassum* sp. for Improving the Cutaneous Wound Enclosure in Zebrafish (Danio rerio). *Jurnal Kelautan Tropis*, 24(3): 385-392. https://doi.org/10.14710/jkt.v24i3.12362.

Kuznetsova, T.A., Andryukov, B.G., Besednova, N.N., Zaporozhets, T.S. and Kalinin, A.V., 2020. Marine algae polysaccharides as basis for wound dressings, drug delivery, and tissue engineering: A review. *Journal of Marine Science and*

Engineering, 8(7): 481. https://doi.org/10.3390/jmse8070481.

Ukaegbu, K., Allen, E. and Svoboda, K.K., 2025. Reactive Oxygen Species and Antioxidants in Wound Healing: Mechanisms and Therapeutic Potential. *International Wound Journal*, *22*(5), 70330. https://doi.org/10.1111/iwj.70330.

Prastanto, H., 2014. Depolimerisasi karet alam secara mekanis untuk bahan aditif aspal. *Jurnal Penelitian Karet*, 32(1): 81-87. https://doi.org/10.22302/ppk.jpk.v32i1.154.

Rahardjo, D. and Prasetyaningsih, A., 2018. Keanekaragaman Spesies dan Kandungan Alginat Sargassum Pantai Sepanjang dan Drini Kabupaten Gunungkidul. *Seminar Nasional Biologi dan Pendidikan Biologi UKSW*, 188-196.

Richardson, R., Slanchev, K., Kraus, C., Knyphausen, P., Eming, S. and Hammerschmidt, M., 2013. Adult zebrafish as a model system for cutaneous wound-healing research. *Journal of Investigative Dermatology*, 133(6): 1655-1665. https://doi.org/10.1038/jid.2013.16,

Rizfa, M.S., Yudiati, E. and Wijayanti, D.P., 2020. Improving the antioxidant activity of sodium alginate from *Sargassum* sp. by thermal heating and chemical methods. *Jurnal Kelautan Tropis*, 23(3): 284-290. https://doi.org/10.14710/jkt.v23i3.8946.

Seo, S.B., Dananjaya, S.H.S., Nikapitiya, C., Park, B.K., Gooneratne, R., Kim, T.Y., Lee, J., Kim, C.H. and De Zoysa, M., 2017. Silver nanoparticles enhance wound healing in zebrafish (*Danio rerio*). *Fish & shellfish immunology*, 68: 536-545. http://dx.doi.org/10.1016/j.fsi.2017.07.057.

Utami, N., 2018. Zebrafish (*Danio rerio*) sebagai hewan model diabetes mellitus. *Biotrends*, 9(1): 15-19.

Xing, M., Cao, Q., Wang, Y., Xiao, H., Zhao, J., Zhang, Q., Ji, A. and Song, S., 2020. Advances in research on the bioactivity of alginate oligosaccharides. *Marine drugs*, 18(3): 144. https://doi.org/10.3390/md18030144.

Yao, Z.A., Xu, L., Wang, B.X., Ye, T.T., Li, Y.F. and Wu, H.G., 2023. Optimization of preparation conditions, molecular structure analysis and antitumor activity of sulfated sodium alginate oligosaccharides. *European Polymer Journal*, 201: 112571. https://doi.org/10.1016/j.eurpolymj.2023.112571.

Yitnosumarto, S. 1990. Percobaan perancangan, analisis, dan interpretasinya. PT Gramedia Pustaka Utama, Jakarta.

Yudiati, E., Pringgenies, D., Djunaedi, A., Arifin, Z. and Sudaryono, A., 2018. Free radicals scavenging activities of low molecular weight sodium alginate (LMWSA) from Sargassum polycystum, produced by thermal treatment. *Aquacultura Indonesiana*, 19(1): 21-27.