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Antibacterial Activity of Polysaccharides and Oligosaccharides Extracted from *Sargassum* sp. against *Vibrio* Species

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Abstract

The aquaculture industry's rapid expansion faces significant challenges in bacterial disease management, particularly infections caused by *Vibrio* species, including *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio harveyi*. Excessive use of antibiotics has led to antibiotic-resistant strains, prompting the search for sustainable alternatives. Polysaccharides and oligosaccharides from marine brown algae, such as *Sargassum sp.*, exhibit promising antibacterial properties. This study investigated the antibacterial activity of polysaccharides and oligosaccharides extracted from *Sargassum sp.* against the three *Vibrio* species. Two extraction methods, stirring and aeration, were compared to evaluate their effects on antibacterial efficacy. Extraction using maximum stirring (100%) produced polysaccharides with the highest antibacterial activity, with inhibition zones of 9.33 ± 1.57 mm, 11.75 ± 2.85 mm, and 12.96 ± 3.62 mm against *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi*, respectively. Similarly, oligosaccharides extracted with 100% stirring showed inhibition zones of 8.38 ± 0.34 mm against *V. parahaemolyticus* and 10.25 ± 0.09 mm against *V. harveyi*. In contrast, aeration-based extractions showed limited antibacterial activity under all conditions tested. The superior efficacy of stirring is attributed to enhanced release of bioactive compounds from the seaweed matrix. These findings highlight the importance of optimizing extraction techniques to enhance the bioactivity of marine-derived compounds. Polysaccharides and oligosaccharides from *Sargassum sp.* present a sustainable, natural alternative to antibiotics for disease control in aquaculture. This research contributes to the development of environmentally friendly strategies that improve productivity while reducing the risks associated with antibiotic resistance.

1. Introduction

The rapid expansion of aquaculture has led to challenges in disease management, particularly bacterial infections caused by *Vibrio* species, such as *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio harveyi* (Manchanayake *et al.*, 2023; Mishra *et al.*, 2024). These pathogens are responsible for significant mortality in shrimp and fish, causing economic losses in the aquaculture industry (de Souza Valente and Wan, 2021; Harrison *et al.*, 2022; Manchanayake *et al.*, 2023). Traditionally, antibiotics have been used to control bacterial infections, but overuse has resulted in the emergence of antibiotic-resistant strains, posing a threat to both ecosystems and human health (Samreen *et al.*, 2021; Arifin and Rahman, 2023; Heriyati and Larasati, 2023; Naryaningsih and Akhmad, 2023). This has led to a growing interest in alternative, sustainable solutions, including the use of natural antimicrobial agents (Liu *et al.*, 2023).

Marine algae, particularly brown algae like *Sargassum sp.*, are rich in bioactive compounds, including polysaccharides, that exhibit antimicrobial properties (Menaa *et al.*, 2021; Flores-Contreras *et al.*, 2023). These polysaccharides have shown effectiveness against a variety of bacterial pathogens, including *Vibrio* species (Yudiati *et al.*, 2022). The extraction method used plays a significant role in determining the bioactivity of these compounds (El-Sheekh *et al.*, 2024), with techniques such as aeration and stirring influencing both the yield and antibacterial potency.

Oligosaccharides, derived from the hydrolysis of polysaccharides, have gained attention due to their smaller

molecular size and enhanced biological activity (Yudiati *et al.*, 2018a). Recent studies suggest that oligosaccharides from marine algae have strong antibacterial properties, making them a promising alternative to synthetic antibiotics in aquaculture (Yudiati *et al.*, 2022; Liu *et al.*, 2023).

This study aims to evaluate the antibacterial activity of polysaccharides and oligosaccharides extracted from *Sargassum sp.* against *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi*. Two extraction methods, aeration and stirring, were compared to determine their effects on the antibacterial activity of the compounds. The findings of this study will provide insights into optimizing extraction techniques for maximizing the antimicrobial efficacy of *Sargassum sp.*-derived compounds, contributing to the development of sustainable, non-antibiotic-based disease control strategies in aquaculture.

By exploring the potential of marine-derived polysaccharides and oligosaccharides, this research supports the growing demand for natural alternatives to antibiotics in aquaculture, which could help improve the sustainability and productivity of the industry while minimizing environmental and health risks.

2. Material and methods

2.1 Materials and Equipment

The materials used in this study include polysaccharides and oligosaccharides extracted from the brown seaweed *Sargassum* sp. This seaweed was selected based on its availability and potential polysaccharide content with antibacterial properties. The raw material, dried seaweed, was ground into fine powder to facilitate the extraction process.

The equipment used in the study included an extraction vessel for dissolving the raw material in distilled water during extraction, a stirrer for mixing the extract, and aeration equipment to provide varying aeration conditions. A pH meter was utilized to monitor and maintain optimal pH during extraction. A spectrophotometer was used for quantitative analysis of active compounds in the extract. Additionally, agar media such as TCBSA (Thiosulfate Citrate Bile Salts Sucrose Agar) was employed for bacterial inoculation, and saline solutions were prepared for bacterial tests.

2.2 Polysaccharide and Oligosaccharide Extraction

Two different methods were employed to evaluate the effect of extraction techniques on yield: aeration and stirring. The powdered seaweed was placed in an extraction vessel and dissolved in distilled water as the solvent. The alginate production process begins with washing and drying the brown seaweed (*Sargassum* sp.) using an air conditioner (AC) for two days. Once dried, the seaweed is cut into smaller pieces to facilitate easier blending during the grinding process. To produce 500 mL of alginate, 500 mL of distilled water is prepared as the solvent. The procedure involves weighing 25 g of sodium carbonate (Na_2CO_3), 20 g of dried *Sargassum* sp., and hydrochloric acid (HCl) in appropriate amounts to adjust the pH to 8.5. These components are combined in an Erlenmeyer flask with a stirrer. The flask is then placed on a magnetic stirrer with a heater and allowed to mix and react continuously for 24 hours (one day) (Yudiati and Isnansetyo, 2017; Yudiati *et al.*, 2018b). Oligosaccharides are derived by heating the sodium alginate for 4.5 hours at a temperature of 145°C. This process facilitates the breakdown of alginate molecules into oligosaccharides, which can later be used for further testing or applications (Yudiati *et al.*, 2018a).

For the aeration method, three aeration conditions were applied: 0% aeration (no aeration), 50% aeration (moderate aeration), and 100% aeration (full aeration). In the stirring method, three stirring conditions were used: 0% stirring (no stirring), 50% stirring (moderate stirring), and 100% stirring (maximum stirring).

The extraction process was conducted at room temperature for 24 hours, with continuous agitation in each treatment. After extraction, the solution was filtered to separate solids from the liquid extract. The liquid extract was then dried to obtain polysaccharides and oligosaccharides, which were prepared for further testing.

2.3 Bacterial Preparation

The bacteria used in this study included *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio harveyi*, which are primary pathogens in aquaculture. The bacteria were isolated from relevant sources and cultured on TCBSA agar media, providing essential nutrients for bacterial growth. After successful culturing, the bacteria were transferred to saline solution to achieve an inoculum concentration of 10^8 CFU/mL, which was calculated using the colony count method after diluting the bacterial suspension with saline solution (Yudiati *et al.*, 2022; Azhar and Yudiati, 2023).

2.4 Antibacterial Activity Test

The antibacterial activity of polysaccharide and oligosaccharide extracts was evaluated using the disk diffusion method. Sterile paper disks were impregnated with the prepared extracts and placed on the surface of agar media inoculated with bacterial suspensions. Each disk was placed on agar media inoculated with *V. parahaemolyticus*, *V. vulnificus*, and *V. harveyi* and incubated at 37°C for 24 hours. After the incubation period, the diameter of the inhibition zones was measured using a caliper. Larger inhibition zones indicated stronger antibacterial activity. Each treatment was performed in triplicate to ensure accurate and reliable results (Bauer *et al.*, 1966; Hudzicki, 2009; Benhabiles *et al.*, 2012; Yudiati *et al.*, 2022).

2.5 Data Analysis

Data obtained from antibacterial tests were analyzed using descriptive statistical methods. The mean and standard deviation were calculated for each extraction method (aeration and stirring) and bacterial strain tested. The results of antibacterial tests were compared based on the diameter of the inhibition zones to determine the antibacterial effectiveness of polysaccharide and oligosaccharide extracts obtained under various extraction conditions. All data were presented in tables and graphs for easy interpretation.

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%, 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.

3.1 Polysaccharides

In the antibacterial activity test of polysaccharide extracts, the extraction condition using 100% stirrer produced the highest antibacterial activity against all three Vibrio species tested: *Vibrio parahaemolyticus*, *Vibrio vulnificus*,

and *Vibrio harveyi* (Figure 1). The polysaccharide extract obtained under maximum stirring (100% stirrer) showed significant antibacterial effects against these bacteria, with inhibition zone diameters as follows: 9.33 ± 1.57 mm against *V. parahaemolyticus*, 11.75 ± 2.85 mm against *V. vulnificus*, and 12.96 ± 3.62 mm against *V. harveyi*.

The extraction condition using 100% stirrer was proven to be more effective compared to other extraction methods, including aeration and lower stirring levels (0% and 50%). In the aeration extraction conditions, whether at 0%, 50%, or 100%, no antibacterial activity was detected against several bacterial species tested. This indicates that the stirring method with maximum agitation yields more consistent and significant results in producing polysaccharide extracts with higher antibacterial activity.

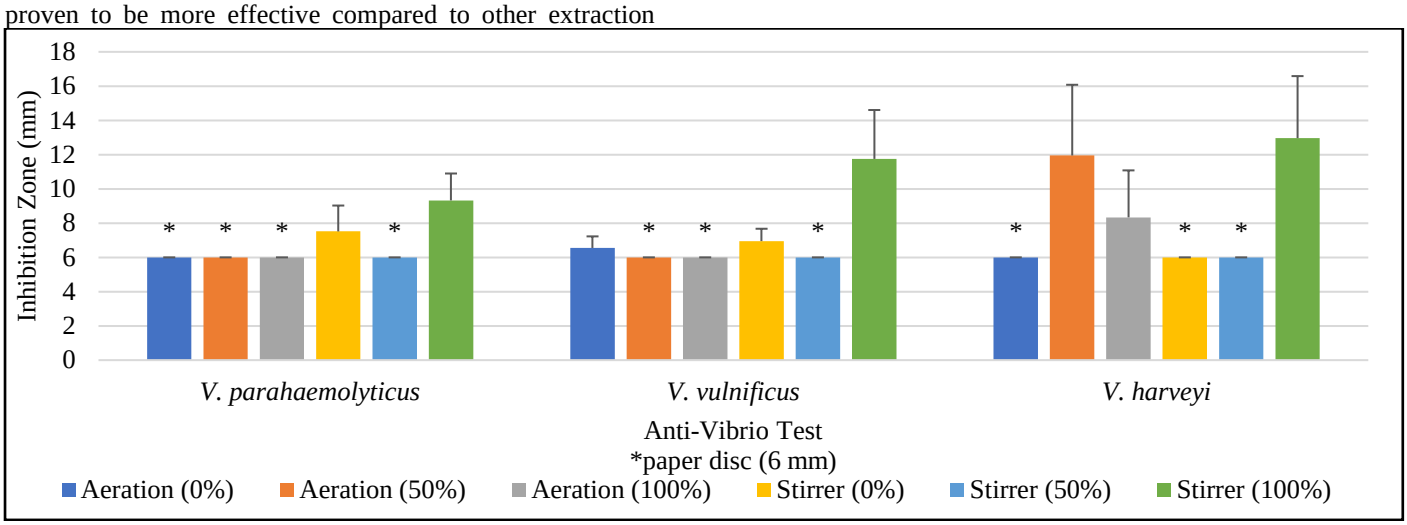

Figure 1. Antibacterial activity test of polysaccharide extracts

3.2 Oligosaccharides

In the antibacterial activity test of oligosaccharide extracts (Figure 2), the extraction condition using 100% stirrer produced the highest antibacterial activity. Oligosaccharide extracts obtained under this condition exhibited antibacterial effects against *Vibrio parahaemolyticus* with an inhibition zone of 8.38 ± 0.34 mm and against *Vibrio harveyi* with a zone of 10.25 ± 0.09 mm. However, these oligosaccharide extracts did not show significant antibacterial activity against *Vibrio vulnificus*, as no inhibition zone was observed for this species.

The extraction condition using 100% stirrer yielded higher results compared to aeration conditions of 0%, 50%, and 100%, where the oligosaccharide extracts failed to show antibacterial activity against certain bacterial species, including *V. vulnificus* and *V. parahaemolyticus* under some aeration conditions. This demonstrates that the stirring method with maximum agitation is more effective in producing oligosaccharide extracts with antibacterial potential, particularly against *V. parahaemolyticus* and *V. harveyi*.

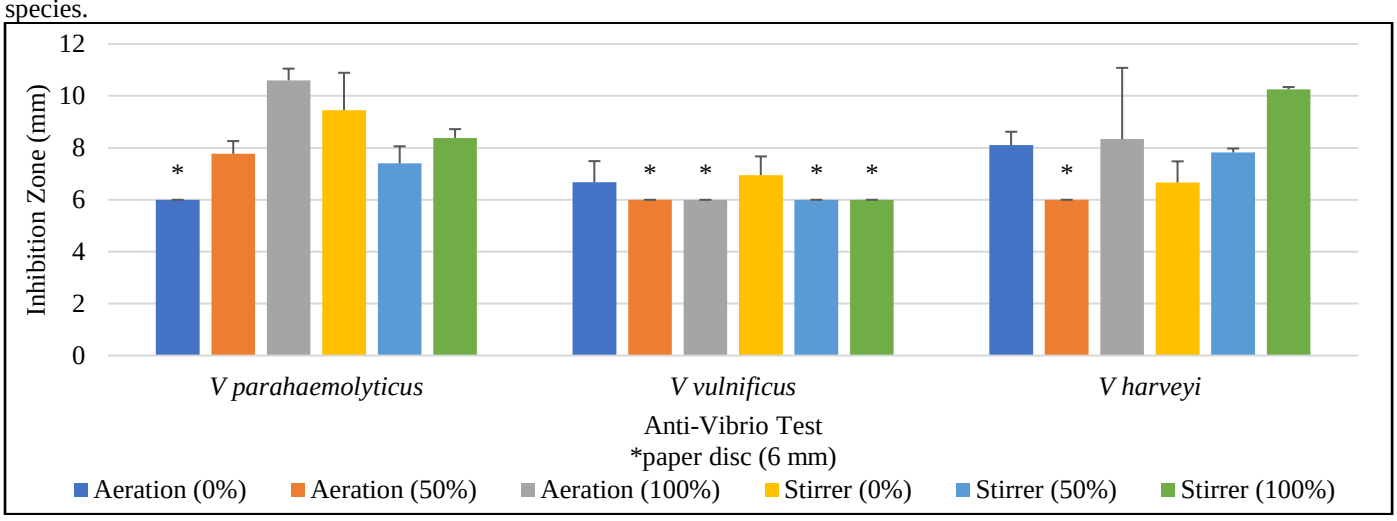

Figure 2. Antibacterial activity test of oligosaccharide extracts

4. Discussion

The chemical composition of algae and their antimicrobial activities vary based on species, physiological status, thallus region, environmental factors (climate, location, salinity, temperature), pollution, growth conditions, collection time, and associated epiphytic organisms (Menaa *et al.*, 2020; Khan *et al.*, 2024). The antibacterial activity of polysaccharide extracts obtained through maximum stirring (100% stirrer) demonstrated significant results against three *Vibrio* species: *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Vibrio harveyi*, with inhibition zone diameters of 9.33 ± 1.57 mm, 11.75 ± 2.85 mm, and 12.96 ± 3.62 mm, respectively. This efficacy indicates that maximal stirring facilitates the release of active polysaccharide components from the seaweed matrix during the extraction process.

Optimizing extraction parameters, such as alkali concentration, temperature, and time, is critical for maximizing the yield and quality of polysaccharides like alginate. Polysaccharides derived from brown seaweed are known to exhibit strong antibacterial properties (El-Sheekh *et al.*, 2024). Their antimicrobial activity depends on various factors, including their distribution, molecular weight, charge density, sulfate content (in sulfated polysaccharides), and structural and conformational characteristics. Additionally,

oligosaccharides obtained through the depolymerization of seaweed polysaccharides provide protection against viral, fungal, and bacterial infections in plants (Viera *et al.*, 2019).

Oligosaccharide extracts produced under 100% stirring conditions exhibited significant antibacterial activity against *V. parahaemolyticus* (inhibition zone: 8.38 ± 0.34 mm) and *V. harveyi* (10.25 ± 0.09 mm) but showed no activity against *V. vulnificus*. Oligosaccharides have a simpler molecular structure than polysaccharides, which may result in different or less effective mechanisms against certain bacteria, such as *V. vulnificus*. Oligosaccharides derived from renewable resources exhibit antimicrobial and prebiotic effects, offering broad-spectrum antibacterial properties through mechanisms like pathogen cell wall destruction and reduced adhesion of harmful microorganisms. They also promote the growth of probiotics, making them promising candidates for the food processing industry (Liu *et al.*, 2023).

The superior antibacterial effects observed under stirring conditions compared to aeration may be attributed to the suboptimal solvent distribution achieved by aeration, which reduces polysaccharide release, whereas uniform heating during stirring ensures better extraction. Research by Salem *et al.* (2019) demonstrated that microwave heating for the condensation of ADA with *o*-phenylenediamine analogs provided higher yields and significantly shorter reaction times than stirring at room temperature. This Schiff-base formation process followed a proposed reaction mechanism, with synthesized products characterized using FTIR and UV spectroscopy. Antimicrobial activity evaluation of these products, along with alginate and precursor amines, highlighted their potential against pathogenic fungi and bacterial species, emphasizing their applicability in antimicrobial treatments.

5. Conclusions

The 100% stirrer extraction method produced polysaccharide and oligosaccharide extracts with higher antibacterial activity compared to the aeration method. Polysaccharide extracts were effective against all three *Vibrio* species, while oligosaccharides exhibited more selective antibacterial activity against *Vibrio parahaemolyticus* and *Vibrio harveyi*.

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

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

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Declaration of competing Interest

None

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