Extract
2026, Vol. 1, No. 1, pp. 7–15

Kognia Prisma Pustaka

Original Article

Molecular Analysis of *Vibrio parahaemolyticus* Based on 16S rRNA GENE Markers and Its Inhibition by *Sargassum aquifolium* Extract

Jatu Maranatha Fimaputra[1], Putu Angga Wiradana[1], Anak Agung Ayu Putri Permatasari[1]*, I Gede Widhiantara[1], Putu Eka Sudaryatma[2], Muhammad Khaliim Jati Kusala[3], Mochammad Aqilah Herdiansyah[4], I Putu Sugiana[5]

[1] Study Program of Biology, Faculty of Health and Science, Universitas Dhyana Pura, Badung, Bali, Indonesia
[2] Badan Pengendalian dan Pengawasan Mutu Hasil Kelautan dan Perikanan (BPPMHKP), Kementerian Kelautan dan Perikanan Republik Indonesia (KKP), Badung, Bali, Indonesia
[3] Research Center of Veteriner, Health Research Organization, Badan Riset dan Inovasi Nasional (BRIN), Cibinong, Jawa Barat, Indonesia
[4] Departement of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Jawa Timur, Indonesia
[5] Pusat Penelitian Lingkungan Hidup (PPLH), Universitas Udayana, Denpasar, Provinsi Bali, Indonesia

ORCID

0000-0002-0139-8781 (Putu Angga Wiradana), 0000-0002-3608-2779 (Anak Agung Ayu Putri Permatasari), 0000-0003-0498-525X (I Gede Widhiantara), 0000-0002-7613-721X (Muhammad Khaliim Jati Kusala), 0009-0006-9383-6203 (Mochammad Aqilah Herdiansyah).

Abstract

Vibriosis is a bacterial disease caused by *Vibrio* spp. that infects pacific white shrimp (*Litopenaeus vannamei*). Brown algae (*Sargassum aquifolium*) is an abundant marine biota found on the coast of Bali, Indonesia. The purpose of this study was to identify *Vibrio* sp. isolates from the hepatopancreas of pacific white shrimp and inhibit their growth using *S. aquifolium* extract. *Vibrio* sp. isolates were isolated from the hepatopancreas of whiteleg shrimp and grown in selective TCBS agar media. Molecular identification includes DNA extraction, amplification, sequencing, and phylogenetic tree construction. Extraction was carried out by maceration with 70% ethanol solvent. Inhibitory power test using the well diffusion method and MIC by measuring turbidity of liquid media with a UV-Vis spectrophotometer. The results identified the Vibrio sp. isolates as *Vibrio parahemolyticus*. At 100% concentration, *S. aquifolium* extract achieved the highest inhibition average of 29.35 ± 1.2 mm, significantly different from other concentrations ($p \leq 0.05$). The MIC of *S. aquifolium* extract has an absorbance value of 0.811±0.055 at 100% concentration, which indicates good antibiotic potential at the highest concentration. That information is useful for further research to evaluate the possibility of *S. aquifolium* extract as an antibacterial agent for controlling *V. parahaemolyticus* infection in pacific white shrimp.

Keywords

Antimicrobial, Brown algae, *Litopenaeus vannamei, Sargassum aquifolium*, Vibrosis.

*Correspondence: Anak Agung Ayu Putri Permatasari

Email addresses:

putripermatasari@undhirabali.ac.id

Received: 20-08-2025; **Accepted:** 25-03-2026; **Published:** 07-04-2026

1. Introduction

Vaname shrimp (*Litopenaues vannamei*) is one of the fisheries sector commodities that has the potential to improve the economy in Indonesia (Scabra et al., 2023). In addition to having a high survival rate, vaname shrimp are also able to adapt to environments that have low temperatures and salinity levels, thus providing a great opportunity to increase the amount of production and development of vaname shrimp farming systems (Hadi et al., 2018). Based on data from the Ministry of Marine Affairs and Fisheries, vaname shrimp production in Indonesia in 2021 reached 953,177 tons, in 2022 increased by 15% to 1,099,976 tons and in 2023 to 1,829,000 tons. This shows that vaname shrimp farming has a major contribution to the fisheries economy in Indonesia because it has high value and market opportunities (Natalia & Nurozy, 2014).

However, the presence of bacterial diseases that attack vaname shrimp has resulted in a decrease in the quality and quantity of vaname shrimp production in Indonesia (Iskandar et al., 2022). Based on data obtained from the Ministry of Marine Affairs and Fisheries (KKP), vaname shrimp production in 2018 decreased from 757,793 tons to 717,094 tons (KKP, 2018). One bacterial disease that often attacks vaname shrimp commodities is vibriosis disease caused by *Vibrio parahaemolyticus* bacteria (Sani et al., 2020). The bacteria are Gram-negative bacteria that are pathogenic with a 100% mortality rate in larvae and adult shrimp (Roza et al., 2017). Clinical symptoms experienced by shrimp that have been infected with these bacteria, namely decreased appetite, look weak, pale in color, melanosis of the skin, necrosis of the tail and the appearance of red spots on the body (Sarjito et al., 2015). This is a serious problem for vaname shrimp farmers because it affects the success rate and sustainability of the farming system.

In general, the treatment of vibriosis disease in vaname shrimp is the administration of synthetic antibiotics, such as chloramphenicol, oxytetracycline, and furazolidone (Aziza & Chaidir, 2024). However, the use of these drugs is very dangerous because it can cause residues in shrimp meat if consumed, then these residues will accumulate in the human body which causes multidrug resistance (Sari et al., 2023). Therefore, efforts to deal with vibriosis disease in vaname shrimp require other alternatives to reduce the use of synthetic antibiotics. One alternative that has minimal side effects is the utilization of natural materials, one of which is macroalgae as a natural antibacterial agent. *Sargassum aquifolium* is one type of brown algae that has potential as an antibacterial because it contains secondary metabolites that are antibacterial (Permatasari et al., 2022a).

Based on research conducted by Pakidi and Suwoyo, (2017) *S. aquifolium* contains secondary metabolites that act as antimicrobials, such as alkaloids, phenols, terpenoids, and steroids. Furthermore, research conducted by Alamsyah et al. (2014) stated that *S. aquifolium* extract processed with ethyl acetate and methanol solvents showed antibacterial activity against *Escherichia coli* and *Staphylococcus epidermidis*. Furthermore, based on research conducted by Yanti et al. (2024), showed the results of phytoconstituents of *Euchema cottoni* red macroalgae extracts that have Hexadecanoic acid and ethyl ester compounds that have potential as antibacterials. Research conducted by Sinurat et al., (2019) reported that the methanol extract of *Gracilaria edulis* seaweed has potential as an antibacterial against pathogenic bacteria *Aeromonas hydrophila*. Research conducted by Alamsyah et al., (2014) reported that *Sargassum cinereum* extract extracted using n-hexane and methanol solvents has antibacterial activity against *Staphylococcus epidermidis* and *Escherichia coli* bacteria.

Based on previous research, there is limited or no research on the specific antibacterial activity of *S. aquifolium* extract against *V. parahaemolyticus* in the context of vaname shrimp aquaculture. The leap from general antibacterial properties to the specific application against vibriosis in shrimp is not clearly established. Thus, it is necessary to conduct further research on the effectiveness of ethanol extract of *S. aquifolium* as a natural antibiotic in controlling vibriosis disease in vaname shrimp farming. This study also conducted molecular identification of Vibrio sp. isolates isolated from vaname shrimp cultivated with super-intensive systems based on 16s rRNA gene markers.

2. Methods

2.1. Materials

Materials used include *S. aquifolium*, *V. parahaemolyticus* bacterial isolate, Phosphate Buffer Saline (PBS), 16S rRNA Primer set, Proteinase K, Gel Solubilization Buffer (GSB), wash buffer, agarose, Tris Borate EDTA, 100bp marker, 70% ethanol, aquadest, Tryptone Soy Agar (TSA), Tryptic Soy Broth (TSB), chloramphenicol, and denatured alcohol.

2.2. Isolation and identification of Vibrio sp. isolate

The *Vibrio* sp. isolate used in this study is the result of isolation from *Vibriosis* positive vaname shrimp cultivated with a super-intensive system. *Vibrio* sp. isolates were isolated from the hepatopancreas of vaname shrimp in accordance with research from Wiradana et al., (2022). In short, the hepatopancreas of vaname shrimp suspected of being infected with *Vibriosis* was weighed as much as 10 grams aseptically and diluted in 90 ml of sterile water, vortexed. Samples were then pipetted as much as 1 ml using a micropipette and cultured on sterile Petri dishes that had been added with Tryptic Soy Agar (TSA) media and homogenized. Petri dishes were then incubated at 37℃ for 24 hours. Bacterial colonies that successfully grew were then purified by taking one loop of colonies using a sterile ose and streaked on TCBS Agar media which is a selective medium, then the isolates were incubated at 37℃ for 24 hours. The growing bacterial colonies will show a bluish-green color which is suspected to be *V. parahaemolyticus*. The growing colonies were observed microscopically by Gram staining and one loop of colony was taken and transferred to a 2 ml microtube that had been added with sterile distilled water to be used for DNA extraction.

2.3. Extraction, amplification, and sequencing of DNA

DNA extraction was performed using the non-organic method with the Genedirex One PCR kit, following the manufacturer's protocol. In short, 200 µl of *Vibrio* sp. bacterial isolate was pipetted into a 1.5 ml microcentrifuge tube containing 200 µl Phosphate Buffer Saline (PBS), then 20 µl Proteinase K was added. Homogenized by pipetting technique, then incubated at 600C for five minutes on a water bath. Cell lysis was performed by adding 200 µl Gel Solubilization Buffer (GSB), then vortexed until homogeneous and incubated again at the same temperature for 2 minutes. Absolute ethanol (96%) was added and vortexed for 10 seconds. Transferred all the mixture into a spin column, centrifuged at 14,000 ×g for 1 minute and removed the collection tube under the spin column and replaced with a new collection tube.

Washing was done by adding 400µl of buffer W1 and then centrifuged for 30 seconds at the same speed then discarded the liquid contained in the collection tube. 600µl wash buffer (Geneid) was added, centrifuged for 30 seconds, then removed the liquid in the collection tube and centrifuged again for 3 minutes. After that, the collection tube was removed and a sterile microcentrifuge was placed at the bottom of the spin column.

Amplification was performed using a PCR machine (DNA thermal Cycler). For PCR amplification, the initial stage of denaturation at 95 ºC for 15 minutes, then 94 ºC for 1 minute. Next, annealing at 55 ºC for 30 seconds, extension 72 ºC for 1 minute for 40 cycles, followed by a final extension temperature of 72 ºC for 5 minutes and 12 ºC ± 30 minutes for storage. The primer types used were forward primer 27f with the sequence 5'AGAGTTTGATCMTGGCTCAG-3' and 1492r with the sequence 5'GGTTACCTTGTTACGGACTT-3'.

The electrophoresis process uses a gel with a concentration of 2% of 0.8 g agarose, mixed with 100 ml, 10 Tris Borate EDTA (100 grams Tris base, 27.5 grams boric acid, 20 ml 0.5 M EDTA pH 8.0 in 1 l water). Then heated to boiling and dissolved. Next, 1 µl of ethidium bromide (0.2 µg/ml) was added and placed in a gel printer that had been fitted with a comb. After agarose solidifies (about 15 minutes), then put into the electrophoresis tank containing 0.5% TBE solution. Entered DNA products that have been mixed with liquid "loading dye" into the wells in a ratio of 2: 1, then entered the 100bp marker after the entire sample is entered. The electrode was connected to the power supply and then turned on for 1 hour. After that, the electrophoresis device was turned off and the gel from the device was taken. The gel was transferred into a UV transilluminator and then the results were observed on a computer. Sequencing in this study was carried out by sending the purified results of PCR products to Genetics Science, Indonesia.

2.4. Extraction of *S. aquifolium*

The samples used in this study were obtained from the Sindhu Beach Waters, Denpasar, Bali at the lowest low tide in the intertidal zone (Rosiana et al., 2022). The selection of the location was based on the abundance and presence of *S. aquifolium* at that location in accordance with previous studies (Permatasari et al., 2022; Yanti et al., 2024). Samples of *S. aquifolium* obtained were collected in sterile plastic clips and washed thoroughly with running water, then dried until there was no more water attached to the sample. The samples were then oven dried at 50ºC for 2 × 24 hours (Alamsyah et al., 2014). The first step of making extracts, namely making powdered simplisia. Samples that have been dried, then blended to get smaller pieces. Next, the sample is continued to be ground until smooth, then sieved to produce a finer simplisia powder (Hidayah et al., 2017).

S. aquifolium simplisia powder was weighed as much as 250 g, then 500 ml of 70% ethanol was added and stirred until homogeneous. During the extraction process, the sample was stirred periodically every day for 3 × 24 hours. After that, the extract was then filtered using sterile filter paper made of gauze to obtain the *S. aquifolium* macerate (Sari et al., 2021). The obtained macerate was then evaporated using a vacuum rotary evaporator at 50 °C to obtain a thick extract.

2.5. Bacterial inhibition test of *S. aquifolium* extract

Inhibition testing of *S. aquifolium* extract was done by well diffusion method. The extract concentrations used were 20%; 40%; 60%; 80%; and 100%, with the positive control being chloramphenicol and the negative control being sterile distilled water. The working method of this test is to prepare a suspension of *V. parahaemolyticus* by growing on TSB media, incubated at 37 °C for 24 hours. After that, the bacterial suspension was diluted to 1.5×108 CFU/ml and the turbidity was compared using McFarland Standard 0.5. A total of 200 µl of bacterial suspension was pipetted and planted on sterile Petri dishes, then TSA media was added, homogenized, and waited to solidify. Petri dishes were then perforated in the center using a cork borer with a diameter of 6 mm. each Petri dish that had been perforated was then added with 20 µl of *S. aquifolium* extract with each concentration that had been prepared. The same thing was also done to the control group. Each concentration and control group was repeated twice. Petri dishes were then covered with plastic wrap and incubated at 37°C for 24 hours. Antibacterial activity is indicated by the appearance of the inhibition zone diameter as an inhibitory response to the growth of *V. parahemolyticus* due to the secondary metabolite profile produced by the extract material (Sari et al., 2021). Measurement of the diameter of the inhibition zone was carried out using the method from the research of Swari et al. (2024), the equation as follows:

$$Inhibition\ diameter\ (mm) = \frac{D1 + D2}{2}$$

Where,

D1: vertical inhibition zone length (mm)
D2: horizontal inhibition zone length (mm)

2.6. Determination of minimum inhibitory concentration (MIC) of *S. aquifolium* extract

MIC testing was carried out by culturing 50 µl of *Vibrio* sp. suspension and 20 µl of *S. aquifolium* extract with various concentrations into a test tube filled with 9 ml of sterile TSB media. The test tube was then vortexed for 1 minute, then incubated at 30°C for 24 hours. The MIC value was determined by measuring the turbidity level of each test tube using a UV-Vis spectrophotometer with a wavelength of 200-800 nm. The higher turbidity level is assumed to be an increase in the number of bacterial cells in the media, thus increasing the absorbance value. The same method was performed on each control. Repetition of measurements was done twice for each treatment.

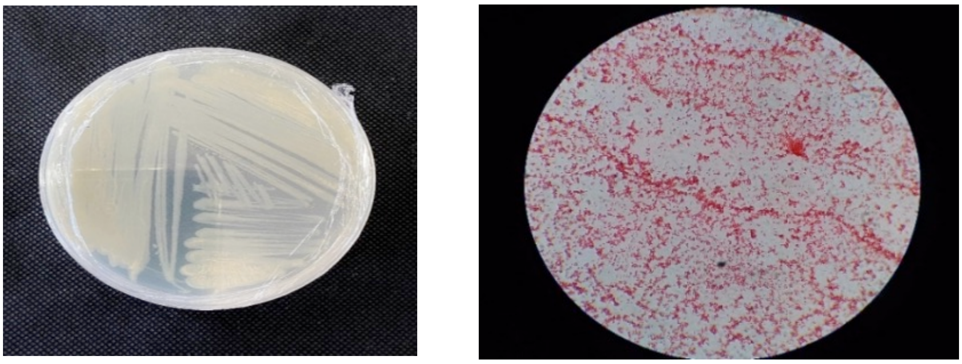
2.7. Data analysis

Data from the inhibition and MIC tests were tabulated using Microsoft Excel and statistically operated using SPSS Version 23.0 software (IBM, USA). Data were analyzed quantitatively by Analysis of Variance (ANOVA) and to determine significant differences in each treatment group of extract concentrations followed by the DUNCAN test with significance indicated by a value of $p < 0.05$. The sequencing results were then BLASTed using NCBI software and continued with nucleotide alignment and phylogenetic analysis performed with MEGA.11 software. The results of statistical analysis are displayed in the form of tables and figures.

3. Results and Discussion

3.1 Molecular identification results of *Vibrio* sp. isolates using the 16S sRNA gene marker

Based on observations made on *Vibrio* sp. isolates successfully isolated from the hepatopancreas of vaname shrimp, showed good growth on TSA media with cream-colored colonies, wrinkle-shaped, and had colony diameters ranging from 3-5 mm (Figure 1A). Based on the results of Gram staining, it can be seen that the *Vibrio* sp. isolate is able to absorb safranin dye so that it produces a red color when observed under a microscope with a magnification of 500×, and confirms that this isolate is Gram negative (Figure 1B). Research conducted by Hidayat, (2014) reported that bacteria isolated from sunu grouper (*Plectropomus leopardus*) were positive for *V. parahaemolyticus*. The characteristics of the identified bacteria are round with flat edges, convex elevation, gray-green color, and smooth texture.



A. B.

Figure 1. Identification results of *Vibrio* sp. isolates isolated from the hepatopancreas of super-intensively farmed vaname shrimp (*L. vannamei*). A: Colonies of *Vibrio* sp. grown on TSA media; B: Gram staining of *Vibrio* sp. isolates at 500× microscope magnification.

The results of phylogenetic analysis show that Vibrio sp. isolates are close to *Vibrio parahamaemolyticus* in the GeneBank database (Figure 2). This phylogenetic tree analysis method is based on the nucleotide sequence process of the partial 16S rRNA gene. Phylogenetic analysis uses the Neighbor Joining method, which is the nucleotide pair that has the smallest change among the sequences that have been compared. The distance value is denoted by a scale line that shows the number of nucleotide substitutions for each sequence position. The distance value of 0.02 in the results of phylogenetic tree construction indicates low nucleotide substitutions in the 16S rRNA sequence.

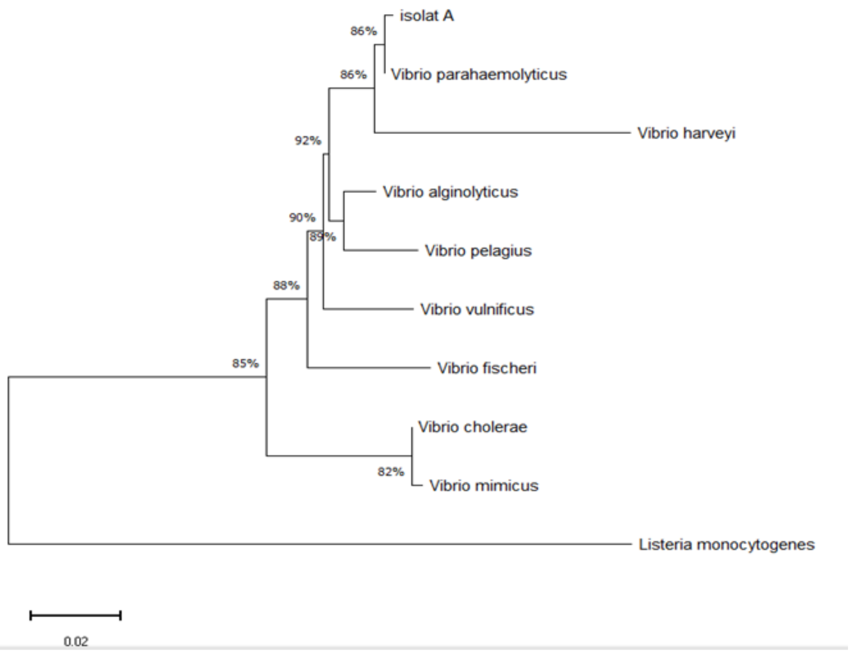


Figure 2. Phylogenetic tree construction results of *Vibrio* sp. isolates with several *Vibrio* genus and outgroups accessed from GeneBank database by Neighbor-Joining method.

Based on the phylogenetic tree analysis, *V. parahaemolyticus* bacterial isolates have the closest kinship with *Vibrio harveyi*. This can be seen from the phylogenetic results that have a close kinship with the formation of one branching point with *Vibrio harveyi* and have a bootstrap value of 860x from 1000x bootstrap is a good bootstrap value. The Bootstrap method is used to test the accuracy of a phylogenetic tree branch point. Clustering stability (robustness) was calculated using a bootstrap with 1,000 replicates.

3.2 Inhibition test results of *S. aquifolium* extract against *V. parahaemolyticus* growth

Inhibition test is a test conducted to determine the potential of a preparation in inhibiting the growth of certain bacteria (Magvirah et al., 2019). The higher the content of active compounds in a preparation, the greater the inhibition zone that will be produced. The presence of an inhibition zone is characterized by the formation of a clear zone or zone that does not grow bacteria around the treatment area (Syarifah et al., 2018). Based on the results of testing the concentration of *S. aquifolum* extract against the growth of *V. parahemolyticus*, the presence of antibacterial activity is indicated by the appearance of a clear zone (Figure 3). The 20% concentration of *S. aquifolium* extract showed negative results or no inhibition of V. parahaemolyticus growth. Meanwhile, the concentrations of 40%, 60%, 80%, and 100% respectively showed positive results or had inhibition with different inhibition zone areas at each concentration. Furthermore, the positive control using chloramphenicol showed positive results of inhibition, while the negative control using sterile distilled water showed negative results or no inhibition.

Resistance of microorganisms to an antibacterial can be done by testing using commercial antibiotics developed by Kirby-Bauer Well Diffusion. The working principle is the ability of antibiotic diffusion given to the well to inhibit the growth of test microorganisms with a marked inhibition zone in the culture medium. The process of inhibition zone formation is influenced by the solubility of bacterial suspensions in TSA solid media, with wells made containing *S. aquifolium* extract of known concentration, causing a clear area around the well which is called the inhibition zone. This shows the sensitivity of bacteria to antibacterial substances in *Sargassum aquifollium* extract, and it can be said that the wider the inhibition zone formed, the more sensitive the bacteria are.

The diameter of the inhibition zone in Table 1 shows that the negative control and 20% concentration of *S. aquifolium* extract had no inhibition zone against *V. parahaemolyticus* bacteria. Meanwhile, the positive control produced an average inhibition zone of 54.35 ± 4.5 mm or significantly higher than the other extract concentration treatments ($p \leq 0.05$). Furthermore, the treatment of 40% concentration of *S. aquifolium* extract produced an average inhibition zone of 25.85±0.2 mm, 60% concentration of 23±1.4 mm, 80% concentration of 21.25±1.06 mm, and 100% concentration of 29.35±4.5 mm. 100% extract concentration showed the highest inhibitory activity significantly when compared to other extract concentrations ($p \leq 0.05$).

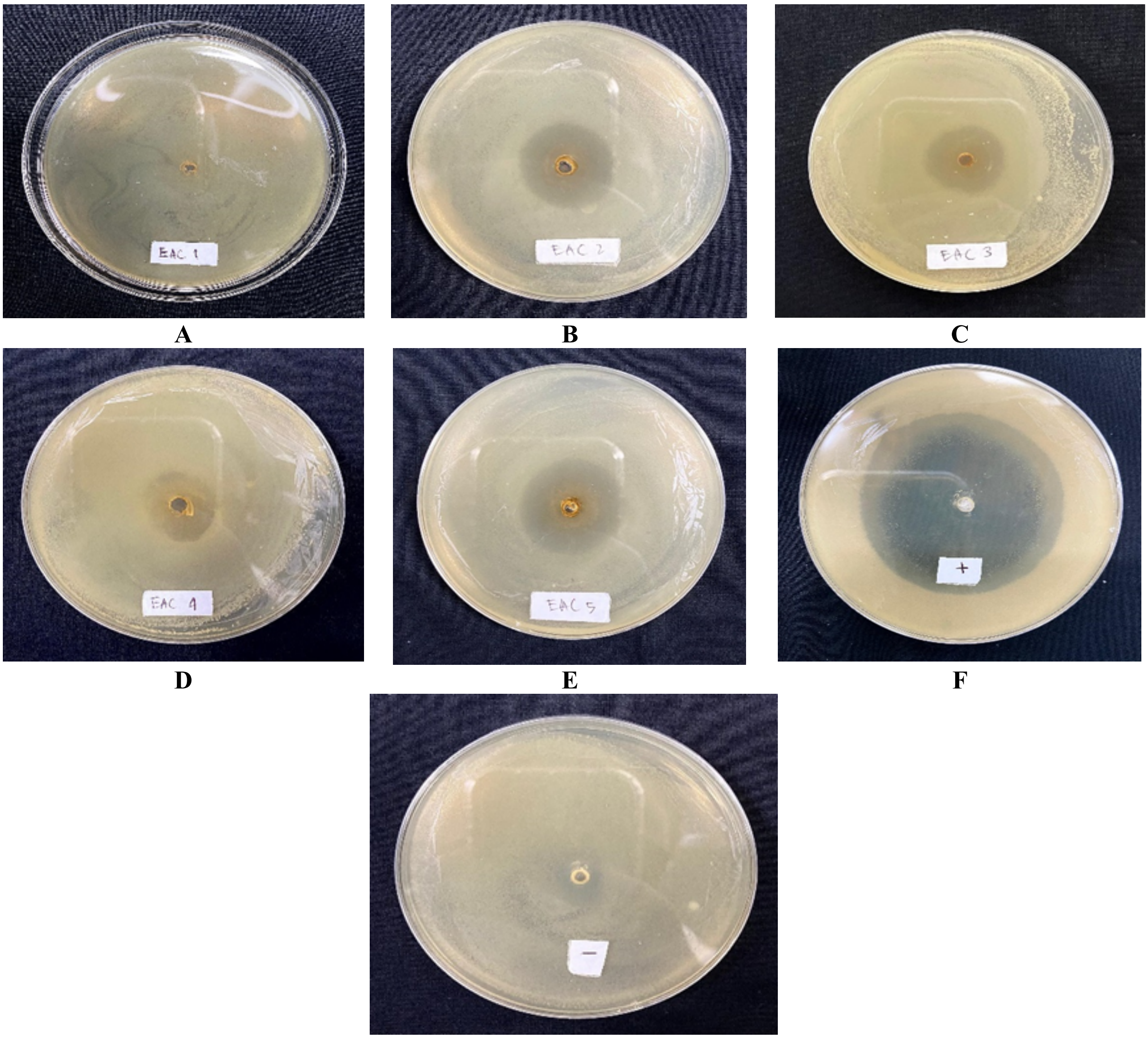


Figure 3. Zone of inhibition of *S. aquifolium* extract against *V. parahaemolyticus* growth. A: 20% extract concentration; B: 40% extract concentration; C: 60% extract concentration; D: 80% extract concentration; E: 100% extract concentration; F: positive control (chloramphenicol); G: negative control (sterile distilled water).

Table 1. Inhibition Zone Diameter of Various Concentrations of Brown Algae Extract *S. aquifolium* against The Growth of *V. parahaemolyticus*

Concentration (%)	Replication		Mean ± Standard Deviation	Inhibition Category
	I	II		
20	0	0	0 ± 0.0^{a}	Not inhibit
40	26	25.7	25.85 ± 0.2^{cd}	Very strong
60	22	24	23 ± 1.4^{bc}	Very strong
80	20.5	22	21.25 ± 1.06^{b}	Very strong
100	30.2	28.5	29.35 ± 1.2^{d}	Very strong
Positive control	57.5	51.2	54.35 ± 4.5^{e}	Very strong
Negative control	0	0	0 ± 0.0^{a}	Not inhibit

Letters with different notations in the same column indicates a significant difference between treatment groups ($p \leq 0.05$) based on Duncan test results.

Based on the inhibition zone diameters, *S. aquifolium* extract at 100% concentration demonstrated a very strong inhibitory effect (29.35 ± 1.2 mm), surpassing other concentrations significantly ($p \leq 0.05$). According to (Rahayu et al., 2019), the diameter category of the inhibition zone of a natural material against test bacteria can be classified as follows, ≤ 5 mm is included in the low category, 6-10 mm in the medium category, 11-20 mm is included in the strong category, and ≥ 21 mm is included in the very strong inhibition category. Based on this classification, the ability of *S. aquifolium* extract in inhibiting the growth of *V. parahaemolyticus* bacteria at concentrations of 40%, 60%, 80%, and 100% includes a very strong inhibition category. Previous research results by (Dolorosa et al., 2017) reported that *Sargassum aquifollium* is a plant that is efficacious as an antibacterial because it has several secondary metabolite compounds. In phytochemical screening that has been done before, *Sargassum aquifollium* contains components that are antibacterial, including flavonoids, steroids and alkaloids.

The antibacterial mechanism of flavonoids inhibits nucleic acid synthesis is the A and B rings which play an important role in the process of intercellation or hydrogen bonding by stacking nucleic acid bases that inhibit the formation of DNA and RNA (Edo et al., 2022). The mechanism of action of flavonoids inhibiting cell membrane function is to form complex compounds with extracellular and soluble proteins so as to damage the bacterial cell membrane and followed by the release of intracellular compounds (Sari, 2015). Another mechanism of alkaloid antibacterial is that alkaloid components are known as DNA intercellators or chemical compounds that occupy the space between DNA pairs and inhibit bacterial cell topoisomerase enzymes (Azzahra & Trimulyono, 2024). Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, causing decreased membrane integrity and altered cell membrane morphology that causes cell lysis (Bonttiura, 2015).

3.3 Minimum inhibitory concentration (MIC) results of *S. aquifolium* extract

Minimum Inhibitory Concentration (MIC) is an important metric in antimicrobial research as it affects the efficacy of antimicrobial drugs. The minimum concentration of an antimicrobial agent required to inhibit bacterial growth that can be observed after incubation for 24 hours (Yasir et al., 2022). The MIC test results measured using a UV-Vis spectrophotometer show that *S. aquifolium* extract has inhibition against *V. parahaemolyticus* bacteria based on the level of turbidity of the liquid media. This is indicated by the difference in absorbance value produced in the negative control with the absorbance value produced in the *S. aquifolium* extract. The results of the MIC test are shown in Table 2 below.

Table 2. Measurement results of minimum inhibitory concentration (MIC) value of *S. aquifolium* extract against *V. parahaemolyticus* by UV-Vis spectrophotometer

Concentration (%)	Replication		Absorbance		Mean ± Standard Deviation
	I	II	I	II	
20	240	240	1.631	1.647	1.639±0.011[a]
40	240	240	1.191	1.522	1.356±0.234[b]
60	240	240	1.525	1.544	1.534±0.013[a]
80	240	240	1.021	1.006	1.013±0.010[c]
100	240	240	0.85	0.772	0.811±0.055[c]
Positive control	240	240	0.242	0.509	0.375±0.188[d]
Negative control	240	240	1.719	1.641	1.680±0.055[a]

The bolded value in the mean section is the MIC value in this study. Letters with different notations in the same column indicate significant differences between treatment groups ($p \leq 0.05$) based on the one way Anova followed by DUNCAN test results.

Based on Table 2 above, the highest absorbance value was found in the negative control at 1.680 ± 0.055 and the 20% concentration of *S. aquifolium* extract at 1.639 ± 0.011. This indicates that the growth of *V. parahemolyticus* in these treatments is very high which is characterized by turbidity of the liquid media. Based on these data, it can be concluded that the MIC value of *S. aquifolium* extract is at a concentration of 100% with an absorbance value of 0.811 ± 0.055 which is produced close to the absorbance value of the positive control of 0.375 ± 0.188. The amount of bacterial cell density can be measured by knowing the turbidity or turbidity of the culture, if the more turbid a culture medium, the higher the number of cells.

Based on the test results, it can be seen that the MIC of *Sargassum auifollium* extract against the growth of *V. parahaemolyticus* bacteria is 100% extract concentration, because this concentration is the lowest extract concentration that shows inhibition of *V. parahemolyticus*. This is in accordance with what Achwandi et al. (2015) stated that the MIC value is the minimum concentration of antimicrobial substances that can inhibit bacterial growth after the incubation process for 24 hours by looking at the level of turbidity in each treatment. MIC parameters are important for assessing new antimicrobial compounds, directing appropriate drug formulations, and designing successful treatment regimens, especially given the growing problem of antibiotic resistance (Chung et al., 2024), including in shrimp aquaculture businesses.

4. Conclusion

This study is the first report on the effectiveness of *S. aquifolium* extract to inhibit the growth of *V. parahaemolyticus* in vitro through measurement of inhibition and MIC value with the highest inhibition value at 100% concentration. This study also highlighted that *Vibrio* sp. isolates successfully isolated from the hepatopancreas of vaname shrimp have similarities with *V. parahaemolyticus* based on molecular identification with the 16S sRNA marker gene. The antibacterial effect exerted by *S. aquifolium* extract suggests the presence of bioactive compounds, warranting further phytochemical analysis. Further research is recommended to assess the in vivo effectiveness of *S. aquifolium* extract in treating *V. parahaemolyticus* infections, focusing on dose optimization, long-term effects, and its potential as an immunostimulant in vaname shrimp.

Supplementary Material

No supplementary materials are associated with this manuscript.

Acknowledgments

Not Applicable.

Author Contributions

Jatu Maranatha Fimaputra: conceptualization, data curation resources, and writing – original draft; **Putu Angga Wiradana**: data curation, methodology, validation, and supervision; **Anak Agung Ayu Putri Permatasari**: methodology, formal analysis, investigation, and supervision; **I Gede Widhiantara:** software, formal analysis, writing – review & editing, and supervision; **Putu Eka Sudaryatma**: formal analysis, writing – review & editing, and supervision; **Muhammad Khaliim Jati Kusala**: formal analysis, writing – review & editing, and supervision; **Mochammad Aqilah Herdiansyah**: formal analysis, writing – review & editing, and supervision; **I Putu Sugiana**: formal analysis, writing – review & editing, and supervision.

Funding

This work is not supported by any external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Achwandi M, Khoiriyati A, Soewito S. 2015. Efektifitas Ekstrak Daun Sirih Merah (Piper crocatum) terhadap Kadar Hambat Minimum dan Kadar Bunuh Minimum Bakteri Salmonella typhi. *IJNP (Indonesian Journal of Nursing Practices)* **2**(**1**): 1–8

Alamsyah HK, Widowati I, Sabdono A. 2014. Aktivitas Antibakteri Ekstrak Rumput Laut Sargassum Cinereum (J.G. Agardh) dari Perairan Pulau Panjang Jepara terhadap Bakteri Escherichia Coli dan Staphylococcus Epidermidis. *Journal of Marine Research* **3**(**2**): 69–78

Aziza RN, Chaidir RRA. 2024. Isolasi Bakteri Vibrio Sp. Resisten Antibiotik pada Sampel Udang Vaname (Litopenaeus Vannamei) dari Pasar Seketeng. *Journal of Life Science and Technology* **2**(**1**): 26–35

Azzahra ANA, Trimulyono G. 2024. Aktivitas Antibakteri Ekstrak Rumput Laut Gracilaria Verrucosa terhadap Bakteri Pseudomonas fluorescens Patogen pada Ikan. *LenteraBio: Berkala Ilmiah Biologi* **13**(**1**): 44–54

Bonttiura S. 2015. Uji Efek Antibakteri Ekstrak Daun Leilem (Clerodendrum minahassae L.) Terhadap Bakteri Streptococcus mutans. *Pharmacon* **4**(**4**)

Chung C-R, Chien C-Y, Tang Y, Wu L-C, Hsu JB-K, Lu J-J, Lee T-Y, Bai C, Horng J-T. 2024. An ensemble deep learning model for predicting minimum inhibitory concentrations of antimicrobial peptides against pathogenic bacteria. *iScience* **27**(**9**): 110718. DOI: 10.1016/j.isci.2024.110718

Dolorosa MT, Nurjanah PS, Anwar E, Hidayat T. 2017. Kandungan Senyawa Bioaktif Bubur Rumput Laut Sargassum Plagyophyllum dan Eucheuma Cottonii Sebagai Bahan Baku Krim Pencerah Kulit. *Jurnal Pengolahan Hasil Perikanan Indonesia* **20**(**3**): 633–644

Edo MYR, Pakan PD, Rini DI. 2022. Test of Antibacterial Activity Of Ethanol 70% Extract Kencur Rhizomes (Kaempferia Galanga Linn) On Streptococcus Pyogenes In Vitro. *Cendana Medical Journal* **10**(**2**): 218–226

Hadi FR, Riyantini I, Subhan U, Ihsan YN. 2018. Efek Cekaman Salinitas Rendah Perairan terhadap Kemampuan Adaptasi Udang Vaname (Litopenaeus Vannamei). *Jurnal Perikanan dan Kelautan* **9**(**2**): 72–79

Hidayah N, Mustikaningtyas D, Bintari SH. 2017. Aktivitas Antibakteri Infusa Simplisia Sargassum muticum terhadap Pertumbuhan Staphylococcus aureus. *Life Science* **6**(**2**): 49–54

Hidayat AS. 2014. Isolasi dan Identifikasi Bakteri Vibrio sp. dari Ikan Kerapu Sunu (Plectropomus leopardus). *Jurnal Teknosains* **8**(**2**): 209–216

Iskandar A, Trianto Y, Hendriana A, Lesmanawati W, Prasetyo B, Muslim M. 2022. Pengelolaan dan Analisa Finansial Produksi Pembesaran Udang Vaname Litopenaeus Vannamei. *Perikanan* **12**(**2**): 256–267

Kementerian Kelautan dan perikanan. 2018. Budidaya Udang Masih Sangat Potensial.

Magvirah T, Marwati, Ardhani F. 2019. Uji Daya Hambat Bakteri Staphylococcus aureus menggunakan Ekstrak Daun Tahongai (Kleinhovia hospita l.). *Jurnal Peternakan Lingkungan Tropis* **2**(**2**): 41–50

Natalia D, Nurozy. 2014. No TKinerja Daya Saing Produk Perikanan Indonesia Di Pasar Global itle. *Jurnal Buletin Ilmiah Litbang perdagangan* **6**(**1**): 69–88

Ni Kadek Yunita Sari, Anak Agung Ayu Putri Permatasari, Sri Puji Astuti Wahyuningsih, Almando Geraldi, Putu Angga Wiradana, I Gede Widhiantara, Novaria Sari Dewi Panjaitan. 2023. An overview of the role of Zingiber officinale as an antimicrobial resistance (AMR) solution and a source of antioxidants. *Indonesian Journal of Pharmacy*. DOI: 10.22146/ijp.5307

Pakidi CS, Suwoyo HS. 2017. Potensi Dan Pemanfaatan Bahan Aktif Alga Cokelat Sargassum Sp. *Jurnal Ilmu*

Perikanan **6**(**1**): 551–562

Permatasari AAA, Rosiana IW, Wiradana PA. 2022a. Extraction and characterization of sodium alginate from three brown algae collected from Sanur Coastal Waters, Bali as biopolymer agent. *Biological Diversity* **23**(**3**)

Permatasari AAAP, Rosiana IW, Wiradana PA, Lestari MD, Widiastuti NK, Kurniawan SB, Widhiantara IG. 2022b. Extraction and characterization of sodium alginate from three brown algae collected from Sanur Coastal Waters, Bali as biopolymer agent. *Biodiversitas Journal of Biological Diversity* **23**(**3**): 1655–1663. DOI: 10.13057/biodiv/d230357

Rahayu S, Rozirwan, Purwiyanto AIS. 2019. Daya hambat senyawa bioaktif pada mangrove Rhizophora Sp. sebagai antibakteri dari perairan Tanjung Api-Api, Sumatera Selatan. *Jurnal Penelitian Sains* **21**(**3**): 151–162

Rosiana IW, Wiradana PA, Permatasari AAAP, Pelupessy YAEG, Dame MVO, Soegianto A, Yulianto B, Widhiantara IG. 2022. Concentrations of Heavy Metals in Three Brown Seaweed (Phaeophyta: Phaeophyceae) Collected from Tourism Area in Sanur Beach, Coast of Denpasar, Bali and Public Health Risk Assessment. *Jurnal Ilmiah Perikanan dan Kelautan* **14**(**2**): 327–339. DOI: 10.20473/jipk.v14i2.33103

Roza D, Zafran, Taufik F, Girsang MA. 2017. Pengendalian Vibrio harveyi secara Biologis menggunakan Bakteri Jenis Lain sebagai Musuh Alaminya pada Larva Udang Windu. *Jurnal Penelitian Perikanan Indonesia* **2**(**4**): 6–14

Sani MD, Maharani AY, Riandy MI, Susilo RJK, Wiradana PA, Soegianto A. 2020. Monitoring of Population Density of Vibrio sp. and Health Condition of Hepatopancreas Pacific White Shrimp (litopenaeus vannamei) Cultivated with Intensive Systems in Bulukumba Regency, South Sulawesi, Indonesia. *Ecology, Environment and ConservationEcology, Environment and Conservation* **26**(**3**): 1271–1275

Sari K. 2015. Kandungan Senyawa Kimia dan Aktivitas Antibakteri Ekstrak Kulit Buah Alpukat (Persea Americana P. Mill) terhadap Bakteri Vibrio Alginolyticus. *Jurnal Kajian Veteriner* **3**(**2**): 203–211

Sari NKY, Deswiniyanti NW, Wiradana PA. 2021. Evaluation of antimicrobial activity and phytochemical screening of red Kamboja (Plumeria rubra L.) extracts. *Biogenesis: Jurnal Ilmiah Biologi* **9**(**2**): 233–240. DOI: 10.24252/bio.v9i2.25409

Sarjito, Apriliani M, Afriani D, Haditomo AHC. 2015. Agensia Penyebab Vibriosis pada Udang Vaname (Litopenaus gariepinus) yang ibudidayakan Secara Intensif di Kendal. *Kelautan Tropis* **18**(**3**): 189–196

Scabra AR, Marzuki M, Yarni BM. 2023. Pengaruh Pemberian Kalsium Hidroksida (Caoh2) dan Fosfor (P) terhadap Pertumbuhan Udang Vaname (Litopenaeus Vannamei) pada Media Air Tawar. *Jurnal Ruaya* **11**(**1**): 39–51

Sinurat AAP, Renta PP, Herliany NE, Negara BF, Purnama D. 2019. Uji Aktivitas Antibakteri Ekstrak Metanol Rumput Laut Gracilaria edulis terhadap Bakteri Aeromonas hydrophila. *Jurnal Enggano* **4**(**1**): 105–114

Swari KKI, Darmayasa IBG, Wibawa IPAH. 2024. Penentuan Minimum Inhibitory Concentration (MIC) minyak atsiri Acorus calamus terhadap Aspergillus flavus. *Jurnal Biologi Udayana* **28**(**1**). DOI: https://doi.org/10.24843/JBIOUNUD.2024.v28.i01.p09

Syarifah R, Fakhrurrazi, Harris A, Sutriana A, Erina, Winaruddin. 2018. Uji Daya Hambat Ekstrak Biji Buah Pala (Myristica fragrans houtt) terhadap Pertumbuhan Bakteri Escherichia coli. *Jurnal Ilmiah Mahasiswa Veteriner* **2**(**3**): 361–372

Wiradana PA, Sani MD, Mawli RE, Ashshoffa FND, Widhiantara IG, Mukti AT. 2022. Monitoring the occurrence of Zoea Syndrome (ZS) in pacific white shrimp (Litopenaeus vannamei) larval from several hatcheries in East Java, Indonesia. *IOP Conference Series: Earth and Environmental Science* **1036**(**1**): 012003. DOI: 10.1088/1755-1315/1036/1/012003

Yanti S, Permatasari AAAP, Sari NKY, Wiradana PA, Rosiana, Wayan IMGSS. 2024. Analisis Fitokonstituen Ekstrak Etanol Makroalga Merah Jenis Euchema cottonii Yang Dikoleksi Dari Petani Rumput Laut Di Pulau Serangan Bali. *Jurnal Kesehatan Terpadu* **8**(**1**): 12–17

Yasir M, Karim AM, Malik SK, Bajaffer AA, Azhar EI. 2022. Prediction of antimicrobial minimal inhibitory concentrations for Neisseria gonorrhoeae using machine learning models. *Saudi Journal of Biological Sciences* **29**(**5**): 3687–3693. DOI: 10.1016/j.sjbs.2022.02.047