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1 SCREENING OF PHYTOCHEMICAL COMPOUNDS AND ANTIBACTERIAL
2 ACTIVITY OF *TENGKAWANG BUKIT* (*Shorea beccariana*) STEM EXTRACT
3 AGAINST *Vibrio parahaemolyticus*
4

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22
23 ABSTRACT

24
25 *Vibrio parahaemolyticus* infection poses a significant challenge in intensive shrimp culture,
26 leading to substantial economic losses. The use of synthetic antibiotics for treatment has raised
27 concerns about antibiotic resistance and residual effects, so a searching for safe and effective
28 natural alternatives becomes essential. *Tengkawang Bukit* (*Shorea beccariana*) stem extract
29 is known to contain antibacterial compounds, including asaric acid, oleanolic acid, and
30 lupanone, which are part of the terpenoid compound group. This study aims to evaluate the
31 antibacterial potential of *S. beccariana* stem extract through phytochemical screening and a
32 disc diffusion test against *V. parahaemolyticus*. The screening results revealed the presence of
33 bioactive compounds such as alkaloids, glycosides, saponins, tannins, and steroids. The disc
34 diffusion test demonstrated an increase in the inhibition zone as the concentration of the extract
35 increased, with inhibition diameters ranging from 9.04 ± 0.48 mm to 10.75 ± 0.26 mm. The
36 extract yield of 17% indicates a rich availability of active compounds. These findings suggest
37 that *tengkawang bukit* stem extract has a potential as a natural antibacterial alternative for
38 controlling *V. parahaemolyticus* in shrimp culture. This approach could help reduce reliance
39 on synthetic antibiotics and promote sustainable fisheries.
40

41 KEYWORDS: antibacterial agents; plant extracts; resistance; *Shorea beccariana*; *Vibrio*
42 *parahaemolyticus*

43
44 ABSTRAK: Skrining Fitokimia dan Uji Aktivitas Antibakteri pada Ekstrak Batang
45 Tengkawang Bukit (*Shorea beccariana*) terhadap *Vibrio parahaemolyticus*
46

47 Infeksi *Vibrio parahaemolyticus* merupakan salah satu kendala utama dalam budidaya udang
48 yang menyebabkan kerugian ekonomi signifikan. Penggunaan antibiotik sintetis sebagai
49 pengobatan telah menimbulkan kekhawatiran terkait resistensi dan residu, sehingga
50 diperlukan alternatif alami yang aman dan efektif. Batang tengkawang bukit (*Shorea*
51 *beccariana*) diketahui mengandung senyawa antibakteri seperti asam asiatik, asam oleanolik,
52 dan lupanon yang tergolong ke dalam senyawa terpenoid. Studi ini bertujuan untuk
53 mengevaluasi potensi antibakteri ekstrak batang *S. beccariana* melalui skrining fitokimia dan

uji difusi cakram terhadap *V. parahaemolyticus*. Hasil skrining menunjukkan adanya kandungan senyawa bioaktif berupa alkaloid, flavonoid, saponin, tanin, dan steroid. Uji difusi cakram memperlihatkan zona hambat yang meningkat seiring dengan peningkatan konsentrasi ekstrak, dengan diameter hambatan berkisar $9,04 \pm 0,48$ mm hingga $10,75 \pm 0,26$ mm. Rendemen ekstrak sebesar 17% menunjukkan ketersediaan senyawa aktif yang melimpah. Hasil riset ini mengindikasikan bahwa ekstrak batang tengkawang bukit berpotensi sebagai alternatif antibakteri alami dalam pengendalian *V. parahaemolyticus* pada budidaya udang, sehingga dapat mengurangi ketergantungan terhadap antibiotik sintetis dan mendukung keberlanjutan perikanan.

KATA KUNCI: agen antibakteri ; ekstrak tanaman ; resistensi ; *Shorea beccariana*; *Vibrio parahaemolyticus*

INTRODUCTION

Marine aquaculture is one of the main sectors in national fisheries development that contributes to food security and the community's economy. As the consumption of animal protein increases, the demand for fish and shrimp continues to rise in both domestic and international markets. Based on data from the Ministry of Marine Affairs and Fisheries (MMAF) (2023), per capita fish consumption in Indonesia increased from 50.69 kg per year in 2018 to 57.61 kg per year in 2023. On the other hand, marine aquaculture production in Indonesia has shown a fluctuating trend in the last five years. Additionally, production in 2019 was recorded at 8.6 million tons. Marine aquaculture production decreased slightly in 2020 to 8.5 million tons, and a more significant decline occurred in 2021 with a total production of 7.3 million tons. The production increased again in 2022 to 7.9 million tons and continued to rise in 2023 to 8.3 million tons. These fluctuations reflect that although the marine aquaculture sector has great potential, there are still various factors and obstacles that affect the stability of its production.

As cultivation intensification increases to meet demand, the challenges to sustainable production are also becoming more complex. One of the main obstacles faced is the high infection rate of diseases caused by pathogenic bacteria (Pramadani, 2022). Disease caused by pathogenic bacteria is one of the main limiting factors in fish and shrimp farming, especially in intensive farming systems that have a higher risk of disease spread. Bacterial infections not

only inhibit growth and reduce the survival rate of cultured organisms, but also have a direct impact on reducing productivity and considerable economic losses for farmers (Semwal *et al.*, 2023). One of the bacteria that is the causative agent of disease in marine fish and shrimp is *Vibrio parahaemolyticus* (Evan *et al.*, 2021).

V. parahaemolyticus is a Gram-negative bacterium that is the causative agent of vibriosis in fish and shrimp. In aquaculture practices, *V. parahaemolyticus* infection is often associated with cases of acute hepatopancreatic necrosis disease (AHPND) in shrimp, which causes severe damage to the hepatopancreas and can cause mass mortality (Evan *et al.*, 2021). Cases of *V. parahaemolyticus* infection have been reported in a number of shrimp farming centers in Indonesia, such as in Sumbawa, East Java, and Lampung, causing a drastic decrease in production and even crop failure (MMAF, 2023). This bacterial infection causes aquatic organisms to become sick with symptoms of swollen, hard abdomen, brown anus, pale liver, swollen intestines, slow movement, decreased appetite, swollen kidneys, hemorrhagic intestines, and skin wounds (Khairiyah *et al.*, 2022). Given the magnitude of the impact, it has become very important to control this bacterial infection, which until now has largely relied on the use of synthetic antibiotics in aquaculture practices.

The use of synthetic antibiotics is one of the steps to prevent bacterial infections. However, the use of synthetic antibiotics is currently limited because it causes bacterial resistance and leaves residues that have the potential to cause serious health problems globally (Alamsjah *et al.*, 2020). To overcome this issue, alternatives to synthetic antibiotics are needed, such as the use of natural ingredients as antibiotics. One potential plant is *Tengkawang Bukit* (*Shorea beccariana*), whose stems contain terpenoid compounds such as asiatic acid, oleanolic acid, and lupanon which are known to be antibacterial (Musa *et al.*, 2024). Until now, there has been no research that specifically examines the antibacterial activity of *tengkawang bukit* plant stems through qualitative phytochemical screening and disc diffusion tests.

⁹This study aims to evaluate the antibacterial potential of *tengkawang bukit* extract through phytochemical screening and disc diffusion test to observe the inhibition zone of the extract against *V. parahaemolyticus*. Phytochemical screening ¹⁹was conducted to identify the content of bioactive compounds such as flavonoids, alkaloids, saponins, tannins, and steroids that are known to have antibacterial activity. Furthermore, ¹²disc diffusion test was used to measure the effectiveness of the extract in inhibiting the growth of the target bacteria (*V. parahaemolyticus*), which is an important pathogen in the shrimp culture. Therefore, the identification and development of natural materials, especially *tengkawang bukit* extract as an alternative antibacterial agent becomes very relevant in supporting disease control efforts in shrimp culture in a sustainable manner. By finding effective antibacterial agents from local natural resources, it is expected that dependence on synthetic antibiotics can be reduced, thus preventing the emergence of antimicrobial resistance and maintaining the sustainability of the fisheries sector.

⁴⁴MATERIALS AND METHODS

²This research was conducted from June to August 2025. Extract preparation activities were carried out at the Organic Chemistry Laboratory, Department of Chemistry, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia. The antibacterial activity test ³⁹was conducted at the Microbiology Laboratory, Faculty of Fisheries and Marine Sciences, Surabaya, Airlangga University.

In this study, the *V. parahaemolyticus* ATCC bacterial isolate was obtained from the Indonesian Agricultural Quarantine Agency, Indonesia. Whereas, *tengkawang bukit* stems were taken from the forest in ²⁹Madurejo, South Arut, West Kotawaringin Regency, Central Kalimantan, Indonesia. These materials were then transported to the Airlangga University for further analysis.

136

137 **Extract Preparation**

138 The extracting process of compounds from *tengkawang bukit* stems was ³carried out based
139 on the method described by Musa *et al.* (2024). The initial stage was begun with the preparation
140 of raw materials, specifically fresh *tengkawang bukit* stems weighing up to 12 kg. The fresh
141 material was washed using clean water and then dried using the room drying method, where
142 the fresh material was allowed to dry in the room at room temperature, without direct sunlight.
143 This drying method was chosen to maintain the stability of bioactive compounds contained in
144 the material, especially phenolic compounds, flavonoids, and tannins that are easily degraded
145 by heat and sunlight (Pujiastuti & Ma'rifah, 2022). Drying is done by placing the material
146 evenly on a mesh tray, flipped daily to ensure good air circulation and prevent the growth of
147 microorganisms. *Tengkawang bukit* stems as a hardwood material are estimated to take 14-21
148 days to reach dry moisture content. The material was considered dry once its weight had
149 remained constant for several days, no longer felt cool to the touch, no longer emitted a damp
150 odor, and showed characteristic textural changes, such as the trunk becoming harder and lighter.
151 (Supriningrum *et al.*, 2018).

152 After complete drying, all materials are ground using a crusher (grinder) into a fine
153 powder or small shavings. This process is done to increase the surface area of the material
154 against the solvent, so as to maximize the efficiency of extraction of active compounds in the
155 next stage. Each type of plant powder material will be extracted separately through maceration
156 method. The dried powder was soaked separately in 30 L of methanol. Methanol was chosen
157 as the extraction solvent in this study because methanol is a universal solvent which can attract
158 non-polar, polar, and semi-polar compounds ¹⁷and has a low boiling point (65°C), so that it is
159 easily evaporated (Supriningrum *et al.*, 2018). This soaking lasted for 72 hours at a temperature
160 of 25-28 °C in room condition, under conditions protected from light. To maximize extraction

and minimize solvent saturation, the methanol solvent will be renewed or replaced three times, every 24 hours. During the maceration stage, intermittent stirring will be applied to ensure even dispersion of the sample (Musa *et al.*, 2024).

After the maceration process, the entire volume of methanol filtrate obtained from the *tengkawang bukit* sample was collected in one container. The methanol extract was then concentrated using a rotary vacuum evaporator at 65°C, 150 mbar pressure, and 120 rpm rotation. This procedure aimed to effectively evaporate methanol, resulting in a more concentrated extract. This concentrated extract is evaporated again using a water bath at 40 °C for 2 hours so that it became a crude extract. The crude extract resulting from this process was stored under suitable conditions before being used into a series of further antibacterial activity tests (Musa *et al.*, 2024).

Yield Calculation

The yield calculation of *tengkawang bukit* stem extract was carried out by weighing the dried extract divided by the initial weight of the sample (initial simplicia) and then multiplying by 100% (Widyastuti *et al.*, 2021). The formula for calculating yield is:

$$\% \text{ Yield} = \frac{\text{Weight of Extract (g)}}{\text{Weight of Simplicia (g)}} \times 100$$

Preparation of Test Bacteria

The test bacteria used is *V. parahaemolyticus*. The bacterial preparation method refers to research performed by Huyyirnah and Fitriyani (2020). Bacteria were inoculated on tryptic soy agar (TSA) + NaCl 3% and propagated on tryptic soy broth (TSB) + NaCl 3%. Bacterial inoculation used a test tube filled with 10 mL of media (TSB + NaCl 3%) then added 500 µL (0.5 mL) of bacteria, and vortexed until homogeneous. The test tube was placed into an incubator (Incucell-MMM, Germany) that has a temperature of 37°C for 18 hours to 24 hours.

Qualitative Phytochemical Analysis

Phytochemical analysis included qualitative alkaloid, flavonoid, saponin, tannin, and steroid tests. Testing of alkaloid compounds was carried out by reacting 2 mL of *tengkawang bukit* stems extract with 1 mL of HCl in three test tubes. Each tube was then added three drops of Mayer, Wagner, and Dragendorff reagents. A positive alkaloid reaction is characterized by the presence of a cloudy white or yellowish white precipitate with Mayer's reagent, brown color with Wagner's reagent, and orange red or reddish brown with Dragendorff's reagent (Affandy *et al.*, 2021).

The flavonoid test of *tengkawang bukit* stem extract was carried out by mixing 0.5 mL of the extract with 10 mL of hot water, then adding 0.25 g magnesium powder and five drops of concentrated HCl. The formation of an orange or bright red color indicates the presence of flavonoids in the extract (Yeti & Yuniarti, 2021).

The saponin test of *tengkawang bukit* stem extract was carried out by mixing 1 mL of the extract with 5-10 mL of warm distilled water, then shaking vigorously for 10 seconds and standing for 15 minutes. The formation of stable foam as high as 1-10 cm, which does not disappear after adding one drop of HCl, shows a positive result for saponins (Kusumawati *et al.*, 2017).

The tannin test of *tengkawang bukit* stem extract was carried out by putting 2 mL of the extract into a test tube, then adding 10 drops of 1% FeCl₃ solution. Changes in color to dark blue or greenish black indicate a positive reaction to tannins (Kusumawati *et al.*, 2017).

Steroid test of *tengkawang bukit* stem extract was carried out by mixing 1 mL of the extract with Liebermann-Burchard reagent, which is a mixture of 2 mL chloroform, 10 drops of acetic acid anhydride, and three drops of concentrated sulfuric acid, then shaken gently and

210 allowed to stand. ⁴⁸ The formation of a green color indicates a positive result for steroids (Yeti &
211 Yuniarti, 2021).

212

213 Disc Diffusion Test

214 The ³antibacterial activity test through the Kirby-Bauer disc diffusion method was carried
215 out ³⁰using a completely randomized design consisting of six treatments and three repetitions.
216 This test procedure refers to research of Ngamsurach and Praipipat (2022). The test was begun
217 with the preparation of extract concentrations according to the treatment given, namely P1 (1
218 mg per disc), P2 (1.5 mg per disc), P3 (2 mg per disc), P4 (2.5 mg per disc), positive control in
219 the form of ampicillin antibiotics, and negative control using sterile distilled water. Treatments
220 P1, P2, P3 and P4 were obtained by dissolving 100, 150, 200 and 250 mg of dry extract,
221 respectively, in 1 mL of distilled water. Subsequently, 10 µL of each extract solution was taken
222 and dropped onto a blank disc paper. Furthermore, pure bacteria were inoculated in TSB + 3%
223 NaCl. The bacterial inoculation was diluted with ²⁵0.85% NaCl to the equivalent of 0.5
224 ^{McFarland standard} turbidity or 1.5×10^8 CFU mL⁻¹ using a turbidimeter (DL Medcall-China).

225

226 The diluted bacterial inoculation was spread onto Mueller hinton agar (MHA) + 3% NaCl
227 using a sterile cotton swab. The next step was placing an antimicrobial disc (paper disc)
228 according to each treatment. Furthermore, the ³³petri dish was incubated for 24 hours at a
229 temperature ^{of} 37°C until ^{the} inhibition zone was seen. The size ¹¹of the inhibition zone was
230 ^{measured} using a digital caliper ⁱⁿ millimeters (mm) (Ngamsurach & Praipipat, 2022).

231

232 Data Analysis

233 Inhibition zone diameter data were analysed using one way ANOVA with IBM Statistics
234 SPSS version 25 at 95% confidence level. If the one way ANOVA test results showed a

significant effect between treatments (p value < 0.05), the analysis was continued with the Tukey test. Observations on the results of qualitative phytochemical tests were analysed descriptively after the data were collected.

RESULTS AND DISCUSSION

The results showed that the yield value of *tengkawang bukit* stem extract was 17%. Phytochemical screening tests were carried out qualitatively on *tengkawang bukit* stem extract to determine the presence of secondary metabolite compounds that have a potential as an antibacterial agent. The results of qualitative phytochemical screening tests can be seen in Table 1. *Tengkawang bukit* is a tall dipterocarp tree reaching up to 60 m, with prominent buttress roots and grey-brown bark that becomes scaly in mature trees (Randa *et al.*, 2022). Its leaves are stiff and elliptic with a bluish underside that turns brown when dried (Vallahayil *et al.*, 2024). The species also produces pink inflorescences and ovoid fruits about 4 cm long (Barstow *et al.*, 2019). The appearance of the *tengkawang bukit* tree is shown in Figure 1.



Figure 1. Tree of *Shorea beccariana*
Source: Balikpapan Botanical Garden (2023).

Table 1. Results of qualitative phytochemical analysis of *tengkawang bukit* (*S. beccariana*) stems extract

Parameter	Result	Description
Alkaloid	+	There is a yellowish-white precipitate with Mayer's reagent, a brown precipitate with Wagner's reagent and a brownish-red precipitate on Dragendorff's reagent
Flavonoid	+	There is a red or orange color change
Saponin	+	Forms a stable foam
Tanin	+	There is a color change to blackish green
Steroid	+	There is a color change to intense green

Note: (+) There is a precipitate or color change, (-) There is no precipitate or color change.

The antibacterial activity of the extract from the stems of *tengkawang bukit* was demonstrated by the formation of an inhibition zone around the paper disc. The measurement results indicated that the extract could inhibit the growth of *V. parahaemolyticus*. The results of the disc diffusion test using the Kirby-Bauer method are presented in Figure 2.

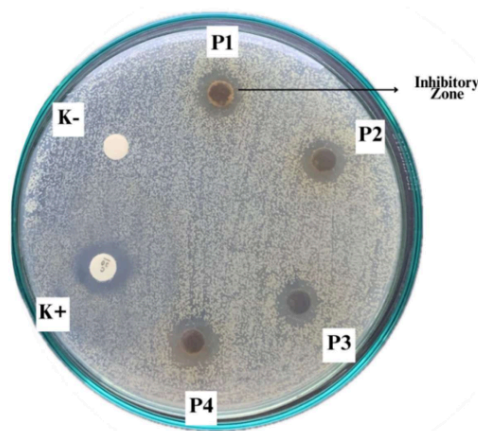


Figure 2. Results of the disc diffusion test of *tengkawang bukit* (*S. beccariana*) stems extract on *V. parahaemolyticus*. P1: 35 mg per disc; P2: 1,5 mg per disc; P3: 2 mg per disc; P4: 2,5 mg per disc; K+: positive control (Ampicillin); and K-: negative control (sterile distilled water)

The results of one way ANOVA test showed that different extract concentrations had a significant effect on the diameter of the inhibition zone formed ($p < 0.05$). The *S. beccariana* stem extract at a concentration of 2.5 mg per disc (P4) produced the highest inhibition zone diameter (10.75 ± 0.26 mm). These results was not significantly different from the positive control (Ampicillin) which had an inhibition zone of 10.94 ± 0.26 mm. A concentration of *tengkawang bukit* of 2 mg per disc (P3) produced an inhibition zone of 9.75 ± 0.24 mm, while P2 and P1 treatments showed lower values (9.17 ± 0.43 mm and 9.04 ± 0.48 mm, respectively) (Table 2).

Table 2. Inhibition zone of *tengkawang bukit* (*S. beccariana*) stems extract against *V. parahaemolyticus*

Treatment	Concentration	Inhibition Zone Diameter (mm)
P1	1 mg per disc	9.04 ± 0.48^b
P2	1.5 mg per disc	9.17 ± 0.43^b
P3	2 mg per disc	9.78 ± 0.26^b
P4	2.5 mg per disc	10.75 ± 0.26^c
K+	Ampicillin 10 mcg	10.94 ± 0.26^c
K-	Sterile water	0.00 ± 0.00^a

Note: Different lowercase letters (a, b, c) indicate significant differences ($p < 0.05$) among treatments. Values are based on mean $\pm$ SD (n=3).

The results of measuring the diameter of the inhibition zone using the disc diffusion test showed that the extract of *tengkawang bukit* stem had strong antibacterial activity against *V. parahaemolyticus*. The diameter of the inhibition zone increased as the concentration of the extract increased. The extract yield obtained through the maceration process was 17%, which was a high amount, thus indicating the availability of bioactive compounds in abundance that support its antibacterial effectiveness. Based on research by Musa *et al.* (2024), *tengkawang bukit* stems contain asiatic acid compounds, oleanolic acid, and lupanon which are classified into terpenoid compounds. Terpenoid compounds are bioactive agents that are effective in antimicrobial activity with the main mechanism of disrupting bacterial cell membranes, inhibiting the quorum sensing (QS) system so as to inhibit communication between bacterial

cells and reduce virulence, and inhibit protein and ATP synthesis. Terpenoid compounds cause ion leakage and direct membrane damage, mainly due to their ability to penetrate and damage the lipid layer of bacterial cells (Ergüden, 2021).

This mechanism is also in line with the finding that membrane-penetrating hygroscopic terpenoid compounds can inhibit the quorum sensing system which is an important pathway for virulence coordination and biofilm formation by *V. parahaemolyticus* (Yu *et al.*, 2022). Terpenoid compounds are also one of the compounds that are effective in antibacterial activity because of their ability to strengthen other antibacterial compounds. In addition to terpenoid content, the results of phytochemical tests on *S. beccariana* stem extract showed the presence of five classes of active compounds, namely alkaloids, flavonoids, saponins, tannins, and steroids.

Alkaloids inhibit bacteria by damaging bacterial cell walls that interact with DNA, inhibit protein synthesis, and prevent the formation of strong cell walls, thus causing cell lysis or destruction (Marbun & Situmorang, 2020). Flavonoids, which are one of the main groups of phenol compounds, are effective against viruses, bacteria, and fungi by damaging cell walls, stopping bacterial movement, and disrupting energy metabolism and bacterial respiration systems (Bontjura *et al.*, 2015). Flavonoids and alkaloids are widely reported as the most effective compounds in antibacterial activity with strong mechanisms of action (Huang *et al.*, 2022).

Steroid compounds act by penetrating the lipophilic layer of the membrane, causing a decrease in the integrity of the membrane structure and disrupting cell morphology, leading to bacterial cell rupture (Kumalasari *et al.*, 2020). Saponins increase the permeability of the cell membrane by interacting directly with sterols in the membrane, leading to leakage of proteins and enzymes from the cell and eventual lysis of the bacteria (Elsa *et al.*, 2023). Tannins are bactericidal by binding to the cell wall and essential proteins, disrupting microbial adhesion

and inactivating vital enzymes in the cell structure (Isnaini *et al.*, 2018). Steroids have a strong mechanism of action in damaging the integrity of bacterial lipid membranes directly, so the absence of steroid compounds in tengkawang bukit extract can reduce the synergy between bioactive compounds (Batubara *et al.*, 2021).

Thus, the higher the concentration of the extract, the more active compounds diffuse into the media, expanding the inhibition zone area and increasing its antibacterial effect. This is in accordance with the results of the research by Wanda *et al.* (2023) which states that the higher the concentration of the extract, the higher the diameter value of the inhibition zone formed. This previous research reported that an inhibition zone of 5.4-6.5 mm was produced by a mangrove stem extract at a concentration of 1-4 mg per disk against *V. parahaemolyticus*, while this study showed a larger inhibition zone, which was between 9.04 ± 0.48 mm to 10.75 ± 0.26 mm at lower concentrations. This difference suggests that tengkawang bukit stem extract has more effective antibacterial potential and could be a natural candidate for the development of new antimicrobial agents.

CONCLUSIONS

Based on the results of the study, tengkawang bukit stem extract was shown to have strong antibacterial activity against *V. parahaemolyticus*, indicated by the formation of a wider zone of inhibition as the extract concentration increased in the disc diffusion test. The high yield of the extract (17%) indicated the availability of significant amounts of bioactive compounds. Phytochemical screening revealed the presence of five major classes of active compounds, namely alkaloids, flavonoids, saponins, tannins, and steroids, in addition to the main content of terpenoid compounds such as asiatic acid, oleanolic acid, and lupanon. This approach could help reduce reliance on synthetic antibiotics and promote sustainable fisheries. To obtain more comprehensive results and support the development of this extract as an

antibacterial agent, further studies are recommended. Quantitative phytochemical analysis of the *tengkawang bukit* extract needs to be conducted to determine the levels of the main bioactive compounds responsible for its antibacterial activity. In addition, in vivo testing on target organisms such as shrimp or fish that are susceptible to *V. parahaemolyticus* is important to evaluate the biological effectiveness and potential direct application of the extracts in aquaculture systems.

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AUTHOR CONTRIBUTION

HA: conceptualization, methodology, investigation, data management, formal analysis, and initial draft writing; ATM: supervision, methodology, and manuscript review and editing; GM: supervision, validation, and manuscript review and editing; MA: conceptualization, methodology, supervision, validation, and review and editing of the manuscript; NSA: conceptualization, methodology, and review.

18 DECLARATION OF COMPETING INTEREST AND USE GENERATIVE AI

The authors declare no conflict of interest.

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