

INHIBITION OF *Vibrio harveyi* BY MENIRAN (*Phyllanthus niruri* L.) LEAF EXTRACT IN LABORATORY TESTS

Inhibisi *Vibrio harveyi* oleh Ekstrak Daun Meniran (*Phyllanthus niruri* L.) dalam Uji Laboratorium

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ABSTRACT

Vibrio harveyi is a Gram-negative bacterium that causes vibriosis in shrimp, which can inhibit growth and trigger mass mortality in the larval phase. Various natural compounds such as flavonoids, alkaloids, saponins, and tannins are known to be able to suppress the growth of pathogenic bacteria. *Phyllanthus niruri* L. leaves have the potential to be antibacterial because they contain various bioactive compounds. This study aims to determine the effect of meniran leaf extract in inhibiting the growth of *Vibrio harveyi* bacteria. This research method used a Completely Randomized Design (CRD) with the concentration of meniran leaf extract (*Phyllanthus niruri* L.) in treatments A, B, C, D, E, and F of 0 ppm, 5,000 ppm, 10,000 ppm, 15,000 ppm, 20,000 ppm and Oxytetracycline 30 μ (control), replications 3 times. The results showed that all treatments of meniran leaf extract were able to inhibit the growth of *Vibrio harveyi* bacteria ($P < 0.05$). The highest inhibitory diameter was obtained by treatment E, namely 13.41 mm, followed successively by D at 12.57 mm; C at 11.29 mm; and the lowest in treatment B at 9.43 mm. Based on ANOVA analysis, meniran leaf extract significantly inhibited *Vibrio harveyi* bacteria.

Keywords: Antibacterial, Extract, *Phyllanthus niruri* L., *Vibrio harveyi*, Vibriosis

ABSTRAK

Vibrio harveyi adalah bakteri Gram-negatif penyebab vibriosis pada udang, yang dapat menghambat pertumbuhan dan memicu kematian massal pada fase larva. Berbagai senyawa alami seperti flavonoid, alkaloid, saponin, dan tanin diketahui mampu menekan pertumbuhan bakteri patogen. Daun meniran (*Phyllanthus niruri* L.) berpotensi sebagai antibakteri karena mengandung berbagai senyawa bioaktif. Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak daun meniran dalam menghambat pertumbuhan bakteri *Vibrio harveyi*. Metode penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan konsentrasi ekstrak daun meniran (*Phyllanthus niruri* L.) pada perlakuan A, B, C, D, E, dan F sebesar 0 ppm, 5.000 ppm, 10.000 ppm, 15.000 ppm, 20.000 ppm dan Oxytetracycline 30 μ (kontrol), ulangan sebanyak 3

kali. Hasil penelitian menunjukkan bahwa semua perlakuan ekstrak daun meniran mampu menghambat pertumbuhan bakteri *Vibrio harveyi* ($P < 0,05$). Diameter daya hambat tertinggi diperoleh perlakuan E yaitu 13,41 mm diikuti secara berturut-turut D sebesar 12,57 mm; C sebesar 11,29 mm; dan terendah pada perlakuan B yaitu 9,43 mm. Berdasarkan analisis ANOVA, ekstrak daun meniran menghambat bakteri *Vibrio harveyi* secara signifikan.

Kata Kunci: Antibakteri, Ekstrak, *Phyllanthus niruri* L., *Vibrio harveyi*, Vibriosis

INTRODUCTION

Shrimp is an important fishery commodity in Indonesia, highly sought after and with high economic value, both for domestic and international markets. Shrimp are popular with the public, especially in South Sulawesi and the surrounding areas. However, shrimp farming often encounters various problems that lead to failure. One of the main causes of failure in cultivation activities is pest and disease attacks (Linayati et al., 2024).

One type of bacteria that most often causes disease and death in aquaculture systems is *Vibrio* sp. According to Hamzah (2019), the *Vibrio* sp. bacteria that most frequently infect shrimp and fish are luminous bacteria, namely *Vibrio harveyi*. These bacteria can trigger vibriosis, which can inhibit the growth of shrimp and fish and even cause death (Mahulauw et al., 2022).

Efforts implemented to treat vibriosis in shrimp include the administration of antibiotics or chemicals. However, the increased use of these substances has actually resulted in the emergence of various new problems. Unknowingly, antibiotics can have negative impacts on the environment, pathogenic organisms, and farmed shrimp or fish. Suryaningtyas et al. (2018) stated that repeated use of antibiotics at inappropriate doses can increase resistance levels in microorganisms. Another impact is that antibiotic residues negatively impact shrimp growth and pollute the environment, resulting in decreased water quality in aquaculture ponds (Nurhayati et al., 2021). Efforts to control bacterial diseases in shrimp farming are focused on the use of natural ingredients that are environmentally friendly, readily available, and have a low risk of resistance and side effects.

The use of natural ingredients as antibacterial agents to control bacterial infections in shrimp farming is gaining attention, along with increasing resistance to synthetic antibiotics. *Phyllanthus niruri* L. is a medicinal plant with antibacterial potential, known to contain bioactive compounds. According to Rivai (2013), phytochemical tests showed that *Phyllanthus niruri* L. leaf extract contains several active compounds, such as flavonoids, steroids, tannins, phenolics, and saponins. Furthermore, meniran is also known to contain various other bioactive compounds, including alkaloids, lignin, and triterpenoids. The flavonoids found are quite diverse, such as quercetin, quercitrin, isoquercitrin, astragalol, rutin, and kaempferol-4. This plant also contains several fatty acids, including ricinoleic acid, linoleic acid, and linolenic acid. Furthermore, meniran is rich in vitamin C, potassium, resin, tannin, geraniol, and saponin, all of which play a role in its biological effects through antibacterial activity (Permadi, 2006). These compounds can function as antibacterials (Auliani & Ridho, 2023).

Meniran leaf extract has been shown to be effective in controlling infectious diseases in African catfish (*Clarias gariepinus*) caused by *Edwardsiella tarda* bacteria, as reported by (Setiaji et al., 2013). Previous research also demonstrated the inhibition of ethanol extract of green phyllanthus herb against the growth of *Staphylococcus aureus* bacteria (Dewangga & Qurrohman, 2019). Therefore, research on the effectiveness of phyllanthus leaf extract in inhibiting the growth of other bacterial species, namely *Vibrio harveyi*, in vitro is necessary.

METHODS

This research was conducted at the Takalar Brackishwater Aquaculture Center, Mappakalombo Village, South Galesong District, Takalar Regency, South Sulawesi Province. The materials used include meniran leaves (*Phyllanthus niruri* L.), *Vibrio harveyi*, 96% ethanol, NA media, MHA media, 100% DMSO, paper discs, aluminum foil, sterile seawater, oxytetracycline, filter paper, and alcohol. The equipment used includes an autoclave, incubator, petri dishes, Erlenmeyer flasks, beakers, measuring cylinders, test tubes, vortex mixers, laminar air flow (LAF) apparatus, vacuum rotary evaporators, digital scales, micropipettes, loop needles, tweezers, hotplates, vernier calipers, and microscopes.

This research employs an experimental approach using a laboratory analytical design. This approach is conducted entirely within a laboratory environment to allow researchers to observe phenomena in a controlled manner. Through this design, the research is directed at assessing and testing the existence of a cause-and-effect relationship, namely by observing how a given treatment can cause changes or produce certain impacts on the variables studied. Thus, this design serves to demonstrate the real effect of each treatment applied during the research process (Effendi, 2013). This study used a Completely Randomized Design (CRD), consisting of six treatments: treatment A without meniran leaf extract (negative control), four treatments using meniran leaf extract at different concentrations (B (5,000 ppm), C (10,000 ppm), D (15,000 ppm), and E (20,000 ppm), and F, the use of oxytetracycline (positive control). Each treatment was replicated three times. The concentrations used were determined based on research conducted by Setiaji et al. (2013) with modifications. The parameter observed in the study was inhibition by measuring the diameter of the clear zone formed around the treated disc.

Equipment sterilization. All equipment used in this study was sterilized prior to use to ensure there was no contamination during the experiment, thus avoiding any potential contamination that could affect the results. For glassware, sterilization was performed using a dry heat method using a laboratory oven. The equipment was heated at approximately 170°C for approximately one hour to ensure all microorganisms and other contaminants were eliminated (Toy et al., 2015).

Extraction preparation. *Phyllanthus niruri* L. leaf extract was prepared using the maceration method. The crushed *Phyllanthus niruri* leaves were soaked in a 96% ethanol solution with a 1:5 ratio of *Phyllanthus niruri* leaf powder to ethanol solvent. The solution was stirred using a spatula for 15 minutes to homogenize the solution, and then stored for 24 hours at room temperature. The residue and filtrate were separated by filtration using filter paper. This soaking process was repeated three times until a third filtrate was obtained, ensuring that all compounds in the *Phyllanthus niruri* powder were completely dissolved. Evaporation is carried out to separate the solvent and meniran leaf extract using a vacuum rotary evaporator.

***Vibrio harveyi* bacterial culture.** Nutrient Agar (NA) media was prepared by adding 1.2 grams of NA powder to 60 mL of distilled water, then homogenizing it by stirring and heating it to boiling using a hotplate. The NA media was then sterilized using an autoclave at 121°C for 15-20 minutes. Once sterilized, the media was poured aseptically into Petri dishes, 10-15 mL per dish, then covered and wrapped with plastic wrap to prevent contamination. After cooling, one loop of *Vibrio harveyi* bacteria was taken and streaked onto the medium. The medium was then stored in an incubator at 37°C for 24 hours. The NA media was poured into the medium and streaked aseptically in a Laminar Air Flow (LAF) chamber (Mantol et al., 2023).

Bacterial suspension preparation. A *Vibrio harveyi* bacterial suspension was prepared by taking approximately two loops and reacting them with a 10 mL test tube containing a physiological NaCl solution at a concentration of 0.9%. Mixing until homogeneous using a vortex, the turbidity was adjusted to the McFarland standard of 0.5%, equivalent to 10⁸ CFU/mL (Masloman, 2016).

A series of extract concentrations was prepared. *Phyllanthus niruri* L. leaf extract was prepared using DMSO as a solvent. Dimethylsulfoxide (DMSO) was chosen as the solvent due to its ability to dissolve polar, semipolar, and nonpolar compounds, particularly peptides (Andayani et al., 2016). A total of 0.4 grams of the concentrated *Phyllanthus niruri* leaf extract was weighed and dissolved in 20 mL of DMSO to achieve the highest concentration, 20,000 ppm. The extract, with a concentration of 20,000 ppm, was then diluted to 15,000 ppm, 10,000 ppm, and 5,000 ppm using the formula $M1 \times V1 = M2 \times V2$.

Preparation of MHA media. 7.6 grams of MHA (Mueller Hinton Agar) powder was placed in an Erlenmeyer flask and 200 mL of sterile seawater was added. The medium was heated on a hotplate while stirring until boiling and a homogeneous solution was formed. The medium was then sterilized by autoclaving at 121°C for two hours to ensure it was free from contaminants. After the sterilization process was complete, the medium was poured into Petri dishes, approximately 10 mL per dish, before being used in the next step.

Inhibitory test. 0.1 mL of bacterial suspension (from a dilution of 10^8 CFU/mL) was evenly inoculated onto the MHA medium using a cotton swab. Paper discs were soaked in each concentration of *Phyllanthus niruri* leaf extract, DMSO as a negative control, and oxytetracycline as a positive control, and allowed to stand for 15 minutes. Each MHA medium inoculated with bacteria was labeled. Next, the treated paper discs were placed on the surface of the medium in a Petri dish. After the discs were placed, the Petri dish was incubated upside down at 37°C for 24 hours to allow optimal and controlled microbial growth. Following incubation, observations were made to observe the appearance of a clear area or zone of inhibition around the disc as an indicator of inhibitory activity. The diameter of the formed zone was then measured using a digital caliper to obtain accurate data regarding the resulting inhibitory power (Maryani et al., 2021).

Data analysis. The test data were statistically analyzed using analysis of variance (ANOVA). This test was conducted to determine the extent to which the treatment affected the size of the inhibition zone formed. This allowed researchers to assess the effectiveness of the treatment in inhibiting the growth of microorganisms in the test medium. If the test results show a significant difference, the analysis is continued using the W-Tuckey test. Data are analyzed using SPSS with a significance level of 95% ($P < 0.05$).

RESULTS

Observations on the effect of *Phyllanthus niruri* L. leaf extract on the inhibition of *V. harveyi* bacteria showed visible inhibition, indicated by the presence of a clear zone around the disk diffusion paper. Each treatment produced a different inhibition zone diameter, depending on the concentration of the extract administered. These results can be seen in Figure 1.

The results showed that after 24 hours of observation, the average inhibition zone value produced by *Phyllanthus niruri* L. leaf extract was obtained. This value illustrates the extract's ability as an alternative antibiotic candidate in suppressing the growth of *V. harveyi* bacteria. The results are presented in Table 1.

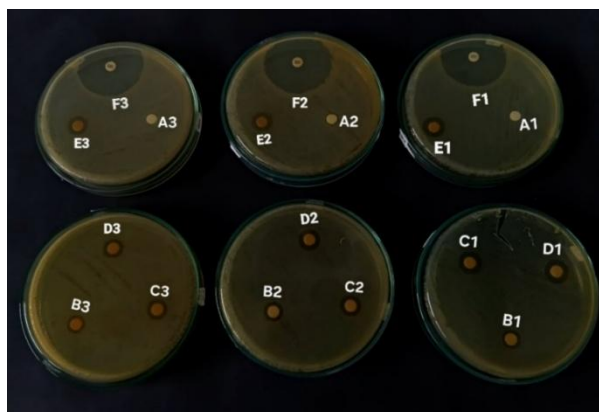


Figure 1. Inhibitory Power of *Phyllanthus niruri* L. Leaf Extract Using the Disc Diffusion Test
 Table 1. Measurement Results of Average Inhibitory Diameter (Clear Zone)

Treatment	Average (mm) \pm SE
A	6.68 \pm 0.33068 ^a
B	9.43 \pm 0.40050 ^b
C	11.29 \pm 0.36768 ^c
D	12.57 \pm 0.50994 ^{cd}
E	13.41 \pm 0.0942 ^d
F	40.85 \pm 0.05774 ^e

Note: The same superscript letter in the same column indicates no significant difference between the treatments.

The inhibitory power of *Phyllanthus niruri* L. leaf extract showed significant differences at different concentrations. The inhibitory power diameter of *Phyllanthus niruri* L. leaf extract in treatment B, at a concentration of 5,000 ppm, had the lowest average inhibition power of 9.43 μ m. At a concentration of 10,000 ppm, the inhibition power increased to 11.29 mm, followed by a more significant increase at 12.57 mm at 15,000 ppm. Treatment E, at a concentration of 20,000 ppm, had the highest average inhibition power of 13.41 mm. Treatment F, a positive control using the antibiotic Oxytetracycline, achieved an average inhibition power of 40.844 mm, while treatment A, without *Phyllanthus niruri* L. leaf extract, as a negative control, had the lowest inhibition power of all treatments, with an average inhibition power of 6.68 mm.

The results of this study indicate a correlation between the diameter of the inhibition zone and the concentration of *Phyllanthus niruri* leaf extract applied. The resulting inhibition zone tends to increase in size as the extract concentration increases.

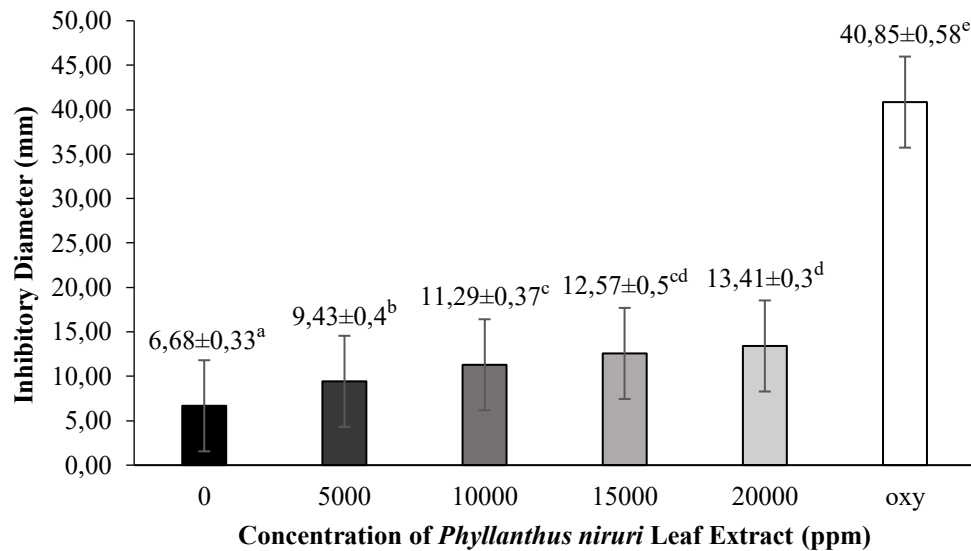


Figure 2. Graph of the Average Inhibition Diameter of *Phyllanthus niruri* L. Leaf Extract Against *V. harveyi* Bacteria

DISCUSSION

Based on the results of the ANOVA analysis, there is a significant effect of the use of meniran extract on the diameter of the inhibition zone of *Vibrio harveyi* bacteria. The results obtained are likely influenced by the activity of antibacterial compounds in meniran leaf extract. These compounds are thought to be able to inhibit and slow bacterial growth. The presence of this antibacterial activity is evident from the appearance of a clear zone around the disc in the disk diffusion test, which indicates an area where bacteria cannot grow due to the effect of the extract. This assumption arose due to research presented by Harmoni et al. (2025) that ethanol extract of meniran leaves (*Phyllanthus niruri* L.) on inhibiting *Stapylococcus epidermidis* bacteria. Where in each treatment has a diameter of inhibition with a very strong category. The effectiveness of inhibition is due to the presence of antibacterial compounds in the ethanol extract of meniran leaves. Meniran leaf extract contains active substances namely alkaloids, phenolics, and steroid compounds (Rakasiwi et al., 2023). Furthermore, Auliani & Ridho (2023) stated that qualitative testing of meniran leaf extract indicated the presence of antibacterial substances, namely alkaloids, flavonoids, tannins, and saponins.

Flavonoids are part of the phenolic compound group known to possess antibacterial properties. Phenolic compounds act as antibacterials by deactivating important proteins or enzymes found in bacterial cell membranes, disrupting cell function and inhibiting bacterial growth. Saponins, on the other hand, also exhibit antibacterial activity, but through a slightly different mechanism: forming complexes with cell membrane components through hydrogen bonds. The formation of hydrogen bonds between bacterial cell proteins and phenolic compounds or saponins can cause changes and damage to the protein structure. This damage then disrupts the stability and permeability of the membrane to ions and important macromolecules, ultimately triggering bacterial cell lysis or rupture (Riyanto & Suhartati, 2019). The antibacterial mechanism of alkaloid compounds involves their components acting as DNA interchelators and inhibiting topoisomerase enzymes in bacterial cells (Karou et al., 2006). Dianita (2011) explained something similar, explaining that alkaloids are known to work by preventing the formation of peptidoglycan cross-links in bacterial cell walls, thus disrupting the structure of the cell wall and ultimately causing some bacterial cells to die. Meanwhile, tannins exert an antibacterial effect by inhibiting the activity of extracellular enzymes needed by bacteria to support their metabolism and growth. Furthermore, these

compounds can reduce or disrupt the availability of substrates needed by bacteria to support their metabolism and growth, thus inhibiting their ability to grow (Widyaningtiyas et al., 2014). These compounds work by attacking the polypeptide structure of bacterial cell walls, disrupting their integrity. This damage can trigger shrinkage or shrinkage of the cell membrane, which then impacts membrane permeability. As a result, the transport of essential substances no longer runs normally, thus inhibiting metabolic activity and bacterial growth. If this disruption continues, bacterial cells can suffer severe damage and ultimately die. (Sari et al., 2017; Pujiastuti & Ermawati, 2013).

Based on the inhibitory response classification according to Kusmarwati & Indriati (2008), it was found that a concentration of 5,000 ppm had moderate bacterial inhibition, while concentrations of 10,000 ppm, 15,000 ppm, and 20,000 ppm had strong inhibition. The positive control (oxytetracycline) had very strong bacterial inhibition, while the treatment without meniran leaf extract (DMSO) as a negative control produced the lowest inhibition, categorized as weak.

The findings in this study indicate that the zone of inhibition that appeared appeared to increase with increasing extract concentration. This increase indicates that the higher the amount of extract used, the stronger the extract's ability to suppress bacterial growth. These results align with the findings of previous researchers, Suyasa et al. (2022), who found that the diameter of the inhibition zone for three single extracts and their combination showed that the lowest classification was at a concentration of 20% and the highest classification was at a concentration of 40%. Similar results were also reported by Zainuddin (2022), who found that *Centella asiatica* leaf extract at concentrations of 5%, 8%, and 10% had inhibition zone diameters of 14.16 mm, 16.63 mm, and 21.44 mm, respectively. These findings indicate that increasing the extract concentration is directly proportional to the number of antibacterial compounds released. The higher the concentration given, the more active compounds work to inhibit bacteria, so that the average diameter of the inhibition zone formed also tends to increase. This can increase the penetration of compounds into bacteria through their respective mechanisms, resulting in an increase in the diameter of the inhibition zone (Andhiarto et al., 2020).

CONCLUSION

Based on the results of the study on antibacterial activity, it can be concluded that meniran leaf extract (*Phyllanthus niruri* L.) has the ability to significantly inhibit the growth of *V. harveyi* bacteria. Variations in extract concentrations provide different inhibitory effects, where the lowest value was obtained in treatment B with a concentration of 5,000 ppm which produced an average inhibitory diameter of 9.43 mm, while the highest value was in treatment E with a concentration of 20,000 ppm, which was 13.41 mm.

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