Effect of Putri Malu (Mimosa pudica Linn) Leaf Extract as an Inhibitor of Vibrio cholerae Growth

Abstract

Indonesia is famous for its diversity of plant sources that are beneficial for health, including the Putri Malu plant (*Mimosa pudica* Linn). Putri Malu leaves contain compounds such as alkaloids, flavonoids, saponins, and tannins, which are antibacterial. This research aims to determine the effect of Putri Malu leaves ethanol extract as an inhibitor against*Vibrio cholerae*. This research is experimental in vivo using the Kirby-Baurer disk diffusion method. The Putri Malu leaf extract concentrations used were 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, and 1.5625 mg/ml. The antibiotic tetracycline 30 mcg was used as a positive control, and distilled water was used as a negative control. The average diameter of the *Vibrio cholerae* inhibition zone was 21.00 mm and 0 mm for the positive control and negative control, respectively. The average diameter of the bacterial inhibition zone for Putri Malu leaf extract was 2.36 mm, 1.26 mm, 0.96 mm, 1.30 mm, 0.80 mm, 0.60 mm, and 0.36 mm, respectively. Based on the data, it can be concluded that Putri Malu leaf extract is less effective in inhibiting *Vibrio cholerae* growth.

Keywords: Putri Malu leaves, *Vibrio cholerae*, antibacterial activity.

Introduction

Since the time of our ancestors, Indonesia has been known for its many spices. Natural ingredients, especially in Indonesia, have been widely used in the health sector for preventive, curative, and rehabilitative purposes against disease. Alternative medicine using plants in Indonesia is currently being used more frequently from year to year, along with the development of technology. The use of plants and other natural ingredients for treatment must be studied further, especially Indonesia's vegetable resources, known for their biodiversity since immemorial[1].

Researchers have carried out many studies to test the contents of various plants or other natural ingredients to determine the efficacy of these natural ingredients. One of them is the Putri Malu plant (*Mimosa pudica* Linn). The phytochemical content in Putri Malu from in vitro and in vivo experiments has been proven to have antibacterial activity, which is beneficial for human body. Research has been tested on the extract of putri malu leaves as an antibacterial in inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Proteus stuarti* in research conducted by Lieken Mehingko et al. in 2010 [2]. The putri malu plant contains alkaloids, flavonoids, saponins, and tannins, inhibiting bacterial growth[3; 4].

The Putri Malu plant has considerable properties for curing various types of diseases. From the leaves to the roots, this plant has medicinal properties as a tranquilizer, expectorant, diuretic, antitussive, antipyretic, anti-inflammatory, and constipation[6; 9].

Putri Malu leaf extract gel has strong antimicrobial power. Based on research conducted

by Abirami et al., Putri Malu extract gel was made in 4 concentrations, namely 30, 60, 90, and 120 ml; the test results showed inhibitory power against fungal and bacterial microbes [10]. The ethanol extract of Putri Malu leaves contains alkaloids, flavonoids, saponins, tannins, triterpenoids, and glycosides[3; 4].

Alkaloids are a group of basic organic substances found in many plants and have pharmacological effects, such as atropine, caffeine, morphine, and nicotine [8]. Alkaloid extract gels obtained from medicinal plants have diverse effects on the host's biological activities, including antimalarial, antimicrobial, antihyperglycemic, anti-inflammatory, and pharmacological effects[7]. Flavonoids are compounds with a specific aromatic core and are widely found in higher plants, often in pigments[9]. Flavonoids have an antimicrobial effect as a plant barrier and a defense response against infections caused by microbes. So, flavonoids are known to have effective antimicrobial effects and can deal with a wide range of microbes[7; 10]. Saponin is a group of glycosides found in plants and will form foam when dissolved in water. Saponin is a detergent-like substance that has antibacterial effects as well as anticancer effects. Saponin has detergent-like properties and can increase the permeability of bacterial cell membranes without damaging them[11;12]. Tannin is an active secondary metabolite compound with antibacterial properties. Tannin is a complex organic substance consisting of phenolic compounds that are difficult to separate and crystallize, precipitate proteins from the solution, and combine with these proteins [13]. Triterpene belongs to the terpenoid class, which chemically has the characteristics of 6 isoprene units with a total of 30 C (carbon) atoms. The hydroxyl group in the A ring of pentacyclic tripene is an important part of antibacterial activity. Pentacyclic tripene contains three A-ring hydroxyl groups commonly found in plants and exhibits significant antibacterial activity [14]. Glycosides are compounds in plants that contain carbohydrates and can be converted through hydrolytic breakdown into sugar and non-sugar components (aglycone) and are named according to the sugar they contain, such as glucoside (glucose), pentoside (pentose), fructoside (fructose) [7]. *Vibrio cholerae*, one of the bacteria most often found in surface water worldwide. Vibrio is an aerobic bacteria that is curved, rod-shaped, motile, and has a flagellum. One example of this type of bacteria is *Vibrio cholerae*serogroups O1 and O139, which cause cholera in humans. These bacteria produce enterotoxins that cause cholera, which is large volumes of watery diarrhea that can cause dehydration and death quickly [15].

Under normal circumstances, *Vibrio cholerae*is pathogenic in humans. Human with normal gastric acidity levels requires an infectious dose of 10 [17] or more bacterial cells in the fluid to cause infection because these bacteria are very sensitive to the acidic atmosphere of the stomach. If bacteria enter with food, the infectious dose becomes 10^2-104 cells because food can neutralize stomach acidity. The treatment process or other conditions that can reduce stomach acidity make a person more sensitive to *Vibrio cholerae*infection[16].

*Vibrio cholerae*produces an enterotoxin with a molecular weight of around 84,000, which consists of subunit A and subunit B. Ganglioside GM1 is a receptor for subunit B, which helps the entry of subunit A into cells. Activated A1 subunit causes an increase in cAMP levels in cells, resulting in prolonged hypersecretion of water and electrolytes. It hampers sodium and chloride absorption by the intestine's microvilli, resulting in diarrhea rich in 20-30 L/day electrolytes, which, if left untreated, will cause dehydration, shock, acidosis, and death[16].

Research Method

This antibacterial effectiveness test study was a descriptive, experimental study using a negative control of sterile distilled water solution and a positive control of 30 mcg tetracycline antibiotic disks as a control for the growth of *Vibrio cholerae*. The serial paper disk diffusion method was used, or the Kirby-Baurer Method. Leaves of Putri Malu (*Mimosa pudica* Linn), which grow wild in the Pangandaran area.This research carried out nine treatments, namely: Negative control, administration of ethanol extract of Putri Malu leaves with concentrations of 1.5625 mg/ml, 3.125 mg/ml, 6.25 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml, and positive control. This treatment was repeated three times. the data was obtained by observing and measuring the inhibition zone on the MHA media using a vernier caliper.

The sample material consists of 1200 grams of Putri Malu plant leaves, which have been cleaned and then dried in the oven for one day at 40°C. After that, grind it with a grinding machine until it becomes powder. The fine powder is then weighed and soaked in 96% ethanol solvent with a ratio of 1:5 while stirring for 2-3 hours, then left for 24 hours, separated and filtered with filter paper, and evaporate the filtrate with a rotary evaporator at temperature 50°C for 3 hours to get Crude extract. To obtain the desired concentration, use serial dilution. To get a 100 mg/ml concentration, take 0.1 gram of thick extract, then mix with 1 ml of sterile distilled water until homogeneous. After that, take 0.5 ml from a concentration of 100 mg/ml, then mix it with 0.5 ml of sterile distilled water to get an extract concentration of 50 mg/ml. Do the same thing to get extract concentrations of 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, and 1.5625 mg/ml.

Making TCBS Agar Media - Sterilize 100 ml distilled water in a 250 ml Erlenmeyer flask, then autoclave for 15 minutes at 121ºC. The TCBS agar medium technically weighed 8.8 g. Then, put it in an Erlenmeyer flask containing it - using a hot plate until it dissolves and shake it until it is homogeneous. Pour 15-20 ml into a Petri dish, wait until the medium hardens, then store the medium in the refrigerator upside down. This media was used to carried out to multiply, rejuvenate, and identify on selective media the *Vibrio cholerae* by taking one cycle of pure culture of the *Vibrio cholerae* into TCBS agar media and then incubating at 37°C for 24 hours in an incubator.

Gram staining was carried out to determine the morphology and type of gram-positive or negative bacteria. The working procedure are: a) Making preparations - Heat the tube over a Bunsen flame and take colonies from TCBS agar media. Physiological NaCl drops on a slide. followed by smear the colony using the loop on the glass preparation and then fix it by passing the preparation over a Bunsen flame 2-3 times; b) Preparing of 0.5% Mac Farland solution - 0.05 ml of 1% BaCl2 solution is mixed with 9.95 ml of 1% H2SO4 solution in a test tube until homogeneous. This solution is used as a standard solution to compare the turbidity of bacterial suspensions. This turbidity is equivalent to or the same as a bacterial suspension of 1.5 x 108 CFU/ml; c) Preparing of bacterial suspension - The culture of Vibrio cholerae on TCBS agar media is taken from the tip of the eye of the tube and suspended in Nutrient Broth, then the turbidity to the 0.5% Mac Farland standard; d) Making MHA media (Mueller Hinton Agar) - 19 grams of MHA powder was put into an Erlenmeyer tube containing 500 ml of distilled water and heated until boiling to dissolve the media. Then, it was sterilized by autoclaving at 121°C for 15 minutes. Then, pour it into a large, sterile petri dish; and e) testing stage is carried out through the following steps: a) Take a bacterial suspension of

0.5 McFarland *Vibrio cholerae* using a sterile cotton swab; b) Then scratch the surface of the MHA media until the bacterial suspension is evenly distributed over the entire surface of the media, then cover the media again; c) MHA media which has been inoculated with *Vibrio cholerae* suspension; d) let stand for a while so that the bacterial suspension seeps into the agar. Each disc has been saturated with varying concentrations of ethanol extract of Putri Malu leaves, concentrations of 1.5625 mg/ml, 3.125 mg/ml, 6.25 mg/ml, 12.5 mg /ml, 25 mg/ml, 50 mg/ml, 100 mg/ml and control, attached to the surface of the agar in the same plate and slightly pressed with tweezers until the disc adheres perfectly to the surface of the media; e) The distance between one disc and another is placed at a distance of ±15 mm and discs that have been attached to the surface of the media must not be moved or shifted; and f) MHA media inoculated with *Vibrio cholerae* bacteria was incubated in an incubator at 37°C for 18-24 hours.

Result and Discussion

Data were obtained descriptively by recording the results of the *Vibrio cholerae* bacteria inhibition test experiment after treatment with Putri Malu (*Mimosa pudica* Linn) leaf extract at various concentrations (1.5625 mg/ml, 3.125 mg/ml, 6.2 mg/ml, 12.5 mg/ml, 25 mg/ml, 50 mg/ml, 100 mg/ml), negative control (sterile distilled water), and positive control (Tetracycline antibiotic). The data was then analyzed in a tabulated form containing details of the size of the inhibition zones formed in several different concentrations of Putri Malu (*Mimosa pudica* Linn) leaf extract and then analyzed and reviewed descriptively. The extraction of 1200 grams of Putri Malu leaves with 96% ethanol solvent resulted in an extract of 31.8 grams.

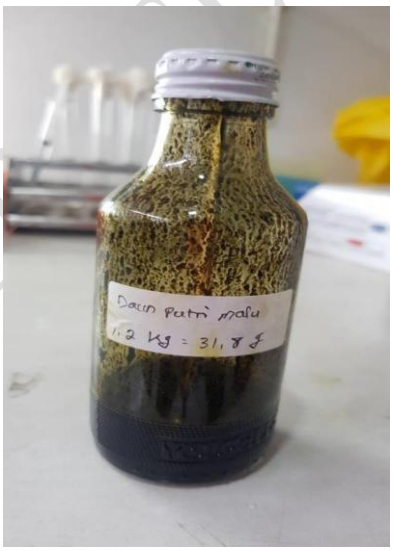

Figure 1. Results of Ethanol Extract of Putri Malu (*Mimosa pudica* Linn) Leaves

Identification of Bacteria on Gram Staining and TCBS Agar Media

a. **Gram Staining** - The morphology of gram-negative rod-shaped bacteria is obtained in gram staining. It can be seen in Figure2.

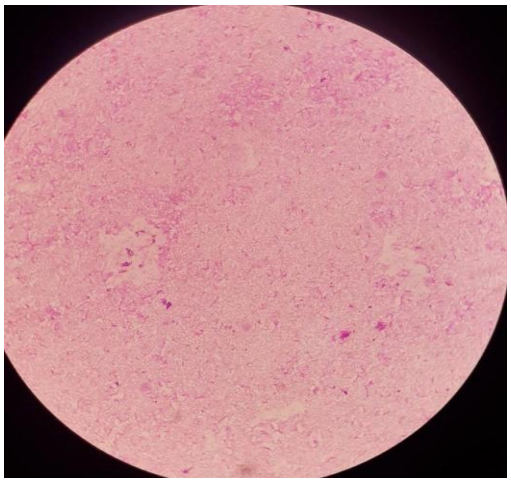

Figure 2. Gram staining of *Vibrio cholerae* (1000x magnificationeeds on TCBS

b. Agar Media - From seeds on TCBS agar media, colonies are round, convex, smooth, and yellow in color. It can be seen in Figure 3.

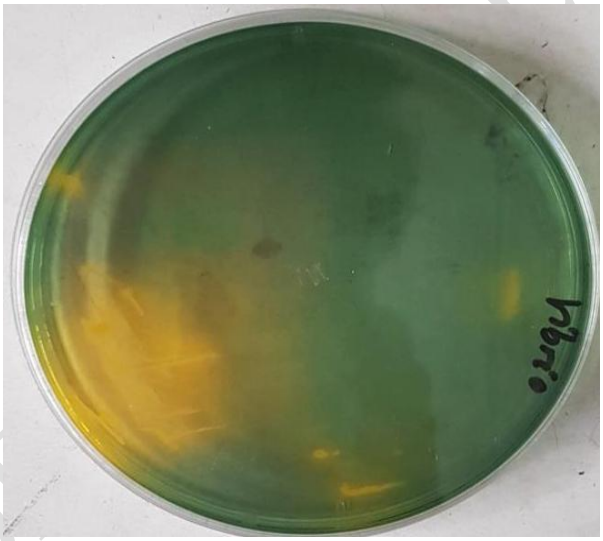

Figure 3. *Vibrio cholerae* colonies on TCBS agar media

c. Antibacterial Activity of Putri Malu Leaf Extract on the Growth of *Vibrio Cholerae*- The antibacterial activity of Putri Malu extract by measuring the diameter of the inhibition zone on Mueller Hinton Agar (MHA) media can be seen in Table 1, and the image of the inhibition zone is shown in figure 4.

Table 1. Average Size of Inhibitory Zone Diameter of Putri Malu Leaf Ethanol Extract in Various Concentrations

Concentration	Average Diameter of the Inhibition Zone Formed (mm)
1,5625	0,36

3,125	0,60
6,25	0,80
12,5	1,30
25	0,96
50	1,26
100	2,36
(+)	21,00
(-)	0

Figure 4. Effectiveness Test of Putri Malu Leaf Ethanol Extract in Various Concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1, 5625 mg/ml) Against *Vibrio cholerae* Inaba.

Discussion

Based on the research results on gram staining and seeding on selective TCBS agar media, the bacteria used in the inhibitory power test of Putri Malu leaf extract were *Vibrio cholerae*. It follows the theory, which states that the morphology of the *Vibrio cholerae* is a gram-negative rod with a characteristic comma shape. However, in long-standing cultures, this bacterium can form a straight rod resembling other gram-negative enteric bacteria. *Vibrio cholerae* grows well on TCBS agar media by forming round, convex, smooth, and yellow colonies (sucrose fermentation) [16]

In this study showed that the largest average diameter of the inhibition zone of Putri Malu leaf extract occurred at a concentration of 100 mg/ml, 2.36 mm and the smallest was at a concentration of 1.5625 mg/ml, 0.36 mm. The inhibition zone formed on the agar medium indicates that the Putri Malu leaf extract has bacteriostatic active compounds.

Flavonoids can damage cell walls and inhibit protein formation, so they can inhibit microbial growth, which results in cell death. Apart from flavonoids, other compounds, such as tannins, can also damage cell membranes. Apart from being antibacterial, tannin compounds also have antifungal properties because they can damage the formation of conidia in fungi. The content of compounds such as alkaloids in putri malu leaves can also denature proteins so that they can interfere with enzyme activity and cause cell death. Saponin compounds include polyphenolic compounds,

which can have antibacterial properties by damaging the cytoplasmic membrane of bacteria. As a result of damage to the cytoplasmic membrane, the necessary food ingredients or nutrients are not distributed properly, so bacterial growth disrupted and can even result in death [20].

It was found that Putri Malu leaf extract with concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.5625 mg/ml has low inhibitory power because the diameter of the inhibition zone formed is less than 10 mm. Factors influencing the formation of inhibition zones include the amount of antibacterial substances in a plant. The antibacterial substances in question include, for example, alkaloids, flavonoids, saponins, and tannins. Many factors influence the amount of substances contained in a plant. According to research by Nurliani Bermawie et al. soil type or regional factors also influence the substance content in a plant [22]. For example, research conducted by Ranjeet Kumar Ranjan et al. conducted phytochemical tests on the leaves and roots of the Putri Malu plant taken in the Kalingavaram area, India. In this research, no alkaloids, flavonoids, and saponins were found. Meanwhile, in another study conducted by Sandhya Madan Mohan et al., they carried out phytochemical tests on the leaves and roots of the Putri Malu plant obtained from the Chhattisgarh area, India. Putri Malu leaves contained flavonoids, alkaloids, saponins, and tannins in the phytochemical test [4; 23].

In general, the increase in the diameter of the inhibition zone is directly proportional to the increase in concentration. In this study, it was found that at concentrations of 50 mg/ml and 25 mg/ml, the average diameter of the inhibition zone formed was smaller than at a concentration of 12.5 mg/ml. This phenomenon was experienced by Aulia Anggita et al. (2018) in research on the antibacterial activity test of ethanol extract of Putri Malu (*Mimosa pudica*) leaves against *Pseudomonas aeruginosa*. According to Ariyanti et al., this phenomenon can occur due to differences in the diffusion of antibacterial compounds in the agar medium, and different types and concentrations of antibacterial compounds also produce different inhibitory zone diameters [20; 24].

The positive control in this study used 30 mcg of tetracycline antibiotic discs and produced the largest average diameter of the inhibition zone, 21.00 mm. According to CLSI (Clinical Laboratory Standards Institute), the level of tetracycline antibiotic resistance is categorized as sensitive if the bacterial inhibition zone diameter is $\geq$ 19 mm, intermediate category if the bacterial inhibition zone diameter is 15-18 mm, and resistant category if the bacterial inhibition zone diameter is $\leq$ 14 mm. Based on this explanation, the antibacterial effect of the tetracycline antibiotic in this study is included in the sensitive category, with the average diameter of the inhibition zone formed being more than 19 mm, namely 21.00 mm. [25] Several previous studies tested Putri Malu leaf extract against several gram-positive and gram-negative bacteria [2; 20; 26].

Table 2. Previous Research Regarding the Antibacterial Activity Test of Putri Malu Leaf Extract

Year	Research Title	Treatment	Results-Conclusions

2010	Testing the Antimicrobial Effect of Putri Malu (*Mimosa pudica* DUCHAAS & WALP) Leaf Extract In Vitro-Lieken Mehingko et al.	In vitro test by administering Putri Malu leaf extract (33%, 50%, and 100%) against *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Proteus stuarti*, and *Escherichia coli* using the Kirby-Bauer method or diffusion test.	The results of the research can be concluded that Putri Malu leaf extract has antimicrobial power against the five types of bacteria tested, namely *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Proteus stuarti*, and *Escherichia coli*.
2015	Inhibition Zone Test of Putri Malu (*Mimosa pudica*) Leaf Extract Against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) Bacteria In Vitro – Nyoman Ririn Chandrika Sari et al	In vitro test by administering putri malu leaf extract (25%, 50%, 75%, 100%) against *Staphylococcus aureus* and MRSA using the Kirby-Bauer method	Putri Malu leaf extract is sensitive in inhibiting the growth of *Staphylococcus aureus* and MRSA bacteria, with the recommended concentration being 25 mcg/ml for *Staphylococcus aureus* and 100 mcg/ml for MRSA
2018	Antibacterial Activity Test of Ethanol Extract of Putri Malu (*Mimosa pudica*) Leaves Against *Pseudomonas aeruginosa* Bacteria – Aulia Anggita et al	In vitro test by administering ethanol extract of putri malu leaves (1%, 2.5%, 5%, 10%) against *Pseudomonas aeruginosa* using the Kirby-Bauer method	Putri Malu leaf extract can inhibit the growth of *Pseudomonas aeruginosa* bacteria and has a weak inhibitory power.

Based on several previous studies, results showed that Putri Malu leaf extract was effective in inhibiting several gram-positive and negative bacteria. However, a study of Putri Malu leaf extract on *Vibrio cholerae* found that the diameter of the inhibition zone formed was less than 10 mm.It can happen because *Vibrio cholerae* produces Cholerae Toxin (CT) and Toxin Coregulated Pilus (TCP), which can protect bacteria from antibacterial compounds [27].

Another factor that could be the cause is that the composition of the *Vibrio cholerae* cell wall consists of three main components, namely outer membrane lipoproteins, which contain protein molecules called porins and lipopolysaccharides. According to Iskandar et al. the porins in the outer membrane of gram-negative bacterial cell walls are hydrophilic, making it more difficult for the component molecules in an extract to enter the bacterial cells. The composition of bacterial cell walls also influences the diffusion of extract compounds into bacterial cells because the cell walls of gram-negative bacteria are more multi-layered than the simpler cell walls of gram-positive bacteria [28].

Conclusion

Based on the results of this research, it can be concluded that the ethanol extract of Putri Malu (*Mimosa pudica* Linn) leaves in various concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, 3.125 mg/ml, 1.5625 mg/ml) is not effective in inhibiting the growth of *Vibrio cholerae* Inaba bacteria. Thus, to develop research regarding the inhibitory test of the ethanol extract of Putri Malu (*Mimosa pudica* Linn) leaves on the growth of *Vibrio cholerae*, it is recommended to carry out a phytochemical test to determine the type and concentration of antibacterial compounds in the ethanol extract of Putri Malu leaves and using different resistance test methods; b) To develop research regarding the inhibition test of Putri Malu (*Mimosa pudica* Linn) leaf extract, different extraction methods, and solvents can be used; and c) To develop research into the inhibitory power of Putri Malu (*Mimosa pudica* Linn) leaf extract, it can be carried out on other pathogenic microorganisms.

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