Quagga
Jurnal Pendidikan dan Biologi

p-ISSN 1907-3089, e-ISSN 2615-5869
Vol 17, No. 1, 2025, pp 24 - 29
Available online https://journal.fkip.uniku.ac.id/quagga
DOI: 10.25134/quagga.v17i1.23

Effect of Water Clover Extract (*Marsilea crenata* Presl.) on the Diameter of the *Staphylococcus epidermidis* Inhibition Zone

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APA Citation: Waluyo, L., Datu, A. L., Nuryady, M. M., & Mahtuti, E. Y. (2025). Effect of Water Clover Extract (Marsilea crenata Presl.) on the Diameter of the Staphylococcus epidermidis Inhibition Zone. *Quagga: Journal of Education and Biology*, 17(1), 24-29. DOI: 10.25134/quagga.v17i1.23.

Received: 17-11-2022 Accepted: 26-08-2024 Published: 04-01-2025

***Abstract**: The incidence of acne in Indonesia reaches 85% with an age range of around 15-24 years. The research aims to analyze the effect of water clover extract (Marsilea crenata Presl.) on the inhibition zone of Staphylococcus epidermidis. This type of research is a true experiment with a quantitative approach. The experimental design is The Post Test-Only Control Group Design. The research population was a pure culture of Staphylococcus epidermidis and the sample used was Staphylococcus epidermidis with a density of 1.5 x 10-8. The research method is the well diffusion method. Data were analyzed using one-way analysis of variance (ANOVA). The results of the research were that the concentration of water clover extract (Marsilea crenata Presl.) 25% volume 30µl, 50µl, 75µl, and 100µl had an average diameter of the inhibition zone, namely 6.41 mm, 7.51mm, 8.36 mm, and 9.70 mm. The conclusion of this research is that there is an influence of water clover extract (Marsilea crenata Presl.) on the diameter of the inhibition zone of Staphylococcus epidermidis and the most effective concentration in inhibiting bacterial growth is 25% extract with a volume of 100 µl with an average diameter of the inhibition zone of 9.70 mm.*

***Keywords**: Acne; Marsilea crenata extract Presl.; Staphylococcus epidermidis; Water clover; Zone of Inhibition.*

1. INTRODUCTION

The incidence of acne in Indonesia reaches 85% with an age range of around 15-24 years (Wibawa & Winaya, 2019). At this age, many people experience acne because the androgen hormone circulating in the blood tends to increase, which can cause the sebaceous glands to produce more sebum. Sebum is an ideal source of nutrition for bacterial growth, so if excess sebum production occurs, bacterial growth will increase and result in acne. According to Lestari et al., (2021), acne can Lestari occur due to several factors including genetic, hormonal, food, cosmetics, stress and one of them is caused by microbial infections.

The *Staphylococcus* genus is the bacteria most found on the surface of the skin and is also found significantly more frequently on skin with acne (Dreno et al., 2017). *Staphylococcus* (S.) *epidermidis* is a species that is often found compared to other *Staphylococcus* species. *S. epidermidis* is found in as many as 70% of acne patients (Rajiv et al., 2013). Other germs that cause acne infections are *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. According to Sari et al., (2020), identification results found *Cutibacterium acnes* in 17 samples (21.2%) in anaerobic culture, while in aerobic culture S. epidermidis was identified in 38 samples (47.5%). Research by Sitohang et al.,

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(2019), stated that the identification results found *S. epidermidis* (50.5%), *S. aureus* (7.7%) in aerobic culture, while *C. acnes* (11%) in aerobic culture. Bacteria from the genus *Staphylococcus*, especially *S. epidermidis*, are most found on normal skin and are significantly more common on acne-prone skin compared to other bacteria (Dreno et al., 2017). The presence of acne not only has a physical impact, but also has a psychological impact on sufferers such as anxiety and depression (Ayudianti & Indramaya, 2014). Given this, it is very important to treat acne.

Efforts to treat acne often use drugs derived from synthetic chemicals. However, inappropriate or excessive use will have a negative impact on the body (Brown & Horswill, 2020). Alternative treatments for acne using herbal medicine are needed. This is because traditional medicines have relatively few side effects compared to synthetic medicines (Sumayyah & Nada, 2017). One alternative for people to treat acne is to use natural ingredients as antibacterials.

The water clover plant (*Marsilea crenata* Presl.) is an aquatic plant that belongs to the type of fern (Pteridophyta). The leaves and stems of water clover contain compounds that can be used as antibacterials (Rachmadiarti, & Trimulyono, 2019). The most abundant bioactive compounds contained in water clover (*M. crenata* Presl.) are compounds that have polar properties (Rachmadiarti, & Trimulyono, 2019). The bioactive components contained in water clover (*M. crenata* Presl.) with 96% ethanol extract include saponins, terpenoids, flavonoids and polyphenols (Putra, 2018). The crude extract of the water clover plant (*M. crenata* Presl.) with the solvent chloroform, ethyl acetate and methanol contain 6 bioactive components, namely alkaloids, steroids, flavonoids, carbohydrates, reducing sugars and amino acids (Rulitasari, & Rachmadiarti, 2020). Some of these bioactive compounds have antibacterial benefits. There has not been much research on the water clover plant (*M. crenata* Presl.) as an antibacterial, so it has the potential to be used as an antibacterial.

This study aims to analyze the effect of water clover extract (*M. crenata* Presl.) on the inhibition zone of S. epidermidis.

2. RESEARCH METHOD

The type of research used is a true experiment (True Experimental Research). The research approach used is a quantitative approach. The experimental design used was The Post Test-Only Control Group Design. The treatment in the experimental method was carried out by administering water clover extract (*Marsilea crenata* Presl.) with different concentration levels to the diameter of the inhibition zone of *Staphylococcus epidermidis*.

The research was conducted at the UPT Herbal Materia Medica Batu Laboratory and the Biomedical Laboratory, Faculty of Medicine, Muhammadiyah University of Malang. The research stages include preparation stage, extract making stage, and testing the inhibitory power of water clover extract (*M. crenata* Presl.)

Preparation and Extract Making Stages

Water clover (*M. crenata* Presl.) cultivated in the Benowo area, Surabaya, East Java. A total of 5 kg of water clover stems and leaves were dried, then ground to obtain water clover simplicia powder. 100 grams of simplicia powder was macerated for 3x24 hours using methanol solvent in a ratio of 1:5 (clover powder: methanol solvent). After that, the maceration results are filtered, and the filtrate is evaporated using a rotary evaporator until a thick extract is obtained.

Inhibitory Test Phase

This inhibition test uses the well diffusion method. Making a bacterial suspension using a cell density meter to obtain a bacterial density of 1.5 x 108 CFU/mL. The *S. epidermidis* used in this research was a pure culture obtained at the Biomedical Laboratory, Muhammadiyah University of Malang.

20 ml of Muller Hinton Agar (MHA) was added to a sterile petri dish. After the agar medium solidified, *S. epidermidis* was inoculated onto the MHA surface using a cotton swab. Next, make holes in the MHA agar media using a cork borer with a hole diameter of 6 mm. Make a concentration of water clover extract (*M. crenata* Presl.) 25%. Add each control treatment (distilled water and minocycline) as well as various concentrations of water clover extract (*M. crenata*

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Presl.) in volumes of 30 µl, 50 µl, 75 µl and 100 µl into the wells using a micropipette and repeat 4 times. Then the petri dish was closed and incubated in an incubator at 37℃ for 1x24 hours. After that, measure the diameter of the inhibition zone that appears in each treatment.

Data Analysis Techniques

After the data was collected, a hypothesis test was carried out which was analyzed using One Way Variance Analysis

3. RESULTS AND DISCUSSION

The results of the *S. epidermidis* inhibition test by administering water clover extract (*M. crenata* Presl.) 25% in various volumes for 1x24 hours are in the following table.

Table 1. Average Inhibition Zone for *Staphylococcus epidermidis*

Treatment	Average Zone of Inhibition (mm)
Minocycline (Control +)	9.88
Aquades (Kontrol -)	0
25% volume 30µl	6.41
25% volume 50 µl	7.51
25% volume 75µl	8.36
25% volume 100µl	9.70

The results of the One-Way Analysis of Variance test showed that there were significant differences in the results of the diameter of the inhibition zone from the different treatments [$F(5,18) = 640.855$, $p < 0.001$]. Based on the test results, it can be said that water clover extract (Marsilea crenata Presl.) influences the inhibition zone of *Staphylococcus epidermidis*.

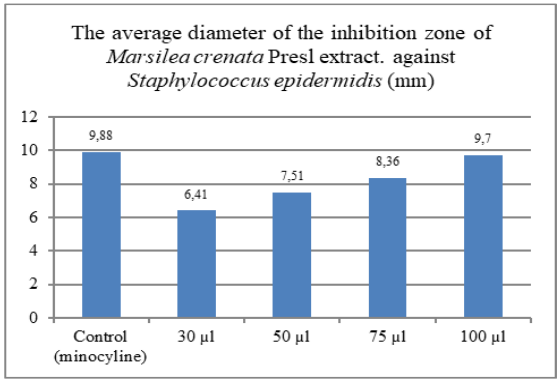

Figure 1. Effect of Water Clover Extract (*Marsilea crenata* Presl.) on the Diameter of the *Staphylococcus epidermidis* Inhibitory Zone

Based on the research results, it shows that water clover extract (*Marsilea crenata* Presl.) has an influence on the diameter of the inhibition zone of *S. epidermidis*. This effect is indicated by the presence or absence of a clear zone that forms around the wells on the agar media. Water clover extract (*M. crenata* Presl.) concentrations of 25% volume, 30µl, 50µl, 75µl, and 100µl, had an average diameter of the inhibition zone, namely 6.41 mm, 7.51 mm, 8.36 mm, and 9.70 mm. The higher the concentration or volume of extract given, the greater the diameter of the inhibition zone formed around the well area on the agar media (Hassanuddin and Salsanus, 2020).

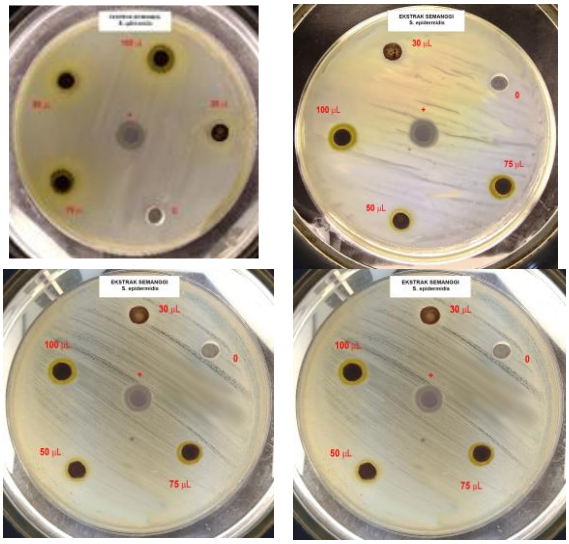

Figure 1. Inhibition Zone of Water Clover Extract (*Marsilea crenata* Presl)

The image above shows that the diameter of the 100 µl treatment inhibition zone is close to that of the antibiotic as a positive control. has maximum preparation in inhibiting bacterial growth. The inhibition zone at a concentration of 25% with a volume of 100 µl is close to the results of the positive control treatment (minocycline) which has a diameter of 9.88 mm. 25% extract with a volume of 100 µl is the treatment with the highest extract volume which has almost the same ability as synthetic chemicals in inhibiting bacterial growth. According to Alam et al., (2017), the higher the concentration or volume of the extract used, the more antibacterial

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compounds it contains. Likewise, the higher the antibacterial content contained, the higher the potential for inhibiting bacterial growth. In this case, the bacteria used as the test is *Staphylococcus epidermidis*.

Water clover extract has the potential to be antibacterial because it contains bioactive compounds, namely secondary metabolite compounds such as alkaloids, flavonoids, steroids (Rachmadiarti & Trimulyono (2019). According to Putra (2018), the bioactive compounds contained in water clover (*M. crenata* Presl.) include saponins, terpenoids, flavonoids and polyphenols. This compound is quite effective in inhibiting bacterial growth. This is because these compounds have antimicrobial activity through various mechanisms and have certain clinical value because their bioactivity generally does not cause resistance (Cheesman et al., 2017).

Alkaloid compounds have several mechanisms for inhibiting bacterial growth, including damaging bacterial DNA and inhibiting the protein synthesis process, reducing the permeability of bacterial cell membranes (Yan et al., 2021). Some of these mechanisms result in damage to the cell membrane and bacterial cell wall.

The mechanism of action of flavonoids as antibacterials is that they form complex compounds with extracellular proteins which then dissolve so that they can damage bacterial cell membranes and reduce cell permeability, followed by the release of intracellular compounds (Amalia et al., 2017). Disruption of the function of the cell wall which gives the cell shape and protects the cell from osmotic lysis will cause bacterial activity to be inhibited.

Steroid compounds have an antibacterial mechanism by damaging lipid membranes, resulting in liposomes leaking which disrupts bacterial activity and causes cells to lyse and become brittle (Sudarmi et al., 2017).

Saponin compounds have a mechanism of action by forming complex compounds on the surface of bacterial cells which cause disruption of the cell membrane, as well as loss of intracellular components (Grzywacz et al., 2020).

Terpenoid compounds work as antibacterials by reacting with transmembrane proteins in the outer membrane of bacterial cell walls, then forming strong polymer bonds resulting in damage (Anggraini et al., 2019).

In this study, water clover extract (*Marsilea crenata* Presl.) can be categorized as an antibacterial with moderate inhibitory power. According to Puspasari et al., (2020), there are 4 categories of inhibition power based on the diameter of the inhibition zone formed, including a weak inhibition zone if < 5 mm, a moderate inhibition zone if 5 mm - 10 mm, a strong inhibition zone if 10 mm - 20 mm, and the zone of inhibition is very strong if > 20 mm.

Based on the mechanism of the compound, types of antibacterials can be classified into 2, namely bacteriostatic and bactericidal. Antibacterials are categorized as bacteriostatic if they can inhibit the growth of bacteria and bactericidal if they can kill bacteria (Wilapangga & Syaputra, 2018).

This antibacterial derived from water clover extract (*M. crenata* Presl.) can be categorized as bacteriostatic and as bactericidal, this depends on the levels of bioactive compounds contained in it. Some antibacterial substances can be bacteriostatic if they contain low levels of bioactive compounds, and bactericidal if they have high levels of bioactive compounds (Dwicahyani et al., 2018).

4. CONCLUSION

The results of the research showed that there was an influence of the concentration of water clover extract (*Marsilea crenata* Presl.) on the diameter of the inhibition zone of Staphylococcus epidermidis, the most effective concentration in inhibiting bacterial growth was 25% extract with a volume of 100 µl with an inhibition zone diameter of 9.70 mm.

5. ACKNOWLEDGEMENTS

The author would like to thank the Directorate of Research and Community Service, Biomedical Laboratory and Biology Education Study Program, University of Muhammadiyah Malang for providing the opportunity to complete this research.

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