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Antimicrobial Potential of Ethanol Extract from Some Plants Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract: The number of reports of bacterial infections that are difficult to treat with antibiotics has led to the need for new alternatives for the treatment of resistant bacterial infections. One alternative is to utilize phytochemical bioactive compounds derived from natural ingredients which contain antimicrobial compounds. Some plants medicine from Sumatra island that should have potential as antimicrobials are the leaves of the matoa plant (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC). The purpose of this study was to discover the antimicrobial properties of the ethanol extracts from the leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L) and jirak (*Eurya acuminate* DC), as well as finding their effective concentrations to inhibit the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. In addition, this study also aimed to find out the active compounds contained in the leaves ethanol extract as antimicrobial against the two test bacteria. Extraction was carried out by maceration method using 96% ethanol solvent. The antimicrobial activity test was done using the Kirby-Bauer disk-diffusion method with concentrations of 10; 20; 30; 40; 50; 60; 70; 80; 90; and 100%. Chloramphenicol was used for positive control and DMSO for negative control. The results show that ethanol extracts from leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) had antimicrobial activity against the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 which was characterized by the formation of an inhibition zone. The ethanol extract from these three plant leaves was effective in inhibiting the growth of the two test bacteria, thus it belongs to a very strong and strong category. Phytochemical screening results show flavonoid compounds, tannins, phenolics and anthraquinones in the ethanol extract from matoa (*Pometia pinnata* Merr) leaves. Ethanol extract from mahang (*Macaranga tanarius* L) leaves contains flavonoids, steroids, tannins, phenolics, and anthraquinones, while jirak (*Eurya acuminata* DC) the contains steroids, tannins, phenolics, saponins, and anthraquinones. Potential ethanol extract of medicinal plants from Indonesia serves as an antibacterial that causes skin infections in *Staphylococcus aureus* and *Pseudomonas aeruginosa* and all extract ethanol of medicinal plants contain metabolite secondary such as tannin, phenolic, flavonoid, steroid, saponins, anthraquinones.

Keywords: Antimicrobial, Inhibition Zone, Ethanol Extract, Phytochemical, *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Introduction

The use of antibiotics with relatively high intensity can cause various problems and it is a global threat to health, especially bacterial resistance to antibiotics. The presence of resistant microbes could be the major cause of infectious disease treatment failure. Indonesia, which is rich in biodiversity, has a great potential for the development of herbal medicine, as an alternative source of antibiotics to suppress infectious diseases and control bacterial resistance to antibiotics.

Based on the diversity of native Indonesian plants, the use of traditional medicines derived from plants has become a hereditary tradition for disease treatment, including infectious wounds, some people believe in modern medical wound treatment, but others still use traditional herbal medicine (Mulatu, 2020). To increase the role of traditional medicine in health services, it is necessary to research, test, and develop the efficacy and safety of a medicinal plant (The potential of metabolite compounds as antimicrobials can be proven by testing the antimicrobial activity of plant extracts against common disease-causing microbes (Mulatu, 2020; Wasihun *et al*., 2023). Examples of microbes that cause wound infections are *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Elisha *et al*., 2017).

Antimicrobial production can be carried out through a chemical synthesis process from plants and microbes. Some plants that have been used for generations as traditional medicine to treat infectious wounds are leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC). Fresh or dried leaves of these plants are finely ground and then smeared on the injured skin. These leaves will speed up the wound drying, stop bleeding, and act as an adhesive. Benefits of these three plants and the increasing number of infectious diseases caused by microbes, it is necessary to conduct research on the test of antimicrobial activity of ethanol extracts from matoa, mahang, and jirak leaves against both *Staphylococcus aureus* and *Pseudomonas aeruginosa in vitro* (Hanafi *et al*., 2020; Sinurat and Alamsjah, 2020; Panda *et al*., 2017).

Several studies have shown that the compounds in plant extracts have the potential as antimicrobials. This is closely related to secondary metabolites contained in these plants, namely steroids, terpenoids, phenol derivatives, flavonoids, and alkaloids (Tunasamy *et al*., 2019). The research objectives are to discover the antimicrobial activity of the ethanol extracts from the leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC), as well as their effective concentrations in inhibiting the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. To find out the active phytochemical compounds contained in the ethanol extract from the leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) as antimicrobials against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Materials and Methods

This research was carried out in several stages, namely preparation and plant leaf sampling, which were taken to the laboratory to make simplisia. Plant leaf extraction was done using 96% ethanol organic solvent. Antimicrobial activity test of the leaves ethanol extract was done against test microbes, which were *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Chloramphenicol was used for positive control and DMSO for negative control.

Plant Leaf Sampling

Leaf samples of matoa (*Pometia pinnata* Merr*)*, mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) in a fresh state were cleaned, then simplicia drying was carried out and extracts were made using the maceration method (Sapiun *et al*., 2020).

Preparation Simplicia and Extraction

The leaf samples were dried, ground using a grinder, and then sieved using a 50-mesh sieve. Next, it was extracted with the maceration method using 96% ethanol solvent and kept at room temperature for 3×24 h while being stirred repeatedly so that the active substance could be extracted perfectly. After 5 days, the extract was filtered and the residue was extracted again using ethanol solvent. This treatment was carried out three times. The extract produced was concentrated using a rotary evaporator to separate the solvent from the active substance to obtain a thick extract. The thick extract obtained was weighed to determine the extract's weight and percentage (Sulaiman *et al*., 2017).

Antimicrobial activity test was done using various concentrations of 10; 20; 30; 40; 50; 60; 70; 80; 90 and 100%. Each extract was further tested for antimicrobial activity in vitro against the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 using the disk-diffusion method Kirby-Bauer.

Antibacterial Activity Test of Ethanol Extracts from Plant Leaves Using the Disk Diffusion Method (*Kirby-Bauer*)

The extracts ethanol of matoa, mahang, and jirak were tested using various concentrations against the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. Bacterial cultures were planted on NA medium, then incubated in an incubator at 37°C for 24 h. A paper disk with a diameter of 0.6 cm was dipped in the plant leaves extract, placed in a petri dish containing the media and culture, then incubated at 37°C

for 24 h. The inhibition zone formed around the paper disk was measured using a caliper. Cloramphenicol solution 30 µL as positive control screening of phytochemical bioactive compounds of ethanol extracts from leaves of matoa (Pometia pinnata Merr), mahang (Macaranga tanarius L) and jirak (Eurya acuminata DC) Qualitatively (Harborne, 1984).

Flavonoids Test

1 mL of leaves ethanol extract was added to 1 mL of 70% ethanol, then added to 0.1 g of mg powder, next 10 drops of concentrated HCl were added. This mixture was shaken vigorously. Observe the color change. A positive test for flavonoids is indicated by the formation of a red, yellow/orange color.

Alkaloids Test

10 mL of leaves ethanol extract was added to 1.5 mL of 2 N HCl, heated for 5 min, and then filtered, into the filter results, 5 drops of Dragendorff's reagent were then added. A positive result for alkaloids is indicated by the presence of orange deposits.

Steroids Terpenoids Test

1 mL of leaves ethanol extract was added to 5 drops of anhydrous acetic acid, then shaken until it was homogeneous. Then 2 drops of concentrated sulfuric acid (H_2SO_4) were added, then shaken and observed for color changes. The positive result for steroids is indicated by the formation of blue green color, while the positive result for terpenoids is indicated by a red color.

Tannins Test

1 mL of leaves ethanol extract was added to 2 mL of distilled water, then 3 drops of 1% $FeCl_3$ were added. The color change was observed. A positive result for tannins is indicated by the solution color change into blackish blue/blackish green.

Phenolic Test

1 mL of leaves ethanol extract was added to 3 drops of 1% $FeCl_3$. A positive result for phenolic is indicated by the colors red, green, purple blue, and dark black.

Saponins Test

1 mL of leaves ethanol extract was added to 1 mL of distilled water, then shaken for 15 min. A positive result is indicated by the formation of a stable foam for 5 min.

Antarquinone Test

Samples of leaves ethanol extract were put in a test tube and then 10% KOH in methanol was added. The color change was observed. A positive result for anatrquinone is indicated by the formation of a yellow-yellow-brown color.

Results

Inhibition Zone Diameters with Disk Diffusion Method (*Kirby-Bauer*)

Antimicrobial activity test results of the ethanol extracts from the leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC), as well as the comparison of commercial antibiotics chloramphenicol against the pathogenic bacteria *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 are presented in Table 1.

Table 1 the ethanol extract from leaves of matoa (*Pometia pinnat*a Merr), mahang (*Macaranga tanariu*s L), and jirak (*Eurya acuminata* DC) all concentrations tested (10; 20; 30; 40; 50; 60; 70; 80; 90 and 100%) had inhibitory activity against the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 the inhibition of bacterial growth was indicated by the formation of an inhibition zone around the disk and the size of the inhibition zone in the media is an indication of bacterial sensitivity response to the test solution.

Table 1: Antimicrobial activity of ethanol extract from leaves of Matoa (*Pometia pinnata* Merr*)*, Mahang (*Macaranga tanarius* L) and Jirak (*Eurya acuminata* DC) *against Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853

No	Ethanol extract concentration (%)	Average inhibition zone diameter (mm) against *Staphylococcus aureus* ATCC 25923			Average inhibition zone diameter (mm) against *Pseudomonas aeruginosa* ATCC 27853		
		Matoa	Mahang	Jirak	Matoa	Mahang	Jirak
1.	10	14.00	17.16	10.75	15.00	19.66	11.00
2.	20	15.00	17.25	11.50	15.00	21.49	11.00
3.	30	15.75	17.33	11.50	17.55	21.33	11.50
4.	40	16.25	17.28	12.50	17.25	22.16	12.50
5.	50	16.50	17.26	12.50	16.25	21.16	13.50
6.	60	18.25	17.75	12.50	16.00	21.66	13.50
7.	70	18.75	17.33	13.00	16.00	21.49	15.00
8.	80	20.75	18.25	15.00	16.75	18.66	15.00
9.	90	19.00	17.16	15.50	18.00	18.66	15.00
10.	100	18.50	18.91	17.50	18.75	19.33	19.00
11.	Control (+)	26.75	31.25	26.25	21.63	30.00	28.75
12.	Control (-)	-	-	-	-	-	-

Table 2: The Results of phytochemical screening of ethanol extracts from leaves of matoa (*Pometia pinnata* Merr), Mahang (*Macaranga tanarius* L) and Jirak (*Eurya acuminata* DC)

No.	Component	Phytochemical test results		
		Matoa leaves	Mahang leaves	Jirak leaves
1.	Alkaloids	-	-	-
2.	Flavonoids	+	+	-
3.	Steroids	-	+	+
4.	Terpenoids	-	-	-
5.	Tannins	+	+	+
6.	Phenolic	+	+	+
7.	Saponins	-	-	+
8.	Anthraquinone	+	+	+

Description: (+) Identified (-) Unidentified

Matoa (*Pometia pinnata* Merr) leaves at 80% concentration of ethanol extract showed the greatest inhibitory activity (20.75 mm) on the growth of *Staphylococcus aureus* ATCC 25923 and the smallest inhibition was obtained at 10% concentration (14.00 mm). For *Pseudomonas aeruginosa* ATCC 27853, the largest inhibition was obtained at 100% concentration (18.75 mm) while the smallest inhibition was obtained at 10 and 20% concentrations (15.00 mm). The ethanol extract of Mahang (*Macaranga tanarius* L) leaves at 100% concentration produced the largest inhibitory activity of 18.91 mm against Staphylococcus aureus ATCC 25923, while the smallest inhibitory activity was obtained at 10% concentration (17.16 mm). The greatest inhibitory activity against *Pseudomonas aeruginosa* ATCC 27853 was obtained at 40% concentration (22.16 mm) and the smallest inhibition was obtained at 80 and 90% concentrations (18.66 mm). The ethanol extract of jirak (*Eurya acuminata* DC) leaves showed the greatest inhibitory activity against *Staphylococcus aureus* ATCC 25923 at 100% concentration (17.50 mm) and the smallest at 10% concentration (10.75 mm). The greatest inhibitory activity against *Pseudomonas aeruginosa* ATCC 27853 was observed at 100% concentration (19.00 mm) while the smallest was observed at 10; 20 and 30% concentrations (11.00 mm).

Based on the extract strength in inhibiting the growth of bacteria, it has been shown that the ethanol extract from matoa leaves at 80% concentration had very strong inhibitory activity against *Staphylococcus aureus* ATCC 25923, while 10; 20; 30; 40; 50; 60; 70; 90 and 100% concentrations were classified in the strong category. As for *Pseudomonas aeruginosa* ATCC 27853, all concentrations of the ethanol extract from matoa leaves had a strong inhibitory activity. In mahang leaves, all extract concentrations were classified in the strong category for *Staphylococcus aureus* ATCC 25923. For *Pseudomonas aeruginosa* ATCC 27853, very strong inhibitory power was observed in 20; 30; 40; 50; 60 and 70% concentrations, while 10; 80; 90 and 100% concentrations were classified as strong. For jirak leaves, all extract concentrations were classified in the strong category, both against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. In the current study, Exract methanol from *Macaranga tanarius* can antibacterial action against Gram positive and Gram-negative bacteria. *S. aureus* and *E. coli* (Chien *et al*., 2022). The provisions of antibacterial power were categorized as follows: Inhibition zone ≥20 mm is in very strong, inhibition zone 10-20 mm is in a strong category, inhibition zone 5-10 mm is in the moderate category, and inhibition zone 5 mm or less is in the weak category (Davis and Stout, 1971; Dharmawan *et al*., 2009; Ouchari *et al*., 2019).

The inhibition zone which is formed against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was due to the presence of secondary metabolites with antibacterial properties. The effect of the antimicrobial agent can be seen from the size of the area that is not overgrown by microbes. The larger the inhibition zone, the greater the ability of ethanol extract from leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Several factors that affect antibacterial activity include antibacterial concentration, the intensity of antibacterial substances, the amount of inoculum, pH of the medium, incubation temperature, the potential of antibacterial substance in the tested solution, and the sensitivity of a bacterium to antibacterial concentration, which can result in size differences of the inhibition zone and the properties of the antibacterial compound. Antimicrobial activity test results of the ethanol extract from leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L) and jirak (*Eurya acuminata* DC) against *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 are shown in Figs 1-2.

The formation of inhibition zones produced by the ethanol extract from leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) occurred due to the presence of active compounds with antimicrobial properties, these compounds play an active role in inhibiting the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. The results of phytochemical screening of ethanol extracts from leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) are presented in Table 2.

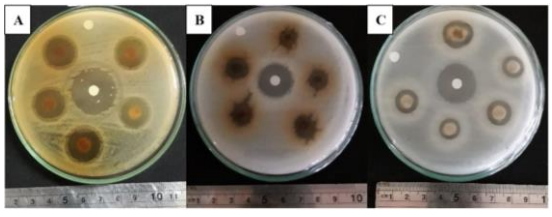

Fig. 1: Inhibitory activity of plant extract against *Staphylococcus aureus* ATCC 25923. Description: (A) matoa leaves extract (*Pometia pinnata* Merr); (B) mahang leaves extract (*Macaranga tanarius* L); (C) jirak leaves extract (*Eurya acuminata* DC)

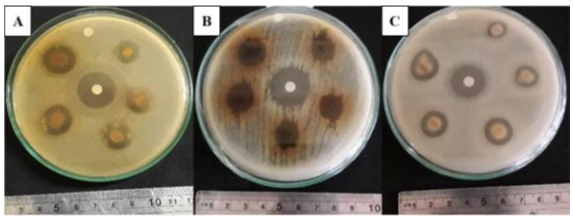

Fig. 2: Inhibitory activity of plant extract against *Pseudomonas aeruginosa* ATCC 27853. Description: (A) matoa leaves extract (*Pometia pinnata Merr*); (B) mahang leaves extract (*Macaranga tanarius* L); (C) jirak leaves extract (*Eurya acuminata* DC)

The results of phytochemical screening in Table 2 showed the presence of secondary metabolites, namely tannins and phenolics in all extracts. Flavonoids were found in ethanol extracts of matoa and mahang leaves, while steroids were found in ethanol extracts of mahang and jirak leaves, saponins in ethanol extracts of Jirak leaves, anthraquinones in ethanol extract of matoa, mahang and jirak leaves. Each of these compounds was proven to have antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Compounds that have antimicrobial activity will diffuse into the agar medium and work according to their respective roles to provide an inhibitory response to the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Discussion

The objective of this study was to screen phytochemicals from some plants in Table 2. Table 2 shows the presence of secondary metabolites as antibacterial. The presence of flavonoid compounds tends to bind proteins which can interfere with metabolic processes by damaging bacterial cell membranes, deactivating enzymes, binding adhesins, and damaging cell membranes (Donadio *et al*., 2021). Flavonoids can also inhibit bacterial metabolism by inhibiting the electron transport chain (Dias *et al*., 2021). The effects of flavonoids on various organisms can indicate why plants containing flavonoids are used in traditional medicine. Phenol compounds cause coagulation or clumping of proteins. Proteins experienced denaturation which resulted in coagulation. In this state, protein can no longer function. phenols coumpound work by denaturing cell proteins and damaging cell membranes (Górniak *et al*., 2019; Donadio *et al*., 2021).

Biharee *et al*., (2020) stated that the cytoplasmic membrane is composed mainly of protein and lipids, which make the membrane susceptible to phenol. Phenol can lower surface tension. When used in high concentrations, phenol works by completely destroying the cytoplasmic membrane and precipitating proteins. In low concentrations, phenol can damage the cytoplasmic membrane which leads to leakage of important metabolites and the inactivation of several bacterial enzyme systems. The action mechanism of steroids in inhibiting microbes is to damage the plasma membrane of microbial cells which can cause leakage of the cytoplasm out of the cell, thus resulting in cell death (Xie *et al*., 2017).

The antimicrobial activity of tannins occurs due to the presence of phenolic hydroxyl groups being able to form stable cross-links with proteins, they can inhibit the work of microbial enzymes (Shamsudin *et al*., 2022). In addition, the antimicrobial effect of tannins is also influenced by their ability to activate microbial adhesion, enzymes, cell membrane transport proteins, and mineral absorption (Sher, 2009; Belhaoues *et al*., 2020). The mechanism of saponin compounds as antibacterial is by lowering the surface tension, resulting in increased permeability or cell leakage, as well as the release of intracellular compounds (Robinson, 1995; Dong *et al*., 2020).

In general, secondary metabolites derived from plants can result in antibacterial activity since they are able to inhibit the work of enzymes used in bacterial metabolism (Othman *et al*., 2019). The use of low concentrations of natural antibacterial extracts will generally interfere with the formation of energy from bacterial cells, but if the concentration is increased, then it will be able to kill bacteria due to the interruption of cell wall proteins and the inhibition of cell wall component formation (Lobritz *et al*., 2015).

The inhibited bacterial growth or bacterial death due to an antibacterial substance can be caused by the inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis, or inhibition of nucleic acid synthesis. Damage to the cell membranes causes disruption of nutrient transport through cell membranes which results in a deficiency of nutrients needed for growth (Li and Xu, 2018).

Factors that affect antimicrobial activity must be considered for the effective use of these antimicrobial substances. In addition, the proportion of each active ingredient produced from the extraction process is also not known for sure. The likelihood is that the active ingredients work alone or all the active ingredients will work together to inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

In this study, chloramphenicol was used as a positive control. This compound is known to have a broad spectrum which effectively inhibits the gram-positive and gram-negative bacteria. The negative control used was Dimethyl Sulfoxide (DMSO) to determine whether there was a solvent effect on the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. DMSO showed no inhibitory response to *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

This has proved that DMSO as a solvent does not have antimicrobial activity and the antimicrobial activity only comes from the test solution, not from the solvent used.

The presence of antimicrobial activity from the ethanol extract of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) leaves are expected to be useful as those ingredients from herbal medicine, especially against antibiotic-resistant pathogenic bacteria.

Conclusion

Based on the results of this study, it can be concluded that the ethanol extracts from leaves of matoa (*Pometia pinnata* Merr), mahang (*Macaranga tanarius* L), and jirak (*Eurya acuminata* DC) contain antimicrobial activity against the growth of *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853. they are classified in the category of very strong and strong. The ethanol extract from the leaves of these three plant species was effective in inhibiting the growth of those two test bacteria because they were classified in the very strong and strong category. Phytochemical screening results showed the present flavonoid compounds, tannins, phenolics, and anthraquinones in the ethanol extract of matoa (*Pometia pinnata* Merr) leaves, flavonoids, steroids, tannins, phenolics, and anthraquinone in mahang (*Macaranga tanarius* L) leaves, as well as steroids, tannins, phenolics, saponins and anthraquinone in jirak (*Eurya acuminata* DC), leaves.

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Author's Contributions

Feskaharny Alamsjah: Ideas in research, participated in all experiments, sample collection coordinated the data analyzed, and contributed to the writing of the manuscript.

Anthoni Agustien: Coordinated the mouse work, and evaluaion data analysis.

Mifthahul Jannah: Designed the research plan and organized the study. Extracting plant sample materials with ethanol solvent using the maceration method, testing ethanol extracts with pathogenic bacteria, testing the content of phytochemical compounds from plant samples, and data analyzed.

Mufidhatul Muqarramah: Sample preparation, making bacterial growth media, making reagents for phytochemical tests observed antimicrobial test results, and phytochemical observations.

Ethics

This article is entirely original and it contains never-before-seen material. The corresponding author certifies that all other authors have read and accepted the work and that there are no ethical contradictions.

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