Phytochemical Screening And Antibacterial Activity Of The Ethanol Extract Of Putri Malu (*Mimosa Pudica* Linn.) Leaves Combined With Klanceng Honey Against *Staphylococcus Aureus* Bacteria

Khairunnisa Dalimunte[1], Anayanti Arianto[2*], Emil Salim[3]

[1]Postgraduate Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[2]Departement of Pharmaceutics, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[3]Departement of Pharmacology Pharmacy, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
*Corresponding Author:
Email: anayanti@usu.ac.id

Abstract.

Mimosa pudica Linn. Leave and honey have been known to have antibacterial activity. The combination of the two is very beneficial as an antibacterial because the hydrogen peroxide content in honey and the flavonoid metabolite compounds in Putri Malu provide a synergistic effect as an antibacterial. The aim of this research was to determine the activity of the ethanol extract of Putri Malu leaves combined with Klanceng honey using the disc diffusion method against Staphylococcus aureus bacteria. The results showed that the resulting extract was thick and blackish-green in colour, with a yield of 25.6%. The results of the phytochemical screening test showed that Putri Malu extract contains alkaloids, flavonoids, glycosides, saponins, and tannins. Characterization results showed that the water content of the extract was 7.28 ± 0.05, water-soluble essence content was 16.03 ± 0.08, ethanol-soluble essence content was 19.65 ± 0.01, total ash content was 4.70 ± 0.00, and essence content acid soluble was 0.17±0.02. Antibacterial testing showed that the best ratio of Putri Malu leaf extract to honey to inhibit Staphylococcus aureus was 9:1, with an inhibitory value of 13.60 ± 0.40 mm. In conclusion, the combination of Putri Malu leaf extract with honey strongly inhibits the growth of Staphylococcus aureus bacteria.

Keywords: *Putri Malu leaves, Klanceng honey, Staphylococcus aureus bacteria and antibacterial.*

I. INTRODUCTION

Antibacterials are compounds that can either inhibit or eliminate the microorganisms that cause infections. Pathogenic bacteria or other germs cause infections by penetrating the body's tissues and multiplying there. Antibacterial substances often work by breaking down cell walls, altering the permeability of membranes, interfering with the creation of proteins, and preventing the function of enzymes. [1].Human pathogenic Gram-positive *Staphylococcus aureus*, are germs that cause illness. *Staphylococcus aureus* is responsible for 70% of nosocomial infections. Invasive skin and soft tissue infections, including pneumonia, osteomyelitis, meningitis, and endocarditis, can be infected by *Staphylococcus aureus*. *Staphylococcus aureus* is resistant to several drugs, such as vancomycin, β-lactamase, methicillin, nafcillin, and oxacillin. [2].

Putri Malu is a plant that belongs to the category of weeds and wild plants. This plant has potential applications in the management of skin infections and wound swelling. This is because the plant contains various chemicals, including tannins, alkaloids, flavonoids, and saponins. The leaves of the Putri Malu plant are frequently employed as a medicinal ingredient due to their high concentration of secondary metabolite chemicals, which have antibacterial properties [3]. Honey's vitamin content is commonly utilised to boost an individual's immune system, making it a natural substance that has been demonstrated to be used as a natural cure. Adding honey to Putri Malu leaves may synergistically inhibit the growth of *Staphylococcus aureus*.

II. METHODS

2.1 Materials

The leaves of *M. pudica* were collected on Jl. Kapten Sumarsono, Helvetia, Medan, Indonesia. *Staphylococcus aureus* ATCC 8027 was obtained from the Microbiology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. Muller Hinton Agar (Merck, Germany), Muller Hinton Broth (Merck, Germany), and dimethylsulfoxide (Merck, Germany) were employed as media.

2.2 Making Simplicia

After being cleaned clean and cleansed from pollutants, samples are chopped into small pieces and dried in a drying cabinet until dry, brown and brittle.

2.3 Making Ethanol Extract of Putri Malu Leaves

The maceration process was used to produce the ethanol extract of Putri Malu leaves at a 1:10 ratio. One part of Putri Malu leaf simplicia powder is mixed with ten parts of 70% ethanol solvent (pa) in a container [4]. Soaked for five days, stirred regularly, then filtered. The filtrate was remacerated with ethanol, steeped for two days, and then evaporated using a rotary evaporator to produce a thick extract [5], [6].

2.4 Phytochemical Screening of Simplicia and Ethanol Extract of Putri Malu Leaves

Phytochemical screening was performed on simplicia and ethanol extracts of Putri Malu leaves, including alkaloid, glycoside, saponin, flavonoid, tannin, and steroid/triterpenoid [7].

2.4.1 Identification of Alkaloids

Simplicia powder was mixed with 2N HCL and distilled water, heated over a water bath, filtered three times, and then placed in a test tube with each of Mayer, Dragendroff, and Bouchardat reagents. If an alkaloid precipitates in two of the three reagents, it is considered positive [8].

2.4.2 Identification of Saponins

Simplicia powder was placed in a test tube, boiling water was added, and shaked vigorously for 10 seconds. Saponin is positive if an excellent foam is formed at least 10 minutes at 1-10 cm high and does not dissolve when 2N HCl is added [9].

2.4.3 Identification of Tannins

Simplicia powder was macerated for 15 minutes in distilled water before being filtered. After diluting the filtrate till it is colourless, two drops of $FeCl_3$10% solution are taken. If the filtrate contains blue and green tints, it is considered positive for tannin [10].

2.4.4 Identification of Flavonoids

Simplicia powder was dissolved in hot water before being boiled and filtered while still hot. Magnesium powder, a strong HCl solution, and amyl alcohol were mixed into the filtrate. If a red or yellow-orange hue develops on the amyl alcohol layer, it is considered positive for flavonoids [11].

2.4.5 Identification of Steroids/triterpenoids

Simplicia powder was macerated and filtered with an n-hexane solution. The filtrate was evaporated, and the remaining liquid was mixed with Liebermann-Burchad reagent through the cup walls. If a red hue forms and then transforms to a greenish blue, it is considered positive for triterpenoids or steroids [12], [13].

2.4.6 Identification of Glycosides

Simplicia powder was dissolved in an ethanol solvent, evaporated in anhydrous acetic acid, and then a small amount of concentrated H_2SO_4 was slowly introduced through the test tube wall. If a blue or green tint forms in the filtrate, it is considered positive for glycosides [14].

2.5 Characterization of Simplicia

The characteristics of simplicia powder were examined, which involves determining the water content, the water-soluble essence content, the ethanol-soluble essence content, the total ash content, and the acid-insoluble ash content [15], [16].

2.5.1 Water Content

The water content was determined using the azeotropy method using toluene. After placing the sample in a 500-mL bottom flask, 200 mL of toluene and 2 mL of water were added, and the sample was distilled for 2 hours [17].

2.5.2 Water soluble essence content

The sample was macerated in chloroform 20 mL for 24 hours, the filtrate was evaporated till dry in an evaporator cup at 105°C until the weight remained constant [18].

2.5.3 The amount of soluble essence in ethanol

After macerating the sample for 24 hours in 96% ethanol, 20 ml of the filtrate was evaporated till dry in an evaporator at 105°C until the weight remained constant [17].

2.5.4 Total ash content

The sample was placed in a heated and tared porcelain and then flattened. The crucible is gradually lit until the charcoal runs out. The crucible is heated at 600°C for 3 hours, then cooled and weighed till a steady weight was attained [19].

2.5.5 Insoluble acid ash content

The ash obtained during the total ash content determination was heated for 5 minutes in 25 mL of 2 N hydrochloric acid. The insoluble component in the acid was collected, filtered through ash-free filter paper, and then washed in a porcelain dish with hot water. The residue and filter paper were heated at 60°C until they attained a consistent weight, then cooled and weighed [19].

2.6 Sterilization of Apparatus and Materials

The glassware used in this study was sterilised in an oven at 170°C for 1 hour using the dry heat procedure. The media used in this study was sterilised for 15 minutes in an autoclave at 121°C using the moist heat method [20], [21].

2.7 Making a test solution with an ethanol extract of Putri Malu leaves and Klanceng honey

The ethanol extract of Putri Malu leaves was mixed with Klanceng honey in the following ratios: Honey: Extract (10:0); (9:1); (8:2); (7:3); (6:4); (5:5); (4:6); (3:7); (2:8); (1:9); (0:10); each combination of sample ratios dissolved in dimethylsulfoxide (DMSO) [1], [2].

2.8 Antibacterial Activity Test

The agar diffusion method was used to assess the antibacterial activity of the combination of ethanol extract of Putri Malu leaves with Klanceng honey. Bacterial inoculum was placed as much as 0.1 mL in a petri dish, and then 15 mL of thawed sterile agar nutrient medium was added, when the temperature reached 45°C, the medium was homogenise and leave until the media hardened [22]. The agar diffusion method was used to assess the antibacterial activity of the combination of ethanol extract of Putri Malu leaves mixed with Klanceng honey. 0.1 mL of bacterial inoculum was put onto a petri dish, and then 15 mL of thawed sterile agar nutrient medium was added, when the temperature reached 45°C, the mixture was homogenised and leave till the media hardened [23].

III. RESULT AND DISCUSSION

3.1 Extraction Results

The maceration of 250 g of putri *Mimosa pudica* Linn. in 70% ethanol resulted in a 50.43 g thick extract with a yield of 25.6% w/w. The yield compares the metabolites obtained after the extraction procedure and the sample weight. If the value is greater than 10%, the yield is considered good. The extracted yield was certified good because it was greater than 10% [24].

3.2 Phytochemical screening

Table 1 shows the results of the phytochemical screening of *M. pudica* Linn. leaves extract.

Table 1. Phytochemical Screening Results of Putri Malu Leaves Ethanol Extract

No.	Secondary Metabolites	Reagents	Results
1.	Alkaloids	Dragendroff Bouchardat Meyer	+ + +
2.	Flavonoids	Mg powder+ Amil Alkohol + Concentrated HCl	+

3.	Glycosides	Molish + H2SO4	+
4.	Saponins	Hot water/mixed	+
5.	Tannin	FeCl3	+
6.	Triterpenoids/Steroids	Lieberman-Bourchat	+

According to Table 1, the ethanol extract of Putri Malu leaves contained flavonoids, glycosides, saponins, tannins, and triterpenoids/steroids. The flavonoid and tannin components found in the samples are antimicrobial [25].

3.3 Examination of Simplicia Powder Characteristics

A characterisation examination was performed on Putri Malu leaves. Table 2 shows the results of water content, water-soluble essence content, ethanol-soluble essence content, ash content, and acid-insoluble ash content of Putri Malu leaves simplicia powder.

Table 2. Data on the Characterization of Simplisia Putri Malu Leaves

No.	Parameter	Results* (%)	Indonesian Herbal Pharmacopoeia Edition II Requirements (MOH RI, 2017)
	Water content	7.28 0.05	10%
2.	Water Soluble Essence Concentrations	16.03 0.08	4.4%
3.	Ethanol Soluble Essence Levels	19.65 0.01	15.4%
4.	Total Ash Composition	4.70 0.00	16.6%
5.	Content of Acid Insoluble Ash	0.17 0.02	0.7%

Information:

(*) : The results represent the mean of three measurements.

Determination of the water content of the simplicia was done to determine the amount of water contained in the simplicia as well as the quality of the simplicia because water content is related to the possibility of fungal or mould growth. The findings of the water content determination was 7.28%. Water with a content greater than 10% can be a favourable medium for the growth of germs, fungi, or insects, accelerating the damage to simplicia [26].The content of simplicia powder was determined using two solvents, namely water and ethanol. Determining the levels of water-soluble essences wa done to determine the level of polar chemical compounds in simplicia, whereas determining the level of soluble essences in ethanol was done to determine the levels of soluble compounds in ethanol, both polar and non-polar compounds. The water-soluble essence percentage of simplicia was 16.03%, while the ethanol-soluble essence level was 19.65%. This demonstrates that non-polar chemicals outnumber polar components in the Putri Malu leaave simplicia [27].The ash content determination was performed to determine the internal mineral content (physiological ash) derived from plant tissue present in simplicial [26]. The acid-insoluble ash content is a measure used to determine the quantity of silicate, particularly sand, in simplicia. This was performed by dissolving the entire ash in hydrochloric acid. The analysis of ash content in simplicia revealed a cumulative ash content of 4.70% and an acid-insoluble ash content of 0.17%.

3.4 Antibacterial activity

Testing of the antibacterial activity of the ethanol extract of Putri Malu leaves combined with Klanceng honey showed an increase in inhibitory activity compared to the ethanol extract of Putri Malu alone or the single Klanceng honey. All concentrations showed effective antibacterial activity because they had an inhibition zone value above 10 mm. The results of antibacterial effectiveness of a combination of an ethanol extract of Putri Malu leaves, and Klanceng honey can be seen in Table 3 below.

Table 3. Inhibitory Zone Diameter of Ethanol Extract of Putri Malu Leaves in Combination with Honey Against *Staphylococcus aureus* Bacteria

Sample	Comparison of Concentrations MK : EEDPM	SA X±SD
A	10:0	10.70 ± 0.44
B	0:10	11.80 ± 0.92
C	1:9	13.60 ± 0.40
D	2:8	13.07 ± 0.15
E	3:7	12.90 ± 0.26
F	4:6	12.73 ± 0.31
G	5:5	12.67 ± 0.21
H	6:4	12.23 ± 0.06
I	7:3	12.07 ± 0.21
J	8:2	12.03 ± 0.12
K	9:1	11.57 ± 0.68
L	Positive Control	29.70 ± 0.26
M	Negative Control	0.00 ± 0.00

Information: The inhibitory zone of the combination of Putri Malu Leaf Ethanol Extract and Honey against *Staphylococcus aureus* bacteria(A = Honey 10, B = Extract 10, C = 1:9, D = 2:8, E = 3:7, F = 4:6, G = 5:5, H = 6:4, I = 7:3, J = 8:2, K = 9:1, L=Positive control, M=Negative control)

The antibacterial test findings revealed an inhibition zone greater than 10 mm. There are three inhibition zone classifications: 5 mm (no response), 5-10 mm (moderate), > 10 mm (strong), and > 20 mm (extremely strong)[28]. Only the 9:1 (M: E) combination has a value less than 12 mm. All concentrations have inhibition zones within the effective range. According to Mehingko et al. (2010), ethanol extract of Putri Malu leaves demonstrated high efficacy against five bacteria, including *Pseudomonas aeruginosa, Enterobacter cloacae, Staphylococcus aureus, Escherichia coli, and Proteus stuarti*, at a concentration of 100% [25].Putri Malu leaves contain secondary metabolite chemicals such as flavonoids and tannins, which can be employed as antibacterials. These compounds can inhibit microbial activity by damaging cell membranes and thus inhibiting bacterial growth; alkaloids bind to cell DNA and disrupt bacterial cell function; flavonoids irreparably denature bacterial cell proteins and cell membranes; and saponins damage the cytoplasmic membrane and then kill bacterial cells. Tannins, flavonoids, and triterpenoids in Putri Malu leaves extract can disrupt the cytoplasmic membrane through various methods of action (Utami et al., 2021).

Flavonoid molecules are lipophilic, which causes membrane damage in bacteria.Flavonoids serve as antibacterials by forming complex compounds with external proteins and soluble proteins to disrupt bacterial cell membranes, followed by the release of intracellular chemicals (Kumalasari et al., 2020).The combination of an ethanol extract of Putri Malu leaves, and Clanceng honey (9:1) is the most effective combination treatment, according to the one-way ANOVA analysis followed by the Tukey test.According to the Indonesian Pharmacopoeia Edition VI, an inhibition zone of 12-14 mm is considered strong. Only the 9:1 (M: E) combination has a value less than 12 mm. All concentrations have inhibitory zones within the effective range. In vitro, testing of Putri Malu leaves extract against five microorganisms showed high activity at 100% concentration against *Staphylococcus aureus*, *Enterobacter cloacae*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus stuarti* [25]. Anggita et al. (2018) discovered that ethanol extract of Putri Malu leaves had antibacterial activity against *Staphylococcus aureus* bacteria at a concentration of 10% and an inhibitory zone diameter of 1.01 mm [29].Honey has been shown to have an antimicrobial impact in expediting wound healing.

Klenceng honey, according to Naureen et al. (2022), is honey that has been shown to have a high antibacterial effect due to the presence of hydrogen peroxide, phenol, and flavonoids [30]. According to Cahyadi et al. (2019), the properties and efficiency of Klanceng honey ointment from *Trigona sp*. bees as an antibacterial and wound-healing agent. Apart from mending wounds, it may also be anti-inflammatory because there is no swelling in the wound area, according to studies [31]. Klanceng honey has a significant

antioxidant activity in addition to wound healing. The combination of Putri Malu leaf ethanol extract and Klanceng honey may have a synergistic impact as an antibacterial and wound-healing agent.

IV. CONCLUSION

The phytochemical screening of the ethanol extract of Putri Malu leaves revealed the presence of secondary metabolite chemicals in the form of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, and triterpenoids. Putri Malu leaves' ethanol extract, combined with Klanceng honey, exhibits strong inhibitory efficacy against *Staphylococcus aureus* (>10 mm in diameter).

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