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Antimicrobial Activity Testing of Ethyl Acetate Fraction from *Mikania micrantha* Leaves Against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT

Bacterial infections remain a major global health issue, worsened by rising antibiotic resistance. This study evaluated the antimicrobial activity of the ethyl acetate fraction of Mikania micrantha leaves against Escherichia coli (Gram-negative) and *Staphylococcus aureus* (Gram-positive) using the disc diffusion method. Phytochemical screening identified flavonoids, tannins, saponins, and steroid/triterpenoids in the fraction. The antimicrobial test showed that this fraction inhibited the growth of both bacteria, with larger inhibition zones observed at higher extract concentrations. At 5% concentration, E. coli showed an inhibition zone of 8.43 mm, while at 15%, the zone increased to 12.26 mm. For S. aureus, the 15% concentration produced a 20.43 mm inhibition zone. Statistical analysis confirmed significant differences between concentrations ($p < 0.05$). These results suggest that the ethyl acetate fraction of Mikania micrantha leaves has potential as an antibacterial agent, especially effective against *Escherichia. coli* at 15%.

Keywords : *Mikania micrantha*, Isolated-fraction, Stigmasterol, Antimikrobial

INTRODUCTION

Bacterial infections remain a serious health problem, particularly with the increasing cases of antibiotic resistance. Therefore, the search for new antimicrobial agents from natural sources, such as medicinal plants, is crucial (World Health Organization, 2020). One such plant with potential is *Mikania micrantha*, a climbing plant from the Asteraceae family commonly found in tropical regions, including Indonesia. *Mikania micrantha* is known as an invasive weed, but in traditional medicine, its leaves have been used to treat wounds, skin infections, coughs, and fevers (Ali Khan *et al.*, 2023). The leaves of this plant contain various active compounds such as flavonoids, tannins, saponins, and alkaloids, which are known to exhibit antimicrobial activity (Perawati, Andriani and Pratiwi, 2018).

Several previous studies have shown that the extract of *Mikania micrantha* leaves has antibacterial activity; however, information regarding the activity of its ethyl acetate fraction is still limited. The ethyl acetate fraction is important to study as it can dissolve semi-polar compounds that often have high biological effects (MC *et al.*, 2010).

This study aims to test the antimicrobial activity of the ethyl acetate fraction of *Mikania micrantha* leaves against Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive) bacteria using the disk diffusion method. The concentration variations used are 5%,

10%, and 20%, to observe the relationship between concentration and antibacterial effectiveness. The research results are expected to provide scientific information on the potential of *Mikania micrantha* as a source of natural antibacterial agents.

METHOD

This study uses an experimental approach to isolate and identify antimicrobial compounds from the leaves of *Mikania micrantha*. The main stages include extraction, isolation of active compounds, antimicrobial activity testing, and structural characterization. The leaves of *Mikania micrantha* obtained for this study were identified and verified at UPT Materia Medica Batu, Malang. This determination process aimed to ensure the accurate identification of the plant species used in the research, confirming its authenticity and suitability for further scientific analysis. Here are the tools and materials used in this study:

Tools: The study utilizes several laboratory instruments essential for extraction, isolation, purification, and antimicrobial activity testing. A distillation apparatus is used for extracting compounds from plant materials, while a rotary evaporator helps in evaporating solvents from the extract to obtain a concentrated form. Buchner funnel is applied in the filtration process, and column chromatography assists in compound separation. To facilitate separation under pressure, an aerator is used. Additional laboratory glassware includes Erlenmeyer vacuum flasks, vials, funnels, and analytical balance for weighing samples accurately. The incubator and Petri dishes support microbial culture growth, whereas the autoclave ensures the sterility of all required equipment and media. Measurement instruments like calipers aid in determining inhibition zone diameters.

Materials: The primary material in this study includes n-hexane, ethyl acetate, and methanol extracts derived from the leaves of *Mikania micrantha* plant. The pure compounds obtained through column chromatography are also tested. Various solvents such as n-hexane, ethyl acetate, dichloromethane (DCM), and methanol are employed for extraction and separation processes. Silica gel 60 GF254 serves as the stationary phase in chromatographic separations. Microbiological tests utilize Nutrient Agar (NA) and Potato Dextrose Agar (PDA) (Merck®) as microbial growth media. Sterile distilled water and ethanol 96% help in media preparation. Physiological NaCl solution (0.9%) is used to prepare microbial suspensions. Antimicrobial tests incorporate chloramphenicol discs for bacterial inhibition.

Work Procedure:

1. **Preparation of *Mikania micrantha* Leaves into Simplisia.** The fresh leaves of *Mikania micrantha* are first cleaned under running water to remove dirt and impurities. After cleaning, the leaves are dried using a suitable drying method, either by direct sunlight or using an oven at a low temperature to preserve the active compounds. Once dried, the leaves are cut into smaller pieces in a cutting process to increase the surface area, making subsequent extraction more efficient. The chopped leaves undergo a second drying step to ensure the moisture content is minimized, improving the stability and shelf life of the simplisia. After the drying process, the leaves are ground using a grinding machine or blender to obtain fine powder suitable for pharmaceutical or research applications. The powdered simplisia is then stored in a sealed container, protected from direct sunlight

and kept in a controlled temperature and humidity environment to maintain its stability and prevent degradation (Perawati, Andriani and Pratiwi, 2018).

Fig 1. *Mikania micrantha*

2. **Extraction and Isolation of Active Compounds.** To investigate the antimicrobial potential of *Mikania micrantha* leaves, particularly the ethyl acetate fraction, a systematic extraction and isolation process is employed to obtain bioactive compounds responsible for antibacterial activity. The dried and finely ground leaves of *Mikania micrantha* are subjected to maceration extraction, starting with n-hexane, followed by ethyl acetate, and finally methanol. This stepwise extraction based on increasing solvent polarity facilitates the separation of compounds according to their polarity. Each extract is then concentrated using a rotary evaporator to remove the respective solvents, yielding crude extracts. The ethyl acetate extract, which is the primary focus of this study due to its potential antimicrobial activity, undergoes further fractionation using vacuum liquid chromatography (VLC) and column chromatography. Silica gel is used as the stationary phase, and elution is performed with a gradient of n-hexane, ethyl acetate, and methanol. Fractions showing similar profiles based on thin-layer chromatography (TLC) are pooled and further purified. The final purification of isolated compounds is achieved through recrystallization, aiming to obtain pure constituents that may contribute to the observed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* (Perawati, Andriani and Pratiwi, 2018).
3. **Sterilization of Equipment and Preparation of Media.** Glassware and laboratory tools are autoclaved at 121°C for 15 minutes. Petri dishes, pipettes, and flasks are also sterilized before use. Nutrient Agar (NA) and Potato Dextrose Agar (PDA) are prepared, heated, and autoclaved before being poured into sterile Petri dishes (Smit *et al.*, 2011).
4. **Preparation of Microbial Test Suspensions.** The test microorganisms include *Escherichia coli, Staphylococcus aureus*. The colonies are resuspended in NaCl physiological solution (0.9%) until the turbidity reaches 25% for bacteria (580 nm) (Chiappim *et al.*, 2021).
5. **Antimicrobial Activity Testing Using Agar Diffusion Method:**
    a. **Preparation of Agar Plates:** Microbial suspensions are spread onto solidified Nutrient Agar for bacteria in Petri dishes.
    b. **Application of Test Samples:** Sterile paper discs impregnated with 10 µL of extract or compound solutions at different concentrations (5%, 10%, 15%).

Positive controls: Chloramphenicol discs for bacteria. **Negative controls:** used aquadest to dissolve the test samples. **Incubation and Measurement:** Bacterial plates are incubated at 37°C for 24 hours, while fungal plates are incubated at 25°C for 48–72 hours. Inhibition zones (clear areas surrounding discs) are measured using calipers, indicating antimicrobial potential.

RESULT

a. **Result Preparation of *Mikania micrantha* Leaves:** The dry simplicia obtained from a 2-day drying process weighed 930 grams. The resulting simplicia powder weighed 500 grams. The moisture content of the powder was measured, and the results are presented in Table 1. The powder was then prepared for the extraction process.

Table 1. Moisture Content of *Mikania micrantha* Leaf Powder

Replicate	Initial Weight (g)	Final Weight (g)	Moisture Content (%)
1	5	4.61	7.7
2	5	4.59	8.2
3	5	4.60	8.0
Average			**7.97 ± 0.26**

b. **Extraction and fractionation of Active Compounds**: A total of 500 grams of simplicia powder was extracted with methanol using the maceration method. The extraction process was carried out for 3 days with regular stirring. The filtrate from the extraction, which had been filtered, was then evaporated using a rotary evaporator. The resulting thick extract was then fractionated using the liquid-liquid method and ethyl acetate as the solvent to obtain the desired compounds. The fractionated product was subsequently evaporated using a rotary evaporator to separate the active compound fraction from the ethyl acetate solvent. From the fractionation process, 46 grams of the ethyl acetate fraction were obtained.

c. **Results of Phytochemical Screening:**

Table 2. Chemical compound identification results of *Mikania micrantha* leaves

Compound	Identification Result	Indicator
Flavonoid	Positive	Yellow-purple spot, UV 366 nm, anisaldehyde
Tanin/Phenol	Positive	Blue-black reaction with $FeCl_3$
Saponin	Positive	Purple spot with vanillin
Steroid/triterpenoid	Positive	Green reaction with Liebermann-Burchard

d. Results of Antimicrobial Activity Test of the Ethyl Acetate Fraction of *Mikania micrantha* Leaves against *Escherichia coli* and *Staphylococcus aureus*

The test microbes used in this study were *Escherichia coli* and *Staphylococcus aureus*. The reason for using *Escherichia coli* is that it is one of the many Gram-negative bacteria, so *Escherichia coli* was selected to represent Gram-negative bacteria (Munita and Arias, 2016). On the other hand, the reason for using *Staphylococcus aureus* is that it is one of the many Gram-positive bacteria, and this bacterium also causes skin diseases such as scabies, boils, and others. Literature has mentioned that *Mikania micrantha* may have the potential to treat skin diseases (Tong *et al.*, 2015). The test microbes were suspended in a physiological NaCl solution to achieve a certain turbidity level. For bacteria, the turbidity was adjusted to 25% transmittance at a wavelength of 580 nm, and for fungi, it was adjusted to 90% transmittance at a wavelength of 530 nm. With this turbidity, the growth of the test microbes was not too dense and was evenly spread. The physiological NaCl solution provided an isotonic environment for the test microbes. In an isotonic environment, the concentration of the surrounding fluid is equal to that of the microbial cells, so fluid does not flow out of the cells, nor does the surrounding fluid enter the cells. The results of the testing can be seen in Table 3.

Table 3. Results of Antimicrobial Activity Test of the Ethyl Acetate Fraction of *Mikania micrantha* Leaves

Concentration	Inhibition diameter (mm)							
	Escherichia coli				*Staphylococcus aureus*			
	R_1	R_2	R_3	R-Average	R_1	R_2	R_3	R-Average
5%	8.4	8.5	8.4	8.43	17	17.1	17.1	17.06
10%	12.1	12.2	12.2	12.16	17.8	17.9	17.8	17.83
15%	12.2	12.3	12.3	12.26	20.5	20.4	20.4	20.43
K (+)	21.1	21	21.1	21.06	20.4	20.5	20.6	20.5
K (-)	-	-	-	-	-	-	-	-

Explanation :
R_1 : First Replication
R_2 : Second Replication
R_3 : Third Replication
K (+) : Positive control (chloramphenicol disk)
K (-) : Negative control (distilled water)

DISCUSSION

Based on the results of the phytochemical screening, the ethyl acetate fraction of *Mikania micrantha* leaves was found to contain several groups of secondary metabolite compounds, namely flavonoids, tannins/phenols, saponins, and steroids/triterpenoids. These four compounds are widely known to have potential biological activities, particularly as antimicrobial agents (Meechai *et al.*, 2016). The flavonoid compound showed a yellow-purple

spot reaction on TLC after being sprayed with anisaldehyde reagent and observed under UV light at 366 nm. Flavonoids are known to exert antibacterial effects by disrupting the microbial cell wall and membrane, as well as inhibiting enzymes crucial for bacterial metabolism. A study by *Pharmacognosy Reviews* confirms that flavonoids have the ability to inhibit the growth of various pathogenic microorganisms (Cheesman *et al.*, 2017). Tannin identification showed a blue-black reaction when tested with $FeCl_3$ solution, indicating the presence of phenolic groups. Tannins act as antimicrobial agents by forming complexes with cell wall proteins and microbial enzymes, leading to denaturation and cell death (Daglia, 2012). Research by Fattouch et al. (2018) demonstrated that tannins exhibit significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, including *E. coli* and *S. aureus*. Saponins showed a purple spot result after reacting with vanillin. Saponins are known to reduce surface tension and damage microbial membrane integrity, causing cell lysis (Dai *et al.*, 2021). This finding is consistent with research by Sparg et al. (2017), which found that saponins in medicinal plants act as antibacterials by altering membrane permeability. Screening using the Liebermann-Burchard reagent resulted in a green reaction, indicating the presence of steroid or triterpenoid compounds. These compounds have been reported to exhibit antibacterial activity through the inhibition of membrane lipid synthesis and disruption of the lipid bilayer structure. According to the *Journal of Applied Microbiology*, triterpenoids from tropical plants are effective against several antibiotic-resistant bacterial strains (Takahashi *et al.*, 2019).

The results of the antimicrobial activity test showed that the ethyl acetate fraction of *Mikania micrantha* leaves has the ability to inhibit the growth of *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive), with the inhibition zone diameter increasing as the extract concentration increased. For *Escherichia coli*, the lowest inhibition zone was recorded at a 5% concentration with an average diameter of 8.43 mm, while the highest concentration (15%) resulted in an inhibition zone of 12.26 mm. This demonstrates concentration-dependent antibacterial activity, where an increase in the ethyl acetate fraction concentration correlates with a higher inhibitory effect on bacterial growth.

Meanwhile, the antimicrobial activity against *Staphylococcus aureus* was stronger compared to *Escherichia coli*. At a 5% concentration, the inhibition zone reached 17.06 mm and increased to 20.43 mm at 15%. The larger inhibition zone for *Staphylococcus aureus* compared to *Escherichia coli* suggests that the ethyl acetate fraction of *Mikania micrantha* is more effective against Gram-positive bacteria. This could be due to differences in cell wall structures between the two types of bacteria, where Gram-negative bacteria have a more complex lipopolysaccharide layer, making them more resistant to antimicrobial compounds.

The results of this test were further supported by statistical analysis using SPSS with the One-Way ANOVA method to test the significance of the differences in inhibition zones between the concentration treatments. The ANOVA test results showed a significance value (p-value) of 0.000 for *Escherichia coli* and 0.001 for *Staphylococcus aureus*, indicating a very significant difference between treatment groups ($p < 0.05$). A subsequent Tukey HSD Post Hoc test was performed to identify the differences between concentrations more specifically. The Tukey test results showed that for *Escherichia coli*, the 15% concentration was significantly different from the 5% and 10% concentrations, while for *Staphylococcus aureus*, the 15%

concentration showed no significant difference from the positive control (chloramphenicol), indicating an almost comparable effectiveness. The positive control using chloramphenicol disks resulted in inhibition zones of 21.06 mm for *Escherichia coli* and 20.5 mm for *Staphylococcus aureus*, which were higher than all extract treatments, confirming that chloramphenicol remains superior as an antibiotic. However, the inhibition zone values from the ethyl acetate fraction, especially at the 15% concentration, were approaching the effectiveness of this antibiotic, particularly against *Staphylococcus aureus*. The negative control (distilled water) showed no inhibition zones, confirming that the antimicrobial activity indeed came from the active compounds in the ethyl acetate fraction, not from the solvent or medium. Overall, these results indicate that the ethyl acetate fraction of *Mikania micrantha* leaves has potential as an antibacterial agent, particularly against Gram-positive bacteria, and are supported by statistical analysis showing significant differences between concentrations.

CONCLUSIONS

The ethyl acetate fraction of Mikania micrantha leaves showed antimicrobial activity against *Escherichia coli* (Gram negative) and *Staphylococcus aureus* (Gram positive). Furthermore, it was found that the ethyl acetate fraction more effectively inhibited the growth of *Staphylococcus aureus* compared to *Escherichia coli* at a concentration of 15%.

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