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ANTIBACTERIAL ACTIVITY TEST OF KETUL LEAF ESSENTIAL OIL (*BIDENS PILOSA* L.) AGAINST *SHIGELLA DYSENTRIAE* AND *SALMONELLA TYPHI* BACTERIA

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ABSTRACT

Diarrhea is a condition of defecating three or more times in one day and the stool that comes out is a watery liquid or slightly frothy. This disease is caused by the bacteria Shigella dysenteriae and Salmonella typhi. This study aims to determine the antibacterial activity of the essential oil of ketul leaves (*Bidens pilosa* L.) against Shigella dysenteriae and Salmonella typhi, and to analyze the composition of chemical compounds in the essential oil. The essential oil was isolated using steam-water distillation. The characterization test of the essential oil was carried out by determining the specific gravity, refractive index, and GC-MS analysis. The antibacterial activity test used the disc diffusion method with concentrations of 20%, 40%, 60%, positive control ciprofloxacin 5 µg/disc and negative control DMSO. The results of the characterization test showed the yield of essential oil was 0.002% (v/w), specific gravity 0.8886 g/ml, and refractive index 1.388. GC-MS analysis identified 51 chemical compounds dominated by sesquiterpenes and benzene. The results of the antibacterial activity test showed that the average inhibitory diameter of essential oils at concentrations of 20%, 40%, and 60% against Shigella dysenteriae bacteria was 8.70 mm; 10.48mm; 11.39 mm, while against Salmonella typhi bacteria it was 8.96 mm; 11.52 mm; 13.25 mm. Ketul leaf essential oil has the potential as a natural antibacterial.

KEYWORDS: *Bidens pilosa* L, Essential oil, *Shigella dysenteriae*, *Salmonella typhi*.

INTRODUCTION

Diarrhea is the passage of loose, watery stools more than three times a day. The prevalence of diarrhea in Indonesia is 6.8%, while the prevalence among toddlers is 11% (Riskesdas, 2018). Diarrhea can be caused by several microbes, one of which is bacterial infection. Some of the microorganisms that are the primary cause of diarrhea are usually normal flora, such as Staphylococcus aureus, Escherichia coli, Shigella, and Salmonella (Jawetz, 2007).

Diarrhea therapy generally given to toddlers is oral rehydration salts (ORS) and zinc. Meanwhile, additional therapy for pain, nausea, and other disorders includes paracetamol syrup, antacid syrup, domperidone, vitamin C, B6, or B complex (Indriani et al., 2019). Treatment of diarrhea caused by bacteria can be given antibiotics. According to Yenny et al., (2007), the Shigella dysentery bacteria show resistance to antibiotics such as ampicillin, co-trimoxazole, chloramphenicol, ciprofloxacin, fluoroquinolones, and tetracycline. The use of antibiotics that do not comply with therapy guidelines will increase the development of bacterial resistance to antibiotics. Therefore, alternative treatments for diarrhea are needed that utilize the effectiveness of active antibacterial ingredients from plants, one of which is the ketul plant (Bidens pilosa L.).

The Bidens pilosa plant is a type of plant belonging to the Asteraceae family. Traditionally, the Bidens pilosa plant has been widely used for generations as a medicine for diarrhea, malaria, diabetes mellitus, inflammation, hypertension, and cancer (Silalahi et al., 2021). The Bidens pilosa plant is also used to treat eye infections, nosebleeds, yellow fever, wounds, and ulcers (Namukobe et al., 2011). Extracts from this plant have biological and pharmacological activities such as antibacterial, anti-inflammatory, antiallergic, antimalarial, T-cell modulator, antihypertensive, anticancer, antiviral, antipyretic, and antioxidant activities (Xuan & Khanh, 2016).

The main parts of the Biden pilosa plant that are utilized are the leaves and stems because they are thought to have higher active compounds, especially the essential oil. The essential oil of the leaves and stems of the Bidens pilosa plant is dominated by sesquiterpenes. The components in the leaves are caryophyllene oxide, β-caryophyllene, humulene oxide, and germacrene D, while the components in the stems are hexahydrofarnesyl acetone, δ-cadinene, and caryophyllene oxide (Ogunbinu et al., 2009). The essential oil of the Biden pilosa plant

also has antibacterial and antifungal activity (Tian et al., 2011).

Based on research conducted by Deba et al., (2008) stated that there are 44 components of essential oils with the main components of Bidens pilosa leaves being b-caryophyllene (10.9%) and s-cadinene (7.82%). Meanwhile, according to Goudoum (2016) stated that the essential oil content in Bidens pilosa leaves consists of 27 compounds, which represent around 97.57% of the total oil content with the main components of the essential oil being α-pinene (14.7%), ε-caryophyllene (13.5%), and β-ocimene (12.8%). The essential oil components also have antibacterial activity, namely against the bacteria Micrococcus flavus, Bacillus subtitulis, Bacillus cereus, Bacillus pumilus, Escherichia coli, and Pseudomonas ovalis with an average diameter of inhibition of 12.7 mm, 17.3 mm, 19.0 mm, 12.3 mm, 13.7 mm and 12.5 mm at a concentration of 40% (Deba et al., 2008). Based on research conducted by Lawal (2015) stated that Bidens pilosa leaf extract has antibacterial activity against S. aureus, S. typhi, E. coli, S. paratyphi, S. typhimurium and P. aeruginosa with an average diameter of inhibition of 20.7 mm, 27.3 mm, 22.7 mm, 21.4 mm, 21.3 mm, and 17.7 mm. Research conducted by Seran (2022) stated that Bidens pilosa leaf extract has antibacterial activity against Escherichia coli bacteria with an average inhibition zone diameter of 3.24 mm, 7.28 mm, and 14.5 mm at concentrations of 20%, 40%, and 60%.

MATERIALS AND METHODS

SAMPLING

Leaf samples from the Bidens pilosa plant were taken in the Kurao Pagang area, Nanggalo District, Padang City, West Sumatra.

Bidens pilosa Leaf Distillation

Two kilograms of chopped dried Bidens pilosa leaves were weighed and placed in a steam distillation apparatus. The distillate was heated at 100ºC for four hours to produce a distillate. The distillate, containing oil and water, was placed in a separating funnel. The water layer at the bottom of the separating funnel was separated, resulting in the Bidens pilosa oil layer (Abdjul et al., 2013).

Evaluation of Essential Oil Components

Specific Gravity

Determination of the specific gravity of a liquid using a Pycnometer. The weight of the empty Pycnometer and the Pycnometer containing essential oil is weighed. The difference in the

weighing is the mass of the liquid at room temperature (25℃) and in constant volume, stated on the Pycnometer, then the specific gravity of the liquid is the mass divided by the volume of the Pycnometer, with units of g/ml (Januarti, 2009).

Refractive index

The refractometer is filled with water to maintain a temperature of 27°C (the operating temperature). The operating temperature is then maintained within a tolerance of ±0.2°C. Before placing the oil in the instrument, it is brought to the same temperature as the measurement. Readings are taken when the temperature has stabilized (Irwanto et al., 2022).

GC-MS

The chemical components of essential oils can be determined using a GC-MS (Gas Chromatography - Mass Spectrometry) device. The GC-MS equipment used for GC will be operated at 60ºC for 4 minutes, then the temperature will be increased to 120ºC with a temperature increase of 2ºC per minute. At 120ºC, the temperature will be maintained for 5 minutes, then the temperature will be increased again with a temperature increase of 50ºC per minute until the final temperature is 290ºC and then maintained for 10 minutes. The total gas flow rate used is 50 ml per minute with a slit ratio of 1: 30, the injector temperature is 300ºC and the amount of sample to be injected through the injector is 0.1 µl. Meanwhile, for MS, the electron energy used is 70 eV with an accelerating voltage of 1.30 kV. The mass range detected was between 40-400 µg/mol with a scanning interval of 1 second (Nurhaen, 2016).

Sterilization of Tools

The tools used were first washed and dried. The equipment was sterilized in an autoclave at 121°C for 15 minutes (Pratiwi, 2008).

Making Nutrient Agar (NA) Media

A total of 5 grams of Nutrient Agar was dissolved in 250 mL of distilled water in an Erlenmeyer flask and heated on a hot plate using a stirring rod until a clear solution was formed. It was then sterilized in an autoclave at 121 °C and 2 atm pressure for 15 minutes (Lay, 1994).

Bacterial Rejuvenation

Bacterial rejuvenation was performed using the streak method. Pure cultures of Shigella dystriae and Salmonella thypi were taken from one loop and inoculated aseptically onto NA

media. The samples were then incubated at 37°C for 24 hours (Khunaifi, 2010).

Making Bacterial Suspension

Allow the bacteria to be removed from the agar medium for 24 hours. Two loops of test bacterial colonies were suspended in 10 mL of sterile 0.9% NaCl in a sterile test tube. Then, they were homogenized by vortexing. Turbidity was compared with McFarland 0.5% (Muljono et al., 2016).

Antibacterial Activity Test

A total of 15 ml of Nutrient Agar (NA) was placed into a sterile petri dish, then 100 µL of test bacteria was added. Then, it was homogenized by shaking the petri dish containing the media. Then, the media was allowed to solidify (Rusdi et al., 2010). Sterile discs were soaked in various concentrations of essential oils of 20%, 40%, and 60%. On the surface of the media, a 5 µg/disc ciprofloxacin disc was also placed as a positive control and a DMSO disc as a negative control. Then, it was incubated at 37°C for 24 hours (Maharaini, 2023). This treatment was repeated 3 times. Then, antibacterial activity was determined by measuring the diameter of the inhibition zone formed using a vernier caliper.

RESULTS AND DISCUSSION

The Bidens pilosa plant is distilled to obtain its essential oil. Distillation is the process of separating the components of a mixture consisting of two or more liquids based on differences in vapor pressure or based on differences in boiling points of the components of the compound (Sari et al., 2018). The yield of essential oil produced from Bidens pilosa leaves is 0.002% (v/w) (Table 1). Meanwhile, research conducted by Paschoal et al., (2025) obtained a yield of Bidens pilosa leaf essential oil of 0.082%. The high or low yield obtained is influenced by the contact between the solvent and the raw material. The more raw material used, the higher the oil content in the material. In the steam-water distillation process, too little raw material mass is also inefficient because it causes more solvent vapor to evaporate directly into the condenser rather than diffusing into the tissue and pushing the essential oil to the surface (Cahyati et al., 2016).

Evaluation of essential oil components includes specific gravity and refractive index. Essential oils are measured for specific gravity using a pycnometer. Specific gravity can be an indicator for determining the purity of essential oils (Hidayati, 2012). The specific gravity results for Bidens pilosa leaf essential oil were 0.8886 g/ml (Table 1). Differences in the

chemical composition of essential oils affect the specific gravity value. In general, essential oils have an average specific gravity of 0.696 - 1.119 (Hidayati, 2012). The number of compounds contained in essential oils determines the specific gravity value. The more compounds with a high specific gravity fraction, the higher the specific gravity of the essential oil (Kartika & Proborini, 2018).

The refractive index of essential oil samples was determined using an Abbe refractometer. The measurement is based on the principle that light passing through a prism can only pass through the boundary between the liquid and the working prism at an angle within certain limits determined by the boundary angle between the liquid and the base (Solarbesain and Pudjihastuti, 2019). The refractive index obtained for Bidens pilosa leaf essential oil was 1.388. The refractive index of essential oils is related to the components composed of the resulting essential oil. The higher the water content in an essential oil, the lower its refractive index value. This is because water easily refracts incoming light. Therefore, essential oils with a high refractive index value are of better quality than those with a low refractive index value (Fitri et al., 2021).

Table 1: Results of the Evaluation of Essential Oil Components of Bidens pilosa Leaves.

No	Evaluation	Results
1.	Yield	0.002% (v/w)
2.	Specific Gravity	0.8886 g/ml
3.	Refractive Index	1,388

The essential oil from Bidens pilosa leaves was identified using GC-MS Gas Chromatography-Mass Spectrometry. The GCMS results of the study showed that Bidens pilosa leaf essential oil has 51 compound components, including the following major compounds: (1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3-methylenetricyclo (31.63%); Benzene, 1,3,5-heptatriyn-1-yl- (21.82%); Caryophyllene (9.91%); Cyclohexene,4-ethenyl-4-methyl-3 (5.73%); (1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo (5.53%); Isospathulenol (2.68%); Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1.69%); 1,4,7,-Cycloundecatriene (1.72%); Naphthalene, 1,2,3,5,6,8a- hexahydro-4,7-dimethyl- (1.47%); 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl- (2.07%). According to research conducted by Deba et al. (2008), from the results of GC–MS analysis of Bidens pilosa leaf essential oil showed that the major components identified included β-caryophyllene (10.9%), τ-cadinene (7.82%), megastigmatrienone (5.35%), β-cubebene (2.23%), diphenylenemethane (1.94%), α-caryophyllene (1.55%), β-cis-ocimene (1.45%), caryophyllene oxide (1.47%), s-muurolene

(1.04%), β-elemene (1.00%), and β-bourbonene (1.10%), α-pinene (0.99%), limonene (0.34%), and β-trans-ocimene (0.55%). The quality and quantity of ketul leaf essential oil are influenced by many factors, both at the pre-harvest and post-harvest stages. One pre-harvest factor that influences the quality and quantity of essential oils is the degree of ripeness, which is why the samples used are not homogeneous. This is because the formation of secondary metabolites in the form of volatile aromatic compounds is a dynamic process, where the concentrations of these compounds tend to vary and change continuously during the ripening process (Assyera et al., 2023).

The antibacterial activity of Bidens pilosa leaf essential oil was tested against Shigella dysentriae and Salmonella typhi. The method used in testing antibacterial activity is the disc diffusion method. A disc containing an antimicrobial agent is placed on an agar medium containing microorganisms that will diffuse through the agar medium. Clear areas indicate that the antimicrobial agent inhibits the growth of microorganisms on the surface of the agar medium. The advantages of this method are its simplicity, relatively low cost, and ease of interpretation (Pratiwi, 2008).

In this study, DMSO was used as a negative control. DMSO is not bactericidal, so it does not affect the results of the antibacterial activity test. Meanwhile, a 5 µg/disk ciprofloxacin disk was used as a positive control. In this study, the average inhibitory diameter of 5 µg/disk ciprofloxacin was 36.6 mm against Shigella dysentriae and 29.27 mm against Salmonella typhi (Table 2). The results showed that 5 µg/disk ciprofloxacin had greater inhibitory power against Shigella dysentriae and Salmonella typhi bacteria when compared to the average inhibitory diameter of Bidens pilosa leaf essential oil. This is because ciprofloxacin is a broad-spectrum antibiotic whose mechanism of action is by inhibiting topoisomerase II and topoisomerase IV in bacteria (Jawetz et al., 2007).

From the results of testing the antibacterial activity of ketul leaf essential oil with a concentration of 20%, 40%, 60% against Shigella dysentriae bacteria, the average inhibition diameter was 8.70 mm ± 0.19; 10.48 mm ± 0.05; 11.39 mm ± 0.27, while against Salmonella typhi bacteria, the average inhibition diameter was 8.96 mm ± 0.11; 11.52 mm ± 0.67; 13.25 mm ± 0.10 (Table 2). Determination of antibacterial criteria based on the strength of antibacterial power can be seen from the diameter of the inhibition formed. Antibacterial strength is classified into four categories, namely very strong, strong, medium and weak categories. Inhibitory power with a very strong category if the diameter of the inhibition

power produced is more than 20 mm. The inhibition power is categorized as strong if the diameter of the inhibition zone produced ranges between 10 mm - 20 mm. The inhibition power is categorized as moderate if the inhibition power ranges between 5 mm - 10 mm and the diameter of the inhibition power is said to be weak if the diameter of the inhibition zone produced is less than 5 mm (Davis and Stout, 1971). The results of the antibacterial activity test of ketul leaves show that ketul leaf essential oil has a strong inhibitory power category at concentrations of 60% and 40%, while at a concentration of 20%, a moderate inhibitory power category was obtained against Shigella dysentriae and Salmonella typhi bacteria.

Table 2. Results of Antibacterial Activity Test of Bidens pilosa Leaf Essential Oil Against Shigella dysentriae and Salmonella typhi Bacteria.

Sample	Concentration (%)	Mean inhibition diameter (mm) ± SD	
		Shigella dysentriae	***Salmonella typhi***
Bidens pilosa Leaf Essential Oil	20	8.70 ± 0.19	8.96 ± 0.11
	30	10.48 ± 0.05	11.52 ± 0.67
	40	11.39 ± 0.27	13.25 ± 0.10
Control + (Ciprofloxacin 5µg/disk)		36.6	29.27
Control – (DMSO)		0	0

The antibacterial activity test results of Bidens pilosa leaf essential oil showed a greater inhibitory effect on Salmonella typhi bacteria compared to Shigella dysentriae bacteria (Table 2). Although both bacteria are gram-negative, they have differences, particularly in their ability to respond to antibacterial substances. Gram-negative bacteria have a high lipid content and porin proteins that act as channels for active substances to enter bacterial cells. The entry of these active substances disrupts enzyme activity in cells and causes cell damage. High lipid levels in cells will increase the permeability of active substances into cells (Purnaningsih et al., 2017).

Based on the results of research that the essential oil of Bidens pilosa leaves can inhibit the growth of Shigella dysentriae and Salmonella typhi bacteria, this is thought to be influenced by secondary metabolite compounds contained in the essential oil of ketul leaves. The higher the concentration of essential oil, the greater the diameter of the inhibition zone against bacteria. The process of bacterial inhibition by essential oils occurs due to their ability to bind to extracellular proteins and bacterial cell walls. The more lipophilic, the more it can disrupt the bacterial cell membrane. The inhibitory mechanism is thought to be through the

destruction of the lipid bilayer of the cell membrane due to its hydrophobic groups (Hanifah, 2018). According to Dewi, (2015) stated that the mechanism of action of essential oils in killing bacteria is by changing the permeability of the cell membrane, eliminating ions in the cell, blocking the proton pump, and reducing the production of adenosine triphosphate (ATP). Essential oils are lipophilic which can pass through the bacterial wall because the bacterial wall consists of polysaccharides, fatty acids, and phospholipids. This can result in damage to the cell walls, which can kill the bacteria.

Terpenoids, such as isopropyl compounds in essential oils, are potent antibacterial agents, resulting in antibacterial agents that can also inhibit bacterial growth. The mechanism of action of terpenoids as antibacterial agents involves membrane damage by lipophilic compounds. Terpenoids can react with porins (transmembrane proteins) in the outer membrane of bacterial cell walls, forming strong polymer bonds and damaging the porins, reducing the permeability of the bacterial cell wall, thus starving the bacterial cells of nutrients, inhibiting their growth or even killing them (Wulansari et al., 2020).

The compound β-caryophyllene is one of the main components of Bidens pilosa essential oil that plays a crucial role in antibacterial activity. This compound belongs to the sesquiterpene group, which is lipophilic, allowing it to interact directly with bacterial cell membranes. The antibacterial mechanism of β-caryophyllene is thought to operate through several pathways, including damaging the integrity of bacterial cell membranes. When this compound infiltrates the phospholipid membrane, membrane permeability increases, causing leakage of cellular contents such as ions, proteins, and other essential components, disrupting osmotic balance and ultimately leading to cell lysis (Bassole & Juliani, 2012). Furthermore, β-caryophyllene is also known to inhibit the activity of enzymes essential to bacterial metabolism, such as cell wall-forming enzymes, thus disrupting the synthesis of vital structural components (Takahashi et al., 2004). This compound is also capable of inducing oxidative stress through the formation of free radicals that cause damage to the DNA and proteins of bacterial cells (Katsukawa et al., 2010).

Benzene-derived compounds such as benzopyran, heptane, and heptatriyne in essential oils are known to have strong antibacterial activity. These compounds have conjugated aromatic rings and functional groups such as hydroxyl or methyl that influence the compound's interaction with the bacterial cell membrane. The main mechanism of antibacterial activity is through the disruption of the microorganism's cell membrane, where the lipophilic nature of

the benzene ring allows the compound to insert into the lipid layer of the bacterial cell membrane, causing damage to the membrane structure, increased permeability, and leakage of cell contents such as electrolytes and proteins (Trombetta et al., 2005). Benzene-derived compounds are effective against both Gram-positive and Gram-negative bacteria, although their effectiveness can vary depending on the concentration and structure of the substituent groups on the benzene ring.

CONCLUSION

Based on the results obtained, the essential oil of ketul leaves (*Bidens pilosa* L.) has antibacterial activity against Shigella dysentriae and Salmonella typhi. While the analysis of the results of GC-MS (Gas Chromatography-Mass Spectrometry) of ketul leaf essential oil obtained 51 compounds, namely with the following major compounds: (1R,2S,6S,7S,8S)-8-Isopropyl-1-methyl-3- methylenetricyclo (31.63%); Benzene, 1,3,5-heptatriyn-1-yl- (21.82%); Caryophyllene (9.91%); Cyclohexene, 4-ethenyl-4-methyl-3 (5.73%); (1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo (5.53%); Isospathulenol (2.68%); Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1.69%); 1,4,7,- Cycloundecatriene (1.72%); Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7- dimethyl- (1.47%); 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-(2.07%).

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