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Antibacterial activity of mandailing traditional plant leaves ethanol extract of *Mikania micrantha*

Mhd. Yusuf Nasution[1], Martina Restuati[1], Ricky Andi Syahputra[1,2] and Ahmad Shafwan S. Pulungan*[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan (Medan State Universiy, Willem Iskandar Street, **Indonesia.**

[2]Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Muslim Nusantara Al-Washliyah, Meda, **Indonesia.**

***Correspondence:** pulungan.shafwan@gmail.com **Accepted:** 31 Jan.2019 **Published online:** 15 Mar. 2019

This study aims to determine the antibacterial activity of the ethanol extract of *Mikania micrantha* leaves. The test bacteria used in this study were *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterobacter aerogenes, Staphylococcus epidermis* obtained from a collection of bacterial cultures of the Medan State University microbiology laboratory. The leaves used in the study were *Mikania micrantha* leaves obtained from Mandailing Natal District, North Sumatra. ethanol extraction method using soxhlet method. The results showed that the best concentration in inhibiting bacterial growth was at a concentration of 30 mg/ml. The largest inhibitory zone formed is *Staphylococcus aureus* bacteria at 9.33 mm. the conclusion obtained in the study is that the greater the concentration level of the *Mikania micrantha* leaf ethanol extract, the greater the inhibition zone is formed.

Keywords: ethanol, antibacterial, medicine, growth, inhibitor

INTRODUCTION

Plant production as a raw material for herbal medicine is a classic approach that was carried out by ancient people. Natural knowledge about the efficacy of medicines derived from plants has existed since ancient times. Plants have been used by the community as traditional medicine and play an important role in the world of medicine (Pan et al., 2013).

The use of natural ingredients as medicine (biopharma) tends to increase with the issue of back to nature and economic crisis which results in a decrease in people's purchasing power towards modern medicines that are relatively more expensive. Drugs from natural ingredients are also considered to have almost no harmful side effects. Alternative antimicrobials can come from various sources, but today the most prevalent research is herbal antimicrobials. This type of antimicrobial is considered safer when used and is relatively difficult to cause resistance. Sembung vine (*Mikania micrantha*) belongs to the family Asteraceae, is a plant native to Central America and South America (Tripathi, Khan, and Yadav, 2012).

Mikania micrantha is an annual weed that grows quickly. *Mikania* is included in important weeds in oil palm which can grow to an altitude of 700 masl. *Mikania* generally grows predominantly in the area of immature oil palm (TBM) so that it can cover/cover all palm fronds/canopy. *Mikania* also produces allelopathic compounds in the form of phenols and flavonoids (Xu, Xie, Xiao, and Wei, 2013)(Wei, Huang, Wu, Cao, and Ye, 2004)(Ishak, Shafie, Esa, Bahari, & Ismail, 2018) (Bravo-Monzón, Ríos-Vásquez, Delgado-Lamas, and Espinosa-García, 2014). Easy to breed through pieces of stems and seeds. Seed viability

reaches more than 60%, while the growth of cuttings can reach 95%.

The great potential possessed by *Mikania Micrantha*, among others, as an antidermatitis antitumor (Dou, Zhang, Sun, Wu, and Li, 2014)(Latha, Jyothilakshmi, & Jyothis, 2015) has been reported. Other studies have shown that water, methanol and chlorophom extracts of *Mikania micrantha* leaves are effective as antimicrobials (Li, Li, Li, Wang, and Cao, 2013). *Mikania micrantha* extract of ethyl acetate is effective as an antioxidant (Ishak et al., 2018). Previous studies also revealed that *Mikania micrantha* is capable of being anti-stress, anticancer. Based on the research of hot water extract from *M. micrantha* leaves (2000 ug / mL) showed antibacterial activity against pathogenic bacteria, namely members of *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Proteus* species (Ghosh, 2008).

Previous research also revealed the antibacterial activity of methanol extract from all parts of *M. micrantha* plants against members of *Klebsiella pneumonia* and *S. aureus* species at 100 µg / 100µL extract (Chethan, Sampath Kumara, Sekhar, & Prakash, 2012). Water extract from *M. micrantha* leaves showed high antibacterial activity against pathogenic bacteria belonging to *S. aureus* species at a concentration of 25g / ml. So far, information on the research of M. micrantha leaf extract on pathogenic bacteria is still limited. Based on previous research, it is necessary to test to see the ability of antimicrobials by using ethanol extract of *Mikania micrantha* leaves.

MATERIALS AND METHODS

The main ingredient used is the leaves of *Mikania micrantha* plant, mandailing natal cultivars collected from Mandailing Natal Regency, North Sumatra. The test microbes used were *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterobacter aerogenes, Staphylococcus epidermis*.

Extraction of the test solution

As much as 50 grams of *Mikania micrantha* leaf powder placed on filter paper was then put into a Soxhlet extraction tool, poured into 95% ethanol, then refluxed to twice the circulation and the colorant was no longer colored. Then filtered, the ethanol extract obtained was concentrated on a water bath to obtain a thick ethanol extract, while the pulp was dried with the help of a fan. Dry dregs are infused by heating the powder with water to 90 C for 15 minutes, then filtering it. Then the infusion obtained was concentrated on a thick water extracted water bath. The production of ethanol extract series is 50 mg / mL; 40 mg / mL; 30 mg / mL; 20 mg / mL; 10 mg / mL.

Preparation of Nutrient Agar (NA) Media

Media is made with a concentration of 2%. A total of 2 grams of Nutrients To be dissolved in distilled water as much as 100 ml, then stirred with heating at 70 ° C. This media is sterilized using autoclave at 121 ° C for 15 minutes. Furthermore, as much as 3 ml of this medium, put into a test tube, placed at a slope of 30-45 ° and allowed to solidify, then stored in a refrigerator.

Antibacterial testing

Antibacterial activity was tested against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterobacter aerogenes, Staphylococcus epidermis* with diffusion agar method with three repetitions of each treatment. The 10 µl test solution was dropped on the paper disk (diameter = 6 mm) and then left to dry. 200 µl of bacterial suspension was mixed with 20 ml of the nutrient medium so that (in warm conditions), homogenized then poured into a petri dish. The media is waiting for a while until it freezes. Paperdisk which contains the test solution is then placed on the surface of the agar media and incubated at 37 ° C for 18-24 hours. A positive control using 10 mg/disk Chloramphenicol antibiotics and negative control paper which has been dripped with DMSO solvents (Perez, C., Paul, M and Bazerque, 1990).

RESULTS AND DISCUSSION

Antibacterial activity test results using agar diffusion method (Kirby and Bauer diffusion) showed that the measurement results of the average zone diameter of inhibition of ethanol extract of Mikania micrantha leaves on *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Enterobacter aerogenes, Staphylococcus epidermis* can be seen in table 1.

Antibacterial activity of ethanol extract of *Mikania micrantha* leaves showed positive results for various types of bacteria. This is indicated by variations in the inhibition zone data formed. The best concentration for all types of bacteria is 30 mg/ml. The amount of inhibition zone for *Escherichia coli* is 8.01 mm, *Staphylococcus aureus* is 9.33 mm, *Salmonella typhi* is 7.96 mm, *Enterobacter aerogenes* is 8.25 mm and *Staphylococcus epidermis* is 9.02 mm (Figure 1).

Table 1. The inhibitory zone of the antibacterial activity of the ethanol extract of *Mikania micrantha* leaves

Microbial Species	Concentration (mg/ml)				
	10	15	20	25	30
Escherichia coli	2,16 ± 0,01	4,54 ± 0,02	5,02 ± 0,01	7,21 ± 0,01	8,01 ± 0,01
Staphylococcus aureus	0,71 ± 0,04	0,91 ± 0,01	2,66 ± 0,02	4,72 ± 0,01	9,33 ± 0,01
Salmonella typhi	0,00 ± 0,00	0,22 ± 0,02	3,45 ± 0,01	5,39 ± 0,07	7,96 ± 0,02
Enterobacter aerogenes	2,01 ± 0,00	3,88 ± 0,01	4,91 ± 0,01	6,46 ± 0,03	8,25 ± 0,04
Staphylococcus epidermis	0,68 ± 0,03	1,33 ± 0,01	3,56 ± 0,03	4,63 ± 0,03	9,02 ± 0,02

Data shows inhibitory zone (mm) ± standard deviation

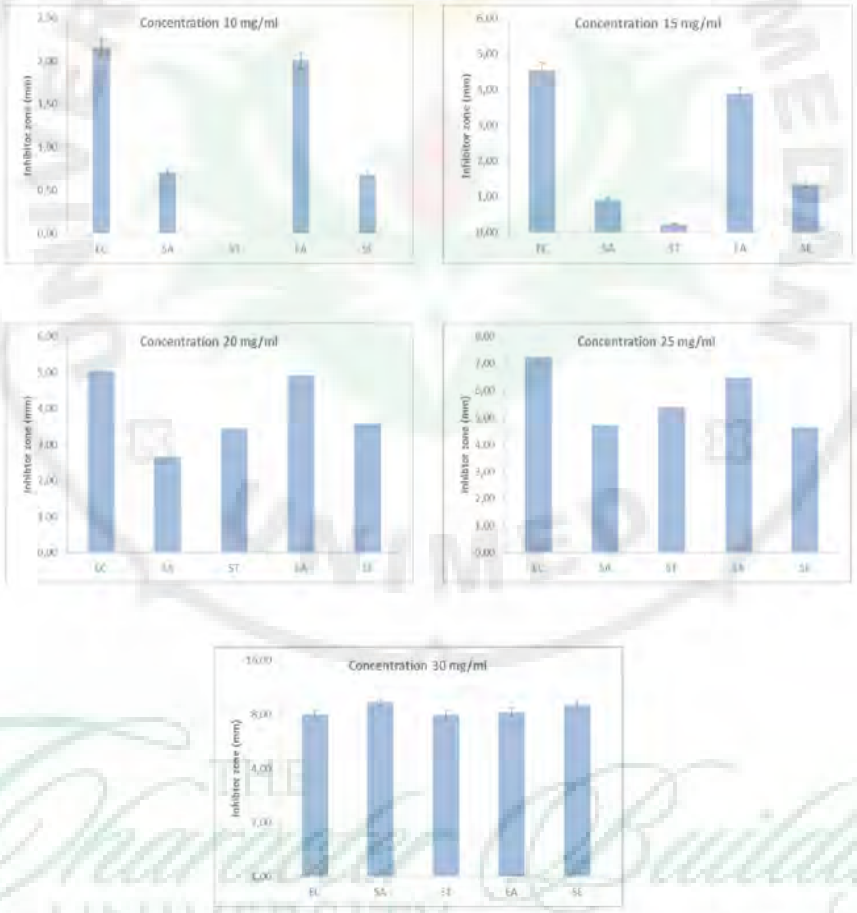

Figure 1. Activity of antimicrobial compounds for each concentration and bacteria; (a) concentration of 10 mg / ml; (b) concentration of 15 mg / ml; (c) concentration of 20 mg / ml; (d) concentration of 25 mg / ml; (e) concentration of 30 mg / ml; EC = *Escherichia coli*; SA = *Staphylococcus aureus*; ST = *Salmonella typhi*; EA = *Enterobacter aerogenes*; SE = *Staphylococcus epidermis*

Based on the Anava test results showed that the inhibition zone data for each concentration was significant with a confidence level of 0.05 with P = 0.0075.

Comparison of effectiveness for each type of bacteria shows variations according to the type of bacteria. The best inhibition zone of *Mikania micrantha* leaf ethanol extract at a concentration of 30 mg/ml is to inhibit the growth of *Staphylococcus aureus*, while the smallest is *Salmonella typhi*. This occurs because of the presence of phenol and flavonoid compounds found in the leaves of *Mikania micrantha* (Xu et al., 2013). Another study showed that *Mikania micrantha* was able to inhibit the growth of *Staphylococcus aureus* and *Streptococcus* group A with ethyl acetate solvents (Facey, Peart, & Porter, 2010). This shows that this research with ethanol solvent is also able to inhibit the growth of pathogenic bacteria.

The mechanism of phenolic compounds as antimicrobials is mostly by affecting cell membranes. Phenol compounds can cause large disturbances because they are able to form complexes with proteins through hydrogen bonds. As a result, the enzyme work can be disrupted. Phenolic components can identify bacterial cell walls so that the presence of phenolic components can inhibit bacterial growth (J. U., S. A., J. D., and O. A., 2013). Various secondary metabolites found in plants have antibacterial activity with various working mechanisms that work synergistically. The efficacy of herbal extracts used in medicine is due to the synergy between the active compounds contained in the extract (Pan et al., 2013). Synergy provides better activity and decreases the potential for toxicity from several single compounds and can prevent drug resistance (Pavarini, Pavarini, Niehues, & Lopes, 2012). The synergy of various secondary metabolites is also claimed to reduce unwanted side effects. The mechanism of action of flavonoids as antimicrobials is to inhibit nucleic acid synthesis, inhibit membrane function and inhibit energy metabolism (Panche, Diwan, & Chandra, 2016)

CONCLUSION

The mechanism of inhibition of bacterial growth by ethanol extract of *Mikania micrantha* leaves showed that the concentration was directly proportional to the inhibition zone formed. The best concentration in inhibiting bacterial growth is a concentration of 30 mg/ml. The higher the concentration of the extract solution, the greater the inhibition zone formed in the growth medium.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed in collecting and analyzing data. All authors participated in writing every part of this study. All authors read and approved the final version.

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