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Antibacterial activity of the ethanolic extract

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Abstract

Sembung rambat (Mikania micrantha Kunth.) is a weed whose presence is detrimental to agriculture because it can prevent agricultural plants from absorbing nutrients, water and light for photosynthesis. But behind that it turns out that sembung rambat has various benefits, one of which can be used as an antibacterial. The antibacterial ability of sembung rambat leaves has the potential to be used as a basis for making alternative antibiotics. This study was conducted to determine the value of Minimum Inhibitory Concentration (MIC) and antibacterial acitivity of leaves of sembung rambat (M. micrantha Kunth.) Which is most effective in inhibiting the growth of B. subtilis. The sampling method was done by purposive sampling, the extract was made by maceration with ethanol 96% ratio of 1:10. Test for antibacterial activity using the diffusion disc method. The antibacterial activity test results of 96% ethanol extract of sembung rambat leaves, inhibited the test of the value that was the most effective at inhibiting the growth of B. subtilis at 6 % with a diameter of 4.63 mm. From the results obtained, it can be seen that the leaves extract of sembung rambat has the potential to be used as an alternative plant-based antibiotic.

INTRODUCTION

Weed is a plant whose presence is unwanted by farmers, weeds cause disturbance and losses for cultivated plants in absorbing nutrients and water from the soil and receiving light for photosynthesis (Pebriani *et al*., 2013). But there are also weeds that are commonly used by the community as traditional medicines to treat various diseases, one of them is sembung rambat (*Mikania micrantha* Kunth).

Sembung rambat (*M. micrantha* Kunth) is a weed that propagates on trees and lives in places with low temperatures, sometimes in highland areas, forests and riverbanks (Susanti *et al*., 2011). This plant contains active compounds in the form of secondary metabolites such as alkaloids, saponins, flavonoids, steroids, tannins, and terpenoids which can be used as antibacterial agents (Narlan *et al*., 2013). Sembung rambat are seen in Barumanis village, Bermani Ulu sub-district, Rejang Lebong district, Bengkulu province. But it has not been used by the local community as an antibacterial drug.

Based on research conducted by Lallianchhunga *et al*. (2016), stated that the leaves of sembung rambat are used to treat fever, rheumatism, influenza, respiratory diseases, make poultices for snake bites, scorpion stings, treat rashes and itchy skin. The leaves of sembung rambat are reported to have antibacterial, anti-tumor, cytotoxic, analgesic, inflammatory, antiproliferative and phytotoxix activity (Li *et al*., 2013).

Infectious disease is a disease caused by pathogenic microbes in human's body (Darmadi, 2008). This disease is caused by microbes *Bacillus subtilis*, a saprophytic Gram-positive bacteria that is in the form of a bacil, found naturally in soil, water, air and plants (Jawetz *et al*., 2008). These bacteria can live in the mesophilic temperature (20-45^{o}C), yellow colonies with a diameter of 3.19 mm and are motile. *Bacillus subtilis* can cause some diseases if the amount is too much in the intestine that transmitted through food contamination (Utami *et al*., 2017), such as diarrhea, meningitis, endocarditis, endophthalmitis, conjunctivitis or acute gastroenteritis when our immune system is weak (Jawetz *et al*., 2008).

One of the efforts to cure diseases caused by pathogenic microbes can be done by administering antibiotics or antimicrobials to treat infectious diseases. Antimicrobial is a compound that has the ability to inhibit the growth of pathogenic microbes. The use of antimicrobials with the right concentration can kill pathogenic microbes in the body but are not toxic / harmful to humans. Antimicrobials work by influencing bacterial nucleic acid metabolism, disrupting ribosome function, acting directly on cell membranes, inhibiting bacterial cell wall synthesis (Setiabudi and Gan, 2007).

The use of antimicrobials with the right concentration can kill pathogenic microbes in the body but are not toxic/harmful to humans. Inappropriate use of antibiotics can cause microorganisms to become resistant. This is what underlies new research to find and produce new antibiotics as alternatives to treat infections with the use of plants as medicine (Nigam and Ponan, 2013). Based on the description above, it is necessary to conduct research on the antimicrobial activity test of sembung rambat leaves extract (M. micrantha Kunth.) From Barumanis Village, Rejang Lebong Regency. This is because sembung rambat is commonly found in Barumanis Village but there is still no data on the benefits of this plant.

MATERIALS AND METHODS

The sample collection was used by purposive sampling method. Before the sample was taken, the abiotic factors were measured, including soil pH, soil moisture, air humidity, light intensity, air temperature, and sample coordinates. The leaves of sembung rambat (*M. micrantha* Kunth) were taken must be fresh, not rotten, damaged or yellowish. The reference bacteria for antagonistic test was *Bacillus subtilis*. This bacteria was obtained from microbiology laboratory, Biology department, University of Bengkulu. The bacteria were incubated for 24 h at 32 ^{o}C on Tripticase Soy Agar (TSA).

One kilograms of sembung rambat leaves (*M. micrantha* Kunth) then sorted wet and washed with running water. Then the wet weight of the sample was noted. The leaves were then dried in a place that was not exposed to direct sunlight until their weight became constant. The dry weight obtained from 1 kilogram of sample is 140 grams. Samples that have been dry were then mashed using a blender and weighed using analytical scales (Departemen Kesehatan RI, 1985).

The extract preparation of *M. micrantha Kunth* leaves was carried out using the maceration method. The blended leaves of sembung rambat were soaked in ethanol 96% each with a ratio of 1:10 (daun sembung rambat 140 gram : 1400 ml ethanol 96 %) at 25-30 °C and protected from the sunlight. The maceration process was carried out for 5x24 hours, and stirred every 1x24 hours using a stirring rod (Direktorat Jenderal POM, 2000). The solution was then filtered using filter paper to obtain macerate, the filtering results were obtained as much as 800 ml.

All macerates from the filtering result were evaporated using a rotary evaporator at temperature of 50 ^{o}C with a rotation speed of 80 rpm until all ethanol 96% evaporate so that a thick extract was obtained as much as 14.5 grams. The viscous extract was put into a sterile container, then stored in a refrigerator.

Determination of Minimum Inhibitory Consentration (MIC) was tested by the disc diffusion method using blank disc paper with a diameter of 6 mm. 1 ml (1 x 10^{8} Cfu/ml) of tested bacterial suspension was inoculated into 100 ml TSA media which was still liquid then homogenized with a magnetic stirrer. After the media was homogeneous, then it was poured into a Petri dish as much as ± 15 ml. Then the disc papers were placed slowly on top of TSA media and were slightly pressed (Hudzicki, 2009). Furthermore, the disc papers were loaded with 8 µl of sembung rambat (*M. micrantha* Kunth) leaves extract according to the predetermined concentration, which were 2.5 %, 5 %, 7.5 %, 10 %, 20 %, 40 %, 80 %, 100 %, positive control using 0,10 % comercial chloramphenicol whereas the negative control using DMSO 10% and steriled ddH_2O. the plates were incubated for 24 hours at 32^{o}C. evidence of clear zone indicates bacterial growth inhibition and diameter were measured in milimeter (mm) using a caliper. The diameter measurement of the inhibition including the diameter of the filter paper disc minus the diameter of the disc paper used (Mulyadi *et al.,* 2013).

From the testing results of MIC determination, the inhibition zone was formed at a lowest concentration which is 5 %. Furthermore, for the antibacterial test, the selected concentration were determined starting from 5 % which was increased every 0.5 % to 8 % so that the concentrations made for the antibacterial activity test were 5 %, 5.5 %, 6 %, 6.5 %, 7 %, 7.5 % and 8 % respectively. Then the inhibition zone testing was carried out for *B. subtilis.* The presence of a clear area around the disc paper indicates an inhibition of the growth of bacteria by antibacterial agents on the surface of the agar medium. Barriers will appear as areas where there is no visible growth of bacteria around the disc.

The research data from the ethanol extract of sembung rambat (*M. micrantha* Kunth.) leaves as an antibacterial were analyzed statistically using a statistical data processing application, namely SPSS with ANOVA. If the F count > F table is obtained, it was continued with Duncan's Multiple Distance Test (Dahlan, 2014).

RESULT AND DISCUSSION

Based on the MIC determination test of ethanol 96% extract of sembung rambat leaves against *B. subtilis* with concentrations of 2.5 %, 5 %, 7.5 %, 10 %, 20 %, 40 %, 80 % and 100 %, the measurement results and test results are obtained as shown in figure 1 and figure 2 following.

Based on figure 1, the average diameter of the inhibition zone at a concentration of 80% is greater than concentration of 100%. This is because when dropping the sembung rambat leaves extract onto disc paper at a concentration of 100% there is still an extract left in the microtipes, so that the volume of the extract dropped is not the same as the extract concentration of 80%.

The initial test results for the determination of the MIC of *M. micrantha* Kunth leaves extract on *B. subtilis* bacteria obtained the best concentration at 5 %. Furthermore, variations in extract concentrations were made from low to higher than the concentration of 5 % with a distance of 0.5 % in order to obtain 10 new concentration treatments that would be carried out by antibacterial acitivity testing, namely 5 %, 5.5 %, 6 %, 6.5 %, 7 %, 7.5 %, 8 %, positive control 0.10% chloramphenicol, 10% DMSO negative control and distilled water as a comparison for *M. micrantha* Kunth leaves extract to be tested. Based on the MIC test of *M. micrantha* Kunth leaves extract on *B. subtilis* bacteria with the average inhibition zone produced after incubation for 24 hours, it can be seen in table 1 below.

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Based on the results obtained in table 1 above, *M. micrantha* Kunth extract has a different inhibition zone in each concentration. The smallest zone of inhibition was found at a concentration of 7 % at 3.03 mm while the largest zone of inhibition was found at a concentration of 6 % at 4.63 mm. But all concentrations of the average diameter of the inhibition zone have a weak category. According to Davis and Stout (1971) the diameter of the inhibition zone was <5 mm in the weak category, 5-10 mm in the moderate category, 10-20 mm in the strong category and >20 mm in the very strong category.

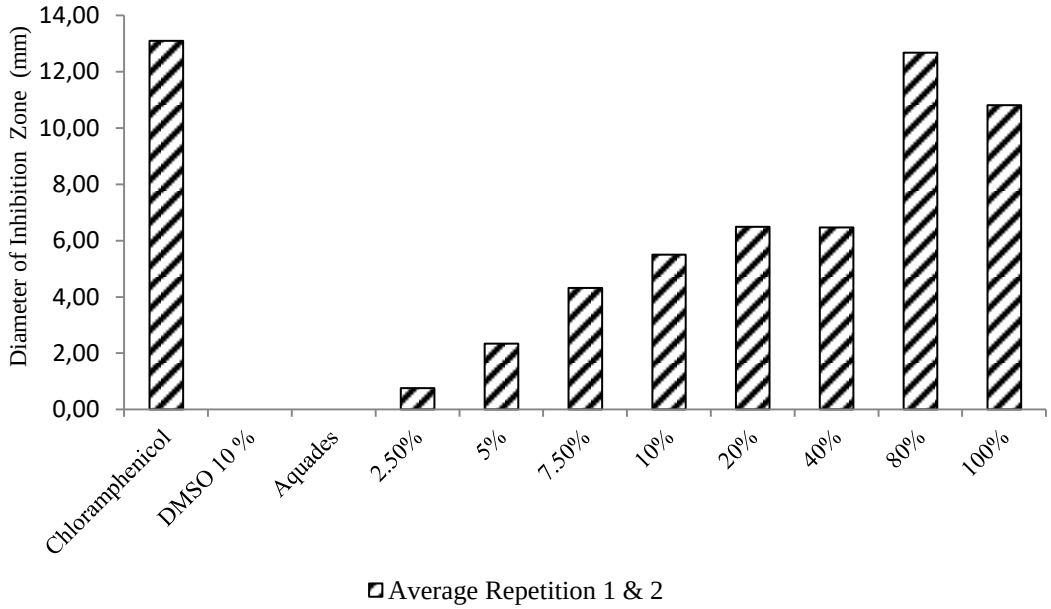

Fig 1. MIC determination results of *M. micrantha* Kunth leaves extract against *B. subtilis*

Based on the results of the Duncan test in table 1 above, it shows that the negative control treatment, namely DMSO 10% and distilled water with notation (a), was significantly different from all treatments, as well as the positive control treatment, namely chloramphenicol with notation (c). Meanwhile, the concentration of 5 – 8 % is not significantly different from notation (b). based on the inhibition zone category according to Davis and Stout (1971) the most effective concentration of sembug rambat leaves extract to inhibit the growth of *B. subtilis* was at 6 %.

Based on Figure 3 above, the formation of an inhibition zone in the antibacterial activity test of *M. micrantha* Kunth leaves extract may have the potential to inhibit the growth of *B. subtilis*. From the results that have been obtained from table 1 above, then analyzed using the ANOVA test to show that the significance value of *M. micrantha* Kunth leaves extract is significantly different to the growth of *B. subtilis* can be seen in table 2.

Based on the ANOVA analysis in table 2, it was found that the F Count in *M. micrantha* Kunth leaves extract was 27,032 greater than F Table, which was 3.02 with a level of 5%. This means that the treatment of *M. micrantha* Kunth leaves extract is accepted at the 95% level, which means that the error is not more than 5%. This shows that positive control and each concentration have an influence on the growth of *B. subtilis*.

96 % ethanol solvent is a polar solvent, where the polar solvent will only bind polar phytochemical compounds. Based on research conducted by Tiwari *et al.* (2011) stated that ethanol solvent can bind phytochemical compounds such as tannins, flavonols, terpenoids, sterols and alkaloids. Where these compounds have the ability, one of which is as an antimicrobial. The mechanism of action of flavonoids inhibits bacteria by inhibiting the function of the cell membrane and the metabolism of energy formation in bacteria. Flavonoids will form complex compounds along with the formation of extracellular proteins so that the cell membrane is damaged. Flavonoids are also able to inhibit the use of oxygen by bacteria so that energy formation metabolism does not occur (Sapara *et al.*, 2016). Tannins as an antibacterial are able to shrink the cell walls causing impaired cell permeability so that they cannot form completely and cause cell death (Dwidjoseputro, 2003). Alkaloids as antibacterial have the ability to interfere with peptidoglycan in bacterial cells. Peptidoglycan is a constituent of the cell wall in bacteria when it is disturbed, it will cause the cell wall to be damaged (Cushnie and Lamb, 2005).

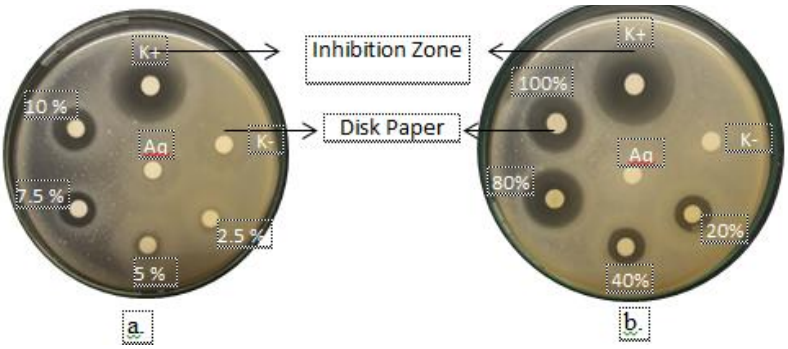

Fig 2. Determination test results of MIC against *B. subtilis*: (a). Concentrations of 2.5 %, 5 %, 7.5 % and 10 %, (b). Concentrations of 20 %, 40 %, 80 % and 100 %.
Note : K+ (Chloramphenicol), K- (DMSO 10 %), Aq (Aquades)

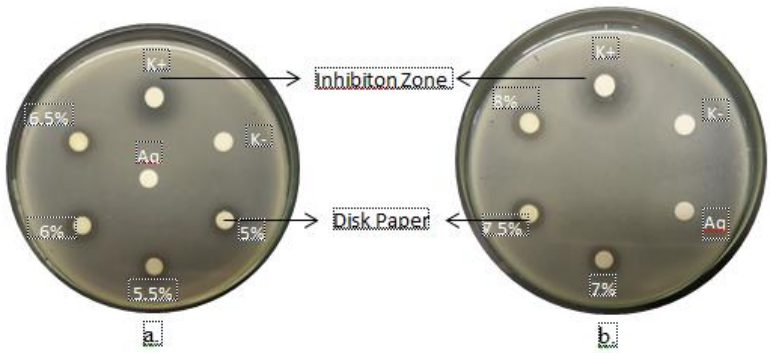

Figure 3. Antibacterial activity test results on *B. subtilis*: (a). Concentrations of 5 %, 5.5 %, 6 % and 6.5 %, (b). Concentrations of 7 %, 7.5 % and 8 %.
Note : K+ (Chloramphenicol), K- (DMSO 10 %), Aq (Aquades)

Bacillus subtilis is included in Gram positive bacteria, the characteristics of Gram positive bacteria are that the cell wall has a simple structure which consists of only one layer, namely peptidoglycan (Pelczar and Chan, 1998). Determination of the 5% concentration for the antibacterial activity test was carried out based on the results of the initial MIC determination test from 2 repetitions, the inhibition zone diameter was the most consistent at a concentration of 5%, so that for the antibacterial activity test the lowest concentrations ranging from 5% to 8% were used. Based on the antibacterial activity test results, it was found that the largest inhibition zone diameter was at a concentration of 6 % of 4.63 mm which is categorized as a weak zone according to David and Stout (1971). The leaves extract of sembung rambat was only bacteriostatic against *B. subtilis*, this was evidenced by the results of the incubation that was carried out. If the bacteria are incubated for more than 48 hours, the inhibition zone formed in the media will slowly fade away and the bacteria that did not initially grow in the area of the disc paper given the extract will grow little by little.

Table 1. The average diameter of the inhibition zone tested for antibacterial activity of *M. micrantha* Kunth leaves extract on *B. subtilis*.

Treatments	Average of Inhibition Zone (mm) ± SD	Notation	Inhibition Zone Category
Chloramphenicol	9.47 ± 0.47	9.46^{c}	Moderate
DMSO 10 %	0.00 ± 0.00	0.00^{a}	Weak
Aquades	0.00 ± 0.00	0.00^{a}	Weak
5 %	3.38 ± 0.58	3.83^{b}	Weak
5.5 %	3.52 ± 0.63	3.52^{b}	Weak
6 %	4.63 ± 0.09	4.63^{b}	Weak
6.5 %	3.92 ± 0.12	3.92^{b}	Weak
7 %	3.03 ± 1.74	3.03^{b}	Weak
7.5 %	3.78 ± 0.44	3.78^{b}	Weak
8 %	3.35 ± 2.60	3.35^{b}	Weak

Table 2. ANOVA analysis of inhibition zone diameter test for antibacterial activity of sembung rambat leaves extract on the growth of *B. subtilis*.

	JK	DB	KT	F_{Count}	F_{Table} 5 %
Treatments	123.789	9	13.754	27.032*	3.02
Galat	5.088	10	509		
Total	128.878	19			

CONCLUSION

Based on the research results obtained ethanol extract of 96% sembung rambat Leaves (*M. micrantha* Kunth.) has the potential to inhibit the growth of *B. subtilis* where the Minimum Inhibitory Concentration (MIC) value is the most effective at a concentration of 6 % with an inhibition zone diameter is about 4.63 mm and can be used as an alternative antibiotic based on plants.

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