Potential of Andalas (*Morus macroura* Miq.) Ethanol Extract in Inhibiting the Microbial Growth

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ABSTRACT
Andalas (Morus macroura Miq.) is a West Sumatra, Indonesia mascot plant. This plant is a Moraceae group, which is widely used traditionally as a medicinal plant. The purpose of this study was to determine the potential of Andalas ethanol extract in inhibiting the growth of pathogen microbes.
Ethanol extraction was done to the roots and stems of Andalas. To see the optimum concentration of ethanol extracts of Andalas roots and stems in inhibiting test microbes, a completely randomized design (CRD) was used. The treatments given in this study were extract concentration of 3.12%, 6.25%, 12.50%, and 25%, respectively. Antimicrobial activity tests were carried out on test microbes (*Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*) using the paper disc diffusion method.
The results showed that the optimum concentration of Andaleh root ethanol extract in inhibiting the growth of *S. aureus* and *E. coli* was at 12.5% and 3.125%. Meanwhile, the best concentration of ethanol extract of Andaleh stem in inhibiting the growth of *S. aureus* and *E. coli* was at 25%. There was no antimicrobial activity of the ethanol extract of Andaleh roots and stem against the growth of *C. albicans*.
Keywords: *Andaleh, Antimicrobial, ethanol extract*

1. INTRODUCTION

Bacterial resistance is one of the causes of increasing cases of deaths due to infections. According to (1), *Staphylococcus aureus* isolated from hospital patients tended to be resistant to doxycycline and tetracycline (72% and 69%). Furthermore, according to(2), Staphylococcus which is resistant to penicillin is not only found in hospitals, but also from the bacteria was isolated from the community. The discovery of new antimicrobial active compounds needs to be done. One of them through the exploration of the Andalas plant (*Morus macroura*)
Andalas has several stilbene-derived chemical compounds, namely Lunularin, Iresveratrol, Andalehin A, together with 2-arilbenzofuran derivatives, M moracin, Coumarin, Umbliferon and β-resolsiladehid derivatives. In addition to these compounds, Andaleh also contains Guangsangos A, Albafuran (Kwanon and Mulberofuran G) and Andalehin B. These chemical compounds have antioxidant, neuroprotective, antiviral, antifungal, and antibacterial activities (3,4)
The growth factor of Andalas which is limited was the reason for the lack of research on the active compounds in these plants. One of the exploration strategies of Andalas plant active compounds is to utilize endophytic microbes that are symbiotic with these plants
Research on the activity of Andaleh antimicrobial active compounds is needed as comparative data that will support the results of the study that was done by (5–7). In theory, endophytic microbes can produce the same active compounds as their host. According to(8), endophytic bacteria can produce secondary metabolites that are the same as their host, even in relatively high amounts. But Nisa's research(9) shows different results. Where the antibacterial activity test of endophytic fungi extract and leaf ethanol extract from Chromolaena odorata against Shigella dysenteriae showed that the inhibitory zone of C. adorata leaf extract had higher activity than of secondary metabolite extracts of endophytic fungi.
For this reason, a research entitled Antimicrobial Activity Test of Andaleh Ethanol Extract (*Morus macroura* Miq.) has been conducted.

2. MATERIALS AND METHODS

2.1. Microbial Strains

Following standard bacterial strains were used in this study belonging to Gram positive and Gram negative species: *Staphylococcus aureus* (ATCC.25923) and *Escherichia coli* (ATCC.EC 25922). They were obtained from mikrobiology laboratotium, Medical Faculty, University of North Sumatra.

2.2. Plant Extracts

Andalas plant were collected from Andalas village, Tanah Datar regency, West Sumatra province, Indonesia. They

were identified by the authors, based on community local knowledge and strengthened by the key of determination for M. macraura. Plant parts taken are roots and stems. Plants were dried at the oven (at a temperature of 45ºC) until a constant weight was obtained. The dried plants parts were ground and then 300 g of the dry plant powder of each part of the plant was macerated for 3 x 24 hours. After the maceration process, the debris of the Andaleh tissue is separated from the ethanol solution by filtering using filter paper. The filter results are then extracted using a vacuum rotary evaporator. The extraction process is carried out until a thick extract is obtained. The obtained residues were kept in the dark and stored at 4 ° C until use.

2.3. Antimicrobial Activity Assay

The research design used to determine the best concentration of Andaleh tissue ethanol extract to inhibit the growth of test microbes is a completely randomized design (CRD). The experiments were carried out on each of the test microbes separately, each using 5 treatments (extract concentration: 25%, 12.5%, 6.25% and 3.123% and antibiotic control). with 3 replications.

The antibacterial activity of tested plant parts was carried out by the disc diffusion method. The extracts were dissolved in DMSO at a concentration of 50%, then serial dilutions were made according to treatment.

The microbial test, which turbidity has been compared with Mcfarland's 0.5 evenly inoculated into NA medium. Furthermore, ethanol extract of Andaleh tissue with different concentrations is dripped on each disc (until it is saturated). The disc is then placed on an agar medium which has been inoculated with the test microbes. Bacterial culture was incubated at 37ºC for 24 hours. Inhibited zones formed are measured to determine the antimicrobial activity produced

3. RESULT AND DISCUSSION

The result of Andaleh root and stem extraction using ethanol solvent was obtained as thick extract as much as 7.92 g and 10.2 g, respectively. The results of the extract antimicrobial activity showed that all concentration was able to inhibit the growth of bacteria. Examples of inhibited zones formed can be seen in Fig 1.

Figure 1: Zone of inhibition around the disc

The optimum concentration of Andaleh ethanol extract in inhibiting the growth of *S. aureus* is based on the lowest concentration that has the same inhibition zone or greater than the control of ampicilli. The results of the analysis of the activities of Andaleh ethanol extract in inhibiting the growth of *S. aureus* can be seen in Table 1.

Table 1. Antimicrobial activity of stem and root Andaleh ethanol extracts against *S. aureus*

No	Concentration **(%)**	Inhibition zone in diameter (cm)	
		Stem	**Root**
1	Ampicilli	1,176 [a]	1.176[b]
2	3,125%	0,800 [c]	0.842[d]
3	6,25%	0,827 [c]	0.977[c]
4	12,5%	0,945 [b]	1.19[b]
5	25%	1,149 [a]	1.377[a]

Values are expressed as Mean (n=3); analysis was performed with One-Way ANOVA followed by DMRT test

Based on statistical analysis ($\alpha = 0.05$), it was known that there are significant differences in the effect of the concentration of the stem and root Andaleh ethanol extract in inhibiting the growth of *S. aureus*. Based on DMRT tests ($\alpha = 0.05$), concentrations of 25% and 12.5% (respectively for the stems and roots Andaleh ethanol extract) was the optimum concentration in inhibiting the growth of *S. aureus*

To find out the extract's ability to inhibit the growth of gram-negative bacteria, an antimicrobial activity test was carried out on *E. coli* bacteria. The results of the effectiveness of stem and root Andaleh ethanol extracts against *E. coli* can be seen in Table 2.

Table 2. Antimicrobial activity of stem and root Andaleh ethanol extracts against *E. coli*

No	Concentration **(%)**	Inhibition zone in diameter (cm)	
		Stem	**Root**
1	Kontrol positif	0,905 [a]	0.905 [c]
2	3,125%	0,696 [c]	0.948 [c]
3	6,25%	0,748 [bc]	1.045 [c]
4	12,5%	0,882 [ab]	1.203 [b]
5	25%	1,015 [a]	1.375[a]

Values are expressed as Mean (n=3); analysis was performed with One-Way ANOVA followed by DMRT test

ANOVA analysis results ($\alpha = 0.05$) (Table 2) can be seen that the concentration of the extract affects its ability to inhibit the growth of *E. coli*. Where, based on the analysis by DMRT test it was found that the optimum

concentration of ethanol extract of stems and roots Andaleh in inhibiting the growth of E. *coli* were 25% and 3.125%, respectively.
The antimicrobial activity of stems and roots Andaleh ethanol extracts was also tested to C. albicans. The test results can be seen in Table 3.

Table 3. Antimicrobial activity of stem and root Andaleh ethanol extracts against *C. albicans***.**

No	Concentration **(%)**	Inhibition zone in diameter (cm)	
		Stem	**Root**
1	+ Control	1,177	1,177
2	3,12%	-	-
3	6,25%	-	-
4	12,5%	-	-
5	25%	-	-

Values are expressed as Mean (n=3); (-) no inhibition zone

Based on the data in Table 3 it can be seen that the two extracts tested were unable to inhibit the growth of *C. albicans*. In this experiment the highest concentration of tests carried out was 25%.
A previous study isolated and tested the ability of endophytic bacteria isolated from Andaleh stem, leaf and root tissue in producing anti-microbial compounds. The study found that endophytic bacteria isolated from different tissues had different antimicrobial activity. Endophytic bacteria that (5,7) isolated from the stem dan leaf (respectivly) have the ability to inhibit *S. aureus* better than other test microbes. In contrast, research conducted by(6), shows that endophytic bacteria isolated from roots can inhibit *C. albicans* better.
Although the research was done by (10)showed the endophytic bacteria can produce secondary metabolites that are the same as their hosts, even in relatively high amounts. Contrariwise, Nisa's research(9) shows that the ability of endophytic bacteria to produce antimicrobial compounds is lower than that of plant hosts. This difference in results can be caused by several factors, one of them is the use of solvents to extract active compounds which are not appropriate. In this research, the solvent used was ethanol, which is polar. According to (11), the type of solvent was a factor that influences the concentration and type of compound to be extracted. The polarity of the solvent is an important thing that influences the antimicrobial activity. Therefore, further experiments need to be carried out using non-polar solvents

4. CONCLUSION

The optimum concentration of Andaleh root ethanol extract in inhibiting the growth of *S. aureus* and *E. coli* was at 12.5% and 3.125%. The best concentration of ethanol extract of Andaleh stem in inhibiting the growth of *S. aureus* and *E. coli* was at 25%. There was no antimicrobial activity of the ethanol extract of Andaleh roots and stem against the growth of *C. albicans.*

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