



ACTIVITY OF ETANOL EXTRACT OF SNAKE LID LEAVES (*Hedyotis corymbosa* (L.) Lamk) ON THE GROWTH OF *Staphylococcus aureus* BACTERIES

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Abstrak

Hedyotis corymbosa or commonly known as pearl grass is one of the plants that has great potential as a traditional medicine, especially in the treatment of infections. The development of infection drugs is very rapid considering that the resistance rate in several countries continues to increase. Some cases of infection that often occur are skin infections caused by *Staphylococcus aureus* bacteria. This study aims to test the ability of ethanol extract of snake tongue leaves in inhibiting the growth of *Staphylococcus aureus* bacteria. This study is an experimental study, consisting of five treatments with three repetitions, using the paper disk method. Samples in the form of snake tongue leaf extract (*Hedyotis corymbosa* (L.) Lamk) were dissolved with aqua pro injection which was divided into several concentration groups, namely 5%, 10%, and 15%. The test results showed that snake tongue leaf extract was able to inhibit the growth of *Staphylococcus aureus* bacteria with an average inhibition area of 13.8 mm (5%), 16.7 mm (10%) and 17.4 mm (15%). Statistical analysis through ANOVA testing obtained results that the three concentrations of snake tongue leaf extract (*Hedyotis corymbosa* (L.) Lamk) have significant differences in inhibiting the growth of *Staphylococcus aureus* bacteria.

INTRODUCTION

Infection is one of the causes of disease that often occurs in tropical climates, especially Indonesia. Pathogenic bacteria are one of the causes of human infection. One infection that often occurs is skin infection caused by *Staphylococcus aureus* bacteria (Siregar et al., 2012).

Staphylococcus aureus is a pathogenic bacterium in humans that can cause ulcers and is a Grampositive bacterium that produces yellow pigments, is facultative aerobic, does not produce spores and generally grows in pairs or groups

with a diameter of about 0.7-0.9 microns (Sudirman, 2014). *Staphylococcus aureus* is found in the upper respiratory tract, face, hands, hair, and vagina. This bacterial infection can cause disease with characteristic signs, namely inflammation, necrosis, appearing as acne, hair follicle infection, and abscess formation. Among the organs that are mutually attacked by *Staphylococcus aureus* bacteria is the skin that is injured and can spread to other people who are also injured (Razak et al., 2013).

According to Siregar et al, (2012) a widely used method to prevent and overcome the attack of bacterial infections on the skin, is by means of treatment using antibiotics. However, over time, bacterial resistance to antibiotic drugs is increasing and also requires a lot of money. Another alternative that is commonly used to overcome infectious diseases, is material from nature or traditional medicine where Indonesia is a country that has thousands of plant species that must be preserved and utilized properly. Some of these plants can be used as traditional medicine. (Wijayakusuma, 2008). Traditional medicines derived from plants and pure natural ingredients, have side effects, levels of danger and risks that are much lower than chemical drugs (Muhlisa, 2005).

One of the medicinal plants that has potential as traditional medicine and is thought to have antibacterial inhibition is snake tongue leaf (*Hedyotis corymbosa* (L.) Lamk) or commonly known as pearl grass. Snake tongue leaves are plants that we can easily find thriving in moist soil on the side of the road, yard and gutter (Ipteknet, 2005). Pearl grass has empirically been widely utilized by people in Indonesia, especially in the Sulawesi region. Some of them are used as drinks that can maintain stamina, skin allergies, normalize high blood pressure, reduce body heat (fever), improve blood circulation and prevent the growth of tumor cells or cancer cells. Based on research by Milah et al. (2012) showed that in

pearl grass extract there are active substances that function as antibacterials contained in the methylene chloride fraction, in this fraction the best activity is produced by *Shigella* Disentry bacteria with a diameter of 27.5 mm inhibition area. Research by Nurhayati et al. (2006) also concluded that pearl grass (*Hedyotis corymbosa*) has the ability to inhibit the growth of microbes that can cause disease in poultry, namely *Salmonella* sp and *Escherichia coli* bacteria, but did not show its activity in inhibiting the growth of *Candida albicans*. The inhibitory activity of pearl grass extract was higher on *Escherichia coli* when compared to its activity on *Salmonella* sp.

RESEARCH METHODS

Tools and Materials

The tools used in the study were Autoclave, Aluminum foil, Stirring rod, Petri dish, Erlenmeyer 250 mL, Beaker, Measuring cup 50 mL, 100 mL, Hot plate, Incubator, Push rod, Needle ose, Flannel cloth, Measuring flask, Spirit lamp, Oven, Tweezers, Tube rack, Rotavapor, Test tube, Analytical scales, Digital scales, Maceration container. The materials used are Aquades, Aqua pro injection (API), *Staphylococcus aureus* test bacteria, Snake tongue leaves, 96% ethanol, NA (Nutrient Agar) media, NaCl 0.9%, Tetracycline.

Research Procedure

1. Tool sterilization

The tools to be used were first washed, then dried and wrapped in paper. Non-scaled tools

were placed in an oven at 180°C for 1-2 hours, while scaled tools were placed in an autoclave at 121°C for 15 minutes.

2. Preparation of NA (Nutrient Agar) media

Weighed as much as 2.8 grams of nutrient agar (NA) and then put into an erlenmeyer, dissolved with 100 mL of distilled water, heated on a hot plate to boil while stirring, then sterilized in an autoclave at 121 ° C for 15 minutes. After the sterilization process is complete, the NA media is placed at room temperature and ready to be used as a growth medium for *Staphylococcus aureus* bacteria.

3. Preparation of test bacteria suspension

Taken as much as 1 ose of rejuvenated *Staphylococcus aureus* bacterial culture on Nutrient Agar (NA) media obliquely, put into a test tube that contains 9 mL of 0.9% NaCl solution, shaken until homogeneous until a bacterial suspension is obtained. The bacterial suspension was then adjusted to the Mac Farland standard.

4. Extract Preparation

Leaves are harvested by picking and then washed and wet sorted. The leaves are drained, then sliced to reduce the outer surface area of the leaves, then dried without exposure to direct sunlight. The dried leaves were then put into a container with solvent liquid for maceration. The liquid was filtered to obtain the macerate which was then evaporated to obtain the extract.

5. Testing the diameter of the inhibition zone of ethanol extract of snake tongue leaves against

the growth of *Staphylococcus aureus* bacteria using the paper disc method

Pipetted as much as 20 mL nutrient agar (NA) into a Petri dish, then allowed to solidify (layer 1), bacteria that have been suspended with NaCl 0.9% are pipetted as much as 1 mL, poured into the first layer (layer 2), place a paper disc which is first dripped with *Hedyotis corymbosa* extract each test solution with a concentration of 5%, 10%, 15%, positive control (tetracycline), and negative control (distilled water). Next, it was placed on the surface of the agar while slightly pressed so that it sticks well, then incubated for 2 x 24 hours at 37°C in an incubator. Next, it was removed from the incubator and observed the clear zone formed and then measured the area of inhibition.

RESULTS AND DISCUSSION

This research is an experimental study that aims to determine whether ethanol extract of snake tongue leaves can inhibit the growth of *Staphylococcus aureus* bacteria using a simple experimental design or design consisting of three treatments, as well as two controls in the form positive and negative with three replications or repetition using paper discs testing method. The sample used in this study was *Hedyotis corymbosa*. Where *Hedyotis corymbosa* are plants that are easily found thriving on moist soil on the side of the road, yard and gutter. Some of the chemical content contained in snake tongue leaves such as secondary metabolite compounds

namely tannins, phenols, terpenoids, saponins, flavonoids, and steroids, which one of these compounds is antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes (flavanoids) (Farida, 2007).

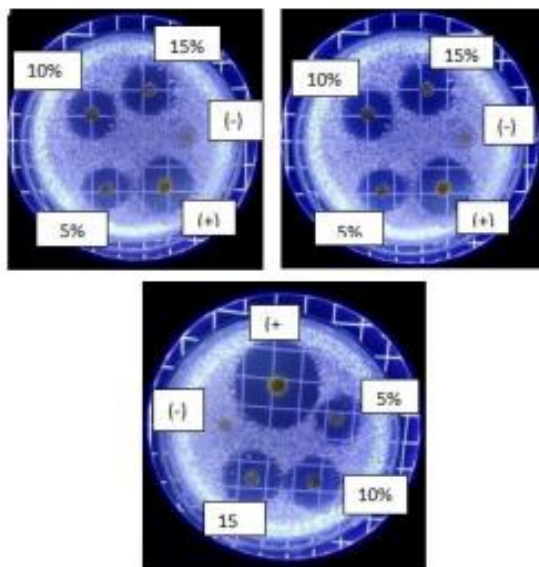
Snake tongue leaves were extracted by maceration method to obtain extracts. The maceration method was chosen due to several considerations including the specifications of the plant to be extracted with a soft texture, some secondary metabolite compounds contained in *Hedyotis corymbosa* leaves that are easily damaged by heating and the selection of solvents used is adjusted to the desired compounds. The solvent used in the extraction is 96% ethanol because of its ability to attract many compounds that are polar, semi-polar, and non-polar. The results of maceration in the form of *Hedyotis corymbosa* leaf extract are then evaporated with the aim of separating the solvent liquid from the extract so that a solution with a higher concentration is obtained. To test the inhibitory power, *Hedyotis corymbosa* leaf samples were made in three concentration variants, namely 5%, 10%, and 15% concentrations by dissolving the *Hedyotis corymbosa* leaf extract using aqua pro injection. The negative control used is aqua pro injection because it does not contain antimicrobial or antibacterial ingredients. The positive control used was tetracycline, because of its antibacterial properties.

Table 1. Inhibition zone measurement results of snake tongue leaves (*Hedyotis corymbosa* (L.) Lamk) against *Staphylococcus aureus* bacteria

No.	Treatment	Extent of Zone of Inhibition (mm)			Average (mm)
		I	II	III	
1	5% Extract	13,4	13,6	14,5	13,8
2	10% Extract	15,8	17,6	16,9	16,7
3	15% Extract	17,8	17,3	17,2	17,4
4	(+) Tetracycline	29,0	29,6	28,1	28,9
5	(-) Aquades	0	0	0	0

(Source: Microbiology Research Data 2017)

The table above shows the results of measuring the inhibition zone area of *Hedyotis corymbosa* leaf extracts. It can be seen that some extracts have differences in the area of the inhibition zone. For *Hedyotis corymbosa* leaf extract with a concentration of 5% has an average inhibition of 13.8 mm, in the extract with a concentration of 10% has an average inhibition of 16.7 mm, in the 15% concentration extract has an average inhibition of 17.4 mm, while for positive control tetracycline has inhibition with an average value of 28.9 mm and for negative control distilled water has no inhibition.



Zone of inhibition or clear area formed

The difference in bacterial inhibition zones can also be seen in the following bar chart :

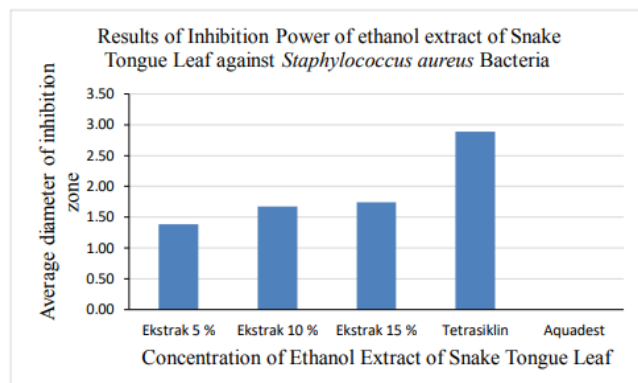


Diagram of the inhibition zone results of ethanol extract of snake tongue leaves against *Staphylococcus aureus* bacteria

Based on the data above, it explains that *Hedyotis corymbosa* leaf extract at a concentration of 10% with the area of inhibition formed shows a weak ability to inhibit the growth of *Staphylococcus aureus* bacteria, while at 15% and 20% concentrations are included in the moderate category in inhibiting the growth of *Staphylococcus aureus* bacteria. Of the three different concentrations, it is inversely proportional to tetracycline which has an

inhibition value of 28.9 mm which is classified into the strong category.

Based on the data from the test results of the inhibition of *Hedyotis corymbosa* leaf extract against the growth of *Staphylococcus aureus* bacteria, data analysis was carried out using the ANOVA analysis method with the SPSS version 20 analyzer, with the following results:

Source Variations	Total Quadratic	Degree of Freedom	Quadratic Middle	F Count	P-value	F table
Treatment	12.836	4	3.2090	872.009	1.14E-12	3.478
Error	0.037	10	0.0037			
Total	12.873	14				

From the results of the analysis using the ANOVA analysis method, it was found that the calculated F value was 872.009 while the F table value was 3.478 at the 0.05 confidence level. The treatment of 3 repetitions in each test sample shows that there is a significant difference (the value of F count is greater than F table), meaning that the average treatment has a very significant difference in inhibiting the growth of *Staphylococcus aureus* bacteria. The results of testing the research hypothesis concluded that snake tongue leaf extract (*Hedyotis corymbosa*) has the ability to inhibit the growth of *Staphylococcus aureus* bacteria, where according to Saptarini (2011) ethanol extract of *Hedyotis corymbosa* leaves contains secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins, and steroids where flavonoid compounds are active compounds that are antibacterial in the sense that they can inhibit microbial growth.

CONCLUSIONS

Ethanol extract of snake tongue (*Hedyotis corymbosa*) leaves has antibacterial activity against the growth of *Staphylococcus aureus* bacteria with an average inhibition area of 13.8 mm (5%), 16.7 mm (10%), and 17.4 mm (15%).

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