

ANTIBACTERIAL TEST OF SQUEEZED WATER OF KATUK LEAF ON STREPTOCOCCUS PYOGENES

Ni Putu S Novitayanti¹⁾, Titien Rahayu²⁾, Sindrawati³⁾

ABSTRACT

Introduction: Many infectious diseases still can be found in developing countries, including Indonesia. The common type of infection is upper respiratory tract infection (URTI), in which one of the diseases is pharyngitis. About 5% - 40% of pharyngitis cases are caused by *Streptococcus pyogenes*. One of the treatments for bacterial infection is antibiotics, but the increasing number of antibiotic resistance causes the need to find other alternatives such as herbal plants for the treatment. The community of Bali, particularly in Singaraja, often consumes the squeezed water of Katuk leaf (*Sauropus androgynus* (L.) Merr) to treat pharyngitis.

Purpose: This study aims to determine the antibacterial effect of squeezed water of Katuk leaf on *Streptococcus pyogenes* by measuring the value of the minimum inhibitory concentration (MIC) and the value of the minimum bactericidal concentration (MBC).

Method: The squeezed water of Katuk leaf was obtained through the squeezing method. The antibacterial activity test used the microdilution method of 96 well plates. The absorbance value was read using a spectrophotometer to determine the MIC value, and streaking was performed to the blood agar strengthened by the results of the spectrophotometric readings to determine the value of MBC.

Result: The results showed that the 300 grams of squeezed water of Katuk leaf had MIC values at a concentration of 10% and MBC values at a concentration of 20%.

Conclusion: It can be concluded that the squeezed water of Katuk leaf has an antibacterial effect on *Streptococcus pyogenes*.

Keyword: Antibacterial, *Sauropus androgynus* (L.) Merr, *Streptococcus pyogenes*

¹⁾ Faculty of Medicine, Widya Mandala Catholic University Surabaya
E-mail: sherlynytaa@gmail.com

²⁾ Department of Clinical Pathology, Faculty of Medicine, Widya Mandala Catholic University Surabaya

³⁾ Department of Pathology Anatomy, Faculty of Medicine, Widya Mandala Catholic University Surabaya

INTRODUCTION

Many people in developing countries still suffer from infectious diseases. A type of infection that is quite common is upper respiratory tract infection. One of the diseases is pharyngitis. 5% - 40% of the causes of pharyngitis are bacteria, one of the bacteria that often causes pharyngitis is *Streptococcus pyogenes* (1,2). Pharyngitis caused by bacteria can cause complications if not treated properly, such as rheumatic fever and glomerulonephritis (3). One of the treatments for bacterial infection is antibiotics, but they must be closely monitored because if used irrationally, they can lead to resistance. According to the Centers for Disease Control (CDC), more than 2.8 million antibiotic-resistant infections occur in the United States each year, and more than 35,000 people die (4). The increasing number of resistance causes the need to look for other alternatives to help cure the infection, which is herbal plants.

Indonesia is a country that has many herbal plants with a total of 9,600 species (5). One of the plants is katuk leaves. To be precise, in Singaraja, Balinese people often consume katuk leaf juice to treat pharyngitis. Katuk leaves contain saponins, tannins, flavonoids, and alkaloids that inhibit bacterial growth (6).

Based on the habits of people in Singaraja, Bali uses squeezed water of katuk leaf to treat pharyngitis. Still, researchers have not been able to find a source that states that squeezed water of katuk leaf has an antibacterial effect. For this reason, researchers are interested in studying the antibacterial effect of the squeezed water of katuk leaf against the bacteria *Streptococcus pyogenes*.

METHOD

This study used an experimental study with a non-equivalent control group design. The research was conducted in the Surabaya Center for Health Laboratory and

Phytochemical-Pharmacognosy Laboratory of the Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya. The samples in this study were selected using purposive sampling technique in a colony of *Streptococcus pyogenes*. The bacteria used was *Streptococcus pyogenes* ATC 19615 obtained from Surabaya Center for Health Laboratory 300 gr leaves of katuk (*Sauropus androgynus* (L.) Merr) in this study were obtained from UPT Materia Medica, Batu City and processed into squeezed water with a blender. Before use, the juice is first sterilized by autoclave at a temperature of 121°C for 15 minutes following the recommendations of the World Health Organization (WHO) (7).

This study used five different concentrations of squeezed water of katuk leaves, 5%, 10%, 12.5%, 15%, and 20%. The antibacterial activity test used the microdilution method to determine the value of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The test used a 96 well microplate, then the optical density (OD) readings were carried out using a spectrophotometer to determine MIC. The MBC value was obtained from streaking agar which was strengthened by the spectrophotometer results. The microdilution test was carried out by inserting 50 µl of Mueller Hinton Broth (MHB) media into treatment wells (P1-P5) and control (K1-K5), 50 µl aquades to control wells (K2 and K4), 50 µl of squeezed water of katuk leaf to treatment wells (P1-P5) and control (K2), 50 µl of 0.12 penicillin to control wells (K5), 50 µl of suspension of *Streptococcus pyogenes* 0.5 McFarland bacteria to treatment wells (P1-P5) and control (K3-K5). The microplate was incubated for 18-24 hours; then the OD value was observed using a spectrophotometer. Determination of MBC by streaking agar from the microplate, which was incubated first.

RESULTS

The graph (Figure 1) shows that the lowest inhibition percentage is 81.12% at a concentration of 5% squeezed water of katuk leaf, and the highest inhibition percentage is 100% occurs at a concentration of 20% squeezed water of katuk leaf. From the results of the inhibition percentage, MIC can be determined at the concentration of 10% katuk leaf solution.

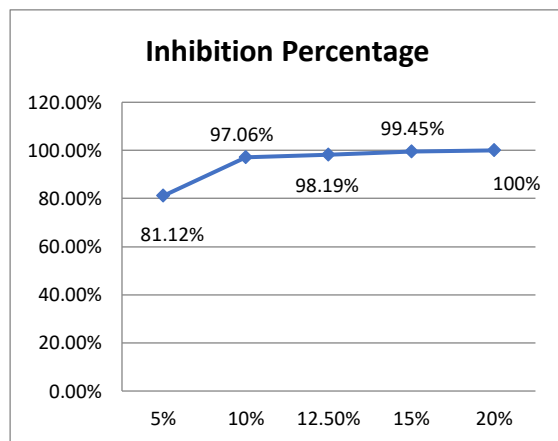


Figure 1. Inhibition Percentage

In the results of agar streaking (Figure 2), it can be seen at a concentration of 20% squeezed water of katuk leaf has not seen any bacterial growth and strengthened with the percentage of inhibition with a result of 100% so that the MBC value can be determined at a concentration of 20%.

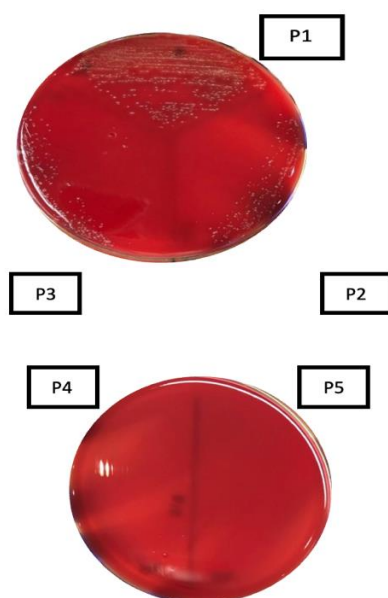


Figure 2. Agar Streaking Results

DISCUSSION

This study used *Streptococcus pyogenes* ATC 19615, obtained from Surabaya Center for Health Laboratory. Before use, the bacteria obtained first performed some biochemistry tests, gram stain, and culture to identify *Streptococcus pyogenes*. In the tested bacteria, the catalase test results were negative, the PYR test was positive, gram staining indicated gram positive bacteria, and the culture test results showed the presence of a hemolysis process. From the results of all tests to identify bacteria, it can be ascertained that the bacteria used is *Streptococcus pyogenes*, and there is no contamination.

The katuk leaves used were obtained from UPT Materia Medica Batu City. This study uses the squeeze method because this method is relatively easy and fast compared to other methods. One of the drawbacks of the squeeze method is that it is easy to grow microbes (8) so that the squeezed water that has been obtained is sterilized first using an autoclave. Sterilization is important for digestion and eliminating microorganisms that can contaminate. There are various types of sterilization, one of which uses an autoclave, as in this study. The use of autoclaves can kill bacteria due to the denaturation of the enzymes and protein structure. The World Health Organization (WHO) recommends using an autoclave for sterilization at a temperature of 121-124°C for 15 minutes (7). In this study, a temperature of 121°C was used for 15 minutes.

The use of autoclave in this study was proven not to eliminate the antibacterial effect of the katuk leaves used, also strengthened by research from Dian Wahyu (2020) (9), who found that the autoclave technique only slightly reduced the content of the sterilized material so that the antibacterial effect is still strong enough to inhibit bacteria.

This study used the microdilution method to test the antibacterial activity. The choice of the microdilution method as an

antibacterial activity test because this method requires only a few antibacterial compounds, can present quantitative data, can be used to determine MIC (10,11), and this method is more sensitive for determining resistance compared to the diffusion method because the microdilution method is measured in a non-visual way, namely using a spectrophotometer so that it can be more sensitive (12).

KHM test in this study was carried out utilizing a spectrophotometer reading. Readings were done after incubation for 24 hours, and the percentage of inhibition was obtained, as shown in Figure 1. From this study, MIC can be found at a concentration of 10% with a percentage of resistance of 97.06%. The determination of KBM was seen based on the results of streaking at the smallest concentration that could inhibit bacterial growth as much as 99.9%. In this study, KBM was found at a concentration of 20% and strengthened by the results of the spectrophotometer, which showed resistance of 100%. These results answered the objectives of this study, namely to determine the antibacterial effect, MIC value, and MBC value of the squeezed water of katuk leaf against *Streptococcus pyogenes* bacteria. The MIC and MBC values found can be used to see the efficacy of the antibacterial solution and determine the bactericidal ability of the tested antibacterials (11).

CONCLUSIONS

This study found the MIC value at a concentration of 10% squeezed water of katuk leaf, and KBM found at a concentration of 20% squeezed water of katuk leaf. From the research results, it can be concluded that the squeezed water of katuk leaf (*Sauropus androgynus* (L.) Merr.) has antibacterial activity against *Streptococcus pyogenes* bacteria.

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