ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF KITOLOD (*Hippobroma longiflora*) LEAVES AGAINST *STREPTOCOCCUS PYOGENES*

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ABSTRACT

Introduction: Antimicrobial resistance to antibiotics has been emerging as a serious issue in healthcare, hence the need to discover and develop new treatment alternatives. Chemical compounds found in medicinal plants can potentially be used to synthesize new antibiotic agents, one of which is the leaves of kitolod (*Hippobroma longiflora*). Phytochemical screening of kitolod leaves showed positive results for secondary metabolites presumed to have antibacterial effects.
Purpose: The purpose of this study was to examine the antibacterial activity of ethanolic extract of kitolod leaves against *Streptococcus pyogenes*.
Method: Antibacterial activity of the ethanolic extract of various concentrations (0,25%, 1,75%, 3,25%, 4,75%, 6,25%) were evaluated by broth microdilution method on a 96-well microplate and by streaking on agar plates.
Results: Minimum Bactericidal Concentration (MBC) of ethanolic extract of kitolod leaves against *Streptococcus pyogenes* was 1,75%. Minimum Inhibitory Concentration (MIC) of ethanolic extract of kitolod leaves against *Streptococcus pyogenes* could not be inferred.
Conclusion: Ethanolic extract of kitolod leaves showed antibacterial activities against *Streptococcus pyogenes*.

Keywords: Antibacterial, Kitolod, *Hippobroma longiflora*, *Streptococcus pyogenes*

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INTRODUCTION

In 2005, WHO estimated that 18.1 million people were suffering from invasive *Streptococcus pyogenes* infections, with 1.78 million incident cases and 517.000 deaths occurring each year.[1] *Streptococcus pyogenes*, a ß-hemolytic streptococci belonging to Lancefield A serogroup (also known as group A streptococci (GAS)), is the most virulent human pathogen among the Streptococcus species.[2] *S. pyogenes* disease manifestations range from asymptomatic carriers; local and superficial infections (e.g. pharyngitis, tonsilitis, and pyoderma); invasive infections (e.g. cellulitis, sepsis, and necrotizing fasciitis); toxin-mediated diseases (e.g. scarlet fever and streptococcal toxic shock syndrome); and autoimmune sequelae (e.g. acute rheumatic fever, rheumatic heart disease, and post-streptococcal glomerulonephritis,).[2,3] The drug of choice for *S. pyogenes* infections is penicillin.[4] Although it has been the drug of choice for several decades due to its efficacy and low toxicity, in vitro isolates of *S. pyogenes* are still susceptible to penicillin.[5]

Patients with a history of allergy or treatment failure with penicillin might be given alternative antibiotics such as macrolides, cephalosporins, lincosamides, and clindamycin.[6] From 355 pediatric patients in Rome, Italy, 127 (35.8%) were resistant to erythromycin.[7] Resistance to clarithromycin, clindamycin, and vancomycin have also been reported.[8]

With the increasing rate of antibiotic resistance, discovering new antibiotic agents is necessary, possibly making use of natural sources such as plants. Various plants have active compounds in the form of secondary metabolites, which could help fight bacterial growth, one of which is the leaves of kitolod.[9] *Hippobroma longiflora* (also known as kitolod, kendali, sangkobak, and Star of Bethlehem) is a wild plant that grows in tropical and subtropic climates.[10] Kitolod leaves have been utilized by the community to help relieve toothache, sore throat, cancer, asthma, and several other diseases. Phytochemical tests showed kitolod leaves contain secondary metabolites which might have antibacterial properties, i.e., flavonoids, tannins, saponins, alkaloids, polyphenols, and terpenoids.[11,12]

METHOD

Kitolod leaves used in this study were Simplicia powder, made in Materia Medica Batu, Malang, Jawa Timur. The plants used were wild plants, grew in damp soil, and harvested after 3-4 months. The leaves were dried and crushed and then were sifted to produce a fine powder. Maceration was the method used for the extraction in this study. 500 g of kitolod leaf Simplicia powder were put into a glass jar and macerated with 70% ethanol at room temperature for 3 x 24 hours, occasionally stirred, and filtered with filter paper. Filtrates were then concentrated using a rotary evaporator at 50°C until the solvent was completely evaporated, producing a thick extract. Extract of 100% concentration was diluted with aqua dest to achieve concentrations of 0,25%, 1,75%, 3,25%, 4,75%, and 6,25%.

The bacteria *Streptococcus pyogenes* ATCC 19615 used in this study was obtained from the Microbiology Laboratory at Balai Besar Laboratorium Kesehatan (BBLK) Surabaya. The bacteria were identified macroscopically, microscopically (with Gram staining), and biochemically (with catalase test, bacitracin susceptibility test, and PYR test)

The samples were divided into two groups: the treatment and control groups. The treatment group consisted of *Mueller Hinton Broth* + *Streptococcus pyogenes* + 0,25% (P1), 1,75% (P2), 3,25% (P3), 4,75% (P4), and 6,25% (P5) ethanolic extract of kitolod leaves. The control group consisted of *Mueller Hinton Broth* (K1), *Mueller Hinton Broth* + 0,25% (K2), 1,75% (K3), 3,25% (K4), 4,75% (K5), and 6,25% (K6) ethanolic extract of kitolod leaves, *Mueller Hinton Broth* + *Streptococcus pyogenes* + Penicillin (K7) as positive control, *Mueller Hinton Broth* + *Streptococcus pyogenes* +

aquadest (K8) as negative control, and *Mueller Hinton Broth + Streptococcus pyogenes* (K9). The Mueller Hinton Broth media in this study was supplemented with 5% sheep blood to meet the growth requirements of *Streptococcus pyogenes*.

The method of antibacterial activity testing in this study was microdilution with a 96-well microplate and by streaking on blood agar plates. The microplate was incubated for 24 hours, followed by optical density (OD) measurement using a spectrophotometer with 600 nm wavelength to determine the Minimum Inhibitory Concentration (MIC), the lowest concentration of extracts to inhibit 90% of bacterial growth. The solution from the microplate was inoculated on Mueller Hinton Blood Agar plates (also supplemented with 5% sheep blood). Streaking on agar plates was done to confirm the presence or absence of viable cells. Viable cells can grow and form colonies. Therefore, the purpose of streaking was to confirm the Minimum Bactericidal Concentration (MBC), the lowest concentration of extracts to inhibit 99.9-100% of bacterial growth, determined by the absence of bacterial colonies forming on agar plates.

RESULTS

Figure 1 Graphics of inhibition percentage of ethanolic extracts of kitolod leaves

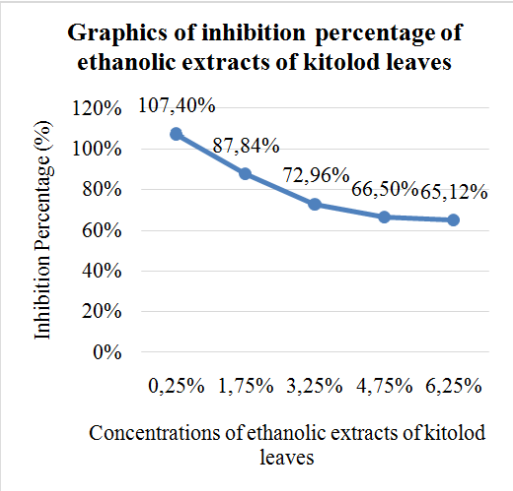

Figure 1 shows the spectrophotometry measurements. An increase did not follow the increase of extract concentrations in growth inhibition (decrease of OD). This might be caused by extract and blood sediments formed at the bottom of the well and the dark color of the extracts. Therefore, the growth of bacteria was not correctly represented by the OD.

Figure 2 Result of the treatment (P1 – P5) group streaking on the blood agar plate

Figure 2 showed bacterial growth inhibition on P1 (*Mueller Hinton Broth + Streptococcus pyogenes* + 0,25% ethanolic extract of kitolod leaves) and on P2-P5 (*Mueller Hinton Broth + Streptococcus pyogenes* + 1,75%-6,25% ethanolic extracts of kitolod leaves) there were no signs of bacterial colony growth. Replication of 5 times produced similar results.

DISCUSSION

Several factors disrupted the spectrophotometry measurements, consequently producing incorrect bacterial growth representation. Those factors were sediments formed at the bottom of the well, the dark color of the extracts, and hemolysis activities of the tested bacteria. For these reasons, the MIC in this study could not be inferred from the experiments. The control group consisting of only Mueller Hinton Broth media (K1) had a greater optical density than the bacterial control (K9) consisting of media + bacteria. Therefore, the optical density of only the bacteria could not be calculated.

Spectrophotometry measurements were followed by streaking on agar plates to confirm the Minimum Bactericidal Concentration (MBC) of ethanolic extracts of kitolod leaves against *Streptococcus*

pyogenes. Based on the results, there were antibacterial effects of ethanolic extracts of kitolod leaves from 1,75% to 6,25% concentration (P2 – P5) on all repetitions, similar to the positive control containing penicillin. MBC was confirmed by the absence of bacterial colony growth by visual inspection. It can be assumed that 1,75% - 6,25% ethanolic extracts of kitolod leaves had bactericidal effects against *S. pyogenes*. Inspected bacterial colony growth on the treatment group containing 0,25% ethanolic extract of kitolod leaves showed that it did not have any bactericidal effects.

The bacteriostatic and bactericidal effects of ethanolic extracts of kitolod leaves come from its secondary metabolites, i.e., flavonoids, tannins, saponins, alkaloids, polyphenols, dan terpenoids.[11,12] Flavonoids denature bacterial cell wall proteins and destruct the cell.[13] It also disrupts the DNA replication process, eventually causing injury and death.[13] Tannins hinder the permeability of bacterial cell walls and inhibit reverse transcriptase and DNA topoisomerase enzymes, thus disrupting their ability to replicate.[13] Saponins increase the cell membrane permeability, causing an imbalance that leads to cell lysis.[9] Terpenoids react with a transmembrane protein called porin. Porins are the entrance and exit for components that alter cell wall permeability. Disruption of porins leads to a lack of nutrition, inducing bacterial growth disturbance.[13]

The results from this study were similar to the previous study conducted by Simanjutak (2020).[9] The previous study tested the antibacterial activity of ethanolic extracts of kitolod leaves against *Staphylococcus aureus* and *Salmonella typhi* with the disk diffusion method. The zone of inhibition produced by 12.5% ethanolic extracts of kitolod leaves against *Staphylococcus aureus* was 7.06 mm, while 6.25% ethanolic extracts of kitolod leaves produced 7.66 mm of inhibition zone.[9] The differences in the MIC of this study from the previous study were speculated to result from one of the disadvantages of the disk diffusion method, which is compounds that are scarcely soluble or insoluble in water prevent uniform diffusion into the media.[14]

CONCLUSION

The result from this study suggests that the MIC value of ethanolic extracts of kitolod leaves against *Streptococcus pyogenes* could not be inferred from the experiments. In contrast, the MBC value is suggested to be 1,75%.

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