Antibacterial Activity of Ethanolic Extract of Kitolod (Hippobromalongiflora) Leaf Against Staphylococcus aureus and Salmonella typhi

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A B S T R A C T

Objective: The purpose of this study was to determine at what concentration of ethanol extract of kitolod leaves is active against Staphylococcus aureus and Salmonella typhi.

Methods: Ethanolic extract of Kitolod leaves was tested for phytochemical screening by using standard protocol. Antibacterial testing was using the diffusion disc method to measure the inhibition zone against the Staphylococcus aureus and Salmonella typhi with various concentration of Kitolod leaves extract (6.25%, 12.5%, 25%, 50%, and 75%).

Results: Phytochemical screening showed that ethanolic extract of Kitolod contains alkaloids, flavonoids and saponins. The antibacterial inhibition of ethanol extract of kitolod leaves against Staphylococcus aureus and Salmonella typhi bacteria at a concentration of 75% had a diameter of 11.3 mm and 12.16 mm with a strong category.

Conclusions: Kitolod leaf could be used as a novel antibacterial agent.

Keywords: Extract, Antibacterial, Kitolod, Hippobromalongiflora, S. aureus, S. typhi

A R T I C L E I N F O

Cite this article as:
Simanjuntak HA, Antibacterial Activity of Ethanolic Extract of Kitolod (Hippobromalongiflora) Leaf Against Staphylococcus aureus and Salmonella typhi, Asian Journal of Pharmaceutical Research and Development. 2020; 8(1):52-54.
DOI: http://dx.doi.org/10.22270/ajprd.v8i1.660

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INTRODUCTION

Medicinal plants have been known to cure various diseases since ancient times. This is due to the potential of chemical compounds found in these plants that can be used to synthesize new conventional medicines, one of which is the plant kitolod Hippobroma longiflora (L.) G. Don¹. Kitolod plants are used to treat toothache, asthma, bronchitis, laryngitis, catarracts, wound medication, and cancer drugs². Ethanol extract of kitolod leaf contain secondary metabolite compounds namely alkaloids, flavonoids, polyphenols, monoterpenoids, sesquiterpenoids, quinins and saponins³. According to Ibrahim explains that the content of secondary metabolites in plants Chitolod has activities such as antioxidants, cytotoxics, anticancer, anti-inflammatory and antimicrobial⁴. Staphylococcus aureus is one of the important pathogenic bacteria associated with toxin virulence, invasive, and the body's resistance to antibiotics. Staphylococcus aureus infection is the main cause of skin, soft tissue, respiratory, bone, joint and endovascular disorders⁵. Salmonella typhi is a pathogen that causes typhoid (enteric) fever, which is a systemic infection in humans caused by Salmonella serotypes, including Salmonella serotype Typhi⁶. This research is expected to provide information on secondary metabolite compounds found in kitolod leaves and the antibacterial activity of ethanol extracts of kitolod leaves against Staphylococcus aureus and Salmonella typhi.

MATERIAL AND METHODS

Plant preparation
Fresh kitolod leaves was collected from local area of from Bakal Julu Village (Dairi Regency North Sumatra Medan, Indonesia), and authenticated by Indonesian Institute of Sciences: Research Center ForBiology. (No. 2347/MEDA/2019). Voucher specimen was deposited in the Pharmacognosy Laboratory, Sekolah Tinggi Ilmu Kesehatan Senior Medan.

Extraction of kitolod leaves
Kitolod leaves simplicia powder was extracted using maceration method with 96% ethanol solvent. Maceration is done by soaking the simplicia of kitolod leaves for 3 days with occasional stirring. The procedure is repeated.
until the color is clear. The results of maceration (macerate) were evaporated using a rotary evaporator to obtain crude extract of Kitolod leaves.

**Phytochemical screening of various lotus leaf extract**

The crude extract of kitolod leaves was screening by using the standard protocol to know the presence of phytochemical compounds.

**Antibacterial test**

Preparation of antibacterial test will begin with sterilizing the tools and materials to be used, rejuvenation of bacteria, making media, making bacterial suspense, making kitolod leaf extract test solutions and making comparative standard solutions. The determination of the antibacterial activity was carried out with sterilized NA media inserted into 20 mL sterile petri dishes each and allowed to condense at room temperature. The media was dropped with 0.1 mL of bacterial suspension test and flattened using an L bar until smooth and dry. Sterile disk paper with a diameter of 6 mm was dropped with ethanol extract 96% of kitolod leaves as much as 10 µL with each concentration of 6.25%, 12.5%, 25%, 50%, and 75% and then placed on the media so that the solid that had been dripped with a test bacterial suspension, DMSO 10% as a negative control, and chloramphenicol as a positive control. Then incubated at 37°C for 24 hours and after incubation the clear zone was measured using calipers, three replications were performed.

**RESULT AND DISCUSSION**

**Phytochemical Screening**

Phytochemical screening results of kitolod leaves ethanol extract showed different phytochemical compounds (Table 1).

**Table 1. Phytochemical screening of Kitolod leaves ethanolic extract**

| No | Screening | Reagent                      | Ethanolic Extract |
|----|-----------|------------------------------|-------------------|
| 1  | Alkaloids | Mayer Positive               | Positive          |
|    |           | Dragendroff Positive         |                   |
|    |           | Bouchardat Positive          |                   |
| 2  | Flavonoids| Mg+HCl+Amyl Alcohol Positive |                   |
| 3  | Saponins  | Foaming Test Positive        |                   |
| 4  | Tannins   | FeCl₃ 1% Negative            |                   |
| 5  | Triterpenoid/Steroids | Liebermann Bouchard | Negative |

**Antibacterial test**

Antibacterial activity testing of ethanol extract of kitolod leaves was carried out using the disk diffusion method, namely the determination of bacterial sensitivity with a particular substance that may have antibacterial activity using disc paper. Antibacterial testing was carried out with various concentrations of 6.25%, 12.5%, 25%, 50%, and 75%, chloramphenicol and DMSO. The results of testing the antibacterial activity of ethanol extract of chitolod leaves against *Staphylococcus aureus* and *Salmonella typhi* can be seen in Table 2 and Figure 1 below.

**Table 2: Antibacterial result of Kitolod leaves ethanolic extract**

| Concentration | Zone of inhibition (mm) |
| ------------- | ------------------------|
|              | *Staphylococcus aureus* | *Salmonella typhi* |
| 75%          | 11.3                    | 12.6                 |
| 50%          | 10                      | 8.9                  |
| 25%          | 8.83                    | 7.6                  |
| 12.5%        | 7.06                    | 7.83                 |
| 6.25%        | 0                       | 7.66                 |
| Chloramphenicol | 28.66                | 30.5                 |
| DMSO         | 0                       | 0                    |

**Figure 1: Antibacterial result of Kitolod leaves ethanolic extract**

Based on Table 2 and Figure 1 it can be seen that 75% concentration of ethanol extract of kitolod leaves is effective in inhibiting the growth of *Staphylococcus aureus* and *Salmonella typhi* with inhibition zone diameters of 11.3 mm and 12.6 mm, which are classified as strong criteria. Whereas the concentration of 6.25% has the ability to inhibit the growth of *Salmonella typhi* bacteria compared to *Staphylococcus aureus*. According to Davis and Stout, explain that the criteria for antibacterial inhibition consist...
of ≥ 20 mm is very strong, 10-20 mm is strong, 5-10 mm is moderate and ≤ 5 mm is weak\(^8\).

In positive controls using standard chloramphenicol with a concentration of 30 mg / ml. Chloramphenicol works by inhibiting protein synthesis in bacterial cells by reversibly binding to the 50 s ribosome subunit\(^9\). The negative control was DMSO with a concentration of 10%. In this study negative control DMSO has no clear zone, so DMSO can be said to not be able to inhibit bacterial growth.

Alkaloids can disrupt the constituent components of peptidoglycan on bacterial cells so that the cell wall layers are not formed intact and cause cell death. Another mechanism of antibacterial alkaloids is that the alkaloid component is known as a DNA accelerator and inhibits bacterial cell topoisomerase enzymes\(^11\).

Flavonoids provide bacteriolytic effects, inhibit protein synthesis, DNA synthesis, RNA and damage cell membrane permeability\(^12\). According to Wu et al, flavonoids have antibacterial activity because of the ability of flavonoids to interact with cell membranes and affect cell membrane bioactivity and it has been reported that flavonoids are able to reduce the fluidity of bacterial cell membranes that is directly related to damage to cytoplasmic membranes or indirect damage through autolysis / weakening of the cell wall and consequently osmotic lysis\(^13\).

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Ethanol extract 96% of active kitolod leaves as antibacterial due to chemical components contained in the extract. Based on phytochemical screening, ethanol extract of kitolod leaves contain alkaloid, flavonoid, and saponin compounds. The mechanism of action of alkaloids as an antibacterial is by inhibiting the synthesis of nucleic acids, because it can inhibit the enzymes dihydrofolate reductase and topoisomerase 1\(^10\). According to Karou et al., the mechanism of action of saponin as an antibacterial causes lysis of the bacterial cell wall and leakage of AKP (Alkaline Phosphate), an increase in saponin concentration causes the protein to dissolve, causing intercellular compounds to diffuse through the outer membrane and cell wall. This causes the cytoplasm to leak out of the cell resulting in cell death\(^14\).

**CONCLUSIONS**

The ethanol extract of Kitolod leaves has a group of secondary metabolites including alkaloids, flavonoids, and Saponins. The ethanol extract of Kitolod leaves has effective antibacterial activity against Staphylococcus aureus and Salmonella typhi bacteria with a concentration of 75% with a strong category.

**ACKNOWLEDGMENTS**

Authors wish to thank to Deby Geovani Hutasoit for her help about this research.

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