Antibacterial Activity Test of Ethyl Ascetic Fraction Ethanol Extract Faloak Tree Bark (Sterculia sp.) on Salmonella Thyposa Bacteria

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Abstract
The bark of the faloak (Sterculia sp.) Tree utilized by the people of Timor Island is limited as a source of medicine. The efficacious substances in faloak bark which are known to have antibacterial properties are flavonoids, alkaloids and saponins. The purpose of this study was to measure the inhibitory power of ethyl acetate fraction of ethanol extract of faloak tree bark (Sterculia sp.) Against the growth of Salmonella thyposa bacteria. Ethyl acetate fraction was obtained from the bark of faloak (Sterculia sp.) Tree which was macerated with 70% ethanol so that the ethanol extract from the bark of faloak tree (Sterculia sp.) Was obtained. The extract was fractionated using a liquid-liquid partition with a mixture of ethanol-water (2: 3) and ethyl acetate. The results of the fraction were identified qualitatively, then antibacterial activity was tested using diffusion method with antibacterial effectiveness parameters based on the diameter of the inhibition zone around the cylinder. Antibacterial activity test carried out on ethyl acetate fraction obtained from fractionation of ethanol extract of faloak (Sterculia sp.) Tree bark was carried out with 3 concentration treatments namely 25%, 50% and 75% and 3 replications for each treatment. The measurement of inhibition zone diameter showed that ethyl acetate fraction of ethanol extract of the bark of faloak (Sterculia sp.) At a concentration of 25% b/v, 50% b/v did not show any antibacterial activity, as seen from the absence of the resulting inhibition zone while at a concentration of 75% b/v showed antibacterial activity against Salmonella typhoid with an area of 15 mm inhibition zone. The conclusion was that ethyl acetate fraction of ethanol extract from the bark of faloak tree (Sterculia sp.) Had antibacterial activity against Salmonella thyposa bacteria at a concentration of 75% b/v.

Keywords: Antibacterial, Faloak (Sterculia sp.), Ethyl Acetate fraction

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INTRODUCTION

Today, Indonesia has extensive tropical forest land with biodiversity, both flora and fauna. We may be proud of the richness of medicinal plants that other countries do not have. More than 30,000 to 40,000 types of plants are spread from Aceh to Papua, from low to highlands, from the tropics to cool regions, even to plants and marine wealth can be used as medicinal ingredients (Wijayakusuma, 2000).

Plants that are used as medicine have been known and used by Indonesian people since a long time ago as ancestral heritage as traditional medicine to overcome health problems. One of the causes of health problems experienced by Indonesian people is caused by bacteria, so that to overcome these health problems, antibacterial substances are needed.

The use of faloak trees by the people in East Nusa Tenggara (NTT) Province is still traditionally used based on hereditary knowledge and experience (Ranta, 2011). In general, faloak bark extract contains saponins, steroids, flavonoids, alkaloids, and triterpenoids which have quite high antimicrobial activity. However, so far scientific research and studies on the characteristics and utilization of faloak as antibacterials are still very limited.

The Sengga study (2013) showed that the ethanol extract of the faloak tree (Sterculia sp) concentration of 100% b/v had a inhibitory effect on Escherichia coli bacteria. This study uses 70% ethanol with high polarity and can attract various flavonoid compounds (Harbone, 1996). However, this study is only limited to the initial extract which is difficult to separate through a single separation technique to isolate a single compound. Therefore, the initial extract needs to be separated into fractions that have the same polarity and molecular size (Mukhriani, 2014). One of the solvents that can be used in the fractionation process is ethyl acetate. Ethyl acetate is a semi-polar solvent and can dissolve semi-polar flavonoid compounds.

This is based on Yulianti's research (2013) entitled "Antibacterial Activity and Bioautography of Ethyl Acetate Fraction Acetone Extract of Cocoa Bark (Theobroma cacao L.) Against
Streptococcus mutans and Bacillus subtilis” proving that the phytochemical test results with KTL (Thin Layer Chromatography) show groups. The compounds of ethyl acetate fraction of acetone extract of cacao fruit peel (Theobroma cacao L.) contain flavonoids.

Flavonoids are the largest group of phenol compounds found in nature and flavonoid compounds consisting of several groups have different polarity (Waji and Sugrani, 2009). Flavonoids also have many benefits, one of which is as an antibacterial which works to inhibit bacterial growth, causing damage to bacterial cell wall permeability (Darsana, et al., 2012).

Based on previous studies of flavonoid compounds from the skin of faloak (Sterculia sp.) Which can be extracted in fractionation with ethyl acetate solvents and efficacious as antibacterial, then encouraged the author to conduct further research on “Antibacterial Activity Test of Ethyl Acetate Fraction of Ethanol Extract of Tree Bark Faloak (Sterculia sp.) Against Salmonella thyposa bacteria”.

MATERIAL AND METHODOLOGY
The type of research used in this study is experimental research with a complete experimental design. The research was conducted at the research of Pharmacognosy Laboratory, Chemistry Laboratory and Microbiology Laboratory, Department of Pharmacy, Kupang Health Polytechnic. In September to November 2017.

The sample in this study was ethyl acetate fraction derived from the ethanol extract of the bark of the faloak tree (Sterculia sp.).

RESULTS AND DISCUSSION
Making Ethyl Asset Acetate Ethanol Extract of Faloak Tree Bark (Sterculia sp.)

The making of the extract starts from the manufacture of Faloak (Sterculia sp.) Tree bark simplicia to obtain simplicia powder. The faloak (Sterculia sp.) Tree Bark powder was extracted using maceration method. Maseration is a simple method of escape. Maseration is done by soaking the powder simplicia with the solvent. The solvent will penetrate the cell wall and
enter the cell cavity containing the active substance, the active substance will dissolve, the difference in concentration between the solution of the active substance in the cell and outside the cell causes the concentrated solution to be pushed out. These events occur repeatedly until a balance of concentration occurs between the solution of the active substance in the cell and outside the cell. The dancer liquid used is 70% ethanol because it has a dielectric constant of 45 (polar) and compounds which are thought to have antibacterial activity on faloak (*Sterculia sp.*) Bark extract in example flavonoids can be extracted with 70% ethanol. This is also based on Harborne (1996) which states that flavonoid compounds can be extracted well using 70% ethanol. In addition, the use of 70% ethanol because it is neutral, selective in producing the optimal number of active compounds, and heat needed for less concentration (Anonim, 1986).

Maserate obtained was separated from the solvent by evaporation with a vacuum rotary evaporator to evaporate 70% ethanol at 68 ° C. The result of evaporation is called aqueous thick extract and the remaining water is removed by evaporating the extract over the waterbath with a temperature below 60 ° C so that the active substance contained in the extract is not damaged by heating to obtain a thick extract with a percentage of yield of 14.66%.

The thick extract extracted is then fractionated by using a liquid-liquid partition. The principle of separation by partitioning is the difference in solubility, and the conditions that must be fulfilled to do this method are two solvents which are not mixed together. The partitioning process uses a mixture of ethanol-water (2: 3) and ethyl acetate solvents. Ethyl acetate is a semi-polar solvent and can dissolve semipolar flavonoid compounds in cell walls such as flavonoid aglycones (Purwanto, 2015). The results of fraksination were then concentrated on a waterbath at 60 ° C to obtain the dried extracts of ethyl acetate fraction ethanol extract from the bark of faloak tree (*Sterculia sp.*) With a yield percentage of 4.3% and 7.4% because fractionation was done twice. Characteristics of extracts and fractions
of faloak (*Sterculia sp.*) Tree bark extract
as shown in Table 1.

**Table 1. Characteristics of ethanol extract from the bark of faloak tree (*Sterculia sp.*) And ethyl acetate fraction of bark extract from faloak tree (*Sterculia sp.*)**

<table>
<thead>
<tr>
<th>No</th>
<th>Type of Extract Characteristics</th>
<th>Characteristics</th>
<th>Concentration</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol Extract</td>
<td>Thicken</td>
<td>Special Chocolate</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ethyl Acetate Fraction</td>
<td>Extract Dry</td>
<td>Dried Chocolate</td>
<td></td>
</tr>
</tbody>
</table>

(Source: Research Primary Data, 2017)

**Qualitative Identification of Ethyl Asset Acetate Ethanol Extract of Faloak Tree Bark (*Sterculia sp.*)**

Before conducting the research, phytochemical screening was carried out to determine whether there was an active compound in the dried extract of faloak (*Sterculia sp.*) Bark from the results of ethyl acetate fraction. The results of identification can be seen in Table 2.

**Table 2. Results of Qualitative Identification of Ethyl Asset Acetate Ethanol Extract of Faloak Tree Bark (*Sterculia sp.*)**

<table>
<thead>
<tr>
<th>No</th>
<th>Compound</th>
<th>Reagent</th>
<th>Changes with Reagent</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>Mg-HCl</td>
<td>-</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Mayer Wagner Hager</td>
<td>Chocolate deposits</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>Steroid</td>
<td>Salkowski’s Test</td>
<td>Chocolate</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Terpenoid</td>
<td>0.1 g + 2 mL etanol + 2 mL CHCl₃ + 3 mL H₂SO₄</td>
<td>No Color change</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>Aquades Formed</td>
<td>Foam</td>
<td>Positive</td>
</tr>
</tbody>
</table>

(Source: Research Primary Data, 2017)
The results of qualitative identification of dried extracts of faloak (Sterculia sp.) Bark from the results of ethyl acetate fraction showed positive results containing flavonoids, alkaloids and saponins. This proves that semipolar flavonoids are contained in ethyl acetate fraction ethanol extract of the skin of faloak tree (Sterculia sp.).

**Antibacterial Activity Test**

Antibacterial activity test of ethyl acetate fraction of ethanol extract of faloak (Sterculia sp.) Tree bark on Salmonella typhoid bacteria was carried out using diffusion method. Cylinders made of glass or stainless steel on medium that have been inoculated with bacteria. Then the cylinder is filled with a test solution that will diffuse on the agar medium. The parameters used in this study were antibacterial effectiveness based on the presence of inhibition zone diameters around the cylinder containing ethyl acetate fraction ethanol extract of the bark of the faloak tree (Sterculia sp.) After incubation for 18-24 hours at 37 °C.

This research was conducted in 3 stages, making Salmonella thyposa bacteria culture, determining the suspension of standard Salmonella thyposa bacteria and antibacterial activity test for ethyl acetate fraction ethanol extract of faloak (Sterculia sp.) Tree bark against Salmonella thyposa bacteria.

Salmonella thyposa bacteria used are bacterial isolates from the BPOM collection in Kupang that have been rejuvenated and enriched in Nutrient media to be tilted. In testing the standard suspension of Salmonella thyposa bacteria was carried out to obtain the number of bacterial colonies of ± 1,000,000 cells / mL by diluting the suspension of Salmonella thyposa bacteria. The results of dilution of Salmonella thyposa bacteria obtained at dilution (10^-5) there was a growth of bacterial colonies ± 1,000 cells / mL, so dilution (10^-3) was used to test the activity of bacterial colonies ± 1,000,000 cells / mL (Appendix 10). After determining the determination of Salmonella thyposa bacterial suspension, antibacterial activity was tested by
diffusion method to use cylinders. The results of the measurement of clear zones with a concentration of 25% b / v; 50% b / v; and 75% b / v ethanol extract of faloak tree bark from ethyl acetate fraction can be seen in Table 3.

Table 3. Results of Measurement of Inhibitory Zones of Ethyl Asset Acetate Ethanol Extract of Faloak Tree Bark (Sterculia sp.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Zone Limits</th>
<th>Amounts (mm)</th>
<th>Average (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I (mm)</td>
<td>II (mm)</td>
<td>III (mm)</td>
</tr>
<tr>
<td>25% b / v</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50% b / v</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75% b / v</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Control (-)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

(Source: Research Primary Data, 2017)

The measurement results of the diameter of the inhibitory zone showed that the ethyl acetate fraction of the ethanol extract of the bark of the faloak tree (Sterculia sp.) At a concentration of 25% b / v; 50% b / v; no, while at a concentration of 75% b / v, the presence of a inhibitory zone showed a inhibition zone against Salmonella thyposa bacteria with an average area of inhibition zone diameter of 15 mm. Because the results of the study are not homogeneous, they cannot be continued statistically. The antibacterial activity of ethyl acetate fraction of ethanol extract from the bark of faloak tree (Sterculia sp.) Was seen at the highest test concentration carried out at 75%. This shows a number of possibilities, one of which is the possibility that the active compounds drawn by ethyl acetate solvents in the fractionation process are very small in number. Where ethyl acetate is used during the fractionation process it is not effective in attracting active compounds that are efficacious as antibacterial. Another possibility is that some active substances that have antibacterial properties from the ethanol extract of faloak tree bark are more soluble in other fractions but this needs to be proven by further research on other fractions, different solvents with different levels of polarity on the ethanol extract of the faloak tree bark.
CONCLUSIONS AND SUGGESTIONS

Based on the results of the study, the ethyl acetate fraction of the ethanol extract of the bark of the faloak tree (*Sterculia sp.*) Had antibacterial activity against *Salmonella thyposa* bacteria at a concentration of 75% b/v.

Suggestions for research are further research using different fractions, different types of bacteria or other pharmacological effects from faloak (*Sterculia sp.*)

REFERENCES


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