Phytochemical screening and antioxidant activities of ethanol extract and ethyl acetic extract of “sikam’s” barks (*Bischofia javanica* BL)

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Abstract. Sikam’s barks (*Bischofia javanica* BL) are used as seasoning by Simalungun People. People use it for nahi nasumbah. Nahi nasumbah is a kind of food that is made from fresh meat or undercooked meat. There have never been reports of food poisoning as a result of consuming it. This research was aimed to evaluate the phytochemical and antioxidant activities of ethanol extract and ethyl acetic extract of *Bischofia javanica* BL barks. The screening was performed for phenolics, flavonoids, saponins, steroids/terpenoids, and alkaloids. Antioxidant capacity was evaluated by DPPH Method. The study of phytochemical screening indicated that ethanol extract contains phenolics, flavonoids, saponins, steroids/terpenoids, and alkaloids. The antioxidant capacity indicated that IC₅₀ ethanol extract of *Bischofia javanica* BL barks was 1,658 ppm and IC₅₀ ethyl acetic extract of *Bischofia javanica* BL barks was 147.553 ppm. The results show that ethyl acetic extract activity is stronger than ethanol extract.

1. Introduction

Sikam (*Bischofia javanica* Blume) is a tree that is commonly found in North Sumatera Province Indonesia. The sikam’s barks used as a natural colouring in the food and non-food [1]. Previous research showed that sikam’s barks contain tannins [2,3], flavonoids [1-3], glycosides and triterpenoids, and has the antioxidant capacity [3]. In addition, the bark also having antimicrobial activities [4,5].

Sikam’s barks (*Bischofia javanica* BL) are used as a seasoning by Simalungun People. People use it for nahi nasumbah, a kind of food that is made from fresh meat or undercooked meat. However, there was no report about having food poisoning as a result of consuming the product.

Meat is a kind of perishable food. The oxidation of lipids causes degradation of fats and proteins, which in turn leads to a decrease in nutrition, as well as acceptance of the colour, taste, and texture of meat.

Lipid oxidation can be reduced by using antioxidants. The existence of natural or synthetic antioxidants in foodstuffs can inhibit fat oxidation, prevent damage, change, and degradation of organic components in food, thereby extending shelf life [6]. However, natural antioxidants are more widely used in food ingredients because of their low degree of toxicity. This study used ethanol and ethyl acetate as solvents in the extraction process due to their low food toxicity.

This research aimed to evaluate the phytochemical and antioxidant of ethanol extract and ethyl acetic extract of *Bischofia javanica* BL barks.
2. Materials and methods

2.1. Materials and chemicals
The materials used in this research were Sikam barks (SB), ethanol (Merck), ethyl acetate (Merck), chloroform 25%, FeCl₃ 1%, MgSO₄, HCl (Merck), cotton, activated carbon, acetate acid anhydrous, H₂SO₄ (Merck), methanol (Merck), aquadest, ascorbic acid (Merck), and DPPH (Sigma Aldrich).

2.2. Plant material
The plant materials or sikam barks were collected from Parluasan Market Simalungun District North Sumatera Province, Indonesia. The freshly cut SB was sorted out by cleaning outer of the bark and dried in the shade at ambient temperature for two weeks. The dried SB were grounded into powder, then packed in a plastic jar, and stored in a dry place.

2.3. Preparation of the ethanol extract and ethyl acetic extract
One hundred grams of dried samples were mixed into 1 L of ethanol (w/v) or 1 L of ethyl acetate (w/v) and submitted to dynamic maceration for 24 hours at room temperature, occasionally stirring in the first six hours. Then, the filtration was filtered by Buchner funnel. After filtration, both extracts were concentrated under reduced pressure of 300-500 mmHg at 70°C and later dried for a day at room temperature. The extract was in powder form.

2.4. Phytochemical screening
Each extract 0.05 g was mixed with 5 mL chloroform and 5 mL aquadest and shaken vigorously. The mixtures allowed to stand for a while until forming two layers. After the separation, the upper layer was used for flavonoids, phenolics and saponins tests. For the phenolics analysis, the upper layer was added with a few drops of FeCl₃(aq) 1%. A blue colour showed the presence of phenolics. Moreover, flavonoids were analysed by added 1-2 drops of upper layer with MgSO₄ and 1-2 drops of HCl concentrated. A yellow – orange to red colour showed the presence of flavonoids. For saponins determination, the upper layer was shaken vigorously. The foam appearance in 3-5 minutes showed the presence of saponins. Meanwhile, the bottom layer was used for steroids/terpenoids tests. The bottom layer was filtered through a dropper topped with cotton wool and charcoal. 2-3 drops of the filtrate were dried and mixed with a drop of acetate acid anhydrous, a drop of H₂SO₄ concentrated. A blue or green colour showed the presence of steroids. 2-3 drops of filtrate were added with Lieberman – Bouchard (2 drops of acetate acid anhydrous and a drop of H₂SO₄ concentrated). A red colour showed the presence of terpenoids. The alkaloids were also assessed in which 40 mg of extracts were moisturized with 5 mL ammonia 25% then were crushed. Then, it was added with 20 mL chloroform, crushed vigorously. The mixture was filtered. The organics solution phase (A solution) was extracted with HCl 1:10 (v/v). The upper layer was formed (B solution). A drop of A solution was dropped to filter paper with Dragendorff reagent. A red or orange colour was showed the presence of alkaloids. B solution was divided into 2 test tubes, one was added with Dragendorff reagent, in which the occurrence of red brick precipitation showed the presence of alkaloids. The other tube was added for Mayer reagent and the presence of alkaloids was shown by the forming of white precipitation.

2.5. Antioxidants activity used DPPH (1,1 - diphenyl - 2- pikrilhidrazil) Method
There was some preparation before doing the DPPH method. Firstly, DPPH 0.2 mM was prepared by adding 8 mg DPPH into 100 mL methanol, stored in a dark bottle. Secondly, 1 mL DPPH 0.2 mM was added with methanol into volumetric flask 5 mL to be used as control. Thirdly, preparing ethanol extract and ethyl acetic extract each was weighed as much as 5 mg, then dissolved in 5.0 ml of methanol; this solution is the mother liquor. A total of 25, 50, 125, 250, and 500 μL of the mother liquor were piped and put into a 5 mL volumetric flask to obtain 5, 10, 25, 50, and 100 μg / mL of samples concentration. Into each tube was added 1.0 mL of DPPH solution and added with methanol up to the 5 mL mark. Fourthly, preparing ascorbic acid as standard antioxidant. Ascorbic acid was weighed approximately 5
mg, then dissolved in 5.0 mL of methanol. This solution is the mother liquor. Pipette 20, 30, 40, 50, and 60 μL of the mother liquor into a 5 ml volumetric flask to reach 4, 6, 8, 10, and 12 μg / mL of concentrations. To each measuring flask was added 1.0 mL of DPPH 0.2 mM and added with methanol up to the 5 mL mark.

The samples and standard antioxidant with various concentrations were incubated into a water bath at 37°C for 30 minutes. The absorbances were determined at 517 nm with Shimadzu UV-1800. The inhibition percentage of sample to free radicals is calculated by using following formula:

\[
\text{Inhibition} \% = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test solution}}{\text{Absorbance of control}} \right) \times 100
\]

(1)

The absorbance of control is t radical activity of blank (without samples or antioxidant standard). The absorbance of the test solution is the activity samples or standard antioxidant. IC_{50} is determined by measuring the concentration of test solution that can get 50% of inhibition by the equation of a straight line

\[
y = a + bx
\]

(2)

where y = 50, x = IC_{50}. The test was carried out in triplicate.

3. Results and discussion

3.1. Extract yield

The extracts obtained were in the form of powder. Table 1 shows that ethanol extract yield was more than ethyl acetic extract. The differences in ethanol and ethyl acetic extracts due to the polarity of the solvent used, where the ethanol solvent is nonpolar, and the ethyl acetate solvent is semipolar. Previous research results [3,7] show different results in ethanol extract due to differences in the extraction process carried out.

| Extract                     | Massa (g) | Yield (%) | Extract Colour |
|-----------------------------|-----------|-----------|----------------|
| Ethanol extract             | ± 10      | 10        | Brownish red   |
| Ethyl acetic extract        | ± 2       | 5         | Yellow         |

3.2. Phytochemical screening

The phytochemical results (Table 2) show that ethanol extract and ethyl acetic extract contain secondary metabolites, i.e., phenolics, flavonoids, saponins, steroids/terpenoids. These results are consistent with the previous studies [1,3,7]. Where the secondary metabolite compounds function as antioxidants, antibacterial, anti-fungal, anti-inflammatory. Ethanol extract contains phenolics, flavonoids and saponins. Ethyl acetic extract contains steroids/terpenoids. According to the result, none of the extracts contains the alkaloids.

| Phytochemical compounds     | Ethanol extract | Ethyl acetic extract |
|-----------------------------|-----------------|---------------------|
| Phenolics                   | +               | -                   |
| Flavonoids                  | +               | -                   |
| Saponins                    | +               | -                   |
| Steroids/ Terpenoids        | -               | +                   |
| Alkaloids                   | -               | -                   |
3.3. Antioxidants activity

The ethanol extract and ethyl acetic extract antioxidant activity of *Bischofia javanica* BL have been assessed by DPPH method using ascorbic acid as a reference standard. The results show that ethanol extract activity is stronger than ethyl acetic extract (Table 3). The smaller the value of IC$_{50}$ is the stronger the antioxidant activity. IC$_{50}$ is the antioxidant concentration required to reduce the DPPH absorbance by half [8]. This study is consistent with the previous studies that SB has antioxidant activity [3].

| Test solution               | IC$_{50}$ (μg/mL) |
|-----------------------------|-------------------|
| Ethanol extract             | 1,658             |
| Ethyl acetic extract        | 147.553           |
| Ascorbic acid               | 27.149            |

4. Conclusions

The sikam bark extracts contain secondary metabolites namely phenolics, saponins, flavonoids, and steroids/terpenoids. Antioxidant activity was expressed as IC$_{50}$ of ethyl acetic extract is lower than ethanol extract but still higher than ascorbic acid as reference standard. The results show that ethyl acetic extract activity is stronger than ethanol extract. For further research, it is recommended to test the toxicity level of ethanol extract and ethyl acetic extract.

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