Phytochemical Screening and Antioxidant Activity of Hippobroma longiflora Extracts

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Abstract. This study aims to identify the secondary metabolite compounds and to test the antioxidant activity of ethanol extract, n-hexane fraction, and chloroform fraction of Hippobroma longiflora leaves. Extraction was carried out by the maceration method using ethanol. The resulting crude ethanol extract was then partitioned with n-hexane and chloroform. Each extract and fraction was then tested by phytochemical screening. Antioxidant activity testing was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The phytochemical screening test showed that the ethanol extract contained flavonoids, saponins, triterpenoids, and alkaloids; the n-hexane fraction contained steroids and alkaloids; and the chloroform fraction contained flavonoids, steroids, and alkaloids. Analysis of the antioxidant activity revealed the following Inhibitory Concentration (IC₅₀) values: 9.57 ppm of ethanol extract, 99.59 ppm of n-hexane fraction, 48.54 ppm of chloroform fraction, and 4.30 ppm of ascorbic acid. Based on these results, the antioxidant activity of the ethanol extract was more potent than the n-hexane and chloroform fractions, but smaller than ascorbic acid.

1. Introduction
The rapid development of technology and industry has changed the behavior and lifestyle of the community. Unhealthy lifestyles such as consuming fast food, lack of time to exercise, and smoking habits as well as environmental factors such as vehicle fumes and solar radiation can cause an increase in the number of free radicals in the body. This condition will trigger oxidative stress [1]. The magnitude of the negative effect of free radicals on human health causes the body to require an intake that contains an antioxidant compound that will be able to capture and neutralize free radicals. It happens because antioxidants can provide electron pairs to free radicals so that they can stop further reactions that cause oxidative stress and cell damage to be avoided [2]–[4].

There are various kinds of food ingredients that have natural antioxidant properties such as apples, tomatoes, and green tea. Besides, there are also synthetic antioxidants that are produced through several chemical reactions, such as Tert-Butyl Hydroxy Quinone (THBQ), Butyl Hydroxy Anisol (BHA), and Butyl Hydroxy Toluene (BHT). However, these synthetic antioxidants have side effects on the body and harmful to human health so that their use must be monitored [5].
Hippobroma longiflora is a plant that has potential as a natural antioxidant and its use has not been widely studied for reducing free radical activity in the body. The classification of H. longiflora plants is as follows:

- **Kingdom**: Plantae
- **Subkingdom**: Tracheobionta
- **Superdivision**: Spermatophyta
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Subclass**: Asteridae
- **Order**: Asterales
- **Family**: Campanulaceae
- **Genus**: Hippobroma
- **Species**: Hippobroma longiflora (L.)
- **Synonym**:
  - Isotoma longiflora (L.)
  - Isotoma longiflora var. runcinata (Hassk)
  - Isotoma runcinata Hassk
  - Laurantia longiflora (L.)
  - Laurantia longiflora var. runcinata (Hassk)
  - Lobelia longiflora L
  - Rapuntium longiflora (L.)
  - Solenopsis longiflora (L.)

The leaves of H. longiflora contain alkaloids, phenolics, flavonoids, triterpenoids, steroids, coumarins, saponins, and tannins [6]-[7]. The activity of alkaloids and flavonoids has been reported to have many pharmacological effects including anti-inflammatory, antioxidant, anticancer, antidiabetic, antibacterial, antimalarial, antitumor, antimicrobial, antifungal, anti-insecticide, and antiseptic [8]-[9]. Flavonoids are a group of polyphenolic compounds that act as antioxidants, namely to capture free radicals [10]. The presence of polar and non-polar compounds in H. longiflora is the reason to research the activity fractionation of leaves of H. longiflora by using three types of solvents, namely ethanol, n-hexane, and chloroform. This study aim to identify the secondary metabolite compounds and test the antioxidant activity of ethanol extract, n-hexane fraction, and chloroform fraction of H. longiflora leaves.

2. Materials and methods

2.1. Materials

The chemicals used for the extraction are ethanol, n-hexane, and chloroform. Dragendorff reagent, Liebermann-Burchard reagent, Mayer reagent, Bouchardat reagent, Wagner reagent, sulphuric acid, sodium hydroxide, magnesium ribbon, hydrochloric acid, and ferric chloride for the phytochemical screening test. Methanol, DPPH, and ascorbic acid for antioxidant activity test. The tools which used in this study included a rotary vacuum evaporator, UV-VIS spectrophotometer, and other glass tools.

2.2. Plant sample

Fresh leaves of H. longiflora were collected from Singaraja Region, Bali, Indonesia. The botanical identification and authentication were confirmed at the Indonesian Institute of Sciences (LIPI) UPT Bali Botanical Garden Eka Karya Bali.
2.3. Preparation of samples

*H. longiflora* leaves are taken from the plant and then washed in running water. Leaves that have been washed are cut into small pieces, then air-dried without being exposed to sunlight at room temperature for 7 days. The dried leaves are then ground to a powder.

2.4. Extraction procedure

The crude powder that has been weighed (100 gm) is macerated with ethanol. Every 24 hours the extract is filtered and replaced with a solvent. This extraction was repeated until the last extract contained no metabolites. The obtained ethanol filtrate is evaporated to produce a crude ethanol extract. Furthermore, the crude extract (10 gm) is dissolved in 100 mL of water. This water extract was partitioned with n-hexane, then separated and the n-hexane layer was evaporated, to obtain the n-hexane fraction (2.7 gm). The residue (water layer) is partitioned with chloroform and separated. The chloroform layer was evaporated to obtain the chloroform fraction (1.1 gm).

2.5. Preliminary phytochemicals screening

Each extract and fraction are tested by phytochemical screening. The test is performed according to various standard methods such as flavonoids, alkaloids, tannins, saponins, and steroids/triterpenoids [11]-[12]. The results are represented as + or – which indicate the presence or absence of classes of compounds in the extract and fractions.

2.6. Antioxidant activity

Antioxidant activity testing used the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method with the ascorbic acid as a positive control. In this research, the DPPH method was described by [13], with minor modification. Five concentration series were created for each extract and fractions. Each concentration was taken 2 milliliters and added as much as 1 milliliter of DPPH (0.1 mM). The blank (DPPH in methanol) and positive controls were prepared in the same way without any extract. Then, the mixture was left to stand for 30 minutes in a dark place and the absorbance was measured at \( \lambda \) 517 nm using a UV-Vis spectrophotometer. The repeated of this treatment is three times. Antioxidant activity can be expressed in units of percent inhibition. This value is obtained by the following equation.

\[
\text{% inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

After the inhibition percentage of each extract and fractions has been obtained, it is followed by a linear regression calculation (x, y) to obtain the IC\(_{50}\) (inhibition concentration) value. x is the concentration (µg/mL), while y is the percentage of antioxidant activity (%). The IC\(_{50}\) value is obtained by the equation \( y = ax + b \).

3. Results and Discussion

The phytochemical screening test showed the ethanol extract which contained flavonoids, saponins, triterpenoids, and alkaloids; the n-hexane fraction contained steroids and alkaloids; and the chloroform fraction contained flavonoids, steroids, and alkaloids. These phytochemical compounds are known to support bioactivity, thus responsible for the antioxidant activities. Phytochemical screening results showed in Table 1.
Table 1. Phytochemical screening of *H. longiflora* extract and its fractions.

| Phytochemical constituents | Reagent | Ethanol extract | n-Hexane fraction | Chloroform fraction |
|---------------------------|---------|-----------------|-------------------|-------------------|
| Flavonoids                | Mg + HCl| +               | -                 | +                 |
| Tannins                   | FeCl₃ 1%| -               | -                 | -                 |
| Saponins                  | Distilled water + HCl 1% | + | - | - |
| Steroid/Triterpenoids     | CH₃COOH + H₂SO₄ | + | + | + |
| Alkaloids                 | H₂SO₄+Meyer | + | - | + |
|                           | H₂SO₄+Wagner | + | - | + |
|                           | H₂SO₄+Dragendorff | + | + | + |
|                           | H₂SO₄+Bounchardat | + | + | + |

Remarks: + = presence; - = absence

Antioxidant activity for each extract and fractions was evaluated using the DPPH method. This method was chosen because it is a simple, fast, and easy method for screening the radical scavenging activity of several compounds. In addition, this method has proven to be accurate and practical [14]. DPPH is a free radical that is stable at room temperature and is purple. If DPPH is reacted with free radical-reducing compounds (for example flavonoids), the intensity of the purple color will decrease and if the amount of free radical-reducing compounds that react is large, the DPPH can change color to yellow. This color change can be measured its absorbance with a UV-Vis spectrophotometer. DPPH provides strong absorption at a wavelength of 517 nm with a dark violet color. The free radical scavenging causes the electrons to pair then causes a decoloration proportional to the number of electrons taken [15].

Table 2. Antioxidant activity of *H. longiflora* extract and its fractions.

| Sample                | Concentration (µg/mL) | % Inhibition | The regression equation | IC₅₀(µg/mL) |
|-----------------------|-----------------------|--------------|-------------------------|------------|
| Crude ethanol extract | 10                    | 52.36        | y = 3.1229x + 20.101    | 9.57       |
|                       | 8                     | 44.43        |                         |            |
|                       | 6                     | 37.80        |                         |            |
|                       | 4                     | 32.48        |                         |            |
|                       | 2                     | 27.11        |                         |            |
| n-Hexane fraction     | 100                   | 50.35        | y = 0.2516x + 24.942    | 99.59      |
|                       | 80                    | 44.66        |                         |            |
|                       | 60                    | 40.07        |                         |            |
|                       | 40                    | 35.16        |                         |            |
|                       | 20                    | 29.94        |                         |            |
| Chloroform fraction   | 50                    | 50.47        | y = 0.5434x + 23.624    | 48.54      |
|                       | 40                    | 45.88        |                         |            |
|                       | 30                    | 40.37        |                         |            |
|                       | 20                    | 33.33        |                         |            |
|                       | 10                    | 29.57        |                         |            |
Ascorbic acid

| Concentration | Percentage Inhibition |
|---------------|----------------------|
| 5             | 53.33                |
| 4             | 48.75                |
| 3             | 43.35                |
| 2             | 39.22                |
| 1             | 33.89                |

\[ y = 4.8417x + 29.184 \]

\[ R^2 = 0.9987 \]

4. Conclusion

To sum up, ethanol extract, n-hexane fraction, and chloroform fraction of *H. longiflora* have the potential and can be developed as a source of natural antioxidants. Further research is needed for the isolation and identification of the compounds responsible for antioxidants.

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