Phytochemical Analysis of the Ethanol Extract of Binahong (Anredera cordifolia (Ten.) Steenis) Leaves by UV-Vis Spectroscopy

Rusdiana Rusdiana\textsuperscript{*1}, Tri Widyawati\textsuperscript{2}, Dina Keumala Sari\textsuperscript{3}, Sry Suryani Widjaja\textsuperscript{1}

\textsuperscript{1}Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
\textsuperscript{2}Department of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
\textsuperscript{3}Department of Nutrition, Faculty of Medicine, Universitas Sumatera Utara, Medan Indonesia.

*Corresponding Author.

Received 10/09/2023, Revised 22/02/2024, Accepted 11/02/2024, Published Online First 20/04/2024

Abstract

Binahong (Anredera cordifolia) leaves contain chemical compounds that can be used to promote anti-obesity. This study aimed to examine the phytochemical and anti-obesity properties of the ethanol extract of binahong leaves. The binahong extract was obtained by maceration of the leaves, followed by ethanol extraction. Phytochemical analysis was performed by UV-Vis spectroscopy to identify and determine the levels of flavonoids, phenolic compounds, alkaloids, and tannins in the extract, using quercetin as a standard for calibration. Absorbance was measured at a wavelength of 430 nm. The flavonoid, glycoside, tannin, saponin, and steroid/terpenoid contents were determined, but alkaloid compounds were not found. The conclusion is, that the large flavonoid content (14.9385 mgQE/g) in the binahong leaf extract has potential to provide anti-obesity effects.

Keywords: Anredera cordifolia, Anti-obesity, Flavonoid, Quercetin, Spectrophotometer.

Introduction

Plants are an important source of therapeutic compounds and drug discovery. A plant’s therapeutic value is derived from the phytochemicals it contains, which have particular physiological effects on the human body. Traditional medicines can be used to prevent diseases, increase physical and mental functions, and help maintain health\textsuperscript{1}.

Natural ingredients have been used as traditional medicines for many generations in numerous communities. Notably, the binahong plant (Anredera cordifolia (Ten.) Steenis) is well known to the Indonesian people and is often used in traditional medicine\textsuperscript{2-3}, for weight loss\textsuperscript{4}, antibacterial activity\textsuperscript{5-6}, healing wounds\textsuperscript{7}, lowering blood pressure\textsuperscript{8}, lowering blood sugar\textsuperscript{9}, and anti-catarract effects\textsuperscript{10}. Binahong can potentially be used to treat various diseases owing to the metabolites (active substances) contained in the leaves. The active substances include flavonoids, saponins, tannins, glycosides, and steroids/terpenoids\textsuperscript{11-13}. Binahong leaves can be used for weight loss because they contain high flavonoid concentrations\textsuperscript{14}. The potential benefits of binahong plants are sufficient for phytopharmac a. However, the quality of the extracted compounds is influenced by differences in the plant origin, the part of the plant,
the planting environment, and the type of solvent used for extraction\textsuperscript{15}. This study aimed to determine the flavonoid contents in the ethanol extract of binahong leaves by UV-Vis spectrophotometry.

Materials and Methods

Materials

Binahong leaves (\textit{A. cordifolia}) were obtained from the upland area of Susuk village, Tiganderket subdistrict, Tanah Karo district, North Sumatra, Indonesia. Ethanol (70\% and 96\%) was purchased from Smart-Lab. Silica gel (G 60 F254) was purchased from Merck®. Aquadest, oxalic acid, boric acid, Dragendorff reagent, Mayer reagent, Hager reagent, Wagner reagent, acetone, ether, anhydrous acetic acid, hydrochloric acid, ethyl acetate, chloroform (Merck®), methanol, and toluene were also purchased from Merck®, all pro-analytical grade. Technical-grade formic acid was purchased from Brataco.

Methods

This experimental study was approved by the ethics committee in the Faculty of Medicine at Universitas Sumatera Utara, under Law No. 726/KEP/USU/2022. Binahong leaf samples were confirmed (identified as an \textit{A. cordifolia} species) at Herbarium Bogoriense, the Biology Research Center at the Indonesian Institute of Sciences, Cibinong-Bogor, Indonesia. The screening tests and concentration measurements of the extract were conducted at the Phytopharmacology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara.

Weighed 250 g of the prepared simplicia, used 5 l of 95\% ethanol as a liquid filter, and stirred for 6 h using a macerator before leaving it for 18 h. Then, the dregs were separated from the extract, and the dregs were macerated twice more. Finally, the solvent was evaporated to produce a dried extract.

Results

Phytochemical Screening of the Binahong Leaf Extract

Phytochemical screening is a qualitative analysis technique used to identify the active chemicals in plants, enabling the development of medications with therapeutic potential. In this study, phytochemical screening was performed to identify the flavonoid compounds, alkaloids, tannins, glycosides, steroids/triterpenoids, and saponins in the binahong leaf extract. Fig. 1.

Screening of Flavonoid Compounds

A total of 0.5 g of the sample powder, 0.1 g of magnesium powder, 1 ml of hydrochloric acid, and 2 ml of amyl alcohol were added to 20 ml of boiling water, heated for 10 min, and then filtered under hot conditions. The mixture was then agitated and allowed to separate in 5 ml of the filtrate. Flavonoids are present if the amyl alcohol layer becomes red, yellow, or orange, Fig. 1a.

Screening of Alkaloid Compounds

Here, 1 ml of 2 N hydrochloric acid and 9 ml of distilled water were added to 0.5 g of the sample powder, which was heated for 2 min using a water bath, followed by cooling and filtering. The filtrate was then subjected to alkaloid screening using the following three trials:

1. Mayer’s reagent: After mixing 3 drops of the test solution (filtrate) with 2 drops of Mayer’s reagent solution, a white or yellow lumpy precipitate appears in the presence of alkaloids.

2. Bouchardat’s reagent: After mixing 3 drops of the test solution (filtrate) with 2 drops of Bouchardat’s reagent solution, a brown to black precipitate appears in the presence of alkaloids.

3. Dragendorff’s reagent: After 2 drops of Dragendorff’s reagent solution are applied along with a drop of the test solution (filtrate), a crimson or orange precipitate appears in the presence of alkaloids.

Precipitation or turbidity occurred in two of the three trials above, but the presence of alkaloid compounds was not confirmed, Fig. 1b.

Screening of Saponin Compounds

A test tube containing 0.5 g of the sample powder was filled with 10 ml of hot distilled water, shaken vigorously for 10 s, and allowed to cool. A steady foam appeared for at least 10 min, rising to a
height of 1 to 10 cm. A drop of 2 N hydrochloric acid was then added, and if the foam did not vanish, saponins were determined to be present, Fig. 1c.

**Screening for Tannin Compounds**

First, 10 ml of distilled water was mixed with 0.5 g of the sample powder. After filtering, the filtrate was diluted with distilled water until it became colorless. The resulting filtrate solution was diluted to 2 ml, and 1–2 drops of FeCl₃ reagent was added. Tannins were considered to be present if a blue or blackish-green hue appears, Fig. 1d.

**Screening of Steroid/Triterpenoid Compounds**

Herein, 1 g of the material was macerated in n-hexane for 2 h before being filtered. The solvent was evaporated in a vaporizer cup. To the remaining material, 2 drops of acetic anhydride and 1 drop of sulfuric acid were added. Triterpenoids were considered to be present if the color turned red, pink, or purple, and steroids were considered to be present if the color turned blue or green, Fig. 1e.

**Screening of Glycoside Compounds**

For this test, 3 g of the sample powder was used for the extraction, along with 30 ml of a solution made up of 7 parts 96% ethanol, 3 parts distilled water, and 10 ml of 2 N hydrochloric acid. The mixture was refluxed for 30 min, followed by cooling and filtering. Then, 20 ml of the filtrate was divided into 20 ml of distilled water and 25 ml of lead (II) acetate, and the mixture was shaken vigorously for 5 min before filtering. Three times, 20 ml of a solution containing 3 parts chloroform and 2 parts isopropanol was used to extract the filtrate. A temperature of no greater than 50°C was used to evaporate the solvents, and 2 ml of methanol was used to dissolve the remaining material. Next, 0.1 ml of the remaining solution was added to a test tube and left to evaporate over a water bath. The remaining substance was dissolved in 2 ml of distilled water with 5 drops of Molish reagent, and 2 ml of sulfuric acid was then gradually added. A purple ring appears in the presence of glycoside, Fig. 1f.

**Figure 1.**

- a. flavonoid analysis (+)  
- b. Alkaloid analysis (-)  
- c. Saponin analysis (+)  
- d. Tannin analysis (+)  
- e. Glycoside analysis (+)  
- f. Steroid/terpenoid analysis (+)

**Identification of Flavonoid Levels in Binahong Leaf Extract (A. cordifolia)**

To determine the flavonoid content in the binahong extract, a quercetin standard solution was first prepared by dissolving 10 mg of quercetin in methanol until a 100 ml solution was obtained with a concentration of 100 ppm. To determine the maximum wavelength of quercetin, a 20 ppm solution was prepared by adding 2 ml of quercetin solution to a 10 mL flask. Next, 0.5 ml of 100 ppm quercetin standard solution, 0.1 ml of 10% AlCl₃, 0.1 ml of 10% CH₃COONa, and 2.8 ml of distilled water were added to the flask. The solution was incubated for 25 min and then the maximum wavelength was measured by UV-Vis spectroscopy within the range of 400–800 nm. To prepare the quercetin calibration curve, 1, 2, 3, 4, and 5 ml of the quercetin standard solution were added to separate 5 ml flasks.
Subsequently, methanol was introduced into every flask, resulting in solutions containing concentrations of 10, 20, 30, 40, and 50 ppm. These steps are designed to ensure that the results obtained are both accurate and reliable. 0.5 ml of each concentration was pipetted into a mixing bottle and mixed with 10% AlCl3, 10% CH3COONa, and 2.8 ml of distilled water. Using UV-Vis spectroscopy, the absorbance was measured after incubation for 25 minutes at 430 nm. The acquired data was subsequently utilized to establish the quercetin calibration curve and linear regression line. Table 1 presents the findings from three distinct measurements of the flavonoid content in the binahong leaf extract, revealing a flavonoid concentration of 14.9385 mgQE/g extract.

<table>
<thead>
<tr>
<th>Sample weight (g)</th>
<th>Sample volume (ml)</th>
<th>FP</th>
<th>Absorbance</th>
<th>Average absorbance</th>
<th>Concentration (µg/ml)</th>
<th>Total Flavonoid content (mgQE/g extract)</th>
<th>Average (mg QE/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0104</td>
<td>10</td>
<td>1</td>
<td>0.304</td>
<td>0.303</td>
<td>14.3190</td>
<td>13.7682</td>
<td>14.9385</td>
</tr>
<tr>
<td>0.0102</td>
<td>10</td>
<td>1</td>
<td>0.343</td>
<td>0.343</td>
<td>16.5891</td>
<td>16.2638</td>
<td></td>
</tr>
<tr>
<td>0.0103</td>
<td>10</td>
<td>1</td>
<td>0.319</td>
<td>0.319</td>
<td>15.2270</td>
<td>14.7835</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

A phytochemical screening was performed in this study to identify the metabolites found in binahong leaves ethanol extract. Phytochemical screening was used in this study to identify the metabolite compounds found in the ethanol extract of binahong leaves. The ethanol extract of binahong leaves contained flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides, but no alkaloids, similar to the results reported by Angeline Salim et al. However, other researchers observed the presence of alkaloids.

This study demonstrates the potential of binahong leaves as an anti-obesity agent, attributed to the flavonoid content found in the binahong leaf extract, with a level of 14.9385 mgQE/g extract.

**Conclusion**

Phytopharmaceutical analysis revealed that binahong leaves (A. cordifolia) contained flavonoids, alkaloids, tannins, steroids/terpenoids, and saponins. The total flavonoid content of the binahong ethanol extract was analyzed by UV-Vis spectroscopy, using a quercetin curve calibration, showing that the extract contains 14.9385 mgQE/g extract, suggesting its potential anti-obesity effects.

**Acknowledgment**

The Ministry of Research and Technology and the Higher Education Republic of Indonesia have generously funded the current study, which the authors gladly recognize. The funding comes from the TALENTA USU research grant for 2023.

**Author’s Declaration**

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for republication, which is attached to the manuscript.
The author has signed an animal welfare statement.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Medical Faculty, Universitas Sumatera Utara approved the experimental protocols of this study (approval No. 726/KEP/USU/2023).

**Author’s Contribution**

All the authors contributed to producing this study. R.R, T.W, D.K.S and S.S.W. contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

**References**

