2024, 21(11): 3446-3451
https://doi.org/10.21123/bsj.2024.9354
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

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

Rusdiana Rusdiana*[1], Tri Widyawati[2], Dina Keumala Sari[3], Sry Suryani Widjaja[1]

[1]Department of Biochemistry, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
[2]Department of Pharmacology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.
[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, Published 01/11/2024

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[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[2,3], for weight loss[4], antibacterial activity[5,6], healing wounds[7], lowering blood pressure[8], lowering blood sugar[9], and anti-cataract effects[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[11-13]. Binahong leaves can be used for weight loss because they contain high flavonoid concentrations[14].

The potential benefits of binahong plants are sufficient for phytopharmaca. However, the quality

2024, 21(11): 3446-3451
https://doi.org/10.21123/bsj.2024.9354
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

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[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 (*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 *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.

The method of making simplicia was modified from a previous report[15]. Briefly, the green binahong leaves were selected, washed with running water, and then dried until brittle in a drying cabinet before being blended into a powder. Next, 500 g of dry binahong leaf powder was macerated for 24 h in 5 l of 70% ethanol. This maceration process was repeated twice. To obtain a thick extract, the macerated material was concentrated using a rotary evaporator at 40°C.

We 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.

2024, 21(11): 3446-3451
https://doi.org/10.21123/bsj.2024.9354
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

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 $FeCl_3$ 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.

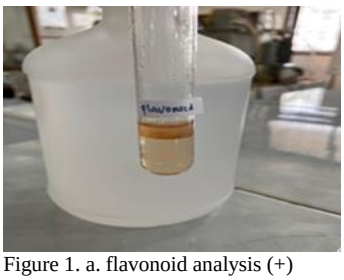
Figure 1. a. flavonoid analysis (+)

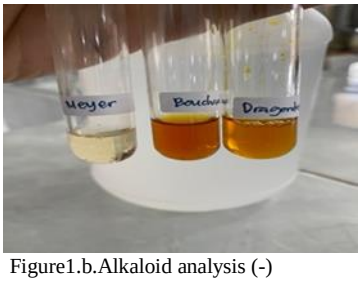

Figure1.b.Alkaloid analysis (-)

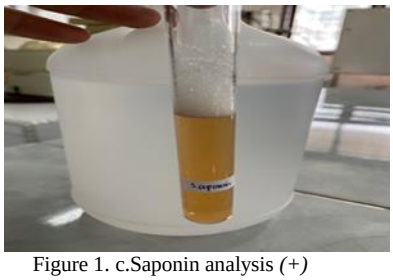
Figure 1. c.Saponin analysis *(+)*

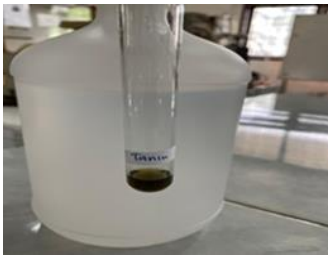
Figure 1.d. Tannin analysis (+)


Figure 1. e.glikoside analysis (+)


Figure 1. f. steroid /terpenoid analysis(+)

Figure 1. Phytochemical screening results of Binahong Leaf Extract (*Anredera cordifolia*)

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

2024, 21(11): 3446-3451
https://doi.org/10.21123/bsj.2024.9354
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

solution was pepared 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% $AlCl_3$, 0.1 ml of 10% CH_3COONa, 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 1. Total Flavonoid Content of Binahong Extracts

Sample weight (g)	Sampel volume (ml)	FP	Absorbance	Average absorbance	Concentration (µg/ml)	Total Flavonoid content (mgQE/g extract)	Average (mg QE/g extract)
0,0104	10	1	0.304 0.303 0.303	0.303	14.3190	13.7682	14.9385
0,0102	10	1	0.343 0.343 0.343	0.343	16.5891	16.2638	
0,0103	10	1	0,319 0.319 0.319	0.319	15.2270	14.7835	

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[16]. However, other researchers observed the presence of alkaloids[17,18].

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 re-publication, 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

1. Alba TM, Pelegrin CM, Sobottka AM. Ethnobotany, ecology, pharmacology, and chemistry of Anredera cordifolia(Basellaceae): a review. Rodriguesia. 2020;13; 71. https://doi.org/10.1590/2175-7860202071060
2. Cahyaningsih R, Magos Brehm J, Maxted NI. Gap analysis of Indonesian priority medicinal plant speciesaspartoftheir conservation planning. Glob Ecol Conserv 2021; 26: e01459. https://doi.org/10.1016/j.gecco.2021.e01459
3. Hasanah Y, Lisa M. Exploration and identification of Anredera cordifolia morphological characters in the highlands and lowlands. Biodiv. 2020; 21(6) : 2759-2766. https://doi.org/10.13057/biodiv/d210652.
4. Rusdiana R, Hamid R Z, Widjaja SS, Fujiati II, Ilyas S, Munir D, et al . Potential Benefits of Ethanol extract of Anredera cordifolia for Antiobesity of High Fat diet-Induced Obesity in White Male Rat Wistar. Baghdad.Sci J. 2023; online first 3. https://doi.org/10.21123/bsj.2023.8666
5. Leliqia N P E, Sukandar EY, Fidrianny I RDA. Antibacterial activities of Anredera cordifolia (Ten.) v. Steenis leaves extracts and fractions. Asian J Pharm Clin Res .2017; 10 (12) : 10-13. https://doi.org/10.22159/ajpcr.2017.v10i12.21503
6. Nasution NA, Artika I M, Safari D. Antibacterial activity of leaf extracts of Anredera cordifolia (Ten.) steenis and Muntingia calabura L. against Streptococcus pneumoniae. Curr Biochem. 2020; 7(1): 1-9. https://doi.org/10.29244/cb.7.1.1
7. Sulfianti A, Firdausi N, Nurhadi N, Ngatinem N, Agustini K, Ningsih S. Antidiabetic activity of Anredera cordifolia (Ten.) Stennis extracts with different ethanol percentages: an evaluation based on in vitro, in vivo, and molecular studies. Pharmacia.2023; 70(1): 39-47. 7. https://doi.org/10.3897/pharmacia.70.e94899.
8. Suliska N, Suryani Insanu M, Sukandar EY. Antihypertensive Activity of Combination of *Anredera cordifolia* (Ten.) V. Steenis and Sonchus arvensis L. Leaves on Epinephrine Induced Male Wistar Rat. J Adv Pharm Technol Res. 2021; 12(4): 384–388. https://doi.org/10.4103/japtr.japtr_91_21
9. Dwitiyanti D, Harahap Y, Elya B, Bahtiar A. Binahong (Anredera cordifolia (Tenore) Steen.) Leaf Extract Modulates Fatty Acids and Amino Acids to Lower Blood Glucose in High-Fat Diet-Induced Diabetes Mellitus Rats. Adv Pharmacol Pharm Sci. 2021; 28: 8869571. https://doi.org/10.1155/2021/8869571.
10. Feriyani F, Darmawi D, Balqis U, Lubis RR. The Analysis of Binahong Leaves Potential (Anredera cordifolia) as an Alternative Treatment of Anticataractogenesis. Open Access Maced J Med Sci. 2020;8: 820-824. https://doi.org/10.3889/oamjms.2020.4849
11. Mali, Priti S., and Pradyuman Kumar. Optimization of Microwave Assisted Extraction of Bioactive Compounds from Black Bean Waste and Evaluation of Its Antioxidant and Antidiabetic Potential in Vitro. Food Chem Adv. 2023;3: p. 100543, https://doi.org/10.1016/j.focha.2023.100543
12. Omer GA., Mohammed LY. Total Phenolic, Flavonoids and Vitamin C Contents with Antioxidant Activity of Urtica dioica L. Leaves Growing in Zakho, Kurdistan Region-Iraq. Baghdad Sci J.2023; online first 5 https://doi.org/10.21123/bsj.2023.8658
13. Firdaus MNA, Kardela W, Ifora I. Phytochemical and Anti-Inflammatory Potential of Anredera cordifolia (Ten): A Review. J Drug Deliv Ther.2022; 12 (2), 121-125. https://doi.org/10.22270/jddt.v12i2.5228

2024, 21(11): 3446-3451
https://doi.org/10.21123/bsj.2024.9354
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

14. Retno M, Endang K, Elena NN. Areca catechu L. and Anredera cordifolia (Ten) *Steenis* supplementation reduces faecal parasites and improves caecal histopathology in laying hens. Int J Vet Sci Med. 2022; 10:1: 52-63. https://doi.org/10.1080/23144599.2022.2090732
15. Dwitiyanti, Harahap Y, Elya B, Bahtiar A. Impact of solvent on the characteristics of standardized binahong leaf (Anredera cordifolia (Ten.) Steenis). Pharmacogn J. 2019; 11(6): 1463-1470. http://www.phcogj.com/v11/i6s.
16. Yuniarti WM, Lukiswanto BS. Effects of herbal ointment containing the leaf extracts of Madeira vine (Anredera cordifolia (Ten.) Steenis) for burn wound healing process on albino rats. Vet world. 2017;10 (7): 808. https://doi.org/10.14202/vetworld.2017.808-813.
17. Souhoka FA, Kapelle IBD, Sihasale E. Phytochemical and antioxidant test of binahong (Anredera cordifolia (Tenore) Steenis) leaves ethanol extract. Fullerene Journal of Chemistry. 2021; 6 (1): 28-33. https://doi.org/10.37033/fjc.v6i1.248
18. Salim A, Dandy FK, Felicia S, Janice ES, Putri AJ, Tiara A, et al. Phytochemical Screening and Therapeutic Effects of Binahong (Anredera cordifolia (Ten.) Steenis) Leaves. Indonesian journal of life sciences. 2021; 43-55. https://doi.org/10.54250/ijls.v3i2.125

التحليل الكيميائي لمستخلص الإيثانول لنبات (Anredera cordifolia (Ten.) Steenis) بواسطة التحليل الطيفي للأشعة فوق البنفسجية

روسديانا روسديانا[1]، تري ويدياواتي[2]، دينا كيومالا ساري[3]، سري سورياني ويدجايا[1]

[1]قسم الكيمياء الحيوية، كلية الطب، جامعة سومطرة أوتارا، ميدان، إندونيسيا.
[2]قسم الصيدلة، كلية الطب، جامعة سومطرة أوتارا، ميدان، إندونيسيا.
[3]قسم التغذية، كلية الطب، جامعة سومطرة أوتارا، ميدان إندونيسيا.

الخلاصة

تحتوي أوراق Anredera cordifolia على مركبات كيميائية يمكن استخدامها كمضاد للسمنة.الهدف من الدراسة هو فحص مستخلص الإيثانول من الخواص الكيميائية النباتية والمضادة للسمنة لأوراق Anredera cordifolia. باستخدام مذيب الإيثانول، تم استخدام عملية النقع لاستخراج أوراق Anredera cordifolia تم التحليل الكيميائي النباتي لمستخلص الإيثانول من أوراق Anredera cordifolia كمضاد للأكسدة بسبب احتوائه على مركبات الفلافونويد والمركبات الفينولية والقلويدات والعفص. تم تحديد محتوى الفلافونويدات في المستخلصات باستخدام مقياس الطيف الضوئي UV-VIS باستخدام كيرسيتين، المذاب في الميثانول للحصول على محلول كيرسيتين بتركيز 100 جزء في المليون وتم قياس قياس الامتصاصية باستخدام مقياس الطيف الضوئي UV-Vis عند نطاق طول موجي قدره 430 نانومتر. أظهرت نتائج الاختبار الكيميائي النباتي محتوى مركبات الفلافونويد والجليكوسيدات والعفص والصابونين والمنشطات/التربينويدات، في حين لم يتم العثور على مركبات قلويدات، في حين تم تحديد مستويات الفلافونويد الكلية باستخدام أداة قياس الطيف الضوئي للأشعة فوق البنفسجية باستخدام كاشف كلوريد الألومنيوم، منحنى كيرسيتين كمعايرة (Y = 0.01762X + 0.0507، r = 0.9939) بحيث تم تحديد ذلك في مستخلص الإيثانول من أوراق Anredera cordifolia يحتوي على 14.9385 ملجم كيو إي / جرام من مركبات الفلافونويد. نستنتج انه يحتوي محتوى الفلافونويد الكبير في مستخلص أوراق Anredera cordifolia على إمكانات كمضاد للسمنة (14.9385 ملجم كيو إي / جم).

الكلمات المفتاحية: أنريديرا كورديفوليا، مضاد للسمنة، فلافونويد، كيرسيتين، مقياس الطيف الضوئي.