Determination Of Flavonoid Levels Of Ethanol Extract 70% Green Binahong Leaf (*Anredera Cardifolia* (*Ten.*) *Stennis* With Uv-Vis Spectrophotometer Method

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Abstract. Binahong leaves are plants that contain saponins, tannins, and flavonoid compounds. Flavonoids are a class of polyphenol compounds that are efficacious as antioxidants. This study aims to determine the levels of flavonoids in ethanol extract of 70% and green binahong *(Anredera cardifolia (Ten) Stennis)* and the content of chemical compounds in ethanol extract in green binahong leaves. This research was carried out by extraction method using maceration with a ratio of 1:5 with 70% ethanol solvent. The identification of chemical compounds was carried out by qualitative tests using phytochemical screening and quantitative tests by determining the flavonoid levels of ethanol extract of binahong leaves using Uv-Vis spectrophotometry. The results of phytochemical screening showed that 70% ethanol extract of binahong leaves contained flavonoids, saponins, tannins and the determination of flavonoid levels showed 18.94%.

Keywords: Binahong, Flavonoid, UV-Vis Spectrophotometry

INTRODUCTION

Indonesia is a country that is famous for its natural wealth, which has various types of plants that can be efficacious as medicine. Therefore, various kinds of research and testing are carried out so that the efficacy of plants as medicine can be more rational and trusted among the community.

One of the plants that has medicinal properties is the green binahong leaf *(Anredera cardifolia (Ten) Stennis).* Binahong plants consist of 2 types, namely red binahong leaves and green binahong leaves. The green binahong plant according to ethnopharmacological studies states that people empirically use the green binahong plant to treat toothache, and headache, reduce postoperative pain and inflammation, migraine, strep throat, gout, rheumatism, and normalize cholesterol levels in the blood. According to research, binahong leaves have pharmacological activities including antibacterial, antifungal, antiviral, antidiabetic, antihypertensive, vasodilator, diuretic, anti-obesity, hypolipidemia, hepatoprotective, anti-inflammatory, analgesic, and wound healing. Secondary metabolites contained in binahong leaves are flavonoid compounds, saponins, and tannins (Febriyanti, 2011).

Flavonoids are polyphenolic compounds containing 15 C atoms consisting of two phenolic nuclei connected by three carbon units. Based on their structure, all flavonoids are parent derivatives of flavones found in plants in the form of white flour (Murni, 2017). Flavonoids are generally classified based on substituting heterocyclic rings containing OH groups. Flavonoids are phenolic compounds so their color changes when a base or ammonia is added. The difference in the OH group in the C3 section will determine the properties, groups, or types of flavonoids, namely anthocyanins, protoanthocyanins, flavonols, flavones, glycoflavones, biflavolyls, chalcones and aurons, flavanons and isoflavones (Dika, 2019). Flavonoids have several pharmacological activities, namely as anti-inflammatory, analgesic, antibacterial, and antioxidant (Khotimah, 2016).

Quantitative analysis of flavonoids can be performed using a UV-Vis spectrophotometer. Ultraviolet absorption spectra and apparent absorption are the single most useful ways to identify flavonoid structures. Flavonoids contain a conjugated aromatic system and can exhibit strong absorption bands in the UV-Vis region (Mukhriani, 2015). A spectrophotometer is a device used to measure energy relative to whether it is transmitted, reflected, or emitted as a function of the wavelength. Spectrophotometry is used to measure the amount of energy absorbed or transmitted. Monochromatic radiation rays will pass through a solution containing substances that can absorb the radiation rays (Pakaya, 2014). UV-Vis spectrophotometers can determine the amount of compounds contained in a substance. One of them is the level of flavonoid compounds contained in green binahong leaves.

Based on the description above, this study was shown to determine the number of flavonoid levels in ethanol extract of 70% green binahong leaves (*Anredera cordifolia (Ten) Steenis)* using UV-Vis Spectrophotometry.

METHODS

1. Making Simplicia

   Fresh Binahong leaves of 3 kg are sorted wet, then washed with running water and stretched to reduce size. The next step is to dry the leaves in a dryer cabinet at a temperature of 40°C. The results of drying are then smoothed using a blender and sifted using a sieve number 40 mesh until a fine powder is obtained.

2. Sample extraction

   The extraction of binahong leaves was carried out by maceration using 70% ethanol solvent. A total of 200 grams of simplicia powder is soaked with 70% ethanol as much as 1000 ml with a ratio of 1:5 is done occasionally stirring. Then filtering is carried out using fannel cloth until the substance is obtained. The results of the maserat are then concentrated using a *Rotary Evaporator* until a viscous extract is obtained.

3. Total flavonoids up to Uji
   a. Manufacture of standard quartz curves

      The standard standard of quercetin is weighed as much as 50 mg dissolved in 50 ml *of ethanol pro analyst* so that a concentration of 1000 ppm is obtained. From a standard solution of 1000 ppm, quercetin is diluted into several concentrations, namely 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Each concentration of quercetin standard solution is taken as much as 1 ml, 1 ml of AICI3 10%, and 1 ml of sodium acetate of 1 M is put into a 10 ml measuring flask, and ethanol p.a is added to the limit mark. Furthermore, it was incubated for 45 minutes and its absorption was measured at a wavelength of 425 nm using a UV-Vis spectrophotometer (Rofida, 2010).

   b. Determination of Total Flavonoid Levels

      A solution of 70% ethanol extract of binahong leaves with a concentration of 1000 ppm was made by weighing 50 mg of ethanol extract of 70% binahong leaves in 50 ml of ethanol p.a to the limit mark. A 1000 ppm solution is diluted to 100 ppm. A 100 ppm solution is taken as much as 1 ml, added with 1 ml of 10 % AlCl3 and 1 ml of sodium acetate as much as 1 ml in a 10 ml measuring flask, and the rest is added with ethanol p.a to the limit mark. then it was left alone for 45 minutes and an absorption reading was taken at a wavelength of 425 nm (Ristanti, 2019).

4. Data Analysis

   The data obtained from this study was analyzed with a linear regression equation using the Microsoft Excel program and then the total flavanoid levels were calculated.

RESULTS AND DISCUSSION

1. Making Simplisia

   Binahong leaf samples that have been dried through a wet sorting process, the goal is to remove soil, stems, or plant parts that are not leaves. Next, washing is carried out using running water to remove dirt that sticks to the leaves, such as soil, sand, or other substances. Then a display is carried out to reduce the size of the leaves and speed up the drying process. The drying process uses a drying cabinet with a temperature of 40°C with the advantage of not being affected by the weather, the drying capacity is larger and the conditions can be controlled in contrast to natural drought which can experience unregulated weather conditions and uncontrolled humidity (Sugiarti & Nafi'ah, 2018). Fresh binahong leaves of 3 kg get 570 grams of simplicia powder with a yield of 19%.

2. Ekstrak binahong ethanol

   200 grams of binahong leaf powder was macerated using 70% ethanol solvent in a ratio of 1:5. The viscous extract obtained was 75.45 grams with a yield of 37.725%.

   The selection of ethanol solvents uses the *principle of like dissolve like* because it can dissolve compounds from polar to semipolar, one of the compounds that can be dissolved in ethanol is

flavonoid compounds because they are polar. Ethanol dissolves flavonoid compounds by degrading the cell wall so that bioactive compounds are easier to exit from plant cells. Ethanol also has a hydroxyl group that can be related to the hydrogen group of the hydroxyl group of flavonoid compounds (Agustina & Wiraningtyas, 2016).

Binahong leaf extraction uses the maceration method. The maceration method is the immersion of dried natural materials (simplisia) in a solvent. The advantages of maceration are that the tool used is simple, the operational cost is quite cheap, and the process is relatively economical, besides that with this technique substances that are not heat-resistant will not be damaged, while the disadvantage of maceration is that the process is long and takes a long time (Yovitasari *et al.*, 2018).

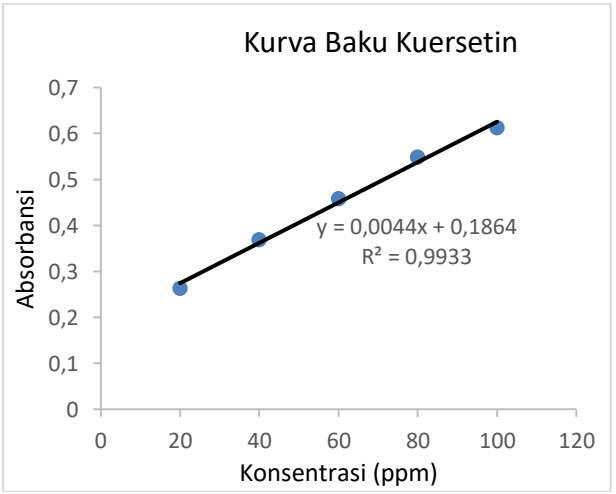
3. Results of determining the standard curve of the quartz

The creation of the standard curve was carried out to calculate the level of flavonoids in the sample based on the absorption produced through the standard curve equation. The standard curve was made using a standard solution of quercetin from several concentration series, namely 20, 40, 60, 80, and 100 ppm then the absorption at a maximum wavelength of 425 nm and incubated for 45 minutes, obtained the linear regression equation y = 0.0044x + 0.1894 with a correlation value (R2) of 0.993. The correlation coefficient (R2) is a number used to determine the strong, moderate, or weak relationship between the variables being studied. The correlation coefficient value of (R2) 0.993 shows that the R2 result is very strong because it is close to 1 and the curve is formed linearly (Winahyu *et al*., 2019).

Table 1. Results of Measurement of Absorbance of Quercetin Raw Solution

Quartz Concentration (ppm)	Absorbance
20	0,263
40	0,368
60	0.458
80	0,548
100	0,612

The results of the measurement of Quartz Absorbance were obtained with the linear regression equation Y = 0.0044x + 0.1864 and R = 0.9933.



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4. Total flavonoid levels results

According to the Ministry of Health of the Republic of Indonesia (2014), the range of total flavonoid levels based on the absorption value ranges from 0.2-0.8. In this study, successive absorbance was obtained in a sample of 70% ethanol extract of binahong leaves which was carried out 3x replication of 0.601; 0.602, and 0.604 so that on average, flavonoid levels were obtained from ethanol extract of 70% green binahong leaves containing flavonoid levels. by 18.94%. Previous research, according to Rusdiati *et al* (2020), the determination of flavonoid levels using 95% ethanol in red binahong leaves produced flavonoids of 25.869 mg. Total flavonoid levels can be an indicator

of the effectiveness of a sample as a free radical scavenger because it has a hydroxyl group that can release protons in the form of hydrogen ions and produce phenoxyl radicals that are stabilized by the resonance effect of the aromatic ring (Widyawati *et al*., 2010).

Table 2. Results of Determination of Total Flavonoid Levels of Ethanol Extract of Binahong Leaves

Kosentrasi (ppm)	Absorbance	Up to Flavonoids (ppm)	Total Flavonoids Up (%)	X ± SD
100	0,601	94,23	18,96	18,94 ± 0,047
	0,602	94,45	18,89	
	0,604	94,90	18,98	

The absorbance results of ethanol extract from binahong leaves were plotted against the standard curve of quartz. They calculated the total flavonoid content in the plant expressed in KE (Quartz Equivalent), which is the amount of quartz equivalent grams per gram of extract. The results of the absorption of total flavonoid levels are presented in Table 2. The determination of the total flavonoid level of binahong leaf extract was obtained by an average of 18.94%.

Flavonoids are the most abundant secondary metabolites found in plant tissues. Flavonoids belong to the group of phenolic compounds with chemical structures C6-C3-C6. Flavonoids are natural compounds that have the potential to be antioxidants that can ward off free radicals (Purnamasari *et al*., 2022).

CONCLUSION

Based on the research results, it can be concluded that binahong leaf ethanol extract contains flavonoid compounds. The average total flavonoid level was 94.53 ppm with a percentage of 18.94%.

REFERENCES

Agustina, S., Ruslan, R. & Wiraningtyas, A. (2016). Skrining Fitokimia Tanaman Obat di Kabupaten Bima, *Cakra kimia Indonesian E-Journal of Applied Chemistry*, 4 (1): 71–76.

Depkes RI. (2014). *Farmakope indonesia* Edisi IV. Departemen Kesehatan. Direktorat Jenderal Pengawasan Obat dan Makanan press: Jakarta. 17 (3): 197–202.

Dika, S, (2019). *Tanaman Obat Indonesia*, Salemba Medika press: Jakarta.

Febriyanti, I. (2011). Faktor–Faktor Yang Berpengaruh Didalam Daun Binahong Peride 2007-229. *Jurnal Ilmu Pengetahuan*. Vol.1, No.3.

Khotimah. (2016). Pembuatan Ekstrak Kering Simplisia Jati Belanda. *Jurnal Farmasi Higea*,5(1);1-8.

Mukhriani., Nonci, F. Y., dan Munawarah, S. (2015). Analisis Kadar Flavonoid Total Pada Ekstrak Daun Sirsak (*Annona muricata* L.) Dengan Metode Spektrofotometri UV-Vis. JF FIK UINAM, 3 (2). 37-42.

Murni, (2017). *Uji Skrining Fitokimia Dari Ekstrak Etanol Daun Binahong*. Maharani Hasibuan Press: Medan.

Pakaya, (2014). Prosedur Penelitian Suatu Praktik. *Jurnal Ilmiah Kedokteran Fakulitas Kedokteran dan Ilmu Pengetahuan*: Jakarta.

Purnamasari, Anita., Zelviani, S., Sahara., Fuadi, N., (2022). Analisis Nilai Absorbansi Kadar Flavonoid Tanaman Herbal Menggunakan Spektrofotometer UV-Vis. Teknosains: Media Informasi Sains dan Teknologi, 16: 1. 57-64.

Ristianti, (2019). Penetapan Kadar Flavonoid Total Daun Binahong Dengan Metode Sprektofotometri UV-Vis. *Journal of Medicine Akademi Farmasi Putra Indonesia.*

Rofida. (2010). Studi Farmakologi Daun Binahong Dengan Metode Sprektrometri. *Jurnal Farmasi Dan Ilmu Kesehatan.*

Rusdiati, Helmidora. (2020). *Pengetahuan tentang Manfaat Kesehatan dan Uji Toksisitas Daun Binahong Merah.* Alfabeta Press*:* Jakarta, 80–90.

Sugiarti, Lilis, dan Luthfiana Nurulin Nafi'ah. (2018). "Potensi Antibakteri Sediaan Gel Handsanitizer Ekstrak Buah Parijoto *(Medinilla speciosa* Blume) Terhadap Bakteri Patogen *Escherichia coli* Dan *Staphylococus aureus*." *Prosiding HEFA (Health Events for All)* 3.

Widyawati, P. S., Wijaya, C. H., Suprapti, P., & Sajuthi, D. (2010). Pengaruh Ekstraksi Dan Fraksinasi Terhadap Kemampuan Menangkap Radikal Bebas DPPH (*1,1-Difenil-2- Prikrilhidrazil*) Ekstrak

Dan Fraksi Daun Beluntas (*Pluchea indica* Less). *Seminar Rekayasa Kimia dan Proses*, 1–7.

Winahyu, D. A., Retnaningsih, A., & Aprillia, M. (2019). Penetapan Kadar Flavonoid Pada Kulit Batang Kayu Raru (*Cotylelobium melanoxylon* P) Dengan Metode Spektrofotometri UV-Vis. *Jurnal Analis Farmasi*, 4(1), 29–36.

Yovitasari, D., A. Retnaningsih, dan V. Elsyana. (2018). Uji Daya Hambat Ekstrak Daun Sirsak (*Annona muricata* l.) terhadap Pertumbuhan Bakteri *Staphylococcus aureus* dan *Pseudomonas aeruginosa* dengan Metode Difusi Agar. *Jurnal Farmasi Malahayati*.