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Determination of Total Flavonoid Content in Bangle Plant (Zingiber Montanum)Extraction Results

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Abstract: The bangle plant (Zingiber montanum) is a plant that has been used traditionally as a medicinal ingredient, both for prevention and treatment by the community. Parts of the bangle plant contain active compounds that act as antioxidants, one of which is flavonoids. This research aims to determine the total flavonoid content in bangle plants from the extraction results measured using a UV-Vis spectrophotometer. The method for separating flavonoid levels used is maceration. The research results showed that positive test results were obtained for flavonoid compounds in bangle rhizomes as evidenced by qualitative test results which produced a red color in the sample extract and the average total flavonoid content in bangle rhizomes from the extraction results measured using a UV-Vis spectrophotometer was 23. 1mg/100g.

Keywords: Bangle rhizomes; distillation; extraction; flavonoids; UV-Vis spectrophotometer.

Introduction

The bangle plant has the Latin name Zingiber montanum, which is a single-seeded plant (monocot) that comes from the Zingiberaceae family (ginger tribe). The bangle plant is a plant that has been used traditionally as a medicinal ingredient for both prevention and treatment by the community, the part of the bangle plant in particular that is commonly used as an ingredient in traditional medicine is the rhizome, which can be used to treat various diseases, including diarrhea, stomach ache, rheumatism , asthma, and hepatitis. Rhizome (rhizoma) is a modified plant stem that grows below the surface of the soil and can produce new shoots and roots from its segments. The shape of the bangle rhizome is slightly round, short and not much branched, with light brown outer skin and light yellow to brownish yellow rhizome flesh (Bestari, 2021). The bangle rhizome plant contains active compounds that act as antioxidants, one of which is flavonoids (Ningsih, 2022).

Flavonoids are a group of polyphenols consisting of flavones, flavonols, flavononols, flavans, and anthocyanins. These compounds have distinctive properties, namely sharp odor, yellow pigments, can decompose at high temperatures, and are soluble in water and organic solvents (Anggrayni & Nasution, 2021; Echeverria et al, 2021). In addition, flavonoids act as effective antioxidants in preventing reactive free radicals and protecting cells from damage. Given the important role of flavonoids, further studies are needed on the flavonoid content in bangle rhizomes through isolation and identification using appropriate separation methods to determine the levels of flavonoids in bangle rhizomes (Supratman, 2020; Musdija, 2021).

This research is important because bangle has great potential in the development of natural health products, especially as a source of antioxidants. Knowing the

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levels of flavonoids in bangle can help evaluate its potential as a traditional medicinal ingredient and ensure its effectiveness in therapeutic use. The method of separating flavonoid levels used is extraction, with the maceration method as one of the most widely used techniques, both on a large scale and in industry (Setiawan, 2022; Chávez-González, 2022). Maceration has the advantage of extracting all active components in the sample without damaging them, although it requires more cost and time due to the use of a lot of solvents (Yulinar & Suharti, 2022; Da Silva et al, 2022).

Determination of total flavonoid levels in bangle rhizomes in this study was carried out by extracting samples using the maceration method, then determining the flavonoid concentration using UV-Vis spectrophotometry (Amri, 2022; Sapiun et al., 2020). This study not only provides scientific insight into the chemical composition of bangle but also supports the development of safe and effective health products based on this plant.

This study aims to determine the total flavonoid content in bangle plants (Zingiber montanum) from the extraction results measured using UV-Vis spectrophotometry. Determination of flavonoid content is important to evaluate the therapeutic potential of bangle plants and support the standardization of natural-based health products.

Flavonoid content analysis can be carried out using UV-Vis spectrophotometry, because flavonoids have a conjugated aromatic system that allows for strong absorption bands in the ultraviolet (UV) and visible light spectrum regions (Harborne, 1987). This method is used for quantitative analysis that measures the amount of flavonoids in the extract by measuring the absorbance value. The relationship between absorbance and flavonoid content is linear; the higher the measured absorbance, the higher the flavonoid content contained in the plant sample (Aminah et al., 2017; Da Silva et al, 2015).

Method

This research is a laboratory experimental research and the tests carried out in this research are qualitative tests and quantitative tests to determine total flavonoid levels in bangle plants (*Zingiber montanum*). The extraction method used in this research was the maceration method.

Extraction

Bangle rhizome extraction was carried out by modifying several procedures carried out by (Kato et al, 2018), namely weighing 50 grams of dried bangle rhizome powder, then extracted using the maceration method with 96% ethanol solvent up to 350 mL for 3 x 24 hours until completely extracted. Then the sample was covered with aluminum foil. Then, stirred using an orbital shaker for 1 hour. Next, it is filtered using filter paper, obtaining the filtrate and residue. Then the filtered filtrate is evaporated using a rotary vacuum evaporator to obtain a concentrated extract of bangle rhizomes.

Total Flavonoid Test

Qualitative Test of Flavonoid Content

Bangle rhizome extract was put into a different test tube as much as 2 mL. Next, add a little magnesium powder and add 3-4 drops of concentrated HCl. The sample was shaken, then left to stand until a color change occurred. If the color changes to dark red, yellow, or orange in the solution, this indicates the presence of flavonoids (Husnul, 2019; Chigayo, 2021).

Quantitative Test of Flavonoid Content

Determination of total flavonoid levels using the colorimetric method using a UV-Vis Spectrophotometer which refers to a procedure with several modifications with quercetin (QE) as the standard (Mangkuasa & Ningsih., 2020, Sapiun et al, 2020; Tristantini & Amalia, 2019).

Determination of Maximum Wavelength (λmax) of Quarcetin

Determination of the maximum wavelength of quarcetin by running a quarcetin solution in the wavelength range 400-500 nm. The running results show that the maximum wavelength of the standard quercetin standard is at a wavelength of 440 nm. This maximum wavelength is used to measure the absorption of samples of bangle rhizome extract (Zingiber montanum),can be seen in Figure 1 below.

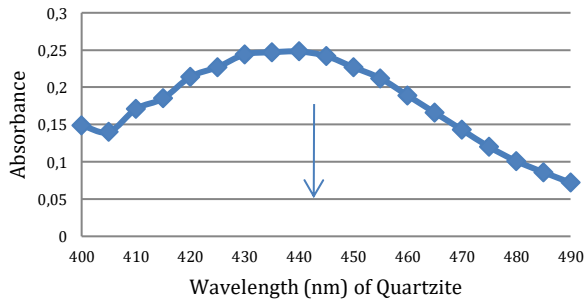

Figure 1. Curvemaximum Wavelength 440 nm

Preparation of Quarcetin Standard Solution

5 mg of quercetin standard was weighed then put into a 50 mL volumetric flask, 96% ethanol was added to the mark (100 mg/L stock solution). Then a series of standard solutions of 2, 4, 6, 8, and 10 mg/L were prepared. Pipette 1 mL of each standard solution, then

add 1.5 mL of 96% ethanol, 0.5 mL of 10% aluminum chloride (AlCl3), 0.5 mL of 1 M potassium acetate and add 2.8 mL of distilled water. After that, it was incubated for 30 minutes at room temperature. Absorbance was determined using the UV-Vis spectrophotometric method, then a calibration curve was created (Grasse et al, 2016; Kato et al, 2018)

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Measurement of Flavonoid Levels

The concentrated extract of bangle rhizome was weighed as much as 5 mg using a digital balance. Next, put it into a 50 mL Erlenmeyer flask. Then 10 mL of 96% ethanol (100 mg/L solution) was added. Next, pipet 1 mL of the solution. Then put them in each test tube. Next, 1.5 mL of 96% ethanol was added, 0.5 mL of 10% aluminum chloride ($AlCl_3$), 0.5 mL of 1 M potassium acetate, and 2.8 mL of distilled water was added to each tube. After that, the solution mixture was shaken and then left for 30 minutes. Next, the solution is put into a cuvette. Measure the absorption value using a UV-Vis spectrophotometer.

Data analysis techniques

Flavonoid levels in herbal samples can be determined by various methods. The method recognized by the Indonesian Ministry of Health is UV spectrophotometry which is based on colorimetric principles. The absorbance of the color formed was measured with a UV spectrometer. Total flavonoid levels were calculated as quercetin levels in the samples. This calculation is based on the Lambert-Beer law which shows a straight relationship between absorbance and analyte levels. To determine flavonoid levels in various types of food based on absorbance values, standard solution data is used. This standard solution data is used to create a regression equation, namely the equation used to calculate flavonoid levels:

$$y = ax + b \quad (1)$$

Where:
y = absorbance value
x = flavonoid content
a,b = constant

Determination of total flavonoid levels using the colorimetric method which refers to the procedure of Chang et al., (2002) and Tommy et al., (2022). Flavonoid levels can be calculated using the Formula 2:

$$F = \frac{c\ x\ V}{m}\ x\ 100 \quad (2)$$

Where:
F = flavonoid content (mg/100g)
c = quercetin equivalent (mg/L)
V = volume (L)
m = sample weight (g)

Result and Discussion

Result

Tabel 1. Qualitative Test Results For Flavonoid Compounds

Qualitative Test	Reactor	Sample Test Results I	Sample Test Results II	Information
Extraction	Mg Metal + Concentrated HCl	Red	Red	There are flavonoids

Tabel 2. Results of absorbance measurements of standard quercetin solutions at wavelengths 440 nm

Quercetin Concentration (ppm)	Absorbance
2	0.026
4	0.1
6	0.158
8	0.238
10	0.31

Creation of a standard curve for Quercetin Standard Solution

The standard curve was obtained from measuring the absorbance of quercetin standard solutions. From standard curve measurements, the regression line equation is obtained, namely y = 0.035x – 0.045. Data from absorbance measurements of standard quercetin solutions can be seen in Figure 2.

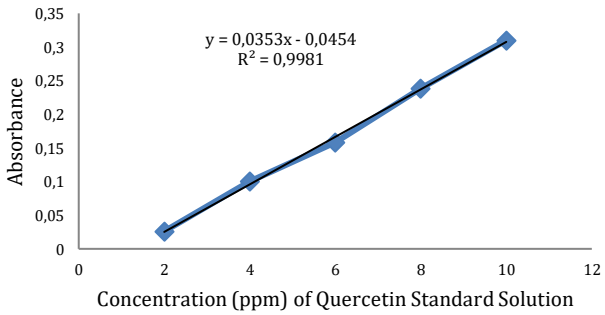

Figure 2. Standard Curve of Quercetin Standard Solution

Tabel 3. Flavonoid Content Analysis Results Bangle Rhizome Extract

Sample	Absorbance	Flavonoid Concentration (mg/L)	Flavonoid Content (mg/100g)	Average Flavonoid Levels (mg/100g)
Bangle Rhizome Extract 1	0.106	4.314	21.4	23.1

Bangle Rhizome Extract 2	0.129	4.971	24.8

Discussion

Qualitative Test of Flavonoid Compounds Resulting from Extraction and Distillation

Qualitative tests were carried out to determine whether or not there were flavonoids in bangle rhizome extract. The presence of flavonoids is known through the color changes to red, yellow and orange in the chloroform layer after being reacted with Mg powder and concentrated hydrochloric acid (HCl). In table. 1 which shows positive test results for flavonoid compounds obtained in a test tube containing red bangle rhizome extract from the extraction results. What is caused by the addition of concentrated HCl and Mg metal is to reduce the benzopyrone nucleus contained in the flavonoid structure so that red or orange flavilium salts are formed. The reaction that occurs between flavonoid compounds with HCl and Mg metal can be seen in Figure 3.

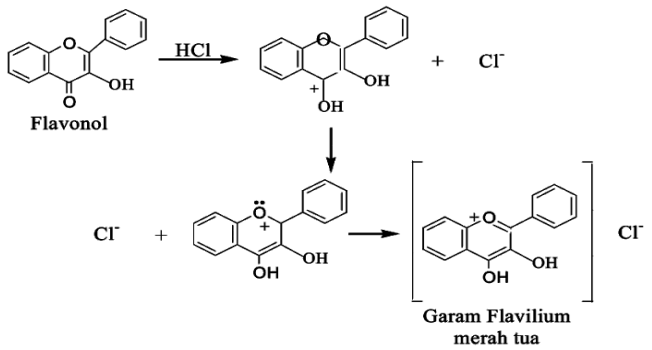

Figure 3. Flavilium Salt Formation Reaction Source. (Yasser et al., 2022).

Quantitative Test of Flaphonoid Compounds Absorbance Measurement of Quercetin Standard Solution

The results of absorbance measurements of standard quercetin solutions as a comparison with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm using a UV-Vis spectrophotometri at a wavelength of 440 nm are in the table 2.

Creation of a standard curve for Quercetin Standard Solution

Measurement of total flavonoid levels in bangle rhizomes (Zingiber montanum) with a maximum wavelength of 440 nm. Flavonoids were calculated using a linear regression equation from a previously measured quercetin calibration curve. The calibration curve is created based on the Lambert-Beer law, namely the linear equation y = ax + b. Where y is the absorbance value, x is the sample concentration, a is a constant and b is the slope of the line. From the measurements, the straight line equation is obtained, namely y = 0.035x – 0.045 with a correlation coefficient (R^2) = 0.998. Because the correlation coefficient (R^2) ≤ 1, the calibration curve obtained is linear.

The results obtained from determining the total flavonoid content of bangle rhizome extract can be seen in table 3. This was repeated twice and the calculation results for the average total flavonoid content of bangle rhizome were 23.1 mg/100g.

Conclusion

Based on the results of the research carried out, positive test results were obtained for flavonoid compounds in bangle rhizomes (Zingiber purpureum Roxb) as evidenced by qualitative test results which produced a red color in the extract and the average results of total flavonoid levels in bangle rhizomes from the extraction results which were measured using a UV spectrophotometri UV-VIS is 23.1 mg/100g.

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Author Contributions

Sri Hastuti Pulukadang, Sitti Rahmawati, Siti Fatimah: conceptualization, analysis, methodology, discussion, conclusion, visualization, investigation, writing original draft, and proofreading; Tri Santoso, Sitti Aminah, Purnama Ningsih Magfirah: editing, paraphrase and review.

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Conflicts of Interest

The authors declare no conflict of interest

References

Aminah, A., Tomayahu, N., & Abidin, Z. (2017). Penetapan kadar flavonoid total ekstrak etanol kulit buah alpukat (Persea americana Mill.) dengan metode spektrofotometri UV-Vis. *Jurnal Fitofarmaka Indonesia,* 4(2), 226-230. https://doi.org/10.33096/jffi.v4i2.265

Amri, A. P. (2022). *Profil Metabolit Sekunder Daun Sirih Merah (Piper Crocatum) Hasil Ekstraksi Maserasi Dan Sokhletasi* (Doctoral dissertation, Poltekkes Tanjungkarang).

Artini, P. E. U. D., Astuti, K. W., & Warditiani, N. K. (2013). Uji fitokimia ekstrak etil asetat rimpang bangle (Zingiber purpureum Roxb.). *Jurnal Farmasi Udayana, 2(4), 279805.* Retrieved from https://ojs.unud.ac.id/index.php/jfu/article/view/7396

Bestari, U. M. (2021). *Implementasi algoritma boyer moore pada chatbot tanaman obat (Doctoral dissertation, Universitas Islam Negeri Maulana Malik Ibrahim)*..

Chang, C. C., Yang, M. H., Wen, H. M., & Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis,* 10(3). https://doi.org/10.38212/2224-6614.2748

Chávez-González, M. L., Sepúlveda, L., Verma, D. K., Luna-García, H. A., Rodríguez-Durán, L. V., Ilina, A., & Aguilar, C. N. (2020). Conventional and emerging extraction processes of flavonoids. *Processes*, *8*(4), 434. https://doi.org/10.3390/pr8040434

Chigayo, K. (2021). *Phytochemical studies of extracts of the Kirkia wilmsii tuber* (Doctoral dissertation).

Da Silva, R. F., Carneiro, C. N., de Sousa, C. B. D. C., Gomez, F. J., Espino, M., Boiteux, J., ... & Dias, F. D. S. (2022). Sustainable extraction bioactive compounds procedures in medicinal plants based on the principles of green analytical chemistry: A review. *Microchemical Journal*, *175*, 107184. https://doi.org/10.1016/j.microc.2022.107184

da Silva, L. A. L., Pezzini, B. R., & Soares, L. (2015). Spectrophotometric determination of the total flavonoid content in Ocimum basilicum L.(Lamiaceae) leaves. *Pharmacognosy magazine*, *11*(41), 96. https://doi.org/10.4103/0973-1296.149721

Echeverria, G., Leclerc, C., Giné-Bordonaba, J., & Romero, A. (2021). Sensorial evaluation and aroma of vegetable oils. *Oil and Oilseed Processing: Opportunities and Challenges*, 245-278. https://doi.org/10.1002/9781119575313.ch12

Grasse, E. K., Torcasio, M. H., & Smith, A. W. (2016). Teaching UV–Vis spectroscopy with a 3D-printable smartphone spectrophotometer. *Journal of Chemical Education*, *93*(1), 146-151. http://dx.doi.org/10.1021/acs.jchemed.5b00654

Kato, E., Kubo, M., Okamoto, Y., Matsunaga, Y., Kyo, H., Suzuki, N., ... & Fukuyama, Y. (2018). Safety assessment of bangle (Zingiber purpureum Rosc.) rhizome extract: acute and chronic studies in rats and clinical studies in human. *ACS omega*, *3*(11), 15879-15889. https://doi.org/10.1021/acsomega.8b02485

Harborne, J.B. (1987). *Phytochemical method* (Padmawinata, K & Soediro, I, Translator). Bandung: ITB.

Husnul, M., & Khusnul, N. (2019). Analysis of total flavonoid content of nut grass (Cyperus rotundus L) tuber extract. *Scientific Journal: J-Hestech*. 2(2) .

Ilmiati Illing, F.N. (2023). Analysis of total flavonoid content of knob grass extract (Hyptis capitata Jacq) using the UV-Vis spectrophotometric method. *Cokroaminoto Journal of Chemical Science*. 5(1). , 20-24. Retrieved from https://science.e-journal.my.id/cjcs/article/view/127

Makuasa, D. A. A., & Ningsih, P. (2020). The analysis of total flavonoid levels in young leaves and old soursop leaves (Annona muricata L.) using uv-vis sepctrofotometry methods. *Journal of Applied Science, Engineering, Technology, and Education*, 2(1), 11-17. http://dx.doi.org/10.35877/454RI.asci2133

Musdja, M. Y. (2021). Potential bangle (Zingiber montanum J. König) rhizome extract as a supplement to prevent and reduce symptoms of Covid-19. *Saudi journal of biological sciences*, *28*(4), 2245-2253. https://doi.org/10.1016/j.sjbs.2021.01.015

Ningsih, H.F. (2022). *Potential of plants in the zingiberaceae family for antihypertensive activity: Systematic review*. (Bachelor's Thesis, UIN Syarif Hidayatullah Jakarta-Fikes).

Santika, F.Y. (2021). *T*he difference in the number of yeast molds on kencur gendong rice jamu in traditional markets and packaged kencur rice jamu in the Bandar Lampung city herbal medicine depot. *Malahayati Medical Journal*. 4(3), , 160-167. https://doi.org/10.33024/jmm.v4i3.3223

Sapiun, Z., Pangalo, P., Imran, A. K., Wicita, P. S., & Daud, R. P. A. (2020). Determination of total flavonoid levels of ethanol extract sesewanua leaf (Clerodendrum fragrans wild) with maceration method using UV-vis spectrofotometry. *Pharmacognosy Journal*, *12*(2). http://dx.doi.org/10.5530/pj.2020.12.56

Setiawan, R. (2022). *Isolation and characterization of marker compounds from bangle* (*Zingiber cassumunar) rhizome extract*. (Doctoral dissertation, Hasanuddin University).

Supratman, S. (2020). *Giving bidara leaf extract (Ziziphus mauritiana) through drinking water as an antioxidant for the performance, hematology, and final part of the digestive tract of quail*. (Doctoral dissertation, Hasanuddin University).

Tommy, M., Pratama, N. P., & Sari, K. R. P. (2022). Perbandingan Kadar Total Fenolik dan Flavonoid Ekstrak Etanol Daun, batang, dan Akar Kirinyuh (Chromolaena odorata L.) dengan Menggunakan Metode Spektrofotometri UV-Vis. *Jurnal Pharmacia Mandala Waluya*, *1*(5), 217-231. https://doi.org/10.54883/jpmw.v1i5.48

Tristantini, D., & Amalia, R. (2019, December). Quercetin concentration and total flavonoid content of anti-atherosclerotic herbs using aluminum chloride colorimetric assay. In *AIP conference proceedings* (Vol. 2193, No. 1). AIP Publishing. https://doi.org/10.1063/1.5139349

Yasser, M., Nurdin, I.M., Amri, Bangnglino, H., Angraini, N., & Said, U.R. (2022). Phytochemical Screening for Flavonoid, Alkaloid, Saponin, Steroid and Terpenoid Compounds From Kopasanda Leaves (Chromoloena odorata L.). *Proceedings of the 6th National Research & Community Service Seminar*, 90-94.

Yulinar, F., & Suharti, H. (n.d). (2022). Selection of the betel leaf extraction process in the pre-design of the betel leaf hand sanitizer factory with a production capacity of 480 tons/year. *Distillates: Journal of Separation Technology*, 8(1), 146-153.