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Total Flavonoid Content in Gedi (*Abelmoschus manihot L.*) Leaf Fraction by Using UV-Vis Spectrophotometry

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ABSTRACT

Gedi (*Abelmoschus manihot* L.) leaves are widely used in traditional medicine because they contain secondary metabolites, particularly flavonoids, which exhibit antioxidant and anti-inflammatory activities. This study aims to analyze the presence of flavonoid compounds and to measure total flavonoid content in Gedi leaf fractions. The extraction process is conducted using a graded maceration method with n-hexane, ethyl acetate, and methanol as solvents to separate compunds based on their polarity. Flavonoid identification is performed through phytochemical screening, while the determination of total flavonoid content is performed using the UV-Vis spectrophotometry method. The measurement result shows that the maximum wavelength (λ Max) is obtained at 415 nm. The result indicate that the ethyl acetate and methanol fractions contain flavonoid compounds. The total flavonoid content in the ethyl acetate fraction is 122.3 mg QE/g while the methanol fraction is 240.7 mg QE/g. The methanol fraction shows the highest flavonoid content compared to the ethyl acetate fraction, indicating that flavonoid compounds in Gedi leaves are more soluble in polar solvents.

Keywords:
Abelmoschus manihot; Gedi Leaves; Extraction; Flavonoid; UV-Vis Spectrophotometry

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1. Introduction

Gedi plant (*Abelmoschus manihot* L.) is one of the plants widely cultivated in Limboto District, Gorontalo Regency, and has long been used as both a vegetable and a traditional medicine. Its leaves are often used because they help in wound healing, reduce inflammation, and maintain digestive health. Scientifically, gedi leaves are known to contain high levels of bioactive compounds, especially flavonoids, compared to other parts of the plant[11].

Flavonoids are secondary metabolites that possess antioxidant, antibacterial, and anti-inflammatory activities [2]. These compounds are capable of protecting cells from oxidative damage and contributing to the healing process. In addition, various studies have also identified flavonoid compounds such as rutin, quercetin, hyperoside, isoquercitrin, and hibifolin in the leaves of gedi [10], and have shown strong antioxidant activity from its flavonoid fraction [13].

The Gedi plant (*Abelmoschus manihot* L) contains various chemical compounds that play an important role in its pharmacological activity. Gedi leaf extract contains a total phenolic content of 10.67 mg GAE/g of extract and a total flavonoid content of approximately 2.33 mg quercetin/g of extract, which demonstrated significant antioxidant activity when tested using the DPPH method. These flavonoids, including quercetin, play a key role in scavenging free radicals, as well as providing anti-inflammatory effects and protection against cellular damage. In addition to flavonoids and phenolics, Gedi leaves also contain alkaloids, steroids, tannins, saponins and glycosides, which further enhance the plant's pharmacological potential, such as its antifungal and antimicrobial activity [16].

Furthermore, according to a study, the part of the Gedi plant most beneficial for medicinal purposes is the leaves. Gedi leaves are known to contain secondary metabolites such as flavonoids, alkaloids, steroids and phenolic compounds, which exhibit biological activities including antioxidant, antibacterial, anti-inflammatory, antidiabetic and analgesic effects. This rich chemical composition makes the Gedi plant a potential source of active ingredients for the development of herbal medicines and health supplements. The consistency of these bioactive compounds is influenced by environmental conditions and extraction methods; therefore, a deeper understanding of the phytochemical characteristics of the Gedi plant is essential[17].

The determination of flavonoid content is greatly influenced by the extraction method. The stepwise maceration method was chosen because it is simple, does not use high temperatures, and is capable of separating compounds based on solvent polarity [9]. The use of n-hexane, ethyl acetate, and methanol has proven effective in increasing the yield and selectivity of the extract [12].

The analysis of total flavonoid content was conducted using UV-Vis spectrophotometry because it is fast, sensitive, and well-validated [8]. Based on this, this research was conducted to analyze the total flavonoid content in the fraction of gedi leaves (*Abelmoschus manihot* L.) as an effort to support its utilization as a natural resource in the pharmaceutical field.

Based on the description, this research was conducted to analyze the total flavonoid content in the gedi leaf fraction (*Abelmoschus manihot* L.) using the UV-Vis spectrophotometry method, with the expectation of providing scientific data that supports the more optimal use of this plant as a natural resource in the pharmaceutical field.

2. Methods

This research is a laboratory experimental study to determine the flavonoid content in the fractions of gedi leaves (*Abelmoschus manihot*) using UV-Vis Spectrophotometry.

Materials and Equipment

The materials used distilled water, ethyl acetate, concentrated HCl, methanol, n-hexane, gedi leaf sample (*Abelmoschus manihot* L.), Mg powder. The equipment used in this study includes a maceration tool, stirring rod, porcelain dish, beaker (Pirex®), measuring cylinder (Iwaki®), filter cloth, label paper, filter paper, fine cloth, coarse cloth, analytical balance (Sojikyo®), Ohaus balance, water bath, dropper pipette, rotary evaporator (Hanvapor®), and UV-Vis Spectrophotometer (Thermo Scientific).

Sample Preparation

Gedi leaf samples (*Abelmoschus manihot* L.) are harvested in the morning when the plants are still fresh, then washed and sorted wet, after that, the samples were dried in an oven at 40°C and then the material was ground into a fine powder using a blender.

Extraction Process

A 250-gram sample of ground Gedi leaves (*Abelmoschus manihot* L.) was extracted using the stepwise maceration method. The process began with a non-polar solvent, namely n-hexane, followed by a semi-polar solvent, namely ethyl acetate, and finally a polar solvent, namely methanol. Each maceration stage involved 4,000 mL of the respective solvent and was carried out for 3 × 24 hours. The filtrate obtained from each solvent was then concentrated using a rotary evaporator at 40°C until a thick extract was obtained; the extraction yield was then calculated using the formula:

$$\%\text{Yield} = \frac{\text{Weight of Extract Obtained}}{\text{Initial Weight of Simplicia}} \text{ X } 100\%$$

Phytochemical Screening

Approximately 1 mg of ethanol extract was placed on a drop plate; 5 drops of methanol were added, followed by 3–4 small pieces of Mg strip and 2 drops of concentrated HCl.

Analysis of Total Flavonoid Content by UV-Vis Spectrophotometry

Preparation of a Quercetin Stock Solution (1000 µg/mL)

Weigh out 10 mg of quercetin and transfer it to a 10 mL volumetric flask; then add methanol up to the 10 mL mark and shake until dissolved to obtain a 1000 µg/mL stock solution.

Preparing Quercetin Standard Solutions (20–100 µg/mL)

Prepare five 10 mL volumetric flasks. For the 100 µg/mL standard, use the stock solution; pipette 10.0 mL of the working solution into a 10 mL volumetric flask. For the 80 µg/mL standard, pipette 8.0 mL of the working solution into a 10 mL volumetric flask and then add methanol to the 10 mL mark; for the 60 µg/mL standard, pipette 6.0 mL and then add methanol to the 10 mL mark; for the 40 µg/mL standard, pipette 4.0 mL; for 20 µg/mL, take 2.0 mL. Shake each flask until homogeneous.

Preparing the Sample Solution

Weigh 10 mg of sample extract from each fraction (methanol and ethyl acetate), then transfer each extract to a separate 10 mL measuring flask, add a small amount of methanol to dissolve, then make up to the 10 mL mark to produce a 1000 µg/mL stock solution. Then, take 1.0 mL of the stock solution and dilute to 10 mL to obtain a 100 µg/mL working sample solution for use.

Colour Reaction Procedure for Standards and Samples

Place 1.0 mL of each standard or sample solution into a test tube. Add 0.5 mL of acetic acid, cap the tube and leave to stand for 5 minutes. Then add 0.5 mL of $AlCl_3$ (10%), stir and leave to stand for 6 minutes. Add methanol to bring the volume up to 10.0 mL. Shake gently and leave to stand for 10–15 minutes.

Preparing the Blank

Add 1.0 mL of methanol to a test tube, then treat the blank with the reagent in exactly the same way as for the sample and standard solution.

Determination of Quercetin Maximum Wavelength (λmax)

The maximum wavelength was determined by reading within the wavelength range of 400-450 nm using UV-Vis spectrophotometry, where λMax was obtained at 415 nm.

Absorbance Measurement

Turn on the spectrophotometer, set the wavelength to 415 nm, insert the cuvette containing the blank to zero the spectro. Measure the absorbance of the standards from the lowest to the highest concentration (done in triplicate), then measure the absorbance of each fraction sample (also done in triplicate). Record all the values and calculate the average.

Creating a Calibration Curve and Obtaining the Line Equation

The average absorbance of each standard solution is used to create a calibration curve graph with concentration on the X-axis and absorbance on the Y-axis. From this graph, a linear regression line is created with the equation $y = ax + b$, and the R^2 value is recorded.

Calculating Sample Concentration from Absorbance

The average absorbance of the sample is inserted into the regression curve equation, so the equivalent Quercetin concentration in the tested solution is calculated using the formula:

$$x = \frac{(y-b)}{a}$$

where y is the sample absorbance, a is the line gradient, and b is the constant of the curve equation.

Conversion to mg QE per Gram of Extract

The original flavonoid concentration is then converted to mg Quercetin equivalent per gram of extract by considering the volume of the solution representing the extract and the weight of the extract used. The formula is:

$$\text{Total Flavonoid Content (mg QE/g)} = \frac{C \times V \times Fp}{m}$$

where C is the concentration, V is the volume of the solution representing the extract, Fp is the dilution factor, and m is the mass of the dissolved extract. In this way, the total flavonoid content for each fraction of the gedi leaf can be accurately obtained.

3. Results and Discussion

Extraction of Gedi Leaves

The Gedi plant (*Abelmoschus manihot* L.) offers a wide range of benefits, particularly in the fields of health and traditional medicine. Several studies have shown that Gedi leaves are rich in bioactive compounds such as flavonoids, alkaloids, tannins, steroids and saponins, which contribute to their antioxidant, anti-inflammatory and antimicrobial properties. The antioxidant activity of Gedi leaves can help prevent oxidative stress associated with various degenerative diseases. Furthermore, the flavonoids they contain are believed to boost the body's immune system and accelerate the wound-healing process[13].

The process of determining flavonoid content is highly dependent on the extraction method used, as the extraction method determines the type, quantity and purity of the compounds from the plant material. The extraction method chosen was stepwise maceration because it is simple, does not require high temperatures that could damage the chemical structure of active compounds such as flavonoids, and is capable

of producing a good yield whilst maintaining the stability of the compounds. Stepwise maceration has advantages over other extraction methods because the process allows for the sequential separation of compounds based on the polarity of the solvents, thereby making extraction more efficient and resulting in a purer and more complete extract[10].

The choice of solvent in stepwise maceration is a key factor determining the success of the extraction process. Among non-polar solvents, N-hexane is selected because it is capable of extracting components that play a vital role in the stability of the extract, such as essential oils, waxes and lipids. The use of n-hexane has been shown to yield a higher extraction yield compared to some other, more toxic non-polar solvents. Among semi-polar solvents, ethyl acetate was selected due to its ability to extract semi-polar metabolites such as certain flavonoids, mild tannins, and some phenolic derivatives that cannot be optimally extracted by non-polar solvents. For the polar solvent, methanol was used as it is the most effective at extracting flavonoids, phenolics and glycosides, which are the main target compounds in gedi leaves. The advantage of methanol lies not only in its polarity, but also in its lower boiling point, which makes it easier to evaporate without damaging the active compounds.

A study by Triyanti et al. (2025) reported a yield of 3.14% for n-hexane, which is higher than that achieved with chloroform, which yielded only around 2.01%. This demonstrates that N-hexane is not only safer but also more efficient at extracting the non-polar components required to produce a clean and stable initial extract. Research by Nor Tiara Sari et al. (2025) showed that the use of ethyl acetate yielded a recovery of 6.40 per cent, higher than that achieved with acetone, which yielded only 4.22 per cent. This higher recovery demonstrates that ethyl acetate is capable of extracting compounds of medium polarity more selectively and efficiently, making it the best choice for the second extraction stage.

Methanol yielded a recovery rate of 10.37 per cent, which is higher than the 8.12 per cent recovery rate achieved with 70 per cent ethanol. This difference confirms that methanol has a greater ability to dissolve polar compounds, thereby yielding a richer and more optimal extract. This is supported by research which also emphasises the use of ethyl acetate, methanol and n-hexane as the primary solvents in stepwise maceration due to their effectiveness and safety in the extraction of plant compounds[16]..

The results of the extraction of gedi leaves (*Abelmoschus manihot* L.) show differences in yield values for each solvent fraction used (**Table 1**). The N-hexane fraction yielded a yield of 8.8% with an extract weight of 22 g, the ethyl acetate fraction yielded 10.8% with an extract weight of 27 g, and the methanol fraction provided the highest yield of 11.20% with an extract weight of 28 g. Based on the yield criteria above 10%, which is categorized as good, the ethyl acetate and methanol fractions can be said to have optimal extraction efficiency[6].

The difference in yield values is influenced by the type and polarity level of the solvent used. Polar solvents tend to dissolve polar compounds, non-polar solvents dissolve non-polar compounds, while semi-polar solvents dissolve compounds with intermediate polarity. This is in accordance with the principle of "like dissolves like" in the maceration process[5]. Higher yields in ethyl acetate and methanol solvents compared to n-hexane indicate that the main components in gedi leaves are more soluble in semi-polar and polar solvents.

Table 1. Results of Gedi Leaf Extraction (*Abelmoschus manihot* L.)

Solvent	Sample Weight (g)	Solvent Volume(mL)	Extract Weight (g)	%Yield
N-heksan	250 g	4.000 mL	22 g	8.8 (Poor)
Etil Asetat			27 g	10.8 (good)
Metanol			28 g	11.20 (good)

Source: Processed primary data, 2026

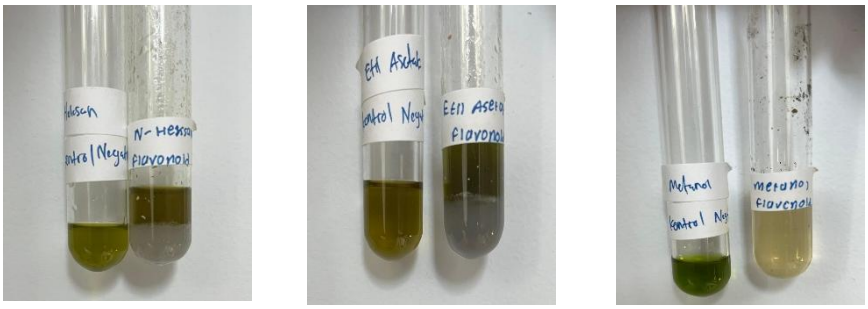
Phytochemical Screening

Based on the results of the phytochemical screening test on the leaf fraction of gedi (*Abelmoschus manihot*), it was found that flavonoid compounds were not detected in the n-hexane fraction, whereas the ethyl acetate and methanol fractions showed positive results (**Table 2 and Figure 1**). These differences in results indicate a variation in the distribution of flavonoid compounds in each solvent fraction used.

Table 2. Results of Phytochemical Screening of Gedi Leaves (*Abelmoschus manihot* L.)

Compound Type	Reagent	N-Hexane Fraction	Ethyl Acetate Fraction	Methanol Fraction
Flavonoid	Mg Powder + Concentrated HCl	- (No Change)	+ (Dark Orange)	+ (Orange)

Source: Processed primary data, 2026



(a) (b) (c)

Figure 1. Results Of Phytochemical Screening Gedi Leaves (a) N-hexane Fraction; (b) Ethyl Acetate Fraction; (c) Methanol Fraction

The results indicate that flavonoids in gedi leaves tend to dissolve more in semi-polar and polar solvents compared to non-polar solvents. This is consistent with the polar nature of flavonoids, making them more soluble in solvents like ethyl acetate and methanol [4]. The absence of flavonoids in the n-hexane fraction shows that non-polar solvents are less effective in attracting these compounds, while solvents with higher polarity levels can extract flavonoids more optimally.

Measurement of Quercetin Absorbance Value

The maximum wavelength was determined by taking readings in the 400–450 nm wavelength range using UV-Vis spectrophotometry. Based on the results shown in the **Figure 2**, it was found that the highest absorbance was obtained at a wavelength of 415 nm.

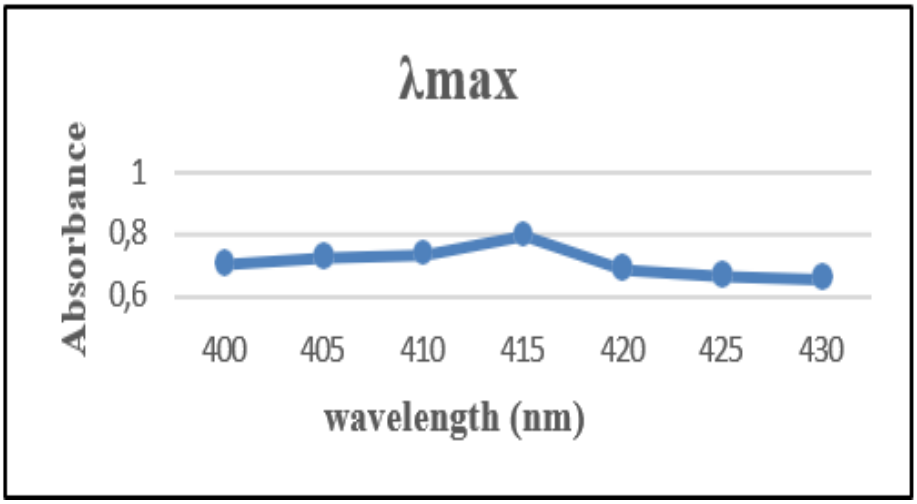


Figure 2. Results of the Determination of the Maximum Wavelength (λmax)

Based on the measurement results of the standard quercetin absorbance value at concentrations of 20, 40, 60, 80, and 100 ppm measured at λ Max 415 nm, the respective values obtained were 0.136; 0.244; 0.492; 0.646; and 0.955 (**Table 3**). The data shows an increase in absorbance values with the increasing concentration of the standard solution, indicating a linear relationship between concentration and absorbance.

Table 3. Measurement Results of Quercetin Absorbance at λMax 415 nm

Concentration (ppm)	Replicates	Absorbance	Average Absorbance
20	1	0.138	0.136
	2	0.136	
	3	0.136	
40	1	0.244	0.244
	2	0.244	
	3	0.245	
60	1	0.489	0.492
	2	0.492	
	3	0.494	
80	1	0.603	0.632
	2	0.646	
	3	0.649	
100	1	0.953	0.955
	2	0.956	
	3	0.957	

Source: Processed primary data, 2026

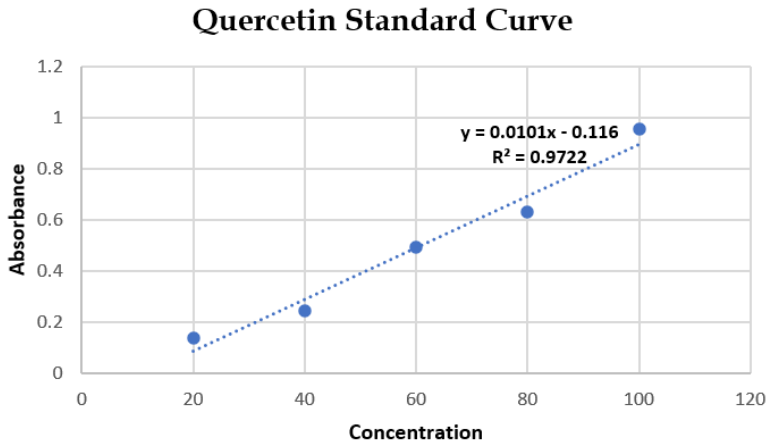


Figure 3. Standard Calibration Curve of Quercetin

Figure 3 showing a linear relationship between concentration and absorbance at 415 nm of quercetin. The linear regression equation for quarsetin was obtained as y = 0.010x – 0.116, with a correlation coefficient (r) of 0.9722. The R^2 value close to 1 indicates excellent linearity, supporting the use of this calibration curve as a reference for quantitative determination of flavonoid in Gedi leaves.

This linearity is consistent with the principle of the Lambert–Beer law, where absorbance is directly proportional to the concentration of the substance in the solution under conditions of constant path length and molar extinction coefficient. In this analysis, quercetin was used as a reference standard because it can form a stable complex with aluminum chloride ($AlCl_3$), which produces maximum absorption at a wavelength of 415 nm, making it suitable for the detection of flavonoid. The obtained calibration curve shows a good linear relationship and can be used as a basis for determining the total flavonoid content[3].

Analysis of Total Flavonoid Content in Gedi Leaves (*Abelmoschus manihot* L.)

The calculation results of total flavonoid content show on **Table 4**, that the methanol fraction has an absorbance value of 0.128 with a total flavonoid content of 240.7 mg QE/g and a percentage content of 0.02407% b/v. Meanwhile, the ethyl acetate fraction shows an absorbance value of 0.007 with a content of 122.3 mg QE/g and a percentage content of 0.01223% b/v. This data indicates that the methanol fraction has a higher total flavonoid content compared to the ethyl acetate fraction.

The results are consistent with research that reports the total flavonoid content in gedi leaf extract (*Abelmoschus manihot* L.) can reach 110.57 mg QE/g, further reinforcing that gedi leaves are a significant source of flavonoids. The difference in concentration between the methanol and ethyl acetate fractions indicates that the distribution of flavonoids is more dominant in polar solvents[7].

Table 4. Results of the Analysis of Flavonoid Content in Gedi Leaves (*Abelmoschus manihot* L.)

Sample	Sample Weight (g)	Vol (mL)	Rep	Absorbance	Concentration (µg/mL)	Average Flavonoid Concentration	Total Flavonoid Content (TFC) (mg QE/g)	Percentage Concentration (% w/v)
Methanol Fraction	0.01	10	1	0.127	23.90	24.07	240.7	0.02407
			2	0.128	24.07			
			3	0.128	24.07			
Ethyl acetate Fraction	0.01	10	1	0.006	12.00	12.23	122.3	0.01223
			2	0.007	12.23			
			3	0.007	12.23			

Source: Processed primary data, 2026

Generally, this is related to the characteristics of flavonoids, which tend to be polar due to the presence of hydroxyl groups, making them easier to extract in polar solvents like methanol. The reliability of the method used is also supported by UV-Vis spectrophotometry validation using $AlCl_3$ complexation, which shows good linearity, precision, and accuracy[1]. Additionally, phytochemical studies also report that gedi leaves contain various flavonoids such as rutin, quercetin, hyperoside, and isoquercitrin, with concentrations that can vary depending on the type of solvent and extraction method used[10].

4. Conclusion

The fraction of gedi leaves (*Abelmoschus manihot* L.) has been proven to contain flavonoid compounds, as indicated by positive results in phytochemical screening tests for the ethyl acetate and methanol fractions, while the n-hexane fraction did not show the presence of flavonoids. The results of the total flavonoid content analysis using the UV-Vis spectrophotometry method with quercetin as the standard showed that the methanol fraction had an average total flavonoid content of 240.7 mg QE/g, higher than the ethyl acetate fraction at 122.3 mg QE/g. Thus, the methanol fraction is the fraction with the highest flavonoid content compared to the ethyl acetate and n-hexane fractions.

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Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this paper.

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