



Microscopic Identification and Determination of Total Flavonoid Content of Moringa Leaves Extract and Ethyl Acetate Fraction (*Moringa oleifera* L.)

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Abstract

This research was conducted to identify simplicia microscopically, phytochemical screening and determination of total flavonoid content of extract and ethyl acetate fraction from Moringa (*Moringa oleifera* L.) leaves using UV-Vis Spectrophotometry method. The experimental design used in this study was to perform microscopic identification of Moringa leaf powder simplicia, make 96% and 70% ethanol extract and ethyl acetate fraction of Moringa leaves from 70% ethanol extract, then carry out phytochemical screening and determination of total flavonoid content with quercetin standards. Phytochemical screening on the ethyl acetate fraction of Moringa leaves included tests for the content of flavonoids, saponins, tannins and alkaloids. The results of microscopic identification of Moringa leaf simplicia showed the presence of calcium oxalate crystals in the form of rosettes, mesophyll and stomata. The result of determination of total flavonoid content in 96% ethanol extract was $16.69 \pm 0.74\%$ (w/w), 70% ethanol extract was $10.84 \pm 0.49\%$ (w/w), Moringa leaf ethyl acetate fraction $14.45 \pm 0.90\%$ (w/w). The highest total flavonoid content was found in the 96% ethanol extract of Moringa leaves in accordance with the Indonesian Herbal Pharmacopoeia 2017, that the thick extract of Moringa leaves containing no less than 6.30% (w/w) total flavonoids was calculated as quercetin.

Keywords: quercetin, *Moringa oleifera*, microscopic, flavonoid

Identifikasi Mikroskopis dan Penentuan Kandungan Flavonoid Total Ekstrak Daun Kelor dan Fraksi Etil Asetat (*Moringa oleifera* L.)

Abstrak

Penelitian ini dilakukan untuk mengidentifikasi simplisia secara mikroskopis, skrining fitokimia dan penetapan kadar flavonoid total ekstrak dan fraksi etil asetat dari daun kelor (*Moringa oleifera* L.) dengan metode Spektrofotometri UV-Vis. Rancangan percobaan yang digunakan dalam penelitian ini dengan melakukan identifikasi mikroskopis pada simplisia serbuk daun kelor, membuat ekstrak etanol 96% dan 70% serta fraksi etil asetat daun kelor dari ekstrak etanol 70% kemudian dilakukan skrining fitokimia dan penetapan kadar flavonoid total dengan standar kuersetin. Skrining fitokimia pada fraksi etil asetat daun kelor meliputi uji kandungan flavonoid, saponin, tanin dan alkaloid. Hasil identifikasi mikroskopis simplisia daun kelor menunjukkan adanya kristal kalsium oksalat berbentuk roset, mesofil dan stomata. Hasil penetapan kadar flavonoid total pada ekstrak etanol 96% sebesar $16,69 \pm 0,74\%$ (b/b), ekstrak etanol 70% sebesar $10,84 \pm 0,49\%$ (b/b), fraksi etil asetat daun kelor $14,45 \pm 0,90\%$ (b/b). Kadar flavonoid total paling besar terdapat pada ekstrak etanol 96% daun kelor sesuai dengan Farmakope Herbal Indonesia 2017, bahwa ekstrak kental daun kelor mengandung flavonoid total tidak kurang dari 6,30 % (b/b) dihitung sebagai kuersetin.

Kata Kunci: kuersetin, *Moringa oleifera*, mikroskopis, flavonoid

1. Introduction

Moringa plant (*Moringa oleifera*) is a species of the genus *Moringa* belonging to the family Moringaceae, which is easy to grow in tropical areas such as Indonesia. Moringa can grow in tropical and subtropical areas on all types of soil and is resistant to dry seasons within six months. Moringa leaves are widely used by the community as traditional medicine, vegetables, beauty and fatty foods. One of the benefits of Moringa leaves as beauty is as an antiaging (premature aging), moisturizing and overcoming dry skin.¹ Moringa leaf extract also has antidiabetic activity², tyrosinase inhibitor³, antibacterial⁴, antioxidant, anti-inflammatory and anti-arthritis.⁵

Moringa leaves contain flavonoids and are rich in pro vitamins A, C, E, especially carotene which will be converted into vitamin A in the body and significantly affect hepatoprotective. The content of glucocyanate and isothiocyanate compounds in Moringa plants is known to the public as hypotensive, anti-cancer, inhibiting bacterial and fungal activity.⁶ Determination of levels and studies on the content profile of secondary metabolites of the flavonoid group can be used as the basis for determining the isolation of potential compounds as antidiabetic, antioxidant, antiaging or other compounds that can become active raw materials for drugs.⁷

Based on previous research, Moringa leaves contain secondary metabolites including alkaloids, flavonoids, tannins and saponins.⁹ Flavonoids are one of the largest natural phenolic antioxidant compounds and are found in all plants, so it can be ascertained that there are flavonoids in these plants. Phytochemical compounds in Moringa leaves

that act as high antioxidant compounds are flavonoids so that the total flavonoid content in Moringa leaves can be determined using a UV-Visible spectrophotometer.¹⁰ Determination of the total flavonoid content of an extract of natural ingredients can use quercetin as a standard. Natural extracts are reacted with AlCl_3 compounds and form complex compounds whose levels can be determined by visible spectrophotometry.^{10,11} The complex reaction between standard quercetin and AlCl_3 is shown in Figure 1. This study aimed to determine the total flavonoid content in the extract and the ethyl acetate fraction of Moringa leaves.⁸

2. Materials and methods

2.1. Materials

The tools used in the research were microscope, maceration vessels, separating funnels, electric stoves, laboratory glassware (pyrex), digital scales, stirring rods, 10 ml measuring pipettes, test tubes, aluminium foil, rotary evaporator, water baths, porcelain crucibles, funnels, filter paper, oven, cream containers, vortex, universal pH, ruler, adhesive and dispersible test equipment and their weights, cuvette and UV-Vis spectrophotometer (Thermo Scientific Evolution 201). The materials used in this study included Moringa leaves (*Moringa oleifera* L.) from Beringharjo Market Yogyakarta, 70% ethanol, 96% ethanol, ethyl acetate, petroleum ether, quercetin (Sigma Aldrich), 10% AlCl_3 and 5% acetic acid.

2.2. Research sites

This research was conducted at the Integrated Chemistry Laboratory and Phytochemical Laboratory, Bachelor of Pharmacy Study Program, Alma Ata University Yogyakarta in May 2021.

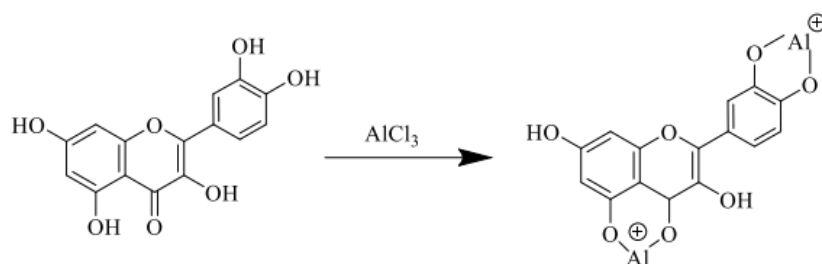


Figure 1. Complex Reaction Between Quercetin and AlCl_3 ⁸

2.3. Microscopic identification of Moringa leaf simplicia

Moringa leaf powder is placed on a glass object, given a solution of chloral hydrate and covered with a cover glass. Then heated on a bunsen fire with tube clamps and kept from boiling, and heated to dry. The preparations were then placed on a microscope and observed with a magnification of 10 times.^{12,13}

2.4. Moringa leaf extract

Moringa leaf powder was weighed as much as 2000 grams for 70% ethanol solvent and 500 grams for 96% solvent were put into different glass jars then added ethanol solvent according to concentration variations as much as 10 L for 70% ethanol solvent and 2.5 L for 96% ethanol to completely submerged and stored for 3x24 hours at room temperature and stirred occasionally to speed up the process of dissolving chemical compounds. The extract obtained was then filtered and separated from the residue, then the residue or dregs obtained were remaceration for 3x24 hours with the same solvent and amount (1:5). The filtrate obtained was then concentrated using a rotary evaporator at a temperature of 60°C until a thick brown extract was obtained. The thick extract was weighed to obtain the extract yield.²

2.5. Fractionation of 70% ethanolic Moringa leaf extract

Moringa leaf ethyl acetate fraction was made by weighing 51.66 grams of EEDK, then dissolved using 500 ml of distilled water and fractionated using petroleum ether (PE) and ethyl acetate in a ratio of 1:1. The ethyl acetate phase containing the moringa leaf extract fraction was evaporated using a rotary evaporator at a temperature of 60°C at a speed of 60 rpm. Next, the semi-viscous extract was put into a porcelain cup and evaporated over a water bath. The formula for calculating the yield of the ethyl acetate fraction of Moringa leaves is = ((Weight of thick ethyl acetate fraction/Weight of thick extract of Moringa leaves) x100%).¹¹

2.6. Moringa leaf ethyl acetate fraction

phytochemical screening

Phytochemical tests were carried out to determine the class of compounds contained in the ethanol extract of Moringa leaves (EEDK) and the ethyl acetate fraction (FEDK). Phytochemical examination was carried out, namely the examination of alkaloid compounds, polyphenols (tannins), flavonoids, and saponins. Polyphenol examination was carried out by taking one ml of EEDK added with 1% FeCl₃ reagent. The reaction is positive if a blackish or dark blue color is formed. For the examination of flavonoids by taking 1.0 ml of EEDK, adding a few drops of 10% NaOH, the reaction will show a positive flavonoid if there is a specific color change. And saponin examination was carried out by adding 1.0 ml of EEDK with hot water and shaking. Positive reaction of saponins when a long-lasting foam is formed.^{11,12}

2.7. Determination of total flavonoid content of Moringa leaf extract and ethyl acetate fraction

2.7.1. Determination of maximum wavelength

1.0 mL of quercetin solution was taken as much as 1.0 mL added with 1 mL of 10% AlCl₃ and 8 ml of 5% acetic acid, then the UV-Vis spectrophotometer readings with a wavelength of 400-500 nm were taken.¹⁰

2.7.2 Determination of operating time

Determination of operating time is done by using a 100 ppm quercetin solution taken as much as 1 mL added with 1 mL of 10% AlCl₃ and 8 ml of 5% acetic acid, the solution obtained is measured by the absorbance of the wavelength obtained at intervals of 0-60 minutes until the absorbance is obtained stable.¹⁰

2.7.3 Determination of the standard curve of quercetin

The 100 ppm quercetin solution was made in series with concentrations of 20, 40, 60, 80 and 100 ppm, 1 mL of each concentration was taken into a volumetric flask, added 1 mL of 10% AlCl₃ and 8 mL of 5% acetic acid, allowed to stand for the optimum time, then

Absorbance readings were carried out using a UV-Vis spectrophotometer at the maximum wavelength.¹⁰

2.7.4. Determination of total flavonoids

The ethanol extract 70%, ethanol 96% and the ethyl acetate fraction of Moringa leaves were weighed as much as 100 mg and added 100 ml of each solvent concentration of ethanol (1000 ppm concentration), then taken 1,0 mL and added 1,0 ml of 10% AlCl_3 and acid 8 mL of 5% acetate, vortexed and allowed to stand for the optimum time and read the absorbance at the maximum wavelength and repeated 3 times.¹⁰

2.8. Research data analysis

The flavonoid content was calculated by measuring 70% ethanol extract, 96% ethanol and the ethyl acetate fraction of Moringa leaves (*Moringa oleifera* L.) and then calculated using the linear regression equation ($y = a + bx$).¹⁰ The statistical test using the LSD (Least Significance Different) test was used to test the average difference between the 3 treatment groups using SPSS 16th Edition software, so this test was used to determine the significant difference in total

flavonoid levels between samples of 70% ethanol extract, 96% ethanol and ethyl acetate fraction of Moringa leaf.¹⁰

3. Result

3.1. Moringa leaf simplicia microscopic identification

Microscopic examination of Moringa leaf powder aims to determine the characteristics of leaf identification fragments using a microscope. The chloral hydrate solution aims to remove cell content such as protein and starch, so that the cell identification fragments on the leaves can be clearly observed with a microscope.¹² Table 1 informs the microscopic results of Moringa leaf simplicia, showing that the presence of calcium oxalate crystals in the form of rosettes, mesophyll and stomata is in accordance with previous research and the Indonesian Herbal Pharmacopoeia Edition II (2017).¹³

3.2. Extract yield and ethyl acetate fraction

The yield of Moringa leaf ethanol thick extract (EEDK) and Moringa leaf ethyl acetate fraction (FEDK) is shown in Table 2. Based on the Indonesian Herbal Pharmacopoeia Edition II, the yield of Moringa leaf thick

Table 1. Moringa Leaf Simplicia Powder Fragment (*Moringa oleifera* L)

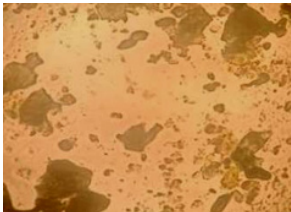
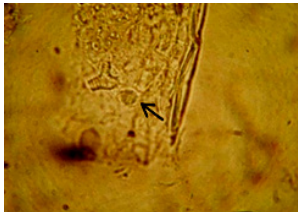
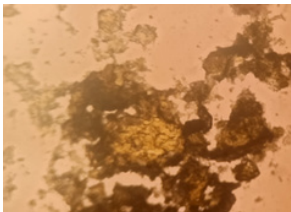
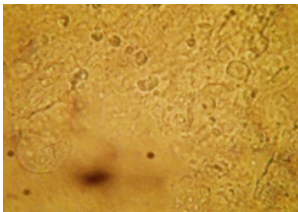
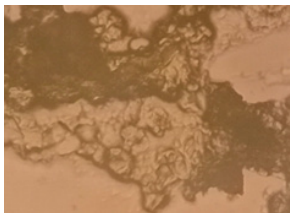
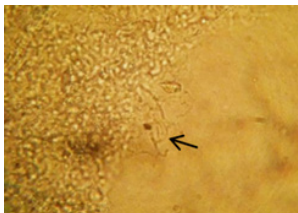
Microscopic Parameter	Observation	Indonesian Herbal Pharmacopoeia Reference ¹³
Rosette-shaped calcium oxalate crystals ¹³		
Mesofil ¹³		
Stomata ¹³		

Table 2. Calculation of Moringa Leaf Extract and Fraction Yield

Method	Thick Extract	Rendemen
Maceration	Moringa Leaf Ethanol Extract 70% (MLEE 70%)	16.09 %
Maceration	Moringa Leaf Ethanol Extract 96% (MLEE 96%)	23.80 %
Fractionation	Moringa Leaf Ethyl Acetate Fraction (EFML)	17.89 %

extract is not less than 9.2% so that all extracts obtained meet the requirements.¹³

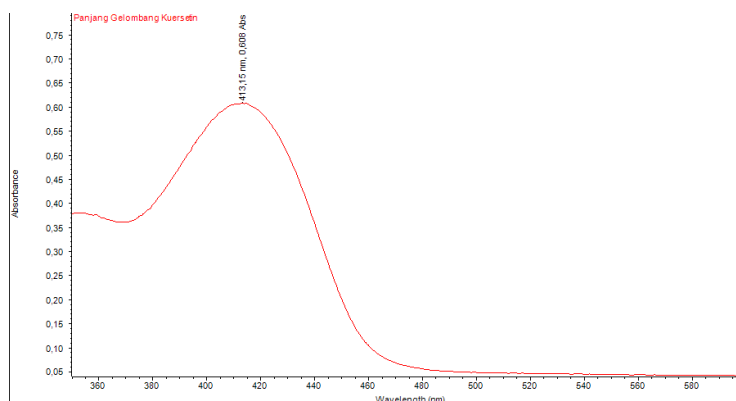
positive for flavonoids, alkaloids, saponins and tanins as shown in Table 3.⁹

3.3. Phytochemical screening moringa leaf extract and ethyl acetate fraction

After obtaining the extract, phytochemical screening was carried out to determine the class of secondary metabolite compounds in the extract. This method is a qualitative analysis of the chemical compounds contained in the Moringa leaf plant. The screening tests carried out were flavonoids, alkaloids, saponins and tanins because this test already represented several groups of compounds contained in the Moringa leaf plant. The results showed that the ethanol extract of Moringa leaves was

3.4. Determination of total flavonoid content of Moringa leaf extract and ethyl acetate fraction

Analysis of the total flavonoid content in this study was carried out using a standard solution of quercetin in Table 4. The readings were carried out at a wavelength of 413.15 nm with an operating time of 30 minutes according to previous studies.¹⁰ The determination of quercetin maximum wavelength shown in Figure 2. Determination of operating time in this study aims to determine the reaction time of flavonoid compounds with $AlCl_3$ can react perfectly so that it is optimum

**Figure 2.** Determination of Quercetin Wavelength in Determination of Total Flavonoid Levels**Table 3.** Phytochemical Screening Moringa Leaf Extract and Ethyl Acetate Fraction

Screening	MLEE 70 %	MLEE 96 %	EFML	Treatment	Results
Flavonoid	+	+	+	Ammonia Vapor	Intensive Yellow
Saponin	+	+	+	Shaking	Stable Foam 30 second
Tanin	+	+	+	FeCl ₃ 1%	Formed blackish green color
Alkaloid	+	+	+	Mayer	Sediment

when the absorption is read with Visible Spectrophotometry.¹⁰ Figure 3 showed the standard curved of quercetin.

The results of the total flavonoid content shown in Table 5 resulted in the highest flavonoid content value in 96% ethanol extract of Moringa leaves compared to 70% ethanol extract and ethanol fraction of Moringa leaves. The flavonoid content obtained for the ethanol extract of Moringa leaves is 70%, ethanolic extract is 96% and the ethanol fraction of Moringa leaves is 10.84%; 16.69% and 14.15%. This shows that the flavonoid components contained in MLEE 70%, MLEE 96% and the ethyl acetate fraction of the 70% ethanol extract of Moringa leaves (EFML) can be said to be quite high and have the potential to be developed and explored further related to biological activity, especially as an antioxidant.⁶

Moringa leaf plants have the characteristics of an oval shape with all parts of the same width, the upper part of the leaf is light green, the edges are split, the underside of the leaves is rounded, the leaf edges are flat, the leaf surface is rough and the leaf spines are pinnate. The cross-sectional sections of

the leaves consisted of the lower epidermis, bundle vessels, collenchyma, spongy tissue, upper epidermis and palisade.¹³ Maceration is an extraction method that is carried out by immersing simplicia powder using a solvent that is suitable for the immersion process, stirring to extract a certain substance. The solvents used in this study were 70% and 96% ethanol. Ethanol is a solvent that is polar, non-toxic and has the advantage of being able to extract more chemical compounds than methanol and water.¹⁴ The choice of solvent used is based on the nature of the compound to be taken (like dissolves like), meaning that if the compound to be taken is polar, the solvent used must be polar so that the active substance will be completely dissolved into the solvent.¹⁵

According to research by Siluh et al., (2015) previously stated that phenolic compounds, namely flavonoids, can be completely dissolved in ethanol solvent¹⁶. The stirring process in the maceration method aims to take a certain substance by breaking the walls and cell membranes due to the difference in pressure outside and inside the cell.¹⁷ The use of solvents with different

Table 4. Quercetin Standard Curve for Determination of Total Flavonoid Level

Quercetin standard (ppm)	Absorbance
20	0.173
40	0.281
60	0.401
80	0.534
100	0.655

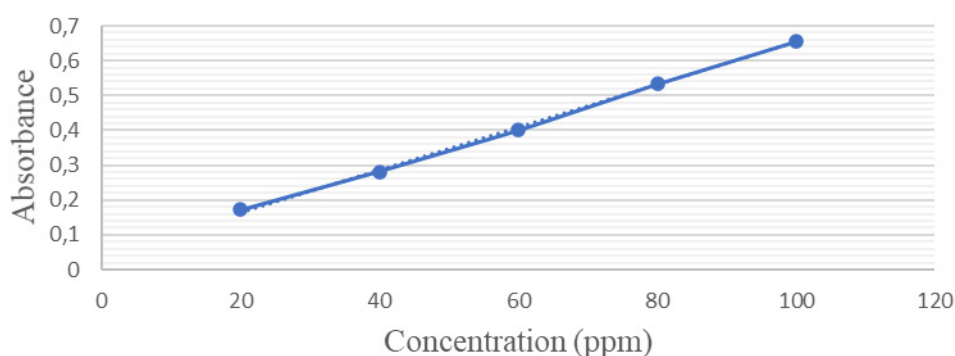


Figure 3. Curve of Quercetin Standard Linear Regression Equation on Determination of Total Flavonoid Level

Table 5. Results of Determination of Total Flavonoid Levels of MLEE and EFML

Sample	Replication	Flavonoid Total (% b/b)	X ± SD (% b/b)
MLEE 70%	1	10.60	10.84 ± 0.49
	2	10.84	
	3	11.09	
MLEE 96%	1	16.66	16.69 ± 0.74
	2	16.34	
	3	17.07	
EFML	1	13.63	14.15 ± 0.90
	2	14.45	
	3	14.37	

concentrations is carried out to determine differences in the acquisition of secondary metabolite content in the extract because the higher the concentration of a solution, the more active substances will be extracted so that the percent yield value of the extract will be greater.¹⁸ Concentration of the filtrate using a vacuum rotary evaporator for 1 hour at a temperature of 60°C at a speed of 100 rpm produces a thick extract which will be tested further. Calculation of the yield of a sample is related to the active compounds contained in the extract so that the greater the yield value, the more active compounds are taken.¹⁹

Figure 3, the curve shows that the higher the concentration of the solution, the higher the absorbance value obtained, with the linear regression equation $y = 0.0061 x + 0.0437$ and the value of $R = 0.9999$. The calculated R value $> R$ table = 0.8783 (degrees of freedom 3; $p < 0.05$), so the linear regression equation can be used to calculate the total flavonoid content in this study.²⁰ The limit of detection (LOD) value is 0.461 ppm and the limit of quantification (LOQ) value is 1.396 ppm. The limit of detection (LOD) states the smallest amount of analyte in the sample that can be detected that still gives a significant response compared to the blank. While the limit of quantization (LOQ) is the smallest amount of analyte in the sample that still meets the criteria carefully and thoroughly and can be quantified with good accuracy and precision.²¹

Determination of total flavonoid levels with standard compounds of quercetin added with $AlCl_3$, resulting in the complex

compounds shown in Figure 1. This test was carried out using UV-Vis spectrophotometry with the principle of complex compound formation reactions, in which there was a shift in the reading of visible light waves marked by the color of the solution. yellow and the addition of acetic acid to maintain the wavelength in the visible light region.¹⁰ The carbonyl hydroxy group contained in flavonoid compounds will react with $AlCl_3$ to form complex compounds.⁸ The hydroxyl group (-OH) in flavonoids, in particular almost always located on ring B at the 3' and 4' positions, can also react with $AlCl_3$ to form flavonoid-aluminum complexes. This research can be an informative basis that the flavonoid group in Moringa leaves plays a role in producing medicinal raw materials derived from natural ingredients with standardized levels of identity compounds.⁷

4. Conclusion

The highest total flavonoid content was found in the 96% ethanol extract of Moringa leaves ($16.69 \pm 0.74\%$ w/w) in accordance with the 2017 Indonesian Herbal Pharmacopoeia, that the thick extract of Moringa leaves contained no less than 6.30% (w/w) total flavonoids calculated as quercetin.

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Moringa Leaf and all participant that we can't mention one by one.

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