THE EFFECT OF SOLVENT TYPE ON FLAVONOID LEVELS OF MORINGA LEAF EXTRACT (*MORINGA OLEIFERA* L.)

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ABSTRACT

This study aims to determine the effect of the type of solvent on the levels of flavonoids in Moringa leaf extract (*Moringa oleifera* L.) and the differences in levels of flavonoids in each solvent. The method used in this study was a laboratory experimental method that included the extraction stage of Moringa leaves using three solvents, namely ethyl acetate, methanol, and 96% ethanol; the phytochemical screening test for the flavonoid compounds of Moringa leaf extract; and the flavonoid compounds test using UV-Vis Spectrophotometry. The results showed that there was an effect of the solvent type on the levels of flavonoid compounds in Moringa leaf extract (*Moringa oleifera* L.), and there were differences in the highest levels of flavonoids obtained in methanol extract of 2708.10 mg/L, 96% ethanol of 1890.24 mg/L, and ethyl acetate of 717.62 mg/L.

Keywords: Flavonoid Levels, Solvent Type, Moringa Leaf Extract, UV-Vis Spectrophotometry

INTRODUCTION

Moringa (*Moringa oleifera* L.) is a plant that grows a lot in Asia, including in Indonesia, such as the Gorontalo region. Moringa or commonly called *Mother's Friend* and the *Miracle Tree* because all parts of the Moringa plant have extraordinary benefits, from leaves, fruit, seeds, flowers, skin, to roots [18]. Moringa (*Moringa oliefera* L.) is a herbaceous plant that contains antioxidant compounds such as flavonoids, saponins, cytokinins, caffeoylquinic acid and contains unsaturated fatty acids such as linoleic (omega 6) and alphalinolenic (omega 3) [17]. In addition, Moringa has several useful ingredients that have the potential to be used in food, cosmetics and industry.

Moringa leaves are also efficacious for treating various complaints caused by vitamin and mineral deficiencies such as vitamin A deficiency (impaired vision), choline deficiency (fat accumulation in the liver), vitamin B1 deficiency (beri-beri), vitamin B2 deficiency (dry and cracked skin). -cracked), vitamin B3 deficiency (dermatitis), vitamin C deficiency (bleeding gums), calcium deficiency (osteoporosis), iron deficiency (anemia), protein deficiency (cracked hair and growth disorders in children). Through research, moringa turns out to contain many important nutrients such as vitamins, minerals, amino acids, beta carotene, antioxidants, nutrients, anti-inflammatory, and omega 3 and 6 fatty acids. [13].

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Research conducted by Yulia, *et al* (2022) states that the ethanol extract of Moringa oleifera L. leaves from the villages of Dolok Sinumbah and Raja Maligas contains secondary metabolites, namely flavonoids, tannins, saponins and steroids. Flavonoid levels of the ethanol extract of Moringa leaves (*Moringa oleifera* L.) between two different villages, namely Dolok Sinumbah Village of 94.1842 mgQE/gr and Raja Maligas 87.5157 mgQE/gr.

Moringa leaf extract contains secondary metabolites in the form of flavonoids, saponins, tannins, terpenoids, and alkaloids [3]. The ethanol extract of Moringa leaves contains flavonoids, alkaloids and saponins [9]. Phytochemical screening of the ethanol extract of Moringa leaves in Dolok Sinumbah Village and Raja Maligas Village showed positive results containing secondary metabolites, namely flavonoids, saponins, tannins, and steroids [22].

Moringa leaves are part of the Moringa plant which has been widely studied for its content and usefulness for health [18]. The content of flavonoids in Moringa leaves provides anti-inflammatory activity which functions to prevent stiffness and pain, as well as reduce pain when bleeding and wound swelling occur [15]. The bioactive compounds contained in Moringa cause this plant to have pharmacological activity, where the active compounds are found in the leaves most compared to the roots, flowers, fruits and seeds [10].

Moringa (*Moringa oleifera* L.) is a plant that contains lots of flavonoid compounds that act as antioxidants, antidiabetics and anticancer [12]. Moringa leaves contain various phytochemical compounds, including tannins, steroids, flavonoids, saponins, and alkaloids, all of which are antioxidants. Moringa leaves also contain high antioxidants in which some of the main phenolic bioactive compounds are a group of flavonoids [2]. Based on research conducted by Saputra (2020) showed that the 96% ethanol extract of Moringa leaves by maceration method obtained secondary metabolites, namely flavonoids (5.17%), alkaloids (3.07%), terpenoids (4.84%), steroids (3.21%).

based on research conducted by Rahmat (2019) the levels of flavonoids in Moringa leaves (499.07 mg) are known to be higher than other leaves such as chayote leaves (203.60 mg), papaya flowers (321.33 mg) and fern leaves (87.53 mg). The withdrawal of secondary metabolites is often influenced by the choice of solvent, so that it will affect the amount of secondary metabolites.

Based on research conducted by Hasanah, et al (2022) the extraction method is known to affect the total secondary metabolite compounds present in these plants. In addition, the selection of solvents can also give different results on the levels of compounds obtained. The selection of extract solvents and the selection of extraction methods need to be considered because they will affect the results of the extract of secondary metabolites. The extracting solution used is adjusted to the polarity of the desired compound. According to the principle of *like dissolves like*, a solvent will tend to dissolve compounds that have the same level of polarity. Polar solvents will dissolve polar compounds and vice versa.

Extraction is a method for separating or withdrawing one or more compounds from a sample using a suitable solvent. Extraction functions to extract all chemical components or secondary metabolites contained in the simplicia. The extraction process is often

used in the withdrawal of chemical compounds by researchers. In general, the process of withdrawal of compounds that are carried out is the withdrawal of secondary metabolites.

There are several factors that can affect extraction, including particle size, type of solvent, temperature, and extraction technique. The selection of the type of solvent is based on the active compounds contained in the material extract. Polar active compounds will dissolve in polar solvents, while non-polar compounds will dissolve in non-polar solvents, according to the concept of *like dissolves like*. Solvents have an important role in the process of extracting chemical compounds. The polarity of the solvent is very influential in extracting the target compound from the raw material.

The solubility of a compound in a solvent depends on the polarity of the compound, and the solvent. Materials from chemical compounds will easily dissolve in solvents that have the same polarity as the material to be dissolved [7]. This study used 96% ethanol, methanol and ethyl acetate because these solvents are easy to obtain, economical, environmentally friendly, and are often used in the pharmaceutical industry. Based on research by Jusnita et al. (2019) said that Moringa leaf extract only dissolves in polar solvents (water, methanol and ethanol) and semi-polar solvents (ethyl acetate) and is insoluble in hexane. Differences in extraction solvents can affect the total content of bioactive compounds. This is due to the difference in polarity of the solvent.

Based on the background above, the researchers were interested in examining how the effect of the type of solvent on the levels of moringa leaf flavonoids. The extraction process will use different types of solvents with different polarity levels, namely ethanol, methanol and ethyl acetate. This is because the choice of solvent can affect the levels of secondary metabolites. The results of this study are expected to provide information about the type of solvent extracted from Moringa leaves which will affect the levels of flavonoids as a raw material for medicine.

RESEARCH METHOD

The research design used was experimental in the laboratory by analyzing the levels of flavonoid compounds using several types of solvents in Moringa leaves. This research will use several extraction solvents, including 96% ethanol, ethyl acetate, methanol. In this study, the simplicia powder of Moringa leaves was extracted using the maceration method. The viscous extract produced by the maceration process using a variety of solvents is evaporated to obtain a viscous extract, then a screening for flavonoid compounds is carried out in each extract and the yield value is calculated. The levels of flavonoids will be determined using a UV-Vis spectrophotometer.

2.1 Tools and Materials

The tools used in this study were a set of glassware (pyrex) in the laboratory, a set of maceration tools, a blender, aluminum foil, an analytical balance, a shaker brand KS 4000, a stir bar, a volumetric flask, a stopwatch, a UV light lamp, a UV-Vis spectrophotometer brand AquaMate 8000, handscoon and tissues.

The materials used in this study were the simplicia of Moringa leaves (*Moringa oleifera* L), ethyl acetate, methanol and 96% ethanol, Mg powder and concentrated HCl,

curcetin, ethanol pa, AlCl3, distilled water, and sodium acetate.

2.2 Ways of Working

2.2.1 Sample Preparation

a. Sampling of Moringa Leaves (*Moringa oleifera* L.)

The sample used is the moringa plant, the part taken is fresh green leaves without any yellow spots. Sampling was carried out in the afternoon at 16.00 - 17.00 WITA by picking. Samples of Moringa leaves were obtained in Kwandang District, North Gorontalo Regency.

b. Processing of Simple Moringa Leaves (*Moringa oleifera* L.)

The samples that have been collected are then cleaned of dirt, washed with running water. Wet sorting was carried out with the aim of removing dirt adhering to Moringa leaves. Furthermore, the sample is chopped for the purpose of chopping a sample, namely to facilitate drying. Then the sample is dried in the wind and covered with a black cloth and not exposed to direct sunlight, the purpose of drying is to reduce the water content in the sample. After the samples were dry, dry sorting was carried out in order to sort out samples that were not suitable for use or samples that were damaged. After the simplicia became dry, the sample was pulverized using a blender and sifted so that the simplicia powder to be used was really smooth.

c. Extraction of Moringa Leaves (*Moringa oleifera* L.)

The simplicia powder was extracted using maceration method with 3 types of solvents namely ethyl acetate, methanol and 96% ethanol with a ratio of 1: 10. The simplicia powder of Moringa leaves (Moringa oleifera L.) was weighed as much as 200 grams then put into each maceration jar and added solvent until all samples are completely submerged and tightly closed. Left for 24 hours protected from light, while occasionally stirring. It is filtered and the dregs and filtrate are separated, then the dregs are macerated again using a new solvent [6]. The liquid extract obtained was collected and evaporated using a modified shaker to obtain a thick extract. Furthermore, the extract was weighed and the yield was calculated using the following formula.

$$\%\text{yield} = \frac{total\ weight\ of\ the\ condensed\ extract\ (g)}{total\ dry\ weight\ (g)} \text{x} 10\ \%$$

d. Flavonoid Screening of Moringa Leaf Extract (*Moringa oleifera* L.)

The thick extract of Moringa leaves was taken as much as 2 ml added with 0.1 gram Mg powder and 5 drops of concentrated HCl. If the sample contains flavonoids, it will change color from orange to red [9].

2.2.2 Testing Flavonoid Compound Levels Using UV-Vis Spectrophotometry

a. Preparation of blank solutions

Take 10 mL of ethanol into a test tube and enter 3 mL of ethanol into the cuvette and insert the cuvette into the UV-Vis Spectrophotometer. Making a blank solution aims to calibrate the device so that the concentration starts from zero.

b. Preparation of Standard Mother Solution

Quercetin as much as 25 mg was weighed and dissolved in 25 mL of ethanol (pa) as a 1000 ppm stock solution. A standard standard solution of quercetin was diluted with a concentration of 100 ppm as much as 25 mL [21].

c. Preparation of quercetin standard series solutions

Preparation of standard solution by pipetting mother liquor as much as 0.25; 0.5; 0.75; 1 and 1.25 mL each into a 25 mL volumetric flask using a micropipette. The volume was added with ethanol up to the mark, so that solutions with concentrations of 10, 20, 30, 40, 50 ppm were obtained.

d. Determination of the Maximum Wavelength of Quercetin

Take 4 mL of the standard standard quercetin solution, then add 0.1 mL of 10% AlCl3, 0.1 mL of 1 M sodium acetate, 2.8 mL of aquadest, and ethanol to 10 mL. Absorbance was measured with a UV-Vis spectrophotometer at a wavelength of 400-500 nm.

e. Creation of calibration curves

A series of 10 mL standard solution levels was made with standard concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm. Pipette 1 mL of each series of levels into a 10 mL measuring flask, add 0.1 mL of 10% AlCl3, 0.1 mL of 1 M sodium acetate, 2.8 mL of aquadest, and 96% ethanol up to the mark [21]. The solution was incubated at room temperature for 30 minutes and then measured the absorbance produced by the concentration at the maximum wavelength obtained and made a curve of the relationship between the standard concentration and the absorbance.

f. Preparation of Extract Standard Solution

Determination of the concentration was carried out by making a solution of Moringa leaf extract weighing 0.5 g of each extract, then dissolving it with a solvent, each of which was labeled Moringa leaf ethanol extract, Moringa leaf methanol extract and Moringa leaf ethyl acetate extract. The solution was stirred using a stir bar, after which it was put into a 50 mL volumetric flask. The beaker is rinsed with solvent and then put into the volumetric flask up to the mark.

g. Determination of total flavonoid compounds

After obtaining the sample solution, take 1 mL of the sample solution and then put it into a 10 mL volumetric flask, add 0.1 mL of 10% AlCl3, 0.1 mL of 1 M sodium acetate, 2.8 mL of aquadest, and 96% ethanol up to the mark, Replication was carried out 3 times [21]. Flavonoid levels were determined based on absorbance interpolation into the standard linear regression equation of quercetin to obtain sample concentrations.

2.2.3 Data Analysis

The data obtained are primary data obtained from the absorbance of the quercetin reference solution, a calibration curve is made and a linear regression equation is obtained. The total compound content was calculated by entering into the linear regression equation y= ax+b, which was obtained from the comparison calibration curve and the results were expressed in units of mg/L. Data analysis was performed using the SPSS (*Statistical Package For Social Science*) statistical test, namely *One Way Anova*, then further testing using *Duncan's test*.

RESEARCH RESULTS

3.1 Descriptive Research Results

a. Yield of Moringa Leaf Extract

The extraction process of *Moringa oleifera* L. leaf extract was carried out using the maceration method. The results of maceration are then calculated yield values to determine the percentage yield of the extract. The yield results can be seen in Table 1.

Table 1. Yield of Moringa leaf extract

Solvent type treatment	**Simplicity heavy**	**Thick extract weight**	**yield**
Ethyl Acetate	200 gram	24 gram	12%
methanol	200 gram	38 gram	19%
Ethanol 96%	200 gram	35 gram	17,5%

Source: Processed Data, 2023

b. The results of the screening of flavonoids in the viscous extract of Moringa leaves

The obtained moringa leaf extract was subjected to a phytochemical screening test to determine the presence of a class of flavonoid compounds shown in Table 2.

Table 2. The results of the flavonoid screening of the thick extract of Moringa leaves (*Moringa oleifera* L.)

Solvent type	compound	Reactor	Results	The color change that occurs
Ethyl Acetate	Flavonoid	Mg and concentrated HCL	+	Reddish orange color
methanol	Flavonoid	Mg and concentrated HCL	+	Reddish orange color
Ethanol 96%	Flavonoid	Mg and concentrated HCL	+	Rosewood

Source: Processed Data, 2023

c. Flavonoid Compound Level Test Results Using UV-Vis Spectrophotometry

After screening for flavonoids, a test for the levels of flavonoid compounds was carried out. Preparation of quercetin standard solutions with concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm, the concentration and absorbance results of the kurcetin standard solutions can be seen in Table 3.

Table 3. Concentration and Absorbance Results of the standard solution of curcetin

Concentration (ppm)	absorbance	Linear regression value
10	0,125	y = 0,021x + 0,094 R^2 = 0,999
20	0,322	
30	0,519	
40	0,746	
50	0,962	

Source: Processed Data, 2023

After obtaining the concentration and absorbance results from the curcetin standard solution, a curve is made of the relationship between the standard concentration and the absorbance. The standard curve linearity graph can be seen in Figure 1.

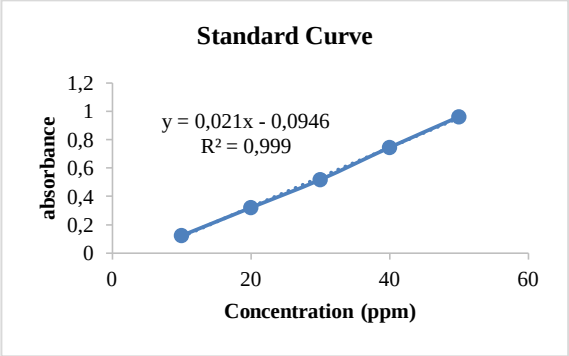

Figure 1. Standard curve linearity

Observation of the results of flavonoid levels in Moringa leaf extract was carried out by UV-Vis spectrophotometry. The results obtained can be seen in Table 4.

Table 4. Flavonoid levels in Moringa leaf extract

Moringa Leaf Extract Solvent Type	Abs	Total Content	Average	Unit
Ethyl Acetate	0,108	723,57	717,62	mg/L
	0,104	709,29		
	0,107	720,00		
Methanol	0,662	2702,14	2708,10	mg/L
	0,664	2709,29		
	0,665	2712,86		
Ethanol	0,433	1884,29	1890,24	mg/L
	0,436	1895,00		
	0,435	1891,4		

3

Source: Processed Data, 2023

3.2 Results of Analysis of the Effect of Solvent Type on Flavonoid Levels of Moringa Leaf Extract (*Moringa oleifera* L.)

Based on parametric statistical tests using One Way Anova with a level of 5% or 0.05, it was found that hypothesis 0 was rejected, indicating that Ho was rejected, meaning that there was a significant influence of the type of solvent on the levels of flavonoids in Moringa leaf extract. Then further tests were carried out using Duncan's analysis test. The results obtained from the One Way Anova test showed that the significance value was p <0.05 (0.000 <0.05) so that it can be concluded that there was an effect of the type of solvent on the levels of flavonoids in Moringa leaf extract (*Moringa oleifera* L.), which means that the conclusion from the hypothesis is that H0 is rejected and H1 is accepted. Furthermore, the Duncan test was carried out, the results of Duncan's Post Hoc test showed that the levels of ethyl acetate extract were significantly different from the levels of ethanol extract and levels of methanol extract. The levels of the ethanol extract were significantly different from the levels of the ethyl acetate extract and the levels of the methanol extract. The levels of the methanol extract were significantly different from the levels of the ethyl acetate extract and the levels of the ethanol extract. The results of Duncan's analysis test can be seen in Table 5.

Tabel 5. Duncan Test Results Effect of Solvent Type on Moringa Leaf Extract (*Moringa oleifera* L.)

flavonoid levels				
Duncan[a]				
solvent type	N	1	2	3
ethyl acetate extract	3	71762.00		
ethanol extract	3		189024.00	
methanol extract	3			270809.67
Sig.		1.000	1.000	1.000

Source: Processed Data, 2023

DISCUSSION

4.1 Yield of Moringa leaf extract

The extract yield results in Table 1. show the percentage yield of 12% ethyl acetate viscous extract, 19% methanol and 17.5% ethanol. These results are in accordance with the requirements of the Indonesian Herbal Pharmacopoeia, namely the yield of Moringa leaf extract is not less than 9.2% [4]. Yield is a comparison between the results of the number of metabolites obtained after the extraction process with the sample weight which is said to be good if the value is more than 10%. The size of the yield obtained is influenced by the effectiveness of the extraction process and the type of solvent used. The high yield of Moringa leaf extract with methanol solvent indicates that the methanol solvent in Moringa leaves is able to extract compounds better, because compound recovery is based on the similarity of polarity to the solvent. Based on research by Jusnita et al. (2019) stated that Moringa leaf extract only dissolves in polar solvents (water, methanol and ethanol) and semi-polar solvents (ethyl acetate) and is insoluble in hexane. Dian Kartikasari (2021) also stated the difference in polarity between ethanol and methanol, it is known that methanol is more polar than ethanol.

4.2 Screening of moringa leaf extract flavonoid compounds

Based on Table 2. the results of the phytochemical screening of the ethyl acetate, methanol and ethanol extracts of Moringa leaves showed the presence of a class of flavonoid compounds. The color change that is formed indicates an interaction between Moringa oleifera L. leaf extract and magnesium powder and concentrated HCl, where the reagent will reduce the benzopirone nucleus contained in the flavonoid structure to form red or orange flavilium salts. These results are in line with the research of Larasati T, et al (2021) ethanol, methanol, ethyl acetate, n-hexane, and aquadest extracts could detect flavonoids, alkaloids, and tannins. In the study by Tutik et al (2018) also mentioned that the phytochemical screening test of the ethanol and ethyl acetate extracts of Moringa leaves positively contained flavonoid compounds.

4.3 Flavonoid Compound Level Test Using UV-Vis Spectrophotometry

Based on Table 3 and Figure 1, the preparation of a quercetin standard curve aims to determine the relationship between the concentration of the solution and the absorbance value. The standard curve obtained has a linear line equation, namely y

= 0.021x - 0.0946, with a correlation coefficient $R^2 = 0.999$. This equation is used to calculate the levels of flavonoids in the sample. Where (y) represents the absorbance and (x) represents the level of flavonoids in the sample. From this curve it can be concluded that the higher the concentration the higher the absorbance.

The absorbance measurement of moringa leaf extract samples was carried out at a wavelength of 418 nm. Flavonoid levels were determined by matching the absorbance to the standard solution concentration curve. Determination of the flavonoid levels of Moringa leaf extract (Moringa oleifera L.) was carried out with 3 repetitions to obtain data accuracy and obtain the results contained in Table 4. Based on these data the results obtained that the highest total flavonoid levels were in Moringa leaf extract (Moringa oleifera L.) obtained from the influence of the type of solvent, namely methanol solvent which is equal to 2708.10 mg/L compared to ethanol solvent 1890.24 mg/L and ethyl acetate 717.62 mg/L. based on Susanty's research (2019) stated that the results of the ethanol extract of Moringa leaves had a flavonoid content of 2457.71 mg/L. methanol solvent obtained higher levels compared to 96% ethanol and ethyl acetate solvents.

High levels of total flavonoid compounds in Moringa leaf extract were obtained using methanol as a solvent because methanol is a polar solvent. Flavonoid compounds are polar so that a polar solvent is needed to extract them. This result is in line with the research of Jusnita et al. (2019) that Moringa leaf extract only dissolves in polar solvents (water, methanol and ethanol) and semi-polar solvents (ethyl acetate) and is insoluble in hexane. Restiani et al, (2019) also said that flavonoids are compounds of the polyphenol group which are widely distributed in plants in the form of glycosides which bind to a sugar. Therefore, flavonoids are polar compounds. Najib A. (2018) states that polar compounds will only dissolve in polar solvents, nonpolar compounds will also only dissolve in nonpolar solvents. Dian Kartikasari (2021) also stated the difference in polarity between ethanol and methanol, it is known that methanol is more polar than ethanol. The more polar the solvent, the more phenolic and flavonoid compounds it can attract in accordance with the principle of like dissolves like. The results of this study indicate that the type of solvent treatment has a significant effect on the levels of flavonoids in Moringa leaf extract, this is because the ability and nature of the solvent to dissolve flavonoid compounds varies depending on the polarity level of the solvent and the compounds extracted.

1. CONCLUSION

Based on the results of research and data processing that has been carried out by researchers, it can be concluded that:

1. There is an effect of the type of solvent on the levels of flavonoid compounds in Moringa leaf extract (*Moringa oleifera* L.).
2. There were differences in the levels of flavonoids in each solvent of Moringa leaf extract (Moringa oleifera L.) The results showed that the flavonoid content with methanol solvent obtained the highest yield of 2708.10 mg/L, ethanol 1890.24 mg/L, and ethyl acetate 717. 62mg/L.

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