Effect of Different Solvents in the Extraction Process of Kelor (Moringa oleifera) Leaves on Bioactive Resources and Phenolic Acid Content

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Keywords : Moringa Leaf, Ethanol, Ethyl Acetate, Phenolic, Maceration

Abstract : Moringa (Moringa oleifera) plants are included in the category of medicinal plants because they contain various bioactive compounds that have the potential to prevent disease. Bioactive compounds can be extracted by maceration method using an appropriate solvent. The determination of the solvent is very influential on the extraction process because it is based on the similarity of the polarity value with the compound being dissolved. This study aims to determine the effect of different solvents on bioactive compounds and phenolic content in moringa leaf extract. The methods used were maceration with 96% ethanol and ethyl acetate solvents, phytochemical screening to determine the class of compounds, UV-Vis spectrophotometry to determine phenolic content, and FTIR to identify compounds. The results of phytochemical screening showed that ethanol extracts of moringa leaves are known to contain flavonoids, saponins, phenolic compounds, and triterpenoids. The results of the phenolic content test showed that the total phenolic content of the ethanol extract of moringa leaves was 34 mg GAE/g, while in the ethyl acetate extract of moringa leaves it was 2.216 mg GAE/g. The results of compound identification with FTIR showed that both moringa leaf extracts contained C-H (2850-2970 cm-1) and C=O (1690-1760 cm-1) functional groups. In addition, the ethanol extract also contained O-H functional group (3000-3500 cm-1).

INTRODUCTION

Moringa plants (Moringa oleifera) are included in the category of medicinal plants because they contain bioactive compounds that have the potential to prevent disease (Ikalinus et al., 2015). Several studies have shown that the compound in moringa have medicinal potential and bioactivity, including having activity as an antioxidant (Benabdesselam et al., 2007), anti-inflammatory (Sashidhara et al., 2009), anticancer (Jayavardhanan et al., 1994), and antifungal (Chuang et al., 2007). In the separation of bioactive compounds, it is important to choose the appropriate extraction method because the separation process will determine the amount of yield produced (Kiswandono, 2011). Extraction or separation of compounds is a process in an effort to withdraw chemical compounds from a plant, where these compounds will be dissolved in the appropriate solvent (Dewatisari, 2020).

One of the commonly used extraction methods is the maceration method. The reason this method is often used is because the treatment is simpler because it does not require expensive equipment, and can avoid damage to thermolabile compounds (Mukhriani, 2014). Maceration is an extraction method by immersing simplisia powder in a filter liquid without heating, otherwise known as cold extraction. Compounds in simplisia are

separated using certain types of solvents (Dewatisari, 2020). Different types of solvents have different efficiencies in the extraction process of certain compounds. Therefore, proper solvent selection is crucial to ensure successful extraction of the desired bioactive compounds (El achkar et al., 2019).

Previous research by Firdiyani et al (2015) has proven that different solvents can significantly affect the content of bioactive compounds extracted from Spirulina platensis. The use of different solvents also affects the solubility of certain compounds and the type of bioactive compounds that are successfully extracted. In addition, research conducted by Agustina (2017) showed that different solvents have an impact on the extraction of bioactive compounds from Tin (Ficus carica linn) leaves that have antioxidant activity. Solvent characteristics have an influence on the solubility of certain compounds and the type of bioactive compounds extracted from plants. Another study conducted by Verdiana et al (2018) showed that solvent selection has a significant impact on the results of lemon peel extraction using ultrasonic waves. The type of solvent used affects the extract yield, vitamin C content, total flavonoids, as well as the antioxidant activity of the extracted lemon peel extract.

Determination of the appropriate solvent is very influential in the extraction process because it is based on the similarity of its polarity value with the compound being dissolved (Leksono et al., 2018). Compounds with polar properties will dissolve in polar solvents, while semi-polar compounds will dissolve in semi-polar solvents, and nonpolar compounds will dissolve in nonpolar solvents. The success of the extraction process is influenced by the type and quality of the solvent used (Sayuti, 2017). Polar solvents are able to extract quaternary alkaloid compounds, phenolic components, carotenoids, tannins, sugars, amino acids and glycosides. While semipolar solvents are able to extract phenol compounds, terpenoids, alkaloids, aglycones and glycosides. While nonpolar solvents can extract chemical compounds such as waxes, lipids and volatile oils (Harborne, 1987). The use of the right solvent can improve the extraction of bioactive compounds and the desired phenolic content, so it is important to consider the type of solvent that is suitable for the compound to be extracted.

The purpose of this study was to determine the effect of different solvents on bioactive compounds and phenolic content in moringa leaf extract.

METHOD

Time and Location of Research

This research was conducted at the Laboratory of Basic Chemistry, Basic Biology, and Instrumentation Campus II Sunan Ampel State Islamic University Surabaya from June to July 2023.

Tools and Materials

The tools used in this research include beaker glass, stirrer, blender, funnel, erlenmeyer, filter paper, rotary evaporator, petri dish, FTIR instrument, measuring flask 100 and 10 ml, measuring pipette, bulb, vortex, cuvette, test tube, spatula, vial bottle, and UV-Vis spectrophotometer. The materials used in this study were Moringa oleifera leaves, ethyl acetate, gallic acid, ethanol 96%, Folin-ciocalteu 0.5 ml, Na2CO3 7%, concentrated HCl 12 M, dragendorf reagent, FeCl3 5%, magnesium, chloroform, and acetic acid.

Extraction

Moringa leaves were cut into small pieces, then finely blended into powder, then the powder was weighed (initial mass). Smooth natural materials were soaked with ethyl acetate solvent until all were submerged at room temperature (25-28°C). This immersion was carried out for 48 hours accompanied by stirring. The extraction results were filtered with filter paper. The filtrate is then evaporated using a rotary evaporator with a certain temperature and pressure to remove the solvent so that the extract of natural ingredients is obtained (Agustina, 2017).

Then a phytochemical screening test was carried out to determine the class of compounds, identification of compounds using FTIR instruments, and phenolic content tests using UV-Vis spectrophotometer instruments.

Phytochemical Screening Test

1. Alkaloid Test

A total of 0.5 grams of sample was put in a test tube and 0.5 mL of HCL 2 M was added, after which 1-2 drops of dragendorf were added. A positive test for alkaloids is indicated by the formation of an orange color (Baud et al., 2014).

2. Flavonoid Test

A total of 200 mg of sample was put into a test tube. Then added 5 mL of ethanol and heated for 5 minutes. After that, a few drops of concentrated HCl were added. Added magnesium as much as 0.2 grams. A positive test for flavonoids is indicated by the formation of a dark red (magenta) color (Sambode et al., 2022).

3. Saponin Test

A total of 0.5 grams of sample was put into a test tube. Then hot water is added and cooled. After the cold is shaken for 10 minutes. A positive test for saponins is indicated by the formation of foam and added HCl 2 M foam remains (Andi et al., 2022).

4. Phenolic Test

A total of 0.5 grams of sample was put into Erlenmeyer and added with 10 mL of ethanol. Taken 1 mL of the solution formed and put into a test tube. After that, 2 drops of 5% FeCl3 solution were added. A positive phenolic test is indicated by the formation of a green or bluish green color (Kartika et al., 2023).

5. Steroid and Triterpenoid Test

A total of 0.5 grams of sample was put into a test tube and added chloroform as much as 0.5 mL and acetic acid as much as 0.5 mL. After that, concentrated H2SO4 was added as much as 2 mL through the test tube wall. A positive test containing triterpenoids is indicated by the formation of a purple-red color. While a positive test for steroids is indicated by the formation of green or blue color (El Kariem and Maesaroh, 2022).

Qualitative test using FTIR instrument.

The FTIR tester was turned on and connected to the software used to analyze. The moringa leaf extract sample is placed into the sample holder. Operate the FTIR instrument so that the FTIR spectrum of the moringa leaf extract sample is produced, then read the FTIR spectra results (Fitriyanti et al., 2018).

Quantitative Test using UV-Vis Spectrophotometer

1. Preparation of Gallic Acid Standard Master Solution

Gallic acid 100 ppm mother solution, gallic acid powder is weighed with an analytical balance as much as 10 mg and then put in a measuring flask (Mukhriani et al., 2019). Then dissolved with ethanol up to 100 ml, after which it is homogenized by shaking.

2. Preparation of Concentration Variations of Gallic Acid Standard Solution

Gallic acid standard solution was made with concentration variations of 10, 20, 30, 40, 50 ppm (Mukhriani et al., 2019).

3. Preparation of 7% Na_2CO_3 Solution

Na2CO3 powder was weighed as much as 7 grams and then dissolved with distilled water to 100 ml (Puspitasari et al., 2019).

4. Determination of Maximum Wavelength

Determination of the maximum wavelength of gallic acid was carried out by measuring gallic acid solution with a concentration of 10 ppm at a wavelength range of 400-800 nm using a UV-Vis spectrophotometer.

5. Gallic Acid Standard Solution Curve

Gallic acid standard solution was made concentration variations of 10, 20, 30, 40, 50 ppm. Gallic acid standard solution of each concentration was taken 1 ml to be put into a test

tube, then added 0.5 ml Folin-ciocalteu and let stand for 8 minutes while shaking, then added with 7% Na2CO3 as much as 4 ml and vortexed for 1 minute (Khadijah et al., 2017). The solution in the test tube was transferred to a centrifuge tube and then centrifuged at 500 rpm for 3 minutes. Measurements were taken at a wavelength of 749 nm.

6. Sample Absorption Measurement

Measurement of sample absorption can use the Folin-ciocalteu method with modifications (Brighente et al., 2007; Saptari et al., 2019). In this study, moringa leaf extract was weighed with an analytical balance as much as 10 mg then the extract was put in a volumetric flask and dissolved with ethanol until the volume of the solution was 10 ml, then homogenized. The extract solution was taken as much as 1 ml to be put into a test tube and added 0.5 ml Folin-ciocalteu then allowed to stand for 4 minutes while being homogenized, the extract was added to 7% Na2CO3 solution as much as 4 ml and vortexed for 1 minute. The solution in the test tube was then transferred to a centrifuge tube and then centrifuged at 500 rpm for 3 minutes, after which the supernatant was taken and transferred to a test tube. The blank solution and extract solution were included in the cuvette and then tested using a UV-Vis spectrophotometer instrument.

7. Total Phenolic Content Test

Based on the research of Candra et al (2021) the total phenol content can be calculated using the following formula:

$$\text{TPC} = \frac{C.V.fp}{g} \qquad (1)$$

Description:

c = phenolic concentration (x value)
v = volume of extract used (ml)
fp = dilution factor
g = weight of the sample used

RESULTS

Qualitative Test of Phytochemical Screening

Phytochemical screening was carried out to provide an overview of the class of compounds contained in ethanol and ethyl acetate extracts of moringa leaves. The components contained in ethanol and ethyl acetate extracts of moringa leaves were analyzed for compounds with color tests using several reagents. The results of phytochemical screening analysis of ethanol and ethyl acetate extracts of moringa leaves can be seen in Table 1. Based on the phytochemical screening test conducted, the ethanol extract of moringa leaves contains alkaloid, flavonoid, phenolic, saponin, and triterpenoid compounds, while the ethyl acetate extract contains phenolic and steroid compounds.

Table 1: Phytochemical Screening Results of Moringa Leaf Extracts

No.	Phytochemical Test	Change	Results	
			Ethanol Extract	Ethyl Acetate Extract
1.	Alkaloids	Orange colour indicates positive alkaloids	+	-
2.	Flavonoids	Dark red (magenta)	+	-
3.	Saponins	Formed froth or stable foam	+	-
4.	Phenolic	Green or bluish green colour	+	+
5.	Steroids	Green or blue colour	-	+
6.	Triterpenoids	Purple-red colour	+	-

Qualitative Test using FTIR

The results of Moringa leaf extract with different solvents were then analyzed by FTIR based on the intensity of infrared light absorbed by some of the extracted compounds. Identification of moringa leaf extract compounds using FTIR aims to determine the functional groups present in the sample. The FTIR spectra of the extracted compounds will show typical absorptions for several functional groups. The qualitative test results of Moringa leaf extract with ethanol and ethyl acetate using FTIR are as follows:

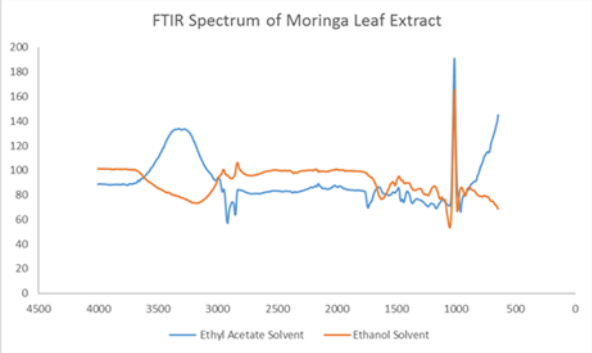

Figure 1: FTIR Results of Moringa Leaf Extract

Tabel 2: FTIR Results of Moringa Leaf Extract

Moringa Leaf Ethanol Extract		Moringa Leaf Ethyl Acetate Extract	
Wavenumber (cm^{-1})	**Functional groups**	**Wavenumber (cm^{-1})**	**Functional groups**
3000-3500	OH	2850-2970	CH
2850-2970	CH	1690-1760	C=O
1690-1760	C=O	675-995	C=CH
1000-1260	C=C		

Quantitative Test using a UV-Vis Spectrophotometer

The measurement for the determination of total phenolic content was carried out at a wavelength of 749 nm with an absorbance value of 0.362. The measurement result of determining the maximum wavelength can be seen in the following figure:

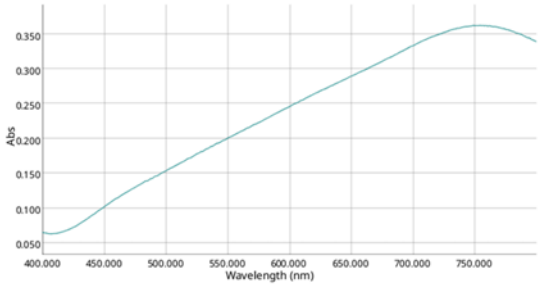

Figure 2: Calibration curve for 10 ppm gallic acid standard solution

The gallic acid standard curve obtained for measuring the total phenolic content is y=0.037x-0.009 with a correlation coefficient (R^2) of 0.999.

The measurement result of galic acid standard solution can be seen in the following figure:

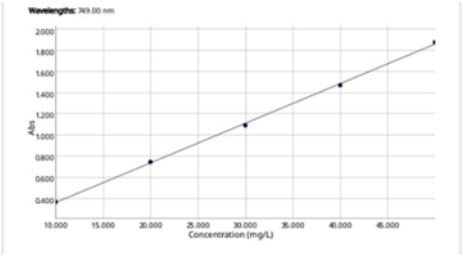

Figure 3. Gallic Acid Standard Standard Curve

DISCUSSION

Extraction is the process of separating mixed materials using a solvent that is suitable for the content in the plant. Several things can affect the efficiency of extraction, namely the plant material used, the choice of solvent, and the method used. The plant material used can be in the form of whole plant parts or those that have gone through the drying process. The selection of methods and solvents used must be appropriate to obtain maximum results (Sarker et al., 2006). Extraction is based on the principle of mass transfer of solute components into the appropriate solvent based on the nature of like dissolves like, where the transfer begins to occur in the layer between the solvent and the solute, namely the solute will diffuse into the solvent.

Generally, active substances in plants are easily soluble in organic solvents (Herbone, 1987).

Moringa leaves were extracted by maceration method using ethanol and ethyl acetate solvents after going through the stages of sorting, washing, drying, and pulverizing. The sorting and washing process aims to separate dirt or other foreign materials. Before drying, clean Moringa leaves are cut into small pieces to facilitate the process of evaporation of water content during the drying process. The drying process aims to remove the water content in the sample, so that H2O molecules will not hinder the distribution of active compounds during maceration, besides that drying is done so that the sample is not easily damaged by mold growth (Agustina, 2017).

The dried Moringa leaves were pulverized to make them into powder. This process aims to increase the surface area of the sample, so that the distribution of compounds in the solvent during maceration runs optimally. Moringa leaf powder is then macerated with the appropriate solvent. In the soaking process, the sample will experience the breakdown of cell walls and membranes due to the pressure difference between inside and outside the cell, so that secondary metabolites present in the cytoplasm will dissolve in organic solvents (Noer, 2016). The maceration process was carried out by soaking moringa leaf powder with ethanol and ethyl acetate solvents for 2x24 hours with occasional stirring.

Ethanol was chosen as the solvent because it has a dielectric constant of 24 (Rafsanjani et al., 2015), the dielectric constant of ethanol is higher than ethyl acetate which has a dielectric constant of 6 (Septian and Asnani, 2012). The dielectric constant shows the degree of polarity, the greater the dielectric constant, the greater the solubility of the solvent, so it can be said that the polarity of the ethanol solvent is higher than the ethyl acetate solvent. The higher solubility of ethanol solvent is useful for dissolving all substances, both polar and semipolar (Agustina, 2017). While the selection of ethyl acetate solvents due to the nature of ethyl acetate has volatile properties that help avoid damage to heat-sensitive compounds during the maceration process (Juwita, 2013). After soaking with ethanol and ethyl acetate for 2x24 hours the sample was then filtered and the filtrate was taken. The filtrate that has been produced is then thickened with a rotary evaporator to evaporate the solvent so that a thick extract of moringa leaves is obtained.

Qualitative Test of Phytochemical Screening

The results of the phytochemical test on the ethanol extract of moringa leaves showed that there were alkaloid compounds indicated by the dragendorf reagent forming an orange red precipitate. The orange red precipitate is the reaction of the dragendorf reagent to form potassium alkaloid (Sangkal, 2021), while the ethyl acetate extract of moringa leaves does not contain alkaloid compounds. This difference is because the ethanol solvent has the same polarity as the alkaloid compound, which is polar, so that in the ethyl acetate solvent the alkaloid compound is negative because the ethyl acetate solvent has an inversely proportional polarity to the alkaloid compound which is polar (Alzanando et al., 2022).

Alkaloids are a class of compounds that are found in nature. Alkaloid compounds are found in leaves that have a bitter taste (Putra et al., 2016). Almost all alkaloid compounds found in nature have certain biological activities, alkaloids can be very toxic and can be useful in medicine such as morphine, stiknin and quinine are alkaloids that have physiological and psychological effects (Batubara et al., 2017). Positive results of flavonoid phytochemical tests were shown in the ethanol extract of moringa leaves, flavonoid testing using concentrated HCL and magnesium powder which will change the color of the sample to a dark red (magenta) color in the amyl alcohol layer. Magnesium is added to form a bond between carbonyl groups in flavonoid compounds, the pair added HCl aims to form flavilium salts that will turn red (Andriyato et al., 2016). Moringa leaf ethyl acetate extract showed a negative reaction. The difference in flavonoid test results is because the ethyl acetate solvent is a semi-polar type solvent

that has a methoxy group so that hydrogen is formed (Romadanu et al., 2014).

Ethyl acetate solvents that have semi-polar properties are not effective in attracting flavonoid compounds that have polar properties and more effective ethanol solvents that have the same polarity properties as flavonoid compounds, namely polar (Putri and Lubis, 2020). Flavonoids are one group of secondary metabolites and are one of the largest classes of phenol compounds produced naturally by plants (Meilanty et al., 2014). Flavonoids are polyphenolic compounds so they are mildly acidic and can dissolve in bases and because polyhydroxy compounds are also polar. So it can dissolve in polar solvents such as methanol, ethanol, acetone, water, butanol, dimethyl sulfoxide, and dimethyl formamide. The presence of glycoside groups bound to flavonoid groups tends to cause flavonoids to dissolve in ethanol. Flavonoids are also solvents that dissolve compounds ranging from less polar to polar (Sari et al., 2016).

Phytochemical test of saponin content in ethyl acetate extract of moringa leaves showed negative results because it did not form foam, while the ethanol extract of moringa leaves showed positive results with the formation of foam. This foam or foam indicates the presence of glycosides that have the ability to form foam in water which is hydrolyzed into glucose and other compounds (Marliana et al, 2005). The addition of HCl to the saponin test causes an increase in the polarity of the saponin compound so that the preparation group changes its location, the situation is that the non-polar group faces inward to form a structure called micelle and the polar group will face outward so that foam is formed which will be a sign that the extract contains saponin compounds (Lubis and Putri, 2020). The difference in saponin test results between ethanol and ethyl acetate solvents is because ethanol solvents are more polar solvents than ethyl acetate solvents, so ethanol solvents are more able to attract saponin compounds in Moringa oleifera leaves because they have the same polarity (Sunnah et al., 2021). Saponins contained in a natural material can be recognized by the presence of bitterness and form a stable foam in liquid solution. Saponins are glycosides of steroids, alkaloid steroids, or steroids with a nitrogen function or triterpenoids that can be found in plants (Putri, 2021).

Phytochemical tests of phenolic content in ethanol and ethyl acetate extracts of moringa leaves showed both were positive with a green color change in samples that had been added FeCl3 which can react with aromatic -OH groups (Ningsih et al., 2020). Phenolic compounds detected in moringa leaves with different solvents, namely ethanol and ethyl acetate, although the ethyl acetate solvent has semi-polarity properties, possibly because propolis H has a long chain so that the phenolic compounds contained in moringa leaves dissolve well in semi-polar solvents such as ethyl acetate (Yuliawan et al., 2021). Phenolic or polyphenolic compounds are natural antioxidant compounds in plants. Phenolic compounds have multi-functional properties that act as antioxidants because these compounds have the ability to reduce and capture free radicals (Mu'nisa et al., 2012).

Ethanol extract of moringa leaves showed positive results containing triterpenoid compounds and negative for steroids, while ethyl acetate extract of moringa leaves showed positive results for steroids and negative for triterpenoids. Ethyl acetate includes compounds that have semi-polar properties. Steroid compounds have non-polar properties so that they can be dissolved in ethyl acetate solvents which have semi-polar properties. Solvents that have polar properties will attract compounds that are polar, as well as non-polar or semi-polar solvents will attract compounds that have non-polar or semi-polar properties (Sunnah et al., 2021). Triterpenoids are compounds whose carbon skeleton comes from six isoprene and is biosynthetically derived from acyclic C30 hydrocarbons, namely skualen. Triterpenoids are generally crystalline, often with a high melting point and difficult to characterize because they do not have chemical reactivity (Purba, 2007). Steroids are a number of lipid compounds that

have the same basic structure and can be considered as derivatives of perhydrocyclopentanophenanthrene, which consists of 3 integrated cyclohexane rings such as phenanthrene forms (rings A, B, and C) and a cyclopentane ring incorporated at the end of the cyclohexane ring (ring D) (Agustina, 2017).

Based on the results of phytochemical tests conducted on moringa leaves using two different solvents, namely ethanol and ethyl acetate, it shows differences in phytochemical results, namely the presence of alkaloids, flavonoids, saponins, phenolics, steroids and triterpenoids in moringa leaves due to differences in solvents used in dissolving moringa leaves and differences in solvent polarity (Purwati et al., 2017). This is in accordance with research conducted by Prabowo (2014) which states that solvents have differences in polarity so that they can cause differences in the amount of compound content obtained.

Qualitative Test using FTIR

The identification process using FTIR is based on vibrations by each functional group. FTIR will produce a spectrum peak display that can show a functional group with a graph comparing the wave number absorption to transmittance. The interaction between energy and molecules in FTIR causes a transition due to molecular vibrations, so that each functional group has a different type of bond and has a distinctive IR absorption. Based on the FTIR results of Ethanol and Ethyl Acetate Extracts of Moringa Leaf (Table 2.) shows that both extracts have bending molecular vibrations or stretching molecules.

The ethanol extract of moringa leaves is known to contain O-H functional groups at wave numbers 3000-3500 cm-1, C-H at wave numbers 2850-2970 cm-1, and C=O at wavelengths of 1690-1760 cm-1. This is in accordance with the research of Bello et al (2017) that moringa leaves have an FTIR spectrum of -OH groups at wave numbers 3000-3500 cm-1 and this is reinforced by the presence of C-O groups in the 1000-1260 cm-1 region. At wave numbers 1650-1800 cm-1 shows the presence of C = C stretching which is characteristic of alkenes. Research by Ningsih et al (2021) also stated that in the ethanol extract of moringa leaves there was a broad peak at wave number 3259,56 cm-1. This proves the existence of stretching vibrations of the -OH functional group. In addition, there are also several functional groups, such as ether, halogen, and so on. Based on some of these functional groups, it can be predicted that moringa leaf ethanol extract contains phenolic or flavonoid compounds.

The ethyl acetate extract of moringa leaves is known to contain C-H functional groups at wave numbers 2850-2970 cm-1, C=O at wavelengths 1690-1760 cm-1, and C=C-H at wave numbers 675-995 cm-1. Based on the research of Salimi et al (2019), the spectra results showed five functional group absorptions. At wave number 3431,0 cm-1 shows the absorption of the O-H group; wave number 1061.2 cm-1 shows the absorption of the C-OH group; wave number 2977,17 cm-1 there is a stretch isolate does not have conjugated carbon which is one of the characteristics of triterpenoid compounds; wave number 1458,70 cm-1 shows the absorption of the aliphatic C-H group. According to Salimi et al (2019) the interpretation of spectral data showing the functional groups -OH bound, C=O, C=C aromatic, C-H aliphatic, C-O alcohol and C-H aromatic is characteristic of flavonoid compounds. Based on the structure of flavonoid types that have aliphatic C-H bonds are chalcone compounds. Thus, the spectra results from the ethyl acetate extract of moringa leaves are known to contain chalcone type flavonoid compounds (Salimi et al., 2017).

Quantitative Test using UV-Vis Spectrophotometer

Quantitative analysis was performed by measuring the total phenolic amount in moringa leaf extract using the Folin-ciocalteu method, which is a common technique for determining phenolic content in plants. Folin-ciocalteu reagent is used because phenolic compounds can react with Folin to form a solution whose

absorbance can be measured (Chun et al., 2003). Before determining the total phenolic content, first determine the wavelength of gallic acid standard solution from the range 600-800 nm using UV-Visible spectrophotometry. The maximum wavelength obtained was 749 nm. The measurement results of determining the maximum wavelength can be seen in Figure 2.

Furthermore, the absorbance of gallic acid standard solution was measured from several concentration variations, namely 10, 20, 30, 40, and 50 ppm. The results of measuring the absorbance of gallic acid standard solution made a calibration curve of the relationship between concentration (C) and absorbance (A) so as to obtain a linear regression equation. The result of the linear regression equation is y=0.037x-0.009 with a correlation coefficient (R^2) of 0.999. This indicates that the curve is linear. The measurement results of gallic acid standard solution can be seen in Figure 3.

As a standard or comparison solution, gallic acid is used which is one of the natural and stable phenolics. According to Ansory et al (2023) gallic acid is included in phenolic compounds derived from hydroxybenzoic acid which is classified as a simple phenolic acid. Gallic acid was reacted using the Folin-ciocalteu reagent to produce a yellow color indicating phenolic content. Furthermore, Na2CO3 solution was added as a base medium.

During the reaction, hydroxyl groups on phenolic compounds react with Folin-ciocalteu reagent, forming a blue molybdenum-tungsten complex with an unknown structure and can be detected with a spectrophotometer. The blue color that is formed will be more intense, equivalent to the concentration of phenolic ions formed, meaning that the greater the concentration of phenolic compounds, the more phenolic ions will reduce heteropoly acid (phosphomolybdat-phosphotungstat) into molybdenum-tungsten complexes so that the color produced is more intense (Nur, 2011).

Determination of total phenolic content was carried out with 2 replications at a concentration of 10 ppm. Absorbance measurements were taken using the maximum wavelength. Total phenolic content of Moringa (Moringa oleifera) leaf extract is expressed in GAE (Gallic Acid Equivalent). GAE is the number of milligram equivalents of gallic acid in 1 gram of sample (Andriani and Murtisiwi, 2018). Based on the results of determining the total phenolic content of moringa leaf extract using ethanol and ethyl acetate solvents, it was positive for phenolic content. The total phenolic content of moringa leaf extract with ethanol solvent was 34 mg GAE/g. While in ethyl acetate solvent amounted to 2.216 mg GAE/g. The level of phenolic compounds is determined by the solubility of the compound itself. Therefore, it is important to choose a solvent that can extract with a broad spectrum (Nur, 2011). This also causes the difficulty of choosing the appropriate extraction procedure to extract phenolic compounds from plants (Naczk and Shahidi, 2004).

CONCLUSION

Based on the results of the study, it can be concluded that the difference in ethanol and ethyl acetate solvents has a significant effect on the composition of bioactive compounds and phenolic content contained in moringa leaf extract. Therefore, the choice of solvent type in moringa leaf extraction needs to be carefully considered to obtain extracts rich in bioactive and phenolic compounds.

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