



PHYTOCHEMICAL CONTENT ANALYSIS OF MORINGA SEED (MORINGA OLEIFERA, L) MACERATION METHOD

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ABSTRACT

Moringa is one of the plants used in traditional medicine by the community as an efficacious medicine utilizing all parts of the plant. Moringa seeds are part of the plant that can be used in medicine. A phytochemical test is a qualitative test of the chemical compound content of secondary metabolites, which is an essential first step in research on medicinal plants or in the search for new active compounds derived from natural materials that can be precursors for the synthesis of new drugs or become prototypes of certain active compounds. This research aimed to identify chemical compounds in Moringa seeds (*Moringa oleifera*, L). This research used the maceration method with 80% methanol solvent and hexane solvent, then identifying secondary metabolites was carried out by phytochemical test. The results showed that the phytochemical test results on the seed extract using the maceration method were alkaloids, flavonoids, and saponins. The results also showed that the seed sample had a moisture content below 10%, which was 9.36%.

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Keywords: Phytochemicals, *Moringa oleifera*, Maceration.

INTRODUCTION

Moringa is one of the plants used as traditional medicine by the community as an efficacious medicine utilizing all parts of the plant. Moringa seeds are part of the plant that can be used in medicine (Simbolan et al, 2007). *Moringa oleifera* L is included in the two major plants that have the highest antioxidant activity (Sato et al., 2002)

A phytochemical test is a qualitative test of the chemical compound content of secondary metabolites, which is an essential first step in research on medicinal plants or in the search for new active compounds derived from natural materials that can be precursors for the synthesis of new drugs or become prototypes of certain active compounds. The most widely used phytochemical test method

is the color reaction and precipitation method, which can be carried out in the field or laboratory (Iskandar & Susilawati, 2012).

Solvent extraction is a technique that is often used to isolate active substances in plants. Solvent extraction is a method of separating components from a mixture using a solvent that aims to attract the active substance in the sample. The solvent used is based on the ability to dissolve the active substance in the maximum amount so that an extract is formed (the extraction product containing various chemical components). The principle of this method is based on the distribution of solutes in a particular ratio between two immiscible solvents. Solvent extraction is done by cold method (maceration). The extraction process by

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maceration technique is carried out several times by shaking or stirring at room temperature. The advantage of this method is that it is easy and does not require heating, so it is less likely that natural materials will be damaged or decomposed. The choice of solvent based on its solubility and polarity facilitates the separation of natural substances in the sample. The extended operation of the maceration method and the stationary state during maceration allow many compounds to be extracted (Istiqomah, 2013). This study aimed to analyze the phytochemical content of Moringa seeds using the maceration method.

LITERATURE REVIEW

General Description of Moringa Plant

Moringa plants are shrubs with a height of 7-11 m. It thrives from the lowlands to an altitude of 700 meters above sea level. Moringa can live in tropical and subtropical areas, on all types of soil and is resistant to dry seasons, with drought tolerance of up to six months (Vani et al., 2021). Moringa is native to the foothills of the Himalayas in northwestern India and is found in several other countries, such as Africa, Arabia, Southeast Asia and South America (Olaborode et al., 2022). In Indonesia, Moringa plants are known by different names in each region including, Sumatra: murong (Aceh), munggai (Padang), kilor (Lampung), Java: moringa (Bandung), moringa (Central Java), marongghi (Madura), Bali: Moringa, Nusa Tenggara: parongge (Bima), kawona (Sumba), Maluku: kirol (Buru), Tarnate and Tidore: kelo (Yanti, 2010). Moringa plant classification Kingdom: Plantae, Subkingdom: Tracheobionta, Division: Spermatophyta, Subdivision: Magnoliophyta, Class: Magnoliopsida, Subclass: Dileniidae, Order: Capparales, Family: Moringaceae, Genus: Moringa, Species: Moringa oleifera Lamk (Narina et al., 2019).

Moringa Seeds

Moringa fruit will produce seeds that can be made with flour or oil as raw materials for the manufacture of high-value drugs and cosmetics (Thapa et al., 2019). Moringa seeds contain phenolic compounds, flavonoids, saponins, terpenoids, proanthocyanidins and cardiac glycosides (Pérez-Rivera et al., 2021).

Moringa seeds are good antioxidants because they can reduce oxidative damage accompanied by ageing and (Narina et al., 2019). Moringa seeds also contain vitamin E (0.01%) and beta carotene (0.014%) (Pérez-Rivera et al., 2021).

METHODS

Materials and Tools

Moringa seed samples were taken in April from North Lombok. The chemicals used were methanol, hexane, phytochemical test reagents (alkaloids, terpenoids, flavonoids, saponins, and tannins), filter paper, distilled water, aluminum foil, fat-free cotton, and tissue. The tools used in this study were a desiccator, analytical balance, a set of evaporator, oven, petri dish, Wattman 42 filter paper, aluminum foil, glassware, blender, incubator, and a set of phytochemical test equipment.

Sample Preparation

Moringa seed samples were obtained, cleaned of impurities, and dried in the open air. The dried samples were then mashed using a blender until the samples became smooth, then the water content was calculated and extracted.

Determination of Water Content

Determination of water content by means of a number of dry sample weights inserted into a porcelain dish whose empty weight is known. Then the cup is heated in the oven at 1050 for 3 hours. After that, it was cooled in a desiccator and weighed to determine the dry weight. The heating of the sample was repeated until a constant weight was obtained, and the water content formula determined the water content.

$$\text{water content (\%)} = \frac{(B - C)}{(C - A)} \times 100\%$$

Information :

A: the weight of the empty cup

B : weight of cup + sample

C : final weight up to

Maceration

A total of 20-25 g of a dry sample that has been mashed is macerated with hexane until it is free of fat. Extraction was carried out 4 times. The resulting extract was filtered, then the filtrate was concentrated using a rotary

evaporation flask at a temperature of 40°C to obtain a crude extract, and the yield was calculated. The dregs were air-dried, and after drying, they have macerated again with 80% methanol in the same way as hexane. The resulting filtrate is then concentrated, the crude extract is obtained, and the yield is calculated.

Phytochemical Test (Harborne, 1987)

Phytochemical tests include alkaloids, flavonoids, saponins, tannins/polyphenols, terpenoids, and steroids. This test was carried out to identify secondary metabolites contained in Moringa seeds

1. Alkaloid Test

The alkaloid test was carried out using Mayer (potassium tetraiodomercurate (II)), Wagner (iodine in potassium iodide), and Dragendorff's (bismuth nitrate in potassium iodide) reagents. Samples containing alkaloids will form orange to brownish precipitate, and a precipitate will form when reacted with each of the three reagents.

2. Flavonoid Test

The flavonoid compounds were identified using magnesium powder (Mg) and concentrated hydrochloric acid (HCl) reagents. Adding Mg powder aims to form bonds with carbonyl groups in flavonoid compounds. Adding HCl aims to form a flavilium salt characterized by a color change to red-orange.

3. Saponin Test

The saponin test was carried out by dissolving the sample in distilled water, heating it for 15 minutes, and shaking it for 10 seconds. If a stable foam is formed for approximately 10 minutes and a few drops of 2 N hydrochloric acid are added, the sample is positive for saponins.

4. Tannin/Polyphenol test

The tannin/polyphenol test was carried out by adding a 5% FeCl₃ solution to the

sample. Samples containing polyphenols will form Fe³⁺-tannin / polyphenol complex compounds with coordination bonds with a change in color to blue-black or brownish green. This happens because the O atom in tannins/polyphenols can donate a lone pair of electrons to Fe³⁺, which has an empty d orbital to form a coordinate covalent bond to become a complex compound.

5. Terpenoid and steroid test

The terpenoid/steroid test was carried out by dissolving the sample with Liebermann Burchard reagent (acetic anhydrous acid and concentrated sulfuric acid). Samples containing steroid group compounds will change color to bluish green. While the triterpenoid group compounds will change color to form a brown or violet ring.

RESULTS AND DISCUSSION

The sample used in this study was Moringa seeds taken from North Lombok. This sample is then dried in a place that is not exposed to direct sunlight so that the secondary metabolite content in the sample is not damaged. The dried samples were then mashed using a blender. The refining process aims to increase the sample's surface area so that the contact between the sample and the solvent becomes large so that the extract in the sample easily dissolves into the solvent.

Furthermore, in sample preparation, it is necessary to determine the water content contained in the sample; this is done because the sample comes from plants that contain relatively high water content. The gravimetric method determines the water content, which is obtained by calculating the weight of the material before and after being dried at a temperature above the boiling point of water so that all water is expected to evaporate at that temperature for a certain period (Harjadi, 1986).

Table 1. Calculation of The Moisture Content of Moringa seeds

Moringa seed sample	Test 1	Test 2	Test 3	Average
	9.71%	9.25%	9.13%	9.36%

Based on the determination of the water content that has been carried out, it was found that the seed sample had moisture content below 10%, namely 9.36%. According to

Winarno (1997), a good sample for long-term storage is if the sample moisture content is less than 10%. The average water content in this

study is below 10%; therefore, the sample can be used for a long time.

The sample was macerated for 3x24 hours with solvent. Maceration is a simple extraction process by immersing the sample into a specific solvent that aims to increase the permeability of the cell wall through three stages; (1) the entry of the solvent into the plant cell wall, (2) the dissolution of the compound in the cell wall into the solvent, (3) the diffusion of the compound by the solvent out of the plant cell wall (Harborne, 1987). Every 1x24 hours, the solvent is replaced with a new one. This solvent replacement aims to reduce the saturation of the solvent by the

solute that comes from within the sample. So it is expected that the substance contained in the sample can dissolve completely. The solvents used in each sample were hexane and 80% methanol. The compounds carried away in this process have a polarity following the solvent used. Then the resulting solution is thickened using a rotary evaporation flask to obtain a thick extract, then the yield is calculated. The dregs from the extraction were then dried and re-extracted using 80% methanol as solvent, with the same treatment, the solution was thickened, and the yield was calculated after obtaining 80% methanol crude extract.

Table 2. Yield of Moringa Seed Samples with Hexane and methanol 80% Solvent

Seed Sample	Yield %	
	Hexane (80%)	Methanol 80%
Test 1	20.127	4.212
Test 2	21.082	4.821
Test 3	21.154	5.235
Test 4	20.782	5.178
Average	20.786	4.861

The average yield obtained from extraction with hexane and methanol 80% solvents were 20.786% and 4.861%, respectively.

Phytochemical test

Phytochemical test was carried out as a qualitative preliminary test to determine the

content of chemical compounds (secondary metabolites) in Moringa seeds. The chemical constituents tested by phytochemicals in Moringa seeds are alkaloids, flavonoids, saponins, tannins/polyphenols and terpenoids/steroids. As presented in Table 3.

Table 3. Phytochemical test results

Phytochemical test	Methanol Solvent	Hexane Solvent
Alkaloids		
- Mayer	++	++++
- Wagner	++	++++
- Dragendorff	++	++++
Flavonoids	-	+
Saponins	+++	++++
Tannins/Polyphenols	-	-
Terpenoids and steroids	-	-

The results of phytochemical tests on samples of Moringa seed powder can be seen in Table 3. From the table, it can be seen that Moringa seed powder only contains alkaloids, flavonoids, and saponins. Alkaloid compounds in the seed powder have a greater intensity than saponins. Based on Table 3, the

yield of hexane solvent is greater than that of methanol solvent. Thus, it can be concluded that the chemical compounds in Moringa seeds contain many non-polar secondary metabolites.

The test results showed that Moringa seeds contain alkaloids, phenol

hydroquinone, flavonoids, and saponins, so Moringa seeds have potential as antioxidants (Pratt & Hudson, 1990). Some researchers also stated that the Moringa plant, or *Moringa oleifera* L is one of the plants that have the potential as an antioxidant (Siddiq et al., 2005; Kumar & Pari, 2003). These compounds act as antioxidants by inhibiting lipid peroxides so that they can protect the body from cancer. Moringa seed samples also have the potential to lower cholesterol and can reduce the risk of atherosclerosis in diabetes because they contain saponins (Rafi, 2003).

CONCLUSION

The moisture content of the average sample of Moringa seeds is 9.36%. Group of chemical compounds in Moringa seed extract by maceration method of alkaloids, flavonoids, and saponins.

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