Extraction And Phytochemical Screening Of Ethanol Extract And Simplicia Of Moringa Leaf (*Moringa Oleifera* Lam.) From Sidikalang, North Sumatera

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Abstract.
Moringa leaf is a plant that has many uses that comes from the family Moringaceae and it is a tropical plant that is familiar to Indonesian people. Pharmacologically, this plant extract is reported to have antimicrobial and fungicide and it is rich in antioxidants. This metabolite compound contained in Moringa leaves has the potential as an antioxidant, antibacterial, functional and others. This study aims to determine the simplicia characterization of Moringa leaves and to determine the secondary metabolites contained in Moringa leaves, both Moringa leaf powder and Moringa leaf extract. The method of this research is experimental including the simplicia making and ethanol extract of Moringa leaves by maceration method, simplicia characterization and phytochemical screening. The results of the simplicia characterization of Moringa leaves for ethanol soluble extract content was 10,9% and water-soluble extract content was 15,8%, ash content was 9,6% and acid insoluble ash content was 0,6% and water content was 8%. The results of this study also showed that the simplicia powder and ethanol extract of Moringa leaves contained flavonoid, tannin, alkaloid, steroid and saponin.

Keywords: *Moringa Leaf, Phytochemical Screening, Characterization*

I. INTRODUCTION

Indonesia has abundant natural wealth. Various kinds of medicinal plants thrive in the nature of Indonesia. This natural wealth give great benefit to the health of its population, even to the world's population. Several studies have proven to the world that Indonesia has the potential as a place for the growth and development of medicinal ingredients for the world community (Fahey, 2005). Indonesia has abundant organic natural resources and contains millions of chemical compounds. Plants have potential as a disease treatment because they contain secondary metabolites. One example of a plant that is usually used as traditional medicine is the Moringa plant.Moringa plant (*Moringa oleifera*) is a plant from the family *Moringaceae* and is a tropical plant that is familiar Indonesian people (Mendieta et al*.,* 2003). Plants generally contain a wide variety of chemical compounds. Chemical compounds in plants are formed and decomposed through two metabolic systems, namely primary metabolism and secondary metabolism. In Halima and Mbulang's 2016 research, this Moringa plant contains active ingredients such as tannin, steroid, triterpenoid, flavonoid and has various potentials. Several studies have shown that compounds in plants such as anti-inflammatory (Sashidhara et al., 2007), antifungal (Chuang et al., 2006) antibiotics and anticancer (Eilert et al., 2007) and antioxidants (Benabdesselam et al., 2007).

Extraction or separation of chemical compounds from plant sources is the beginning of the process of isolating bioactive compounds present in plants, including leaves, seeds, roots, and stems. In carrying out, this extraction will assist by a solvent. This solvent must be selected. What must be considered is the selectivity, toxicity and ease of evaporation. Extraction is used to obtain the content of chemical compounds that are soluble in solvents. There are several types of extraction commonly used in the process of separating bioactive compounds from plants to determine the yield produced, namely cold extraction and hot extraction (Kiswandono, 2011).One approach to research plants containing secondary metabolites is the screening of chemical compounds called phytochemical screening contained in plants. This method is used to detect the

presence of a group of alkaloid compounds, flavonoid, phenolic, tannin, coumarin, quinone, steroid, terpenoid, and other compounds (Halima and Mbulang, 2016). Phytochemical screening is an analytical method to determine the type of secondary metabolites found in plants because of their characteristic that can react specifically with specific reagents (Harbone, 1987). The content and activity of secondary metabolites depend, one of which is how the process is extracted and the state of the plant is seen (fresh Moringa leaves and Moringa leaf simplicia). It is very necessary to research to find out what secondary metabolites are int these two ingredients, namely extracts from fresh Moringa leaves and extracts from Moringa leaf simplicia (dried leaves).

II. METHODS

2.1 Tools

Tools used in this study were beakers, tube racks, blenders, analytical balances, rotary evaporators, porcelain dishes, maceration vessels, furnace, water bath, and oven.

2.2 Materials

Materials used in this study were simplicia and Moringa leaf extract *(Moringa oleifera)*, aquadest, 96% ethanol, magnesium powder, concentrated HCl, Iron (III) Chloride 10%, 2N HCl, Libermann-Bourchard reagent, Bouchardat reagent, Mayer reagent, Dragendorff's reagent, amyl alcohol, and toluene.

2.3 Samples

Moringa *(Moringa oleifera)* leaves were taken using a purposive sampling technique, taking samples intentionally from one area without comparing them with other areas. In this study, Moringa leaves were obtained from Sidikalang Village, Dairi, North Sumatra.

2.4 Method

2.4.1 Extraction

Weighed Moringa (*Moringa oleifera*) leaf powder 100 grams, put in a dark bottle, added with 1500 mL of ethanol solvent, stirred, covered with aluminum foil, and stored for 3 days protected from sunlight. During 1 x 24 hours of immersion, the shaking was carried out, after 5 days, filtered using a cloth flannel to separate the macerate from Moringa leaf *(Moringa oleifera)* (macerate I) and residue, then the residue was macerated again with 1000 mL of ethanol, stored for 2 days to avoid contamination of sunlight. During 1 x 24 hours of immersion, the shaking was carried out, after 2 days, it was filtered again (macerate II), combined with macerate I, and then evaporated using a rotary evaporator until a thick extract was obtained. The extract yield was calculated (Ditjen POM, 1995).

2.4.2 The Simplicia Characterization Examination

Characterization examination includes determination of water content, determination of water-soluble extract content, determination of ethanol-soluble extract, determination of total ash content and determination of acid-insoluble ash content (Ditjen POM, 1995).

2.4.3 Determination of Moisture Content by Azeotropic Method

A total of 200 ml of toluene was put into a round bottom flask, then 2 ml of distilled water was added, then azeotrope was installed and distilled for 2 hours. The distillation was stopped and allowed to cool for ± 30 minutes, then the volume of water in the receiving tube was read to an accuracy of 0.05 ml.Then into the flask 5 g of simplicia powder which has been weighed carefully, then heated carefully for 15 minutes, after the toluene boils, the drop rate is set to 2 drops per second until most of the water is distilled, then the distillation speed is increased to 4 drops per second. After all the water is distilled, the inside of the cooler is rinsed with toluene. Distillation was continued for 5 minutes, then the receiving tube was allowed to cool at room temperature. After the water and toluene were completely separated, the volume of water was read to an accuracy of 0.05 ml. The difference between the two volumes of water contained in the material being examined. The water content is calculated in percent (Ditjen POM, 1995).

2.4.3 Determination of Water Soluble Extract Content

A total of 5 g of powder that has been dried in the air, macerated for 24 hours in 100 ml of water-chloroform (2,5 ml of chloroform in distilled water precise 1 L) in a corked flask while occasionally shaking for the first 6 hours, then left for 18 hours, then filtered. A number of 20 ml of the first filtrate evaporated to

dryness in a flat-bottomed evaporation dish that has been heated and tared. The leftover is heated at 105°C until the weight remains. The concentration in percent of water-soluble juice calculated from the material that has dried in the air (Ditjen POM, 1995).

2.4.5 Determination of Essence Content Soluble in Ethanol

A total of 5 g of simplicia powder which has dried in the air, macerated for 24 hours in 100 ml of 96% ethanol in a corked flask while shaking occasionally for the first 6 hours, then left for 18 hours, then filtered quickly to avoid ethanol evaporation. A number of 20 ml of the filtrate was evaporated to dryness in a flat-bottomed evaporation dish that has been heated and tared. The leftover is heated at 105ºC until the weight remains. The concentration in the percentage of 96% ethanol-soluble extract is calculated from the material that has been dried in the air (Ditjen POM, 1995).

2.4.6 Determination of Total Ash Content

A total of 2 g of simplicia powder was weighed carefully and put in a porcelain crucible that had been burnt and tared. Burn crucible slowly until charcoal runs out, burns in 500-600°C for 3 hours then cooled and weighed to obtain the weight remains. Ash content is calculated on the dried material (Ditjen POM, 1995).

2.4.7 Determination of Acid Insoluble Ash Content

Ash obtained from the determination of total ash content, boil with dilute hydrochloric acid for 5 minutes, collect the insoluble part in acid, filtered through ash-free filter paper, then washed with hot water, burn until constant weight, weighed. Calculate the ash content that is not soluble in acid against the material that has dried in the air (Ditjen POM, 1995).

2.5 Phytochemical Screening Test

2.5.1 Alkaloid Examination

Simplicia powder and extract weighed 0.5 g added 1 ml HCl 2 N added 9 ml distilled water, then heated on a water bath for 2 minutes, cooled and filtered the filtrate used for alkaloid examination:

1. 3 drops of filtrate are added with 2 drops of Mayer reagent, a white or yellow lumpy residue will be formed.
2. 3 drops of filtrate are added with 2 drops of Bouchardat reagent, a brown to black residue will be formed.
3. 3 drops of filtrate are added with 2 drops of Dragendroff reagent to form brown or orange.

If there is a residue or turbidity in at least 2 test tubes in the above experiment, the alkaloid is positive (Ditjen POM, 1995).

2.5.2 Flavonoid Examination

A total of 10 g of simplicia powder and extract were weighed and then added 100 ml of hot distilled water, boiled for 5 minutes, and filtered while its hot, into 5 ml of the filtrate added magnesium powder and 1 ml of concentrated HCl and 2 ml of amyl alcohol, shaken vigorously and let it separate. The presence of flavonoids indicated by the presence of a red, yellow, or orange color on the amyl alcohol layer (Ditjen POM, 1995).

2.5.3 Tannin Examination

A total of 1 g of simplicia powder and extract were extracted with 10 ml of distilled water and filtered. The filtrate was diluted with distilled water until it was colorless. 2 ml of the solution was taken and added with 1-2 drops of 1% iron (III) chloride reagent. If a blackish-blue or blackish-green color occurs, it indicates the presence of tannins (Ditjen POM, 1995).

2.5.4 Saponin Examination

A total of 0,5 g of simplicia powder and extract were put into a test tube, then 10 ml of hot distilled water was added and cooled, then shaken vigorously for 10 minutes. If the foam is formed with a height of 1-10 cm which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2 N HCl, it indicates the presence of saponins (Ditjen POM, 1995).

2.5.5 Steroid/Triterpenoid Examination.

Simplicia powder and extract of 1 g of solution were macerated with 20 ml of n-hexane for 2 hours, then filtered. The filtrate evaporated in an evaporating dish to dryness, then added 2 drops of acetic

anhydride and 1 drop of concentrated sulfuric acid. If the color changes to purple or red and turns green or blue, indicating the presence of steroid/triterpenoid compounds (Harbone, 1987).

III. RESULTS

In this study, the extraction method used was the maceration method. This method was chosen because the process is easy, the equipment used is simple, and it does not damage the compounds contained in the test sample. The solvent used in this maceration process is ethanol. The choice of ethanol as a solvent is because ethanol can dissolve almost all secondary metabolites in polar test samples (Prima and Ivan, 2013f).

Table 1. Results of the Characterization of Moringa Leaf Simplicia

No.	Parameter	Result (%)	Terms
1.	Moisture content	8%	<10%
2.	Ash content	9,6%	<11%
3.	Acid Insoluble Ash content	0,6%	<1%
4.	Ethanol Soluble Extract Content	10,9%	>5%
5.	Water Soluble Extract Content	15,8%	>5%

Based on the results of the simplicia characterization of Moringa leaves in Table 4.1. The extract content was carried out to see the number of soluble compounds in polar and non-polar solvents. The ethanol soluble extract content was 10,9% and the water soluble extract content was 15,8%. Examination of ash content is useful to see the mineral content of simplicia. The ash content was 9,6% and the acid insoluble ash content was 0,6%. The water content was carried out to see the number of water contained in the simplicia. For the water content obtained 8%. From the results of the determination of simplicia characterization, it shows that the results meet the requirements and are guaranteed quality based on Materia Medika Indonesia (MMI).

Table 2. Results of Characterization of Moringa Leaf Simplicia

No.	Group of Chemical Compounds	Moringa Leaf Powder	Ethanol Extract of Moringa Leaves
1.	Alkaloid	+	+
2.	Flavonoid	+	+
3.	Tannin	+	+
4.	Saponin	+	+
5.	Steroid	+	+

Description:

(+) = contains the substance examined

(-) = no contains substances examined.

Based on the results of the phytochemical screening examination, Moringa leaf powder and ethanol extract of Moringa leaves in Table 4.2 contain metabolites of alkaloid, flavonoid, tannin, saponin, and steroid.The results above indicate that the powder and extract of Moringa leaves are positive compounds flavonoid, alkaloids, saponins, tannins, and steroid. In the alkaloid test, Moringa leaf extract showed positive results containing alkaloid compounds in 2 out of 3 tests which characterized by the formation of a white residue after adding the Mayer reagent, a brown residue after the addition of Bouchardat reagent and no red-orange residue formed when the Dragendorff reagent added. Furthermore, the flavonoid test also obtained positive results that indicated a yellow-orange color change in the alcohol layer. From the results of the examination of flavonoids, the addition of concentrated hydrochloric acid to Mg powder and amyl alcohol formed a yellow-orange color layer on the amyl alcohol layer. It shows that the ethanol extract of Moringa leaves contains flavonoid (Depkes, 1989).

On examination, the compounds of the saponin group were declared to contain saponins due to the presence of stable foam after administration of hydrochloric acid. Saponins are a glycoside form of sapogenins then they are polar. Saponins are compounds that are surface active and can cause foam when shaken in the water. The presence of foam in the saponin test indicates the presence of glycosides which can form foam in the water which is hydrolyzed into glucose and other compounds. The saponin compounds will tend to be attracted by semi-polar solvents such as ethanol (Astarina et al., 2013). In the tannin test, a

positive result is indicated by a change in the color of the filtrate to blackish-green or balckish-blue. In the tests that have been carried out, the results obtained are blackish-dark green, so the sample is declared positive for tannins. And the steroid/triterpenoid examination showed positive steroid was indicated by the appearance of a green color ((Ditjen POM, 1995; Saputro, 2015).

IV. CONCLUSION

The results of the simplicia characterization of Moringa leaves for ethanol soluble extract content obtained 10,9% and water soluble extract content obtained 15,8% ash content obtained 9,6% and acid insoluble ash content obtained 0,6% and water content obtained 8%. The results of this study also showed that the simplicia powder and ethanol extract of Moringa leaves contained flavonoid, tannin, alkaloid, steroid and saponin.

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