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Freezed Drying of Kelor Leaves Extract (*Moringa oleifera* Lam.)

Yonathan Tri Atmodjo Reubun[1,*], Shirly Kumala[1], Siswa Setyahadi[1,2], Partomuan Simanjuntak[1,3]

[1]Faculty of Pharmacy, Pancasila University, Jakarta.
[2]Center for Bioindustrial Technology, Agency for the Assessment and Application of Technology, Serpong
[3]Center for Biotechnology, Indonesian Institute of Sciences, Cibinong.
* Corresponding author: jonathanreubun90@gmail.com

Abstract

Moringa oleifera Lam. is a plant that is widely used by people in Indonesia because it is believed to be one of the medicinal plants for various diseases such as diabetes, diuretics, and has the potential to prevent Alzheimer's disease. The purpose of this research is to obtain kelor leaves extract which is more stable in storage and durable, namely by using the *freeze drying* method, which in this method aims to remove the moisture content in an ingredient or extract. The initial stage of making the extract begins with the extraction of kelor leaves using the maceration method using ethanol as a solvent. The results of maceration are collected and concentrated using a *rotary evaporator* until a thick extract is obtained and followed by *freeze drying* which aims to obtain a thick, stable extract. The results showed that 467.12g of kelor leaves extract from 1,000g simplicia was obtained where the yield obtained was 46.71%. the extract yield after freeze drying was 286.03g. After that the extract was tested for phytochemical screening where the results showed that the Moringa leaf extract contained alkaloids, saponins, tannins, phenolics, flavonoids, glycosides, and steroids.

Keywords: Moringa oleifera, ethanolic extract, freeze drying

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1 Introduction

Traditional medicine is a national cultural heritage that needs to be preserved and developed to support health. Traditional medicine plays a very large role in public health services in Indonesia, therefore traditional medicine is made to be developed. Indonesia has many medicinal plants because Indonesia has the second largest biodiversity after Brazil. Although there are many plants that can be used as medicinal ingredients, they have not been fully utilized by the people of Indonesia [1].

Kelor (*Moringa oleifera* Lam.) is a tropical plant that grows in tropical areas such as

Indonesia. Kelor plants are plants with a height of 7-11 meters and thrive from the lowlands to an altitude of 700 m above sea level. Moringa can grow in all types of soil and is resistant to dry spells with drought tolerance of up to 6 months [2]. Kelor leaves (*Moringa oleifera* Lam.) Contain antioxidants including alkaloids, saponins, phytosterols, tannins, phenolics, polyphenols and flavonoids. The levels of polyphenols and flavonoids in kelor leaves are known to be higher than other leaves such as pumpkin leaves and fern leaves [3].

Freeze drying or lyophilization is a process in which water is frozen to be removed from the sample, initially this process begins with a sublimation process (primary drying) and then continues with a desorption process (secondary drying). Freeze drying is a drying process in which water is sublimated from the sample after freezing. This drying process is applicable to the manufacture of certain pharmaceuticals and biological processes that are thermolable or unstable in aqueous solutions for long storage periods, but which are stable in dry conditions [4].

This mechanism is different from the usual drying process, where drying usually occurs through an evaporation mechanism (evaporation) which usually occurs at high temperatures. Difference between freeze drying and normal drying The drying process usually occurs through the evaporation mechanism at hot temperatures, so that the dry parts of the food will undergo chemical changes (starch gelatinization, sugar caramel, and protein denaturation) which cause crust to form on the surface; which will provide a barrier for the diffusion of vapor from the wet part to the ambient air. As a result, the drying process will be hampered and stopped, resulting in a product that is dry on the outside, even too dry and becomes a crust - but the center is still wet. Such cases are often referred to as casehardening. The freeze-drying process occurs through a sublimation mechanism that occurs in cold temperatures. Therefore, the processes of gelatinization, caramel, and denaturation do not occur, so that the dry parts of the food do not change in scale formation. Thus, water vapor can diffuse well from the wet part to the ambient air, so that a dry product can be produced properly [5].

The purpose of this research is to obtain the results of kelor leaves extract that are stable in storage and become the best solution in the development of natural medicinal products where the kelor leaves extract obtained does not cause the surface to wrinkle and is easily refreshed and makes the extract more durable in storage.

2 Methods

2.1 Tool

Tools used in research include rotary evaporator (IKA), freeze drying (New Brunswick), glassware.

2.2 Material

The test material was *Moringa oleifera* leaves obtained from Moringa Organic Indonesia, Blora, Central Java and was determined at LIPI Cibinong. The chemicals used are ethanol (Merck), 2N hydrochloric acid, mayer reagent, Bouchardat reagent, Dragendorf reagent, FeCl3 (Merck), NaOH (Merck), amyl alcohol, glacial acetic acid, n-hexane (Merck), Liebermann's-Burchard reagent..

2.3 Sample preparation

The 1,000 g of kelor leaves are washed, cut and dried. After drying, it is pollinated using a 100 mesh sieve to become a simplicia powder.

2.4 Moringa leaf extraction

The leaves of Moringa (*Moringa oleifera* Lam.) Obtained from Moringa Organic Indonesia, Blora were extracted using maceration method. Simplicia weighing 1,000g was immersed in 1,000ml of ethanol for 24 hours and filtered. Maceration was carried out eleven times until the filtrate was obtained which did not contain the active substance. The maceration result is then concentrated with a rotary evaporator until a thick extract is obtained. Then freeze dry the thick extract to get a stable extract. then the extract was weighed to calculate the yield (%).

2.5 Moringa leaf extract freeze drying

Lyophilization or freeze drying is carried out at temperature and pressure conditions

below three points or 0 ° C, to allow sublimation of ice. The whole process is carried out at low temperature and pressure, therefore it is suitable for drying thermolable compounds. The steps involved in lyophilization start from sample preparation followed by freezing, primary drying and secondary drying, to obtain the final product which is dried to the desired moisture content [6]. The moisture concentration gradient between the drying front and the condenser is the driving force for water removal during the lyophilization process. The water vapor pressure increases with increasing temperature during primary drying. Therefore, the primary drying temperature must be kept as high as possible, but below the critical process temperature, to avoid loss of its structure. This temperature is the temperature of the collapse for amorphous substances, or eutectic melting for crystalline substances. During freezing, the ice crystals begin to separate until the solution is maximally concentrated. On further cooling, a phase separation between the solute and ice occurs [4].

2.6 Phytochemical screening

2.6.1 Alkaloid identification

The test sample was weighed as much as 0.5 g then added 1 ml of 2N hydrochloric acid and 9 mL of distilled water, heated over a water bath for 2 minutes, cooled and filtered. The filtrate obtained is used for the alkaloid test, 3 test tubes are taken, then 0.5 mL of the filtrate is inserted into them. Each of the first test tubes added 2 drops of Mayer reagent will form a white or yellow precipitate. The second test tube is added with 2 drops of Bauchardat reagent to form a brown precipitate. The third test tube is added with 2 drops of Dragendorf reagent to form a white precipitate. The sample is said to contain alkaloids if there is sedimentation or turbidity in at least two of the three experiments above.

2.6.2 Saponin identification

The test sample was weighed as much as 0.5 g and put into a test tube and then added 10 mL of hot water, cooled then shaken vigorously for 10 seconds. If it is foamy and does not disappear by adding 2N hydrochloric acid, it indicates the presence of saponins.

2.6.3 Tannin Identification

The test sample was weighed as much as 1 g, boiled for 3 minutes in 100 mL distilled water then cooled and filtered. The solution is taken 2 mL and added 1-2 drops of 1% iron (III) chloride reagent. If there is a dark blue or blackish green color, it indicates the presence of tannins.

2.6.4 Phenolic Identification

Identification of phenolic compounds can be done by adding sodium hydroxide. Samples containing phenolic compounds are shown by the appearance of a red color.

2.6.5 Flavonoid Identification

A total of 10 g of the test sample was added with 10 mL of hot water, boiled for 5 minutes and filtered in a hot state, into 5 mL of the filtrate was added 0.1 g of magnesium powder and 1 mL of concentrated hydrochloric acid and 2 mL of amyl alcohol, shaken and allowed to separate. The sample is said to contain flavonoids if there is a red color on the amyl alcohol layer.

2.6.6 Glycoside Identification

The identification of glycosides was carried out by adding glacial acetic acid and then adding iron (III) chloride and adding concentrated sulfuric acid and shaking it. The sample is said to contain glycoside compounds indicated by the appearance of a purple ring.

2.6.7 Triterpenoid / Steroid Identification

A total of 1 g of sample was macerated for 2 hours with 20 mL of non-polar n hexane and filtered. The filtrate is evaporated in a steam cup. Add 3 drops of Liebermann-Burchard reagent added to the remaining filtrate. The appearance of a green color indicates the presence of steroid compounds and a red or purple color which is said to contain triterpenoid compounds.

3 Results and Discussion

Moringa leaf extract weighing 1,000g was macerated with 96% ethanol for 11 days. Obtained data as below (Table 1).

Table 1: Moringa leaves extracted with ethanol

Days-	Amount of solvent	macerate
Day 1	3.000 mL	450 mL
Day 2	2.000 mL	2.000 mL
Day 3	2.000 mL	2.200 mL
Day 4	2.000 mL	2.000 mL
Day 5	2.000 mL	2.000 mL
Day 6	1.800 mL	1.800 mL
Day 7	2.000 mL	1.500 mL
Day 8	2.000 mL	1.500 mL
Day 9	2.000 mL	1.800 mL
Day 10	2.000 mL	1.700 mL
Day 11	2.000 mL	1.300 mL
Total	22.800 mL	18.250 mL

In this extraction, it was obtained that the perfect kelor leaves extract was shown by the filtrate that did not contain active substances by testing it in thin layer chromatography. The macerated liquid extract obtained was continued using a rotary evaporator to obtain a thick extract. The thick extract obtained is as follows (Table 2).

Table 2. The result of the thick extract of kelor leaves

Name	Total
Container weight + kelor leaves extract	766.80 g
Contained weight	299.68 g
Kelor leaves extract weight	467.12 g

Based on table 2, the results of the thick extract of kelor leaves are 467.12 g from 1,000 g with a yield percentage of 46.71%. These results are said to meet the requirements of the literature on the Indonesian Herbal Pharmacopoeia where the yield of kelor leaves extract is not less than 10%.

The thick extract is evaporated and then the freeze drying stage is carried out with the aim of maintaining the quality of an extract so that it can be made into a stable preparation in storage. From the test results, freeze drying extract results were obtained as Table 3.

Table 3. The results of Moringa leaf extract using freeze drying method

Name	Total
Container weight + kelor leaves extract	467.42 g
Contained weight	181.39 g
Kelor leaves extract weight	286.03 g

Based on table 3 the results of the kelor leaves extract using the freeze drying method, the extract weight was 286.03 g with a yield percentage of 28.60%. These results are said to meet the requirements of the Indonesian Herbal Pharmacopoeia where the extract yield is not less than 10%.

The extract produced from the freeze dry method is a stable extract which has a low density and has the advantage of being made into a dosage product which has a longer storage time. This starts with fresh products being converted into frozen products and the sublimation process removes moisture content in frozen products to become freeze-dried products [4].

Phytochemical screening was carried out with the aim of knowing the group of compounds contained in the test extract used. Testing of the chemical content of Moringa leaf extract includes testing for alkaloids, saponins, tannins, phenolics, flavonoids, glycosides, triterpenoids, and steroids. The results of phytochemical screening obtained the data as Table 4.

Table 4. Phytochemical screening of kelor leaves extract

No	Compound	Kelor leaves extract
1	Alkaloid	+
2	Saponin	+
3	Tanin	+
4	Phenolic	+
5	Flavonoid	+
6	Glikoside	+
7	Triterpenoid	-
8	Steroid	+

The choice of ethanol in this study is because it is universal in that this solvent can dissolve almost all organic compounds in the sample, both polar and non-polar compounds [7]. Based on the results of phytochemical screening, it shows that gotu kola herbal extract gives positive results for the alkaloid compound group , saponins, tannins, phenolics, flavonoids, glycosides, and steroids.

The results of the phytochemical screening of kelor leaves on flavonoids include quercetin and kaempferol. Quercetin is a powerful antioxidant with a strength 4-5 times higher than vitamin C and vitamin E which are known

as potential antioxidants [8]. The flavonoid content found in kelor leaves provides several medicinal effects, namely protection of endothelial function by inhibiting platelet aggregates, thereby reducing the risk of coronary heart disease. In addition, flavonoids have a hypotensive effect by inhibiting the activity of Angiotensin I Converting Enzyme (ACE), as well as a diuretic [9].

4 Conclusion

Based on the extraction of *Moringa oleifera* Lam. Leaves from 1,000 g of simplicia, it was found that the thick extract using a rotary evaporator was 467.12g with a yield of 46.71%. Meanwhile, in freeze drying kelor leaves extract using freeze dry amounted to 286.03g with a yield of 28.60%. these results are in accordance with the requirements set by the Indonesian Herbal Pharmacopoeia regarding the yield of kelor leaves extract.

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