Jurnal Tumbuhan Obat Indonesia
Vol. 17, No 1, Juli 2024, hal 1-13

ISSN: 1979-892X (print)
ISSN: 2354-8797 (online)

Chemical Characterization of *Moringa oleifera* Lam. from Six Growth Locations in Central of Java: An Initiation of Standardization

Karakterisasi Kimia *Moringa oleifera* Lam. dari Enam Lokasi Tumbuh di Jawa Tengah: Inisiasi Standardisasi

Mery Budiarti[1*)], Nur Rahmawati Wijaya[1)], Wahyu Jokopriyambodo[1)]
[1]Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Bogor, Indonesia
[*]e-mail: mery.budiarti.supriadi@brin.go.id

ABSTRACT

Moringa oleifera Lam. is in high demand as a raw material for pharmaceutical constituents and dietary supplements, particularly for producing stunting supplements. However, recommendations regarding M. oleifera quality standards have not supported this claim as scientific evidence of its safety and effectiveness. This research intends to expand knowledge regarding the quality standards of M. oleifera leaves, particularly their chemical properties. Height-variant samples of M. oleifera were collected from six locations in the Central Java, Indonesia. A hierarchical cluster analysis (HCA) was performed to group the most each parameter's values. The parameters included water-soluble extract, ethanol-soluble extract, total ash content, acid-insoluble ash, total flavonoid content was quantified as quercetin, and thin-layer chromatography (TLC) fingerprint. The suggestion of specific value for each parameter as a future reference for M. oleifera, including water- and ethanol-soluble extract content of at least 41% and 19%, and a total- and acid-insoluble ash content of a maximum of 11% and 4%, respectively. The minimal quercetin contains as the chemical marker compound of M. oleifera was a minimum of 1.285%. The chromatogram profile of TLC suggested 7 (254 nm) and 8 (366 nm) spots.

Keywords**:** *Chemical profile, cluster analysis, moringa, quality standard, standardization*

ABSTRAK

Kelor (*Moringa oleifera* Lam.) sangat diminati sebagai bahan baku bahan farmasi dan suplemen makanan, khususnya untuk memproduksi suplemen stunting. Namun, ketersediaan rekomendasi mengenai standar kualitas *M. oleifera* yang dapat mendukung klaim keamanan dan efektivitasnya belum tersedia. Penelitian ini bertujuan untuk memperluas pengetahuan mengenai baku mutu daun *M. oleifera*, khususnya sifat kimianya. Sampel *M. oleifera* dengan varian tinggi dikumpulkan dari enam lokasi di Jawa Tengah, Indonesia. Analisis klaster hierarki (HCA) dilakukan untuk mengelompokkan sebagian besar nilai setiap parameter. Parameternya meliputi ekstrak larut air, ekstrak larut etanol, kadar abu total, abu tidak larut asam, kandungan total flavonoid yang diukur sebagai kuersetin, dan sidik jari kromatografi lapis tipis (KLT). Usulan nilai spesifik untuk setiap parameter sebagai acuan *M. oleifera* di masa mendatang, antara lain kadar sari larut air dan etanol minimal 41% dan 19%, serta kadar abu total dan tidak larut asam maksimal 11 % dan 4%, masing-masing. Kandungan minimal kuersetin sebagai senyawa penanda kimia *M. oleifera* minimal sebesar 1,285%. Profil kromatogram KLT menunjukkan 7 (254 nm) dan 8 (366 nm) spot.

Kata kunci: Profil kimia, analisis klaster, kelor, kontrol kualitas, standarisasi

Received 14-11-2023
Revised 20-02-2024
Accepted 28-06-2024
Publish 01-07-2024

DOI: 10.31002/jtoi.v16i2.885

INTRODUCTION

Moringa oleifera Lam., one of the most prominent plants, is a dietary and medicinal supplement. This plant is renowned for its anti-diabetes, anti-inflammatory, anti-cancer, anti-bacterial, anti-fungal, antioxidant, hepatoprotection, cardiovascular, wound healing, and anti-ulcer, anti-inflammatory, and contraceptive properties (Priyanshu, Nancy, & Patil, 2020). Recent scientific advancements indicate that biomolecular or nutrigenomic, supplements manufactured from *M. olerifera* can overcome the Indonesian health problem of stunting (Putra, Setiawan, Sanjiwani, Wahyuniari, & Indrayani, 2021). Evidence shows that *M. oleifera* supplements can increase a toddler's weight and height (Abidin & Liliandriani, 2021).

Therefore, *M. oleifera* is known as the "Miracle Tree". The increased use of *M. oleifera* leaves increases the market demand. Indonesia's tropical environment and fertile soil are ideal for establishing a production center for *M. oleifera* raw resources. In contrast, the geography of Indonesia consists of numerous islands with various genetic resources. Therefore, it can potentially have moringa variants (Riastiwi, Damayanto, Ridwan, Handayani, & Leksonowati, 2018; Ridwan, Hamim, Suharsono, Hidayati, & Gunawan, 2021). This reality required developing quality standards for *M. oleifera*, mainly used as a pharmaceutical ingredient. Information on the *M. oleifera* plant quality parameter criteria is still limited.

Standardized raw material procedures must serve as the foundation for the quality of herbal medicines. The standardization procedures provide quality consistency in terms of safety and efficacy. Additionally, it improves confidence in the purity and utility of medicinal raw materials. Observation of a minimum of three samples from distinct places should be used to establish quality parameter guidelines for herbal components (Bata, Wijaya, & Setiawan, 2018). The diversity of pharmaceutical substances should be examined, and significant profiles might serve as benchmarks or references for quality aspects.

Karanganyar Regency is in Central of Java, Indonesia, with coordinates 7^{o} 35` 45.96`` S, 110$^{o}$ 57` 2.88`` E. Karanganyar is positioned in a basin between Mount Lawu and the Kendeng mountains. Therefore, the topography is hilly, with an elevation maximum and minimum, respectively of 3,232 and 32.93 masl. Karanganyar gains some benefits from this morphology, one of them is the wide variety of soil types, such as regosol, Mediterranean, andosol, litosol, and grumusol (Priyono, Jumadi, Saputra, & Fikriyah, 2020). This geographical condition makes Karanganyar one of the major regions for developing medicinal plants and makes Central Java the center of traditional medicine, or *jamu*, in Indonesia (Karanganyar, 2017; Syafitri & Azeriansyah, 2019). These facts become one of the considerations in this study's decision to use *M. oleifera* samples from the Karanganyar region.

Classification or grouping is a fundamental procedure for establishing scientific standards. Classification plays a crucial role in forecasting the similarity of the properties possessed by an entity. Several classification methods include mapping using principle component analysis (PCA), k-means, hierarchical cluster analysis (HCA), and neural networks (Drab & Daszykowski, 2014; Leal, Llanos, Restrepo, Suárez, & Patarroyo, 2016). Based on the ISI web of data science, HCA has become a common method for classifying entities based on their chemical constituents. It is the rationale behind using HCA in drug development, including standardizing drug raw materials (Leal et al., 2016). Standardization of natural-based pharmaceutical ingredients is required to reduce the reality of variety (Muyumba, Mutombo, Sheridan, Nachtergael, & Duez, 2021; Saleem et al., 2014).

The Pharmacopoeia is one of the guidelines for ingredient quality requirements, including a monograph of naturopathic substances in the form of dry material or extracts for certain standardized plant species. It lists physicochemical and phytochemical parameters as quality indicators for natural-based product ingredients. These two characteristics are necessary to assert the pharmacological action of herbal medicinal components (WHO, 2021). The physicochemical properties represent the purity and efficacy of medical raw materials. Total ash, acid soluble ash, water soluble extractive value, and alcohol soluble extractive value are standard methods for physicochemical properties of a natural-based product ingredient (Fatima, Khan, Ahmad, Badruddeen, & Akhtar, 2024; Gaddaguti et al., 2015). Numerous chemical components in plant-based raw materials are referred to as phytochemical characteristics. These chemical's expression relies on abiotic, biological, and genetic circumstances and taxonomic identity (species, varieties, cultivars).

However, Pharmacopoeia has not previously provided a standard reference for *M. oleifera* based drug source materials. Similar studies related to the diversity of physicochemical and phytochemical of *M. oleifera* each utilized three samples from different growing locations (Bata et al., 2018). The scope of the two research was limited to a descriptive evaluation of the range of values for essence content, ash content, and total flavonoids, with no detailed data processing. This condition is possible due to the limited number of samples for classification. Therefore, this study uses more samples so that classification can be carried out to analyze the homogeneity of each variable. It is essential because it is critical to determine the reference value of a pharmaceutical ingredient derived from natural sources. This study aimed to assess the reference value of *M. oleifera* from six growth locations in the Karanganyar Regency with varying elevations.

METHOD

Materials

The sample used in this study was dried *M. oleifera* from six different elevations of growth location in Karanganyar Regency, Central Java (Figure 1). Table 1 shows a more detailed sample identity (IDs).

Table 1. Identity of Moringa (*Moringa oleifera* Lam.) samples

No	Origin of sample location	Code	Elevation (masl)
1	Jaten	Jt	100
2	Karanganyar	Kr	240
3	Matesih	Mt	380
4	Karangpandan	Kp	500
5	Ngargoyoso	Ng	790
6	Tawangmangu	Tw	1,100

The materials used for the analysis were absolute ethanol per analysis (Merck), absolute methanol pro analysis (Merck), chloroform (Merck), aluminium (III) chloride /$AlCl_3$ (Merck), quercetin standard (Sigma Aldrich), aquadest (Ikapharmindo), hydrochloric acid/HCl pro analysis (Merck), 96% ethanol, and distilled water.

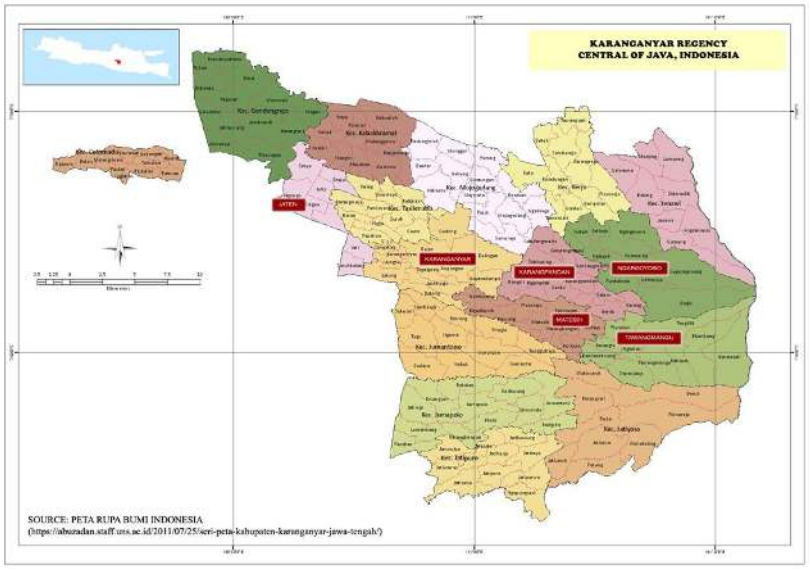

Figure 1. Six growth locations of samples

Methods

The age of the samples included in this study was six months. The sampling occurred in March, followed by analysis from April through September at the Laboratory of Medicinal Plants and Traditional Medicinal Research and Development Center (MPTMRD), Tawangmangu. Laboratory analysis can be divided into dried sample preparation, water- and ethanol-soluble extractive value, total ash and insoluble-acid ash levels, chemical marker compound content, and thin layer chromatography (TLC) fingerprint profiles.

Sample Preparation

The harvested *M. oleifera* plants are sorted, rinsed, and dried in an oven at 40°C until their moisture content falls below 10%, particularly for leaves based on handling requirements for natural-based product ingredients. The sample was crushed and filtered through a 40-mesh filter after being dried.

Water- and ethanol-soluble extractive value

The determination of the water- or the ethanol-soluble extractive value was based on the gravimetric principle, modified slightly from Pharmacopeia guidelines (Kementerian Kesehatan, 2017). 5 grams of each sample material were weighed, and 100 mL of the chloroform water mixture was added. A shaker homogenized the mixture for 6 hours, then deposited at room temperature for 18 hours. After settling, the filtrate was filtered through filter paper, and 20 mL of it was transferred to a pre-weighed porcelain cup. The extract filtrate was evaporated, and the dried extract filtrate was heated in an oven at 105°C for 2 hours. After 2 hours, the porcelain cup was transferred to a desiccator until the temperature reached room temperature. The following step is to measure and record the weight of the porcelain cup containing the dry extract filtrate. Heating and weighing are accomplished until a consistent weight is achieved thrice.The experiment was performed in triplicate.

Except for the solvent, the steps to determine ethanol-soluble extractive value are the same as for water. A mixture of water and chloroform was used as a solvent for determining the water-

soluble extractive value, whereas ethanol solution was utilized to determine the ethanol-soluble extractive value. The necessary variables are entered into the appropriate formula to determine the extractive value, whether it is water- or ethanol-soluble, in the final stage.

The final step is calculating the value of the water- or ethanol-soluble extractive content by entering the required variables into a specified formula (Eq.1).

$$\text{Extractive value } (\%) = \frac{\text{final weight (G)- initial weight (G)}}{\text{sample weight (G)}} \times \text{dilution factor} \times 100\% \quad \textbf{(Eq.1)}$$

Information:

Extractive content (%)	=	percentage of water- or ethanol-soluble extractive value
Final weight (gram)	=	weight of porcelain and filtrate of the dried extract by heating
Initial weight (gram)	=	weight of porcelain before adding extract

Total ash content

The standard procedure for assessing total ash and acid-insoluble ash content is based on gravimetry (Kementerian Kesehatan, 2017). Two grams of sample powder were weighed and deposited in a silica crucible. In a furnace at 500 ^{o}C, burn the sample powder in the crucible until the ash is burned. Cooling the remaining crucible in a desiccator to room temperature and weighing it gives a steady weight. Adding hot water and filtering using ash-free filter paper is the next step if the remaining ash cannot be eliminated. Once the ash has been filtered and adhered to the filter paper, it is returned to the crucible for further burning in a furnace set at 500 ^{o}C. After cooling in a desiccator, it is weighed until it reaches a constant weight.

A sample was replicated three times for the experiment. In the final step, the total ash and acid-insoluble ash content is calculated by putting the value of the dependent variable into the given equation (Eq.2).

$$\text{Ash content } (\%) = \frac{\text{final weight (G)- initial weight (G)}}{\text{sample weight (G)}} \times 100\% \quad \textbf{(Eq.2)}$$

Information:

Ash content (%)	=	percentage of total ash or acid-insoluble ash content
Final weight (gram)	=	weight of crucible and ash
Initial weight (gram)	=	weight of the empty crucible

Acid-insoluble ash content

The acid-insoluble ash content is determined by extending the total ash content determination process. The residual ash from the previous stage was added to 25 mL of 10% HCl solution, heated for 5 minutes, and filtered through ash-free filter paper (Whatman no. 41). The acid-insoluble ash that adheres to the filter paper is subsequently rinsed with hot water until it is free of the 10% HCl solution. The remaining acid-insoluble ash is placed in a crucible previously used, heated in a furnace, and incinerated until a consistent weight is obtained. Filter paper is also used in this process. Calculate acid-insoluble ash content using formula (Eq.2). The experiment also involved replicating a sample three times.

Total Flavonoid Content

The total flavonoid content assay method is likewise based on the Pharmacopoeia, with a few minor adjustments to the sample test preparation. Spectrophotometry is used to measure total flavonoid content (Kementerian Kesehatan, 2017). The development of a standard curve is crucial in considering the total flavonoid content. In this research, quercetin was utilized to measure the total flavonoid concentration. Ten ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm quercetin standard solutions were developed. Each concentration was adjusted to a volume of 2 mL, and 1 mL of 10% $AlCl_3$ and 2 ml of distilled water were added. The blank solution consisted of a mixture of 2 mL of each quercetin standard solution concentration and 3 mL of distilled water. Then, both mixes were incubated for 30 minutes at room temperature. Determine the absorbance of the concentration series of the quercetin solution at 427 nm. The calibration curve was generated by connecting the absorbance value (y) and solution concentration (x), and the linear regression equation was calculated to be $y = a + bx$.

The test sample was created in triplicate. Each powder sample of *M. oleifera* was weighed at 100 mg, 10 mL of ethanol was added, the sample was sonicated for 15 minutes, and it was left at room temperature for 12 hours. Then, 5 mL of the filtrate was collected and dried at 60 °C. The dried filtrate was mixed with 5 mL of methanol, stirred, and then allowed to precipitate at room temperature for 12 hours. 2 mL of sample filtrate, 1 mL of a 10% $AlCl_3$ solution, and 2 mL of distillation water are the components of test samples for UV-visible spectrophotometry measurements. The blank solution contained 2 mL of sample filtrate and 3 mL of distilled water. Following 30 minutes of incubation at room temperature, the absorbance at 427 nm was measured. The total flavonoid content was calibrated using the linear regression equation on the calibration curve and represented as a percentage of milligram equivalent to quercetin per gram of sample.

TLC Chemical Fingerprint Profile

Chemical fingerprint profiling was performed using TLC devices and CAMAG software. A 100 mg sample of *M. oleifera* powder was dissolved in 1 mL of a methanol solution and left at room temperature for 12 hours. The filtrate was filtered using Whatman no. 40 filter paper to remove residue. Next, spot each acquired filtrate to a volume of 5 µl and elute using a chloroform: methanol (8:2) mixture. Rf measurements were conducted at wavelengths 254 and 366 nm after the elution results were dried and documented.

Data Analysis

The data for each variable were analyzed using IBM SPSS 26 software with a Hierarchical Cluster Analysis (HCA) method.

RESULTS AND DISCUSSION

Several physicochemical characteristics, including water- and ethanol-soluble extract value, total ash and acid-insoluble ash content, were evaluated for this study. In addition, phytochemical characteristics included total flavonoid content as a chemical marker and TLC fingerprint profiles. Table 2 provides the mean values for each parameter for every sample of *M. oleifera*.

Table 2**.** Physicochemical and phytochemical analysis of *M. oleifera* Lam.

Parameter (%)	Samples code (elevation (masl))						Reference value
	Sk (100)	Kr (240)	Mt (380)	Kp (500)	Ng (790)	Tw (1,100)	
Water-soluble extractive	41,68 ± 0,43 *(2)*	47,09 ± 0,91 *(1)*	44,33 ± 1,49 *(2)*	47,32 ± 0,43 *(1)*	36,61 ± 0,47 *(3)*	43,38 ± 0,14 *(2)*	**≥ 41**
Ethanol-soluble extractive	24,62 ± 0,76 *(2)*	27,35 ± 0,83 *(1)*	19,69 ± 0,20 *(2)*	28,36 ± 0,30 *(1)*	23,12 ± 0,28 *(2)*	21,50 ± 0,62 *(2)*	**≥ 19**
Total ash	9,69 ± 0,00 *(2)*	7,96 ± 0,03 *(1)*	11,19 ± 0,05 *(2)*	7,81 ± 0,03 *(1)*	10,46 ± 0,03 *(2)*	10,05 ± 0,21 *(2)*	**≤ 11**
Acid-insoluble ash	3,25 ± 0,05 *(1)*	1,50 ± 0,26 *(2)*	3,30 ± 0,05 *(1)*	2,33 ± 0,25 *(2)*	3,70 ± 0,43 *(1)*	4,24 ± 0,18 *(1)*	**≤ 4**
Total Flavonoid	1,665 ± 0,107 *(2)*	1,285 ± 0,079 *(1)*	1,375 ± 0,085 *(1)*	1,020 ± 0,086 *(3)*	1,380 ± 0,087 *(1)*	1,423 ± 0,072 *(1)*	**≥ 1,285**

Note: HCA reveals that the average number followed by the same number for each parameter are clustered together

Water- and ethanol-soluble extractive value

The *M. oleifera* utilized as a sample in this research exhibits a wide range of extractive values. The water- and ethanol-soluble extract values reflected the level of extract solubility of the dried material. (Bhargava, Saluja, & Dholwani, 2013). Extraction values obtained with different solvents are used to assess quality, purity and detect adulteration of expired and inappropriate drugs process (Hait, 2021). Upon determining the water-soluble extractive value, it was determined that the Karangpandan and Karanganyar samples had the highest values, 47%. At the same time, Ngaryoso's water-soluble extractive value was the lowest (Table 2). Three clusters emerged from a hierarchical cluster analysis of water-soluble extractive value. The largest cluster comprises three individuals, Jaten, Matesih, and Tawangmangu, with water-soluble extract percentages between 41% and 43%. Therefore, the relevant water-soluble extractive value for reference is at least 41%.

According to the ethanol-soluble extractive value, Matesih and Karangpandan samples exhibited the lowest and highest values, 19% and 28%, respectively (Table 2). HCA result was utilized to classify each ethanol-soluble extractive value. We can conclude from this that there are two clusters, with the primary cluster containing four members: Jaten, Matesih, Ngargoyoso, and Tawangmangu. The percentage of ethanol-soluble extracts value in the primary groups varied between 19% and 24%. In conclusion, the reference value for the ethanol-soluble extractive value of dried *M. oleifera* should be greater than 19%. The reference values for the dried plant material's water- and ethanol-soluble extractive values are established using the Indonesian Pharmacopeia recommended minimal limits.

Bata, Wijaya, and Setiawan (2018), using samples of *M. oleifera* from Bogor, Batu, and Pacet, recommend water- ≥ 33% and ethanol-soluble extractive values ≥ 21% as a reference

standard. This number was derived from the two samples with the lowest yield, Bogor and Batu. The variation in extractive value may be attributed to a fundamental difference, namely the environment in which the sample grew (Bata et al., 2018).

Both research results indicated that the water-soluble extractive value of *M. oleifera* is higher than the ethanol-soluble extractive value, indicating that the sample contains chemical compounds with a polarity similar to that of water. Rachmawati and Suriawati (2019) revealed that *M. oleifera* extracted with water contain secondary metabolites such as flavonoids, phenols, triterpenoids/steroids, tannins, and saponins (Rachmawati & Suriawati, 2019). Moringa also contains minerals, vitamins (especially B and C), and carbohydrates that are soluble in water (Karmakar, Chandra Mondal, & Horijan, 2024; Singh, Kaushik, Jyothsna, Ameta, & Kumar, 2024).

Total ash and acid-insoluble ash content

The results for total ash and acid-insoluble content varied in Table 2. The Matesih sample had the highest total ash content (11.19%), while the Karangpandan sample had the lowest (7.81%). HCA result of the total ash content revealed two clusters. The main cluster consists of four samples, Jaten, Matesih, Ngargoyoso, and Tawangmangu, whose total ash concentration ranges from 9.69% to 11.19%. The analysis concluded that the recommended total ash content for dried *M. oleifera* maximum is 11%.

Table 2 also revealed that the Tawangmangu sample had the highest acid-insoluble ash level (4.24%), whereas the Karanganyar sample had the lowest acid-insoluble ash content (1.5%). The HCA result of each acid-insoluble ash value in each of the six samples revealed the presence of two clusters. Four samples from Jaten, Matesih, Ngargoyoso, and Tawangmangu make up the main cluster. The range of acid-insoluble ash levels in main clusters was 3.25% to 4.24%. Consequently, the standard for acid-insoluble ash content is not to exceed 4%. The content of total ash and acid-insoluble ash is adjusted to fulfill the specifications of the Indonesian Pharmacopoeia. This guideline specifies the maximum value or the value not to exceed.

In contrast, Bata et al.'s research on *M. oleifera* sample obtained reference values of 10% and 2% for total ash and acid insoluble, respectively. This value is determined by the highest value received from the three samples, the Bogor and Batu sample (Bata et al., 2018). According to the findings of these two research, the average percentage of total ash in dried *M. oleifera* was greater than the percentage of acid-insoluble ash. This observation implies that the dried *M. oleifera* has high mineral content. Pre-harvest and post-harvest treatments affect the mineral content of dried material as measured as ash. Pre-harvest mineral content is closely related to plant species naturally occurring mineral elements. One of the factors impacting the macro and micro levels of the mineral elements naturally carried by plant species is the geography and properties of the soil. Post-harvest treatments that can impact ash include poor cleaning processes for harvested material, drying, and storage that allow contamination of other chemicals. This issue is the primary explanation for the variation in the recommended ash content reference values between these two studies.

Calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), manganese (Mn), iron (Fe), zinc (Zn), and heavy metals are minerals typically found in *M. oleifera* (Fakankun OA, Babayemi, & Utiaruk JJ, 2013). Oxalic acid and phytic acid are naturally contained in plants which bind minerals and act as anti-nutritional factors which reduce nutrient availability (Feizollahi et al., 2021; Joseph, Uzoma, Juliana, & Precious, 2024; Nagraj, Chouksey, Jaiswal, & Jaiswal, 2020). Ibraheem et al. (2019) reported that *M. oleifera* contain low quantities of oxalic and phytic acids.

Both substances are vulnerable to forming acid-insoluble crystals, such as calcium oxalate and magnesium oxalate, which inhibit the absorption of micronutrient minerals in the digestive system. Generally, the concentration of these crystals is detectable as acid-insoluble ash contents (Ibraheem et al., 2019). Further analysis needs to be carried out to determine the mineral elements in the *M. oleifera* samples involved in this study.

Total Flavonoid Content

Flavonoids are secondary metabolites commonly found in plants, fruits, and vegetables. The major flavonoids of *M. oleifera* are myricetin, quercetin, and kaempferol. Quercetin is one type of flavonoid group with the classification of flavanol compounds. Most quercetin compounds found in plant tissues are in the form of glycosides (Laksmiani, Widiantara, Adnyani, & Pawarrangan, 2020; Makita, Chimuka, Steenkamp, Cukrowska, & Madala, 2016; Vergara-Jimenez, Almatrafi, & Fernandez, 2017). According to Vergara-Jimenez, Almatrafi, and Fernandez (2017), quercetin is generally found in dried *M. oleifera*, commonly within the shape of quercetin-3-O-β-d-glucoside with median attention of 100 mg/100 g dry leaves (Vergara-Jimenez et al., 2017). Therefore, in this study, quercetin was used as the standard.

In Table 2, Karangpandan had the lowest total flavonoid content (1.020%), whereas Jaten had the highest (1.665%). The results of the HCA reveal three clusters, with Matesih, Ngargoyoso, Tawangmangu, and Karanganyar being the main cluster (Figure 2). The reference value for the total flavonoid compound concentration in the dried sample of *M. oleifera* can be derived using the minimum value of the main cluster member, Karanganyar. Hence, quality parameter data of the level of the marker compound in dried *M. oleifera* can be used at least 1.285%, according to Pharmacopeia, where the marker value applies a minimum limit.

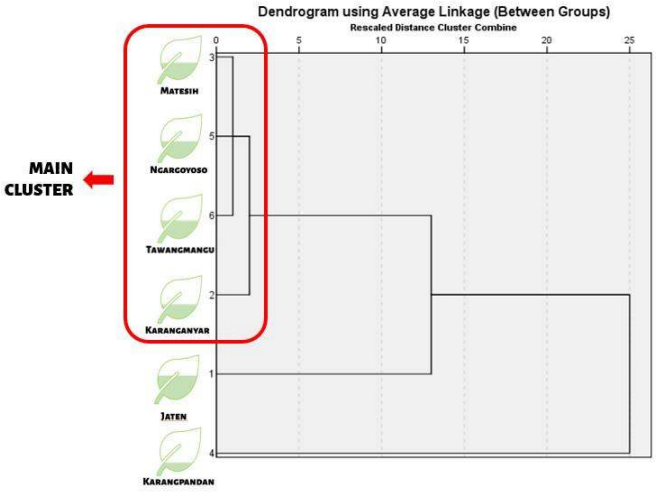

Figure 2. HCA Results of Total Flavonoid Content of Dried *M. oleifera* Lam.

In previous research by Bata, Wijaya, and Setiawan (2018) and Vergara-Jimenez, Almatrafi, and Fernandez (2017), the total flavonoid content of each sample was 0.5% and 0.1%, respectively (Bata et al., 2018; Vergara-Jimenez et al., 2017). In this study, the total flavonoid contents of each dried *M. oleifera* was greater than 1 percent. In this study, the total flavonoid content of the six *M. oleifera* samples from Karanganyar Regency is relatively high than those of the previous research. The amount of bioactive compounds in *M. oleifera* is influenced by genotypic features, location, season, harvest time, post-harvest procedure, and storage conditions (Sulastri et al., 2018).

Thin-Layer Chromatography Fingerprint Profile

A chemical fingerprint profile is one method for identifying and recognizing natural products, particularly those used as pharmaceutical raw materials. Chemical fingerprints are a profile of secondary metabolites found in a natural source. TLC is a simple procedure that is often used for both the qualitative evaluation of low levels contamination and the quantitative analysis of natural medicine that are listed within various pharmacopeias (Giri et al., 2020; Sonam, Singh, & Saklani, 2017). Additionally, it is a rapid and low-cost procedure (Kartini, Andriani, Priambodo, Jayani, & Hadiyat, 2021). The Pharmacopoeia provides chromatogram data from TLC as guidelines for the quality of pharmaceutical raw materials.

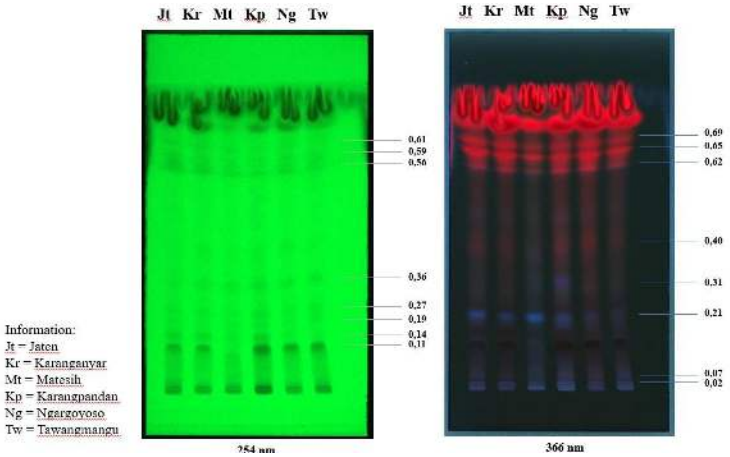

Figure 3. TLC Chemical Fingerprinting of *M. oleifera* Lam.

Figure 3 is a chromatogram of the dried *M. oleifera* used in this research as a sample. On the 254 nm chromatogram, Karanganyar, Karangpandan, Ngargoyoso, and Tawangmangu had more compound spots than the other two samples. There were eight spots with Rf values. The Karangpandan chromatogram at 366 nm contained seven spots, whereas the other samples contained six. The profiles of chromatograms for each sample can vary based on the concentrations of substances. The expression of the thickness of the compound spots at a particular Rf value also influences the concentration.

The Rf showing in each sample, either at 254 or 366 nm, was subjected to HCA result, yielding four clusters. The main cluster has the most members, with samples from Ngargoyoso, Tawangmangu, and Karanganyar (Figure 4). The chemical fingerprint profiles of the three samples generated eight spots at 254 nm and six spots at 366 nm when analyzed by TLC. The reference for the chemical fingerprint profile employed for the simplisia of Moringa leaves about to is at least comparable to these three samples' common chromatograms.

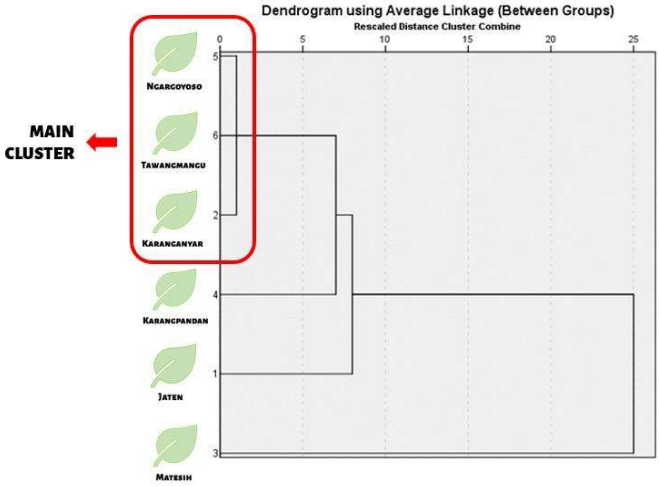

Figure 4. HCA Results of TLC Fingerprinting of *M. oleifera* Lam

The location at which *M. oleifera* grows affects physicochemical and phytochemical properties. Specific quality standards are essential for it to be used as a raw material for pharmaceuticals or dietary supplements. Standardization is closely related to safety and efficacy claims. Therefore, availability is crucial. Recommended references for the quality of dried *M. oleifera* include water- and ethanol-soluble extractive values of at least 41% and 19%, total ash and acid-insoluble ash contents of maximum 11% and 4%, and total flavonoid component content of minimum 1.285%. Then, at least seven (254 nm) and eight (366 nm) spots make up the standard chromatogram pattern.

CONCLUSION

The location at which *M. oleifera* grows affects physicochemical and phytochemical properties. Specific quality standards are essential for it to be used as a raw material for pharmaceuticals or dietary supplements. Standardization is closely related to safety and efficacy claims. Therefore, availability is crucial. Recommended references for the quality of dried *M. oleifera* include water- and ethanol-soluble extractive values of at least 41% and 19%, total ash and acid-insoluble ash contents of maximum 11% and 4%, and total flavonoid component content of minimum 1.285%. Then, at least seven (254 nm) and eight (366 nm) spots make up the standard chromatogram pattern.

ACKNOWLEDGMENT

The authors are grateful to the Medicinal Plants and Traditional Medicinal Research and Development Center (MPTMRD), Ministry of Health of Indonesia in Tawangmangu, for funding and facilitating this research. Great appreciation is given to all laboratory assistants in MPTMRD, Tawangmangu.

REFERENCES

Abidin, U. W., & Liliandriani, A. (2021). Moringa oleifera sebagai makanan pendamping asi pada balita stunting. *J Kesmas*, *7*(1), 40. https://doi.org/10.35329/jkesmas.v7i1.1759

Ajazuddin, & Saraf, S. (2010). Evaluation of physicochemical and phytochemical properties of safoof-e-sana, a unani polyherbal formulation. *Phcog Res*, *2*(5), 318. https://doi.org/10.4103/0974-8490.72332

Alegantina, S., Isnawati, A., & Widowati, L. (2013). Kualitas ekstrak etanol 70% daun kelor (Moringa oleifera Lam.) dalam ramuan penambah asi. *JKI*, *3*(1), 1–8.

Bata, M. H. C., Wijaya, S., & Setiawan, H. K. (2018). Standarisasi simplisia kering daun kelor (Moringa oleifera) dari tiga daerah berbeda. *J Pharm Sci & Pract*, *5*(1), 45–52.

Bhargava, V., Saluja, A., & Dholwani, K. (2013). Detection of heavy metal contents and proximate analysis of roots of Anogeissus latifolia. *Phytojournal*, *1*(6), 61–65.

Dinas Lingkungan Hidup dan Kehutanan. (2007). Status lingkungan hidup daerah kabupaten karanganyar tahun 2007. In *Dinas Lingkungan Hidup dan Kehutanan Karanganyar*.

Drab, K., & Daszykowski, M. (2014). Clustering in analytical chemistry. *J AOAC Int*, *97*(1), 29–38. https://doi.org/10.5740/jaoacint.SGEDrab

Fakankun OA, Babayemi, J., & Utiaruk JJ. (2013). Variations in the mineral composition and heavy metals content of Moringa oleifera. *Afr J Range Forage Sci*, *7*(6), 372–379. https://doi.org/10.5897/ajest12.228

Ibraheem, A. S., Nuhu, A., Musa, M., & Ashade, N. O. (2019). Evaluation of proximate content and vitamin profile of Moringa oleifera seed hull. *Int J Livest Prod*, *10*(8), 188–191. https://doi.org/10.5897/ijlp2018.0512

Karanganyar, P. K. (2017). Kabupaten Karanganyar. Retrieved September 17, 2022, from Kabupaten Karanganyar website: https://www.karanganyarkab.go.id/category/profile/kabupaten/

Kartini, K., Andriani, Y. A., Priambodo, W., Jayani, N. I. E., & Hadiyat, M. A. (2021). Validating and developing tlc-based fingerprinting for Curcuma longa L. *J Pharm Pharmacogn Res*, *9*(5), 704–715.

Kementerian Kesehatan. (2017). *Farmakope herbal indonesia edisi II*. Kementerian Kesehatan RI.

Laksmiani, N. P. L., Widiantara, I. W. A., Adnyani, K. D., & Pawarrangan, A. B. S. (2020). Optimasi metode ekstraksi kuersetin dari daun kelor (Moringa oleifera Lam.). *Jurnal Kimia*, *14*(1), 19. https://doi.org/10.24843/jchem.2020.v14.i01.p04

Leal, W., Llanos, E. J., Restrepo, G., Suárez, C. F., & Patarroyo, M. E. (2016). How frequently do clusters occur in hierarchical clustering analysis? a graph theoretical approach to studying ties in proximity. *J Cheminform*, *8*(4), 1–16. https://doi.org/10.1186/s13321-016-0114-x

Makita, C., Chimuka, L., Steenkamp, P., Cukrowska, E., & Madala, E. (2016). Comparative analyses of flavonoid content in Moringa oleifera and Moringa ovalifolia with the aid of uhplc-qtof-ms fingerprinting. *S Afr J Bot*, *105*, 116–122. https://doi.org/10.1016/j.sajb.2015.12.007

Muyumba, N. W., Mutombo, S. C., Sheridan, H., Nachtergael, A., & Duez, P. (2021). Quality control of herbal drugs and preparations: the methods of analysis, their relevance and applications. *Talanta*, *4*, 100070. https://doi.org/10.1016/j.talo.2021.100070

Nicoletti, M. (2011). Hptlc fingerprint: a modern approach for the analytical determination of botanicals. *Rev Bras Farmacogn Braz J Pharmacogn*, *21*(5), 818–823. https://doi.org/10.1590/S0102-695X2011005000131

Priyanshu, J., Nancy, J., & Patil, U. (2020). Phytochemical and pharmacological profile of Moringa oleifera Lam. *IJPSR*, *11*(12), 5968–5973. https://doi.org/10.13040/IJPSR.0975-8232.11(12).5968-73

Putra, A. I. Y. D., Setiawan, N. B. W., Sanjiwani, M. I. D., Wahyuniari, I. A. I., & Indrayani, A. W. (2021). Nutrigenomic and biomolecular aspect of moringa oleifera leaf powder as supplementation for stunting children. *J Tropical Biodiversity Biotechnology*, *6*(1), 1–15. https://doi.org/10.22146/jtbb.60113

Rachmawati, S. R., & Suriawati, J. (2019). Characterization of moringa (Moringa oleifera Lam.) leaf water extracts by chemical and microbiology. *SANITAS*, *10*(2), 102–116.

Riastiwi, I., Damayanto, I. P. G. P., Ridwan, R., Handayani, T., & Leksonowati, A. (2018). Moringa oleifera distribution in java and lesser sunda islands which is attributed with annual rainfall. *Biosaintifika*, *10*(3), 613–621. https://doi.org/10.15294/biosaintifika.v10i3.16115

Ridwan, Hamim, Suharsono, Hidayati, N., & Gunawan, I. (2021). Drumstick (Moringa oleifera) variation in biomass and total flavonoid content in indonesia. *Biodiversitas*, *22*(1), 491–498. https://doi.org/10.13057/biodiv/d220159

Saleem, U., Hussain, K., Ahmad, M., Bukhari, N. I., Malik, A., & Ahmad, B. (2014). Physicochemical and phytochemical analysis of Euphorbia helioscopia (L.). *Pak J Pharm Sci*, *27*(3), 577–585.

Soetan, K., Olaiya, C., & Oyewole, O. (2010). The importance of mineral elements for humans, domestic animals and plants: a review. *Afr J Food Sci*, *4*(5), 200–222.

Sulastri, E., Zubair, M. S., Anas, N. I., Abidin, S., Hardani, R., Yulianti, R., & Aliyah. (2018). Total phenolic, total flavonoid, quercetin content and antioxidant activity of standardized extract of Moringa oleifera leaf from regions with different elevation. *Pharmacogn J*, *10*(6), S104–S108. https://doi.org/10.5530/pj.2018.6s.20

Sumbul, S., Ahmad, M. A., Asif, M., Akhtar, M., & Saud, I. (2012). Physicochemical and phytochemical standardization of berries of Myrtus communis Linn. *J Pharm Bioallied Sci*, *4*(4), 322–326. https://doi.org/10.4103/0975-7406.103266

Syafitri, A. K. N., & Azeriansyah, R. (2019). Pemanfaatan geoportal dalam visualisasi peta ancaman bencana tanah longsor kabupaten karanganyar berdasarkan simulasi tingkat intensitas curah hujan. *Jurnal Geodesi Dan Geomatika*, *02*(01), 1–8. Retrieved from https://ejournal2.undip.ac.id/index.php/elipsoida/article/view/3438

Vergara-Jimenez, M., Almatrafi, M. M., & Fernandez, M. L. (2017). Bioactive components in Moringa oleifera leaves protect against chronic disease. *Antioxidants*, *6*(4), 1–13. https://doi.org/10.3390/antiox6040091

Wahyuni, W. T., Saharah, M., Arif, Z., & Rafi, M. (2020). Thin layer chromatographic fingerprint and chemometrics analysis for identification of Phyllanthus niruri from its related species. *J Idn Chem Soc*, *3*(1), 47. https://doi.org/10.34311/jics.2020.03.1.47

WHO. (2021). WHO expert committee on specifications for pharmaceutical preparations. In *World Health Organization - Technical Report Series*. Geneva: World Health Organization.