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Evaluation of Maja Leaf Simplisia (*Aegle marmelos* L.) for Herbal Standardization: Physicochemical and Microbial Testing

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ABSTRACT

Standardization aims to determine the quality of maja leaf simplicia (Aegle marmelos L.) on non-specific parameters, which include drying shrinkage test, moisture content test, ash content test, specific gravity test, total plate count test, yeast mold count test, and heavy metal contamination test. This study uses descriptive qualitative and quantitative analytical methods. The sample in this study was taken from the village of Kedamean, Gresik Regency. The standardization results on non-specific parameters showed that the drying shrinkage was 0.423%, the moisture content was 0.77%, and the ash content was 6.62%. The total plate number test results were 0.6 × 107 colonies/mL, while the total yeast contamination number was 326.6 colonies/mL. Then, for testing for heavy metal contamination, Pb did not find contamination, and Cd obtained results of 4.60 mg/kg, Cu 268.21 mg/kg, and Zn 10.89 mg/kg. Based on the research conducted, maja leaf simplicia powder on non-specific parameters, namely drying shrinkage, water content, total ash content, specific gravity, total plate number, and heavy metal contamination Pb, Cd, and Zn has fulfilled the requirements and conditions indicating that the simplicia has good quality. The results suggest that the maja leaf simplisia met most non-specific parameter standards, except for yeast and copper levels, which exceeded the acceptable limits.

Keywords:
Maja leaves; Non-Specific Parameters, Aegle Marmelos; Simplisia Quality; Heavy Metal Contamination; Microbial Count; Standardization Test

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1. Introduction

Aegle marmelos L., or maja, is widely recognized in traditional medicine. According to Bhar *et al*. (2019), the leaves of maja (*Aegle marmelos* L.) the leaves contain various bioactive compounds with antioxidants, antibacterial, antifungal, antidiarrhoeal, antidiabetic, immunomodulatory and to heal wounds. Research conducted by Balakumar *et* al., 2011 shows that *Aegle marmelos* can be used as a treatment in the Ayurvedic system, a natural medicine system originating from India. In India, Maja leaves are traditionally used as medicinal mixtures for various diseases, especially for treating asthma, hypoglycemia, febrifuge, and hepatitis, and are also used as an analgesic and antifungal [1,2].

Quality maja leaves are needed to develop herbal medicines. Therefore, preparing simplisia raw materials from maja leaves must meet the requirements of simplisia standards. The quality requirements of simplisia are carried out to determine

the quality of simplisia such as the leaves, if squeezed, will be destroyed, do not contain excessive pathogenic microbes, and moisture content test which is not more than 10% according to the Indonesian Herbal Pharmacopoeia 2017, ash content test which is less than 11% according to Materia Medika Indonesia Sixth Edition, the number of total plate numbers 5<× 10^7 colonies, the number of yeast mold numbers 5<× 10^5 colonies, and the test for heavy metal levels that do not exceed the predetermined limits according to the Food and Drug Administration No 32 of 2019. Quality requirements and standardization can guarantee the quality of traditional medicine raw materials, which will later be used as preparations. One of the standardization parameters is non-specific parameters that are not directly related to pharmacological activity but can affect the safety and stability aspects of the simplisia used by Mustapa *et al*. (2020). Non-specific parameters include drying shrinkage, moisture content, total ash content, contamination, and other contaminants [3,4,5].

2. Methods

This research used an experimental method by conducting non-specific parameter tests, which include drying shrinkage test, moisture content test, ash content test, total plate number test, yeast mold number test, and heavy metal test.

Plant Determination

Determination of maja leaves was conducted at the Department of Health UPT Laboratory Herbal Materia Medica Batu, East Java, to ensure the truth of the plants used in this study.

Simplisia Preparation

A total of 10 kg of maja leaves from Gresik Regency were washed under running water until clean, drained, then aerated and weighed. Then, it dried in the oven at a temperature of 50°C, sorted dry, and weighed dry weight. Dry samples were blended, sieved, and stored in plastic containers [6]. After that, it was calculated with the formula:

$$\text{Percent yield} = \frac{Weight\ of\ simplicia}{Weight\ of\ fresh\ leaves} \times 100\%$$

Non-specific Parameter Testing

Drying Shrinkage

A total of 2 grams of sample was weighed using a closed weighing bottle, which was previously heated at 105°C for 30 minutes and cooled in a desiccator for 15 minutes. Before weighing, the sample was leveled on the weighing bottle until flat. Then, put it in the oven, open the weighing cap, and leave the weighing bottle cap in the oven. Heat at 105°C for 1 hour, then weigh and reheat until the weight remains constant [7]. Then calculated by the formula:

$$\text{Drying shrinkage} = \frac{a-b}{a} \times 100\ \%$$

Description:
a = Weight of sample before heating (gram)
b = weight of the sample after heating (gram)

Water Content

This test was carried out using the gravimetric method, namely by weighing 1 gram of the sample and then weighing the cup that had been previously weighed. Dried in an oven at 105°C for 5 hours and weighed. Then, continue drying and weighing at a

distance of 1 hour until the difference between 2 consecutive weighings was no more than 0.25% [7]. Furthermore, it was calculated by the formula :

$$\text{Percent water content} = \frac{a-b}{a} \times 100\ \%$$

Description:
a = Weight of sample before heating (gram)
b = weight of the sample after heating (gram)

Ash Content

This test was done by weighing 0.1 grams of the sample and putting it into a test tube with a label. Add 1.5 mL of concentrated HNO_3 (in the acid cabinet). Then, heat slowly at 30-95°C for 30 minutes. Then, heat until the smoke is brownish yellow, cool, and add 1 mL of H_20_2 drop by drop through the test tube wall. Then, heat until the temperature reaches 95°C for 5 minutes. The mixture was filtered into an Erlenmeyer containing 15 mL of distilled water. The precipitate on the filter paper was washed with distilled water until the filtrate was clear. The filter paper was folded and dried in an oven at 105°C for 30 minutes. After drying, the filter paper was placed in a desiccator for 10 minutes, then weighed and repeated until a constant weight was obtained [8]. Then, the percentage of total ash content was calculated using the formula:

$$\text{Ash content} = \frac{a-\text{b}}{\text{c}} \times 100\%$$

Description:
a = Dry weight
b = Filter paperweight
c = Initial weight of the sample

Total Plate Count (TPC)

A total of 15 mL of *Nutrient Agar* (NA) media was poured into a Petri dish and shaken until evenly distributed. Furthermore, as much as 2 grams of the sample was dissolved with 18 mL of distilled water and then shaken until homogeneous until a dilution of 10^{-1} was obtained. Then prepared, 5 test tubes and each tube was filled with 9 mL of distilled water. From dilution 10^{-1}, 1 mL was taken and put into the tube until dilution 10^{-2} was obtained and shaken until homogeneous. Dilutions up to 10^{-6} were made. From each dilution, 1 mL was taken and put into a petri dish and immediately shaken to distribute evenly. After the media solidified, Petri dishes were incubated at 35 - 37°C for 24 hours in an inverted position [9]. The number of colonies that grew was observed and calculated using the formula:

$$\text{ALT} = \text{Number of colonies} \times \text{dilution factor}$$

Mold Yeast Count

A total of 15 mL of *Potato Dextrose Agar* (PDA) media was poured into a Petri dish, and 1% chloramphenicol was added as much as 1 mL. Then, as much as 2 grams of the sample was diluted with 18 mL of distilled water and then shaken until homogeneous until a dilution of 10^{-1} was obtained. Then prepared, 3 test tubes and each tube was filled with 9 mL of distilled water. From dilution 10^{-1}, 1 mL was taken and put into the tube until dilution 10^{-2} was obtained and shaken until homogeneous. Dilutions up to 10^{-4} were made. From each dilution, as much as 0.5 mL was poured on the surface of the PDA media and immediately shaken so that it was evenly distributed. To determine the sterility of the media and diluent, a blank test was made containing only the media which was allowed to solidify. All Petri dishes were incubated upside down at 25°C for 5-7 days [9]. The number of growing colonies was observed and counted using the formula:

$$\text{AKK} = \text{number of colonies} \times \text{dilution factor}$$

Heavy Metal Contamination

The first thing to do was to make a standard solution of Cadmium (Cd) with a concentration of 0.6, 1.2, 2.4, and 3.0 ppm and a standard solution of Lead (Pb) with a concentration of 0.2, 0.4, 0.6, 0.8, and 1.0 ppm. Then, weighed simplisia as much as 3 grams, then put into the Kjedahl flask and added HNO_3, concentrated 65% p.a as much as 25 mL to dissolve the metals in the sample. Heated for 30 minutes, after which the heating was stopped briefly. Then, five drops of H_2O_2 30% and heating continued, and added H_2O_2 repeatedly until the solution was clear and then cooled. Then, it was put into a 25 mL volumetric flask and diluted using aquabidest to the limit mark. Then, it was analysed using atomic absorption spectrostometry (AAS) to determine the levels of each metal contamination Cd and Pb [10].

Data Analysis

The data analysis used is descriptive data analysis that explains and describes the non-specific parameter tests, including drying shrinkage, moisture content, ash content, Total Plate Number (ALT), Chamomile Mould Number (AKK), and heavy metal contamination in simplisia. The data obtained were compared with the quality standards of simplisia with the Indonesian Herbal Pharmacopoeia (FHI), Materia Medika Indonesia (MMI), and the Food and Drug Administration Number 32 of 2019.

3. Results and Discussion

Determination of Maja Leaf (*Aegle marmelos* L.)

Maja leaf sampling was carried out in Kedamean Village, Gresik Regency. As shown in **Figure 1**, the determination of Maja leaf plants was conducted at UPT Laboratorium Herbal Materia Medica Batu, East Java, with the letter number 067/158/102.20/2023. The identification results show that the Maja leaf plants used are from the maja plant species (Aegle marmelos L.).

Figure 1. Maja leaf (*Aegle marmelos* L.) collected from Kedamean Village, Gresik Regency. Source: Personal documentation, 2022.

The quality evaluation of Maja leaf (Aegle marmelos L.) was carried out through various tests to assess key parameters such as drying shrinkage, water content, ash content, microbial contamination, and heavy metal levels. The results of these evaluations are summarized in **Table 1**, which presents the test outcomes and compares

them to the standard quality requirements. As shown in Table 1, most parameters met the required standards, indicating that the Maja leaf simplisia used in this study is of good quality, except for the yeast mold count and copper contamination which exceeded the permissible limits.

Table 1. Quality Evaluation Results for Maja Leaf Simplisia (Aegle marmelos L.)

No.	Observation Description	Observation Result ± SD	Specifications	Description
1.	Drying Shrinkage	0,423%±0,158	≤10% (FHI, 2017)	Eligible
2.	Water Content	0,77%±0,105	≤10% (FHI, 2017)	Eligible
3.	Ash Content	6,62%±0,829	≤11% (MMI, 1995)	Eligible
4.	Total Plate Count (ALT)	$0.6\times10^{7}\pm 0.360$	$\leq5\times10^{7}$ (BPOM, 2019)	Eligible
5.	Mould Yeast Count (AKK)	$326.6\times10^{5}\pm 120,312$	$\leq5\times10^{5}$ (BPOM, 2019)	Not Eligible
6.	Heavy Metal Contamination			
	Cadmium (Cd)	Not Detected	≤ 0.3 mg/kg	Eligible
	Lead (Pb)	4.60 mg/kg	≤ 10 mg/kg	Eligible
	Copper (Cu)	268.21 ± 11.16 mg/kg	0.1-150 mg/kg	Not Eligible
	Zinc (Zn)	10.89 ± 0.05 mg/kg	2.0-100 mg/kg (BPOM, 2019)	Eligible

Drying Shrinkage

Drying shrinkage testing on maja leaf simplisia powder aims to provide maximum limits on the number of compounds lost in the drying process so that it can determine the quality of the simplisia [11]. Drying shrinkage on maja leaf simplisia powder from Gresik Regency obtained an average of 0.423%. This shows that the water content and compounds lost in maja leaf simplisia powder are 0.423%.

The results obtained meet the Indonesian Herbal Pharmacopoeia 2017 standard requirements, which is no more than 10%. Weight shrinkage or drying shrinkage is a parameter used to maintain the quality of simplisia and avoid fungal growth [12]. The Maja leaf simplisia powder is of good quality; this can allow the content of secondary metabolite compounds to evaporate a little when heating, so the percentage of drying shrinkage is low.

Water Content

Testing the water content of simplisia aims to determine the remaining water after the drying process. The principle of the water content test is the percentage of water contained in simplisia. The water content test on maja leaf simplisia powder uses the gravimetric method. The results obtained with an average of 0.93% means that it meets the Indonesian Herbal Pharmacopoeia 2017 standard requirements. The factor that can affect the moisture content of simplisia is drying because drying aims to reduce the moisture content so that the simplisia is not damaged and can be stored for a long time.

So that the water content can meet the requirements. If the moisture content of simplisia is more than 10%, it will cause enzymatic processes and damage caused by microbes.

Simplisia stored for a long time will change the chemical content formed into other products by enzymes so that it does not have a pharmacological effect like the original compound. This will not happen if the dried material has a low moisture content value [13]. Moisture content can also be influenced by air humidity. If the air humidity is high, there will be water vapor moisture so that the moisture content will be higher [14].

Ash Content

Ash content is related to minerals in a material that comes from the initial process until the formation of the extract. The principle is to give specific chemical reagents to the material before ignition and then weigh the substances left after combustion. The amount of ash content in maja leaf simplisia indicates that the simplisia contains many minerals.

The test results of ash content in maja leaf simplisia powder obtained an average of 6.381%. According to the standards listed in the sixth edition of the Indonesian medical material book, a good total ash content is less than 11%, so the ash content of maja leaf simplisia meets the predetermined standards. Because if the ash content obtained is high, the mineral content in the material is also higher. Humans need minerals such as calcium, phosphorus, and magnesium for bone growth. But it differs from toxic minerals (heavy metals) such as mercury, lead, copper, Cadmium, and strontium. Because heavy metals in the human body for an extended period can interfere with the circulatory system [15], heavy metal mineral contamination in simplisia can be in silicates derived from soil or soil sand and metal elements silver, lead, and mercury. Ash content can also be influenced by the type of plant and where the plant grows [14].

Total Plate Count (TPC)

Total plate number testing aims to assure that the simplisia does not contain bacteria exceeding the specified limit. Sources of bacterial contamination can be caused by a lack of cleanliness of the place of manufacture, storage, and length of storage time [16]. The results of the total plate number research on maja leaf simplisia powder were obtained, namely an average of 0.6 × 10 7 colonies. It can be said to meet the requirements of the Food and Drug Administration standard Number 32 of 2019 [4].

The total plate number test results obtained are by previous research conducted by Sinaga, 2021 for the determination of the total plate number obtained results of 4.8 x 10^{5} cfu / ml and met the BPOM standard in 2019, which is ≤5×$10^{7}$and also research conducted by Puskesmas & Ahmad (2022). the average total plate number test results of 4.3×10^{three} also meet the predetermined standards. A traditional medicine preparation must meet the requirements because if there are too many bacteria, it can harm health because bacteria can cause disease, for example, vomiting, diarrhea, fever, and infection [17,18,19]. Testing the total plate number in maja leaf simplisia meets the requirements because drying simplisia meets the criteria for water content, which is no more than 10%, which also affects the quality of raw materials, so bacteria will be challenging to grow. The less water content, the more difficult it is for the bacteria to grow because water is one of the basics needed for the growth of bacteria. So, the smaller the water content in

a product can extend the storage period of the product because bacterial growth is inhibited.

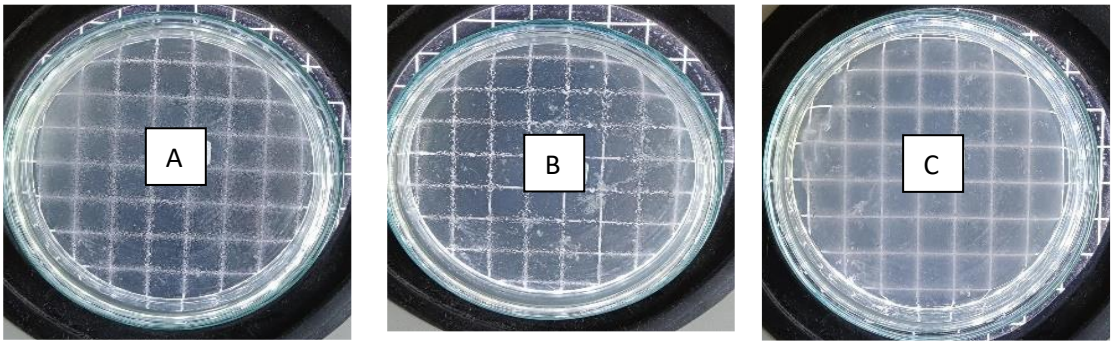

Figure 2. TPC test results A. replication 1 B. replication 2 C. replication 3

The image in **Figure 2** shows the results of TPC testing, consisting of three replications. The TPC test results for Maja leaf simplisia powder indicate that the colonies obtained have an average of 0.6×10^{7} bacterial colonies. These results meet the standards set by the Food and Drug Supervisory Agency Number 32 of 2019.

Mould Yeast Count (MYC)

In the study, the yeast mold number test aims to see fungi's presence microbiologically and assure that the simplisia used does not contain fungi beyond a predetermined limit. The yeast mold number test is a group of microorganisms that include fungi. This test uses *potato dextrose agar* media. The research results on yeast mold numbers in maja leaf simplisia powder are obtained colonies with an average of 326.6×10^{5} yeast-shaped fungal colonies.

The difference between molds and yeasts can be seen from their shape. Molds are cotton-like and usually white, with a black core in the center. Meanwhile, yeast is an ordinary colony without filaments or fibers [20]. In the maja leaf simplisia powder, the resulting fungus is in the form of yeast. The results exceeded the limit set by the Food and Drug Administration Number 32 of 2019, $\leq 5\times10^{5}$. This is because making Maja leaf simplisia is less straightforward so fungi can contaminate it. In addition, factors that can cause microbial contamination, such as high humidity, can also trigger high numbers of fungal contamination [21]. According to research by Wulandari et al., 2012), bacteria can be contaminated through water, dust, air, and soil. To reduce mold and yeast contamination in simplisia powder, it is necessary to wash the leaf samples repeatedly until they are clean. The storage method of leaf powder that has been made is also essential. The powder must be placed in a closed container at room temperature without direct sunlight exposure [20].

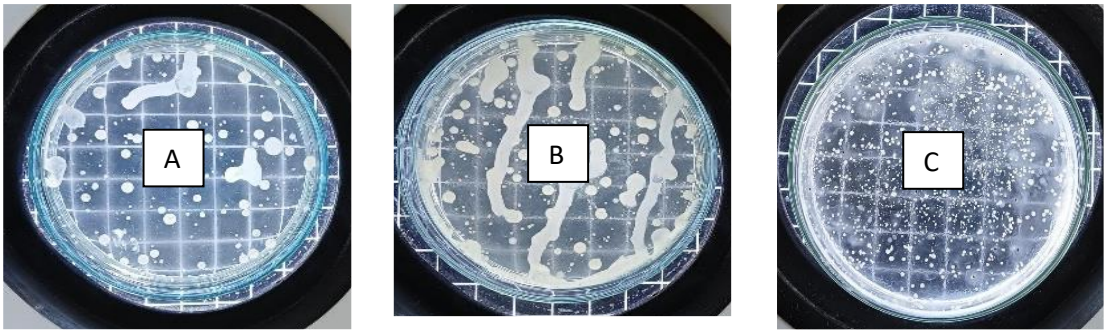

Figure 3. AKK test results A. replication 1 B. replication 2 C. replication 3

The image in **Figure 3** shows the results from the MYC test, consisting of three replications. The study of the number of yeast molds aims to detect the presence of fungi microbiologically and provide assurance that the simplisia used does not contain fungi beyond the predetermined limit. The research results on the number of yeast molds in Maja leaf simplisia powder were obtained from colonies with an average of 326.6×10^5 mushroom colonies in the shape of yeast. The difference between mold and yeast can be seen in the shape. The mold is cotton-like and usually white with a black core in the center, while yeast forms an ordinary colony without filaments or fibers.

Heavy Metal Contamination

Testing for heavy metal contamination ensures that simplisia or extracts do not contain heavy metals such as Pb, Cd, Cu, and Zn beyond predetermined limits. Lead (Pb) is a metal that is harmful to humans. This metal usually comes from Pb-contaminated food, Pb-contaminated dust, and contact with skin or eyes. Lead is also one of the primary pollutants in the environment and is commonly used for fuel additives.

Cadmium (Cd) is a hazardous metal. In adults, Cadmium can cause breast cancer, reproductive failure, and cardiovascular or lung disease. From the research conducted, no cadmium (Cd) levels were found in maja leaf simplisia powder and for lead (Pb) levels, namely 4.60 mg/kg, which means that it meets the requirements set by the Food and Drug Administration Number 32 of 2019, namely for Cadmium (Cd) levels ≤ 0.3 mg/kg and for lead (Pb) levels ≤ 10 mg/kg. Zn levels in maja leaf simplisia powder obtained 10.89 mg/kg results also meet the Food and Drug Administration requirements. The Cu content was 268.21 mg/kg, which means it does not meet the requirements

Factors that can cause heavy metal contamination in the environment vary. For example, the geological conditions of the soil where the plants are cultivated, the condition of the water used for watering, the presence of specific heavy metal contaminants originating from industry if the cropping location is close to industrial sites, and even unexpected disasters. Heavy metal pollution of copper and zinc can occur during the pre-harvest process, namely during planting and maintenance. It can also be caused by using copper and zinc fertilizers [23]. The Maja leaf growth is on the agricultural land of residents, which is far from industrial and household activities and motorized vehicles. Motor vehicle fumes can pollute the environment where materials grow, such as urban waste disposal activities, smelting, mining, and metal

manufacturing, which can increase Cd concentrations and cause human cancer [24]. Other factors that can affect the accumulation of heavy metals in plants are the period of plant contact with heavy metals, the type of metal, and plant species [5].

The primary source of heavy metal contaminants is air and water, which pollute the soil. All plants that grow on soil that heavy metals have polluted will accumulate in all parts of the plant, namely roots, stems, leaves, and fruit. Metals will accumulate in body tissues and can cause poisoning in humans, animals, and plants if they exceed predetermined limits [25].

This study has several limitations that should be acknowledged. The samples of *Aegle marmelos* L. leaves were collected exclusively from Kedamean Village in Gresik Regency, which may not represent the full variability of the plant's quality across different regions, environmental conditions, or cultivation methods. As a result, the findings may have limited generalizability. Furthermore, this research focused only on non-specific parameters such as drying shrinkage, moisture content, ash content, microbial contamination, and heavy metal levels. It did not assess the specific active compounds or pharmacological activities essential in evaluating herbal raw materials' therapeutic potential. Additionally, the study did not investigate the influence of post-harvest processing methods, drying techniques, or storage conditions, which could significantly affect the long-term quality and safety of the simplisia.

4. Conclusion

Based on the results of this study, the maja leaf (Aegle marmelos L.) simplisia from Kedamean Village meets the required standards for most non-specific parameters, including drying shrinkage, moisture content, ash content, total plate count, and heavy metal levels of Pb, Cd, and Zn. However, the yeast and mold count and copper (Cu) contamination exceeded the permissible limits, indicating a need for improved hygienic practices and stricter control of environmental exposure during cultivation and processing. It is recommended that future studies explore improved drying, storage, and handling methods to reduce microbial and metal contamination. In addition, a broader sampling from different regions and seasons is suggested to assess the consistency and generalizability of quality parameters.

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Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this article.

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