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Analysis Specific and Non-Specific Parameters of African Leaf Extract (*Vernonia amygdalina*) from Mantup, Lamongan

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Abstract

Indonesia has a wide range of biodiversity that is widely used in traditional medicine. One such plant is the African leaf (*Vernonia amygdalina*), which is known to have antidiabetic, anticancer, and antimalarial activities. To support its use as a quality and safe traditional medicine, efforts to standardize the quality of the extract are needed. This research aims to analyze the specific and nonspecific parameters of African leaf extract originating from the Mantup area, Lamongan Regency. This research is an experimental study, the method used is maceration with 96% ethanol solvent. The results showed that the specific parameter analysis of the African leaf extract had a yield of 16.24%. The characteristics of the extract were dark green color, distinctive odor, bitter taste and a viscous consistency. Phytochemical screening tests showed positive flavonoids. For non-specific analysis, the water content was 9.6% (according to the Indonesian Herbal Pharmacopoeia standard). The total ash content of 19.5% and the acid insoluble ash content of 11.4% did not conform to the requirements. Non-conformities are caused by contamination with inorganic compounds such as soil, dust, or heavy metals, both during post-harvest and processing, and are influenced by the location where the plants are grown. **Keywords:** Maceration, Water content, Total ash content

Introduction

Indonesia has a rich biodiversity that is utilized as traditional medicine, that came from plants, animals, minerals, or their extracts that have been used for generations according to health standards (Menkes RI, 2018). One of the medicinal plants is the African leaf (*Vernonia amygdalina*) from the Asteraceae family which grows abundantly in East Kalimantan and is

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also known in Africa, especially Nigeria (Rahmadani et al., 2021) . In Indonesia, this plant has believed to have antidiabetic, antimalarial, and anticancer properties (Febrianti et al., 2017) with secondary metabolites in the form of alkaloids, flavonoids, and tannins that support its pharmacological activity (Fatimah & Sundu, 2020). Technological developments in Indonesia have encouraged the use of medicinal plant extracts in healthcare services, dried, viscous, or liquid form according to the characteristics of their active compounds. However, the quality and safety of raw materials are often unstable due to environmental factors such as temperature, climate, and growing location (Marjoni, 2016).

To ensure quality, BPOM Regulation No. 32 of 2019 emphasizes that every traditional medicine, both raw materials and finished products, must safe and suitable with quality standar as stated in the plant monograph (BPOM, 2019). Standardization of medicinal plant raw materials is important to ensure uniformity and quality, so that pharmacological activity remains consistent in final products such as simplicia, extracts, and herbal preparations (Widiyastuti & Putranti, 2019) . This process refers to specific and non-specific parameters of the Indonesian Herbal Pharmacopoeia, including organoleptic tests, water content, ash content, acid-insoluble ash content, and flavonoid content (RI, 2017) . The main objective is to improve quality, guarantee safety, and ensure the pharmacological effects of herbal products (Saifudin et al., 2011) . In the study, standardization was applied through the extraction of African leaves using the maceration method with 96% ethanol solvent because chosen for its characteristics as a universal solvent capable of attracting polar to nonpolar compounds (Ambaro et al., 2020)

African leaf plants (*Vernonia amygdalina*), obtained from the Mantup area of Lamongan Regency, East Java, generally grow wild along roadsides and have not been optimally utilized by the community. To ensure their suitability as a raw material for herbal medicine, characterization studies of specific and nonspecific parameters of the extract are required. This standardization process aims to ensure product quality, safety, and consistency so that processed African leaf products can suitable whit standards and be marketed as standardized herbal medicines (Saifudin et al., 2011). Based on the background explained, the research problem formulation in this study is how to test specific and non-specific quality parameters on African leaf extract (*Vernonia amygdalina*) grown in the Mantup area, Lamongan Regency. The purpose of this study is to determine the results of the specific and non-specific quality parameter tests of the African leaf extract.

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Research and Methods

Types of Research and Research Design

This study used an experimental method to analyze the specific and nonspecific quality parameters of African leaf extract (*Vernonia amygdalina*). The research activities were carried out at the Pharmaceutical Laboratory of the University of Muhammadiyah Gresik from November 2024 to June 2025, with data collection carried out between February and May 2025. The variables studied were the results of quantitative analysis of specific and nonspecific quality parameters of African leaf extract, which aimed to ensure its quality and consistency as a raw material for herbal medicine (Saifudin *et al.,* 2011).

Materials and tools

The materials used in this study included African leaf (*Vernonia amygdalina*) collected from Mantup, Lamongan Regency, East Java. Aquadest (*Water One*), 96% ethanol (*Technical*), magnesium powder (*Merck*), Hcl 2N and dilute hydrochloric acid. The tools used in this study included a maceration vessel, blender (*Cosmos*), knife, tray, 500 mL Erlenmeyer flask (*Herma*), 600 mL beaker glass (*Herma*), 100 mm glass funnel (*Herma*), mesh no. 60 sieve (*Retsch*), oven (*Mito*), analytical balance (*Centarus Scale*), rotary evaporator (*Vitalab Jaya Mandiri*), aluminum foil, porcelain crucible, wooden clamp, filter paper, ash-free filter paper, 250 mL measuring cylinder (*Herma*), porcelain dish, wooden spatula, glass stirring rod, 100 mL volumetric pipette (*Herma*), furnace, test tube and dropper pipette, hot plate.

Research Procedures 1. *Sample Preparation*

Preparation of African leaf (*Vernonia amygdalina*) samples was carried out using 4 kg of fresh leaves. The leaves were first subjected to wet sorting to remove twigs, yellowed leaves, and damaged leaves, followed by thorough washing under running water. The cleaned leaves were then drained, chopped and dried in an oven at 40 °C. After drying, a dry sorting process was conducted to eliminate impurities. The resulting dried simplicia was weighed, ground using a blender and sieved through mesh no. 60 to obtain uniform powdered simplicia (Widiyastuti & Putranti, 2019).

2. *Making African Leaf Extract*

150 grams of African leaf (*Vernonia amygdalina*) was macerated with 96% ethanol in a

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1:10 ratio for three days in a vessel covered with aluminum foil to prevent compound damage, with daily stirring. After that, the extract was filtered, evaporated using a rotary evaporator until a thick extract was obtained and the yield was then calculated to determine the extraction results (Hakim et al., 2024).

3. *Extract Test*

The specific parameter tests of African leaf (*Vernonia amygdalina*) extract included organoleptic examination, carried out using sensory observation to describe the form, color, odor, and taste of the extract. In addition, phytochemical screening to identify secondary metabolites, particularly flavonoids. The flavonoid test was conducted by adding concentrated HCl and magnesium powder to the extract, a positive result was indicated by the formation of a yellow color, with quercetin used as a reference standard (Ikalinus *et al.,* 2015)..

Nonspecific parameter testing of African leaf extract (*Vernonia amygdalina)* was carried out using the oven method at 105 °C for 5 hours, followed by cooling in a desiccator until a constant weight was obtained. The moisture content was calculated as a percentage of the initial extract weight, with the purpose of evaluating the stability and storage quality of the extract (Andasari *et al.,* 2021).

In addition, total ash content was determined using the ash method at 800 ± 25 °C until a constant weight was obtained, to evaluate the inorganic mineral content of the extract. Acid-insoluble ash content was measured by boiling the ash with dilute HCl to measure the remaining insoluble ash, so that its relate to impurities of materials (Menkes RI, 2017).

4. *Data analysis*

Research on African leaf extract (*Vernonia amygdalina*) produced data in the form of yield percentage, organoleptic test, phytochemical screening, and nonspecific parameters including water content, total ash content and acid-insoluble ash content. The yield was calculated based on the ratio of extract weight to the initial simplicia weight. Organoleptic evaluation showed that the extract was dark green, distinctive odor, a bitter taste and a viscous form. Phytochemical screening confirmed the presence of flavonoids, indicated by the formation of a yellow color, consistent with the properties of secondary metabolites that contribute to pharmacological activity (Ikalinus *et al*., 2015).

The water content test showed results according to the Indonesian Herbal Pharmacopoeia (FHI) standards, where a water content of ≤12.5% indicates that the stability of the extract is maintained. Meanwhile, a total ash content of ≤10.2% indicates the amount of mineral residues

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that are still in accordance with the provisions, and an acid-insoluble ash content of ≤0.6% indicates a good level of material purity. Thus, African leaf extract meets the required specific and non-specific parameter standards, so it has the potential to be used as a raw material for standardized herbal medicines (Andasari *et al.*, 2021).

Result and Discussion

Sample Preparation

In this study, 4 kg of fresh African leaves (*Vernonia amygdalina*) were obtained from Mantup, Lamongan, then wet sorted to separate unsuitable parts such as twigs, yellowed leaves, damaged leaves, as well as dirt and insects, then washed with running water and drained (Ningsih, 2016). The sample was then chopped, dried using an oven at 40°C for 13 hours to obtain 595 g of dry simplicia, then dry sorted to separate foreign materials. The simplicia was blended and shieved using mesh no. 60 to obtain 423 g of powder, where the reduction in particle size aims to increase the surface area allowing the solvent to penetrate the cells more efficiently and release secondary metabolites (Rifkowaty & Wardanu, 2016).

Extraction procedure from Africa leaf

The extraction process was carried out using the maceration method using 150 g of African leaf powder (*Vernonia amygdalina*) and 1,500 mL of 96% ethanol solvent. The maceration method was chosen because it is simple, easy to do with simple equipment, and able to extract active compounds in larger quantities without damaging thermolabile compounds (Endarini, 2016) . Maceration was carried out for 3 days with stirring twice a day and the container was covered with aluminum foil to prevent damage to the compounds due to sunlight. After filtration on the third day, the filtrate was evaporated using a rotary evaporator at 50°C and then continued with a water bath until a viscous dark green extract was obtained (Hakim *et al.,* 2024). The thick extract obtained from evaporation was 24,37 grams.

Table 1. Results Randemen Extract Leaf Africa

Solvent	Weight Simple ingredients	Weight extract	Condition (FHI)	Yield %
Ethanol 96%	150 g	24,37 g	≥ 11,8%	16,24%

The results showed a yield of 16,24% of the concentrated African leaf extract, which meets the Indonesian Herbal Pharmacopoeia (FHI) standard of at least 11,8%. This indicates

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that the extraction method used was appropriate and effective in producing optimal yield. The yield is influenced by several factors, such as extraction time, solvent type, and sample size. The smaller the particle size, the greater the contact with the solvent, thus increasing the interaction and extraction results (Sineke et al., 2016) .

Standardization Extract

Standardization is the process of establishing quality parameters of an extract through chemical, physical, and microbiological analyses to ensure safety and prevent potential toxicity, thereby allowing herbal products to meet established standards (Saifuddin *et al.,* 2011). In this study, standardization was carried out through organoleptic testing, phytochemical screening, determination of moisture content, total ash content and acidinsoluble ash content.

Organoleptic test was carried out to evaluate the characteristics of African leaf extract (*Vernonia amygdalina*) using sensory observation, including form, color, odor, and taste, as the initial quality parameters of the extract (Ahmadita, 2017).

Table 2. Test Results Organoleptic Extract leaf Africa

Parameter	Condition (FHI)	Observation result
▪ Color	Dark green	Dark green
▪ Odor	Distinctive	Distinctive
▪ Taste	Bitter	Bitter
▪ Form	viscous	viscous

Table 2 shows extract of African leaf (*Vernonia amygdalina*) exhibited a dark green color due to the presence of extracted chlorophyll, distinctive odor and a bitter taste derived from sesquiterpene lactones. The viscous consistency resulted from the evaporation process. (Nainggolan *et al*., 2018)..

Phytochemical screening

African leaf extracts showed positive flavonoids, indicated by a change in the color of the extract solution from dark green to yellow. Previous research also showed that phytochemical screening of African leaf extracts contained flavonoids (Rahmadani et al., 2021). Flavonoid

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compounds are a group of secondary metabolites commonly found in medicinal plants and serve an important role in various pharmacological activities, including antioxidants, anti-inflammatory, antidiabetic, antihypertensive and anticancer activities (Panche *et al*., 2016) .

Water Content Test

Table 3. Results of the Water Content Test of African Leaf Extract

Level Results Water	Level	Condition (FHI)	Average	Information
▪ R1	10%	≤ 12,5%	9,6%	Fulfil condition
▪ R2	9%			
▪ R3	10%			

Based on the table above, the water content of African leaf extract shows an average value of 9.6% across the three replications. This value is still below the maximum water content limit set by the Indonesian Herbal Pharmacopoeia (FHI), which is ≤ 12.5%, The water content of the extract is highly influenced by the drying time of the herbal medicine, where the more thoroughly the material is dried, the lower the residual moisture content will be (Najib *et al*., 2017) .

Ash Content Test

Table 4. Results of Ash Content Test Extract African Leaf

Level Results Ash Total	Level	Condition (FHI)	Average	Information
▪ R1	19,4 %	≤ 10,2 %	19,5%	No fulfil
▪ R2	19,5 %			condition
▪ R3	19,7 %			

Table 4 shows that the total ash content of African leaf extract. In this study reached an average of 19.5%, while the maximum limit set by the Indonesian Herbal Pharmacopoeia is ≤10.2%. This result indicates a high ash content. The elevated ash level is influenced by the extraction duration, where 72 hours of extraction produced a higher ash content compared to 24 hours of extraction, which was only 8.0214±0.4802% (Afidah, 2020). It could be concluded that longer extraction results in higher ash content. The condition of the Vertisol soil where the plants grew, containing cations such as Ca^{2+}, Mg^{2+}, K^{+}, and $NH4^{+}$, also contributed to the ash content level (Prasetyo, 2007).

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Acid Insoluble Ash Content Test

Determination level ash No late sour done with method dissolve The results of determining the ash content in a heated hydrochloric acid solution were then filtered with ash-free filter paper and the residue was incandescent to a constant weight. The acid-insoluble ash content test was carried out on African leaf extract (*Vernonia amgdalina*).

Table 5. acid-insoluble ash content Test Extract African Leaf

Level Results Ash No Acid Soluble	Level	Condition (FHI)	Average	Information
▪ R1	13,1%	≤ 0,6 %	11,4%	No fulfil
▪ R2	10,9%			condition
▪ R3	10,4%			

The average acid-insoluble ash content of African leaf extract was 11.4%, far exceeding the maximum limit of the Indonesian Herbal Pharmacopoeia (≤0.6%), thus not fulfilling quality requirements. This high value indicates the presence of inorganic impurities such as silicates, sand and heavy metals (Ag, Pb, Hg) that are insoluble in acid, possibly originating from soil or environmental dust (Guntarti et al., 2015). The use of HCl is intended to dissolve internal plant minerals (Irsyad, 2013).

Picture 1. Result of Acid Insoluble Ash Content

In this research, metal particles were found on the filter paper, presumed to be external inorganic matter contributing to the high acid insoluble ash content and derived from the soil or environmental conditions where the plant grows.

Conclusion

African leaf extract (*Vernonia amygdalina*) from Mantup, Lamongan have a yield of 16.24%, with dark green color, distinctive odor, bitter taste and a viscous consistency. water

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content of 9,6% suitable with standart of the Indonesian Herbal Pharmacopoeia (FHI), while the total ash content of 19,5% and acid-insoluble ash of 11,4% do not conforms the standards. Phytochemical screening confirmed the presence of flavonoids.

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