

From Ethnomedicine to Potential Herbal Product: Standardization of *Uvaria Rufa* Blume Bark

Burhan Ma'arif¹, Maximus M. Taek^{2*}, Zakiya Q. 'Aini¹, Mayu Rahmayanti¹,
Novia Maulina¹, Helmi S. Iskandar¹

¹Department of Pharmacy, Faculty of Medicine and Health Science, Maulana Malik Ibrahim State Islamic University, Locari Street, Junrejo District, Batu City, East Java 65151, Indonesia

²Department of Chemistry, Faculty of Science and Technology, Widya Mandira Catholic University, Jendral Achmad Yani Street No. 50-52, Kota Lama District, Kupang City, East Nusa Tenggara 85225, Indonesia

*Presenting author: maximusmt2012@gmail.com

ABSTRACT

Uvaria rufa Blume, locally known as lelak, is an ethnomedicinal plant traditionally used in East Nusa Tenggara, Indonesia, to treat conditions such as fever, bleeding, and skin allergies. To support its development as a raw material for herbal medicines, standardization is crucial to ensure its safety, efficacy, and quality. This study aimed to establish a standardization profile to ensure the consistent quality of *U. rufa* bark sourced from East Nusa Tenggara. A descriptive experimental method was used to determine specific and non-specific parameters of the bark. Specific parameters included identification of the plant part (bark), macroscopic characteristics (8–10 cm long, dark brown exterior, yellow interior, fibrous texture), microscopic features (parenchyma with stone cells, sclerenchyma, cork tissue), and organoleptic properties (light brown color, slightly astringent, aromatic, fine powder). The water-soluble extractive value was $12.0160\% \pm 0.0090$, and ethanol-soluble was $22.475\% \pm 0.0111$. Non-specific parameters assessed safety and stability: loss on drying ($7.26\% \pm 0.8723$), moisture content ($9.4\% \pm 0.2000$), total ash content ($2.601\% \pm 0.0002$), and heavy metal levels (Pb: 0.0472 ppm, Cd: 0.0014 ppm). All results met the general requirements for herbal standardization, supporting *U. rufa* bark's potential as a standardized herbal medicine ingredient.

Keywords: East Nusa Tenggara; Ethnomedicine; Standardization; *Uvaria rufa* Blume.

INTRODUCTION

Indonesia is a country rich in natural resources, including those derived from plants, animals, and microorganisms. One of these resources is medicinal plants, which have been widely developed and utilized for therapeutic purposes (Setyani et al., 2021; Hermawati et al., 2023). A native Indonesian plant commonly used in traditional medicine is lelak (*Uvaria rufa* Blume) from the Annonaceae family. People in East Nusa Tenggara, in particular, have long used this plant for medicinal purposes.

In traditional medicine, *U. rufa* is known to exhibit pharmacological effects that can help treat various health conditions (Alimboyoguen et al., 2023). Different parts of the *U. rufa* plant have

medicinal applications: the bark and roots are used to reduce fever, decoctions of the fruit are used to treat skin allergies and intestinal ulcers, and decoctions of the stem are traditionally used by Thai communities to treat benign prostatic hyperplasia (BPH) (Buncharoen et al., 2016). Its flavonoid compounds are reported to have the potential to inhibit the formation of advanced glycation end products (AGEs), and its alkaloid content is believed to have anticancer potential (Thang et al., 2014).

Given its therapeutic uses, the development of herbal medicines from natural ingredients holds great potential. However, the development of plant-based medicines requires a standardization process to ensure the quality, safety, and efficacy of the medicinal raw materials (Faramayuda et al., 2021). Standardization is a crucial step in the development of natural medicines to ensure consistency in chemical profiles, pharmacological activity, and quality assurance during production (Setyani et al., 2021).

The potential use of *U. rufa* bark as a medicinal plant highlights the need for standardization. To date, no standardization has been conducted for this material, and it has not yet been included in the official monograph published by the Indonesian Ministry of Health (Andini & Putri, 2021). Therefore, this study aims to conduct standardization of specific and non-specific parameters of *U. rufa* bark simplicia to determine the values of each parameter according to the standardization procedures established by the Ministry of Health of Indonesia (Depkes RI, 2000; Andini & Putri, 2021).

METHODS

1. Materials

Powdered *U. rufa* bark were obtained from Kupang, East Nusa Tenggara, and identified in Phytochemistry Laboratory, Widya Mandira Catholic University. Aquadest, chloroform, 70% ethanol, nitric acid, and chloral hydrate were purchased from Merck (Darmstadt, Germany).

2. Instrumentations

Fume hood, Atomic Absorption Spectrophotometer (AA240 Varian), Oven (Binder), Moisture Analyzer (HC103), Hot Plate (Cimarec Thermo Scientific), Analytical Balance (Pioneer Ohaus), Porcelain Cup, Erlenmeyer Flask (250 mL, Schott Duran), Beaker Glass (100 mL and 250 mL, Herma), Measuring Cylinder (100 mL, Iwaki), Glass Funnel, Microscope Slide, Stirring Rod, and Volumetric Flask (25 mL, Iwaki).

3. Research Prosedures

3.1 Preparation of Simplicia

The preparation of simplicia involved two main steps: drying and powdering. The *U. rufa* bark was first cut into smaller pieces, then dried by sun exposure and air circulation for several days. Once subjectively assessed as dry, the material was sorted and ground into a fine powder to achieve a uniform simplicia consistency. Upon completion, the simplicia was ready for standardization.

3.2 Standardization of Simplicia

Standardization was carried out based on procedures from the Indonesian General Standard Parameters for Medicinal Plant Extracts (Indonesian Ministry of Health, 2000), covering both specific and non-specific parameters.

4. Specific Parameters

4.1 Identity

The identity test involves determining the botanical name, the plant part used, and its common Indonesian name.

4.2 Organoleptic Test

This test was conducted by evaluating the sample's shape, smell, taste, and color using the senses. A small amount of the sample was placed in a porcelain cup, and descriptive observations were recorded.

4.3 Macroscopic Observation

Macroscopic characteristics such as shape, size, and texture were observed with the naked eye. Measurements were taken using a ruler and the findings were documented.

4.4 Microscopic Observation

Microscopic examination was performed using a trinocular microscope connected to a computer. A thin layer of the powdered simplicia was placed on a slide, moistened with chloral hydrate, covered with a cover slip, and examined. Visible structural fragments were identified and documented.

4.5 Water and Ethanol-Soluble Extract Content

Two grams of simplicia were placed into an Erlenmeyer flask containing 100 mL of either water-chloroform (2.5 mL chloroform in 1 L water) or 70% ethanol. The mixture was shaken for 6 hours and then soaked for 18 hours. Twenty milliliters of the filtrate were evaporated at 105 °C to constant weight. The yield was calculated according to Equation 1.

5. Non-Specific Parameters

5.1 Loss on Drying

Two grams of simplicia were weighed and placed into a moisture analyzer (Mettler Toledo HC103), set at 105 °C. The sample was heated until a constant weight was reached. Results were expressed as percentage weight loss and the test was repeated three times (Ma'arif et al., 2023).

5.2 Moisture Content

Using the gravimetric method, 2 g of powder were weighed into a pre-weighed porcelain cup. The sample was dried at 105 °C for 5 hours and reweighed. Drying and weighing were repeated every hour until a constant weight was achieved, defined as less than 0.25% difference between weighings.

5.3 Ash Content

The crucible was first ignited in a furnace at 600 °C to a constant weight. Then, 2 g of sample were placed into the crucible and incinerated in the furnace. After ashing, the crucible was cooled in a desiccator for 15 minutes and reweighed to a constant weight. The test was performed in triplicate.

5.4 Heavy Metal Contamination

Levels of heavy metals including lead (Pb) and cadmium (Cd) were determined using an AAS. Sample preparation was carried out using wet destruction method. Then the preparation of standard solutions of Pb was made in concentrations of 0.2; 0.4; 0.8; 1.2; 2 ppm and Cd was made in

concentrations of 0.2; 0.4; 0.6; 0.8 ppm from a standard solution of 50 ppm of each metal. The absorbance was measured at a wavelength of 217.0 nm (Pb) and 228.8 nm (Cd).

RESULTS AND DISCUSSION

The process of standardizing simplicia is carried out by referring to the standard operating procedures adapted from the Indonesian General Standard Parameters for Medicinal Plant Extracts (Indonesian Ministry of Health, 2000) and the Indonesian Herbal Pharmacopoeia Second Edition 2017. The standardization includes specific parameters and non-specific parameters. Standardization is done to ensure that the quality of simplicia is consistent and effective for natural medicine; this way, the active compounds can be reliably measured across different treatments, keeping the simplicia stable in terms of effectiveness and safety (Ningsih et al., 2022).

1. Specific Parameter Determination Results

1.1 Identity Test

Identity testing of simplicia is very important in the introduction as an initial introduction and part of the plant used by describing the plant's name, here including the name of the simplicia used, the Latin name of the plant, the plant part used, and the Indonesian name of the plant (Indonesian Ministry of Health, 2000; Mustapa et al., 2020). The results of the identity of the plant obtained from the literature are that this plant is known as lelak with the Latin name *Uvaria rufa* Blume with the part of the plant used is the stem bark (Isnayanti, 2020).

1.2 Organoleptic Test

U. rufa bark simplicia have a light brown color, a tasteless taste that tends to be astringent, a typical aromatic odor, and a fine powder (**Figure 1**). The distinctive aroma of plants comes from secondary metabolites of the terpenoid group (Ma'arif et al., 2023). Based on the Indonesian Herbal Pharmacopoeia Edition II 2017, the interpretation of organoleptic test results stating "typical aromatic odor" or others is only descriptive and cannot be considered as a standard of purity of the material concerned.



Figure 1. *U. rufa* bark powder

1.3 Macroscopic Test

Based on **Figure 2**, *U. rufa* bark has a dark brown color like stem bark in general, with the texture of the bark fibrous, thick, and shaped like pieces. The size of the *U. rufa* bark has a length of approximately 8-10 cm. In general, the morphology of *U. rufa*, which is in the Annonaceae family, can be recognized by its characteristics, such as skin that has fibers (Erkens et al., 2022). Another feature

that can be useful in identifying Annonaceae is the thick, hard skin, but it peels off easily in one piece with a distinctive bright yellow inner side of the skin and brown on the outside (Couvreur et al., 2022).

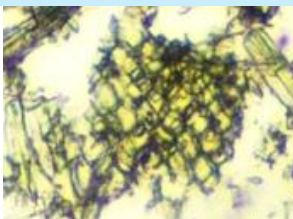
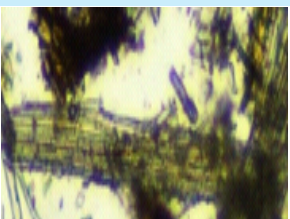
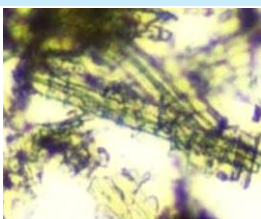


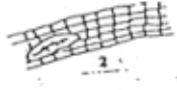


Figure 2. *U. rufa* bark

1.4 Microscopic Test

The results of microscopic observations with a magnification of 10/0.25 (**Table 1**) show that the bark powder from *U. rufa* contains fragments of cortex parenchyma with stone cells, sclerenchyma tissue, and cork tissue. Plant stem bark is composed of collenchyma and parenchyma (Rahayu and Kurniawan, 2020). In addition, the skin consists of epidermal tissue, cork cambium, cortex, and phloem. Stem bark used as a medicinal material can consist of secondary phloem formed from cambium with or without cork (Hayati et al., 2019).

Table 1. Comparison of microscopic test results with literature

	Picture A	Image B	Picture C
Research Results			
Literature			

(Hayati et al., 2019)

2. Test for Soluble Essence Content in Certain Solvents

Tests of soluble essence content in certain solvents were carried out on *U. rufa* bark. Testing the content of water-soluble juice and ethanol is shown to know the initial picture of some of the content in it. This test can estimate the amount of active compounds that have polar properties (soluble in water) and semi-polar-nonpolar properties (soluble in ethanol). The test results (**Table 2**) show the water-soluble essence content in the sample amounted to $12.016\% \pm 0.0090$, while the ethanol-soluble juice content amounted to $22.475\% \pm 0.0111$. This shows that the *U. rufa* bark contains more compounds soluble in ethanol organic solvents than compounds soluble in water solvents. Ethanol as a solvent has a wide polarity range from semi-polar to non-polar so that it can attract alkaloid compounds that are semi-polar, non-polar flavonoids, and triterpenoids, which are compounds included in terpenoids that have non-polar properties (Putri et al., 2022).

Table 2: Water and ethanol soluble essence test results

Testing parameters	% soluble essence content $\bar{x} \pm SD$
Water Soluble	$12.016\% \pm 0.0090$
Ethanol Soluble	$22.475\% \pm 0.0111$
Requirements	Not available

Description: SD value = Standard deviation of $n = 3$

3. Non-specific Parameter Determination Results

Non-specific parameter testing resulted (**Table 3**) in a drying shrinkage of $7.26\% \pm 0.8723$ and a moisture content of $9.4\% \pm 0.2000$. Moisture content that has a value below 10% will minimize damage to the simplicia caused by the growth of fungi and molds so that the shelf life becomes durable and improves the quality of *U. rufa* bark (Sagita, 2021). The determination of total ash content (Table 3) shows that the simplicia has an ash content of $2.601\% \pm 0.0002$. In simplicia, ash content can come from soil contamination that is carried in the simplicia; the higher the ash content, the more inorganic content due to contaminants such as soil, sand, or dust. Conversely, the lower the ash content, the simpler it is within reasonable limits, and there are no contaminants, so the purity level of the simplicia as raw material will be higher (Falah and Sa'diyah, 2024).

Table 3: Loss on drying and moisture content test results

Testing parameters	% test result $\bar{x} \pm SD$
Drying Shrinkage	$7.26\% \pm 0.8723$
Water Content	$9.4\% \pm 0.2000$
Ash content	$2.601\% \pm 0.0002$

Description: SD value = Standard deviation of $n = 3$

4. Heavy Metal Contamination

Metal contamination levels in *U. rufa* bark (**Table 4**) showed Pb metal content of 0.0472 ppm and Cd metal of 0.0014 ppm. These metals can come from the soil, where plants have the ability of phytoextraction, or absorption and accumulation of polluting substances through other plant parts, so that they can capture metal particles (Rusmalina et al., 2023). Naturally, heavy metals can come from rock weathering and atmospheric deposition, but also from anthropogenic sources, including agriculture,

livestock, and industry (Patty et al., 2018). Heavy metals can endanger health if they enter the body's metabolic system in amounts that exceed the threshold (Setyaningrum et al., 2018).

Table 4: Results of metal contamination levels in *u. rufa* bark

Type of Heavy Metal	Level (ppm)	Requirements (BPOM, 2019)	Description
Pb	0.0472	<10 ppm	Qualified
Cd	0.0014	<0.3 ppm	Qualified

CONCLUSIONS

The identification results confirm that the sample is *U. rufa* bark, characterized by macroscopic features (8–10 cm in length, dark brown exterior, yellow interior, fibrous texture), microscopic attributes (parenchyma with stone cells, sclerenchyma, cork tissue), and organoleptic properties (light brown color, slightly astringent, aromatic, fine powder). The water-soluble extractive value was $12.0160\% \pm 0.0090$, whereas the ethanol-soluble value was $22.475\% \pm 0.0111$. Non-specific criteria evaluated for safety and stability include loss on drying ($7.26\% \pm 0.8723$), moisture content ($9.4\% \pm 0.2000$), total ash content ($2.601\% \pm 0.0002$), and heavy metal concentrations (Pb: 0.0472 ppm, Cd: 0.0014 ppm). All outcomes satisfied the overarching criteria for herbal standardization, substantiating *U. rufa* bark's viability as a standardized herbal medicinal material.

ACKNOWLEDGEMENTS

The authors would like to sincerely thank the Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, for the research funding assistance (Contract No. 136/E5/PG.02.00/PROTOTIPE/2024). We also thank all laboratory staff and students who have greatly assisted in this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between all parties involved. The authors have full freedom in designing and carrying out this research. The results of this study will be published openly, regardless of the outcome. The authors have minimized any potential bias by taking appropriate measures.

REFERENCES

- Alimboyoguen, A.B., Castro-Cruz, K.A.D., Shen, C.C., Tsai, P.W., 2023. Anti-mitotic activities of ethanolic extract and glutinol from *Uvaria rufa* Blume. *Indian Journal of Pharmaceutical Education and Research*, 57, 526–530.
- Buncharoen, W., Saenphet, S., Saenphet, K., 2019. Relaxant activities of extracts from *Uvaria rufa* Blume and *Caesalpinia sappan* L. on excised rat's prostate strips. *Journal of Pharmaceutical Research International*, 29, 1–12.
- Couvreur, T.L.P., Dagallier, L.P.M.J., Ballings, P., Bissiengou, P., Chatrou, L.W., et al., 2022. Flora of Cameroon - Annonaceae Vol 45. *PhytoKeys*, 207, 1–532.
- Department of Health of the Republic of Indonesia (Depkes RI), 2000. *General Standard Parameters for Medicinal Plant Extracts*. Directorate General of Food and Drug Administration, Jakarta.
- Erkens, R.H.J., van den Berg, R.G., van Zanten, B.T., Maas, P.J.M., Chatrou, L.W., et al., 2022. Spatial distribution of Annonaceae across biomes and anthromes: Knowledge gaps in spatial and ecological data. *Plants, People, Planet*, 5, 520–535.
- Falah, M.N.A., Sa'diyah, K., 2024. Effect of tofu dregs ratio on the quality of tilapia feed products. *Jurnal Teknologi Separasi*, 10, 171–179.
- Faramayuda, H., Prasetyo, E., Kurniawan, B., 2021. Improving the product quality of Jamu Manunggal Association of Cimahi City through standardization of raw materials. *AJAD: Jurnal Pengabdian Kepada Masyarakat*, 2, 123–130.
- Hayati, S., Ahmad, R., Fitriyani, L., 2019. *Pharmacognosy Practicum Module*. Prof. Dr. Hamka Muhammadiyah University, Jakarta.
- Isnayanti, I., 2020. Isolation and identification of endophytic bacteria from leaves and stem bark of lelak plant (*Uvaria rufa* Blume) as antibacterial substances. Thesis. Universitas Airlangga, Surabaya.
- Miller, J.N., Miller, J.C., Miller, R.D., 2018. *Statistics and Chemometrics for Analytical Chemistry*. 7th ed. Pearson Education Limited, Harlow.
- Ministry of Health (MOH), 2017. *Indonesian Herbal Pharmacopoeia*. 2nd ed. Directorate General of Pharmaceuticals and Medical Devices, Jakarta.
- Mustapa, M.A., Abdulkadir, W., Halid, I.F., 2020. Standardization of specific parameters of methanol extract of kebiul seed (*Caesalpinia bonduc* L.) as standardized herbal medicine raw material. *Jurnal Syifa Science and Clinical Research*, 2, 49–58.
- Ningsih, A.W., Azizah, M.N., Sinaga, B., 2022. Standardization of moringa leaf (*Moringa oleifera* L.) simplisia from Luwung Sidoarjo village using food dehydrator drying. *Journal of Pharmacy and Herbs*, 5(1), 1–10.
- Patty, J.O., Siahaan, R., Maabuat, P.V., 2018. Presence of heavy metals (Pb, Cd, Cu, Zn) in water and sediments of Lowatag River, Southeast Minahasa - North Sulawesi. *Jurnal Biologos*, 8, 15–20.
- Putri, F.A., Diharmi, A., Karnila, A., 2022. Identification of secondary metabolite compounds in brown seaweed (*Sargassum plagyophyllum*) by fractionation method. *Jurnal Teknologi dan Industri Pertanian Indonesia*, 15, 41–46.
- Rahayu, R.B., Kurniawan, A., 2020. *Core Science Materials: Physics, Chemistry, Biology for SMP/MTs*. Genta Group Production, Sidoarjo.
- Riswanto, F.D.O., Windarsih, A., Lukitaningsih, E., Rafi, M., Fadzilah, N.A., Rohman, A., 2022. Metabolite fingerprinting based on ¹H-NMR spectroscopy and liquid chromatography for the authentication of herbal products. *Molecules*, 27(1198), 1–17.

- Rusmalina, S., Haryadi, D., Putri, A.P., Fitriyah, N., 2023. Quantitative analysis of metal contaminants in the production of acidic turmeric instant powder herbal medicine through the formation of metal-ditizone complexes. *Jurnal Pharmaceutical Science*, 2, 73–84.
- Setyaningrum, E.W., Hasan, M., Arifin, Z., 2018. Analysis of heavy metal content of Cu, Pb, Hg and Sn dissolved in the coast of Banyuwangi Regency. In: *Proceedings of the National Seminar on Marine and Fisheries IV 2018*.
- Thang, T.D., Dai, D.N., Le, A.T., Ogunwande, I.A., 2014. Constituents of essential oils from the leaves and stem barks of *Uvaria rufa* and *Uvaria cordata* (Annonaceae) from Vietnam. *Journal of Essential Oil Bearing Plants*, 17, 427–434.