

## Standardization of Simplicia and Ethanol Extract of Purun Danau (*Lepironia articulata* (Retz.) Domin) Rhizome

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Standardization

### Abstract

Purun Danau (*Lepironia articulata* (Retz.) Domin) has been shown to have antimalarial and antioxidant activity. This study aimed to standardize simplicia and extract of *L. articulata* by determining the value of its specific and non-specific parameters. A sampling of *L. articulata* rhizome was carried out in Guntung Manggis, Haur Gading, and Halat. The standardization method used was based on the Indonesian Herbal Pharmacopoeia and the General Standard Parameter of Extract. The organoleptic observations showed that *L. articulata* simplicia was reddish-brown in color, chelated taste, and had a specific odor. Microscopic observation showed parts of this plant: epidermis, cortex, endodermis, parenchyma, bundle vessels, and scalariform vessels. Ethanol-soluble extract content was 10.00-12.66%, water-soluble extract content 8.03-10.87%, drying shrinkage 7.10-7.33%, total ash content 2.03-2.52%, acid-insoluble ash 0.33-0.42%, Pb content 5.698-9.989 ppm, Cd content 0.300-0.500 ppm, Hg content 0.070-0.090 ppm. Ethanol extract of *L. articulata* rhizome contained alkaloids, flavonoids, tannins, glycosides, and saponins. The yield obtained was 8.05-11.23%, total ash content was 1.58-1.67%, acid-insoluble ash was 0.23-0.33%, and water content was 7.10-8.50%. Standardization of simplicia and ethanol extract of *L. articulata* rhizome has met the criteria.

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## INTRODUCTION

One of the plants known to be efficacious as medicine is purun danau (*Lepironia articulata* (Retz.) Domin), especially the rhizome. Our previous study showed that the ethanolic extract of the rhizome of *L. articulata* contained alkaloids, flavonoids, tannins, saponins, and anthraquinone compounds<sup>1</sup>. The rhizome of *L. articulata* has antimalarial and antioxidant activity<sup>2</sup>. Traditional medicinal raw materials from plants are often constrained by scientific research in clinical trial research and standardization of these traditional medicinal raw materials<sup>3</sup>.

The *L. articulata* plant is generally used as raw material for making hands woven by utilizing its stems. The plants are widespread in several countries such as Sri Lanka, India, China, Indonesia, Malaysia, Micronesia, Australia, New Caledonia, and Fiji<sup>4</sup>. The plant can live in a swamp or brackish land, which is included in the Cyperaceae group so that *L. articulata* may contain chemical compounds of alkaloids, flavonoids, steroids, and tannins<sup>5</sup>. The dominant plant part of *L. articulata* widely studied because it is considered efficacious is the rhizome. *Lepironia articulata*, emergent aquatic to approximately 200 cm tall, grow in Esk River, New South Wales, Australia. *Lepironia articulata* (grey-green plant to

left-hand side foreground) growing with *Machaerina articulata* (right-hand side) and naturalized *Nymphaea caerulea* (mid-foreground). Pseudo-lateral inflorescence at the functionally female stage, past functionally male phase with spider and grey mold and fully mature, with most fruits and bracts fallen<sup>6</sup>.

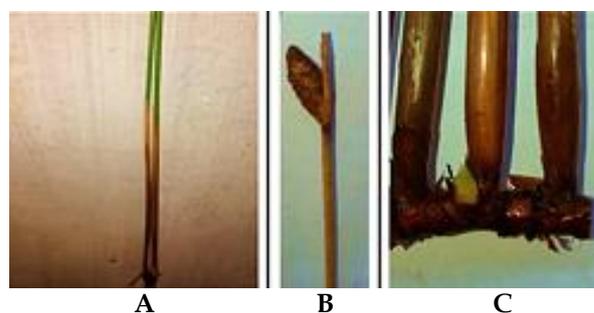
The development of Indonesian traditional medicines continues to be carried out towards obtaining standardized herbal medicinal products and phytopharmaceuticals, so the raw materials for these medicines must meet the quality standards of materials expected to guarantee their safety and efficacy<sup>7</sup>. The thing that can be done to meet the quality standards of medicinal raw materials is to standardize the raw materials of the drug. Standardization is a series of procedures or tests carried out physically, chemically, and biologically to ensure the quality of drugs and the quality of raw materials is obtained<sup>8</sup>. This study aims to determine the standardization of simplicia and ethanol extract of *L. articulata* rhizome consisting of specific and non-specific parameters. Specific parameters include organoleptic, microscopic, ethanol-soluble extract content, water-soluble extract content, description of extracts, yield, and phytochemical screening. Non-specific parameters include drying shrinkage, total ash content, acid insoluble ash content, and metal contamination.

## MATERIALS AND METHODS

### Materials

The tools used were furnace (Ney-Vulcan D-550), hot plate stirrer (Stuart), atomic absorption spectrophotometer, microscope, oven (Vinco), silica gel plate 254, analytical balance (Pioneer), water bath (SMIC). The materials used in this study were fresh rhizome of *L. articulata* (Retz.) Domin (**Figure 1**) obtained from three sites in South Kalimantan: Guntung Manggis in the city

of Banjarbaru (A), Haur Gading in the Hulu Sungai Tengah Regency (B), and Halat in the Balangan Regency (C), as presented in **Figure 2**. The determination of this plant was carried out at the Indonesian Institute of Sciences, Bogor, Indonesia, with specimen number B-43/IV/DI.01/2021. The reagent used was hydrochloric acid, nitric acid, sulfuric acid, ethanol, ethyl acetate, FeCl<sub>3</sub>, 10% gelatin, potassium hydroxide, chloroform, n-hexane, sodium hydroxide, methanol, Dragendorff's reagent, Liebermann Burchard's reagent, Mayer's reagent, magnesium powder, and toluene.

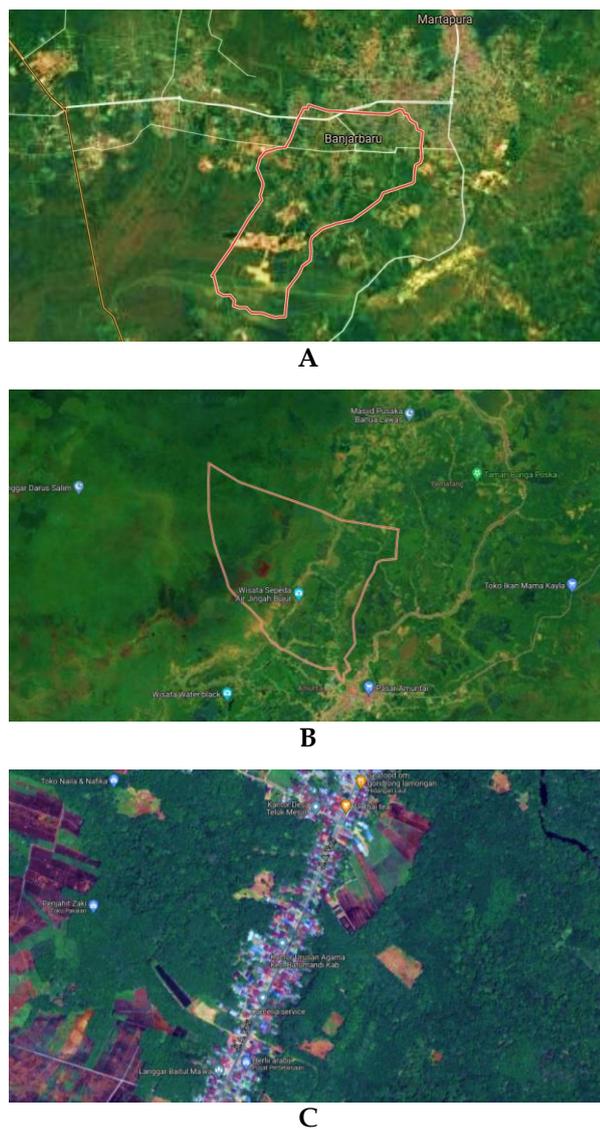


**Figure 1.** Stem (A), flower (B), and rhizome (C) of *L. articulata*

### Methods

#### *Simplicia preparation*

*Lepironia articulata* was obtained from swamp area by purposive sampling method. The determination of the three selected locations was based on the consideration of the size of the growing *L. articulata* population and the condition of the geographical area. The fresh *L. articulata* rhizomes were sorted and cleaned, and then chopped. The rhizome of *L. articulata* was dried using an oven at a temperature of 55°C until the sample was dry with a water content of less than 10%. This temperature range was ideal for drying herbal raw materials, because higher drying temperatures could destroy the active components in simplicia. Furthermore, the simplicia was powdered with a number 25 sieve until a fine powder was obtained<sup>9</sup>. *Lepironia articulata* rhizome simplicia obtained weighing about 600 g from 1000 g of fresh samples.



**Figure 2.** Map of sampling areas in Guntung Manggis (A), Haur Gading (B), and Halat (C)

#### Ethanol extract preparation

As much as 400 g of *L. articulata* simplicia powder was put into the macerator, then 96% ethanol solvent was added until all simplicia was submerged. The filtering process was carried out for five days, in which every 24 hours, the solvent was changed and continuously stirred every six hours. The filtrate was then evaporated using a water bath until a thick extract was obtained. The method used for solvent evaporation is rotary evaporation. The extract obtained was weighed, and the yield was expressed in percentage w/w<sup>10,11</sup>.

#### *Simplicia* standardization (Specific parameters)

##### 1. Organoleptic test

Organoleptic tests were carried out using the five senses to determine the form, color, smell, and taste of the *L. articulata* simplicia. Observations were made after the simplicia came into contact with air for 15 minutes<sup>12</sup>.

##### 2. Microscopic test

Microscopic tests were carried out using a microscope with a magnification of 10x, with transverse and longitudinal preparations.

##### 3. Ethanol-soluble extract content

As much as 5 g of *L. articulata* simplicia powder was macerated with 100 mL of 96% ethanol for 18 hours. The 20 mL filtrate was evaporated until dried in a porcelain dish. The remaining filtrate was heated at 105°C until the weight remained constant.

##### 4. Water-soluble extract content

As much as 5 g of *L. articulata* simplicia powder was macerated with 100 mL of chloroform saturated distilled water in Erlenmeyer for 18 hours. The 20 mL filtrate was evaporated to dryness in a porcelain dish. The remaining filtrate was heated at 105°C until the weight remained constant.

#### *Simplicia* standardization (Non-specific parameters)

##### 1. Drying shrink

The porcelain crucible was heated at 105°C for 30 minutes then added with 1-2 g of *L. articulata* simplicia. The silicate crucible containing simplicia was left open in the oven at 105°C for 30 minutes until the weight remained constant.

##### 2. Total ash content

Simplicia powder (2 g) in a crucible that had been tared was put at incandescent at 800°C for 6 hours, then cooled and weighed. Total ash content was calculated to the initial powder weight in %w/w.

### 3. Acid-insoluble ash content

Five grams of simplicia powder was macerated with 100 mL of 96% ethanol for 18 hours. The 20 mL filtrate was evaporated to dryness in a porcelain dish. The remaining filtrate was heated at 105°C until the weight remained constant.

### 4. Metal contamination of Pb, Hg, and Cd

Thirty grams of simplicia powder was placed in a porcelain cup then ignited in a furnace. The temperature was gradually increased to 100°C every 30 minutes until the temperature reached 450°C and allowed to stand until the ash turned white. The ash is then cooled at room temperature. After cooling, 5 mL of 6 M HCl was added while shaking until the ash dissolved. The solution was evaporated on a hotplate at 100°C until dried. The sample was added with 10 mL of 0.1 M HNO<sub>3</sub> and then cooled for an hour. The solution was added 0.1 M HNO<sub>3</sub> to 100 mL, then filtered with Whatman paper, and the filtrate was read using an atomic absorption spectrophotometer (AAS)<sup>13</sup>.

#### Extract standardization (Specific parameters)

##### 1. Description of extract

The description of the extract used the five senses in terms of describing color, smell, and taste<sup>11</sup>.

##### 2. Yield

The yield was the percentage of the extract obtained from simplicia.

##### 3. Phytochemical screening

Harborne developed the standard procedure to analyze biologically active compounds, including alkaloids, saponins, flavonoids, tannins, anthraquinones, phenolics, glycosides, triterpenoids, and steroids<sup>14</sup>.

###### a. Alkaloids

As much as 0.2 g of the thick extract of the rhizome of *L. articulata* was dissolved in 2 mL of ethanol

and then dripped with 2 N HCl then divided into two test tubes of 1 mL each. In the first tube, ten drops of Mayer's reagent were added, in which a positive result was indicated by the formation of a yellow or white precipitate. In the second tube, ten drops of Dragendorff's reagent were added, in which the presence of an orange precipitate indicated alkaloid's existence<sup>15</sup>.

###### b. Triterpenoids and steroids

As much as 0.1 g of the thick extract of the rhizome of *L. articulata* was dissolved in 1 mL of ethanol, then ten drops of Liebermann Burchard reagent were added. A color change indicated the positive results, as triterpenoids would appear red-purple while steroids would appear blue-green<sup>16</sup>.

###### c. Saponins

As much as 0.1 g of the thick extract of the rhizome of *L. articulata* was added with 10 mL of hot water, then two drops of 2 N HCl were added. Saponins were indicated by the formation of stable foam as high as 1-10 cm for not less than 10 minutes<sup>17</sup>.

###### d. Flavonoids

As much as 0.1 g of the thick extract of the rhizome of *L. articulata* was dissolved in 1 mL of ethanol. The solution was then added with Mg powder and a drop of concentrated HCl. A change in color to orange, red, or yellow indicated positive results<sup>18</sup>.

###### e. Tannins

As much as 0.1 g of the thick extract of the rhizome of *L. articulata* was dissolved in 1 mL of ethanol. The test solution was added with 1 mL of 10% gelatin. The appearance of a white precipitate indicated positive results<sup>19</sup>.

###### f. Anthraquinone

As much as 0.1 g of the thick extract of the rhizome of *L. articulata* was dissolved in 1 mL of ethanol,

then added with ten drops of 10% KOH (in methanol). A change in color to yellow or yellow-brown indicated the presence of anthraquinone<sup>20</sup>.

g. Phenolic

As much as 0.1 g of the thick extract of the rhizome of *L. articulata* was dissolved in 1 mL of ethanol, then added with five drops of 10% FeCl<sub>3</sub>. A change in color to blue-black indicated phenolics presence<sup>21</sup>.

h. Glycoside

As much as 0.1 g of the thick extract of the rhizome of *L. articulata* was dissolved in 1 mL of ethanol, then added with ten drops of chloroform and concentrated sulfuric acid. A change in color to brown indicated positive results<sup>22</sup>.

*Extract standardization (Non-specific parameters)*

1. Water content

Measurement of water content was carried out using toluene distillation. Five grams of *L. articulata* thick extract was placed in a round bottom flask and then added toluene solvent that had been saturated with 200 mL of water. The solution was heated for 100 minutes until the toluene boiled. After it was boiled, then the solution was cooled to room temperature. The volume of water was read after the toluene and water had separated. The water content was determined in percentage.

2. Total ash content

Two grams of thick extract in a crucible ignited at 800°C for six hours, then cooled and weighed. Total ash content was calculated to the initial powder weight in %w/w.

3. Acid-insoluble ash content

The ash obtained from the total ash content test was boiled with 25 mL of dilute HCl for five minutes. The ash was then filtered using ash-free filter paper and then washed with hot water to collect acid-insoluble

ash, then ignited with a porcelain crucible in a furnace to obtain ash with a constant weight. The content was calculated from the initial weight of the powder, expressed in %w/w<sup>23</sup>.

*Data Analysis*

The results obtained showed in qualitative and quantitative data. Qualitative data were the results of organoleptic, microscopic, and phytochemical screening. In contrast, quantitative data resulted from drying shrinkage testing, water content, total ash content, acid-insoluble ash content, water-soluble extract content, ethanol-soluble extract content, and heavy metal contamination levels. The data was made in tabular form along with the documentation and analyzed descriptively.

**RESULTS AND DISCUSSION**

*Ethanol extract preparation*

The results obtained from each growing place were different, as presented in **Table I**. This result was more than our previous study, in which from 400 g of *L. articulata* rhizome powder using the maceration method for 3x24 hours and the solvent used was 96% ethanol, the yield was 8.232%<sup>1</sup>. Sampling was carried out in three different places with different environmental factors that affected the yield. Metabolism in plants is influenced by environmental factors such as soil, altitude, light, temperature, humidity, organic compounds, and inorganic compounds. Specimens of the same plant species grown under different environmental conditions showed significant differences in the production and accumulation of primary and secondary metabolites. Chemical interactions between plants and their environment are mediated primarily by the biosynthesis of secondary metabolites, which carry out their biological roles, as a plastic adaptive response to their environment<sup>24</sup>.

**Table I.** Yield of *L. articulata* rhizome ethanol extract

Area	Powder weight (g)	Thick extract weight (g)	Yield (%)
Guntung	400	44.90	11.23
Manggis			
Haur	400	37.81	9.45
Gading			
Halat	400	32.18	8.05

**Simplicia standardization (Specific parameters)***Organoleptic test*

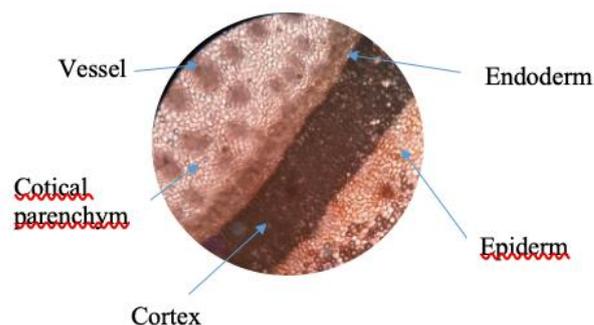
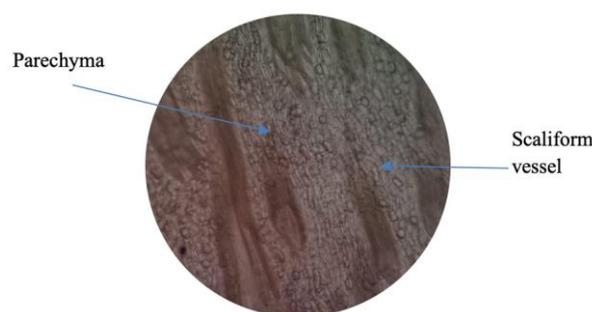
The organoleptic test was carried out with the help of five panelists who explained the shape, color, smell, and taste of the simplicia, as presented in **Table II**. *Lepironia articulata* simplicia powder has a characteristic odor, with a chelate taste and reddish-brown color. The simplicia of *L. articulata* had a chelate taste with a reddish-brown color because it contains tannins and polyphenols<sup>25</sup>.

**Table II.** Organoleptic properties of *L. articulata* simplicia

Area	Smell	Taste	Color	Form
Guntung	Specific	Chelate	Reddish-brown	Coarse powder
Manggis				
Haur	Specific	Chelate	Reddish-brown	Coarse powder
Gading				
Halat	Specific	Chelate	Reddish-brown	Coarse powder

*Microscopic test*

Microscopic tests were carried out using an optical microscope with a magnification of 10 times to observe the constituent elements of a sample. Microscopic observations on the rhizome of *L. articulata* were carried out transversely (cross) and longitudinally. The results of cross-sections of *L. articulata* rhizomes from the three locations showed the same results covering the epidermis, cortex, endodermis, cortical parenchyma, and bundle vessels (**Figure 3**). The cross section of *L. articulata* rhizome from the three locations also showed similar results, consisting of parenchyma cells and scaliform vessels (**Figure 4**), which results were also found in longitudinal sections.

**Figure 3.** Cross section of *L. articulata* rhizome**Figure 4.** Longitudinal section of *L. articulata* rhizome*Ethanol and water-soluble extract content*

The results obtained in determining the levels of dissolved compounds are relatively small, as presented in **Table III**. The results were different because the samples used in this study were taken from three different places. Differences in plant growth area affect the percentage composition of chemical compounds in a plant<sup>26</sup>. The water solvent used aims to dissolve polar compounds, and ethanol solvent is used to dissolve polar-nonpolar compounds<sup>27</sup>.

**Table III.** Determination of water-soluble and ethanol-soluble extract content of *L. articulata* simplicia

Area	Water-soluble extract content (%)	Ethanol-soluble extract content (%)
Guntung	10.87 ± 0.15	12.66 ± 0.05
Manggis		
Haur	8.80 ± 0.10	10.73 ± 0.15
Gading		
Halat	8.03 ± 0.15	10.00 ± 0.10

**Simplicia standardization (Non-specific parameters)**

The results of non-specific parameter testing of *L. articulata* rhizome simplicia were presented in **Table IV**. The results of the drying shrinkage test of *L. articulata*

rhizome simplicia meet the standards set by the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM RI), which is less than 10%<sup>28</sup>. Dry shrinkage is generally related to the water content contained in simplicia, so the smaller the drying loss, the better the results obtained. The smaller the water content contained in simplicia, it can reduce the growth of molds and fungi as well as enzymatic reactions that can damage the quality of simplicia<sup>29</sup>.

Data on total ash content and acid-insoluble ash content in the simplicia rhizome of *L. articulata* did not differ. The higher the total ash content obtained, the higher the mineral content contained in the simplicia. Humans need minerals such as calcium, phosphorus, and magnesium for bone formation, sodium, and chloride for body fluids, and iron to form hemoglobin and red blood cells. Minerals can also be harmful if they accumulate in the human body over a long time, which can interfere with the circulatory, nervous, and kidney systems such as mercury, lead, copper, cadmium, and strontium<sup>30</sup>. The acid-insoluble ash content reflects the contamination of minerals or metals that are not acid soluble in the simplicia. The high content of acid-insoluble ash in simplicia indicates the presence of impurities such as soil or sand, metallic elements silver, lead, and mercury<sup>15,23</sup>.

Measurement of metal content ensures that the samples do not contain heavy metals that can endanger health if they exceed the specified requirements<sup>12</sup>. Based on the results obtained, the Cd levels from the three sites did not meet the specified requirements<sup>31</sup>. The results obtained can be influenced by genetic factors and environmental factors. The simplicia processing process can also cause high levels of cadmium metal from sampling to sample processing, such as in the sample washing process, which may be less clean because the sample used itself is a rhizome so that the risk factor is greater. The use of metal materials during sample processing can affect the results

obtained—in the sample processing, chopping the rhizome using a machete made of iron so that metal contamination is possible. Therefore, it can be reviewed more carefully in terms of simplicia processing.

**Table IV.** Non-specific parameter testing of *L. articulata* simplicia

Standardization Parameters	Result			Requirements <sup>28</sup>
	Guntung Manggis	Haur Gading	Halat	
Drying shrinkage (%)	7.78	7.10	7.33	<10
Total ash content (%)	2.52	2.03	2.05	-
Acid-insoluble ash content (%)	0.43	0.33	0.42	-
Pb contamination (mg/kg)	9.989	5.698	6.297	≤10
Cd contamination (mg/kg)	0.499	0.500	0.300	≤0.03
Hg contamination (mg/kg)	0.070	0.079	0.090	≤0.5

**Extract standardization (Specific parameters)**

*Description of extract*

Extract description is the initial stage in the introduction, which is carried out as simply and objectively as possible by using the five senses with the help of panelists who are asked to describe the shape, color, smell, and taste of the *L. articulata* rhizome extract, as presented in **Table V**. Based on the results presented by the panelists, the ethanolic extracts of *L. articulata* rhizomes in three places all had a thick extract form, a characteristic odor, and a chelating taste. The data obtained from the panelists are collected and converted into quantitative data (quantification) using 'coding' so that conclusions can be drawn from the organoleptic results. Towaha reported that the taste of chelates in plants is because it contains tannins and polyphenol compounds<sup>32</sup>. The smell of the *L. articulata* rhizome ethanol extract from the three places obtained a distinctive and more pungent odor than simplicia because the compounds contained in the *L. articulata* rhizome had been extracted. The color of the *L. articulata* rhizome ethanol extract from the three places

was reddish-brown which was more concentrated than the liquid extract due to the concentration process.

**Table V.** Organoleptic examination of *L. articulata* ethanol extract

Area	Parameters				Extract profile
	Form	Color	Smell	Taste	
Guntung Manggis	Thick Extract	Reddish-brown	Specific	Chelate	
Haur Gading	Thick Extract	Reddish-brown	Specific	Chelate	
Halat	Thick Extract	Reddish-brown	Specific	Chelate	

### Phytochemical screening

Screening of phytochemical content of ethanolic extract of *L. articulata* rhizome aims to determine the content of active ingredients, which are secondary metabolites in plants. These active ingredients can function as a plant's self-defense against the environment, disease, and insects<sup>33</sup>. The results of phytochemical screening of *L. articulata* rhizome extract at three locations indicated the presence of alkaloids, flavonoids, saponins, anthraquinones, glycosides, tannins, and phenolics, as presented in **Table VI**. This is consistent with our previous study, which also reported the presence of alkaloids, flavonoids, tannins, saponins, and anthraquinones<sup>1</sup>.

**Table VI.** Phytochemical screening of *L. articulata* ethanol extract

Phytochemicals	Guntung Manggis	Haur Gading	Halat
Alkaloids	+	+	+
Terpenoids/steroids	-	-	-
Flavanoids	+	+	+
Saponins	+	+	+
Anthraquinones	+	+	+
Glycosides	+	+	+
Tannins	+	+	+
Phenolics	+	+	+

Notes: (+) presence of the phytochemicals; (-) absence of the phytochemicals

### Extract standardization (Non-specific parameters)

The results of non-specific parameter testing of *L. articulata* rhizome ethanol extract included water content, total ash content, and acid-insoluble ash content, as presented in **Table VII**. The total ash content and acid-insoluble ash obtained from the three sites did not show a significant difference. This value has met the requirements set by BPOM RI, which states that the water content contained in the extract should not be more than 10%.

**Table VII.** Non-specific parameter testing of *L. articulata* ethanol extract

Standardization Parameters	Results			Requirements <sup>33</sup>
	Guntung Manggis	Haur Gading	Halat	
Water content (%)	8.50	7.10	7.60	≤10
Total ash content (%)	1.67	1.58	1.63	-
Acid-insoluble ash content (%)	0.33	0.22	0.23	-

## CONCLUSION

The results of specific and non-specific parameters of simplicia and ethanol extract of *L. articulata* rhizome at three different places (Guntung Manggis, Haur Gading, and Halat) can be used as a standard for using *L. articulata* rhizome as raw material for traditional medicine.

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## AUTHORS' CONTRIBUTION

**Arnida:** conceived and design the analysis, writing of the paper. **Maulidia:** conceived and design the analysis. **Amalia Khairunnisa:** collected data, contributed data or

analysis tools. **Sutomo**: conceived and design the analysis, writing of the paper. **Faisal**: collected data, contributed data or analysis tools.

## DATA AVAILABILITY

None.

## CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

The author is fully responsible for the content and writing of this article.

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