Jurnal Ilmiah Farmasi (Scientific Journal of Pharmacy) 21(1) January-May 2025, 66-77
ISSN: 1693-8666
available at http://journal.uii.ac.id/index.php/JIF

Determination of specific and non-specific parameters of saluang belum (*Luvunga sarmentosa* (Blume) Kurz.) root extract and quantification of its total flavonoid content

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Abstract

Background: Saluang belum (*Luvunga sarmentosa* (Blume) Kurz.) is an endemic plant native to the Kalimantan region. The Dayak people traditionally consume a decoction of *L. sarmentosa* root to improve stamina. Previous research has confirmed that the roots of *L. sarmentosa* possess antioxidant properties.

Objective: This study aims to determine the characterization values of both specific and nonspecific parameters of the ethanol extract from *L. sarmentosa* roots, as well as to assess the total flavonoid content.

Method: The research samples were collected from Timpah Village, Central Kalimantan. The methods for standardization employed the general standard parameters for Indonesian Herbal Extracts and the Pharmacopoeia. Specific parameters include the characteristics of the extract, phytochemical screening, and thin-layer chromatography profiles, while nonspecific parameters consist of determining water content, total ash, acid-insoluble ash content, and heavy metal contaminants (Pb, Cd, and Hg). The total flavonoid content was determined using a colorimetric method with $AlCl_3$ and quercetin as the standard.

Results: The extract was characterized as a thick, yellowish-brown substance with a strong, distinct odor and a bitter taste. It contains alkaloids, flavonoids, tannins, terpenoids, and steroids. The TLC profile revealed four distinct spots using a non-polar eluent and six spots with a polar eluent. The extract yield was 8.57%, with an average water content of 2.43±0.15%, total ash content of 1.50%, and acid-insoluble ash content of 1.00±0.87%. Heavy metal contamination levels were determined as follows: Pb < 0.001 mg/kg, Cd 0.446 mg/kg, and Hg 2.077 mg/kg. The total flavonoid content of the extract was quantified at 6.9147 ± 0.0083 mg QE/g extract.

Conclusion: All specific and non-specific parameters of the extract meet the requirements except for the heavy metal contaminants of Hg and Cd, which are still within the threshold limits set by BPOM. Meanwhile, the total flavonoid content measured was 6.9147 ± 0.0083 mg QE/g.

Keywords: Saluang belum, *Lavanga sarmentosa*, total flavonoid, Dayak tribe, extract standardization

1. Introduction

Indonesia is one of the countries with a tropical climate, allowing it to possess a diversity of advantageous biological natural resources. It is home to an abundance of plant species with immense potential as sources of medicinal compounds. Ancient civilizations have utilized medicinal plants for therapeutic purposes, drawing upon empirical knowledge. One of the plants commonly used for medicinal purposes is saluang belum (Marjoni, 2022).

Saluang belum (*L. sarmentosa*) is an endemic plant species found exclusively in Central and South Kalimantan. Local communities have historically recognized and employed it as traditional medicine for various of diseases, including back and kidney pain, as well as vitality enhancer (Anggriani, 2018). The Dayak people traditionally consume boiled water infused with the roots of *L. sarmentosa* once daily to enhance male fertility, sexual desire, and endurance (Wardah & Sundari, 2019). Previous studies have established that saluang belum root has antioxidant properties with an

IC_{50} of 80.33 ppm. It is undeniably impacted by the concentration of secondary metabolites, including phenol and flavonoid compounds (Wathan & Rizki, 2020). The highest total flavonoid content in *L. sarmentosa* roots was found in the extract using 96% ethanol as the solvent, measuring 6.56 ± 0.006 % w/w QE, compared to 4.10 ± 0.084 % w/w QE with methanol and 5.36 ± 0.012 % w/w QE with ethyl acetate (Wathan *et al*., 2023). According to the research by Musfirah *et al*. (2016), histopathological examinations of testicular organs treated with a 70% ethanol extract of *L. sarmentosa* roots indicate that spermatogenesis occurs normally in mice and that administration of the extract does not harm the testes. Applying a 70% ethanol extract of *L. sarmentosa* influences the formation of spermatocyte and spermatid cells, which indicates an increase in male fertility.

It is imperative to evaluate the character of traditional medicine with respect to its safety, effectiveness, and overall quality (Aulani, 2018). The regulatory framework governing the quality of traditional medicine is specified in regulation No. 32 of 2019, which is issued by the Indonesian Food and Drug Monitoring Agency (BPOM RI). Two parameters are used to standardize extracts, namely specific and nonspecific parameters (Najib *et al.*, 2017). Specific parameters refer to the aspect of both qualitative and quantitative chemical analysis that pertain to the concentrations of active compounds and the pharmacological activity of an extract. Nonspecific parameters refer to the physical, chemical, and microbiological analyses pertaining to the stability and safety of an extract (Marpaung & Septiyani, 2020). Determining the parameters of extract of *L. sarmentosa* is essential for ensuring consistent quality, efficacy, and safety, especially for its future development as a pharmaceutical product. This research highlights the importance of creating standardized parameters that can help validate and optimize the extract's therapeutic potential, supporting its reliable use in medicine. Determining total flavonoid content in *L. sarmentosa* extracts is critical for guaranteeing consistent extract quality and supporting its development as a standardized herbal medicine.

2. Method

2.1. Sample preparation

The roots of *L. sarmentosa* were collected in January 2023 from Timpah Village in Kapuas Regency, Central Kalimantan Province. The roots were extracted from mature *L. sarmentosa* plants, approximately 2 meters in height, exhibiting acceptable conditions, and identified as "male" due to the presence of thorns on the stem and leaf axils. A two kilograms sample was gathered, and the complete plant was submitted for identification to the Laboratorium Dasar at the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University.

2.2. Extraction

The fresh *L. sarmentosa* roots were wet-sorted and then thoroughly washed under flowing water and chopped. Subsequently, it was desiccated at 55°C in a drying cabinet. Using a blender, the root simplicia was subsequently ground into powder form (Kemenkes RI, 2017). The extraction of *L. sarmentosa* roots was performed using the maceration technique. A total of 250 g of *L. sarmentosa* root powder (simplicia) was weighed and transferred into a maceration vessel. An ethanol solvent was then added to cover the powder, reaching a depth of 1 to 2 cm above its surface. The extraction process was carried out over three cycles, each lasting 24 hours, with agitation every six hours. The solvent was replaced after each 24-hour period. The resulting macerate was filtered and evaporated using a water bath (50°C) to complete the extraction procedure.

2.3. Method and result analysis

2.3.1. Specific parameters

a) Extract description

The five senses describe the shape, color, odor, and flavor of the extract. It aims to provide an objective and straightforward introduction to the extract (Depkes RI, 2000). The shape, color, odor, and flavor of the extract can be described to a group of panelists.

b) Yield percentage

The yield is calculated as the proportion of the extracted substance to the initial simplicial (Depkes RI, 2000). The following formula is utilized to determine the percentage yield.

$$\% \text{ yield} = \frac{\text{weight of extract obtained (g)}}{\text{weight of simplicial prior to extraction (g)}} \text{ x } 100\%$$ (Wijaya *et al*., 2018)

c) Phytochemical screening

Phytochemical screening was conducted to identify key bioactive compounds in the plant extract, and it allowed for a preliminary assessment of the extract's bioactive components. Phytochemical screening includes the identification of alkaloids using Dragendorff reagent, flavonoids using $AlCl_3$, foam tests for saponin, tannins with $FeCl_3$ reagent, quinones using H_2SO_4, and terpenoid/steroid compounds using Liebermann Burchard method (Yuda *et al*., 2017).

d) Profile of thin-layer chromatography

One hundred milligrams of thick ethanol extracts from *L. sarmentosa* root were measured. The extract was solubilized in 10 mL of ethanol and subsequently filtered using filter paper. The *L. sarmentosa* roots extract solution was spotted onto a GF254 silica gel plate and further eluted with polar ethyl acetate eluent:n-hexane (7:3) and a non-polar ethyl acetate eluent:n-hexane (3:7) solvent system. Next, the eluted plates were examined under UV light at 254 nm to reveal the stains.

2.3.2. Non-specific parameters

a) Determination of the water content

The water content was determined using the toluene distillation technique. Toluene is first saturated with water, agitated until homogeneous, and then allowed to settle until it separates into two layers (the toluene phase and the water phase). The toluene layer is used as the solvent to determine the water content. After weighing 1 g of *L. sarmentosa* root extract, 40 mL of saturated toluene was added to a round-bottom flask. Following 100 minutes of boiling distillation, the mixture was cooled to room temperature. The observed volume of water was then converted into a percentage (Depkes RI, 2008). The maximum limit of water content is ≤ 10% (BPOM RI, 2019).

b) Determination of the overall ash content

The amount of 2 g of extract, which had been meticulously weighed and crushed, was placed in a heated silicate crucible and leveled. Then, it was cooled after slowly heating the charcoal until it was gone prior to weighing it. When this technique fails to eliminate the charcoal, heated water is introduced, and the solution is filtered via ash-free filter paper. Filter paper and the residual paper are both heated in the identical crucible. The filtrate was placed to the crucible, then it was evaporated, heated, and weighed to a fixed weight. The ash content is calculated through the air-dried material (Depkes RI, 2000).

c) Determination of acid insoluble ash

The ashes acquired during ash content analysis were subjected to boiling for 5 minutes in 25 mL of diluted hydrochloric acid. The insoluble fraction of the acid was gathered, and the ash was filtered using ash-free filter paper and subsequently washed with hot water. The residue and filter paper were burnt until a constant weight was attained. The acid-insoluble ash content was determined using the air-dried sample (Depkes RI, 2000).

d) Determination of heavy metals contamination

Heavy metal contamination (Pb, Cd, and Hg) can be analyzed using the atomic absorption spectrophotometry (AAS) method. A total of 1 g of extract was added to 10 mL of concentrated HNO_3. The mixture was then heated until it becomes thick or dry. Once the extract was thick and cooled, 10 mL of distilled water and 5 mL of perchloric acid were added. The mixture was re-heated again until thick and then filtered into a 50 mL volumetric flask. Distilled water is added up to 50 mL, and the sample is measured using the AAS method (Depkes RI, 2000). Lead (Pb) contamination is limited to ≤ 10 mg/kg or mg/L, cadmium (Cd) to ≤ 0.3 mg/kg or mg/L, and mercury (Hg) to ≤ 0.5 mg/kg or mg/L, as per BPOM RI regulation (2019).

2.3.3. Total flavonoid determination

Quantitative analysis was performed using UV-Vis spectrophotometry to determine the total flavonoid content of the 70% ethanol extract of *L. sarmentosa* root. The total flavonoid content was determined using a colorimetric method with $AlCl_3$ as a reagent and quercetin as the standard.

3. Result and discussion

The identification of the *L. sarmentosa* sample confirmed that the saluang belum plant from Timpah Village, Kapuas Regency, Central Kalimantan, belongs to the species *Luvunga sarmentosa* (Blume) Kurz and is classified under the Rutaceae family, as stated in certificate 065/LB.LABDASAR/II/2023. Two kilograms of *L. sarmentosa* roots, from mature plants at least 2 meters tall, were collected, washed, sliced, and dried at 55°C for eight hours. The dried roots were then ground into powder (simplicia) and stored in an airtight container, protected from direct sunlight. From the initial collection, 1201.5 grams of simplisia powder were obtained, equating to a yield of 60.075%. The simplicia powder was further subjected to maceration using 96% ethanol. A total of 250 grams of the powdered roots were processed, resulting in 21.44 grams of extract, which represents an extraction efficiency of 8.57%.

3.1. Specific parameters

3.1.1. Characteristics of the extract

Extract description (**Table 1**) was conducted by administering a questionnaire regarding the shape, color, odor, and flavor of the *L. sarmentosa* root extract sample to 5 panelists. The viscous extract was procured through the process of evaporation, employing a rotary evaporator in conjunction with a water bath maintained at a temperature of 50°C. The hue of the resultant extract manifests as a yellowish-brown shade. The alteration in colour can be attributed to the oxidation reactions prompted by the thermal effects of the filtrate's evaporation into extracts (Ramayani *et al*., 2021). The scent and taste generated by the thick extract of *L. sarmentosa* roots can be ascribed to the secondary metabolites contained within, which include bitter-tasting substances like flavonoids and alkaloids (Hasby *et al*., 2019).

Table 1. Results of the description of *L. sarmentosa* root extract

Figure	Shape	Color	Odor	Flavor
	viscous	yellowish brown	strong, distinct	bitter

3.1.2. Phytochemical screening

Table 2. Phytochemical result of *L. sarmentosa* roots extract

No	Phytochemical compounds	Test results	Conclusion
1.	Alkaloid	Sediment orange and yellow	Positive
2.	Flavonoid	Color of solution red yellowish	Positive
3.	Saponins	Not formed foam	Negative
4.	Tannin	Green-black color of the solution	Positive
5.	Quinone	No color solution changed	Negative
6.	Steroid	Formed ring blue greenish	Positive
7.	Terpenoid	Formed ring brownish	Positive

The phytochemical screening test (**Table 2**) shows that viscous extracts of *L. sarmentosa* roots contain alkaloids, flavonoids, tannins, terpenoids, and steroids. The identification of steroid compounds was carried out using the thin layer chromatography (TLC) method. The Liebermann-Burchard reagent sprayed on a plate coated with *L. sarmentosa* root extract, followed by heating in an oven at 105°C for 5 minutes. The TLC results showed a green stain, indicating the positive presence of steroids. Positive steroid reactions are confirmed by the appearance of blue-green stains (Yuda *et al*., 2017). According to Anggriani's research (2018), the roots of *L. sarmentosa* also tested positive for steroids and flavonoids using TLC. Additionally, another study identified alkaloids, terpenoids, flavonoids, tannins, and steroids in the roots of *L. sarmentosa* using LC-MS (Syarpin *et al*., 2023). Qualitative testing is based on observing color changes that occur after the sample is treated with specific reagents. A negative test result, indicating the absence of a compound, may be due to the compound being present in very low concentrations in the extract.

3.1.3. Thin layer chromatography (TLC) profile

The TLC profile of *L. sarmentosa* root extracts (**Figure 1**), utilizing a nonpolar mobile phase of ethyl acetate:n-hexane (3:7) and examined under UV illumination at 254 nm, exhibited four spots with Rf values between 0.56 and 0.88 (**Table 3**). The TLC profile utilizing a polar mobile phase of ethyl acetate:n-hexane (7:3) under identical UV light conditions exhibited six spots with Rf values between 0.58 and 1.08. The spots and Rf values were examined under a 254 nm UV light, generating a luminous background, while the sample manifested as dark spots. The identification extract utilizing TLC seeks to observe the separation of samples through characteristic chromatogram patterns, which arise from polarity discrepancies between the sample and the solvent (eluent), thereby providing insights into the initial chemical composition based on the chromatogram pattern (Depkes RI, 2000). The Rf value was ascertained for the purpose of compound identification. The Rf value is the ratio of the distance travelled by the eluent to the phase movement on the TLC plate, serving as a comparative metric among samples (Sopiah *et al*. 2019).

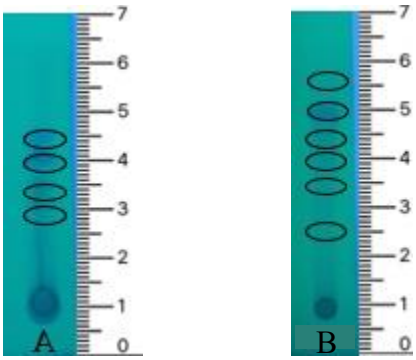

Figure 1. TLC results under $UV_{254\ nm}$ with (**A**) ethyl acetate:n-hexane (3:7) v/v and (**B**) ethyl acetate:n-hexane (7:3) v/v as mobile phase.

Table 3. Rf value of *L. sarmentosa* root extract using n-hexane:ethyl acetate mobile phase under 254 nm UV light

Non polar mobile phase/ ethyl acetate:n-hexane (3:7)	Polar mobile phase/ ethyl acetate:n-hexane (7:3)
$Rf_1 = \frac{2,8}{5} = 0.56$	$Rf_1 = \frac{2,9}{5} = 0.58$
$Rf_2 = \frac{3,3}{5} = 0.66$	$Rf_2 = \frac{3,6}{5} = 0.72$
$Rf_3 = \frac{3,9}{5} = 0.78$	$Rf_3 = \frac{4,0}{5} = 0.8$
$Rf_4 = \frac{4,4}{5} = 0.88$	$Rf_4 = \frac{4,5}{5} = 0.9$
	$Rf_5 = \frac{4,8}{5} = 0.96$
	$Rf_6 = \frac{5,4}{5} = 1.08$

3.2. Nonspecific parameters

3.2.1. Determination of the water content

This study used the toluene distillation method, which fundamentally utilizes toluene-saturated water, to ascertain the water content. Toluene is saturated, thereafter mixed with water, agitated until homogeneous, and allowed to stand until two distinct layers are created (aqueous and toluene phase). Layer toluene is used as a solvent in the determination of water content. Weigh the extract root *L. sarmentosa* as much as 1 g, then enter it into a round-bottom flask, and 40 mL of toluene was added to saturation. Distil for 100 minutes until boiling, then cool down until reaching room temperature. The volume of water obtained was observed and calculated in percentage form (Depkes RI, 2008). The result of determining the water content of ethanol extracts of *L. sarmentosa* roots is 2.43 ± 0.15%. The stipulations outlined in this requirement have been satisfied, as the water content is below 10%. Water content determination is conducted to assess the residual water remaining after the drying or concentration process, with the aim of establishing a permissible range or maximum value for the extract's moisture content. This determination is crucial because high water levels can provide a favourable environment for bacteria and fungi, which may degrade the

extract's constituent compounds (Wahyuni & Anggelina, 2021). Additionally, water content is linked to the extract's purity. Excessively high-water content (>10%) can promote microbial growth, which in turn reduces the stability of the extract (Utami *et al.*, 2017).

3.2.2. Determination of total ash content and acid insoluble ash content

Determination of ash content has the principle of oxidizing inorganic substances at high temperatures (600°C) and then weighing the substances left behind during the ashing process; the higher the ash content, the more mineral content in the extract (Maulana, 2016). The total ash content of the ethanol extract from *L. sarmentosa* roots was determined to be 1.50% ± 0, meeting the requirement of less than 1.9% (Kemenkes RI, 2017). This result satisfies the specified guidelines. Higher total ash content indicates a greater presence of minerals in the material or sample, which may include calcium, phosphorus, magnesium, sodium, chloride, or heavy metals such as mercury, lead, copper, and others (Utami *et al.*, 2017). Additionally, the acid-insoluble ash content of the *L. sarmentosa* root extract was found to be 1.00% ± 0.86, also meeting the requirement of less than 1.5% (Kemenkes RI, 2017). This result complies with the outlined stipulations. Acid-insoluble ash content serves as an indicator of metal or mineral contamination that is insoluble in acid. Elevated acid-insoluble ash content suggests the presence of silicates from sand or soil, as well as metals such as silver, lead, and mercury (Utami *et al.*, 2017).

3.2.3. Determination of heavy metal contamination (Pb, Cd, and Hg)

Natural components in the soil, known as heavy metals, are incapable of degradation or destruction. These compounds can infiltrate the human body through food, drinking water, and air (Hardani *et al*., 2022). Heavy metal contamination is a quality requirement that needs to be considered in the manufacture of traditional medicines (Susanti *et al*., 2023). The test results for heavy metal contamination in *L. sarmentosa* root extract showed a lead (Pb) content of <0.001 mg/kg. The permissible limit for Pb contamination is ≤10 mg/kg or mg/L, so the results comply with the established requirements. However, the cadmium (Cd) contamination level in the root extract was found to be 0.446 mg/kg, exceeding the permissible limit of ≤0.3 mg/kg or mg/L, meaning the result does not meet the requirements. Additionally, the mercury (Hg) content was 2.077 mg/kg, also exceeding the allowed limit of ≤0.5 mg/kg or mg/L, indicating the result does not comply with the regulations (BPOM RI, 2019). Based on this data, *L. sarmentosa* root extract is contaminated with Cd and Hg heavy metals. This contamination may be attributed to natural processes or pollution from human activities. Cd contamination can originate from the combustion of domestic waste, coal, or other fossil fuels (Syamsuddin & Rasjid, 2022). Mercury is a naturally occurring element found in the environment in metallic form, as mercury salts, and as organic mercury. It enters the environment

through the natural breakdown of minerals in rocks and soil due to exposure to wind and water. However, human activities such as fossil fuel combustion, mining, smelting, and solid waste incineration cause the majority of Hg pollution (Irianti *et al.*, 2021). The solution to this problem is to cultivate *L. sarmentosa* in protected environments that are shielded from heavy metal pollution. The procedure includes maintaining controlled soil conditions and pH levels to minimize the heavy metal content in saluang belum, ensuring it is safe for consumption or for processing into herbal products.

3.3. Total flavonoid determination

The determination of total flavonoid content in *L. sarmentosa* used an extract with 70% ethanol as the solvent. According to Riwanti *et al*. (2020), flavonoid compounds, which are polar in nature, tend to dissolve more effectively in 70% ethanol, resulting in a higher flavonoid content compared to 96% ethanol.

The standard curve determination aims to calculate sample concentration using a regression equation based on the known absorbance of a standard. When concentration and absorbance are directly proportional, the r value indicates a linear curve (Suharyanto & Hayati, 2021). A quercetin standard solution with concentrations of 20, 40, 60, 80, and 100 ppm was used. Absorbance was measured with a UV-Vis spectrophotometer at 433.40 nm after 30 minutes of incubation. This is because the reaction between quercetin and $AlCl_3$ produces a yellow solution that absorbs light in the blue wavelength range of 400-435 nm (Kent, 2012). The standard curve for quercetin was replicated three times for each concentration, resulting regression equation y = 0.0066x – 0.0183 with r value of 0.9979.

Table 4. Results of the total flavonoid content analysis from the ethanol 70% extract of *L. sarmentosa* root

Replication	Abs. sample	Total flavonoid content (%w/w QE)	$\bar{x}$ total flavonoid content (%w/w QE) ± SD	RSD (%)
1	0.4381	6.9152		
2	0.4375	6.9061	6.9147 ± 0.0083	0.12
3	0.4386	6.9227		

The total flavonoid content from the 70% ethanol extract of *L. sarmentosa* root, based on three replications (**Table 4**), averaged 6.9147 ± 0.0083 %w/w QE, which is slightly higher than the findings of a previous study by Wathan *et al*. (2023) using a 96% ethanol extract, with a content of 6.56 ± 0.006 %w/w extract. The RSD values of the three replications met the criteria for satisfactory RSD (<2%) due to their low variability, indicating consistent and reliable results.

4. Conclusion

All specific and non-specific parameters of the extract meet the standards, except for the heavy metal pollutants Hg and Cd, which remain under the threshold levels established by BPOM. Consequently, we must conduct an analysis to ascertain the concentration of metallic weight present in the root of *L. Sarmentosa*. The total flavonoid content was determined to be 6.9147 ± 0.0083% w/w QE. This research should be extended to investigate additional characteristics not addressed in this work, including microbial contamination, arsenic as a heavy metal contaminant, and the identification of specific chemicals.

Acknowledgement

The researchers would like to extend sincere appreciation to the Laboratorium Dasar of FMIPA ULM for providing the facilities that ensured the smooth progress of this research.

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