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Determination Of Lead And Cadmium Metal Content In Rambai Leaf Simplicia (Baccaurea Motleyana Müll.Arg.) Using Atomic Absorption Spectrophotometry Method

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Abstract

Rambai leaf (Baccaurea motleyana Müll. Arg.) is traditionally used to treat smallpox, diarrhea, and bruises, but it has the potential to be contaminated with heavy metals such as lead (Pb) and cadmium (Cd) which are toxic and accumulative. This study aims to determine the levels of Pb and Cd in rambai leaf and compare them with the safe limits of BPOM RI and WHO. The type of research is descriptive quantitative with Atomic Absorption Spectrophotometry (AAS) method using an air-acetylene flame system. The study population was all rambai plants in Nanga Kantuk Village, West Kalimantan, with purposive sampling. The main instrument was SSA contrAA 800, while data analysis was carried out through a linear calibration curve (R> 0.998), regression, and calculation of levels (mg/kg). The results showed Pb levels of 4.29 mg/kg and Cd <LOD (0.0131 mg/L). These results indicate that Pb metal was detected, while Cd was not detected quantitatively. In conclusion, the levels of Pb and Cd in rambai leaf simplicia are still below the safe limits of BPOM RI (Pb ≤ 10 mg/kg; Cd ≤ 0.3 mg/kg), so it is safe to use as a traditional medicine ingredient from the aspect of heavy metal contamination.

Keywords: Atomic Absorption Spectrophotometry, Baccaurea motleyana, Cadmium, Lead, Medicinal Plants.

INTRODUCTION

Indonesians have long used medicinal plants traditionally to maintain health and treat various ailments, including as an affordable and accessible alternative treatment. To this day, the use of simple, dried, traditional medicinal ingredients remains popular. The rambai plant (Baccaurea motleyana Müll. Arg.) is one example, widely used empirically in various regions of Indonesia to treat smallpox, diarrhea, and bruises, as reported in an ethnobotanical survey in East Kalimantan involving 390 respondents.

Medicinal plant raw materials can potentially contain heavy metal contamination, such as lead (Pb) and cadmium (Cd), due to absorption from soil, water, and air during growth. These heavy metals are toxic, non-biodegradable, and tend to accumulate in plant tissues, posing health risks to consumers. In Indonesia, the use of medicinal plants contaminated with heavy metals has become a global issue because it can compromise the safety of traditional medicines.

The main problem arises from the ability of rambai plants to accumulate heavy metals, as shown by previous studies that reported its potential as a biosorbent for mercury (Hg) up to 121.95 mg, indicating a similar risk for Pb and Cd. Studies on vegetable plants in polluted lands showed Pb levels reaching 1.58-2.54 mg/kg and Cd 0.07-0.65 mg/kg, which are close to the maximum limits set by the Indonesian Food and Drug Authority (BPOM RI) (Pb 10 mg/kg, Cd 0.3 mg/kg). This contamination often comes from industrial, traffic, and agricultural pollution, thus requiring close monitoring of rambai leaf simplicia.

Chronic exposure to lead (Pb) causes hypertension, nephropathy, anemia, peripheral neuropathy, and encephalopathy in adults, while cadmium (Cd) triggers obstructive pulmonary disease, emphysema, kidney damage, and bone deformities. In children, the accumulation of heavy metals in traditional medicines can cause gastrointestinal disorders such as nausea, vomiting, and diarrhea, as well as reduced intelligence and neurobehavioral development. This risk is further exacerbated by long-term consumption without safety evaluation, as found in herbal medicines in Banda Aceh with Pb levels exceeding the threshold.

This study aims to determine the levels of Pb and Cd in rambai leaf (Baccaurea motleyana Müll. Arg.) simplicia using Atomic Absorption Spectrophotometry (AAS), and compare them with the safe limits of BPOM RI and WHO. The urgency of this research lies in the need to ensure the safety of traditional medicinal ingredients amidst the widespread empirical use, in order to prevent toxic effects on the community. The novelty of this study is the specific analysis of rambai leaf simplicia from West Kalimantan, complementing previous data on its accumulation potential and supporting the standardization of safe herbal medicines.

RESEARCH METHODS

This research is a quantitative descriptive study aimed at determining the levels of lead (Pb) and cadmium (Cd) in rambai leaf (Baccaurea motleyana Müll.Arg.) using the Atomic Absorption Spectrophotometry (AAS) method with an air-acetylene flame system. The quantitative approach was chosen because it allows for accurate and objective numerical measurements of metal levels through calibration curves and linear regression analysis, in accordance with the principles of quantitative research methods that emphasize hypothesis testing and generalization of results. The AAS method was chosen because it has high sensitivity, good selectivity, and low interference for heavy metal analysis in plant matrices, as described in the pharmaceutical analysis literature. In addition, this study follows a positivist paradigm with a focus on verifiable empirical data, as recommended by Sugiyono in the latest edition of quantitative research methodology. Sudaryono emphasized that the quantitative descriptive design is ideal for studies measuring chemical parameters such as contamination levels, with systematic standard procedures to ensure reliability.

The main instrument in this study was a contrAA 800 Atomic Absorption Spectrophotometer (AAS) with an air-acetylene flame system, equipped with a hot plate, analytical balance, oven, and laboratory glassware such as a volumetric flask and Whatman No. 42 filter paper for pre-analysis. Chemicals included HNO3 and HClO4 for wet digestion of samples, as well as Pb and Cd standard solutions (1000 ppm) for calibration curves at wavelengths of 217 nm (Pb) and 228.8 nm (Cd). Data analysis techniques included the creation of a linear calibration curve ($R > 0.998$), calculation of concentration using the regression equation $y = bx + a$, and conversion to mg/kg levels with the formula $C \times V / m$, followed by comparison with the limits of BPOM RI No. 32/2019 ($Pb \leq 10$ mg/kg, $Cd \leq 0.3$ mg/kg). This technique was validated through linearity testing, limit of detection (LOD), and triplicate measurements for precision, in accordance with Emzir's instrumental analysis guidelines, which emphasize instrument validity in quantitative research. Cresswell added that integrating analytical instruments with software such as Aspect CS ensures data triangulation for reliable results.

The population of this study was all rambai leaf (Baccaurea motleyana Müll.Arg.) simplicia growing in Nanga Kantuk Village, Embaloh Hulu District, Kapuas Hulu Regency, West Kalimantan, as a potential source of traditional medicine ingredients. Samples were taken purposively with the criteria of fresh leaves, not wilted, free from visible contaminants, as much as 5.3 kg wet weight which was processed into 2 kg of simplicia powder (yield 37.73%) through sorting, drying (dry cabinet 40°C), grinding (blender), and sieving (mesh 40). The selection of this single representative sample is in accordance with the non-probability sampling technique for initial chemical analysis studies, where sample homogeneity is prioritized over large size, as described by Sugiyono in the quantitative sampling strategy. Sudaryono emphasized that for natural populations such as medicinal plants, purposive samples from endemic locations are adequate for quality inference, with replication of analysis ($n=3$) to reduce bias.

The research procedure began with the collection of fresh rambai leaf samples, followed by taxonomic determination at the Biology Laboratory of Tanjungpura University, wet sorting, washing, slicing, drying (40°C to constant), dry sorting, grinding, and sieving for powdered simplicia. Next, standardization of simplicia through organoleptic tests, water content (moisture analyzer 105°C, ≤10%), ash content (furnace 600°C, ≤8%), and phytochemical screening; then wet digestion (1-2 g sample + 10 mL HNO3 + 5 mL HClO4, hot plate until clear), filtration, and preparation of test solution (25 mL). The creation of a calibration curve from a series of standards (0.05-3 mg/L), SSA calibration, absorbance measurement of triplicate samples, calculation of content, and evaluation against BPOM standards were carried out sequentially to ensure traceability. This procedure follows a logical analytical descriptive flow, with complete documentation for reproducibility, as recommended by Emzir in a structured quantitative methodology. Cresswell and Sudaryono emphasize this systematic sequence to maintain external validity in laboratory design.

RESULTS AND DISCUSSION

Sample Collection and Plant Determination

Plant Determination and Sample Collection

The rambai leaves used as material in this study were taken from Nanga Tidur Village, Empangan District, Kapuas Hulu Regency, West Kalimantan Province. which had previously been determined in the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Tanjung Pura University. The determination process was carried out to determine the truth of the rambai leaf sample (Baccaurea motleyana Müll.Arg.) that would be used for the study in order to avoid errors in collecting materials and the possibility of mixing with other plants. From the determination results, it is known that the plant used in this study is indeed rambai leaves (Baccaurea motleyana Müll.Arg.).

Making Simple Drugs

Table 1. Simple Drugs

Wet weight	Powder weight	Yield (%)
5300	2000	37.73%

From the results of table 1, it was found that the shrinkage of the kirinyuh leaf simplicia was 37.73% with a powder yield of 2000 grams.

Standardization of Simplex for Determining Drying Loss

Drying loss is a measurement of the remaining substance after the drying process using an oven at a temperature of 105°C for 30 minutes and obtaining a constant weight.

Table 2. Drying Loss

Sample	Results	Average	Terms (Library)
Replication I	8.83%		
Replication II	8.96%	8.7%	<10 (FHI, 2017)
Replication III	8.42%		

Determination of Water Content of Simple Drugs

Determining the water content of simple drugs aims to provide a minimum limit or range of water content in the material and to determine the durability of a material during storage.

Table 3. Water Content

Sample	Results	Average	Condition (Library)
Replication I	7.49%		<10% (FHI, (2017)
Replication II	5.95%	6.61%	
Replication III	6.39%		

Ash Content

Determining the ash content is a test where sample heated ontemperature, at which organic compounds and their derivatives are destroyed and evaporated

Table 4. Ash Content

Sample	Results	Average	Condition (Library)
Replication I	4.19%		<8% (FHI, (2017)
Replication II	3.14%	2.79%	
Replication III	1.04%		

The ash content obtained in the Kirinyuh leaf simplicia was 14.78%, which means that the Kirinyuh leaf simplicia has met the standard parameter requirements.

Phytochemical Screening

Table 5. Phytochemical Screening

Compound	Reagent	1	2	3	Results	Reference
Alkaloid	Wagner	+	+	+	Sediment Chocolate	Brown sediment (+)
	Mayer	-	-	-	Sediment Green	White/yellow (+)
	Dragendorf	+	+	+	Sediment Orange	Orange precipitate (+)
Flavonoid	Mg + HCl Powder	+	+	+	Solution orange red	Red/orange yellow (+)
Saponin	Aquadest	+	+	+	Stable foam	Stable foam (+)
Tannin	FeCl3 1%	+	+	+	Blackish green solution	Blue/blackish green (+)
Steroid	Liebermann Burchard	+	+	+	Ring Greenery	Greenish blue ring (+)
Terpenoid	Liebermann Burchard	+	+	+	Brownish ring	Brownish/violet ring (+)

Tool Optimization

The content of Pb and Cd metals in rambai leaf simplex can be determined using the Atomic Absorption Spectrophotometry flame method using an acetylene-air fuel mixture. The Atomic Absorption Spectrophotometry tool must first be optimized to obtain good and perfect analysis results. Optimization conditions for the analysis of Pb and Cd metals using the Atomic Absorption Spectroscopy flame method are carried out to obtain the largest population of atoms at the ground level in the flame passed by radiation. Atoms will absorb the radiation energy that is unique to these atoms and then change to an excited state. The more atoms in the ground state, the more radiation will be absorbed, under optimum conditions maximum absorption will be obtained. The determination of Lead and Iron Metal content in sardines is carried out at wavelengths of 217 nm and 228.8 nm. This wavelength is the wavelength that most strongly absorbs lines for electronic transitions from the ground level to the excited level. If atoms at the ground state energy level are given the appropriate energy, then the energy will be absorbed and the atoms will be excited to a higher energy level (excited state), the atoms are unstable so they will return to the ground energy level by releasing a certain amount of energy in the form of light. The optimum wavelength for lead (Pb) and cadmium (Cd) is respectively: 217 nm and 228.8 nm.

Calibration Curve Optimization

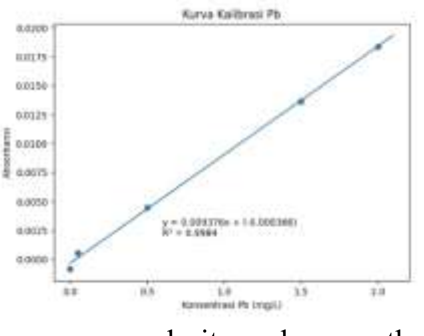
The calibration curve is the relationship between the concentration of the analyte and the absorbance value measured by the instrument. This relationship follows the Lambert–Beer law which states that absorbance (A) is directly proportional to the concentration (c), the light path length (b), and the molar absorptivity coefficient (ε), which is expressed in the equation $A = \varepsilon bc$. In analysis using Atomic Absorption Spectrophotometry (AAS), the light path length and the ε value are considered constant, so that absorbance is directly proportional to concentration. Therefore, the relationship between absorbance and concentration can be expressed in the form of a linear regression equation, namely $y = bx + a$, then a straight line can be drawn. The linear relationship between absorbance and concentration is evaluated through the coefficient of determination (R^2). An R^2 value close to 1 indicates that the relationship between the two variables is very linear and the calibration curve is suitable for use in quantitative analysis.

Standard pb solution

The standard Pb solution was prepared from a stock solution of $Pb(NO_3)_2$ with a concentration of 1000 ppm, then diluted to several working concentrations. The absorbance measurement of the standard solution was carried out using an Atomic Absorption Spectrophotometer (AAS) with a flame system. The resulting data

Table 6. Standard pb solution

No	Pb concentration (mg/L)	Absorbance (Mean±(SD)
1.	0.00	-0.00086± 0.00056
2.	0.05	0.00053± 0.00086
3.	0.50	0.00444± 0.00072
4.	1.50	0.01365± 0.00009
5.	2.00	0.01838± 0.00008



Based on the calibration curve graph, it can be seen that the higher the Pb concentration, the greater the absorbance value produced. The relationship between concentration and absorbance shows a linear pattern. From the results of the linear regression analysis, a linear regression equation $y = bx + a$ was obtained, namely $y = 0.009376x - 0.000366$ with a coefficient of determination (R^2) of 0.9984. The R^2 value approaching 1 indicates that the relationship between Pb concentration and absorbance has very good linearity, so this calibration curve is suitable for quantitative determination of Pb concentration in samples. In this equation, y represents absorbance, x represents Pb concentration (mg/L), b is the slope that indicates the sensitivity of the method to changes in concentration, and a is the intercept that describes the instrument's response at concentrations approaching zero.

Standard solution cd

A standard Cd solution was prepared from a stock solution of $Cd(NO_3)_2$ at a concentration of 1000 ppm, then diluted to several working concentrations. The absorbance of the standard solution was measured using a flame-assisted AAS (Analysis of the Sun) system. Absorbance indicates the

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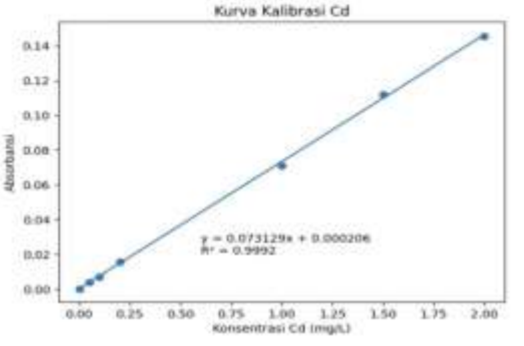
ability of Cd atoms to absorb electromagnetic radiation at the maximum wavelength specific to the element. The measurement data for the standard Cd solution are presented in Table 7.

Table 7. Standard solution cd

No	Pb concentration (mg/L)	Absorbance (Mean±(SD)
1.	0.00	0.00006±0.00029
2.	0.05	0.00410±0.00100
3.	0.10	0.00697±0.00022
4.	0.20	0.01588±0.00078
5.	1.00	0.07119±0.00042
6.	1.50	0.11218±0.00224
7.	2.00	0.14574±0.00417



Based on the calibration curve graph, it can be seen that the higher the concentration of cadmium (Cd), the greater the absorbance value produced. The relationship between concentration and absorbance shows a linear pattern. From the results of the linear regression analysis, a linear regression equation was obtained y = bx + a, with y = 0.073129x + 0.000206 and a coefficient of determination (R^2) value of 0.9992. The R^2 value approaching 1 indicates that the relationship between Cd concentration and absorbance is very strong and linear, so that the obtained calibration curve is suitable for use in quantitative analysis. In this equation, y is the absorbance, x is the Cd concentration (mg/L), b is the slope that indicates the sensitivity of the method, and a is the intercept that describes the instrument's response at concentrations close to zero.

Sample Preparation

The sample analyzed using an atomic absorption spectrophotometer must be in liquid form, so the powdered simplicia is prepared to form a solution. In this study, the sample preparation method using the wet destruction method was chosen because the procedure using an acid reagent as an oxidizer causes less loss of material compared to using dry destruction carried out with high heating, in addition the wet destruction process is relatively simpler and slightly more cost-effective. (Ningru et al., 2019). In this study, the method In the wet destruction process, HNO3 and HCl were added. This is because in hot conditions this acid is a strong oxidizer that can dissolve almost all metals and can prevent element precipitation. By heating to boiling, the destruction process can take place more quickly. The destruction process begins with the appearance of brownish foam and steam. When the breakdown has been completely completed, a clear solution indicates that all components have dissolved and the sample has been properly destroyed. Next, the clear solution resulting from the destruction was filtered using Whatman paper no. 42, the purpose of filtering is to make the solution clear and free from impurities that can interfere with the reading with the SSA tool. Whatman filter paper no. 42 was chosen because it has small pores so that a clear solution is obtained and impurities can be separated perfectly, after that the determination of lead and cadmium levels in rambai leaf simple powder was carried out using atomic absorption spectrophotometry (Pratiwi, 2021).

Sample Measurement by Atomic Absorption Spectrophotometry (AAS)

The filtered digestion sample solution was then analyzed using an Atomic Absorption Spectrophotometer (AAS) with a flame system. Measurements were carried out at specific wavelengths for each element, namely 217 nm for Pb and 228.8 nm for Cd. The selection of these wavelengths was based on the maximum absorption wavelength of each element, thus optimizing the analysis sensitivity. Each sample was measured three times (triplicate) to increase the accuracy and precision of the analysis results. The triplicate measurements aimed to minimize random errors and ensure the consistency of the instrument readings. The absorbance values from the three measurements were then averaged and used as the basis for calculating metal concentrations using a linear regression equation from the calibration curve. The average absorbance value was then entered into the linear regression equation to obtain the metal concentration in the sample solution. The obtained concentrations were then used to calculate the metal content in the solid sample.

Table 8. Absorbance Readings of Pb and Cd Metal Samples

No.	Absorbance 1	Absorbance 2	Absorbance 3	Mean ± SD
Pb	0.00088	0.00119	0.00180	0.00129 ± 0.00047
CD	0.00093	0.00039	0.00052	0.00061 ± 0.00028

Based on Table 8, the sample absorbance values for Pb metal show relatively consistent results with an average value of 0.00129. Meanwhile, the Cd absorbance value is very low and approaches zero, indicating that the Cd content in the sample is at a very small concentration.

Determination of Metal Concentration in Sample Solution

The average absorbance value obtained from the sample measurement results was then used to determine the concentration of Pb and Cd metals in the sample solution. The concentration determination was carried out by entering the absorbance value into the linear regression equation obtained from the calibration curve of each metal. Based on the results of the calibration curve, a linear regression equation was obtained for Pb metal, namely: y = 0.00937x-0.000366y while for Cd metal the equation was obtained y = 0.073129x - 0.000206 The average absorbance value of the sample was then substituted into the equation to obtain the metal concentration in mg/L units. The results of the calculation of the concentration of Pb and Cd metals in the sample solution are presented in Table 9 below.

Parameter	Average Absorbance	Regression equation	Concentration (mg/L)	Information
Pb	0.00129	y=0.00937x-0.000366y	0.1767	Detected
CD	0.00061	y=0.073129x-0.000206	< LOD	Not detected

Based on Table 10, it is known that Pb metal was detected in the sample solution with a concentration of 0.1767 mg/L. Meanwhile, although the instrument showed a Cd concentration value of 0.0056 mg/L, this value was below the method detection limit (LOD) obtained at 0.0131 mg/L. Therefore, quantitatively the Cd content in the sample cannot be reported. This concentration value was then used to determine the metal content in the solid sample by calculating the final volume of the solution and the initial sample mass.

Determination of Metal Content in Solid Samples

After obtaining the concentrations of Pb and Cd metals in the sample solution (mg/L), the next step is to calculate the metal content in the solid sample. This calculation aims to determine the amount of metal contained in each unit mass of the sample, so that the analysis results can be compared with applicable quality standards. The metal content calculation is carried out by calculating the final volume of the digestion solution and the initial mass of the sample used. The formula used to determine the metal content in a solid sample is as follows:

$$\text{Kadar (mg/kg)} = \frac{C\ X\ V}{G}$$

It is known:

C = solution concentration (mg/L)
V = final volume of solution (L)
m = sample mass (kg)

The concentration values obtained in the previous step are then entered into the equation to obtain the metal content in mg/kg. The complete calculation is presented in Table 10.

Parameter	Concentrati on (mg/L)	Solution volume (L)	Sample mass (Kg)	Level (Mg/kg)	Information
Pb	0.1767	0.025	0.0010296	4.29	Detected
CD	< LOD	0.025	0.0010336	< LOD	Not detected

Based on Table 10, it is known that the lead (Pb) metal content in the rambai leaf simple sample is 4.29 mg/kg. This value indicates that Pb metal was detected in the sample at a certain concentration. Meanwhile, cadmium (Cd) metal is below the method's detection limit, so it cannot be quantified and is reported as <LOD.

Comparison of Analysis Results with Maximum Limits of Metal Contamination

Analysis of the Pb and Cd metal levels in the samples was then compared with the maximum limits for heavy metal contamination set by regulatory bodies. This comparison aims to determine whether the detected metal levels are still within safe limits or have exceeded the permitted threshold. The determination of the maximum limits for heavy metal contamination in food and traditional medicine raw materials generally refers to regulations issued by the Food and Drug Monitoring Agency (BPOM) or the Indonesian National Standard (SNI). The presence of heavy metals in excessive amounts can cause toxic effects on the human body, especially if consumed continuously over the long term. The results of the comparison of Pb and Cd metal levels in the samples with the maximum contamination limits are presented in the following table.

Table 11. Comparison of Metal Content with Maximum Pollution Limits

Parameter	Sample content (mg/L)	Maximum limit	Information
Pb	4.29 mg/kg	10 mg/kg	Not exceeding
CD	< LOd	0.3 mg/kg	Not exceeding

Based on Table 11, it is known that the lead (Pb) content in the sample is 4.29 mg/kg. This value indicates that the Pb metal meets the heavy metal contamination requirements set by the Indonesian Food and Drug Authority (BPOM RI) and the WHO, which is ≤ 10 mg/kg for Pb. The presence of Pb in natural materials can originate from environmental factors, such as soil, water, and air pollution around the plant growth location. Meanwhile, the results of the cadmium (Cd) analysis showed that the levels were below the method detection limit (<LOD). This indicates that the Cd content in the sample is very low and cannot be accurately quantified using the method used. Thus, analytically, Cd is declared to meet the heavy metal contamination requirements set by the Indonesian Food and Drug Authority (BPOM RI) and the WHO, which is ≤ 0.3 mg/kg for Cd, so it is safe to use as a traditional medicine ingredient from the aspect of heavy metal contamination. This comparison is important to assess the safety level of the sample against possible heavy metal exposure. These results are the basis for determining the suitability of the sample as a safe material for consumption or use as a raw material for traditional medicine.

CONCLUSION

This study successfully determined that the lead (Pb) metal content in rambai leaf (Baccaurea motleyana Müll.Arg.) simplicia was 4.29 mg/kg, while the cadmium (Cd) content was below the detection limit (<LOD), both of which met the safe limits of BPOM RI (Pb ≤ 10 mg/kg, Cd ≤ 0.3 mg/kg) and WHO. These findings confirmed the safety of the simplicia from heavy metal contamination, with a strong linear calibration curve (R^2 Pb 0.9984; R^2 Cd 0.9992) and the standardization process of the simplicia (drying loss 8.7%, water content 6.61%, ash content 2.79%) that met the FHI 2017 standards. This simplicia is potentially safe as a traditional medicine ingredient for smallpox, diarrhea, and bruises, supported by positive phytochemical screening for alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids. The study included the use of a single sample from one location (Nanga Kantuk, Kapuas Hulu), which limits generalizability to a wider population, and reliance on the SSA method with a Cd LOD of 0.0131 mg/L, which may be less sensitive for very low levels. Suggestions for further research include multi-location analysis, ICP-MS methods for more precise detection, and evaluation of other metals such as Hg and As. Practically, these results support the standardization of herbal medicines by the pharmaceutical industry and the Indonesian Food and Drug Authority (BPOM), encouraging public education about contamination testing before long-term consumption to prevent the risk of chronic toxicity.

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