

Minerals and heavy metals in *Labisia pumila* var. *alata folia* of Selected Geographic Origins

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Abstract

Labisia pumila var. *alata* leaves (LP) with a long history of use folk remedy and endemic to the Malay Archipelago, is now supplied worldwide as ingredient of functional foods and beverages. Minerals and heavy metal concentrations in *Labisia pumila* var. *alata folium* (LP) of selected geographic origin based on microwave-assisted sample digestion and inductively coupled plasma mass spectrometry (ICP-MS) were determined. Fifteen elements comprising minerals (Ba, Ca, Cr, Co, Cu, Fe, K, Mg, Na, Ni, Rb and Zn) and heavy metals (As, Cd and Pb) were analyzed with an inductively coupled plasma – mass spectrometer (ICP-MS). The highest nutrient concentration was measured in LP from Tilu Mountain (Cu, Na, K, Mg and Zn). The highest values of Ba, Ca, Co, Cr and Fe were detected in LP from Raub. Ni and Rb were highest in LP from Cibeunday Village. As was highest in LP from Raub (0.04 ± 0.00 mg/kg). The highest Pb contents were in LP from Tilu Mountain (2.90 ± 0.10 mg/kg) and LP from Halimunan-Salak Mountain (3.12 ± 0.03 mg/kg), all of which were well within the permissible limits as specified by the U.S. FDA for edible plant parts.

Keywords: heavy metals, inductively coupled plasma-mass spectrometry, *Labisia pumila* var. *alata folia*, microwave-assisted sample digestion, minerals

1 Introduction

Plants constitute an important link in the transfer of minerals and heavy metals from soil to man. A beneficial level of these elements in humans is required to maintain good health. Since raw plant materials are increasingly used as ingredients of dietary supplements, data on minerals and heavy metal contents are required by regulatory agencies due to nutritional as well as toxicological implications [1]. Quality control monitoring should ensure the safety, efficacy and quality of the raw plant material [2]. While, there are no standards for such raw plant materials, the Food & Drug Administration of the United States (USFDA) mentions maximum permissible levels in edible plant parts for lead, cadmium and arsenic which amount to 10, 0.3 and 1.0 mg/kg, respectively [3].

Plant materials have been analyzed for different elements using techniques such as atomic emission spectrometry (AES), atomic absorption spectrometry (AAS), induced coupled plasma atomic emission (ICP-AES) and induced coupled plasma mass spectrometry (ICP-MS). Obtaining reproducible results present a challenge as analytes are present in low levels in high matrix samples such as plants. ICP-MS is becoming one of the most widely used techniques for the determination of elements at trace and ultra-trace level (ppb, ppt), owing to low detection limits, multi-elemental capacity and wide linear range. A recent validation study based on selected criteria of performance demonstrated that the use of both microwave digestion in closed-vessel for sample preparation and the ICP-MS for detection permitted an accurate determination of matrices of animal and plant origin [4].

Labisia pumila (Malay: *kacip fatimah*) is a herbaceous plant, endemic to the rainforests of the Malay Archipelago. Traditionally, *Labisia pumila* var. *alata folia* (LP) is used in the form of a decoction as postpartum tonic [5]. Today, the demand for LP has expanded beyond the regional dietary supplements market, increasing the need for several sources of supply. Results of a recent study found that extracts of LP affect 11- β dehydrogenase 1 expressions in both adipose and liver tissues while decreasing circulating corticosterone [6]. This implies the potential of LP extracts as a natural remedy for weight

management. There is, however, a paucity of published studies on nutrient and heavy metal constituents in LP. In the present work, minerals (Ba, Ca, Cr, Co, Cu, Fe, K, Mg, Na, Ni, Rb and Zn) and heavy metals (As, Cd and Pb) in LP were determined by means of the ICP-MS method.

The objectives of this study were to determine the distribution of minerals and toxic elements in LP, to determine whether the safety limits had been exceeded and to compare these findings with results on LP collected from different geographic origins between Malaysia and Indonesia (Table 1).

Table 1. Sources of *Labisia pumila* var. *alata*.

Code	Altitude (msl)	Location
LPR	300-400	Raub, Pahang
LPB	600	Halimun-Salak Mountain, Bogor District, West Java
LPT	600-700	Tilu Mountain, Bogor District, West Java
LPA	300	Cibeundey Village, South Aceh District, Aceh Province
LPL	150	Pekandangan Village, Middle-Lampung District, Lampung Province

msl: meters above sea level

2 Material and Methods

2.1 Reagents

Reagents used were of analytical grade. Plant digests were prepared using HNO₃ (Merck, Darmstadt, Germany) and 30% v/v H₂O₂ (Merck, Darmstadt, Germany). Hydrophilic PVDF Millipore Millex-HV filters were used. Doubly deionized water was obtained using an ELGA Purelab Option-Q DV 25 (UK). Plastic bottles were cleaned by soaking in 10% v/v HNO₃ for 24 hours and rinsed abundantly in doubly deionized water before use. Teflon materials were immersed in freshly prepared 20% v/v HNO₃ overnight and thoroughly rinsed with 5% v/v HNO₃ prior to use. Stock standard solutions of individual metals (1000 mg/L) were supplied by Merck.

2.2 Instrumentation and parameters

Elements in LP in this study were determined by a quadrupole-based ICP-MS (ICP-QMS, Agilent 7500a Series). The plant samples were investigated with respect to accumulation and distribution of the nutrient elements Al, Ba, Ca, Cr, Co, Cu, Fe, K, Mg, Na, Ni, Rb and Zn and heavy metals As, Cd and Pb under optimized experimental parameters as summarized in Table 2.

Table 2. Operating conditions for ICP-MS

Instrument	Agilent 7500a
Nebulizer	Babington type
Spray chamber	Scott-type
Plasma	
RF generator	Frequency: 10 MHz, Power output 1300 W
Ar flow rate (1/min)	Plasma: 15, auxiliary: 0.9, nebulizer: 1-1.1
Solution uptake rate	1.8 ml/min
Interface	
Sample cone	Nickel, id 1.1 mm
Skimmer	Nickel, id 0.9 mm
Vacuum	Interface: 4 torr, quadrupole: 2x10 ⁻⁵ torr
Data acquisition	Peak hopping, replicate time 200 ms, dwell time 200 ms, sweeps/reading 3, readings/replicate 3
Analytical masses	²³ Na, ²⁴ Mg, ³⁹ K, ⁴⁰ Ca, ⁵³ Cr, ⁵⁷ Fe, ⁵⁸ Ni, ⁵⁹ Co, ⁶⁰ Cu, ⁶⁶ Zn, ⁷⁵ As, ⁸⁵ Rb, ¹¹¹ Cd, ¹³⁸ Ba, ²⁰⁸ Pb



2.3 Sample preparation

LP in this study was collected from various study sites between Malaysia and Indonesia. Plant samples were identified by Herbarium Bogoriense (Indonesian Institute of Sciences). LP were rinsed to remove debris, dried at 40°C for 3 days and ground into powder.

2.4 Sample digestion

Samples were prepared by accurately weighing approximately 0.5 g LP powder into a modified Teflon digestion vessel, to which was added exactly 7 ml of 65% solution of HNO₃ and 1 ml of 30% solution of H₂O₂. Samples were acid digested with microwave-assisted irradiation using the Milestone Ethos One ATC-FO 300. Duration of heating was 20 minutes at 300W. Upon cooling, the vessel content was transferred into a 50mL volumetric flask. Samples were diluted and filtered through a Hydrophillic PVDF Millipore Millex-HV prior to analysis. Standard metal solutions were prepared directly from the 1000 mg/l stock of 2% v/v HNO₃.

2.5 Method detection limit

Six blank samples were digested and each of the samples analyzed for metal concentration by ICP-MS. The standard deviations (3σ) for each element were calculated from the six blank

measurements (measured three times) to determine detection limit of the method.

2.6 Statistical analysis

The experimental results are expressed as mean standard deviation (SD) of replicate measurements. The results were processed using Microsoft Excel 2013 and the data were subjected to one way analysis of variance (ANOVA) and the significance of differences between sample means were calculated by Duncan's multiple range test using SPSS for Windows (Standard Version 15.00, SPSS Inc., Chicago, IL). P values ≤ 0.05 were regarded as significant and P values ≤ 0.01 as very significant.

3 Results and Discussion

3.1 Minerals and heavy metals

Determination of minerals and heavy metals in this study, were carried out using Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). A calibration graph was constructed using five concentrations (0-500 µg/L) covering the ranges expected in the various samples. Based on data obtained, the quantities of the minerals and heavy metals contribution by each sample were compared. Table 3 lists concentration of minerals of LP sourced from different geographic origins between Malaysia and Indonesia.

Table 3. Mean concentration of minerals in different samples

Minerals	LPR (µg/kg)	LPB (µg/kg)	LPT (µg/kg)	LPA (µg/kg)	LPL (µg/kg)	RNI# (per day)
Essential micromineral						
K	8102.0	6016.0	11280.0	8720.0	6625.0	3500 mg
Mg	2104.0	2789.0	4761.0	3491.0	2946.0	350 mg
Ca	2658	1459.0	1789.0	1910.0	1340	1000 mg
Na	61.49	132.6	202.0	42.19	32.89	2400 mg
Cr	9.29	0.29	0.64	0.20	2.65	120 µg
Fe	426.1	141.9	346.7	59.62	69.37	15 mg
Co	1.78	0.39	0.37	0.57	0.53	n.a.
Cu	3.46	2.9	4.58	3.83	3.78	2 mg
Zn	10.69	15.37	23.2	9.63	13.70	15 mg
Non-essential trace element						
Rb	19.64	21.71	33.05	41.1	34.09	n.a.
Ba	21.38	7.91	7.62	16.47	12.04	n.a.
Ni	5.61	0.83	2.17	4.64	4.09	<1 mg

Recommended nutrient intake (RNI) for adults ages 19-65 years old (WHO)
Measurements: average (n=6); n.a. = not available

The abundance of K, Mg and Ca, in the result of this analysis, was in agreement with previous findings that these three metals represent the most abundant metal constituents of many plants [7]. The average content of potassium (K) ranged between 601.6 – 1128.0 mg/100g. The average content of calcium (Ca) ranged between 134.0-265.8 mg/100g. LPR contained the highest concentration of calcium (265.8 mg/100g). The average content of magnesium (Mg) ranged between 210.4 to 476.1 mg/100g. LPT (476.1 mg/g) contained the highest concentration of magnesium. The average content of sodium (Na) in LPT was relatively high (20.2 mg/100g). LPT (1128 mg/100g) contained the highest potassium. Sodium and potassium are the principle cations in intracellular fluid, regulating nerve and muscle function, and Na^+/K^+ -ATPase; while calcium and magnesium, the constituents of bones and teeth and magnesium, an enzyme cofactor [8].

Fe content ranged between 59.62 mg/100g in LPA to 426.1 mg/100g in LPR. The average content of iron (Fe) in LPR was highest (426.1 mg/100g). Fe is a constituent of heme enzymes such as hemoglobin and cytochromes [9]. WHO (2002) mandates the RNI for consumption of Fe as 15-20 mg per day as deficiency will cause anemia. Cr ranged from 0.20 mg/100g in LPA to 9.29 mg/100g in LPR. The average content of chromium (Cr) in LPR was highest (9.29 mg/100g). Cr is implicated in sugar metabolism in human [10]. Trivalent Cr is a constituent of glucose tolerance factor which binds to and potentiates insulin [8].

The average content of copper (Cu) ranged from 2.90 mg/100g in LPB to 4.58 mg/100g in LPT. The recommended nutrient intake (RNI) for adults ages 19-65 years old (WHO) is 2 mg/day. Cu is a constituent of oxidase enzymes and plays a role in iron absorption [8]. The average content of cobalt (Co) ranged between 0.27 mg/100g in LPT to 1.78 mg/100g in LPR. LPR contained the most Co at 0.18 mg/100g. Co is an essential constituent of vitamin B₁₂. The amount of cobalt

in blood is dependent on the amount that is in the soil, and where foods are grown [11]. The average content of zinc (Zn) ranged between 0.96 mg/100g in LPA to 2.32 mg/100g in LPT. LPT contained relatively high amount of Zn at 2.32 mg/100g. The recommended nutrient intake (RNI) for adults ages 19-65 years old (WHO) is 15 mg/day. Zinc is required in the diet for a wide range of biological functions such as components of enzymatic and redox systems, regulating synthesis and transcriptions proteins [12].

Non-essential minerals are other trace minerals not yet recognized by the health authorities, but which are believed to be essential for human health. The average content of Rb in LPA was highest (41.10 $\mu\text{g/g}$) compared to the other samples. The average content of Ba ranged between 7.62 $\mu\text{g/g}$ (LPT) - 21.38 $\mu\text{g/g}$ (LPR). The average content of Ni ranged 0.83 $\mu\text{g/g}$ in LPB to 5.61 $\mu\text{g/g}$ in LPR.

Among the heavy metals, lead (Pb), cadmium (Cd) and arsenic (As) are especially toxic and are harmful to humans even at low concentrations. They have an inherent toxicity with a tendency to accumulate in the food chain and a particularly low removal rate through excretion [13]. Exposure to heavy metals above the permissible level can cause high blood pressure, fatigue, as well as kidney and neurological disorders. Heavy metals are also known to cause harmful reproductive effects [14]. Like all plant raw materials, LP is regulated in terms of standard limitation of heavy metal content. The concentration of heavy metals is one of the criteria according to which raw plant materials can be used in the production of functional foods and beverages. Table 4 shows that all samples under investigation accumulated heavy metals (As, Cd, and Pb) at a level appreciably below the permissible level in edible plant parts as mentioned by the Food & Drug Administration of the United States (USFDA). Based on the results obtained, LP may be included as an important source of minerals for daily consumption.

Table 4. Mean concentration of heavy metals in different samples

Heavy Metal	LPR ($\mu\text{g/kg}$)	LPB ($\mu\text{g/kg}$)	LPT ($\mu\text{g/kg}$)	LPA ($\mu\text{g/kg}$)	LPL ($\mu\text{g/kg}$)	USFDA (mg/day)
As	0.04	<0.00	<0.00	<0.00	<0.00	1.0
Cd	<0.00	<0.00	<0.00	<0.00	<0.00	0.3
Pb	1.10	3.12	2.9	0.41	1.67	10



3.2 The limit of detection

Microwave digestion and ICP-MS analysis has been established and used for the determination of 12 minerals and 3 heavy metals, in samples of LP of selected geographic origins. Samples collected were found to give distinct distribution patterns of trace elements.

The method has been validated using certified reference material, assessing its quality in terms of accuracy, repeatability and detection limit (Table 5). The results obtained show the validity of the method used.

Table 5. LOD of methods in determining minerals and heavy metals

Minerals & Heavy Metals	Method detection limit ($\mu\text{g}/\text{kg}$)
K	0.015
Mg	0.004
Ca	0.060
Na	0.005
Cr	0.003
Fe	0.074
Co	0.058
Cu	0.042
Zn	0.012
Rb	0.050
Ba	0.090
Ni	0.030
As	0.022
Cd	0.012
Pb	0.046

4 Conclusion

The highest nutrient concentration was measured in LP from Tilu Mountain (Cu, Na, K, Mg and Zn). The highest values of Ba, Ca, Co, Cr and Fe were detected in LP from Raub. Ni and Rb were highest in LP from Cibeunday Village. As was highest in LP from Raub (0.04 ± 0.00 mg/kg). The highest Pb contents were in LP from Tilu Mountain (2.90 ± 0.10 mg/kg) and LP from Halimunan-Salak Mountain (3.12 ± 0.03 mg/kg), all of which were well within the permissible limits as specified by the U.S. FDA for edible plant parts. The method has been validated using certified reference material, assessing its quality in terms of accuracy, repeatability and detection limit.

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