AGRIVITA VOLUME 34 No. 1 *FEBRUARY-2012* *ISSN : 0126-0537*

PHYSICOCHEMICAL, MINERALS AND ANTIOXIDANT PROPERTIES OF *Labisia pumila* var. *alata* OF SELECTED GEOGRAPHIC ORIGINS

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Received: January 10, 2012/ Accepted: April 20, 2012

ABSTRACT

In this study, relationships between physic-chemical, mineral and antioxidant properties of water-soluble extracts of *Labisia pumila* var. *alata* are proposed. A wide range of analytical parameters were studied including physic-chemical viz., proximate composition, yield, pH, total solid, total acidity, solubility; minerals content (Ca, Fe, and Zn) and heavy metals (Cd, Hg, As, and Pb); total phenolic content (TPC), tannin content, gallic acid (GA) content, and free scavenging activity of DPPH (EC_{50}). Data were analyzed by ANOVA and principal component analysis (PCA). Based on PCA analysis, TPC, GA content, moisture content, crude protein, and zinc content were found to have a significant positive correlation (r close to 1) to antioxidant activity. Cluster analysis (CA) resulted in four groups of *L.pumila* (LP). These results provide useful data about the relationships and chemical patterns in LP leaves.

Keywords: physicochemical, minerals, antioxidant activity, *Labisia pumila* var.*alata,* principal component analysis (PCA)

INTRODUCTION

Functional food and beverage companies are touting the presence of antioxidants in their products in response to consumer interest in the potential health benefits of antioxidants in the diet. *Labisia pumila* (LP) or "Kacip Fatimah" is a popular plant and supplement in the Malaysian functional food and beverage market. According to Burkill (1935), there are about 550 genera of tropical plants, containing over 1,300 species possessing medical values in Malaya Peninsular, one of which is LP.

There are three varieties of LP, namely, *L. pumila* var. *alata*, *L. pumila* var. *pumila* and *L. pumila* var. *lanceolata.* In Indonesia*,* LP can be found in Sumatera, Kalimantan and Java Island. In West Java, LP has been found to grow well in the Halimun-Salak Mountain, Bogor Indonesia (Setiawan, 2005) and on Sumatera Island, where LP thrives in Jambi, Riau, and Aceh (Rahayu *et al.*, 2007).

The search for antioxidant from natural sources has received much attention. LP is a herb rich with phenolic compounds, synthesized via secondary metabolism. Water has been used as the extraction solvent to extract the hydrophilic antioxidants present in LP. In previous studies, antioxidant activities have been reported for *Labisia pumila* var. *alata* (Norhaiza *et al*., 2009). Ali *et al.* (2009), reported that methanolic extracts of LP contained phenolic compounds, glycerogalactolipid, cerebrosides, alpha-tocopherol, sterols and lipids. Meanwhile Yusoff and Wan Mohamud (2011) reported the presence of gallic acid, a well known antioxidant, in water soluble extracts of LP.

Many literature reports showed a simple relationship between phenolic compounds and the antioxidant activity of plant extracts, but their contribution to antioxidant activity was not clear (Goffman and Bregman, 2004). Maisuthisakul *et al.* (2008) Biosynthesis of phenolic compounds and related substances are derived from proteins, including tyrosine and tryptophan via the shikimic pathway. Maisuthisakul *et al.* also reported that plants which had high total phenolic content had strong antioxidant activity. Betancur *et al.* (2004), indicated that minerals, protein, carbohydrates, vitamin and fiber also contribute to the antioxidant activity. However, to the best of our knowledge, relationships between physicochemical composi-

Accredited SK No.: 81/DIKTI/Kep/2011

http://dx.doi.org/10.17503/Agrivita-2012-34-1-p094-104

tion, minerals and antioxidant properties of water soluble extracts of LP was not well understood.

This study was therefore carried out to evaluate physicochemical composition, minerals and free scavenging activity of water soluble extracts of LP from different geographical origins and to determine possible relationships between physicochemical composition, minerals and antioxidant properties of LP using principal component analysis (PCA).

MATERIALS AND METHODS

Plant Material and Sample Preparation

Fresh *Labisia pumila* var. *alata* leaves (LP) were collected from Raub Pahang, Malaysia (LP-R); Salak Halimun Mountain, Bogor, Indonesia (LPB); Tilu Mountain, Bogor, Indonesia (LPT); Cibeundey Village, Aceh, Indonesia (LPA) and Pekandangan Village, Lampung, Indonesia (LPL). Identification of plant species was carried out by a botanist from Indonesian Institute of Sciences (LIPI).

LP leaves were rinsed to remove debris, dried at 40^{o}C for 3 days and ground into powder. After soaking in ultrapure water (1:6) overnight, it was extracted for 6 hours (twice) by Soxhlet with ultrapure water. Filtrates were consolidated and lyophilized in a freeze dryer. For analysis, 0.06 g of freeze dried material was weighed into a centrifuge tube, to which was added 10 mL of ultra pure water. The sample was shaken for 15 minutes and then centrifuged. The supernatant was then transferred into a 10 ml volumetric flask, diluted to the mark with ultra pure water and mixed well by shaking for 15 minutes.

Physicochemical Properties

Physical properties, *viz*. yield, total acidity, pH, total solid, solubility (cold and hot water) were performed in triplicates. Proximate composition were determined in accordance with AOAC (1990), namely, moisture content by direct heating (oven); ash by muffle furnace; protein by semi micro-Kjeldahl nitrogen with conversion factor of 6.25; fat by Soxhlet method; crude fiber by gravimetric methods; and carbohydrate by Luff Schrool method. Energy was measured by direct application of the Atwater factor (1 g carbohydrate = 4 kcal; 1 g lipid = 9 kcal; 1 g protein = 4 kcal).

Determination of Minerals and Heavy Metals

Determination of minerals (calcium, iron and zinc) and heavy metals (cadmium, arsenic, lead, and mercury) were carried out on an Inductively Coupled Plasma – Mass Spectrometer (ICP-MS; Agilent 7500a, USA) and Mercury Analyzer (RA-3000 Nipon, Japan). Lyophilized plant extracts were weighed, approximately but accurately (0.5 g) into a Teflon digestion vessel, to which was added exactly 7 mL of HNO_3 (65%) and 1 mL of H_2O_2 (30%). The vessels were then placed into a rotor segment and inserted into the microwave cavity. The samples were radiated for 20 minutes. Upon cooling, the vessels were uncapped and solutions transferred into volumetric flasks (50 mL). A five-point calibration curve was constructed using five different concentrations (0-500 µg L^{-1}), covering the concentration ranges expected in the various samples. HNO_3 was used as a blank and all analyses were performed in triplicates. The limit of detection (LOD) for the methods used to determine minerals and heavy metals were Ca (1.32 µg kg^{-1}), Fe (0.03 µg kg^{-1}), Zn (2.72 µg kg^{-1}), Cd (0.02 µg kg^{-1}), As (0.11 µg kg^{-1}), Pb (0.94 µg kg^{-1}) and Hg (0.38 µg kg^{-1}).

Total Phenolic Content (TPC)

The total phenolic content of LP was determined using a modified Folin-Ciocalteu assay (Waterman and Mole, 1994). To a lyophilized LP extract redissolved in ultrapure water (0.1 mL) or ultra pure water blank or gallic acid standard solution (0; 25; 50; 100; and 200 µg/ml) was added ultra pure water (2.8 mL) and sodium carbonate (2%, 2 mL) and left standing for 4 minutes. Then, 100 µL Folin-Ciocalteu was added and the solutions were left standing for another 30 minutes. Measurement was conducted on a spectrophotometer (λ= 760 nm) against a blank. Total phenolics was derived using expression (1) and expressed as mg gallic acid equivalent (GAE) in g of dry weight of lyophilized plant extract (R^2=0.9939). Samples were analyzed in triplicates.

TPC = 0.008 gallic acid (µg) + 0.083 (1)

Remarks:
TPC: Total phenolic content

Determination of Tannin Content

The tannin content of LP was determined using a modified vanillin-HCl method assay (Morrison *et al*., 1995). To a lyophilized LP extract redissolved in ultrapure water (1 mL) or blank or catechin standard solution (0; 12.5; 50.0; 100.0; and 1000 µg/mL) was added methanol (9 mL) and vanillin reagent (5 mL; equal volume of vanillin (1%) and HCl solution (8%) in methanol). Upon adding HCl solution (4%) the solution was left standing in a water bath for 20 minutes at 30ºC. Measurement was conducted on a spectrophotometer (λ= 500 nm) against a blank. The tannin content was derived from expression (2) and expressed as mg catechin equivalent (CE) in g of dry weight of lyophilized plant extract (R^2:0.9995). Samples were analyzed in triplicates.

TC = 0.0158 catechin (µg) + 0.0582 (2)

Remarks:
TC= Tannin Content

Free Scavenging Activity Assay

The free radical scavenging activity of the extracts, based on activity of the stable 2,2-dipheynil 1-picrylhydrazyl (DPPH) free radical, was determined by a method described by Kumaran and Kuranakaran (2006). An accurately weighed sample of aqueous extracts of LP prepared by Soxhlet extraction was placed in a volumetric flask (10 mL) and diluted to the mark with ultra pure water and subjected to sonication to make up a stock concentration of 1000 µg/mL. The curve for lyophilized extracts was constructed using six different concentrations (0; 25; 50; 100; 200; and 300 µg mL^{-1}). To the lyophilized extract solutions of different concentrations (1 mL each) or blank or standard was added 3 mL of 0.004% DPPH methanolic solution which was left standing in the dark for 30 minutes. Measurement was conducted on a spectrophotometer (λ = 517 nm) against a blank. The data was derived using expression (3) and reported as concentration of antioxidant required for 50% scavenging of DPPH radicals in a specified time period (EC_{50}). Next, EC_{50} was measured with α-tocopherol as a standard. Antioxidant activity expressed as mg trolox equivalent (TE) in g weight of lyophilized plant extract. Samples were analyzed in triplicates.

$$\% \text{ inhibition} = \left[\frac{(Ac-As)}{Ac}\right] \times 100 \quad (3)$$

Remarks :
A_c = absorbance control or blank,
A_s = absorbance with sample or standard

Determination of Gallic Acid Content (GA)

1. HPLC assay

Profiling and quantification GA were carried out by liquid chromatography. Instrumental parameters used were as described by Yusoff and Wan Mohamud (2011). Gallic acid (GA) was used an internal standard and chlorogenic acid (CGA) as external standard. The HPLC (Agilent 1260) system used comprised vacuum degasser, quaternary pump, autosampler and diode array detector fitted with a reversed phase, Zorbax column (250 mm x 4.6 mm x 120 °A). The mobile phase: acetonitrile (A), 0.25% phosphoric acid (B), and methanol (C). 0-19 minutes: A/B = (1/99); 20-29 minutes: A/B = (5/95); 30-54 minutes: A/B/C = (12/85/3); 55-59 minutes: A/B/C = (1/97/2); 60-80 minutes: A/B = (1/99). Injection sample volume was 50 µL at ambient column temperature. Flow rate was 1.0 $mL.min^{-1}$, pressure 400 Bars and detection wavelength was 254 nm.

2. GA measurement corrected by CGA

The recovery of CGA in the sample was calculated by expression (4), where A is the actual result obtained of CGA in samples with addition of CGA standard (mg), B is the actual results obtained of CGA in samples without addition of CGA standard (mg), and C is the CGA standard (mg). The corrected value of GA in LP samples was calculated using expression (5).

$$\% \text{ recovery of CGA in samples} = \left[\frac{(A-B)}{C}\right] \times 100 \quad (4)$$

$$\text{GA value (mg)} = \frac{\text{actual results obtained of GA}}{\% \text{ recovery of CGA}} \times 100 \quad (5)$$

Statistical Analysis

Data were first subjected to analysis of variance (ANOVA), and then tested for normality. Significant differences between mean values were determined using Duncan's Multiple Range test (P=0.05) following one-way ANOVA. Principal component analysis (PCA) was used to correlate the relationships between physic-chemical composition, minerals and antioxidant

properties by Microsoft Excel 2007-XL STAT 2010 and Minitab 15.

RESULTS AND DISCUSSION

Physicochemical Properties and Mineral Content of LP

Physicochemical properties and mineral content of LP are shown in Table 1.

Table 1 shows the moisture content of LP ranged from 7.52% in LPR to 10.11 percent in LPT. Moisture content of each sample differed significantly (Sig. 0.000, p <0.05). LPT was found to have the highest moisture content. Jamal (2006) reported that the average moisture content of LP from Malaysia was 11%. Moisture variations in LP could be attributed to the prime material characteristics and operations (drying) (Neri *et al*., 2010). LPR had the highest ash content (9.87%), followed by LPL (8.81%), LPB (8.62%), LPT (8.13%) and LPA (7.76%). There was a significant difference (Sig. 0.000, p<0.05) in ash content of LP of different geographic regions. The crude protein content of LP ranged from 5.50% in LPR to 9.87% in LPT. The crude protein content of different LP samples also differed significantly (Sig. 0.000, p <0.05). LPT and LPB had the highest crude protein content.

Meanwhile, the carbohydrate content of LP ranged from 10.43% in LPB to 23.16% in LPR. The carbohydrate content in each LP sample differed significantly (Sig. 0.000, p <0.05). LPL and LPT were found to contain the least lipid content (1.97% and 2.01%, respectively) while LPR was the richest source (2.75%), followed by LPB (2.72%), LPA (2.43%) and LPT (2.01%). There was a significant difference (Sig. 0.001, p<0.05) in lipid content of LP of different geographic regions. LPR contained the least amount of crude fiber (31.30%) while LPB was the richest source (47.18%), followed by LPT (43.47%), LPA (41.98%) and LPL (39.04%). There was a significant difference (Sig. 0.000, p<0.05) in crude fiber content of LP of different geographic regions. The energy content of LP ranged from 101.30 kcal in LPL to 139.38 kcal in LPR. The energy content of each LP sample differed significantly (Sig. 0.000, p <0.05).

Table 1 also shows that the total solid content of lyophilized plant extracts ranged between 1.31 – 1.33 °Brix. The total solid in each sample did not differ significantly (Sig. 0.458, p > 0.05). Table 1 also shows that acidity content of lyophilized plant extracts ranged between 58.33% - 77.78% (mL 1 M NaOH per 100 gram). There was no significant difference (Sig. 0.09, p>0.05) in acidity content in the lyophilized plant extracts of various geographic origins.

Table 1 shows that solubility ranged between 78.43 – 93.48% in cold water and 78.64 – 93.81% in hot water. Statistical ANOVA showed that, there are no significant differences for solubility of lyophilized plant extracts in cold or hot water due to geographic origins (σ 0.342 (cold); σ 0.054 (hot), p > 0.05). Meanwhile, the extract from Halimun-Salak Mountain, Bogor (LPB) was found to have the highest pH value (Table 1). The average pH content of the extract ranged from 5.20 in LPT to 5.87 in LPB. There was a significant difference (Sig. 0.00, p<0.05) in pH value of the extracts. Jamal (2006) had reported a pH range of 3 – 11 was required to maintain plant phytochemicals in extracts. Shahidi and Naczk (2004) reported that antioxidant activity of hydroxyflavones in plants was influenced by pH.

Herbals have previously been reported to contain heavy metals such as cadmium (Cd), arsenic (As), lead (Pb) and mercury (Hg) (Obiajunwa *et al*., 2002). Lead and cadmium caused both acute and chronic poisoning, adversely affecting the kidney, liver, heart, vascular and immune systems (FAO, 2002). LP, like all herbal raw materials are regulated in terms of standard limitation of heavy metal content. Table 1 shows that all samples passed the standard maximum heavy metal (Cd, As, Hg, and Pb) limit except LPT (3.02 µg/g) and LPB (2.90µg/g) whose lead content exceeded the standard maximum (< 2µg/g) for food safety slightly.

TPC, Tannins and Free Scavenging Activity

Phenolics are very important constituents of plants. Their free radical scavenging ability is attributed to hydroxyl groups. Total phenolic content (TPC) was measured using an established method employing the Folin-Ciocalteu reagent. The principle of this method is the reduction ability of the phenol functional group. Oxidation and reduction reaction of the phenolic ion takes place in basic conditions. Reduction of the phosphortungstat-phosphor-molybdenum complex (Folin-Ciocalteu reagent) by phenolic ions changes the reagent to dark blue (Prior *et al.,* 2005). The color becomes darker, absorbing at progressively higher wavelengths as

reduction ability increases with increasing phenolic compounds as is the case with LP. Meanwhile, lyophilized plant extracts of LP also showed a concentration-dependent free radical scavenging activity by inhibiting the DPPH radical and values are expressed as EC_{50}. The DPPH method is based on the reduction of methanolic DPPH solution in the presence of hydrogen donating antioxidant through a formation of the non-radical form, DPPH-H. The scavenging effect increases with increasing concentration of the lyophilized plant extracts. As the extracts reduce the stable DPPH radical, the solution changes color from purple to yellow, arising from diphenylpicrylhydradzine. The lyophilized plant extracts possess hydrogen donating capabilities and acts as free radical scavengers. TPC, tannin content and antioxidant activity in lyophilized extracts of LP of various geographic origins are shown in Table 2.

Table 1. Physicochemical properties and mineral content in samples of various geographic origins

Content	**LPR**	**LPB**	**LPT**	**LPA**	**LPL**
Proximate composition					
Moisture (g/100g)	7.52±0.15^{d}	8.54±0.13^{c}	10.11±0.06^{a}	9.39±0.25^{b}	9.35±0.28^{b}
Ash (g/100g)	9.87±0.19^{a}	8.62±0.13^{b}	8.13±0.17^{c}	7.76±0.11^{d}	8.81±0.07^{b}
Crude Protein (g/100g)	5.5±0.16^{c}	9.58±0.35^{a}	9.87±0.18^{a}	7.88±0.16^{b}	8.56±0.38^{b}
Carbohydrates (g/100g)	23.16±0.52^{a}	10.43±0.21^{d}	13.18±0.29^{b}	13.28±0.18^{b}	12.33±0.21^{c}
Lipid (g/100g)	2.75±0.25^{a}	2.72±0.27^{a}	2.01±0.16^{b}	2.43±0.07^{ab}	1.97±0.13^{b}
Crude fiber (g/100g)	31.3±1.43^{e}	47.18±0.16^{a}	43.47±0.61^{b}	41.98±0.29^{c}	39.04±0.36^{d}
Calories (Kcal)	139.38±4.92^{c}	104.52±4.66^{a}	110.29±3.25^{a}	106.54±1.93^{b}	101.30±2.95^{b}
Minerals					
Calcium (mg/100g)	265.8±4.67^{a}	145.90±1.11^{d}	178.90±5.80^{c}	191.00±6.20^{b}	134.0±4.02^{e}
Iron (mg/100g)	42.61±0.75^{a}	14.19±0.11^{c}	34.67±1.13^{b}	5.96±0.20^{e}	6.94±0.21^{d}
Zinc (mg/100g)	1.07±0.02^{d}	1.54±0.01^{b}	2.32±0.10^{a}	0.96±0.03^{d}	1.37±0.04^{c}
Heavy Metals					
Lead (µg kg^{-1})	1.10±0.02	3.22±0.03	2.90±0.10	0.41±0.02	1.67±0.05
Cadmium (µg kg-1)	<0.00	<0.00	<0.00	<0.00	<0.00
Arsenic (µg kg^{-1})	0.04±0.00	<0.00	<0.00	<0.00	<0.00
Mercury (µg kg^{-1})	0.60±0.02	0.89±0.01	1.72±0.07	1.52±0.04	0.70±0.02
Physical properties					
Yield (%)	10.72	9.91	10.31	10.11	10.27
Total acidity (M NaOH/100 g)	72.22±9.62^{a}	58.33±8.33^{a}	61.11±4.81^{a}	63.89±4.81^{a}	77.78±9.02^{a}
Total solid (Brix)	1.33±0.01^{a}	1.33±0.00^{a}	1.31±0.00^{a}	1.33±0.00^{a}	1.33±0.00^{a}
Solubility:					
Hot water (%)	91.46^{a}	92.21^{a}	93.81^{a}	92.17^{a}	93.31^{a}
Cold water (%)	86.08^{a}	84.98^{a}	93.48^{a}	85.31^{a}	80.26^{a}
pH	5.72±0.01^{b}	5.87±0.01^{a}	5.20±0.01^{e}	5.26±0.01^{d}	5.44±0.01^{c}

Remarks: values are mean ± SD of triplicate. a>b>c>d>e, same alphabets = no difference

Table 2. TPC, tannin content and antioxidant activity in lyophilized extracts of LP of various geographic origins

No.	Sample	TPC (mg GAE g^{-1})	Tannins (mg CE g^{-1})	EC_{50} (µg mL^{-1})	AOA (mg TE g^{-1})
1	LPR	86.875 ± 0.00^{e}	1.00±0.06^{c}	244.32	24.58^{e}
2	LPB	117.5 ± 0.00^{c}	1.88±0.00^{a}	231.65	25.92^{d}
3	LPT	140.49 ± 0.12^{a}	1.16±0.00^{b}	78.79	76.21^{a}
4	LPA	113.96 ± 0.00^{d}	0.65±0.00^{e}	145.11	41.38^{c}
5	LPL	127.36 ± 0.12^{b}	0.76±0.00^{d}	137.54	43.66^{b}

Remarks: values are mean ± SD of triplicate. a>b>c>d>e, same alphabets = no difference GAE: gallic acid equivalent; CE: catechin equivalent; TE: Trolox equivalent

Table 2 shows that TPC ranged between 86.88 - 140.49 mg GAE/g of dry weight of lyophilized plant extract and a significance of 0.000 < α. This indicates significant differences in TPC of the different extracts. Duncan's multiple range test showed that LPT (140.49 mg GAE/g dry weight of lyophilized plant extract) contained the highest average of TPC followed by LPL (127.36 mg GAE/g dry weight of lyophilized plant extract), LPB (117.50 mg GAE/g dry weight of lyophilized plant extract), LPA (113.96 mg GAE/g dry weight of lyophilized plant extract), and LPR (86.88 mg GAE/g dry weight of lyophilized plant extract). Phenolic compounds in food and nutraceuticals originate from one of the main classes of secondary metabolites in plants derived from phenylalanine and tyrosine (Shahidi, 2002). In LP, TPC could be affected by geography, specific climatic condition and solvent.

Table 2 also shows that tannin content ranged between 65.03 – 188.13 mg CE/100 g of dry weight of lyophilized plant extract and a significance of 0.000< α. This indicates significant differences in tannin content of the different extracts. Duncan's multiple range test showed that LPB (188.13±00 mg CE/100g dry weight of lyophilized plant extract) contained the highest average of tannin content followed by LPT (115.53±0.00 mg CE/100g dry weight of lyophilized plant extract), LPR (100.01±0.61 mg CE/100g dry weight of lyophilized plant extract), LPL (75.55±0.00 mg CE/100g dry weight of lyophilized plant extract) and LPR (65.03±0.00 mg CE/100g dry weight of lyophilized plant extract).

Table 2 shows EC_{50} of LPR is computed to be 244.32 µg mL^{-1}, followed by LPB (231.65 µg mL^{-1}), LPA (145.11 µg mL^{-1}), LPL (137.54 µg mL^{-1}) and LPT (78.79 µg mL^{-1}). Antioxidant activity was observed when EC_{50} of extracts ranged between 24.58 to 76.21 mg TE g^{-1} dry weight of lyophilized plant extract. ANOVA analysis showed a significance of 0.000 < α. It can be concluded that there were significant differences in antioxidant activity of LP of different geographic origins. Duncan's multiple range tests showed that LPT (76.21 mg TE g^{-1} dry weight of lyophilized plant extract) contained the highest average of antioxidant activity. This is followed by LPL, LPA, LPB and LPR.

Gallic Acid (GA) Content

1. Chemical profiling

Gallic acid is known to have anti-inflammatory, anti mutagenic, anticancer, and antioxidant activity. A HPLC connected to a UV-vis DAD was employed to profile and quantify gallic acid and chlorogenic acid in lyophilized LP extracts. Concentrations were determined by calculating the HPLC peak areas which are proportional to the amount of analytes in a peak. Chromatograms of authentic standard gallic acid and chlorogenic acid are shown in Figure 1.

Gallic acid had been identified in the water soluble extract of LP according to its retention time against those of the standard, as well as by spiking the samples with the standard GA and standard CGA (Figure 2).

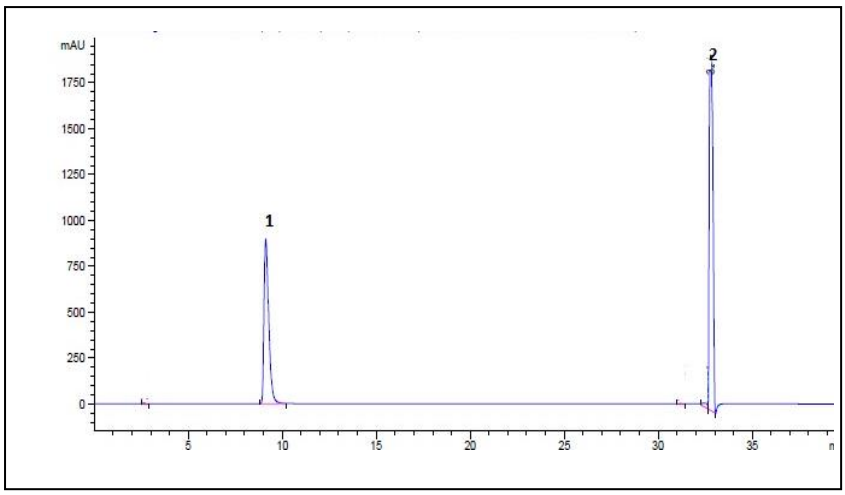

Figure 1. HPLC chromatogram containing both (1) GA (RT: 9.11 min) and (2) CGA (RT:32.78 min) (λ = 254 nm)

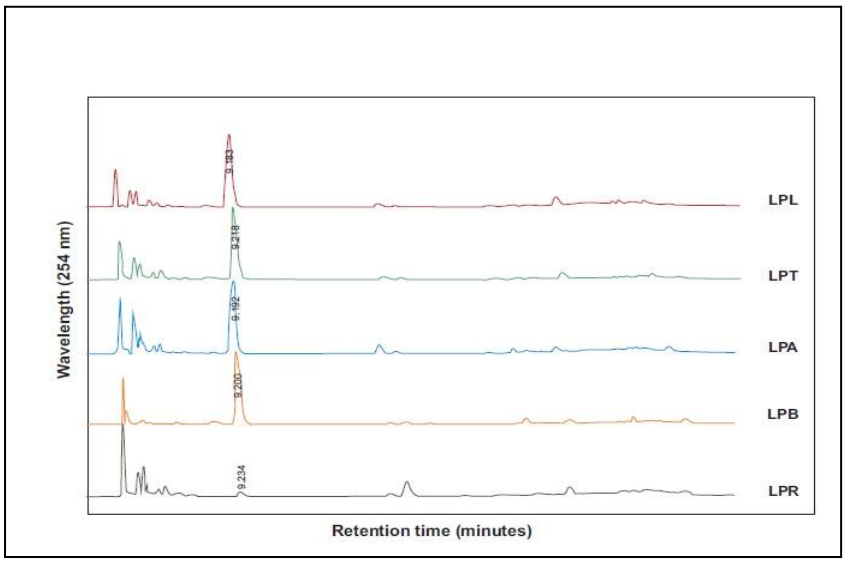

Figure 2. HPLC chromatogram GA (RT: 9.18-9.24 min) (λ = 254 nm) in samples of various geographic origins

2. Quantitative measurement of GA

In order to determine the GA content in the lyophilized plant extract, an HPLC method was used. The GA content was corrected by an external standard (CGA). The average content of GA in lyophilized plant extracts of various geographic origins are shown in Table 3.

Table 3 shows that GA of lyophilized plant extracts of various geographic origins ranged over 0.12% in LPR to 1.86% in LPT. ANOVA analysis showed there was a significant difference (Sig. 0.00, $p<0.05$) in the amount of GA due to different geographic origins. Duncan's multiple range tests showed that LPT was found to have the highest GA value (1.86%) followed by LPB (1.47%), LPL (1.46%), LPA (1.31%), and LPR (0.12%).

Table 3. Average GA value (%w/w) corrected by CGA in samples of various geographic origins

Sample	Gallic acid, GA (%w w^{-1})
Raub, Pahang-Malaysia (LPR)	0.12
Halimun-Salak Mountain, Bogor Indonesia (LPB)	1.47
Tilu Mountain, Bogor-Indonesia (LPT)	1.86
Cibeundey Village, South Aceh-Indonesia (LPA)	1.31
Pekandangan Village, Lampung-Indonesia (LPL)	1.46

Principal Component Analysis (PCA)

A principal component analysis is often performed before a regression, to avoid using correlated variables. It was used to classify samples and find variables, *viz*., proximate composition, physical properties, mineral, GA content, TPC, tannins and antioxidant activity of LP that will contribute to differentiation. Factor analysis was used to understand the correlations between variables instead as the dimension of the variables were small. Varimax methods were used to produce orthogonal transformations to the reduced factor so as to better identify the high and low correlations. Based on the theoretical arguments of PCA as described by Hair *et al.* (2005), the significant factor loading values higher than or equal 0.7 were used to identify the most important variables and observations in each dimension, or principal components (PC). Kaiser (1974) recommends accepting values greater than 0.5.

Factor loading values are the correlation of each variable with the PC. They are represented as vectors (position) in space resulting from the axes of the biplots (Figure 3) .

Figure 3 also shows TPC, GA content, DPPH (EC_{50}), moisture, crude protein, crude fiber, zinc, tannins, and pH were all significantly positively correlated (r is close to 1). The first two factors (F1 and F2) accounted for 61.63% and 19.51%, respectively. The loading factors of F1 had a positive correlation with antioxidant activity (DPPH EC_{50}), TPC, moisture, GA content, crude protein, and zinc. The strong positive loadings of F2 are tannin content and pH value. The correlation of TPC with protein can be explained via the biosynthetic pathway of phenolic compounds and related substances, which are derived from proteins (Zhang and Hamauzu, 2004; Maisuthisakul *et al*., 2008). Maisuthisakul *et al.* (2008) reported that plants which exhibit strong antioxidant activity also tend to have high TPC. The mineral of zinc also has a positive correlation with antioxidant activity which may explain the function of zinc as antioxidant and protein stabilizer, without which, the protein will react with highly unstable iron or cooper (Smith, 2008). The loading factors of the first five principal components of PCA are shown in Table 4.

Figure 3 shows how samples of different geographical origins are differently structured according to their physicochemical composition, minerals and antioxidant properties. Cluster analysis (Fig. 3) resulted in four groups. The first group comprised LPR. According to the cluster centroid, this group presented lower values for fiber and protein, but the highest values for ash, carbohydrate, energy and calcium content. The second group comprised LPB, which presented higher values for tannin content, crude fiber, lipid content and pH. LPL and LPA together form a third cluster, characterized by high fiber, protein and intermediary values for other variables. Finally, a fourth group comprising LPT, presents as its main characteristics, higher values of DPPH, TPC, GA content, moisture content, iron content, zinc content and lower values for carbohydrate and lipid contents.

Table 4. Loading factors of the first five principal components from PCA

	F1	F2	F3	F4	F5
TPC	0.962	-0.149	0.176	0.123	0.071
Tannin	0.078	0.739	0.658	-0.048	-0.109
Gallic acid	0.989	0.045	0.079	-0.011	-0.004
DPPH(EC_{50})	0.689	-0.649	0.310	-0.067	0.047
Moisture	0.912	-0.366	-0.051	-0.087	0.055
Ash	-0.826	0.066	0.251	0.474	0.140
Protein	0.919	0.245	0.279	-0.013	0.083
Carbohydrates	-0.904	-0.408	0.123	-0.001	-0.009
Lipid	-0.648	0.550	0.083	-0.462	0.236
Crude Fiber	0.811	0.486	0.131	-0.273	-0.053
Energy	-0.901	-0.282	0.298	-0.106	0.053
Ca	-0.829	-0.396	0.157	-0.348	-0.096
Fe	-0.527	-0.408	0.742	-0.056	-0.040
Zn	0.611	-0.207	0.758	0.084	0.021
pH	-0.555	0.765	0.235	0.215	-0.040

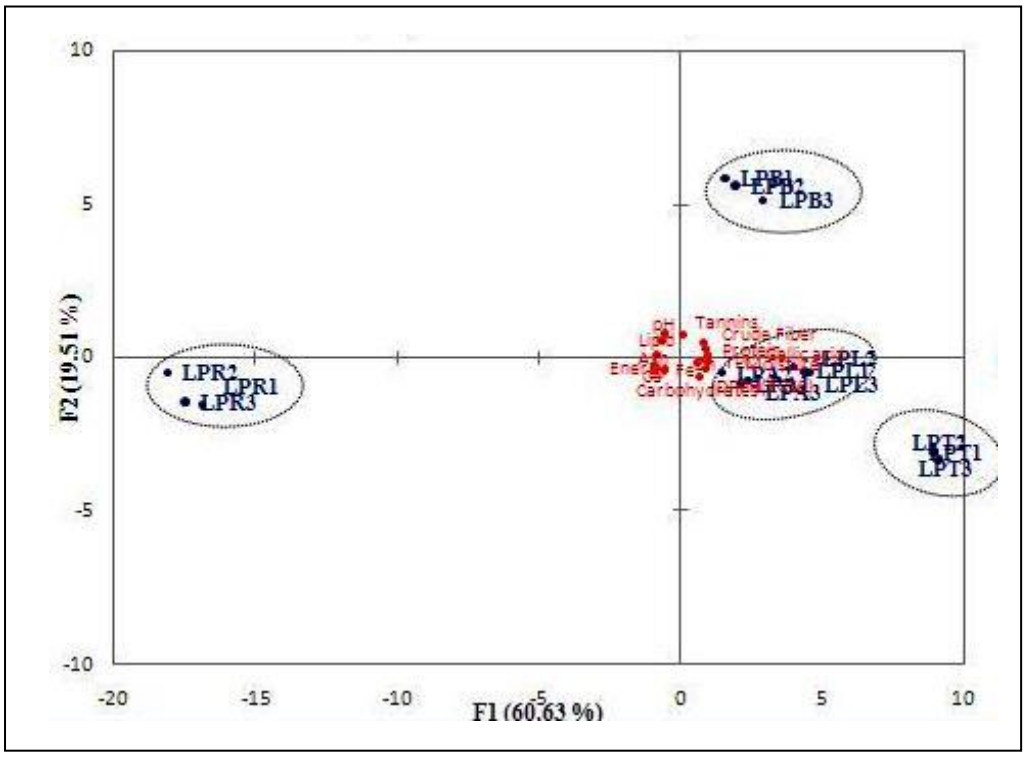

Figure 3. Biplot obtained from PCA of variables comprising physicochemical composition, mineral and antioxidant activity

CONCLUSIONS AND SUGGESTIONS

In this study, we have successfully examined and found correlations between physicochemical composition, minerals and antioxidant properties of *Labisia pumila* var. *alata* of selected geographic origin. Based on the results, LPT was found to contain the highest average of TPC (140.49 mg GAE g^{-1} dwt), DPPH (76.21 mg TE g-1 dwt), GA (1.86%), moisture content (10.11±0.06%), crude protein (9.87±0.18%), and zinc (2.32±0.10 mg/100g). PCA analysis showed TPC, GA, moisture content, crude protein, and zinc content were significantly positively correlated (r close to 1) to antioxidant activity. Cluster analysis (CA) resulted in four groups of LP. The first group comprised of LPR. According to the cluster centroid, this group presented the highest values for ash, carbohydrate, energy and calcium content. The second group comprised LPB, which presented higher values for tannin content, crude fiber, lipid content and pH value. LPL and LPA together form a third cluster, characterized by high fiber, protein and intermediary values for other variables. Finally, a fourth group comprising LPT, presents as its main characteristics, higher values of DPPH, TPC, GA content, moisture content, iron and zinc contents.

ACKNOWLEDGEMENTS

The authors are grateful to Research Management Center (RMC), Universiti Malaysia Pahang for financial assistance (GRS090312) and to the Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang (UMP) and Indonesian Institute of Sciences (LIPI) for providing access and technical support. The authors also thank Ms. Tay Joo Hui for useful discussions on method validation and method performance characteristics.

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