

## ***In vitro* antiplasmodial activity, macronutrients and trace metals in the medicinal plants: *Phyllanthus* spp. and *Alpinia conchigera* Griff.**

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**Abstract.** An antiplasmodial screening of *Phyllanthus debilis* and *Phyllanthus urinaria* was carried out. The medicinal plants were extracted and evaluated for *in vitro* antiplasmodial activity against D10 (chloroquine-sensitive, CQS) and Gombak A (chloroquine-resistant, CQR) strains of *Plasmodium falciparum*. The methanolic crudes from the soxhlet extraction were active against both strains however, *P. urinaria* (IC<sub>50</sub> 8.9 µg/ml with CQR strain) exhibited better anti-malarial activity compared to *P. debilis* (IC<sub>50</sub> 12.2 µg/ml with CQR strain). Furthermore, the methanolic crude of *P. urinaria* obtained by the cold extraction has good anti-malarial activity towards CQS (IC<sub>50</sub> 4.1 µg/ml). The concentration of macronutrients (calcium and magnesium) and trace metals (copper, manganese, iron and zinc) from three *Phyllanthus* species i.e. *P. debilis* Klein ex Wild., *Phyllanthus niruri* L., *P. urinaria* L. and *Alpinia conchigera* Griff. were determined using microwave digestion method and analyzed by Flame Atomic Absorption Spectroscopy. Standard Reference Material 1547 (peach leaves) was used to validate the method throughout this study. The recovery values were in the range of 80% to 120% which were in very good agreement with the certified values. The three *Phyllanthus* species and leaves of *A. conchigera* showed the highest concentration of calcium compared to other metals and macronutrients studied. The significant presence of all the important macronutrients and trace metals which are essential for human health and well-being substantiate their use medicinally in traditional practices.

### INTRODUCTION

Active compounds of the plants are metabolic products of plant cells and a number of trace elements play an important role in the metabolism (Rajurkar & Damame, 1997). In the past decade, studies have shown that medicinal plants are consumed worldwide for the treatment of several diseases such as diabetes (Ebrahim *et al.*, 2012), high blood pressure (Subramanian *et al.*, 2012) and hepatitis B (Ong & Nordiana, 1999). In addition, medicinal plants are important raw materials in pharmaceutical industries i.e.

for the production of phytopharmaceuticals (Ajasa *et al.*, 2004). Recently, medicinal plants play vital role in traditional medicine and are widely consumed as home remedies. Most herbal medicines have low side effects compared to the synthetic drugs and as well as the cost is cheaper than the conventional drugs (Ajasa *et al.*, 2004). Rai *et al.* (2001, 2005) have studied macronutrients and trace element content in *Phyllanthus* spp. species and in *Alpinia* species, as well as heavy metal accumulation in *A. galanga*. The macronutrients and trace metal concentration in *Phyllanthus niruri* have been reported by

Subramanian (2012) and Bempah (2013), *Phyllanthus amarus* by Anupama (2012) and Okolo (2012), *Phyllanthus emblica* by Gajalakshmi (2012) and *Phyllanthus acidus* by Leterme (2006).

*Phyllanthus* is the largest genus in *Phyllanthaceae* family which comprise over 700 species distributed throughout the tropical and subtropical regions of the world (Reveal *et al.*, 2007). There are several species of *Phyllanthus* such as *P. amarus* Shum. & Thonn, *P. niruri* Linn, *Phyllanthus fraternus* Webster, *Phyllanthus virgatus* Forst, *Phyllanthus debilis* Klein ex. Willd, *P. urinaria* L. and others which are similar in appearance. Common vernacular names 'Bhumyamalaki' in Sanskrit or 'dukung anak' by the local Malays assigned to these *Phyllanthus* species create confusion in species identification (Bagchi *et al.*, 1992). It has been traditionally used to cure a variety of diseases including malaria. In Ayurvedic medicine of India, *Phyllanthus* is prescribed for jaundice, gonorrhoea and diabetes (internal use) as well as poultices, skin ulcer and other skin problems (external use) (Subramanian *et al.*, 2012). Some of the medicinal *Phyllanthus* species are also used for dye and tanning purposes (e.g. *Phyllanthus emblica*, *Phyllanthus reticulatus*), as edible fruits (e.g. *P. acidus*, *Phyllanthus emblica*), and as ornamentals (*Phyllanthus pulcher*) (Patel *et al.*, 2011). In Brunei, a leaves poultice is applied with coconut milk and applied to small pox (Holthoon, 1999) while in some other studies, *P. urinaria* extracts presented inhibitive effects on duck hepatitis B virus (DHBV) polymerase, whereas *P. amarus* and *Phyllanthus maderaspatensis* extracts were found to lack antiviral activity against DHBV (Lee *et al.*, 1996; Figueira *et al.*, 2006). Hout *et al.* (2006) also reported the methanol and water extracts of *P. urinaria* has shown the anti-plasmodial activity against *Plasmodium falciparum*. In Malaysia, the juice of *P. urinaria* is used to clean a child's tongue and to stimulate a child's appetite.

Malaria is one of the major health problems in tropical and sub-tropical regions. A dramatic recrudescence of malaria is ongoing due to the increasing resistance of

*P. falciparum* against classical anti-malarial drugs. The Malays use 'dukung anak' vicariously, internally for diarrhoea, kidney trouble, gonorrhoea, syphilis, as an emmenagogue and after a miscarriage and childbirth. Its young leaves are used to treat coughs especially in children (Burkill, 1935). There are no records of the plant being used traditionally in the treatment of fever or malaria by the Malay communities up to present, this study was undertaken to investigate the possibility of *P. debilis* Klein ex. Willd and *P. urinaria* L. which are grown in Malaysia in exhibiting anti-malarial properties.

*Alpinia* is the largest genus which belong to *Zingiberaceae* family and also known as Ginger which embraces 230 species distributed throughout the world. *Alpinia conchigera* Griff. is also known as *lengkuas ranting*, *lengkuas kecil*, *lengkuas getting*, *lengkuas padang* or *chengkenam* in Malay communities (Burkill, 1966; Janssen & Scheffer, 1985; Kress *et al.*, 2005). The plant can be found in Eastern India through continental Asia to peninsular Malaysia and Sumatra (Burkil, 1966; Larsen *et al.*, 1999). Many studies have reported on the medicinal values of *A. conchigera* Griff. especially in the treatment of skin diseases due to fungal infections (Ong & Nordiana 1999; Wannissorn *et al.*, 2005; Ibrahim *et al.*, 2009; Aziz *et al.*, 2013). *Alpinia conchigera* also possesses cytotoxic activity (Hasima *et al.*, 2010) and it is also consumed as food condiment especially in northern states of peninsular Malaysia (Ibrahim *et al.*, 2000). Despite of numerous studies of these medicinal plants, there is no report on their metal content except for *P. niruri* (Subramanian *et al.*, 2012).

The presence of organic compounds and essential macronutrients were influenced by the pharmacological activities. Macronutrients such as calcium and magnesium and trace metals such as iron, copper, zinc and manganese are essential metals and nutrients which play an important role in biological systems. Trace metals, if consumed more than the recommended levels can cause morphological abnormalities, stunted growth and increase

mortality and mutagenic effects in human (Onianwa *et al.*, 2001). Cu is an essential metal to human body as constituent of some metalloenzymes and it is required in haemoglobin synthesis and in the catalysis of metabolic oxidation (Onianwa *et al.*, 2001). Zn is an essential metal for the normal functioning of various enzyme systems. Therefore, Zn deficiency might lead to loss of appetite, growth retardation particularly in children, weakness and even stagnation of sexual growth (Saracoglu *et al.*, 2009; Subramanian *et al.*, 2012). The permissible limit for Cu and Zn in agricultural products should be within 4 to 15 ppm and 15 to 200 ppm, respectively (Allaway, 1968; Ajasa *et al.*, 2004).

According to FAO/WHO (2002), the maximum tolerable daily intake of zinc for an adult man is 45 mg/day and for children is 23-28 mg/day while for copper, the daily dietary limit is 0.5 mg/kg of body weight (World Health Organization, 1982; National Coordinating Committee on Food and Nutrition, 2005; Subramanian *et al.*, 2012). The Recommended Dietary Allowance compiled by Food and Nutrition Board of United States government advocated an average intake of 800, 350, 10, 15 mg per person/day for Ca, Mg, Fe, Zn, respectively. But for Cu and Mn, they are between 1.5 to 3 mg and 2 to 5 mg per person/day respectively. This daily nutrient intake is likely to pose no risk of adverse effects (Food and Nutrition Board, 1989). The information could justify their medicinal properties as many studies have shown the importance of metals in a balanced and healthy human biological system. Therefore, there is a need to study the macronutrients and trace element of these medicinal plants in Malaysia.

In this study, the antiplasmodial screening and the analysis of the levels of macronutrients and trace metal in three *Phyllanthus* species namely *P. debilis* Klein ex Wild., *P. niruri* L., *P. urinaria* L. were carried out. In addition, the macronutrients and trace metal content of medicinal anti-fungal plant (Ibrahim *et al.*, 2009) *A. conchigera* were also analysed.

## MATERIALS AND METHOD

### Plant material and preparation of extraction

Three *Phyllanthus* spp. which are *P. debilis*, *P. niruri* and *P. urinaria* were obtained from Station MARDI Telong Bachok Kelantan. *Alpinia conchigera* were collected from Jeli, Kelantan, Malaysia. The entire plants were dried and milled and subsequently extracted by means of soxhlet extraction and cold extraction, respectively. For pre-treatment of samples digestion, all the plant samples were ground and sieved through a 0.5 mm diameter sieve. The pulverized and powdered plant samples were kept in plastic bottles and placed in desiccator. The standard reference material NIST 1547 (Peach leaves) was purchased from the National Institute of Standards and Technology.

### Soxhlet extraction

The samples of *P. debilis* (70 g) and *P. urinaria* (70 g) were separately extracted sequentially with *n*-hexane, dichloromethane and methanol exhaustively for 17 hours. Each of the supernatant was concentrated under reduced pressure, yielding a dark green residue.

### Cold extraction

The samples of *P. debilis* (150 g) and *P. urinaria* (150 g) were separately extracted at room temperature sequentially with *n*-hexane for three days, dichloromethane for seven days and methanol for ten days. Thereafter, the solvent from each extract was evaporated to dryness under reduced pressure to afford a dark green coloured sludge.

### Dry ashing digestion

About 2 g of plant samples were weighed into the quartz crucible. The samples were dried and charred slowly on a hot plate. Complete ashing was achieved once the samples stop smoking. Five ml of deionised water was added and the samples were evaporated to dryness using a hot plate. The sample was further heated in a muffle furnace for 24 hours

at 450°C. One to two ml of deionized water is added and evaporated to dryness followed by heating in the muffle furnace for 2–3 hours at 450°C to remove carbon residues. The ash was then dissolved in 10 ml of suprapur nitric acid (1+9) (Merck) and the mixture was warmed gently on hot plate for 15 minutes. The sample was filtered with Whatmann No. 1 (110 mm pores size) filter paper, transferred into a 25 ml volumetric flask and diluted to the volume with deionized water. Finally, the solution was transferred quantitatively into plastic bottle and kept in refrigerator prior to analysis.

#### **Microwave digestion**

0.2 g of sample was placed in microwave vessels. The sample was added to 8 ml of 65% suprapur nitric acid (Merck) and followed by 2 ml of 30% suprapur hydrogen peroxide (Merck). All the mixtures were then left for an hour. After that, the samples were placed in the microwave oven (Microwave Accelerated Reaction System, MARS 5 by CEM Corporation, North Carolina USA). The oven temperature was increased in 15 minutes to 200°C and held for 15 minutes and then the temperature was increased in a minute to 210°C and held for one minute. The final temperature was increased in one minute to 220°C and held for one minute. The sample was cooled down to room temperature and let to stand overnight. Later, the sample was filtered through Whatman No. 1 (110 mm pores size) filter paper, transferred into a 25 ml of volumetric flask and deionized water was added up to the calibration mark. The sample was kept in the refrigerator before analysing using FAAS.

#### **Screening for anti-malarial activity**

The anti-malarial assay utilized is an adaptation of the parasitic lactate dehydrogenase (pLDH) assay developed by (Makler *et al.*, 1993) using a 96 well microplate assay protocol with two *P. falciparum* strains [D10 (chloroquine sensitive, CQS) and Gombak A (chloroquine resistant, CQR)]. Two strains of *P. falciparum* were used. The CQS sensitive strain, D10, was obtained from the Institute of Medical Microbiology, University of Milan, courtesy

of Professor Donatella Taramelli. Gombak A is a local isolate and known to be resistant to CQS (Makler *et al.*, 1993; Makler *et al.*, 1993). It was originally isolated from an 'orang asli' (aborigine) patient who was admitted to the Gombak Hospital in 1982.

#### **Antiplasmodial testing**

Briefly, continuous culture of the CQ sensitive, D10, and a local strain of the CQ resistant parasite, Gombak A, were maintained in a suspension consisting of RPMI 1640 cultured medium supplemented with HEPES (25 mM), sodium bicarbonate (0.2%) and gentamycin (40 µg/ml) at pH 7.4 and A or O type blood cells. For each pLDH test, a blood suspension of 1% parasitaemia and 2% hematocrit were prepared. Controlled readings of parasitised red blood cells devoid of plant extract/drugs and non-parasitised red blood cells were done simultaneously. After the plate has been prepared, it was placed in a candle jar and incubated for 72 h at 37°C. After 72 h, 100 µl of Malstat (Flow Inc., Portland, OR) was dispensed into a new microtitreplate and 25 µl of NBT-PES (Sigma Chemicals, USA) mixture was added. Twenty µl of blood suspension was transferred into the plate containing Malstat and NBT-PES. Any air bubbles were eliminated as it could interfere with the absorbance readings. Absorbance was read at 630 nm using ELISA plate reader (MRX Microplate Reader, Dynex Technologies, USA).

#### **Analysis of results of antiplasmodial activity and analytical procedure of macronutrients and trace metals analysis**

The percentage inhibition of parasite viability was determined and the mean of three IC<sub>50</sub> values was calculated using the curve fitting analysis in Grafit. (Grafit v. 4.09, Erithacus Software Limited).

Seven subsamples of each material and one blank were digested using dry ashing and microwave digestion method. All measurements were run in triplicates for each sample and all elements were determined by Flame Atomic Absorption Spectrometer model (Perkin Elmer AAnalyst 400 equipped with WinLab 32 Version 6.5). Perkin Elmer single-element hollow cathode lamps (HCL)

for Ca, Cu, Fe, Mg, Mn and Zn were used for determination of macronutrients and trace elements content with air-acetylene flame. The parameters used for the determination is given in Table 1. The method validation was applied to find the best method for sample preparation in this study. Furthermore, the standard deviation (S.D) and coefficient variation (CV) were calculated to obtain the precise data. The t-test and analysis of variance (ANOVA) are the statistical inference to analyse the significant different within two or more variables and samples.

## RESULTS AND DISCUSSION

### Anti-malarial activity

The percentage of yield obtained from soxhlet extraction and cold extraction technique are presented in Table 2. Only the methanol extracts obtained from the soxhlet extraction of both plants and the cold extraction of *P. urinaria* showed anti-malarial activity. The results obtained from the screening are shown in Table 3. The methanol crude from the soxhlet extraction of *P. urinaria* (IC<sub>50</sub> 8.9 µg/ml with Gombak A - chloroquine resistant,

CQR strain) exhibited better anti-malarial activity compared to that of *P. debilis* (IC<sub>50</sub> 12.2 µg/ml with CQR strain). Furthermore, the crude from the cold extraction of *P. urinaria* with methanol has good anti-malarial activity towards D10 - chloroquine sensitive, CQS strain (IC<sub>50</sub> 4.1 µg/ml).

### Digestion method validation

Dry ashing and microwave digestion methods were carried out using certified reference material NIST 1547 for method validation. Table 4 shows the microwave digestion method gave quite similar results to the certified concentration of elements, indicating the method employed was acceptable to apply for the real samples.

The recovery results are in good agreements with the validation values ranging from 80% to 120%, contrary to dry ashing method which gave the percentage recovery values between 18% and 142%. Therefore, the microwave digestion was applied for the samples. Furthermore, it is a safer technique compared to dry ashing because it was conducted in a close vessel while dry ashing was conducted in an open system. In addition, there is less chance of sample contamination, more reproducibility and more rapid.

Table 1. Experimental conditions for macronutrients and trace element analysis using Perkin Elmer AAnalyst 400

Element	Ca	Mg	Mn	Fe	Zn	Cu
Wavelength (nm)	422.7	285.2	279.5	248.3	213.9	324.8
Slit width (nm)	0.7	0.7	0.2	0.2	0.7	0.7
Sensitivity check (mg/L)	4	0.3	2.5	5	1	4
Working range (µg/L)	3–5	0.18–0.25	0.6–1.0	2–3	0.3–0.75	1.3–1.6

Table 2. Percentage of yield from soxhlet extraction and cold extraction

Plants	Solvents	Distillation period (hours)	% of yields (w/w)	Cold extraction period (days)	% of yields (w/w)
<i>Phyllanthus debilis</i>	Hexane	17	3	3	1
	Dichloromethane	17	1	7	1
	Methanol	17	8	10	8
<i>Phyllanthus urinaria</i>	Hexane	17	1	3	1
	Dichloromethane	17	0.4	7	1
	Methanol	17	5	10	9



Table 3. Parasite lactate dehydrogenase test (pLDH) for anti-malarial *in-vitro* drug screening results

Plant Sample Method Crude	<i>Phyllanthus debilis</i> Klein ex. Willd		<i>Phyllanthus urinaria</i> L.	
	Cold Extraction	Soxhlet Extraction	Cold Extraction	Soxhlet Extraction
Hexane	NA	NA	NA	NA
Dichloromethane	NA	NA	NA	NA
Methanol	NA	(+)ve D10 IC <sub>50</sub> 14.2 µg/ml	(+)ve D10 IC <sub>50</sub> 4.1 µg/ml	(+)ve D10 IC <sub>50</sub> 14.2 µg/ml
		(+)ve Gombak A IC <sub>50</sub> 12.2 µg/ml	(-)ve Gombak A	(+)ve Gombak A IC <sub>50</sub> 8.9 µg/ml

Upper limit for pLDH IC<sub>50</sub> is 8 µg/ml

Two *Plasmodium falciparum* strains used: i) D10 (chloroquine sensitive, CQS); ii) Gombak A (chloroquine resistant, CQR)

NA: Not Active to both strains; (+)ve: positive test; (-)ve: negative test

Table 4. Method validation for dry ashing digestion and microwave digestion method (µg element /g dry weight ± SD)

Element	Dry ashing digestion			Microwave digestion		
	Found (%w/w)	Certified value (%w/w)	Recovery (%)	Found (%w/w)	Certified value (% w/w)	Recovery (%)
Ca	1.97×10 <sup>4</sup> ±0.1	1.56×10 <sup>4</sup> ±0.02	126.28	1.59×10 <sup>4</sup> ±49.64	1.56×10 <sup>4</sup> ±0.02	101.92
Mg	0.406±1.307	0.432±0.008	93.98	0.497±0.01	0.432±0.008	115.04
Cu	4.60±0.1	3.70±0.4	124.32	3.09±0.13	3.70±0.4	83.51
Fe	40±21	2.18×10 <sup>2</sup> ±14	18.35	1.88×10 <sup>2</sup> ±0.34	2.18×10 <sup>2</sup> ±14	86.24
Mn	139±0.1	980±3	14.18	887±2	980±3	90.51
Zn	194±5.3	179±0.4	108.38	151±0.1	179±0.4	84.36

Table 5. Limit of detection (LOD) and limit of quantitation (LOQ) of Ca, Mg, Cu, Fe, Mn and Zn with n=6; (µg/L)

Element	Ca	Mg	Cu	Fe	Mn	Zn
LOD	1.543	1.208	0.002	0.003	0.111	0.137
LOQ	5.143	4.028	0.007	0.110	0.372	0.457
Correlation coefficient (R <sup>2</sup> )	0.9942	0.9995	0.9966	0.9989	0.9997	0.9990

Consequently, microwave digestion method was applied for the leaves and rhizomes sample of *A. conchigera* and the plant of *P. debilis*, *P. niruri*, and *P. urinaria*. The coefficient of variation (CV) of microwave digestion for intraday precision and interday precision was below than 5%, indicating the repeatability and reproducibility of the data collected for microwave digestion method compared to dry ashing method. The limit of detection (LOD) and the limit of quantitation (LOQ) of the data were calculated and the

mean of blank solution were determined for 6 replicates blank solution (n=6). As shown in Table 5, the LOD is in the range 0.002 µg/L to 1.543 µg/L and the LOQ is in the range of 0.007 µg/L to 5.143 µg/L for all the element studied. The correlation coefficient (R<sup>2</sup>) of the standard solution for the entire element tested were >0.9900.

#### ***Phyllanthus spp.***

The composition of macronutrients and trace metal in three species of *Phyllanthus spp.* is

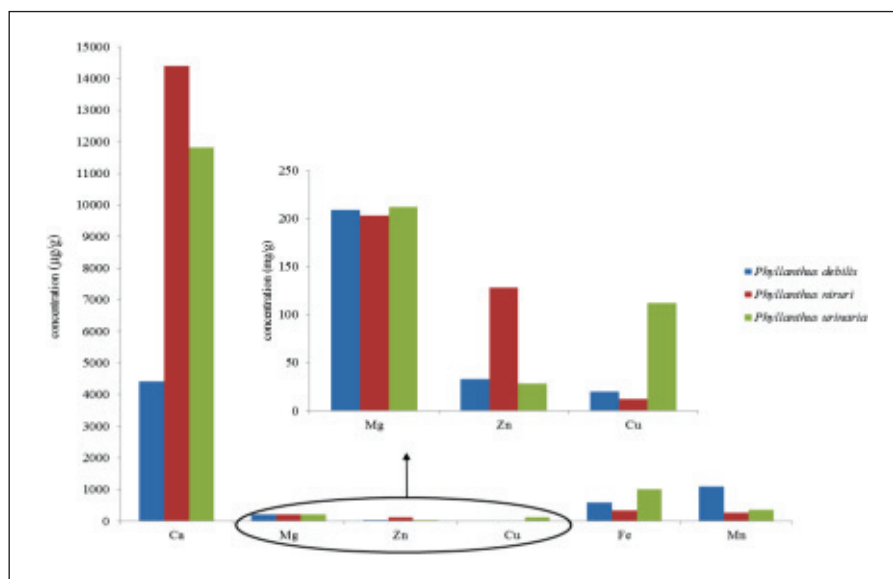


Figure 1. The mean concentration of macronutrients and trace metals in *Phyllanthus debilis*, *Phyllanthus niruri* and *Phyllanthus urinaria*.

presented in Figure 1. Ca showed the highest content in these three *Phyllanthus spp.* species especially in *P. niruri*, 14403.71 µg/g compared to the other two species; *P. debilis*, 4430.23 µg/g and *P. urinaria*, 11821.50 µg/g. Our study showed potent anti-plasmodial activity in *P. urinaria* in which the Ca content was higher than *P. debilis*. Singh *et al.* (2012) have reported that high Ca content is vital to prevent the malaria victims from hypocalcaemia which can cause prolonged Q-Tc interval that could be a risk factor for quinine cardiotoxicity and sudden death. Therefore, these findings showed that a high Ca content could be beneficial in the treatment of malaria patients, hence *P. urinaria* could be a good alternative source for anti-malarial treatment. The lowest trace metal concentration is Cu which are found in *P. debilis*, 9.29 µg/g and *P. niruri*, 6.28 µg/g respectively while *P. urinaria* contain the lowest concentration of Zn with 28.12 µg/g. *P. niruri* comprises the lowest concentration of Mg (45.62 µg/g) and Mn (80.44 µg/g) compared to *P. debilis* and *P. urinaria*. On the other hand, *P. urinaria* contained the highest level of Mg (195.04 µg/g), Cu (112.30 µg/g) and Fe (997.68 µg/g). *Phyllanthus debilis* presented the highest concentration

of Mn (483.45 µg/g). The concentration of Fe and Mn of all *Phyllanthus spp.* studied were slightly higher compared to the results reported by Subramanian (2012). However, Mg concentration was lower than the value reported by Subramanian (2012).

As a result, the concentration of macronutrients and trace metals were obtained in decreasing order of Ca>Mn>Fe>Mg>Zn>Cu for *P. debilis*, *P. niruri* has Ca>Fe>Mn>Mg>Zn>Cu and *P. urinaria* has Ca>Fe>Mn>Mg>Cu>Zn. The one-way ANOVA showed there is a significant difference within *P. debilis*, *P. niruri* and *P. urinaria* for all the trace elements analysed where the F calculated value is bigger than F critical value (3.15) with degree of freedom 2 and 60 and P<0.05. Therefore, the three *Phyllanthus spp.* species indicate different metal composition in each species which were high in Ca.

#### ***Alpinia conchigera* Griff.**

The concentration of macronutrients and trace metal in rhizomes and leaves of *A. conchigera* are illustrated in Figure 2. Ca is the highest macronutrients in leaves of *A. conchigera* (3929.32 µg/g) while Mg is dominant in the rhizomes (4036.53 µg/g). The

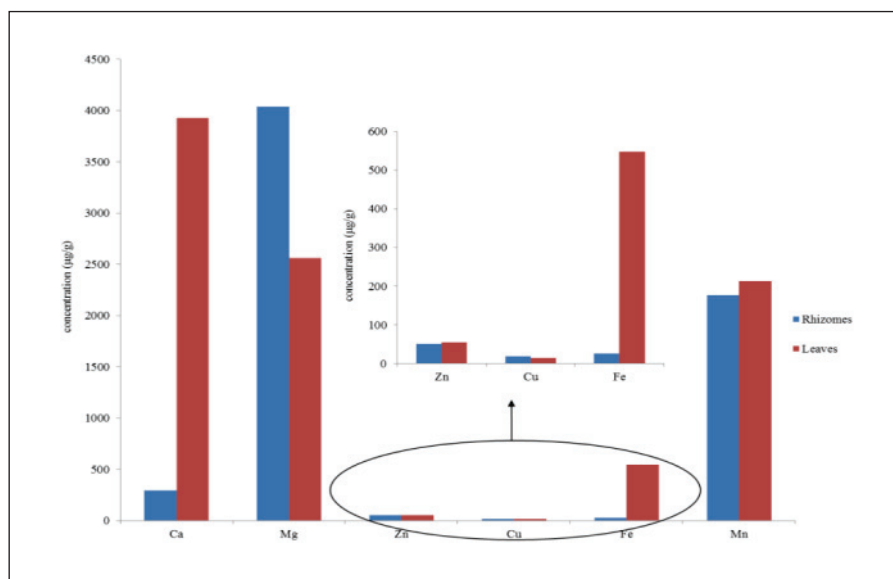


Figure 2. The mean concentration of macronutrients and trace metal in rhizomes and leaves of *A. conchigera*.

trace metal concentration in leaves of *A. conchigera* showed increasing order of Cu (14.37 µg/g), Zn (55.52 µg/g), Fe (547.90 µg/g), Mn (2334.01 µg/g), Mg (2558.56 µg/g) and Ca (3929.32 µg/g). On the other hand, rhizomes of *A. conchigera* gave decreasing order of Mg (4036.53 µg/g), Mn (2201.44 µg/g), Ca (295.27 µg/g), Zn (51.02 µg/g), Fe (25.64 µg/g) and Cu (18.10 µg/g). The abundance of Mn (2334 µg/g) in leaves of *A. conchigera* are in agreement as reported by Ra•ia•e, S. (2005) which is Mn composition in leaves is the highest but less in roots and stems. The presence of Mn in *A. conchigera* could be correlated with therapeutic properties against diabetic to stimulate insulin action and cardiovascular diseases (Chizzola & Franz 1996; Lavilla *et al.*, 1999; Ajasa *et al.*, 2004). Both part of *A. conchigera* showed the lowest concentration of Cu. The t-test for leaves and rhizomes of *A. conchigera* showed a significant difference for all macronutrients and trace metal except for Cu and Mg. It is because the t calculated value is higher than t critical (2.021) (t-calculated < t-critical) with P<0.05 and sample number, N: 21. Therefore, the mineral composition between two parts of *A. conchigera* are significantly different and

this may be due to the environment, mineral in soil, fertilizer used and the climate conditions (Subramanian *et al.*, 2012).

As a conclusion, Ca is the most abundant macronutrients in *P. niruri* as well as for *P. debilis* and *P. urinaria*, whereas *P. urinaria* contained high concentration of Fe, Mg and Cu compared to *P. debilis* and *P. niruri*. Ca has been found to be rich in the leaves of *A. conchigera* and high concentration of Mg was found in the rhizomes of *A. conchigera*. The methanol extracts of *P. debilis* and *P. urinaria* showed antiplasmodial activity. These confirmed the use of *Phyllanthus* species or 'dukung anak' in several regions in the world for the treatment of malaria. Although the plant is not used in Malaysia, both species can be further investigated as anti-malarial agents. In addition, *A. conchigera* is used as a medicinal fungal infection and we have reported previously its anti-microbial compounds from the rhizomes (Ibrahim *et al.*, 2009; Aziz *et al.*, 2013). The significant presence of all the important macronutrients and trace metals coupled with phytochemicals which are essential for human health and well-being substantiate their use medicinally in traditional practices.



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