



Original Paper

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Trace and heavy metals analysis of *Phyllanthus amarus* and *Phyllanthus fraternus* in Ghana

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ABSTRACT

Ethno-pharmacological uses and information about *Phyllanthus* species have prompted this work. The aim was to investigate the presence and levels of aluminum (Al), magnesium (Mg), iron (Fe), manganese (Mn), lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), chromium (Cr) and nickel (Ni) in *Phyllanthus amarus* (PA) and *Phyllanthus fraternus* (PF) in Ghana. Three different extracts (hot aqueous, hot ethanol and cold ethanol) were prepared from dried powdered samples of these plants. These extracts, including the dried samples of the plants were analyzed for the presence and levels of Al, Mg, Fe, Mn, Pb, Cd, Cu, Zn, Cr, and Ni using Atomic Absorption Spectrophotometry (AAS). The levels of Ni (0.43 ± 0.24 ppm), Cr (0.35 ± 0.04 ppm) and Cd (0.18 ± 0.10 ppm) in both plant species were found to be very low in the dried samples plants and below the FAO/WHO maximum limits for vegetables, but were below the detectable limits of our AAS in the extract. Pb was not detected in the dried plant samples and in the extracts of the PA and PF; hence the plants may not pose serious health threat to consumers. PA and PF contain appreciable amounts of trace metals though they were all below the FAO/WHO maximum permissible limits in vegetables. The level of Fe in PA and PF (145.11 ± 11.69 ppm and 179.94 ± 14.60 ppm respectively) was found to be relatively high compared to the other elements analyzed. This finding makes the two plants suitable candidates for use in formulating effective remedies against iron deficiency diseases besides conferring some nutritive value to the patients.

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INTRODUCTION

A number of the *Phyllanthus* species have been reported to have extensive history in medical systems. They have been found to be of high medicinal value and are widely distributed in all tropical regions of the planet

(Adukpo et al., 2010; Sittie et al., 1998). Substantial number of species of the genus is used widely in traditional medicine for the treatment of flu, dropsy (edema), diabetes, jaundice, gall and bladder calculus and liver diseases (Dhiman and Chawla, 2005). Most

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of these species are known to have important pharmacological properties. For example, *Phyllanthus amarus* has demonstrated *in vitro* antibacterial actions against *Staphylococcus*, *Micrococcus* and *Pasteurella* bacteria as well as *in vivo* and *in vitro* antimalarial properties, which validates other traditional uses of the genus (Veeramuthu et al., 2006). Extracts of *Phyllanthus* have been used as antiviral source to treat hepatitis B (Mdlolo et al., 2008). The methanol extracts of five *Phyllanthus* species from India was reported to have strong antioxidant activity (Kumaran and Karunakaran, 2007). Mostly the aqueous or alcoholic extracts of the plant species are used for these treatments.

In Ghana, *Phyllanthus amarus* and *Phyllanthus fraternus* are very common and widely distributed (Adukpo et al., 2010). These plants grow in areas where there are a lot of human activities especially along road sides and may have the ability to accumulate some amount of poisonous metals (heavy metals) from the environment which might also endanger the lives of the people who use it. The major source of human exposure to (toxic/heavy) trace metals from the environment is from food (Bagalkotkar, 2006). Human activities such as mining, industrial use, sewage disposal and hydro-projects have greatly increased the mobilization and bioavailability of metals to plants and increased the chance of exposure of harmful concentrations to humans (Wang and Rene, 2009). On the other hand, some amount of trace metals which can be helpful to the body can be accumulated in the plants thus enhancing its nutritional value. If the plant has the ability to accumulate high concentrations of heavy metals, it could also be exploited to extract toxic metals from the soil where they have higher concentrations (Edeoga et al., 2005; Kumar et al., 1995).

Trace metals play an important role in chemical, physiological, biochemical, metabolic, catabolic and enzymatic reactions

in the living cells of plants, animals and human beings (Ron Kennedy and Santa Rosa, 2010). They have great significance due to their tendency to accumulate in vital human organs over prolonged period of time. Iron is an important element in human body metabolism which acts as a catalyst and is present in greater amount than any other trace element. According to an estimate 57.6% of the body iron in human is contained in Hemoglobin and 8.9% in myoglobin, whereas approximately 33% in non-heme iron complexes, including ferritin and haemosiderin (Hashmi et al., 2007). A deficiency of Fe limits oxygen delivery to the cells, resulting in fatigue, poor work performance, and decreased immunity. On the other hand, excess amounts of Fe can result in toxicity and even death

The main aim of this study was to investigate the presence and levels of the following metals; aluminum (Al), magnesium (Mg), iron (Fe), manganese (Mn), lead (Pb), cadmium (Cd), copper (Cu), zinc (Zn), chromium (Cr) and nickel (Ni) in the extracts of *P. amarus* and *P. fraternus* as these are utilized by human beings for food and medicine.

MATERIALS AND METHODS

Digestion of samples was done with Milestone microwave Labstation (ETHOS 900) and the digestate were analysed with VARIAN AA240FS Atomic Absorption Spectrophotometer in an acetylene-air flame. The whole plant of *Phyllanthus fraternus* and *Phyllanthus amarus* were collected from University of Cape Coast (UCC), Cape Coast and authenticated at the School of Biological Science herbarium, University of Cape Coast, Ghana.

Sample treatment and extraction

The fresh weights of the plant samples were determined and then air dried for 7 days until constant weights were obtained. The dried plant samples were milled. For each

sample, three extractions were made using cold ethanol, hot ethanol and hot distilled water. About 20 g each of the powdered samples were weighed into 500 ml round bottom flask. Cold extraction in 250 ml of 85% ethanol was done on each sample for three days using the mechanical shaker (Heidolf, Vibramax 100 and Germany). The mixtures were filtered and concentrated using rotatory evaporator (Heidolph type VVI rotary evaporator (Normschiff Geratelbau Wertheim, Federal Republic of Germany) to form the crude cold ethanol extracts. Another 20 g of each sample was again weighed and extracted with hot 85% ethanol for 3 days. The third batch of extraction was done with hot distilled water on 20 g of each sample. Cold and hot ethanol extracts were filtered and concentrated as before.

Digestion and AAS analysis

Three pairs of each crude extract samples were obtained and used for analysis in addition to a sample each of dried powdered samples of the two *Phyllanthus* species. All the samples were digested and analyzed for the presence of trace and heavy metals. About 0.5 g each of the powdered dried samples and 10 ml of each crude extract were transferred into Teflon beakers. Concentrated HNO₃ (65%), 6 ml and 1 ml H₂O₂ (30%) each were added. These were digested in a closed vessel, high-pressure microwave digester, (Milestone microwave Labstation, ETHOS 900) according to the thermal programme described previously (Milestone, 1996, Lippo and Sarkela 1995). Atomic Absorption Spectrophotometer (AAS) Model VARIAN AA240FS in an acetylene-air flame was used to identify and determine the metal concentrations. In all, eleven (11) elements namely; Fe, Mg, Ca, Zn, Cu, Mn, Al, Cr, Ni, Pb and Cd were analyzed

Statistical Analysis

Statistical analysis was performed using the t-test. The values are means \pm SD for three replicate values in each group. P values $<$ 0.05 were considered to be statistically significant.

RESULTS

The results of the analysis are presented in Tables 1-2. Generally, acceptable recoveries were obtained in all case (values above 95%) which validates the experimental process and the efficiency of the AAS. There were very little amounts of the heavy metals detected in the dried plant samples as shown in Table 1. The concentrations of Cr, Ni, Al and Cd were 0.35 ± 0.04 ppm, 0.43 ± 0.24 ppm, 3.36 ± 1.70 ppm and 0.18 ± 0.10 ppm respectively for *P. fraternus*. Corresponding values for *P. amarus* were 0.51 ± 0.27 , 0.14 ± 0.13 , 4.67 ± 0.37 and 0.20 ± 0.03 ppm respectively. All these elements were however not detected in the extract formulations i.e. all had a recovery rate of 0% according to our analytical procedure. The amount of Pd in the two samples was below our instrumental detection limit of 0.01 ppm. The heavy metals such as Cr, Pd, Ni, Cd and Al were not detected in the extracts.

Iron registered the highest concentration in all the extracts among the metals. Its concentration was found to be 145.11 ± 11.69 ppm in *P. fratenus* and 179.94 ± 14.60 ppm in *P. amarus*. From Table 2, the mean recovery rates for Fe, by the three methods detailed in the experimental from the milled plants, was 21.78% for *P. fratenus* compared with 16.88% for *P. amarus* ($r = 0.74$). Similarly, the mean recovery rate for Cu was 62.47% for *P. fratenus* and 36.78% for *P. amarus* ($r = 0.71$).

Table 1: Concentration (ppm) of metals in dried sample and extracts of *P. fraternus* and *P. amarus* compared with FAO/WHO maximum daily intake limit.

Plant	Treatment	Fe	Ca	Mg	Zn	Mn	Al	Cu	Ni	Cr	Cd	Pd
PF	Dried plant	145.11 ±11.69	59.23±3.02	37.34±4.21	11.86±2.10	6.27±1.97	3.36±1.71	1.75±0.62	0.43±0.24	0.35±0.04	0.18±0.10	ND
	Cold EtOH ext.	31.44	1.14	0.51	ND	0.92	ND	1.04	ND	ND	ND	ND
	Hot EtOH ext.	32.56	3.73	2.09	0.24	1.00	ND	1.28	ND	ND	ND	ND
	Hot water	28.20	2.89	2.21	0.28	1.12	ND	0.96	ND	ND	ND	ND
PA	Dried Plant	179.94 ± 14.60	62.05±3.61	19.26±1.71	8.24±1.26	18.00±5.25	4.67±0.37	1.65±0.21	0.14±0.13	0.51±0.27	0.20±0.03	ND
	Cold EtOH ext.	30.12	2.60	1.3	ND	0.60	ND	0.67	ND	ND	ND	ND
	Hot EtOH ext	30.92	0.96	0.60	ND	0.45	ND	0.68	ND	ND	ND	ND
	Hot Water	30.07	1.75	0.56	ND	0.77	ND	0.36	ND	ND	ND	ND
WHO Limit		250.0	250.0	150.0	99.4	50.0	5.0	5.0	5.0	2.3	0.3	0.3

PF - *Phyllanthus fraternus*; PA - *Phyllanthus amarus*; ext. – extract; ND - below detectable limit. Uncertainty is given at 90 % confidence level.

Table 2: Comparison of recovery rates of elements extracted from two plants by the stated methods.

Element	<i>P. fraternus</i>		<i>P. amarus</i>		Correlation
	Mean	Var.	Mean	Var.	
Fe	21.18	2.43	16.88	0.07	0.74
Cu	62.48	90.56	36.77	137.77	0.71
Mg	4.29	6.46	4.26	4.67	- 0.99
Ca	4.36	4.98	2.85	1.75	- 0.98

These were found to be statistically significant at 95% confidence interval ($p=0.05$). It appears, from Table 2 that, the recovery of Cu was more favourable in both plants than that of Fe. While the recovery of Cu in *P. fraternus* was 62.48% and 36.77 for *P. amarus*, the corresponding values for Fe were 21.18 and 16.88% respectively.

The concentrations of magnesium and calcium were also higher in the dried samples of the two species. The concentration of calcium was 59.23 ± 3.20 ppm for *Phyllanthus fraternus* and 62.05 ± 3.61 for *P. amarus* while that of Magnesium was 37.34 ± 4.21 ppm and 19.26 ± 1.71 for *P. fraternus* and *P. amarus* respectively. The recovery rates of these two elements were quite low ranging from 4.29% to 6.46% (Table 2) for both plants and significantly reduced the concentration of these elements in the extracts. For instance Ca values ranged from 1.14 to 3.73 ppm and 0.96 to 2.6 ppm in extracts of *P. fraternus* and *P. amarus* respectively while values for Mg in the extracts ranged from 0.51 to 2.21 ppm and 0.56 to 1.3 ppm in *P. fraternus* and *P. amarus* respectively.

The concentrations of trace elements such as Manganese, Copper, and Zinc in the whole plants were 6.27 ± 1.97 ppm, 1.75 ± 0.62 ppm and 11.86 ± 2.10 ppm respectively for *P. fraternus* with corresponding values for *P. amarus* being 18.00 ± 5.25 , 1.65 ± 0.21 and 8.24 ± 1.26 respectively. Even though the concentration of zinc in the whole plants of both plants was quite appreciable, the recovery rate of

the element in the extracts was very low (Table 2), and even below the detectable limits of our analytical tool.

DISCUSSION

Phyllanthus species have long history of medical usage in folklore, especially in traditional Asian medicine. In recent years, substantial interest has been placed on the chemical and pharmacological properties of these species (Igwe et al., 2007). It is believed that the presence of metals in these plants have placed significant roles in their pharmacological and micronutrient properties. All the three extracts (hot aqueous, cold and hot ethanol) registered very low metal concentrations for some of the metals as compared to the dried plant samples. All the values obtained in both the dried samples and the extracts were below the maximum limits of the FAO/WHO recommended values for vegetables.

The average metal concentrations of the non-heavy metals were high as compared to that of the heavy metals. It is evident from Table 1 that in the whole plants, iron, had the highest concentration compared with the other metals analyzed. However, its levels in the extracts decreased substantially but could be enough to explain why *Phyllanthus* is used to formulate blood tonics to build up blood and improve the innate defense system of the body (Igwe et al. 2007).

Plants are thought to be good sources of non-heme iron which is a major micronutrient in the human system (Lammi-Keefe et al. 2008).

From Table 2, the mean recovery rates for Fe and Cu, by the three methods detailed in the experimental from the milled plants suggests that for the different modes of

extraction employed, the recovery of Fe and Cu were more efficient for *P. fratenus* than it was for *P. amarus*. It seems likely that as all plants are known to contain nicotianamide which chelates metal cations (Curie et al. 2009) the biomass of *P. fratenus* is richer in Fe and Cu chelators that are more soluble in the test solvents under the conditions studied than *P. amarus* so as these metal chelates are extracted into the solvents, they co-extract the metals in question thus enhancing their recovery. It appears from Table 2 that the recovery of Cu was more favorable in both plants than that of Fe. This trend might be explained by the fact that Cu is able to form more complexes with chelates than Fe considering their positions on the periodic table.

The concentrations of magnesium and calcium were also higher in the dried samples of the two species. The recovery rates of these two elements were quite low (Table 2) for both plants and significantly reduced the concentration of these elements in the extracts. Given that the plants are administered in the extract form, the fact that Ca levels dropped in the extracts could be good for Fe uptake since Ca is known to be a mild inhibitor of Fe assimilation into the body (Lammi-Keefe et al., 2008). Furthermore, calcium is good for important functioning of the body especially in the skeletal system hence the levels maintained in the extracts should be adequate to support this course assuming that most of the measured Ca is biologically available.

The concentrations of trace elements such as Manganese, Copper, and Zinc in the whole plants were quite appreciable. Even though the concentration of zinc in the whole plants of both *plants* was quite high, the recovery rate of the element in the extracts was very low (Table 2), and even below the detectable limits of our analytical tool. Thus consuming the whole plant may be useful as far as the supply of Zinc to the body is concerned given that the plant does not

contain substantial levels of substances that limit bioavailability of Zn to the body. Zn is an important trace element and one of the several important micronutrients that is essential for the proper functioning of the body. It acts as a co-factor for enzymes in the body and also takes part in the synthesis of DNA, proteins, and insulin. Zn is known to govern the contractibility of muscles and helps to avoid prostate problems (Ron Kennedy and Santa Rosa, 2010). A formulation of the plants in the extract form may, however, not be a vital source of Zn to the body as the recovery rate for this metal is rather very low.

There were very little amounts of the heavy metals detected in the whole plants, that is, Chromium, Nickel, Aluminium and Cadmium. All these elements were however not detected in the extract formulations i.e. all had a recovery rate of 0% according to our analytical procedure. The amount of Lead in the two samples was below our instrumental detection limit of 0.01 ppm. The low levels of heavy metals showed the sites where the plants were obtained were either not polluted by heavy metals or that the plants under study do not have the ability to accumulate these toxic elements into their system. The site where the plants were collected was probably not a polluted and the values obtained confirmed this. It will be instructive in future studies to do a comparative study of plant and soil metal content analysis to be able to properly offer a scientific explanation for these postulates.

Conclusion

Trace and heavy metals are natural components of the earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies *via* food, drinking water and air (as trace elements). The ethno-pharmacological uses of *P. amarus* and *P. fratenus* can be one source of introducing these metals into the body. The study has revealed that these plant species contain

appreciable amounts of iron which may explain their use traditionally in the formulation of blood tonics and in the treatment of liver disorders.

Although, folk history does not have a record of *P. amarus* being used as a vegetable, it can be recommended as such since eating the whole plant is likely to provide enough amount of non-heme iron to the body. This may be the reason why the plant's extract is allegedly used to formulate tonic. The concentrations of calcium and magnesium observed in the whole plants and the extracts might explain the traditional use of the plant extract in the treatment of oedema and kidney problems thus maintaining the osmotic pressure and water distribution in the The very low or non detectable amounts of the heavy metals Al, Ni, Cr, Cd and Pb in the extracts is suggestive that the plant extract contains little on no toxic element and may pose no danger to consumers and therefore safe to use. In conclusion, the low levels of the heavy metals found make the plants good candidates for both medicinal and other stated purposes.

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