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Phytochemical screening and nutrient analysis of *Phyllanthus amarus*

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ABSTRACT

This study was carried out to evaluate the phytochemical properties and nutrient analysis of Phyllanthus amarus. The whole plant was analysed for proximate and mineral element compositions. The results indicate that the plant contains useful nutrients for use in dietary preparations of functional foods. Carbohydrate (65.28 ± 0.04%) was found to present in highest concentration followed by crude proteins (10.50 ± 0.15%). Among the mineral elements determined, Calcium was present in the highest concentration (2209 ± 0.50ppm) while Chromium was the least (15.25 ± 0.13ppm). The phytochemical screening of the plant extracts revealed the presence of alkaloids, saponins, glycosides, tannins, steroids, terpenes, flavonoids and carbohydrate.

Keywords: Phytochemicals, proximate composition, mineral element, *Phyllanthus amarus*

INTRODUCTION

Plants have been and are still being processed and utilized as raw materials for pharmaceutical applications. Many of our modern drugs and processed scientific medicines are of plant origin [1]. Man has been using herbs for the treatment of many diseases [2]. In this respect, *P. amarus* is usually used as infusion and drunk by Nigerian for health maintenance and it is considered as a wonder- working herb and has great economic importance. Therefore this research aimed at determining the phytochemical and nutritive properties of this medicinal plant in order to evaluate its medicinal implications and establish the safety of the plant extract on human.

The plant *Phyllanthus amarus* belongs to the family Euphobiaceae and of the genus phyllanthus and the species is amarus [1]. The plant is a common weed of cultivated fields and spreads widely in West Africa and other parts of the world and has been discovered some years ago in Akwa Ibom State [3].

The Euphobiaceae is a large family of about 300 genera and more than 6000 species. Most members of this family are trees or shrubs and few are herbs. The flowers are unisexual and regular with perianth leaves, the stamens are numerous and are either free or united. *Phyllanthus amarus,* as a member of this family *is* a wide spread tropical herb employed widely in traditional medicine preparations [4], [5], [6].

Phyllanthus amarus is closely related in appearance, structure and constituents to two other weeds also common in cultivated fields. These are *Phyllanthus urinaria* and *Phyllanthus niruri.* The difference between the two and *Phyllanthus amarus* is that *P. amarus* has a tiny greenish leaves, stems and fruits while *P.niruni* have larger leaves and stems that are red and green respectively. The fruits *P. urinaria* are warlike and the plant is bigger.

P. amarus is one of the most important herbs discovered recently in Nigeria and Akwa Ibom State in particular. It is known among Ibibios and Efik's as ''oyomokiso aman ke edem'', Yoruba as "eyin olobe", Hausa as "geeron tsutsaayee" and Igbo as "Ite knwonwa nazu" and in English as "leaf flower" or "chamber bitter" [7], [8].

This plant is found distributed in some parts of Nigeria especially southern, eastern and western regions, example Aba, Uyo, Ibadan etc. [9], [10]. It is also found distributed in other countries example India, Tanzania, New Zealand and others.

MATERIALS AND METHODS

2.1 Sample collections, preparations and preservation

The entire samples comprising the stem, root, leaves, fruit, seeds and flowers of the plant *P. amarus* was collected from cultivated farmland in Uyo Capital City, Akwa Ibom State. The plant was identified traditionally by the chief herbalist, Mr. Abia Williams in the Faculty of Pharmacy, scientifically by Mr. Bala Danladi and Mr Okon Etefia, technologists in the department of Pharmacognosy, Faculty of Pharmacy, University of Uyo. The plant was separated from other weeds and dirt, washed with ordinary water and rinsed with distilled water. It was sundried for 5 days and then ground with pestle and mortar into coarse powder and packed in an air tight plastic container for further analysis.

2.2 Sample Preparations

About 150g of the sample was weighed into a 2 litre conical flask and was extracted with 70% ethanol at room temperature (27^oC) for 72 h with occasional shaking. Aqueous extract was obtained by extracting the weighed sample in distilled water directly. Fresh sample, crushed and uncrushed dried samples were also treated as above. Each extract was filtered using a clean muslin cloth and coloured filtrates were obtained. The filtrates were concentrated at temperature between 40 – 50 ^oC using water bath. The raw extracts were labelled accordingly, cooled and stored in a desiccator for phytochemical analysis. Part of the plant sample was preserved for chemical analysis.

2.3 Phytochemical Analysis: The raw material, aqueous and alcoholic extract were chemically tested for phytochemical constituents using standard procedures recommended by Trease, and Evans, [1] and Harbone, [11]. The sample used were numbered 1 to 9.The phytochemicals indicates the presence or otherwise of some secondary metabolites in the sample [12]. The phytochemical tests carried out in this work include alkaloids, saponins, glycosides, tannins, steroid, terpenes, flavonoids, and carbohydrate.

2.4 Nutritive Analysis of the Plant

The plant was analyzed for proximate compositions and mineral elements.

- ***Proximate Compositions*** : Proximate compositions constitute the different classes of nutrients present in samples such as carbohydrate, protein, fat, crude fibre, ash and moisture as well as caloric value calculated from the values of carbohydrate, protein and fat. All the methods used in estimating the proximate composition of the plant samples were standards methods of the Association of Official Analytical Chemists [13]
- ***Estimation of Mineral Elements:*** The mineral elements estimation indicates the quantity of inorganic element present in the sample. The determination was done following the standard procedures of the Association of Official Analytical Chemists [13]. In this determination, the sample was first ashed and the resultant solution aspirated into an air-acetylene flame. The mineral elements determined were iron (Fe), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), calcium (Ca), sodium (Na), potassium (K), phosphorus (P) and chromium (Cr) by spectroscopic methods.
- Flame Emission Spectrophotometer (FAS) was used for Na and K determination while Atomic Absorption Spectrometer (ASS) was used for the determination of other elements.

RESULTS AND DISCUSSION

The result for the phytochemical screening of *P. amarus* as shown in Table 1 revealed the presence of alkaloids, saponins, glycosides, tannins, steroids, flavonoids and carbohydrates. From these results, the level of detection of these components depended on the method of preparation of the sample, solvent used and the temperature. The results showed that the dry crushed sample gave high levels of detection. The fresh uncrushed sample showed very low levels of detection of the components. From table 1, sample 1 – 6 which include (1) ethanolic extract (dry uncrushed sample), (2) aqueous extract (dry uncrushed sample) , (3) ethanolic extract (dry crushed sample), (4) aqueous extract (dry crushed sample), (5) ethanolic extract (fresh uncrushed sample),and (6) aqueous extract (fresh uncrushed sample) indicated high level of detection of almost all the components tested for. While most of the components were not detected in sample 7 – 9 which were (7) raw dry sample (crushed), (8) dry sample (uncrushed) and (9) fresh sample (uncrushed); this could be attributed to insufficient extraction and absorption of solvent by the sample used.

3.1 Phytochemical Screening of the Extracts

Table 1: Result of phytochemical screening of the extract of *Phyllanthus amarus*

Test	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
Alkaloids	+	+	++	+	+	+	+	+	+
Saponins	+	++	+	++	+	+	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+
Tannins	++	++	++	+	+	+	-	-	-
Steriods and Terpenes	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	-	-	-
Carbohydrate	+	++	+	+++	+	+	+	+	+

+=low, ++ = moderate, +++ = high, - = not detected

The components detected have been shown to exhibit various therapeutic properties such as the astringent action of tannins, anti-inflammatory and anti-allergic effect of flavonoids. Some carbohydrate, flavoniods, steroids and alkaloids have been shown to exhibit anti-diabetic action [1], [14], [15].The detection of most of these plant constituents in *P. Amarus* have suggested the possibility of using the plant in the treatment and management of many aliments including non-insulin dependent diabetes mellitus[5], [8]. Saponins inhibit sodium efflux by blocking the entrance of the sodium ions into the cell [16], hence activating sodium-calcium antiporter producing elevated cytosolic calcium which strengthens the contractions of heart muscle and thus reducing congestive heart failure.

3.2 Proximate Composition of Phyllanthus amarus

The result of the proximate composition of *Phyllanthus amarus* are presented in Table 2 and illustrated in Figure 1.

Moisture content

The proximate analysis showed the moisture content of *P. amarus* to be 69.96 ± 0.05% (wet weight). This result indicated that the shelf life of this plant while fresh is low and long storage lead to spoilage due to its susceptibility ot microbial attacks. This justifies the practice of storage in dry form by users. Moisture content is one of the most important and widely used measurements in the processing, preservation and storage of foods and drugs [17].

Table 2: Proximate Composition of *Phyllanthus amarus (% dry matter)*

Component	Concentration (%)
Moisture (wet weight)	*69.96 ±0.05*
Ash content	*11.20 ±0.17*
Crude fibre	*6.95±0.03*
Crude protein	*10.50 ±0.15*
Lipids	*6.07 ±0.03*
Carbohydrate	*65.28 ±0.04*
Caloric value (kcal/100g)	*357.75 ±0.03*

The results are expressed as mean of three determination ±SD

Ash Content

The result obtained for ash was 11.20 ± 0.17 % (dry matter). This result is comparable with those reported by [18] and [19] for some other plants and plant parts. The ash content reflects the mineral content of *P. amarus* [20].

Crude Fibre

The value of crude fibre obtained for *P. amarus* was 6.95 ± 0.03% (dry matter). Crude fibre in foods or plants is an indication of the presence of non digestible carbohydrate and lignin. The low value obtained for *P. amarus* is considered appropriate, studies have shown that crude fibre aids in reducing peaks of blood glucose following a meal due to delayed gastric emptying [22]. The low crude fibre content of this plant is advantageous in absorption of glucose and fat. Although crude fibre enhances digestibility, its presence in high levels can cause intestinal irritation, lower digestibility and decreased nutrient utilization [19].

Crude Protein

The crude protein in *P. amarus* was 10.50 ± 0.15% (dry matter). Hence the plant is a moderate source of protein. [21], suggested that protein from plant sources have lower quantity, but their combination with many other sources of protein such as animal protein may result in equivalent nutritional value. The recommended dietary allowance (RDA) for protein is 56g for individual weighing 70kg and 46kg for adult weighing 50kg. Children may consume 2g/kg/ day [21], [22].

Crude Lipids

The crude lipids content was 6.07 ± 0.03 (dry matter). Many body functions depend on lipids. Lipids provide excellent source of energy and enhance transport of fat soluble vitamins, insulate and protect internal tissues and

contribute to vital cell processes [21], [22]. It has been suggested that enough lipid (fat) be included in the diet to account for at least 20- 25% of the total caloric intake [23].

Carbohydrate Content

The carbohydrate content of *P. amarus* was 65.28 ± 0.02 (dry matter). This carbohydrate may be one of the contributing factors for the efficacy of *P. amarus* as an anti-diabetic agent [1]. Pemela *et al.*, observed that some carbohydrate containing foods have a rapid rise followed by a slow decline in blood glucose concentration.. The RDA for carbohydrate is 130g/kg [21].

Caloric value

The caloric value of *P. amarus* was 357.75 ± 0.03kcal/100 g dry matter. An average person requires 2000- 3000 kcal/day [22]. The value obtained shows that *P. amarus* could contribute to energy requirement of its consumers.

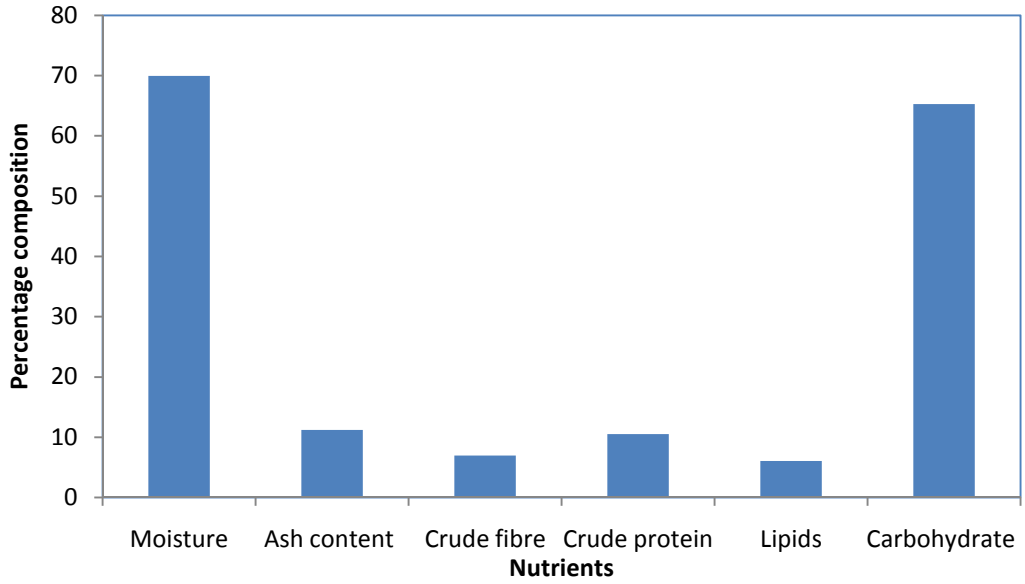

Figure 1: Proximate Composition of *P. amarus*

3.3 Mineral Element Composition

The mineral element compositions of *P. amarus* are presented in Table 3 and illustrated in figure 2. The mineral elements determined were iron (Fe), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), calcium (Ca), sodium (Na), potassium (K), phosphorus (P) and chromium (Cr)

Iron (Fe)

The iron content of *P. amarus* was 172.73 ± 0.07ppm. Iron is essential for transport of oxygen in haemoglobin and also involves in energy metabolism. Deficiency of iron results in anaemia. This plant from the result obtained can be used in improving the anaemic condition in iron deficient diabetic patients. The recommended daily requirement of iron for man is 6-40 mg/kg [24], [25].

Table 3: Mineral Element Composition in *Phyllanthus amarus*

Element	*Comcentration (ppm)*
Iron	*172.73 ±007*
Copper	*6.25 ±0.01*
Zinc	*61.75 ±0.01*
Magnesium	*327.00 ±0.20*
Calcium	*3467.00 ±0.01*
Manganese	*35.75 ±0.01*
Potassium	*2209.00 ±0.50*
Sodium	*78.50 ±0.25*
Phosphorus	*96.25 ±0.05*
Chromium	*15.25 ±0.13*

The results are expressed as mean of three determination ±SD

Copper (Cu)

The value obtained for copper was 6.25 ± 0.001 ppm. Copper is essential in the diet because, it is involved in the proper utilization of iron and essentially for the synthesis of cytochrome oxidase, which contains both iron and

copper [22]. The value obtained is appropriate and is considered to be safe for the body. The daily recommended allowance (RDA) for copper is 3.5mg/kg [22].

Zinc (Zn)

The zinc content of *P. amarus* was 61.75 ± 0.01ppm.. Zinc is essential in the activation of certain enzymes, these include dehydrogenase, alkaline phosphatise and carboxypeptidase. Zinc containing organic compounds is employed as astringent and anti- fugal agents. Zinc aids wound healing and metabolism of nuceic acid and insulin [22] [25], the RDA for zinc is 13mg [22].

Magnesium (Mg)

The magnesium content of *P. amarus was 327.00* ± 0.20 ppm. Magnesium is essential for enzyme reaction in the metabolism of ingested carbohydrate, example of such enzymes are α-amylases, maltase, sucrase and lactase. It is essential in electrical breakdown of nutrient and other materials within the cells. The RDA for magnesium in human is 250- 380 mg/kg [24].

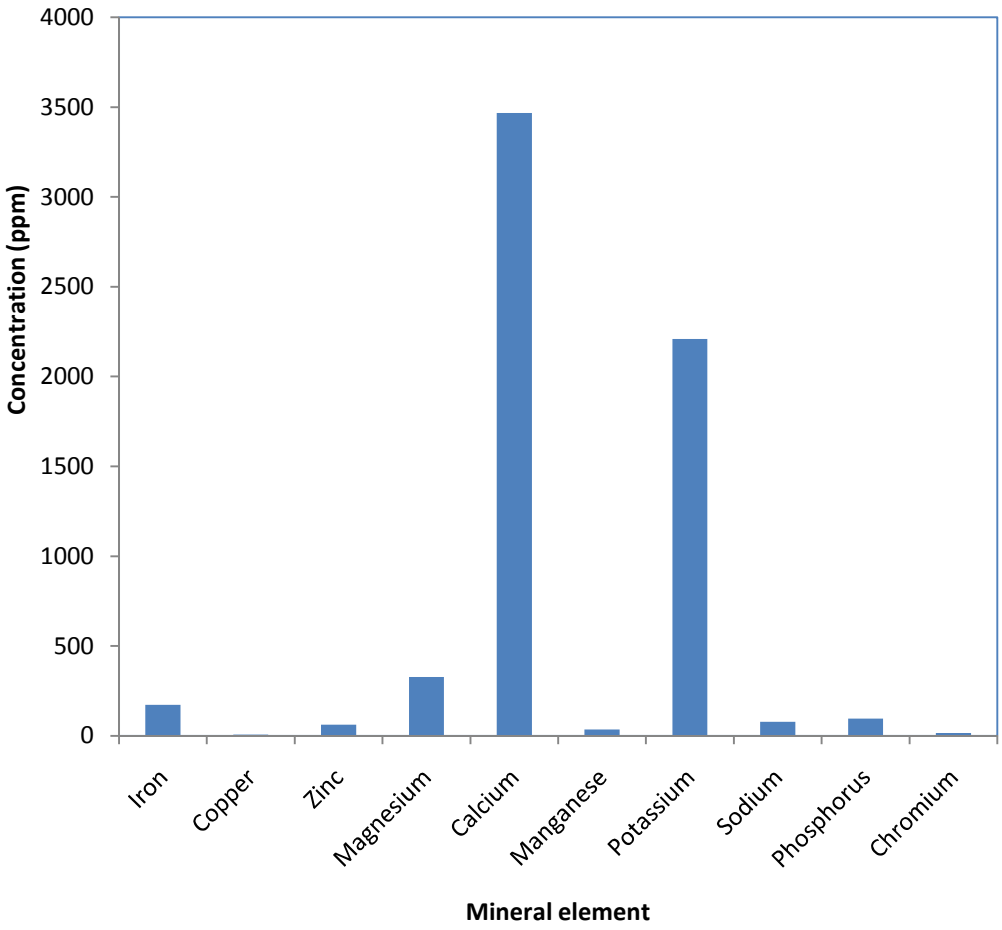

Figure 2: Mineral Element Composition of *P. amarus*

Calcium (Ca)

The calcium content was found to be high 3467.00 ± 0.28ppm. Calcium is necessary for the strong bones and teeth. It is relatively high in cereals, nuts and vegetable [26], [27]. The RDA value of calcium is 600-1400mg/kg [24].

Manganese (Mn)

The manganese content of *P. amarus* was 35.75 ± 0.01ppm. Manganese like iron and zinc has low order of toxicity in mammals. Trace level of Mn are essential to good health. Compounds containing Mn are employed as tonic and used in treating anemia, enzymes using manganese include ascorbate and transketolase [25]. The RDA for manganese varies between 2mg to 8mg/kg [24].

Potassium (K)
The potassium content of *P. amarus* was 2209.00 ± 0.05 ppm. Potassium is responsible for nerve action and is very important in the regulation of water and electrolyte balance and acid – base balance in the body. The high level of potassium in *P. amarus* is a good indication that its consumption will enhance the maintenance of the osmotic pressure and acid-base equilibrium of the body [28].The recommended daily allowance of potassium is 1875 – 5625mg/kg for adult [23].

Sodium (Na)
The sodium content was 79.50 ± 0.25ppm. The recommended daily value for sodium is 1100- 3300mg/kg for adults [24]. Sodium is the principal exteacellular cation and is used for acid – base balance and some osmo-regulation in the body fluid [28].

Phosphorus (P)
The phosphorus content was 96.25± 0.05ppm. Phosphorus is a constituent of bone and teeth, nucleoprotein, phospholipids, enzymes and high energy compounds. The RDA for phosphorus is 1,400mg/kg [22].

Chromium (Cr)
The chromium content was 15.25 ± 0.13ppm. This may be considered high but chromium with other mineral elements, such as potassium, zinc and calcium play important roles in the maintenance of normal glucose tolerance in the release of insulin from the β-cell [16]. The RDA for chromium is 0.15mg [22].

CONCLUSION

The results obtained have shown that *Phyllanthus amarus* contained considerable amount of some important chemical compounds. Nutritionally it is interesting to note that *Phyllanthus amarus* contain protein, lipid, ash, fibre and carbohydrate. Also mineral elements such as iron manganese, magnesium, zinc, calcium, potassium, phosphorus, copper and chromium were found in appreciable amount, with calcium present in the highest concentration. The phytochemical analysis of *P. amarus* revealed the presence of alkaloids saponins glycosides, tannins, steroids, flavonoids and carbohydrates. This confirms its therapeutic potentials as claimed by ethnobotanical users.

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