Phytochemical Composition, Mineral Contents and Amino Acid Profile of *Phyllanthus amarus* leaves

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Abstract

Leaves of Phyllanthus amarus was analyzed for phytochemical, mineral and amino acid constituents using standard methods. The data obtained indicated that aqueous extract of P. amarus leaves contained alkaloids (28.5 mg/L), flavonoids (13.6 mg/L), saponins (11.2 mg/L), steroids (8.4 mg/L), anthraquinones (3.8 mg/L), tannins (2.7 mg/L), and terpenes (2.3 mg/L). Anti-nutrients found in the plants are phytate (8.74 mg/ L), phytin phosphorus (2.74 mg/ L), oxalate (2.52 mg/ L), cyanide (0.84 mg/ L) and polyphenol (0.57 mg/ L). Mineral analysis of the leaves revealed the presence of Calcium (352.13 mg/100 g) > Potassium (239.29 mg/100 g) > Sodium (171.48 mg/100 g) > Magnesium (47.61 mg/100 g) > Iron (36.17 mg/100 g) > Phosphorus (34.29 mg/100 g) > Manganese (19.18 mg/100 g) > Zinc (12.47 mg/100 g) > Chromium (8.19 mg/100 g) > Copper (6.53 mg/100 g). Lead and Cadmium which are toxic elements to humans and animals were not detected. A total of 17 amino acids were detected in P. amarus leaves including glutamine which was the highest (10.60 g/ 100 g) and Methionine was the most limiting (1.32 g/ 100 g). Since the leaves contain various secondary metabolites in varying concentrations, the synergetic effects of these constituents could make the leaves a potential source of drug.

Keywords: *Phyllanthus amarus*, Euphorbiaceae, Phytochemicals, Amino acid profile, Anti nutrients, Minerals.

1.0 Introduction

The use of plants as medicine has been in existence from time immemorial allowing them to be employed as a valuable precursor for drug development. Most people now rely heavily on herbal medicine for their primary healthcare, not only because they are cheap and readily available, but have also been shown to be fast acting and less toxic compared to the synthetic orthodox medicines [1]. Despite its popularity and continued use in many countries, research in herbal medicine has not been accorded due attention and support. Over the years, due to reorganization of natural products and processes in sustaining the importance of medicinal plants, secondary plant metabolites (phytochemicals) have been extensively investigated as a source of medicinal agents and they have made significant contribution in maintaining human health [1].

Phyllanthus amarus (Euphorbiaceae), also known as *eyin olobe* (Yoruba), *geeron tsutsaayee* (Hausa), *Ngwu ite kwowa nasu* (Igbo) and *ebe-benizo* (Benin), is a widely distributed, small, erect tropical annual herb that grows 30 – 40 cm high and have slender, leaf-bearing branchlets, distichous leaves which are subsessile elliptic-oblong, obtuse, rounded base. The plant has been found in Philippine, Cuba, India, Nigeria among others as a weed in cultivated and waste lands. *Phyllanthus* means "leaf and flower" because the flower, fruit and leaf appear fused [2]. *Phyllanthus amarus* leaves has been acclaimed in herbal medicine and literatures to have diverse therapeutic uses such as the management of urinogenital disorders, jaundice, intermittent fevers, dropsy, dysentery, diarrhoea, gonorrhea, pain, swelling, sores, wounds, scabies, stomach pain, ulcers, ringworm, colic, snake bite, menorrhagia, leucorrhoea and constipation [3-7]. Studies have reported that the aqueous extract possess anti-diarrhoeal [8], anti-carcinogenic and anti-mutagenic [9], anti- nociceptive and anti- inflammatory [10], antidiabetic and antilipidemic [11] and anti-viral [12,13] properties. The medicinal properties of plants are mainly attributed to the presence of secondary plant metabolites (bioactive agents and other nutrients). Thus, this study investigated the phytochemicals, mineral composition and amino acid profile of *P. amarus* leaves to enrich the available scientific data on the phytochemistry and efficacy of *P. amarus* in Nigeria.

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2.0 Materials and Methods

2.1 Plant Materials and Authentication

Fresh *Phyllanthus amarus* leaves were collected within the premises of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria, and it was authenticated at the University of Ilorin Herbarium, Ilorin, Nigeria, where a voucher specimen (UIH 001/1109) was deposited.

2.2 Reagents and Chemicals

All reagents and chemicals used were of analytical grade and were prepared using distilled water before storing in neat and airtight bottles.

2.3 Sample Preparations

The fresh leaves collected were plucked separately from the stalks and rinsed with distilled water. The leaves were later air dried at room temperature for 15 days and pulverized in a blender (Mikachi Blender, Model MK-1830, China). The powdered sample was then transferred into an airtight labeled jar and kept in the refrigerator prior to phytochemical analysis.

2.4 Phytochemical Analysis

Powdered sample of Phyllanthus amarus leaves (10 g) was extracted in 100 ml of distilled water for 48 hours after which the resulting suspension was filtered using Whatmann No. 1 filter paper. The extract was concentrated using a Rotary Evaporator to give a yield of 1.10 g. The dried plant material (1 % w/v) was screened for the presence of some secondary metabolites as described for alkaloids [14, 15], steroids, anthraquinones, cardenolides [16], saponins [17], phenolics and flavonoids [18], cardiac glycosides [19], tannins and terpenoids [20].

2.5 Estimation of Mineral Elements

Powdered sample of *Phyllanthus amarus* leaves (5 g) was ashed at 550°C in muffle furnace for 5 hours; the residue was dissolved in 100 ml of deionized water, and the resultant solution aspirated into an air-acetylene flame. Flame Emission Spectrophotometer was used for sodium (Na) and potassium (K) determination while Atomic Absorption Spectrometer was used for the determination of iron (Fe), magnesium (Mg), copper (Cu), zinc (Zn), manganese (Mn), calcium (Ca), phosphorus (P), lead (Pb), cadmium (Cd) and chromium (Cr). The determination was done following the standard procedures of the Association of Official Analytical Chemists [21].

2.6 Amino Acid Analysis

The amino acid profile was determined using methods described by Spackman *et al.* [22]. A small portion (2 g) of the powdered plant sample was defatted (with chloroform/methanol sample mixture), hydrolyzed (at 105 ± 5^{o}C for 22 hr), evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi sample (TSM) amino acid analyzer (Technicon Instrument Corporation, New York).

2.7 Statistical Analysis

Results were expressed as mean ± SEM of three determinations. Statistical Package for Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA) was used to carry out the statistical analyses.

3.0 Results

The phytochemical analysis of the aqueous extract of *P. amarus* leaves revealed the presence of alkaloids, saponins, flavonoids, anthraquinones, tannins, terpenes and steroids, while cardenolides, phenolics and cardiac glycosides were not detected (Table 1). Quantitative analysis of the phytochemicals revealed that alkaloids has the highest concentration (28.5 mg/L) followed by flavonoids (13.6 mg/L), saponins (11.2 mg/L), steroids has (8.4 mg/L), anthraquinone has (3.8 mg/L) and tannins has (2.7 mg/L), while terpenes have the least concentration (2.3 mg/L) (Table 1).

Table 1: Phytochemical constituents of the aqueous extract of *Phyllanthus amarus* leaves

Phytochemicals	Inference	Concentration (mg/L)
Alkaloids	Present	28.5 ± 1.18
Tannins	Present	2.7 ± 0.11
Saponins	Present	11.2 ± 0.54
Flavonoids	Present	13.6 ± 0.61
Anthraquinones	Present	3.8 ± 0.19
Steroids	Present	8.4 ± 0.46
Terpenes	Present	2.3 ± 0.08
Phenolics	ND	ND
Cardenolides	ND	ND
Cardiac glycosides	ND	ND

n =3 ± SEM; ND= Not detected

The anti-nutrient and mineral constituents of aqueous extract of *P. amarus* leaves are presented in Tables 2 and 3. The extract contained phytate (8.74 mg/ L), phytin phosphorus (2.74 mg/ L), oxalate (2.52 mg/ L), cyanide (0.84 mg/ L) and polyphenol (0.57 mg/ L) as anti-nutrients (Table 2). Mineral analysis of the extract revealed the presence of Calcium (352.13 mg/100 g) > Potassium (239.29 mg/100 g) > Sodium (171.48 mg/100 g) > Magnesium (47.61 mg/100 g) > Iron (36.17 mg/100 g) > Phosphorus (34.29 mg/100 g) > Manganese (19.18 mg/100 g) > Zinc (12.47 mg/100 g) > Chromium (8.19 mg/100 g) > Copper (6.53 mg/100 g) while Lead and Cadmium were not detected (Table 3).

Aqueous extract of *P. amarus* leaves contained 17 amino acids (in grams of amino acid per 100 g of recovered amino acid) with glutamine (10.60 g/ 100 g) having the highest concentration followed by leucine (9.56 g/ 100 g), asparagine (7.53 g/ 100 g), alanine (6.41 g/ 100 g), valine (6.47 g/ 100 g), phenylalanine (6.06 g/ 100 g), arginine (5.96 g/ 100 g), lysine (5.92 g/ 100 g), glycine (5.62 g/ 100 g), isoleucine (5.42 g/ 100 g), serine (5.25 g/ 100 g), threonine (5.16 g/ 100 g), tyrosine (4.42 g/ 100 g), proline (4.35 g/ 100 g), cysteine (4.12 g/ 100 g), histidine (2.63 g/ 100 g) and methionine (1.32 g/ 100 g) (Table 4).

Table 2: Anti-nutrients values of the aqueous extract of *Phyllanthus amarus* leaves

Anti-nutrients	Concentration (mg/ L)
Cyanide	0.84 ± 0.02
Phytate	8.74 ± 0.42
Oxalate	2.52 ± 0.11
Phytin phosphorus	2.76 ± 0.18
Polyphenol	0.57 ± 0.01

n =3 ± SEM

Table 3: The mineral content of the aqueous extract of *Phyllanthus amarus* leaves

Minerals	Concentration (mg/ 100 g)
Calcium	352.13 ± 10.46
Potassium	239.29 ± 8.53
Iron	36.17 ± 2.52
Magnesium	47.61 ± 2.84
Copper	6.53 ± 0.37
Zinc	12.47 ± 0.68
Manganese	19.18 ± 0.52
Sodium	171.48 ± 6.12
Phosphorus	34.29 ± 2.41
Lead	ND
Cadmium	ND
Chromium	8.19 ± 0.23

n =3 ± SEM; ND= Not detected

Table 4: Amino acid composition of the aqueous extract of *Phyllanthus amarus* leaves

Amino acids	Concentration (g/100 g)	Amino acids	Concentration (g/100 g)
Arginine	5.96±0.28	Methionine	1.32±0.28
Alanine	6.41±0.39	Leucine	9.56±0.67
Asparagine	7.53±0.52	Phenylalanine	6.06±0.41
Cysteine	4.12±0.21	Serine	5.25±0.22
Glutamine	10.60±0.73	Tyrosine	4.42±0.19
Glycine	5.62±0.25	Threonine	5.16±0.24
Lysine	5.92±0.32	Valine	6.47±0.38
Histidine	2.63±0.09	Proline	4.35±0.17
Isoleucine	5.42±0.23		

n =3 ± SEM

4.0 Discussion

Medicinal plants form an integral part of human health which is used for the management of many diseases [1]. The medicinal values of plants are due to the presence of bioactive compounds and other chemical constituents. Medicinal plants have been and are still being processed and utilized as synthetic materials for pharmaceutical applications since most modern drugs and processed scientific medicines are of plant origin [2]. In this regard, *P. amarus*, which is usually considered as a wonder- working herb was screened for its bioactive principles in order to evaluate its medicinal implications.

The ethnopharmacological activities of most medicinal plants are dictated by their secondary metabolites (phytochemicals) and other chemical constituents [23]. The presence of alkaloids, saponins, flavonoids, anthraquinones, tannins, terpenes and steroids in this plant may account for its therapeutic properties. Alkaloids and their synthetic derivatives are used as basic medicinal agents and have been implicated to possess anti-malaria, analgesic properties [24], anti-cholinergic and anaesthetic properties [25].

Flavonoids protect against allergies, platelet aggregation, ulcer, tumor and viruses [26]. The rejuvenating effects of flavonoids on cells and tissues can also contribute a substantial role to the fertility enhancing potentials of the plant. Flavonoids have also been reported to possess many pharmacological properties such as: anti-oxidant, anti- cancer, anti-inflammatory and anti-microbial activities [10, 11, 27, 28, 29]. Tannins, which denature protein constituents of the intestinal mucosa and form protein- tannates causes astringent activity [24]. Tannins hasten healing of wounds and inflamed mucous membranes [24]. They can also be used medicinally as antidiarrheal, hemostatic, and antihemorrhoidal agents [30]. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Tannins also participate in oxidation–reduction reaction of ascorbic acid and its antibacterial properties have been reported [30].

Saponins, due to their amphiphilic nature, dissolve in water to form a stable soapy froth [31]. Saponins have been found to possess hypocholesterolemic property [31] which is important for the control of high blood lipids. Saponins are used as an expectorant, emetic, contraceptive and for the treatment of excessive salivation, epilepsy, chlorosis, and migraines [31]. Saponins inhibit some kinds of cancer cell tumor growth in animals, particularly lung and blood cancers, without killing normal cells [32]. Saponins possess aphrodisiac properties and act as a source or precursor of sexual hormones like testosterone, corticosterone, aldosterone, progesterone, estrogens and androgens. The saponins present may be responsible for the medicinal properties accorded the plant [32].

Anthraquinones have been reported in scientific literatures to possess antiviral, antibacterial, and cytotoxic properties [33]. They found wide application as immunosuppressive, immune-stimulant, antiulcer, antioxidant, antitumor, cardiac stimulant and anti-microbial activities [34]. Natural anthraquinone derivatives also tend to have laxative effects [35]. Plant terpenoids are used extensively for their aromatic qualities. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery, and in traditional and alternative medicines such as aromatherapy.

Anti-nutrients such as cyanide (0.84 ± 0.02) and polyphenol (0.57 ± 0.01) are present in this plant in low amount such that they do not pose much serious health threat to consumers. Since the levels of cyanide and polyphenol were not above the lethal dosage (values > 1 mg/L are considered toxic) approved by standard bodies like National Agency for Food and Drugs Administration and Control (NAFDAC) in Nigeria, consumption of the plant extract may be considered to be non-toxic and may not elicit any adverse effect on the nutritional values of the leaves [36]. High levels of phytate (8.74 ± 0.42) and phytin phosphorous (2.76 ± 0.18) as reported in this study have been reported to have complicated effects in human system including indigestion of food and flatulence [37]. They have also been known to exert substantial effect on bioavailability of minerals in foods by forming complexes with minerals (such as Ca, Zn and Mg), thereby preventing efficient absorption by the body systems [38]. However, extensive and proper processing methods are needed to reduce some of these anti-nutrients. High oxalate concentration as reported in this study is known to cause great risk of renal absorption and also possess the ability to chelate divalent minerals and prevent their absorption by the body systems. However, the levels of oxalate in the leaves can be reduced when subjected to Heat treatment (cooking) [39].

Generally, minerals from plant sources are less bio-available than those from animal sources [40]. However, they play essential roles in metabolism and serves as co-factors and co-enzymes for enzymatic reactions. The concentration of the minerals found in *P. amarus* leaves were comparatively higher than those obtained from the leaves of *Pterocarpus Mildbraedii* [41] and *melanthera Scanden* [42]. From this study, *P. amarus* leaves are fairly good sources of minerals, which are involved in diverse metabolic functions. For example, sodium and potassium are involved in body water balance and acid-base balance [43]. Sodium plays an important role in nervous transmission as well as in the osmoregulation of the body fluid, while potassium plays an important role in ionic basis of muscle excitability and also as cofactor for several reactions in carbohydrate metabolism [43].

Iron is an essential trace element for haemoglobin formation and in the oxidation of carbohydrate, protein and fats [43]. The presence of iron in *P. amarus* leaves makes it suitable for use in improving the anaemic condition in iron-deficient patients. Zinc is involved in normal functioning of immune system, activation of certain enzymes and it also plays a special role in the production of testosterone, thereby enhancing male fertility [44]. Available data showed that *P. amarus* leaves are rich sources of Mg, Mn, Cu, Ca and P. Magnesium is an essential cofactor for many enzymatic reactions in the body while Manganese is a required trace mineral for living organisms which functions as a cofactor for a large variety of enzymes. Copper is also

essential to all living organisms as a trace dietary mineral because of its diverse roles in biological electron transport, oxygen transportation as well being a key constituent of the respiratory enzyme complex - cytochrome c oxidase [44]. Calcium and phosphorus are involved in teeth, bone and muscles development [45]. Relatively high concentration of these minerals in *P. amarus* makes the leaf excellent antioxidant.

The biological and nutritive value of a protein is dependent upon its constituent amino acids and its ability to meet the nitrogen and essential amino acids requirements. Aside methionine and histidine, the concentrations of other amino acids (both essential and non-esssential amino acids) detected in *P. amarus* leaves are appreciably high. Phenylalanine is the precursor of some hormones and the pigment melanin in hair, eyes and tanned skin [46], while methionine is needed for the synthesis of choline which in turn forms lecithin and other phospholipids in the body [46]. Aspartic acid is needed in the body to generate adenosine triphosphate (ATP), the fuel that powers all cellular activity. Leucine is used extensively by body builders and other athletes to promote muscle recovery. Apart from structural functions, amino acids are the main precursors for the manufacture of many important substances in the body of living organisms and could also serve as valuable sources of energy especially in the absence of carbohydrate and fats in the body [46].

5.0 Conclusion

The present study revealed the phytochemicals, mineral contents and amino acids present in *P. amarus* leaves. Since the leaves contain various secondary metabolites in varying concentrations, the synergetic effects of these constituents could make the leaves an important source of phytomedicine. The presence of some bioactive principles necessitates further scientific investigation to establish the medicinal potentials of the leaves and thus can be potential sources of useful drugs.

6.0 Conflict of Interest

The authors report no conflict of interest in the publication of this article. The authors alone are responsible for the content and writing of the paper.

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