American-Eurasian Journal of Toxicological Sciences 7 (4): 321-327, 2015
ISSN 2079-2050

DOI: 10.5829/idosi.aejts.2015.7.4.9699

A Comparative Study of Nutritional and Phytochemical Composition of *Phyllanthus amarus* Leaf and Seed

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Abstract: *Phyllanthus amarus* is valued in many countries its broad spectrum of pharmacological activities for a variety of ailments. The study evaluated and compared the nutritional and phytochemical components of the dried leaf and seed of the plant. Proximate analysis revealed that both leaf and seed are rich in carbohydrate and energy, low in fat, ash, crude fibre, with moderate protein as well as adequate moisture content for preservation. The leaf has significantly higher protein and ash, but lower values of carbohydrate and energy than the seed, with no significant difference in their moisture and fat contents. For the minerals and vitamin contents, both seed and leaf are rich in Mg^{2+}, Ca^{2+}, K^{+}, PO_4^{3-} and ascorbic acid, moderate level of Fe^{2+}; but low in Zn^{2+}, thiamin, niacin and riboflavin. The seed has significantly higher contents of Mg^{2+}, Ca^{2+}, K^{+}, PO_4^{3-} Fe^{2+}, thiamin, ascorbic acid and riboflavin, but lower in Zn^{2+} and thiamin than the leaf. Phytochemical evaluation revealed that both seed and leaf have high contents of alkaloids and saponin, moderate in tannins, with appreciable antioxidant level, but low in phytates, oxalate, protease inhibitor and total phenol. The seed, however, has significantly higher values of alkaloids, tannins, antioxidant, protease inhibitor and total phenol; but lower saponin content than the leaf. There was no significant difference obtained for the phytates and oxalate content of the leaf and seed. The present study demonstrated that *P. amarus* is a valuable plant for naturaceuticals and dietary supplement. The differential distributions of the chemical constituents in the leaf and seed, needs to be explored when targeting isolation of a specific chemical so as to reduce cost of research.

Key words: *Phyllanthus amarus* · Phytochemicals · Nutritional · Evaluation · Naturaceuticals and Dietary Supplement

NTRODUCTION

Phyllanthus amarus (Schumm and Thonn) is one of the medicinally important plants belonging to family Euphorbiaceae commonly known as "stone breaker". The plant is locally called gyerontsuntsaye (Hausa), eyin Olobe (Yoruba), ngwu (Igbo) and sheffapitu (Marghi) all in Nigerian languages [1]. The plant has been valued in many countries for its medicinal properties and its broad spectrum of pharmacological activities for a variety of ailments such as asthma/bronchial infection [2], jaundice and hepatitis B and other viral infections [3, 4]. It exhibits inhibitory effect on human immune virus (HIV) and reverse transcriptase activity [5]. Hypoglycaemic and hypocholestrolemic effect of *P. amarus* extract on hepatocytes of diabetic rats has been reported by Nwanjo *et al.* [6], hypotensive effect [7], while Nwankpa *et al.* [8] reported the antioxidative effect of the plant extract on *S. typhi* induced oxidative stress in rats. The *in–vitro* and antimicrobial activity of the plant extract against *Staphylococcus, Micrococcus* and *Pasteurella* spp have also been reported [9, 10]. In rural communities in Nigeria, *P. amarus* is used for the management of typhoid fever and related cases [11, 12]. It was reported to have anti-plasmodial, [13] and anti-diarrheal [14] properties.

Phyllanthus amarus is known to have a number of active constituents which are related to biological activity including lignans, glycosides, alkaloids, flavonoids, ellagitannins and phenylpropanoids found in the leaf, stem and root of the plant along with common lipids, sterols and flavonols tetracyclic triterpenoids volatile oil [15-17].

Nine (9) components from *P. amarus* leaves have been identified. The prevailing components in the ethanolic extract of leaves were reported to be; 3,5-di-t-butylphenol (1.2%), methyl 14-methyl pentadecanoate

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(1.4%), palmitic acid (hexadecanoic acid) (11.8%), 10-octadecanoate (5.5%), 9-hexadecenal (9.0%), glycerol 1, 3-dipalmitate (5.7%), 2, 13-octadecadiene-1-ol (8.2%), dioctytl ester (10.1%) and heptanoic acid (9-dece-1-yl ester), (4.6%) [1].

In general, compound isolated from the plant have shown a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective, nephroprotective and diurectic properties [1, 15-17]. Locally, the whole plant is often used in aqueous form for the treatment of different ailments. Most literatures have only been able to demonstrate the efficacy of the whole plant and different reports have laid claims on the usage of different part of the plant. Meanwhile, there is no report to ascertain the active constituents peculiar to each part. This work therefore focuses on comparative study of the phytochemical mineral components and proximate composition present in the leaf and the seed of the plant.

MATERIAL AND METHODS

Collection of Plant Samples: The whole plant of *Phyllanthus amarus* were collected from the vicinity of Afe Babalola University Ado-Ekiti, in the month of July 2015. The harvested plant materials were air dried at room temperature carefully separated into its leaves and seeds and each part ground into a fine powder using an electric blender and stored in sterile containers.

Quantitative Phytochemical Analysis: Tests for the presence of the following plant secondary metabolites including alkaloids, flavonoids, steroids, saponins, phenols, tannins, glycosides and cardiac glycosides and phytates were carried out on the powdered samples following the methods described by Harbone and Mayuri [18, 19].

Test for Flavonoids: One gramme of the sample was weighed into 10ml of 80% Methanol; left to stand for 2 hours, filtered into a weighed Petri dish and left to dry in the oven at 40°C until it attained a constant weighed. The weight of the Petri dish was recorded.

Determination of Total Flavonoids: A volume of 0.5ml of 2% $AlCl_3$ Methanol solution was added to 0.05ml sample solution. After 1hr at room temperature, the absorbance was measured at $A_{420.}$ Calculate Flavonoids content was obtained with the aid of a calibration graph.

Tannins: One gramme of the sample was extracted with 25ml of the solvent mixture of 80:20 Acetone: 10% Glacial Acetic Acid for 5hours. The supernatant was filtered and the Absorbance of the filtrate as well as the reagent blank measured at 500nm Absorbance. A standard graph was produced with 10, 20,30,40,50 mg/100g of Tannic Acid. The concentration of Tannin was read off taking into consideration dilution factors.

Alkaloids: One gramme of the sample (W) was added to 20ml of 10% Acetic Acid in Ethanol, shaken, allowed to stand for 4 hours and filtered. The filtrate was allowed to evaporate to about a quarter of its original volume and one drop of concentrated Ammonia added. The precipitate formed was filter through a weighed (W1) filter paper. The filter paper dried in the oven at 60°C, weighed when it has attained a constant weight (W2).

$$\%\text{Alkaloids} = \frac{W2 - W1}{W} \times 100$$

Phenols/Phenolics and Antioxidants – (Extraction): Extract 2g with 20ml of 80:20 Acetone: 0.5% formic Acid for 2min and filter.

For Phenols/Phenolics/Phenolic Acids: 2ml of the extract is mixed with 0.5ml of Folin-Ciocalteau Reagent and 1.5ml Sodium Carbonate (20%). Mix for 15 sec and allow to stand at 4°Cc for 30min to develop colour. Measure $A_{765.}$ Express as GAE/g (Gallic Acid Equivalent).

Saponins: One gramme of sample was added to 5ml of 20% Ethanol in a conical flask and placed in a water bath at 55°c for 4hours. This was followed by filtering and washing the residue with 20% Ethanol twice and reducing the extract to about 5ml in the oven. The extract was further treated successively Petroleum Ether, butanol and 5% Sodium Chloride.

Steroids: Five gramme of the sample was added to 100ml of water and drops of 0.1M Ammonium Hydroxide was added to take the pH to 9.1. then 2ml Pet Ether, 3ml Acetic Anhydride and conc. H_2S0_4 were added and the absorbance measured at 420nm.

Cardiac Glycosides/Cardenolides: One gramme of the sample was extracted with 40ml water and place in the oven 100°c for 15min. add 1ml extract plus 5ml water to 2ml Glacial Acetic Acid plus one drop of $FeCl_{3.}$ Add 1ml Conc. H_2S0_4. Measure the absorbance of the resulting solution at 410nm.

Phytates: Extract One gramme of the sample was added to 10ml 3% TCA. The Phytate was precipitated as Ferric Phytate with 0.1% Ammonium Ferric Sulphate. The Ferric Phytate was converted to Ferric Hydroxide and Sodium Phytate by adding 10ml 0.5M Sodium Hydroxide. The precipitate was boiled and dissolved with the addition of dilute acid. The absorbance of the solution was taken at 519nm.

Protease Inhibitor Determination: A 2% solution of egg albumin and 0.1% solution of Bromelain were prepared, both in pH 7 Phosphate buffer. Then 5ml of the egg albumin substrate and 1ml of the Bromelain enzyme were incubated at 55°C for 10 min. A 5ml of 10% TCA was added to stop the reaction. The precipitate was filtered off with Whatman No 1 Filter paper and the absorbance of the filtrate was measured at 280nm As.

The entire procedure was repeated but incubating with the enzyme and substrate mixture 1ml of the extract of the material for Protease Inhibitor determination. Also the absorbance of the filtrate was measured at 280nm (Ai).

$$\% \text{ Protease Inhibition is: } \frac{As - Ai \times 100}{As}$$

Determination of Antioxidants (Oxygen Radical Scavenging Activity): The oxygen radical scavenging activity was carried out by method described by Cuatrecasas and Anfisen [20] and Anosike *et al*. [21] with some modifications. The method is based on measurement of loss of colour of DPPH solution by the change of absorbance at 517nm caused by the reaction of DPPH with the test sample. To 0.2ml extract add 2.8ml of freshly prepared 20mg/dm^3 DPPH in Methanol, incubated for 20min at room temperature. Inhibition in% RSC (Radical Scavenging Capacity) as follows:

$RSC\% = (1 - A_{sample}/A_{blank}) \times 100\%$

$A_{blank}$is Abs for Control while $A_{sample}$is Abs for test sample.

Proximate Analysis: The methods described in official methods of Association of Official Analytical Chemists [23] were used for the proximate analysis.

Ash Content: A dry ashing method was used to determine the ash content. First, the crucible was measured; then, the weight of the sample + crucible was taken before the sample was incinerated in a Muffle furnace at 400°C. The remaining inorganic material was cooled in a desiccator, weighed and the ash content was determined.

$$\% \text{ Ash} = \frac{\text{weigh of sample remaining}}{\text{weigh of original sample}} \times \frac{100}{1}$$

Determination of Moisture Content: Moisture content was measured using oven dry method following official methods of Association of Official Analytical Chemists, AOAC [22]. The oven was used to dry the samples till constant weight then removed and measured. The percentage of moisture content was calculated as: $\% \text{ moisture} = (1\frac{\text{moisture}}{\text{weight of sample}} \times 100)$

Determination of Crude Fiber: This is the non-digestive component of food sample. This test involves two stages, acid and alkaline solution using method described by AOAC [23] and the crude fibre is calculated thus:

$$\% \text{ crude fibre} = \frac{(\text{weight of sample} - (\text{weight of sample} + \text{weight of crucible})}{\text{weight of sample}} \times 100$$

Determination of Protein: The crude protein was determined using the Kjeldahl Nitrogen method described by AOAC [23] (1990).$\% \text{Nitrogen} = \frac{v2 - v1 \times ca \times 0.014 \times 100}{ws \times 10\ (\text{ml of sample})} \times 100$

Mineral Composition Test: The mineral contents were determined on aliquots of the solution of the dried leaf sample by UV/Visible and atomic absorption spectrophotometers [22]. A NOVA 400 atomic absorption spectrometer (Analytik Jena AG, Jena, Germany) with an air/acetylene flame and respective hollow-cathode lamps was used for absorbance measurements. Wavelengths, slits and lamp current used for the determination of six elements were 213.9 nm, 0.5 nm, 4.0 mA (zinc); 422.7 nm, 1.2 nm, 4.0 mA (calcium); 248.3 m, 0.2 nm, 6.0 mA (iron) and 766.5 nm, 0.8 nm, 4.0 mA (potassium), respectively. The results for mineral contents were expressed as mg/100 g DW.

RESULTS AND DISCUSSION

The chemical properties of plants have been shown to be responsible for their nutritional and healing properties [23, 24]. Proximate analysis revealed that both leaf and seed are rich in carbohydrate and energy, low in fat, ash, crude fibre, with moderate protein, as well as, adequate moisture content for preservation. The leaf has significantly higher protein and ash, but lower values of carbohydrate and energy than the seed and no significant difference in their moisture and fat contents (Table 1).

Table 1: Proximate composition of *Phyllanthus amarus*

Parameters	Leaf	Seed	Significance (*p* Value)
Moisture Content%	9.00±0.10	9.37±0.10	0.093
Protein%	18.77±0.15	11.33±0.25	0.001*
Ether Extract (Fat)%	2.47±0.06	2.13±0.153	0.063
Ash%	3.67±0.15	2.93±0.15	0.048*
Crude Fibre%	9.13±0.15	5.53±0.15	0.002*
Carbohydrates (By Difference)%	56.97±0.42	68.70±0.60	0.002*
Metabolizable Energy (Kcal/Kg)	2891.0±15.21	2912.2±22.41	0.068

*Significant difference between mean values along rows p ≤0.05

Table 2: Minerals and vitamins composition of *Phyllanthus amarus*

Parameters	Leaf	Seed	Significance (*p* Value)
Fe^{++} (mg/100g)	7.57±0.21	11.40±0.26	<0.001*
Zn^{++} (mg/100g)	0.27±0.06	0.47±0.056	0.074
Mg^{++} (mg/100g)	40.00±5.00	75.00±5.00	0.007*
Ca^{++} (mg/100g)	178.33±7.64	263.33±2.89	0.001*
K^{+} (mg/100g)	45.00±5.00	73.33±2.89	0.023*
PO_4^{---} (mg/100g)	216.67±7.64	386.67±2.89	0.001*
Ascobic Acid mg/100g)	18.80±0.32	25.53±0.25	0.001*
Thiamin mg/100g)	0.10±0.01	0.12±0.02	0.074
Niacin mg/100g)	0.08±0.02	0.14±0.01	0.027*
Riboflavin mg/100g)	0.03±0.01	0.08±0.01	0.026*

*Significant difference between mean values along rows p ≤0.05

Food fibers have been shown to aid absorption of dietary minerals as well as reduce absorption of cholesterol. The crude protein content observed in the leaf (18.77%) and the (11.33%) compared favorably with and in most cases surpassed those reported for most medicinal plants [25].This signifies the healing properties of the plant as proteins are essential for the synthesis/repair of body tissues and as enzyme [26]. The moisture content of are relatively the same.

For the minerals and vitamin contents, both seed and leaf are rich in Mg^{2+}, Ca^{2+}, K^{+}, PO_4^{3-} and ascorbic acid, moderate level of Fe^{2+}; but low in Zn^{2+}, thiamin, niacin and riboflavin. The seed has significantly higher content of Mg^{2+}, Ca^{2+}, K^{+}, PO_4^{3-} Fe^{2+}, thiamin, ascorbic acid and riboflavin, but lower in Zn^{2+} and thiamin than the leaf (Table 2).

The traditional use of the plant to prevent oedema, kidney problems and oliguria [27] may be linked to the high concentrations potassium and magnesium ions phosphate ion in the both the leaf and seed which may serve the purpose of the maintenance of osmotic pressure, water balance and pH in the body. The level of calcium in the seed and leaf may be adequate for the removal of the anti-nutritional factors (oxalate) and modulate oxalate intoxification by forming complexes with oxalate. Oxalates from plant sources have been known to cause irreversible oxalate nephrosis when ingested in large doses. It is an anti- nutrient and prevents the absorption of some vital nutrients in food, especially divalent metals (Ca^{2+}, Mg^{2+} etc.) and fatty acids by forming salts. Oxalate intoxification (high ingestion of oxalate) causes malabsorption syndromes leading to steatorrhoea, in which fatty acids are not absorbed, causing formation of insoluble calcium salt of fatty acid [28]. Complex formation between calcium and oxalate makes more calcium unavailable, but ensures excretion of the oxalates. For this reason, synergistic pharmacological effect of the plant might be gotten when used as a whole plant, but the seed could be better preferred when used as drug or for nutritional purpose, as it contains lesser quantity of oxalate 36.7g when compared to that of leaf 58.33g (Table 3).

Presence of minerals such as zinc, iron and magnesium in the leaf extract may possibly contribute to immunomodulatory action, since these substances have been implicated in immune modulation [29, 30] and may also enhance the activities of antioxidant enzymes, since these elements serve as cofactors for such enzymes and ultimately modulate the immune system [31], thus be an important activity that could be attributed to its neuroprotective property coupled with the presence ascorbic acid in both seed (25.53 mg/100g) and leaf (18.8 mg/100g). High intake of ascorbic acid correlates with reduced gastric cancer [32].

Table 3: Photochemical analysis

Parameters	Leaf	Seed	Significance (*p* Value)
Alkaloids (mg/100g)	871.67±12.58	1631.17.56	0.001*
Cyanogenic Glycosides (mg/100g)	ND	ND	
Phytates (mg/100g)	55.00±5.00	70.00±5.00	0.095
Tannins (mg/100g)	381.67±7.64	455.00±15.00	0.013*
Saponins (mg/100g)	545.00±10.41	225.00±5.00	<0.001*
Oxalates (mg/100g)	58.33±7.64	36.67±2.89	0.069
Protease Inhibitor (mg/100g)	0.02±0.01	0.05±0.01	0.035*
Total Phenols (GAE/g)	44.17±0.21	52.73±0.15	0.001*
Antioxidant (ORAC) (% Inhibition)	37.43±0.21	48.43±0.21	<0.001*

*Significant difference between mean values along rows p ≤0.05

ND : Not determined

Sorting the phytochemicals in the plant, the two plant parts revealed the presence of the same components but differ in quantity. The plant is rich in alkaloids, tannins, saponins in high quantity and devoid of cyanogenic glycosides. This coincides with the report of Oyewo *et al*. [28], that alkaloids were the highest in terms of concentration, while the least was cyanogenic glycosides. Saranraj and Sivasakthivelan [17] in a qualitative determination reported the presence of alkaloids, flavonoids, phenols and triterpenes in the plant. Except for saponins and oxalate that are more present in the leaf, all other secondary metabolites are located more in the seed especially alkaloids and tannins (Table 3) with good significance difference at $P<0.05$. High levels of alkaloids and saponins present in both leaf and seed of *P. amarus* (Table 3) is suggesting that the plant might have good blood glucose reducing activities by reducing the absorption of dietary glucose in the gastrointestinal tract [33] and may have marked effects on the modulation of immune system and the prevention of oxidative stress concertedly [34]. According to Oyewo *et al*. [28], some bitter alkaloids in plants are metabolised in the liver into dimethylxanthine and finally methyl uric acid bycytochrome P450 oxygenase systems [35]. Methyl uric acid in the liver stimulates the expression of tumor necrosis factor (in the endothelia cells of the liver by macrophages), which modulates the immune system [35]. Saponins are expectorants, cough suppressants and administered for hemolytic activities [36, 37].The intake of the leaf could have a positive role in cholesterol metabolism due to the presence of saponin [33, 38]. Saponin, being the highest phytochemical component present in the leaf (545.00 mg/100g) could result in the permeabilization of plasma membranes due to the bipolar structure of saponins, in which the lipophilic components integrate easily and complex with the lipid fraction of plasma membranes, while the hydrophilic glycosidic portion forms complexes with transmembrane proteins, thereby causing irreversible disorder and disruption of the plasma membrane [39], while tannins, being the highest phytochemical component of the seed (455mg/100g) are well known for their antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, as anti-inflammatory and dieresis [40].

The plant also showed some level of antioxidant property as it has an oxygen radical absorption capacity of about 37.43% in the leaf and 48.43% in the seed. The presence of phenols and flavonoids in the extract may help among others, in preventing oxidative stress by scavenging free radicals and bioactivation of carcinogens for excretion in the liver [41, 42]. This shows that the plant could be very useful in fighting diseases that can lead to cell injury like neuroinflamation due to presence of free radicals and could also be useful to fight the aging process.

CONCLUSION

The present study has equally demonstrated that *P. amarus*, based on the findings on the nutritional and phytochemical evaluation of its leaf and seed, is a valuable plant for naturaceuticals and dietary supplement. The differential distributions of the chemical constituents in the leaf and seed, needs to be explored when targeting isolation of a specific one so as to reduce cost.

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