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Vitamin Content of *Phyllanthus amarus* Leaf

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Abstract
Vitamins are a group of organic compounds present in small amounts in food that the body requires for its normal metabolism. This study evaluated the vitamin composition of the leaf extract of Phyllanthus amarus (ngwu). The vitamin contents were analyzed using standard method and the results of vitamin contents were vitamin A (7.67±0.01mg/kg), vitamin B_1 (16.15±0.00mg/kg), vitamin B_2 (0.014±0.00mg/kg), vitamin B_3 (0.59±0.01mg/kg), vitamin B_6 (0.17±0.03mg/kg), vitamin B_9 (4.03±0.01mg/kg), vitamin C (70.54±0.01mg/kg), vitamin D (1.45±0.01mg/kg) and vitamin E (16.15±0.01mg/kg). The result indicates that Phyllanthus amarus leaf extract contained a considerable amount of some vitamins that could be useful in both as food nutrient and as a nutraceutical.

Key words: *Vitamins, Phyllantus amarus, metabolism, extract*

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I. Introduction

The shift in the attention to the use of herbal plants to treat diseases and infections is due to their minimum side effects, their improved safety, reliability when compared to synthetic drugs and high nutrient composition (Joseph and Raj, 2011). The survival of man throughout history has depended on harnessing these unique gifts found in plants to the full. Herbal plants are vital storehouses of bioactive compounds and nutrients, including minerals and vitamins (Adnan *et al*., 2010). Herbal plants are the cheap and reliable sources of vitamins, minerals and proteins in most developing countries and their medicinal properties is a bonus (Achi *et al*., 2017). Vitamins are organic compounds essential in small quantities for normal physiologic and metabolic functioning of the body (Kamangar and Emadi, 2012).

Vitamins are a large family usually grouped as micronutrients; they are mostly derived from diets with few exceptions and unlike other food substances, this family of micronutrients usually exist in complexes with one another and thus cannot be obtained from a single dietary source. Functionally, vitamins are involved in various metabolic processes where they serve usually as coenzymes in various biochemical reactions associated with proper functioning of the whole organism (Abrha *et al*., 2016). They thus catalyze organic reactions by participating in the formation of hormones, cells, chemical structures of the nervous system, composition of genetic material and a host of other biological processes. They also combine with proteins to form enzymes which participate in various body reactions including in the development of body's immune system.

Probably because vitamins are present in small quantities, in the past, diseases of vitamin deficiencies were treated using various vitamins supplementary in their management; however, advancement in science has led to many biochemical and biological methods that are appropriately used in the identification, measurement and diagnosis of diseases associated with many of the known vitamins. (Danladi *et al*., 2018).

Phyllanthus amarus, commonly called "dobisowo" in Yoruba culture; and "ngwu" among the Igbo tribe, is a plant of the family of *Euphorbiaceae* with approximately 800 species spread over the Australian, American, African and Asian continent (Joseph and Raj, 2011; Iranloye *et al*., 2010). It is a branching glabrous annual herb, grows 30-40 cm in height, with small leaves and yellow, whitish or greenish flowers which has five white sepals and an apical anther (Verma *et al*., 2014, Danladi *et al*., 2018). There are multiple medicinal uses of the plant, which are still in practice till date. For example, ethic tribes of India and other Asian countries have been known to use various parts of the plant in traditional home remedies for treating urinary tract infections, diabetes, hypertension and wounds (Patel *et al*., 2011). Various chronic diseases such as cancer, hepatitis and diabetes mellitus have been well treated with *P. amarus* extract in traditional medicine systems in China. The cardio-protective activity was ascribed to the synergic presence of flavonoids, phenolic compounds, saponins, phyllanthin and hypophyllathinlignans in the plant (Putakala *et al*., 2017). *P. amarus* has also been reported to possess hepatoprotective, antiviral, antimicrobial, antimutagenic and tumor suppressive properties (Joseph and Raj, 2011).

Though, valuable pieces of information about *Phyllanthus amarus* abound in literatures, however, the leaf extract of *Phyllanthus amarus* within the literatures reviewed and where such information exists, it is usually scanty. Thus, this project research work is aimed at determining the vitamin composition of *Phyllanthus amarus* leaf extract with the view of recommending it as a plant rich in micronutrient.

II. Methods

2.1 Sample Collection

Fresh leaves of *Phyllanthus amarus* were obtained from Akegbe-Ugwu in Nkanu West Local Government of Enugu State, Nigeria. It was conveyed to Department of Botany, UNIZIK Akwa in a black polyethylene bag where it was identified as *Phyllanthus amarus*(Ngwu) and named in with code NAUH-202[A].

2.2 Sample Preparation

Fresh leaves of *Phyllanthus amarus* were thoroughly were plucked and slightly rinsed in cold tap water to remove sand, dirt and dust. The leaves were thoroughly air dried at room temperature for three weeks at room temperature. The dried sample was ground into powder using mortar and pestle and subsequently into fine powder using an electric blender, sieved through muslin cloth. One hundred grams (100 g) of the powdered sample was obtained and then kept in an air-tight container prior to analysis.2.3 Sample Analysis. The vitamin contents of the samples were determined using the modified method of AOAC (2010).

2.4 Determination of Beta Carotene Concentration

A quantity (5 g) of the sample was weighed into the test tube and 20 ml of petroleum spirit was added and shaken for 5 min. The supernatant was decanted into another test-tube and the absorbance read at 450 nm.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coeficient}}$$

Path-length =1(constant).

2.5 Determination of vitamin B_1 (Thiamine)

A quantity (1 g) of the sample was homogenized with 50 ml of ethanolic sodium hydroxide solution and filtered into a 100 ml flask. Filtrate (10 ml) was pipette into a beaker and 10 ml potassium dichromate added for color development.

A blank sample was prepared and the absorbance was taken at 560 nm. The concentration of each sample was extrapolated from a standard curve.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coeficient}}$$

Path-length =1(constant).

2.6 Determination of vitamin B_2 (Riboflavin)

Each sample (5 g) was extracted with 100 ml of 50 % hydrogen peroxide and allowed to stand for 30 min. Thereafter, 2 ml of 40 % sodium sulphate was added to makeup to 50 ml mark. The absorbance at a wavelength of 510 nm was read in a spectrophotometer.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coeficient}}$$

Path-length =1(constant).

2.7 Determination of vitamin B_3 (Niacin)

Each sample (5 g) was added 50 ml sulphuric acid and shaken for 30 min. Thereafter, 3 drops of ammonia solution were added to the mixture and filtered. Potassium cyanide (5 ml) was added to 10 ml volumetric flask and the mixture acidified with 0.02 M H_2SO_4. The absorbance was read at a wavelength of 470 nm in a spectrophotometer.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coeficient}}$$

Path-length =1(constant).

2.8 Determination of vitamin B_6 (Pyridoxine)

A quantity (1 g) of each sample was extracted with 500 ml of distilled water for 1 hour and filtered. Then, 2 ml of distilled water, 0.4 ml of 50% sodium acetate, 0.1 ml of diazotized reagent and 0.2 ml of 5.5 Sodium Carbonate was added to 1 ml of the filtrate and mixed thoroughly. The absorbance of the solution was read at a wavelength of 540 nm.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coefficient}}$$

Path-length =1(constant).

2.9 Determination of vitamin B_9 (Folic Acid)

A quantity, 1 g of each sample was weighed into a beaker and extracted with 100 ml of distilled water with slight heat. The mixture was shaken thoroughly and filtered after cooling. The absorbance of the filtrate was read spectrophotometrically at a wavelength of 325 nm.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coefficient}}$$

Path-length =1(constant).

2.10 Determination of vitamin C (Ascorbic Acid)

A quantity (1 g) of each sample was macerated with 20 ml of 0.4 % oxalic acid for 10 min and centrifuged for 5 min. The supernatant (1 ml) was transferred into test tubes to which 9 ml of 2,6-dichlorophenol indophenols (12 mg/l) had been mixed thoroughly by shaking. The absorbance of the resulting solution was taken at 520 nm at 15 sec and 30 sec against corresponding blank.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coeficient}}$$

Path-length =1(constant).

2.11 Determination of vitamin D

A quantity (1 g) of each sample was weighed into a beaker and macerated with 20 ml ethanol for 10 min and filtered. Thereafter, 0.5 ml of Conc. Sulphuric acid was added over a period of 1 min of the filtrate and diluted to 2.5 ml with ethanol. Then, 1 ml Concentrated Sulphuric acid was added over a period of 1 min and mixed thoroughly. The absorbance was read after 2 min at a wavelength of 525 nm.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extinction\ coeficient}}$$

Path-length =1(constant).

2.12 Determination of vitamin E (α- Tocopherol)

A quantity (1 g) of each sample was macerated with 20 ml of petroleum ether for 10 min and allowed to stand for 1 hour with intermittent shaking at every 1 min, and centrifuged for 5 min. supernatant (3 ml) was transferred into triplicate test tubes, evaporated to dryness and the residue re-dissolved with 2 ml ethanol and shaken. A known volume, 1 ml of 0.2 % ferric chloride in ethanol and 1 ml of 0.5 % α-dipyridyl in ethanol were added to the resulting solution and then made up to 5 ml with ethanol. The mixture was thoroughly shaken and the absorbance taken at a wavelength of 520 nm against corresponding blank.

$$\textbf{Cocnentration } (\textbf{mg/g}) = \frac{\boldsymbol{absorbance\ x\ dilution\ x\ pathlength}}{\boldsymbol{Extraction\ coeficient}}$$

Path-length =1(constant).

III. Results

Table 4.1: Results of Vitamin Composition of *Phyllanthus amarus* leaf extract

S/N	Vitamins	Concentrations (M±SD) (mg/kg)
1	A	7.67±0.01
2	B_1	0.03±0.00
3	B_2	0.01±0.03
4	B_3	0.59±0.01
5	B_6	0.17±0.03
6	B_9	4.03±0.01
7	C	70.54±0.01
8	D	1.45±0.01
9	E	16.15±0.00

Data are presented as mean ± standard error of mean (SEM) (n = 2).

IV. Discussion

Vitamins are a group of organic compounds present in small amounts in food that the body requires for its normal metabolism (Ankar and Kumar, 2019). They are essential substances for the normal functioning and development of the body.They are mostly derived from diets with few exceptions and unlike other food substances, this family of micronutrients usually exist in complexes with one another and thus cannot be obtained from a single dietary source.

The result of the study showed that the ethanolic extract of *Phyllanthus amarus* contained vitamin A, B_1, B_2, B_3, B_6, B_9, C, D and E at varying concentrations. The Fat soluble vitamins (Vitamin A, D, E and K) are stored in the fat tissues and liver. When the body requires them, they are transported to the area where they are required within the body with the help of special carriers. Water soluble vitamins (B-vitamins and Vitamin C) are stored in the body like the fat-soluble ones. They travel in the blood stream and need to be replenished everyday (Womb, 2005).

Vitamin A has been found to enhance immune system function by supporting and promoting activities of white blood cells as well as other immune related cells. It also helps to inhibit free radicals and their damaging effects. Vitamin A is essential for vision and immune system health.

Vitamin B_1 (Thiamine) can be found in a variety of food; pork, sunflower, seeds, yeast, peas and wheat (Ezekiel *et al*., 2019).Very little thiamine is stored within the body and must be consumed on a regular bases. A deficiency may result in weakness, loss of appetite, nerve degeneration and irritability. It serves as component of a coenzyme in carbohydrate (known as Thiamine Pyrophosphate) metabolism and supports normal nerve function (Carpenter, 2020).

VitaminB_2 (Riboflavin) works with the other B vitamins. It is important for body growth and the production of red blood cells (Ezekiel *et al*., 2019). Vitamin B_3 (Niacin) is a B vitamin that helps maintain healthy skin and nerves. It also has cholesterol-lowering effects at higher doses (Fagbohun *et al*., 2011). Vitamin B_6 (pyridoxine) helps form red blood cells and maintains brain function. The vitamin also plays an important role in the proteins that are part of chemical reactions in the body. The more protein one takes the more pyridoxine one requires (Snehal and Jignasha, 2015). Vitamin B_9 (Folate) works with vitamin B_{12} to help form red blood cells. It is needed for the production of DNA which controls the tissue growth and cell functions.

Vitamin C (Ascorbic Acid) is water soluble anti-oxidant essential for human health. It has been proven necessary for health responses, wound healing, non-hemi iron absorption (coming from grains and vegetable), reduction in allergic responses and development connective tissue components such as collagen and for the prevention of disease (Omoyeni and Aluko, 2010). Vitamin C is important for cardiovascular health, reducing free- radicals production and free radical damage, good cognitive health and performance (Fagbohun *et al*., 2011).

Vitamin D helps the body absorb calcium which is needed for normal development and maintenance of healthy teeth and bones. Vitamin E is an antioxidant also known as Tocopherol. It helps the body form red blood cells. Therefore, consumption of adequate quantities of this plant will help to meet the daily requirement for both adult and children (Asaolu and Asaolu, 2010).

V. Conclusion and Recommendations

The results of the study showed that the extract of *Phyllanthus amarus* contains some vitamins, it could be useful medicinally.

References

[1]. Abrha, T., Girma, Y., Haile, K., Hailu, M. andHailemariam, M. (2016). Prevalence and associated factors of clinical manifestations of vitamin a deficiency among preschool children in asgede-tsimbla rural district, North Ethiopia, a community based cross sectional study. Archaeology Public Health; 74(1):4-41.

[2]. Achi, N. K., Onyeabo, C.,Ekeleme-Egedigwe, C. A. and Onyeanula, J. C. (2017). Phytochemical, proximate analysis, vitamin and mineral composition of aqueous extract of Ficus capensis leaves in South Eastern Nigeria. Journal of Applied Pharmaceutical Science;**7**(3): 117-122.

[3]. Adnan, M., Hussain, J., Shah, T. M., Shinwari, Z. K., Ullah, F., Bahader, A., Khan, N., Khan, A. L. and Watanabe, T. (2010). Proximate and nutrient composition of medicinal plants of humid and sub-humid regions in North-west Pakistan. Journals of Medical Plants Research;4(4): 339-345.

[4]. Ankar, A. and Kumar, A. (2019) Vitamin B12 Deficiency (Cobalamin) [Updated 2019 Jan 11]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2019 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441923/

[5]. Asaolu, S.S. and Asaolu, M.F. (2010). Trace metal distribution in Nigeria leafy vegetables. Pakistan Journal of Nutrition; 9(1): 91-92.

[6]. Danladi, S., Idris, M. A. and Umar, I. I. (2018) Review on pharmacological activities and phytochemical constituents of Phyllanthusniruri (Amarus). The Journal of Phytopharmacology;**7**(3): 341-348

[7]. Ezekiel, T.W., Nachan, T. and Umaru, A. (2019). Chemical Composition and Nutritional Value of Cassia occidenatlis Seeds. International Journal of Biochemistry Research and Review; 27(3):1- 8.

[8]. Fagbohun, E.D., Lawal, O.U. and Ore, M.E. (2011). The proximate, Mineral and Phytochemical Analysis of the leaves of Ocimumgratissilium L. MelantheraScandens.International Journal of Applied Biology and Pharmaceutical Technology; 3(1):15-22

[9]. Iranloye, B., Oyeusi, K. and Alada, A. (2010). Effect of Aqueous Extract of Phyllanthus amarus Leaves on Implantation and Pregnancy in Rats. Nigerian Journal of Physiological Science;25: 63-66.

[10]. Joseph, B. and Raj, S. J. (2011). An overview: Pharmacognostic properties of Phyllanthus amarus Linn. International Journal of Pharmacology; 7: 40-45.

[11]. Kamangar, F. And Emadi, A. (2012). Vitamin and mineral supplements: do we really need them? International Journal of Preventive Mediccine 3(3):221

[12]. Omoyeni, O.A. and Aluko, B.T. (2010). Qualitative Determination of Chemical and Nutritional Composition of Cissuspetiolata leaves. Electronic Journal of Environmental, Agricultural and Food Chemistry; 9:436-440.

[13]. Patel, J. R., Tripathi, P., Sharma, V., Chauhan, N.S. and Dixit, V.K. (2011). Phyllanthusamarus: Ethnomedicinal uses, Phytochemistry and Pharmacology: A review. Journal of Ethnopharmacology, 138: 286-313.

[14]. Putakala, M., Gujjala, S., Nukala, S., Bongu, S. B. R., Chintakunta, N. and Desireddy, S. (2017). Cardioprotective effect of Phyllanthus amarus against high fructose diet induced myocardial and aortic stress in rat model. Biomedicine & Pharmacotherapy; 95: 1359-1368

[15]. Snehal, S.P. and Jignasha, K.S. (2015). Systemic review of plant steroids as potential anti-inflammatory agents: Current statua and future perspective. The Journal of Phytopharmacology; 4(2): 121- 125.

[16]. Verma, S., Sharma, H. and Garg, M. (2014). Phyllanthus Amarus: A Review. Journal of Pharmacognosy and Phytochemistry, 3(2):18-22.

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