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Nutraceuticals And Antinutritional Factors Assessment Of Ethanol Leaf Extract Of *Phyllanthus Amarus*

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Abstract
This study evaluated the proximate composition, amino acids profile, vitamins, minerals and antinutrients contents of *Phyllanthus amarus* ethanol leaf extract. The leaves were collected, processed and the extract was prepared for the analysis. The research was carefully conducted following the standard analytical procedures. Proximate compositions showed high content of carbohydrate (77.39±0.43 %), followed by moisture (9.52±0.11 %), crude protein (8.43±0.13 %), crude fibre (3.36±9.19 %) and low fat (0.79±0.02%) and ash (0.51±0.04 %). Amino acids components of the extract showed abundant glutamic acid (9.38±2.03 g/100g protein), moderate aspartic acid (7.83±1.07 g/100g) and leucine (7.09±0.78 g/100g) and fairly contents of arginine (5.04±0.30 g/100g) and alanine (4.82±0.75 g/100g) and others were in smaller quantities. The abundance of vitamins in the extract in descending order was vitamin C (31.87 ± 0.02mg/100g), vitamin A (7.02 ± 0.11mg/100g), vitamin E (4.70 ± 0.03 mg/100g), vitamin B_3 (1.65 ± 0.00 mg/100g), vitamin B_1 (0.70 ± 0.02mg/100g), vitamin B_6 (0.61 ± 0.00mg/100g), vitamin B_2 (0.41 ± 0.00mg/100g) and vitamin B_9 (0.25 ± 0.01mg/100g). Minerals recorded high contents of K (270.85±0.20mg/100g), Ca (181.30±0.05mg/100g), P (153.15± 0.05), Mg (113.09±0.49mg/100g) and Na (51.63±0.00mg/100g). Other minerals include Fe (6.42±0.05mg/100g), Zn (4.59±0.01mg/100g), Cu (1.82±0.00mg/100g), Se (2.70±0.06mg/100g) and Mn (2.80±0.05 mg/100g). The anti-nutrients analysis revealed high content of total phenol (516.94±0.17mg/100g), followed by phytate (100.14±0.16 mg/100g), moderate tannins (22.22±0.41 mg/100g) and low saponins (0.86±0.02mg/100g) and cyanides (0.73±0.03 mg/100g) contents. This study suggests that ethanol extract of *Phyllanthus amarus* leaves is a good source of essential nutrients that can contribute to food supplements which could be harnessed for its potential nutritional benefits.
Keywords: *Phyllanthus amarus*, amino acids, Proximate, vitamins, minerals, antinutrients,

I. Introduction

Essential nutrients are crucial in supporting a person's good health, growth and reproduction. These essential nutrients are divided into two categories, micronutrients and macronutrients. Micronutrients are required in small doses and consist of vitamins and minerals. Although the body only needs them in minute quantities, their deficiencies can cause ill health condition (Gombart *et al.*, 2020). Conversely, macronutrients are required in larger amounts and they include water, proteins, carbohydrates, and fats. These nutrients are essential for normal body function and for growth. The body utilizes protein for the maintenance and repair of tissues, for growth and energy (Srivastava *et al.*, 2014).

Phyllanthus amarus (Schumm and Thonn) is a medicinal plant known for its various therapeutic abilities which come from its nutritional and phytochemical compositions. *Phyllanthus amarus* belongs to the family Euphorbiaceae commonly known as "stone breaker". The plant is commonly called "dobisowo", "ehin olobe" or "ehin olubi sowo" in Yoruba, "ngwu" by the Igbo tribe and "buchi oro" in Asaba, all in Nigeria (Iranloye *et al*., 2010). *Phyllanthus amarus* has been valued in many countries for its broad spectrum of pharmacological activities for the treatment of variety of diseases which include asthma/bronchial infection, jaundice and hepatitis B and other viral infections. It exhibits inhibitory effect on human immune virus (HIV) and reverse transcriptase activity (Notka *et al.*, 2003).

Nutritional science refers proximate composition as the basic nutritional components of a food or plant material. In the case of *Phyllanthus amarus* leaves extract, the proximate composition typically includes moisture content, protein content, lipid (fat) content, carbohydrate content, and ash content. The moisture content determines the water content in the extract, while the protein content reflects the presence of amino acids. Lipid content indicates the amount of fats present, and the carbohydrate content represents the source immediate energy, fibres, and starches present. Lastly, ash content gives an estimate of the mineral content of the extract (Anuforo *et al.*, 2017). Amino acids are the building blocks of proteins and play a crucial role in numerous physiological processes. Researches revealed that there are essential amino acids which cannot be synthesized by the human

body and must be obtained through the diet and the non-essential amino acids which can be synthesized in the body (Elufioye and Agboade, 2009). *Phyllanthus amarus* is valued for its medicinal properties and it also contains some antinutrients which are compounds found in plants that may impede the absorption or utilization of certain nutrients. Some antinutrients include phytic acid, which can reduce the absorption of minerals like calcium, iron, and zinc.

Understanding the proximate composition, amino acid profile, vitamins, minerals and antinutrient content of *Phyllanthus amarus* extract provides valuable insight into its nutritional value and potential health benefits.

II. Materials And Methods

Sample Collection and Extract Preparation

Phyllanthus amarus herb was collected from Ebonyi Local Government Area in Ebonyi State. The plant was authenticated by a taxonomist in the Department of Applied Biology, Ebonyi State University, Nigeria. The leaves were cleaned and kept for 25 days to dry at room temperature. Using a mechanical blander, the dried sample was grounded and sieved to obtain fine powder, after which it was weighed. Extraction was carried out by soaking 400g of the sample powder in 1000ml of 98% absolute ethanol for 72 hours with intermittent shaking. Then the extract was filtered and concentrated to dryness using a rotary evaporator.

Determination of Proximate Compositions

The protein, lipid, fibre and ash contents of the extract were determined using the methods of AOAC (2006). Moisture content was determined using the official method of analysis of the Association of Analytical Chemists (2005). Percentage nitrogen free extract (NFE) or carbohydrate was calculated according to Pearson (1976). Thus, the carbohydrate content of the sample was determined by taking the sum of ash, protein, moisture, fibre, fat and oil from 100.

Determination of Amino Acid Profile

The amino acid profile of *P. amarus* leaf extract was determined using the methods described by Benitez (1989). The sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the applied Biosystems PTH amino acid analyzer. The defatting of the sample was done using chloroform/methanol mixture of 2:1 ratio. About 500mg of the sample was put in extraction thimble and extracted for 15 hours in soxhlet extraction apparatus according to the method of AOAC (2006).

Determination of Vitamin Content of the Extract

Vitamin A (retinol), vitamin B_3 (niacin), and vitamin C (ascorbic acid) contents of the extract were determined by AOAC (2010) method while vitamin B_1 (thiamin), B_2 (riboflavin) and B_6 (pyridoxine) contents were determined as described by AOAC (2005). Vitamin E (tocopherol) content of the extract was determined using the method of Rutkowski *et al.* (2005) and vitamin B_9 (folate) was determine according to the method of Association of Vitamin Chemists (1966).

Determination of Mineral Content of the Extract

Exactly 0.5 g of the extract was weighed into a 250 mL conical flask. Then 20 mL of a mixture of nitric acid and perchloric acid was added and allowed to stand overnight. Thereafter, the solution was evaporated to a near dryness. After cooling, 100 mL of distilled water was added and the content filtered using Whatman N0 42 filter paper and the filtrate was used for the mineral determination.

The levels of manganese and selenium in the extracts were determined using Atomic Absorption Spectrophotometer (AAS) method described by Maida *et al.* (2005). Potassium and sodium contents were determined by running the extract solution filtrate in a flame photometer with the standard of potassium and sodium respectively according to the method of AOAC (2010).

Magnesium and calcium components were determined by EDTA titration method of AOAC (2010). While iron and zinc were determined by applying EDTA complexometric titration methods of AOAC (2010). Copper content was determined by ferrocyanide method of AOAC (2005) and iron was determined using the orthophenothrolin method described by AOAC (2010). Lastly, the concentration of phosphorus in the extract was determined by the ascorbic method of AOAC (2010).

Statistical Analysis

All results obtained were subjected to statistical analysis, using analysis of variance (ANOVA). Means were compared at $p \leq 0.05$.

III. Results

Proximate Composition of *Phyllanthus amarus* Extract

The result showed that *Phyllanthus amarus* extract of the leaves has high carbohydrate content (77.39±0.43 %), followed by moisture (9.52±0.11 %), crude protein (8.43±0.13 %), and crude fibre content (3.36±9.19 %), but very low in ash and fat contents (0.51±0.04 % and 0.79±0.02 % respectively) as shown in Figure 1.

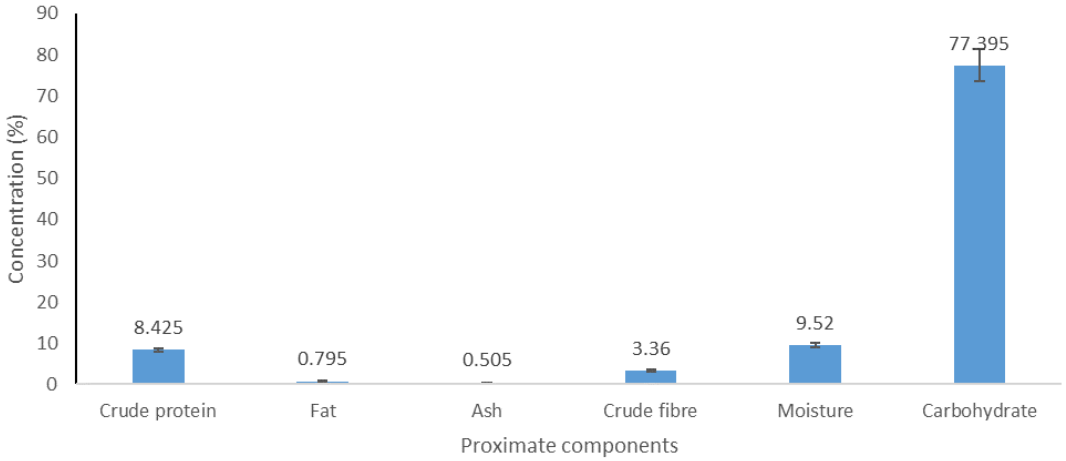

Figure 1: Proximate composition of *P. amarus* extract

Amino Acids profile of *Phyllanthus amarus* of the Leaf Extract

The result of amino acid composition of the extract recorded the most abundant amino acid as glutamic acid (9.38±2.03 g/100g protein), followed by aspartic acid (7.83±1.07 g/100g protein), leucine (7.09±0.78 g/100g protein), arginine and alanine (5.04±0.30 g/100g protein and 4.82±0.75 g/100g protein) respectively. Others were in moderate concentrations ranges from 4.12 g/100g protein to 1.64 g/100g protein. However, amino acids with least concentrations were methionine (1.26±0.09 g/100g protein) and tryptophan and (0.62±0.09 g/100g protein) as shown in Figure 2.

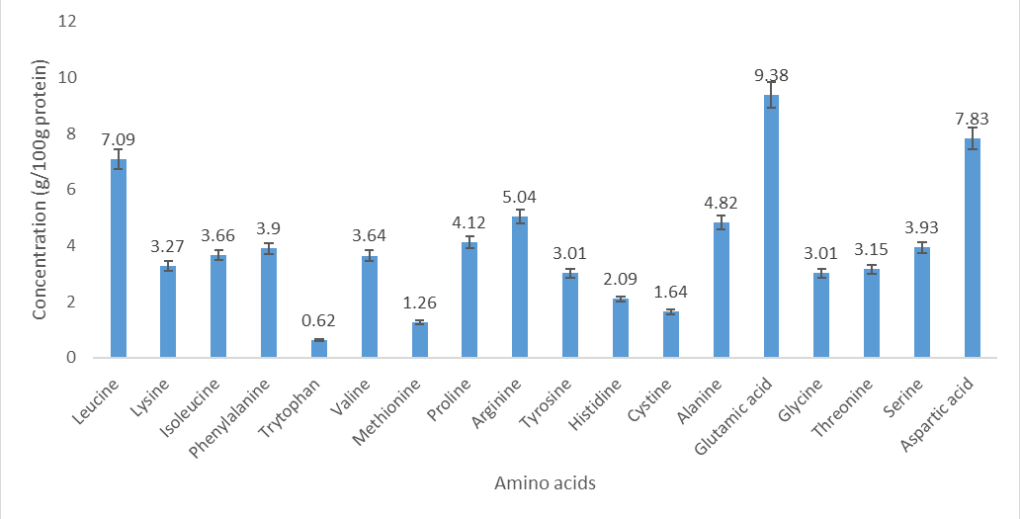

Figure 2: Amino acids compositions of *Phyllanthus amarus* extract of the leaves

Vitamin content of ethanol leaf extract of *Phyllanthus amarus*

The vitamin contents of the ethanol leaf extract of *Phyllanthus amarus* showed that vitamins A, B_1, B_2, B_3, B_6, B_9, C, and E were present in the extract. The record showed that among the vitamins identified, the most abundant was vitamin C (31.87 ± 0.02mg/100g), followed by vitamin A (7.02 ± 0.11mg/100g), vitamin E (4.70 ± 0.03 mg/100g), vitamin B_3 (1.65 ± 0.00 mg/100g), vitamin B_1 (0.70 ± 0.02mg/100g), vitamin B_6 (0.61 ± 0.00mg/100g), vitamin B_2 (0.41 ± 0.00mg/100g) while vitamin B_9 recorded the least concentration (0.25 ± 0.01mg/100g) as shown in Table 1.

Table 1: Vitamin content of ethanol leaf extract of *Phyllanthus amarus*

S/N	VITAMIN CONTENTS	CONCENTRATIONS (mg/100g)
1	Retinol (A)	7.02 ± 0.11
2	Ascorbic acid (C)	31.87 ± 0.02
3	Tocopherol (E)	4.70 ± 0.03
4	Thiamine (B_1)	0.70 ± 0.02
5	Riboflavin (B_2)	0.41 ± 0.00
6	Niacin (B_3)	1.65 ± 0.00
7	Pyridoxine (B_6)	0.61 ± 0.00
8	Folic acid (B_9)	0.25 ± 0.01

Mineral Content of Ethanol Leaf Extract of *Phyllanthus amarus*

The result of the mineral content showed that the most abundant mineral in the sample was potassium (270.85±0.20mg/100g) followed by calcium (181.30±0.05mg/100g), phosphorus (153.15± 0.05), magnesium (113.09±0.49mg/100g), sodium (51.63±0.00mg/100g). Others include iron (6.42±0.05 mg/100g), zinc (4.59±0.01mg/100g), copper (1.82±0.00mg/100g), selenium (2.70±0.06mg/100g), and manganese (2.80±0.05 mg/100 g) as shown in Table 2.

Table 2: Mineral content of ethanol leaf extract of *Phyllanthus amarus*

S/N	MINERAL CONTENTS	CONCENTRATIONS (mg/100g)
1	Potassium (K)	270.85±0.20
2	Calcium (Ca)	181.30±0.05
3	Phosphorus (P)	153.15± 0.05
4	Magnesium (Mg)	113.09±0.49
5	Sodium (Na)	51.63±0.00
6	Iron (Fe)	6.42±0.05
7	Zinc (Zn)	4.59±0.01
8	Copper (Cu)	1.82±0.00
9	Manganese (Mn)	2.80±0.05
10	Selenium (Se)	2.70±0.06

Antinutrient Composition of *Phyllanthus amarus* Leaf Extract

The antinutrient composition of *Phyllanthus amarus* extract is presented in Figure 3. It revealed high content of total phenol (516.94±0.17mg/100g), followed by phytate (100.14±0.16 mg/100g) and tannins (22.22±0.41 mg/100g) but very low in saponins (0.86±0.02 % mg/100g) and cyanides (0.73±0.03 mg/100g) contents.

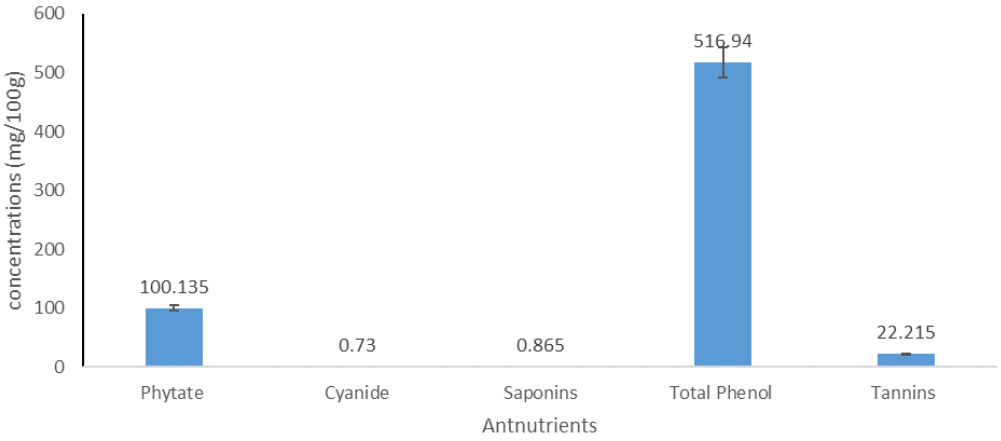

Figure 3: Antinutrient Composition of ethanol Extract of *P. amarus*

IV. Discussion

This research analyzed the nutritional and antinutrient composition of *Phyllanthus amarus* ethanol leaf extract. The results revealed high carbohydrate content (77.39±0.43 %), followed by moisture (9.52±0.11 %), crude protein (8.43±0.13 %), and crude fibre content (3.36±9.19 %), but very low in ash and fat contents (0.51±0.04 % and 0.79±0.02 %) in the extract. Carbohydrates and fats are the major sources of energy. Fats aid in the absorption of fat-soluble vitamins and they are required for growth, immune function and reproduction (Princewill-Ogbonna *et al*., 2019). This study revealed that the leaves of *P. amarus* contain some ash, making it a good source of plant minerals required for normal metabolic activity of body tissues as well as the proper assimilation of vitamins (Umoh *et al*., 2013). Diets rich in fibre helps to prevent constipation, supports the health

of the digestive tract as well as avert colon cancer (Umoh *et al*., 2013). Soluble fibre also lowers cholesterol levels and helps to maintain blood sugar (Dhingra *et al*., 2012). This further suggests why the plant extract could be used in the prevention and management of diseases such as coronary heart diseases, cancer and diabetes (Egbon *et al*., 2017). The findings are in line with the previous report of Owusu-Apenten and Nii-Trebi (2016), on the proximate composition of *Phyllanthus amarus* leaf extract. Water is a very important part of nutrition as the body is made up of 50 to 75% water. Water forms the basis of blood, digestive juices, urine and perspiration, and is contained in lean muscle, fat and bones. The moderate moisture content observed in this study is suggestive that the extract could also serve as a good source of natural water for proper metabolism.

The high concentration of glutamic acid (9.38±2.03 g/100g) in the amino acids profile of *Phyllanthus amarus* extract exposed the nutritional value of the extract. Glutamic acid is an important amino acid involved in various physiological processes. It plays a vital role in neurotransmission in the central nervous system and also a precursor for the synthesis of the neurotransmitter gamma-aminobutyric acid (GABA) (Akiko and Junichi, 2021). Additionally, glutamic acid is a key component in protein synthesis and serves as an energy source. Followed by aspartic acid (7.83±1.07 g/100g), a non-essential amino acid that is involved in the urea cycle, where it plays a role in the elimination of ammonia from the body. It is also a precursor for the synthesis of other amino acids and is important for the production of energy. Aspartic acid is also needed in the body to generate adenosine triphosphate (ATP), the fuel that powers all cellular activity (Formon, 1974). Leucine which is one of the essential amino acids was present in the extract with the value of (7.09±0.78 g/100g), and it is crucial for protein synthesis. It is a branched-chain amino acid (BCAA) that plays a key role in muscle protein synthesis, making it important for muscle growth and repair. Leucine works closely with insulin to regulate the blood sugar levels and stimulates wound healing (Fitch and King, 1987). The amino acids profile also revealed the presence of arginine and alanine with values 5.04±0.30 g/100g and 4.82±0.75 g/100g respectively. Arginine is a semi-essential amino acid involved in the synthesis of proteins, nitric oxide, and urea. It has been associated with cardiovascular health and immune function. Alanine is a non-essential amino acid that plays role in glucose metabolism and is important for maintaining blood sugar levels. Meanwhile, tryptophan and methionine were found to have the lowest concentration of 0.62±0.09 g/100g and 1.26±0.09 g/100g respectively in the extract. Tryptophan is an essential amino acid that serves as a precursor for the synthesis of serotonin, a neurotransmitter that regulates mood and sleep (Olusanya, 2008). Methionine is also an essential amino acid and is important for protein synthesis and the production of sulfur-containing compounds in the body. Methionine is needed for the synthesis of choline which in turn forms lecithin and other phospholipids in the body (Olusanya, 2008). The amino acid composition of *Phyllanthus amarus* suggests that it is a good source of essential and non-essential amino acids, indicating its potential as a nutritional supplement. Amino acids play crucial roles in various physiological processes, including protein synthesis, neurotransmission, and overall metabolic function. This study supports the work of Ong *et al*. (2013), who also observed that *Phyllanthus amarus* leave extract contains significant amounts of essential and non-essential amino acids such as leucine, isoleucine, valine, and phenylalanine.

Analysis of vitamin compositions of the extract was also part of the nutraceutical assessments of the extract. Vitamins are group of organic compounds that are required by the body for normal metabolic process at minute quantities (Borel and Desmarchelier, 2018). They are one of the essential nutrients for the normal functioning and development of the body. This family of micronutrients usually exist in complexes with one another and thus cannot be obtained from a single dietary source. Among the vitamins studied, vitamin C recorded highest concentration (31.87 ± 0.02mg/100g). Ascorbic acid is a water soluble antioxidant essential for human health. It has been proven that vitamin C facilitates quick healing of wounds, non-heme iron absorption and reduction of allergic responses, development of connective tissue components such as collagen and offers antioxidant protections against diseases (Bechara *et al*., 2022). Vitamin C is important for cardiovascular health, reduces production free radicals and scavenges reactive substances, good cognitive health and performance (Morelli *et al*., 2020).

The second most abundant vitamin in the extract was vitamin A (7.02 ± 0.11 mg/100g). 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 (Gombart et al., 2020). Vitamin E is another antioxidant and third most abundant found in the extract. It helps the body for the formation of red blood cells. Thus, this plant extract could be a supplement for daily requirements of vitamin E for both adult and children (Rizvi *et al*., 2014).

The presence of niacin, pyridoxine phosphate and folic acid in the extract has given the plant more nutritional value. Niacin is required in the body for the synthesis of NAD and NADP, which are important biological coenzymes and are also involved in DNA synthesis and repair (Kennedy, 2016). Niacin also helps to maintain healthy skin and nerves. It also has cholesterol lowering effects at higher doses (Fagbohun *et al*., 2011). Pyridoxine phosphate and folic acid participate in red blood cells production and maintains brain function. The vitamins also play important role alongside with some body proteins (Ali *et al*., 2022). Vitamin B9 is also required for the production of DNA which controls the tissue growth and cell functions.

Vitamin B1 (Thiamine) serves as component of a coenzyme in carbohydrate metabolism and supports normal nerve function (Martel *et al*., 2024). Vitamin B2 (Riboflavin) is involved in the metabolism of macronutrients and the production of some other B-complex vitamins. The nutrient participates in redox reactions in the metabolic pathways through cofactors Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN), derived from riboflavin, by acting as electron carriers (Peechakara *et al*., 2024).

The research shows that the extract composed of significant amount of potassium, calcium, phosphorus, magnesium, sodium and iron. The significance of these mineral elements in human diets cannot be overemphasized. The absence of mineral elements in the diet is detrimental and could result in deficiency diseases based on the lacking element (Kiani *et al*., 2022). Most deficiency diseases can be treated by increasing the nutritional sources of the minerals in the diet. This study shows that the extract of *Phyllanthus amarus* leaves is a rich source of essential elements required for proper metabolic processes in the body as presented in Table 2. The results suggests that *Phyllanthus amarus* can be a remedy for iron deficient anaemia. Potassium was the most abundant mineral in the extract, followed by calcium, phosphorus and magnesium. The presence of magnesium, calcium, potassium, and sodium indicates the leaves could be a good source of nutrients for the body system. Phosphorus is an essential element for ATP production which is the most important energy molecule for all the activities of the living cells and for DNA metabolism (Kiani *et al*., 2022). Zinc, copper and nickel are very essential cofactors of numerous enzymes required for various metabolic processes in the body and they are required at their minute quantities (Bhattacharya *et al.*, 2016). The presence of these micronutrient in less concentrations in the extract qualifies the plant as a supplement for human dietary requirement.

On the other hand, the result showed some antinutrient compositions of the plant extract at different concentrations. The antinutrient content of *Phyllanthus amarus* extract in Figure 3, provided valuable insights into its chemical profile. The concentrations of various antinutrients revealed that the extract exhibited the most significant amount of total phenol. Phenols are known for their antioxidant properties and potential health benefits, such as anti-inflammatory and anti-cancer effects. This finding aligns with previous studies that have highlighted the antioxidant capabilities of *Phyllanthus amarus* (Adeyemi *et al*., 2011). However, extremely high concentrations of total phenols, may be associated with plant toxicity. Consuming excessive amounts of phenolic compounds beyond what is typically found in a balanced diet may lead to adverse reactions (Adeyemi *et al*., 2011). The second abundant antinutrient was phytate which are often considered antinutrients due to their ability to bind minerals, potentially reducing their absorption. However, it is also important to consider the overall potential positive effects of phytates on human health, such as anticancer properties and lowering the risk of certain diseases (Adeyemi *et al*., 2011). Tannins were also present in the extract and can be traced to their positive and negative effects on the health. While they can inhibit the absorption of certain minerals, they also possess antioxidant and anti-inflammatory properties. The concentrations tannins reported in this study could contribute to the overall health benefits associated with the plant. It is also worthy to note that the extract contains some levels of saponins and cyanides at lesser amounts. Saponins, despite being considered antinutrients, have been studied for their potential health-promoting effects, including anti-inflammatory and immune-modulating properties (Adeyemi *et al*., 2011). The low levels reported in this study may suggest a minimal impact on their potential negative effects. The low concentration of cyanides is particularly reassuring, as cyanide compounds can be toxic. This findings underscore the safety profile of the leaf-extract of *Phyllanthus amarus* and agrees with the study by Adeyemi *et al*. (2011), who also have conducted antinutrient investigations on the extract. While some antinutrients could be of health benefits, their excessive intake may hinder nutrient absorption or cause other side effects in human. Fortunately, the levels of antinutrients in this plant extract are generally low and the potential negative effects can be mitigated through proper processing techniques to enhance their nutrient bioavailability (Srivastava *et al.*, 2014). These findings provided valuable information for researchers, nutritionists, and healthcare professionals, contributing to a comprehensive understanding of the nutritional and potential therapeutic aspects of *Phyllanthus amarus*.

V. Conclusion

In conclusion, *Phyllanthus amarus* ethanol leaf extract is nutritionally rich, particularly in terms of protein content and essential amino acids; vitamins and minerals. Its low lipid and antinutrient content make it a promising ingredient for the development of functional foods and dietary supplements. The extract could potentially contribute to meeting nutritional requirements and may have beneficial effects on overall health and well-being.

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References

[1] Adeyemi, A. O., Smith, J., Johnson, A., Davis, B., Wilson, C., Thompson, E. And Peterson, G. (2011). Antioxidant And Antimicrobial Properties Of Phyllanthus Amarus Schum & Thonn. International Journal Of Current Research, 3(9): 138-142.

[2] Akiko, S. And Junichi, I. (2021). Amino Acid Metabolism And Autophagy In Skeletal Development And Homeostasis. Bone, 146: 115881.

[3] Ali, M. A., Hafez, H. A. Kamel, M. A. Ghamry, H. I., Shukry, M. And Farag, M. A. (2022). Dietary Vitamin B Complex: Orchestration In Human Nutrition Throughout Life With Sex Differences. Nutrients, 14(19):3940.

[4] Anuforo, P. C., Ngozi, K. A., Egbuonu, A. C. C. And Egu, E. U. (2017). Proximate Analysis And Determination Of Some Selected Page 2 Of 4 Vitamins And Minerals Contents Of Terminalia Catappa Endocarp Flour. Journal Of Nutrition Health And Food Science, 5(5):1-4.

[5] Aoac (Association Of Official Analytical Chemists), (2006). Official Method Of Analysis Of The Aoac (W. Horwitz Editor) Eighteenth Edition. Washighton D.C, Aoac.

[6] Aoac. (2005). Official Methods Of Analysis Of The Association Of Official Analytical Chemists International 18th Ed. Published By The Association Of Official Analytical Chemists International, Wilson Boulevard, Arlington, Virginia. Usa

[7] Aoac (Association Of Official Analytical Chemists) (2010). Official Method Of Analysis Of The Aoac (Ed.): Horwitz, W. 20th Edn. Association Of Official Analytical Chemists Inc., Washighton D.C., Usa. Pp. 1321-1337.

[8] Association Of Vitamin Chemists (1966). Methods Of Vitamin Assay. 3rd Edition, New York: Interscience Publishers, 197–208.

[9] Bechara, N.; Flood, V.M.; Gunton, J.E. (2022). A Systematic Review On The Role Of Vitamin C In Tissue Healing. Antioxidants, 11: 1605.

[10] Benitez, L. V. (1989). Amino Acid And Fatty Acid Profiles In Aquaculture Nutrition Studies, P. 23- 35. In S.S. De Silva (Ed.) Fish Nutrition Research In Asia. Proceedings Of The Third Asian Fish Nutrition Network Meeting. Asian Fisheries Society, Manila Philippines. Society Special Publication, 4:166

[11] Bhattacharya, P. T., Misra, S. R. And Hussain, M. (2016). Nutritional Aspects Of Essential Trace Elements In Oral Health And Disease: An Extensive Review. Scientifica (Cairo), 2016:5464373

[12] Borel, P. And Desmarchelier, C. (2018). Bioavailability Of Fat-Soluble Vitamins And Phytochemicals In Humans: Effects Of Genetic Variation. Annual Review Of Nutrition, 38: 69-96.

[13] Dhingra D., Michael, M., Rajput, H. And Patil, R. T. (2012). Dietary Fibre In Foods: A Review. Journal Of Food Science And Technology. 49(3): 255-266.

[14] Egbon, E. E., Olayioye, E. Y., Olayioye, S. A., Adesuyi, A. T. And Owolabi, T. (2017). Phytochemical Screening And Proximate Composition Of Phyllanthus Amarus. International Research Journal Of Plant Science, 8(1): 9-12.

[15] Elufioye, T. O. And Agboade, O. A. (2009). Amino Acid Composition And Proximate Analysis Of Phyllanthus Amarus Leaves. Journal Of Medicinal Plants Research, 3(2):066-070.

[16] Fitch, W. L. And King, J. C. (1987). Protein Turnover And 3-Methylhistigline Excretion In Nonpregant, Pregnant Any Gestational Diabetic Women. Human Nutrition And Clinical Nutrition, 41c: 327- 339.

[17] Formon, S. J. (1974). Infant Nutrition, 2nd Ed. W.B. Saunders, Philadelpia. Pp 575.

[18] Gombart, A. F., Pierre, A. And Maggini, S. (2020). A Review Of Micronutrients And The Immune System–Working In Harmony To Reduce The Risk Of Infectio. Nutrients, 12(1): 236.

[19] Iranloye, B., Oyeusi, K. And Alada, A. (2010) Effect Of Aqueous Extract Of Phyllanthus Amarus Leaves On Implantation And Pregnancy In Rats. Nigeria Journal Of Physiological Sciences, 25: 63 – 66

[20] Kennedy, D. O. B. (2016). Vitamins And The Brain: Mechanisms, Dose And Efficacy-A Review", Nutrients, 8(2): 68.

[21] Kiani, A. K., Dhuli, K., Donato, K., Aquilanti, B., Velluti, V., Matera, G., Iaconelli, A., Connelly, S. T., Bellinato, F., Gisondi, P. And Bertelli, M. (2022). Main Nutritional Deficiencies. Journal Of Preventive Medicine And Hygiene, 63(2):E93-E101.

[22] Maida, A., Farooq, A., Raziya, N., Umer, R., Kazi, T G. And Nadeem, M. (2005). Mineral Composition Of Moringer Oleifera Leaves And Pods From Different Regions Of Punjab, Pakistan. Asian Journal Of Plant Sciences, 4(4): 417-421.

[23] Martel, J. L., Kerndt, C. C., Doshi, H., Sina, R. E. And Franklin, D. S. (2024). Vitamin B1 (Thiamine). In: Statpearls [Internet]. Treasure Island (Fl): Statpearls Publishing Llc, Https://Www.Ncbi.Nlm.Nih.Gov/Books/Nbk482360/

[24] Morelli Mb, Gambardella J, Castellanos V, Trimarco V, Santulli G.(2020). Vitamin C And Cardiovascular Disease: An Update. Antioxidants, 9(12):1227.

[25] Notka, F. Meier, G. R. And Wagner, R. (2003). Inhibition Of Wild-Type Human Immunodeficiency Virus And Reverse Transcriptase Inhibitor-Resistant Variants By Phyllanthus Amarus. Antiviral Research, 58 (2): 175-186

[26] Olusanya, J. O. (2008). Proteins, In: Essential Of Food And Nutrition, Apex Books Limited. Pp. 13-21

[27] Ong, H. C., Mahli, T. M. I. And Masjuki, H. H. (2013). Amino Acid Composition And Some Biochemical Properties Of Phyllanthus Amarus And Phyllanthus Urinaria. Tropical Journal Of Pharmaceutical Research, 12(3): 343-349.

[28] Owusu-Apenten, R. And Nii-Trebi, N. I. (2016). Proximate And Mineral Composition Of Edible Wild Plant Phyllanthus Amarus Schum. And Thonn. Journal Of Applied Biology And Biotechnology, 4(01): 001-005.

[29] Pearson, D. (1976). Chemical Analysis Of Food. 7th Edition, Edinburgh: Churchill Livingstone. 488 – 496.

[30] Peechakara, B. V., Sina, R. E. And Gupta M. (2024) Vitamin B2 (Riboflavin). In: Statpearls [Internet]. Treasure Island (Fl): Statpearls Publishing, Https://Www.Ncbi.Nlm.Nih.Gov/Books/Nbk525977/

[31] Princewill-Ogbonna, I. L., Ogbonna, P. C. And Ogujiofor, I. B. (2019). Proximate Composition, Vitamin, Mineral And Biologically Active Compounds Levels In Leaves Of Mangifera Indica (Mango), Persea Americana (Avocado Pea), And Annona Muricata (Sour-Sop). Journal Of Applied Sciences And Environmental Management, 23(1): 65–74.

[32] Rizvi, S., Raza, S. T., Ahmed, F., Ahmad, A., Abbas, S. And Mahdi, F. (2014). The Role Of Vitamin E In Human Health And Some Diseases. Sultan Qaboos University Medical Journal, 14(2):E157-65.

[33] Rutkowski, M., Grzegorczyk, K. And Paradowski, M.T. (2005). Kolorymetryczna Metoda Oznaczania Całkowitej Witaminy E W Osoczu Krwi – Modyfikacja Własna Metody Tsena (Colorimetric Method Of Blood Plasma Total Vitamin E Determination – The Own Modification Of Tsen Method). Diagnostic Laboratory, 41:375-379.

[34] Srivastava, S., Mishra, A., Chaudhary, A., Yadav, N. P. And Shukla, S. (2014). Nutritional And Medicinal Properties Of Phyllanthus Amarus: A Review. International Journal Of Pharmaceutical Sciences And Research, 5(3): 739-748.

[35] Umoh, E. D., Akpabio, U. D. And Udo, I. E. (2013). Phytochemical Screening And Nutrient Analysis Of Phyllanthus Amarus. Asian Journal Of Plant Science And Research, 3(4):116-122.

[36] Unuofin, J. O., Otunola, G. A. And Afolayan, A. J. (2017). Essential Oil Composition, Nutrient And Anti-Nutrient Analysis Of Vernonia Mespilifolia Less. Research Journal Botany, 12(2): 38-45.