Phytochemical, Nutritional and Mineral Evaluation of Aqueous and Ethanol Extract of *Phyllanthus amarus* Leaves

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ABSTRACT: This present study was conducted to investigate the phytochemical, nutrient and mineral composition of the aqueous and ethanolic extract of the leaves of *Phyllanthus amarus* collected from the University of Benin botanical garden using standard procedures. Phytochemical studies carried out on the aqueous and ethanolic leaf extract of *Phyllanthus amarus* revealed the presence of bioactive compounds namely, cardiac glycosides, coumarins, saponins, reducing sugar, tannin, flavonoids, steroids, alkaloids and phenolic compounds. The proximate analysis of the leaves shows that it is a rich source of fibre (36.3%) which is essential for the health of the digestive tract, prevention of cancer and diabetes. It is also an adequate source of energy from carbohydrates (13.15%) and lipids (19.01%). However, the amount of moisture (16.91%) and protein (1.13%) was quite undesirable. The mineral content showed the presence of calcium (160ppm), potassium (107.25ppm), magnesium (50ppm), sodium (32.95ppm), iron (5.78ppm), zinc (0.47ppm), copper (0.13ppm), lead (0.04ppm) and nickel (0.01ppm). This result suggests that the leaves of *Phyllanthus amarus* hold great potential for bone formation, maintenance of electrolytic balance, and iron source for anaemic patients. Also, lead and nickel are present in small amount hence pose no great harm to the body. The presence of these phytochemicals, nutrients and minerals in the leaves of *Phyllanthus amarus* supports the therapeutic uses of the plant in different parts of the world. The result is also suggestive that the leaves of *Phyllanthus amarus* holds great promise in the biopharma industry as a source of novel drug.

Keywords: *Phyllanthus amarus*, phytochemicals, nutrient, minerals, therapeutic uses, novel drug

I. INTRODUCTION

The shift in the attention to the use of medicinal plants to treat diseases and infections is due to their minimum side effects and their improved safety and reliability when compared to synthetic drugs (Joseph and Raj, 2011). The survival of man throughout history has depended on harnessing these unique gifts found in plants to the full. Nowadays, modern medicine follows the example of traditional medicine by using active ingredients from the plants to produce effective drugs with fewer side effects. Medicinal plants are also vital storehouses of bioactive compounds and nutrients, including minerals and vitamins (Adnan et al., 2010). Vegetables are the cheapest and most reliable sources of vitamins, minerals and proteins in most developing countries and their medicinal properties is a bonus (Achi et al., 2017). Phytochemicals are bioactive compounds found naturally occurring in plants, and their presence accounts for the unique colour, flavour and aroma of the plant. They also function to protect plants against invasion, disease and infection. When consumed by man, they transfer their biologically active constituents to man, who exploits them in the prevention and treatment of his own illnesses (Adnan et al., 2010; Achi et al., 2017; Iranloye et al., 2010).

*Phyllanthus amarus*, commonly called “Jamgli amli” in Hindi; “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 (Danladi et al., 2018; Verma et al., 2014). Ayurvedic literature has recorded 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 has been well treated with P. amarus extract in traditional medicine systems in China. The presence of the isolates viz; phyllanthin and hypophyllanthin of P. amarus has been found to be responsible for the anti-hepatic viral activity of the plant in different types of in vitro cultures (Muhammad et al., 2018). Aqueous extract of P. amarus was also found to be effective against high-fructose diet induced insulin resistance causing cardiac and aortic tissue damage in rats. This cardioprotective activity was ascribed to the synergic presence of flavonoids, phenolic compounds, saponins, phyllanthin and hypophyllathin lignans 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). Bearing in mind the above uses of P. amarus in folk medicine, this present study was conducted to evaluate the phytochemical, nutritional and mineral composition of its leaves as a possible explanation for its therapeutic potential.

II. MATERIALS AND METHODS

2.1. Collection, Identification and Preparation of P. amarus Leaves

Fresh leaves of P. amarus were collected from the University of Benin botanical garden. The plant was identified by a Botanist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin city. The collected plant leaves were rinsed in clean water and air-dried at room temperature (24°C) for three weeks. The air-dried leaves were then pulverized using mortar and pestle, the fine powder obtained was weighed and small portions of the crude powdered leaves were used to prepare the extracts, as well as for the nutritional and mineral analyses.

2.2 Extraction of P. amarus Leaves

Ethanol extract of the plant leaves was prepared by soaking 1000g of the dried powdered leaves in 2 Litres of ethanol at room temperature for 48hrs with constant maceration. The extract was then filtered using a fine cheese-cloth, the filtrate was thereafter concentrated using a rotary evaporator into a clean conical flask, and the concentrate was then freeze-dried. Similarly, the aqueous extract of the plant leaves was made by soaking 1000g of the dried powdered leaves in 3 Litres of distilled water at room temperature, and the mixture was allowed to stand for 72hrs with constant maceration. After that, it was filtered, and the filtrate was concentrated and freeze-dried. The freeze-dried extracts were later stored at 4°C. Stock solutions of 10mg/ml were prepared by dissolving exactly 0.5g of the freeze-dried extracts in 50ml of their respective extraction solvent. The filtrate obtained in each case was used for the phytochemical screening.

2.3 Qualitative Phytochemical Screening

Simple chemical tests were carried out on the aqueous and ethanol extract according to standard procedures to identify the phytochemical constituents. Dragendorff’s test for alkaloids, alkaline reagent test for flavonoids and foam test for saponin (Tiwari et al., 2011). Ferric chloride test, libermann burchard’s test, and sodium hydroxide test were carried out for tannins, steroids and coumarins respectively (Jayapriya and Shoba, 2014). Finally, Cardiac glycosides, phenolic compounds and quinones were identified by the Kellar-Kiliani test, Folin-Ciocalteau test and concentrated sulphuric acid test respectively (Rajesh et al., 2014).

2.4 Determination of Nutritional Composition

The dry matter, moisture, ash, crude fat, crude protein, carbohydrate and crude fibre contents of the leaves of P. amarus were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000).
The quantitative determination of the anti-nutrient composition such as oxalate and phytate of *P. amarus* leaves was performed using the procedures described in Unuofin et al., (2017) and Ifemeje et al., (2014).

### 2.5 Determination of mineral content

The AOAC method was used to determine the mineral content. Sodium (Na) and potassium (K) levels of the crude powdered leaves of *P. amarus* were ascertained using a flame photometer. At the same time, other metals such as calcium (Ca), magnesium (Mg), Copper (Cu) Iron (Fe), Lead (Pb), Nickel (Ni) and Zinc (Zn) were determined by atomic absorption spectrometry method (Omotosho et al., 2018).

### III. RESULT AND DISCUSSION

#### 3.1. Phytochemicals analysis

*Phyllanthus amarus* is a useful medicinal plant found in several African and Asian countries. The leaves of *P. amarus* have been shown to possess anticarcinogenic, antitumour, antioxidant, antibacterial, antidiabetic, antifungal, and antiviral activities (Gupta and Vaghela, 2019). These medicinal properties in *P. amarus* can be attributed to the presence of its phytochemicals. Table 1. below shows the result of the qualitative phytochemical analysis of the aqueous and ethanol extract of Phyllanthus amarus leaves.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
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<tbody>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Quinone</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key:** + = present - = absent

The cardiac glycosides found presents in the leaves of *P. amarus* exerts a positive effect on the heart in cardiac failures by increasing the capacity of the heart muscles to pump blood (Aldred, 2009). They have been used for centuries as essential drugs in treating heart failure and cardiac rhythm disorders. By inhibiting the Na+/K+ ATPase on cardiomyocyte membranes, cardiac glycosides cause a fall in the intracellular potassium concentration and a rise in the extracellular sodium concentration, leading to the accumulation of intracellular calcium ion via the Na+/Ca2+ exchange system. The overall outcome of these events is increased contractility of the cardiac muscle (Aldred, 2009). Coumarins have been shown to display anticoagulant, antifungal, antibacterial, antifungal and is most likely responsible for the activities earlier reported to be present in the leaves of *P. amarus* (Matos et al., 2013; Gupta and Vaghela, 2019). Tannins have astringent properties and so are remarkable in the treatment of stomach ulcers and diarrhoea. They form a protective layer over wounds and so preventing it from infections (Ashok and Upadhyaya, 2012). The presence of alkaloid and terpenoids in the plant support its use in the possible treatment of malaria, hypertension and cancer (Achi et al., 2017). Due to their free radical scavenging activities, flavonoids have been associated with the prevention of diseases that involve oxidative stress (Huang et al., 2010). Saponins are natural antibiotics which fight infections and microbial invasions. They also have hypcholesterolemic properties, which could offer some chemoprotection against heart diseases to human consumers (Okwu and Emenike, 2006). The most striking prospect for saponins is how they inhibit the growth of cancer cells without posing any significant risk on
normal cells, as is the mode of some cancer-fighting drugs. Cancer cells have more cholesterol-type compounds on their membranes than normal cells. Saponins bind these cholesterol-like compounds and thus interfere with their growth and division (Okwu, 2005).

3.2. Nutritional composition

The result of the nutritional and anti-nutrient content of *P. amarus* leaves is presented in Figure 1. The result obtained revealed high values of fibre (36.3%), moisture (16.91%) and ash (13.5%) with moderate levels of fat (19.01%) and carbohydrate (13.15%). However, the plant was seen to be relatively deficient in protein (1.13%). The anti-nutritional factor of *P. amarus* leaves was also found to be relatively low as observed in its oxalate (0.95%) and phytate (0.21%).

![Figure 1. Nutritional composition of Phyllanthus amarus leaves](image)

Moisture content is a major determinant in the handling, safeguarding and sustenance of food and drug. A high moisture value will promote the activities of spoilage microorganisms and result in reduced shelf life (Unuofin et al., 2017). Mineral elements are the essential constituents of ash. This study revealed that the leaves of *P. amarus* are rich in ash, making it a good source of plant minerals required by man 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. Soluble fibre also lowers cholesterol levels and helps to maintain blood sugar (Dhingra et al., 2012). This further justifies the use of the plant in the prevention and management of diseases such as coronary heart diseases, cancer and diabetes (Egbon et al., 2017). The carbohydrate and fat value of *P. amarus* leaves were found to be (13.15%) and (19.01%) respectively. These biomolecules can serve as an interchangeable source of energy for man, In addition, fat aids absorption of fat-soluble vitamins and are required for growth, immune function and reproduction (Princewill-Ogbonna et al., 2019). The crude protein content was found to be very low; this implies that the leaves of *P. amarus* is deficient in protein. It is therefore advisable that the plant is consumed alongside other food items rich in protein such as legumes in order to complement this deficiency. Protein deficiency in the diet may lead to muscle wasting, prolong wound healing, as well as increased susceptibility to infection (Unuofin et al., 2017). The presence of oxalate and phytate in food is known to interfere with the assimilation of nutrients, decrease the nutritive value of food and at high doses may have adverse effects on human health (Gemede and Ratta, 2014). These anti-nutrient factors act by posing a negative impact on the bioavailability of divalent and trivalent mineral ions, such as calcium, zinc, iron, magnesium, copper and manganese, thereby causing nutritional deficiencies (Unuofin et al., 2017). However, the concentrations of these anti-nutrients,
namely; oxalate (0.95%) and phytate (0.21%) recorded in this study are within tolerable limit and may not elicit toxic effect when consumed. It is interesting to note that these anti-nutrients could easily be removed by soaking, blanching, steaming, boiling or frying (Unuofin et al., 2017).

3.3. Mineral analysis:

The significance of mineral elements in human diets cannot be overemphasized. Vegetables are excellent sources of minerals and so should be regularly incorporated in the diet to improve the body’s metabolic processes (Achi et al., 2017). The absence of mineral elements in the diet is detrimental and could result in deficiency diseases based on which particular mineral element is deficient. Most deficiency diseases can be treated by merely increasing the consumption of foods containing the deficient nutrient (Ojo et al., 2015; Johnson et al., 2018). Figure 2. below shows the mineral composition of the leaves of P. amarus.

![Figure 2. Mineral Composition of Phyllanthus amarus leaves](image)

The present result shows that the leaves of *P. amarus* can be recommended for anaemic patients as it contains high amounts of iron. Calcium was found to be the highest mineral present, followed by potassium and magnesium. The presence of magnesium, calcium, potassium and sodium indicates the leaves to be an excellent source of nutrient for boosting the immune system (Johnson et al., 2018). Zinc, copper and nickel are essential elements in nutrition where they function as an integral part of numerous enzymes required for various metabolic processes in the body. The presence of the toxic element lead (0.01ppm) in this plant is negligible so it cannot pose any health hazards in consumers. The safe limit for the presence of lead in vegetables is 3mg/100g (Ojo et al., 2015).

IV. CONCLUSIONS

The present study revealed that the leaves of *P. amarus* contain several phytochemicals with profound therapeutic usage in diabetics, cardiovascular diseases and cancer. Like other common vegetables, the leaves of *P. amarus* is rich in fibre, lipids and ash. Hence, it should be incorporated into the diet. Interestingly, the anti-nutrient content was found to be lower than that found in most common vegetable. Therefore, it will not interfere with the bioavailability of nutrients when incorporated into the diet. Furthermore, *P. amarus* is rich in several minerals and can serve as a supplement to many mineral deficiencies. Further studies are therefore recommended to harness the nutritional and pharmaceutical potential of this plant as well as isolate and characterize the bioactive compounds present.
V. REFERENCES


