Phytochemical, nutritional and mineral evaluation of aqueous and ethanol extract of *Phyllanthus amarus* leaves

**ABSTRACT**

This present study was conducted to investigate the phytochemical, nutrient and mineral composition of the ethanol and aqueous 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. It may be implied that the presence of these phytochemicals, nutrients and minerals in the leaves of *Phyllanthus amarus* supports the medicinal uses of the leaves in different parts of the world.

**Key words:** *Phyllanthus amarus*, Phytochemical, nutrient, minerals, medicinal uses

**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 the 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 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. *Phyllanthus amarus*, commonly called “Jamgliamli” 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 (Iranloye et al., 2010; Joseph and Raj, 2011). It is a branching glabrous annual herb, grows 30-40 cm in height, with small leaves and yellow, whitish or greenish flowers which have five white sepals and an apical anther (Danladi et al., 2018; Verma et al., 2014). The Ayurvedic literature has recorded multiple medicinal uses of the plant, which are still in practice to 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). *Phyllanthus amarus* has also been reported to posses hepatoprotective, antiviral, antimicrobial, antimutagenic and tumor suppressive properties (Joseph and Raj, 2011). Bearing in mind the above uses of *Phyllanthus 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.

**MATERIALS AND METHODS**

**Collection, identification and preparation of Phyllanthus amarus leaves**

Fresh leaves of *Phyllanthus amarus* were collected from the University of Benin botanical garden, on May 2017. 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.

**Extraction of Phyllanthus amarus leaves**

Ethanol extract of the plant leaves was prepared by soaking 1000 g of the dried powdered leaves in 2 Litres of ethanol at room temperature for 48 hrs 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 1000 g of the dried powdered leaves in 3 Litres of distilled water at room temperature and the mixture was allowed to stand for 72 hrs 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 10 mg/ml were prepared by dissolving exactly 5g of the freeze-dried extracts in 50 ml of their respective extraction solvent. The filtrate obtained in each case was used for the phytochemical screening.

**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 sapoin(Tiwari et al., 2011). Ferric chloride test, Liberman-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 quinines were identified by the Kellar-Kilian test, Folin-Ciocalteau’s test and concentrated sulphuric acid test respectively (Rajesh et al., 2014).

**Determination of nutritional composition**

The dry matter, moisture, ash, crude fat, crude protein, carbohydrate and crude fibre contents of the leaves of *Annonamuricata* 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 *Phyllanthus amarus* leaves was performed using the procedures described by Unuofin et al., 2017 and Ifemeje et al., 2014 respectively.

**Determination of mineral content**

The AOAC (2000) method was used to determine the mineral content. Sodium (Na) and potassium (K) levels of the crude powdered leaves of *Annonamuricata* 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 (AAS) method (Omotosho et al., 2018).
**Table 1**: Qualitative phytochemical analysis of aqueous and ethanol extract of *Phyllanthus amarus* leaves.

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Aqueous extract</th>
<th>Ethanol extract</th>
</tr>
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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

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**RESULT AND DISCUSSION**

**Phytochemicals analysis**

*Phyllanthus amarus* is a useful medicinal plant found in several African and Asian countries. The leaves of *Phyllanthus amarus* have been shown to possess anticarcinogenic, antitumour, antioxidant, antibacterial, antidiabetic, antifungal, and antiviral activities (Gupta and Vaghela, 2019). These medicinal properties in *Phyllanthus amarus* can be attributed to the presence of its phytochemicals. Table 1 shows the result of the qualitative phytochemical analysis of the aqueous and ethanol extract of *Phyllanthus amarus* leaves. The cardiac glycosides found presents in the leaves of *Phyllanthus 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. Coumarins have been shown to display anticoagulant, antifungal, antibacterial and is most likely responsible for the activities earlier reported to be present in the leaves of *Phyllanthus 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. Saponins are natural antibiotics which fight infections and microbial invasions. They also have hypocholesterolemic 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-like compounds on their membranes than normal cells. Saponins bind these cholesterol-like compounds with and thus interfere with cell growth and division (Okwu, 2005).

**Nutritional composition**

The result of the nutritional and antinutrient content of *Phyllanthus 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 antinutritional factor of *Phyllanthus amarus* leaves was also found to be relatively low as observed in its oxalate (0.95%) and phytate (0.21%). Moisture content is a major determinant in the handling, safeguarding and sustenance of food and drug. A high moisture value of 16.91% 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 *Phyllanthus amarus* are rich in ash (13.5%), 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 (Eghon et al., 2017). The carbohydrate and fat value of Phyllanthus 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 Phyllanthus amarus are 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 antinutrient 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 antinutrients, 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 antinutrients could easily be removed by soaking, blanching, steaming, boiling or frying (Unuofin et al., 2017).

**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. 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 number of foods containing the deficient nutrient in the diet. Figure 2 shows the mineral composition of the leaves of *Phyllanthus amarus*. The present result shows that the leaves of *Phyllanthus 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 (Ojo et al., 2015). 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 (0.01 ppm) 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 3 mg/100g (Johnson, 2012).

**CONCLUSION**

The present study revealed that the leaves of *Phyllanthus amarus* contain several phytochemicals with profound therapeutic usage in diabetics, cardiovascular diseases and cancer. Like other common vegetables, the leaves of *Phyllanthus amarus* is rich in fibre, lipids and ash. Hence, it should be incorporated into the diet. Interestingly, the antinutrient 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, *Phyllanthus 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.
plant as well as isolate and characterize the bioactive compounds present.

REFERENCES


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