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Elemental, proximate and phytochemical evaluation of *Phyllanthus muellerianus* (Kuntze) Excell leaves

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ABSTRACT

Phyllanthus muellerianus (Kuntze) Excell (Euphobiaceae) is a popular member of genus "Phyllanthus" in Africa. The use of herbal remedies has been largely employed for the management and treatment of some health challenges and diseased conditions which has led to the experiment on *Phyllanthus muellerianus* leaves. The objective of the study is to evaluate the nutritional characteristics which includes the mineral content, proximate, as well as the phytochemicals present in the leaves of this plant. The plant part studied was examined for the minerals, proximate and phytochemical components present in them using standard laboratory techniques. Screening of the elemental content using Atomic Absorption Spectrophotometer showed the presence of Magnesium (Mg) (3157.50 mg/kg), Manganese (Mn) (189.50 mg/kg), Iron (Fe) (122.00 mg/kg), Copper (Cu) (10.30 mg/kg), Zinc (Zn) (28.50 mg/kg), however, elements Chromium (Cr), Nickel (Ni), Cobalt (Co) and toxic elements Lead (Pb) and Cadmium (Cd) were found absent. Proximate components such as moisture content (9.58%), fat (2.40%), crude protein (23.42%), crude fibre (13.39%) and ash (7.67%) were also found present. Phytochemical screening revealed the presence of tannins, saponins, alkaloids, flavonoids and terpenoids while cardiac glycosides, terpenoids and steroids were found absent. The results obtained from this study indicates that there are essential nutritional and phytochemical components in the leaves which are beneficial and could complement protein and mineral deficiencies and which could also serve as a lead in the quality and safety assurance of this plant.

***Keywords*:** *Phyllanthus muellerianus*, mineral content, phytochemicals, nutritional

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1. INTRODUCTION

The use of plants for the treatment and management of various diseases dates as far back as the existence of man [1] and this practice has continued to rapidly grow across the world with many people now resorting to the use of these products for treatment of various health challenges [2]. Within the last century, plants have been identified to be the major source of lead compounds for therapeutic purpose [3, 4]. Thus, it is of great importance that many plants globally utilized in various systems of traditional healing should be screened for their medicinal properties [5].

Phyllanthus muellerianus is a member of the spurge family (Euphorbiaceae) and it is the most popular herbal drugs of genus "Phyllanthus" in Africa, particularly widespread in the tropical region of West Africa. It is popularly known as "mijiriyarkurumi" in Hausa (Nigeria), "oguazu" in Igbo (Nigeria), and "nkanga" in Efik (Nigeria) [6]. It is a scandent shrub with numerous stems radiating from the base, 2.5-12m tall, monoecious, evergreen, completely glabrous, spiny with spreading branches, reddish tinged; leafy plagiotropic shoots and greyish-brown bark. Foliage leaves distichous; petioles 2–5 mm long; stipules 2 mm long, subulate, chaffy, brownish. Leaf blades 1.5–8 × 1–4.5 cm, broadly ovate to ovate-lanceolate, usually subacute or obtuse, sometimes shortly acuminate, cuneate, rounded or truncate at the base, thinly to firmly chartaceous, dark green and shiny above, paler and duller. It is occasionally found in deciduous and secondary forests from Guinea-Bissau and Mali to West Cameroon and widespread in other areas of Tropical Africa. It occurs from Senegal and Guinea-Bissau east to Sudan and Kenya and south to Northern Angola and Northern Mozambique [7]. It has a widespread usage in the treatment of intestinal troubles [8]. In West Africa, the leaves are locally used for wound healing [7].

In Sierra Leonne, the leaf decoction is employed for the treatment of constipation. In Ghana and Nigeria, the roots are sometimes cooked with maize meal to treat severe dysentery, also, the leaves when boiled with palm fruit are given to women after delivery as a general tonic. In Ubangi, the leaf decoction serves as a mouth wash for toothache, In Nigeria, the young root with young leafy twigs, is given for jaundice and to treat dysentery and urethral discharge [9]. In Cote d'Ivoire, the leaves are eaten, together with young leaves of *Funtumia elastic* (Preuss) Stapf, to improve male fertility [10]. In Gabon, roasted powdered twigs are eaten with plant ash to treat dysmenorrhoea [8]. Its bark commonly called "Mbolongo" in Cameroon, is used by Pygmies as a remedy for tetanus and wound infections and its extracts are also antimicrobial [11, 12].

Local medicinal uses abound especially in the treatment and management of intestinal problems, body pain and as an antiseptic. Preliminary phytochemical screening of the leaves showed the presence of tannins, flavonoids, saponins, alkaloids and anthraquinones [6]. Reported pharmacological activities of *P. muellerianus* leaves include antimalarial [13, 14], antiviral, anti-inflammatory, antidiabetic [15], antimicrobial [12], antioxidant [16]. Compounds that have been isolated from *P. muellerianus* are Corilagin, caffeic acid, furosin, geranin, gallic acid, isoquercitrin, quercitrin, β-sitosterol, chlorogenic acid [17]. As the global use of herbal medicinal products continues to grow and many more new products are introduced into the market, public health issues, and concerns surrounding the quality and safety are also increasingly recognized [18]. It is therefore important to ensure that all plant medicines are of acceptable quality and safety. Thus, the objective of this work is to evaluate the chemical

content and nutritional values of *P. muellerianus* leaves as a major contribution to the knowledge on the plant.

2. MATERIALS AND METHOD

2. 1. Plant collection and authentication

The leaves of *Phyllanthus muellerianus* was collected along the Biological garden, Obafemi Awolowo University, Ile Ife, Osun State. The plant was collected under sterile conditions but however, it was identified by a taxonomist at the Forest Herbarium of Forestry Research Institute of Nigeria (FRIN), Ibadan. After proper identification, the sample was dried, blended and stored in an appropriate container for storage until required for use.

Figure 1. *Phyllanthus muellerianus* in its natural habitat

2. 2. Elemental analysis

Elemental analysis was done according to the method of Isaac and Korber, [22]. Briefly, 0.5g of the powdered sample was weighed into a 50 ml beaker and 10 ml of an acid mixture in the ratio 2:1 (HNO_3:$HClO_4$) was added and allowed to undergo heating on a hot panel placed in a fume cupboard for about 30 minutes until a clear solution(digest) was obtained. The digest was allowed to cool and the volume adjusted to 20ml, this was read on an atomic absorption spectrophotometer (Buck Scientific, model210/211 VLP) to determine the heavy metal composition of the sample. The elements were determined at various wavelengths viz. Zn = 213.9 nm, Fe = 248.3 nm, Cu = 324.8 nm, Cr = 357.9 nm, Co = 324.8 nm, Mg = 285.2 nm, Mn = 279.5 nm, Ni = 341.5 nm, Cd = 228.9 nm, Pb = 283.3 nm.

2. 3. Proximate Analysis

The proximate analysis of powdered plant materials was carried out using the AOAC, [21] in the laboratory of the Department of Human Nutrition, Faculty of Medicine, University of Ibadan. The powdered plant sample was analysed for proximate compositions: moisture content, crude protein, crude fat, ash, crude fibre and carbohydrate.

2. 4. Phytochemical Screening

2. 4. 1. Test for Saponins

1g of the powdered sample was boiled in 5ml of distilled water in a water bath and filtered. 5 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The persistent frothing was taken as the evidence for the presence of saponins [19].

2. 4. 2. Test for Tannins

2g of the dried powdered samples was boiled in 10 ml of water in a test tube and then filtered. 2-5 few drops of 0.1% ferric chloride solution was added. The occurrence of a blue-black, green or blue-green precipitate indicates the presence of tannins [19].

2. 4. 3. Test for Anthraquinones

2g of the plant sample was extracted with 5ml of chloroform, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet color in the ammoniacal (lower) layer indicated the presence of free anthraquinones. Combined anthraquinones were tested for by boiling 2.5g of each extract with 2.5 ml of benzene, the benzene layer separated and half its own volume of 10% NH_3 solution added. A pink, red or violet coloration in the ammonia lower phase indicated the presence of derivatives of anthraquinone [19].

2. 4. 4. Test for Cardiac Glycosides (Keller-Killiani Test)

1g of the plant sample was extracted with 10 ml of 80% ethanol for 5 minutes on a water bath. The extract was filtered and equal volume of distilled water was added to it. A few drops of lead acetate solution were added, shaken and the precipitates were filtered off. The filtrate was extracted with three aliquots of chloroform. The chloroform extract was divided into two portions in evaporating dish and evaporated to dryness on a steam bath [19].

a. **Keller–Killiani's Test:** To the first portion, 3ml of ferric chloride reagent (0.3 ml of 10% $FeCl_3$ in 50 ml glacial acetic acid) was added in a clean test tube. This was then underlayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicates the presence of a deoxy sugar characteristic of cardenolides. A violet ring appeared below the brown ring while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer [20].

b. **Kedde test:** The second portion of the residue was mixed with 1 ml of a 2% solution of 3,5-dinitrobenzoic acid in ethanol and 1ml of a 5% aqueousNaOH. An immediate violet color indicated the presence of cardenolides in the extract, the color fading gradually through reddish-

brown to brownish-yellow with the precipitation of a whitish crystalline solid. This test indicates the presence of a lactone ring in the cardenolides [19].

2. 4. 5. Test for Flavonoids

Shinoda's test: 0.5g of each of the powdered sample was extracted in ethanol by boiling in a water bath for 5min, filtered and cooled. To the filtrate was added four pieces of magnesium filings followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids.

Sodium hydroxide Test: 5ml of 10% NaOH was added to an equal volume of the ethanolic extract. A yellow colouration indicates the presence of flavonoids [19].

2. 4. 6. Test for Alkaloids

About 1g of the plant powder was extracted with 5 ml of 1% aqueous hydrochloric acid on a water bath. The pH of the filtrate was adjusted to about 6. To about 0.5 ml of the filtrate few drops of Mayer's reagent, Dragendorff's reagent, Wagner's reagent, 1% Picric acid solution and 10% Tannic solution were added. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract evaluated [19].

2. 5. Statistical analysis

Data collected were expressed as mean ± standard deviation of triplicate analysis.

3. RESULTS

Elements Mg, Mn, Fe, Copper and Zinc were present at different concentrations in Mg/kg while elements Zn, Co, Cr, Cd, Pb and Ni were not detected in the understudied sample as presented in Table 1. Magnesium has the highest concentration of 3157 mg/kg, followed by manganese (189.50 mg/kg), then Iron which has 122 mg/kg. Zinc has 28.50 mg/kg while Copper which has the least value gave 10.30 mg/kg

Table 1. Elemental analysis of *P. muellerianus* leaves

Element	***P. muellerianus* (mg/kg)**
Magnesium (Mg)	3157.50
Manganese (Mn)	189.50
Iron (Fe)	122.00
Copper (Cu)	10.30
Zinc (Zn)	28.50
Cobalt (Co)	ND

Chromium (Cr)	ND
Cadmium (Cd)	ND
Lead (Pb)	ND
Nickel (Ni)	ND

*Not detected

Table 2 presents the proximate content of *P. muellerianus* leaves with moisture content (9.58%), Crude Protein (23.42%), Ether (2.40%), Crude fibre (13.39%) and Ash value (7.67%).

Table 2. Proximate analysis of *P. muellerianus* leaves.

Parameters	***P. muellerianus* (%)**
Moisture content	9.58 ± 0.07
Crude Protein	23.42 ± 0.31
Ether	2.40 ± 0.01
Crude Fibre	13.39 ± 0.04
Ash	7.67 ± 0.08

*Values are expressed as Mean ± SD

Table 3 shows the result of the qualitative phytochemical analysis. The phytochemical screening carried out on the leaves of *P. muellerianus* showed that it contains saponins, flavonoids, tannins, terpenoids and alkaloids while anthraquinones, cardiac glycosides and steroids were found absent.

Table 3. Phytochemical screening of *P. muellerianus* leaves.

Constituents	***P. muellerianus***
Alkaloids	+
Anthraquinones	-
Tannins	+
Saponins	+

Cardiac glycosides	-
Terpenoids	+
Steroids	-
Flavonoids	+

***** + (Positive), - (Negative)

4. DISCUSSION

In recent times, there has been a tremendous surge in the acceptance and public interest in natural methods of treatment both in developing and developed countries with these herbal remedies being available not only in drug stores but also in food stores and supermarkets [23]. Experimental evaluation of these plants is, therefore, important in order to determine the quality and safety of these plant materials as well as to establish the active constituents of these herbal remedies. The mineral element compositions of *P. muellerianus* leaves as presented in Table 1 shows the elements determined were Iron (Fe), Magnesium (Mg), Copper (Cu), Zinc (Zn), Manganese (Mn), Cobalt (Co), Chromium (Cr), Cadmium (Cd), Lead (Pb) and Nickel (Ni).

The Magnesium content of *P. muellerianus* leaves was 315.75 mg/kg which is slightly more than the value obtained for *Synsepalum dulcificum* (Sapotaceae) (300.8 mg/kg) as reported by Awotedu and Ogunbamowo, [24]. Magnesium is widely distributed in foods, particularly nuts, seafood, green leafy vegetables, other fruits and vegetables, black beans and whole- grain products. Magnesium influences bone metabolism and helps prevent bone fragility [25]. The Recommended Dietary Allowance (RDA) for Mg in human is 250-380 mg/kg, thus from the result obtained, we can suggest that *P. muellerianus* leaves can substitute for regular Magnesium drugs or foods. Iron content in the leaves of *P. muellerianus* is 122.0mg/kg which is slightly lower than that of *P. amarus* as reported by Umoh *et al.,* [26] and over two times lower than the report of Omolola, [27] in *Tithonia diversifolia* leaves (291.5mg/kg). Iron is involved in energy metabolism as well as in the transport of oxygen in haemoglobin. Iron is found only in animal foods, such as meat, chicken and fish. Excellent animal sources of dietary iron include liver, heart, lean meat, oysters, clams and dark poultry meat.

The recommended daily requirement of Iron for man is 6-40 mg/kg [28]. 10.30 mg/kg Copper content was discovered in *P. muellerianus* leaves which is higher than its content in *P. amarus* (6.25 mg/kg), as reported by Umoh *et al.,* [26] and 2.1 mg/kg in *Euphorbia heterophylla*, a member of the same family as *P. muellerianus as reported by* James and Friday, [29]. Copper is an essential mineral whose function is closely associated with the function of iron in oxygen metabolism. It is widely distributed in foods and is high in seafood, meats, nuts, beans, and whole-grain products. The major deficiency symptom is anemia, but osteoporosis, neurological defects, and heart disease may also develop. The RDA for copper is 0.90 mg, for adults age 19-50, but amounts vary for other age groups [25].

The Zinc content of *P. muellerianus* was 28.50 mg/kg which is three times lesser than the Zinc content in *Euphorbia hirta* (83.0 mg/kg) as reported by Ghosh and Chatterjee [30] and eight times lesser than 235.7 mg/kg found in *E. heterophylla as researched by James and Friday,* [29]. Zinc is found virtually in all tissues in the body and is required for the activity of more than 300 enzymes and a wide variety of other body functions such as protein synthesis,

the growth process, bone formation and wound healing [25]. The RDA for Zinc is 13 mg. Manganese **(**Mn) content of *P. muellerianus* was 189.50 mg/kg which is slightly higher than 134 mg/kg for *Euphorbia hyssopifolia* leaves [31]. They function in the body in energy metabolism, bone formation and fat synthesis. Its deficiency results in poor growth, weakness, nervous system problems, mental confusion. The major food sources of manganese are wholegrain products, dried peas and beans, leafy vegetables, bananas. However, Elements, Cobalt (Co), Chromium (Cr), Lead (Pb), Cadmium (Cd) and Nickel (Ni) were found absent in the leaves of *P. muellerianus*. Reports suggest that there is a close relationship between cases of declining reproductive health and environmental pollutants like lead [32]. Also, it has been documented that metals such as lead, cadmium, arsenic and mercury can affect male reproductive functions including sperm motility and morphology [33] and spermatogenesis [34]. Thus, the absence of these toxic heavy metals in this plant part further corroborates the safety of the plant.

Table 2 represents the proximate composition of *P. muellerianus* leaves. The result revealed that the ash value of *P. muellerianus* leaves is 7.67%, this is double the content in *P. amarus* leaves (3.67%) and three times in *P. amarus* seeds (2.93%) in the works of Okiki *et al*., [35], this value falls in the acceptable range of 22% for any standard drug [36] and this gives us an idea on the mineral content of the plant. Higher ash denotes higher mineral content, thus, we can say the plant sample understudied has a higher mineral content than that of *P. amarus*. The determination of the crude protein content of any sample is important because of their nutritive values and because protein has been proven to be of paramount importance for the survival of man [37]. *P. muellerianus* leaves shows a high Crude protein content of 23.42% which indicates that it can be a very good source of protein. The result obtained for Crude protein in this study is twenty times more than 1.54% obtain for *P. amarus* by Egbon *et al*., [38]. Proteins help improve the immune system and plays a major role in cell division and growth [39]. The moisture content of *P. muellerianus* leaves is 9.58% which is 8 times lesser than 75.69% recorded for *E. hirta* leaves [25] and slightly higher than 6% obtained for *P. muellerianus* leaves by Olalekan *et al.*, [40].

This implies that there is a moderate amount of moisture in the understudied plant since the normal range is 14% by African Pharmacopoeia, (41). Excessive moisture in a given sample encourages microbial growth or contamination [42], therefore we can say the understudied sample has limited chances of microbial invasion. Crude fibre content was 13.39% which is slightly lower than 14.99% obtained for *P. amarus* whole plant as researched by Egbon *et al.*, [38]. Fibres function by reducing the rate at which glucose is being absorbed into the blood stream thus reducing chances of having hyperglycemia [42]. Fat composition was 2.40% which is more than the fat content of 1.10% in *E. heterophylla as recorded by James and Friday*, [33]. Fats provide excellent source of energy and enhance transport of fat soluble vitamins, insulate and protect internal tissues and contribute to vital cell processes [43]. It has been suggested that enough fat be included in the diet to account for at least 20-25% of the total caloric intake [26].

Medicinal plants always contain some bio-active substances that have been widely reported to contribute their metabolic, physiologic and protective effects to humans [44]. The result gotten in this study as clearly expressed in Table 3 shows that Tannins, saponins, alkaloids, flavonoids, terpenoids were found present in the leaves of *P. muellerianus* while anthraquinone, cardiac glycoside and steroids are absent. This result also aligns with that obtained on *Phyllanthus fraternus as researched by* Menta *et al.*, [45]. This result obtained is also in accordance with the report of Obianimi and Uche, [46] for *Phyllanthus amarus* leaves

except that Steroids were found present while absent in *P. muellerianus* leaves. Evaluation of the phytochemicals in *P. muellerianus* leaves by Olalekan *et al.*, [40] also revealed the presence of alkaloids, saponins, tannins, terpenoids and flavonoids except for steroids and cardiac glyosides which were present in their own evaluation but absent in the sample used for this study. Therapeutically, when tannin is present in plants, it cures diverse range of diseases, it serves as a potent antidote for many poisons [47], antibacterial [48] and anti-parasitic [49]. It also and serves as immediate relief for people with sore throat, diarrhea and dysentery and wounds [50]. Presence of saponin in plants helps the body system to fight against viruses and bacteria and parasitic infections. However, the occurrence of saponin in this study suggests it for use in fighting against infections and recommending it for soap making properties because of its foamy abilities [51-57].

Alkaloids have been known to possess antimalarial, analgesic, antihypertensive, anti-inflammatory, antifungal, anti-fibrogenic and micro-biocidal effect if present in plants [58, 59]. Flavonoids which are widely distributed in plants are known to have natural antioxidant properties such as free radical scavenging, anti-inflammatory and anti-carcinogenic effects [60]. Terpenoids, which presence inhibits cholesterol synthesis and also contains pharmacological activities such as anti-viral, anti-bacterial, anti-inflammatory and anti-malarial [61]. Hence, the presence of these phytochemicals in the leaves of *P. muellerianus* tends to support its medicinal use as a plant of high medicinal credence.

5. CONCLUSION

The results obtained in this study are all quality and safety indices necessary to achieve standardization and it further confirms its therapeutic potential as reported by ethnobotanical users. Thus making us appreciate the fact that the plant can be greatly used for the management and treatment of diseases because of its high nutritive and safety index.

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