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Phytochemical screening and proximate composition of methanol leaf extract of *Phyllanthus niruri*

Ayo Victoria Ifeoluwa¹, Moses Adondua Abah^{2*}, Ebenezer Morayo Ale³, Victoria Oduh Onyemowo⁴, Paula Paul Shingu⁵, Akinyemi Damilola⁶, Anih David Chinonso⁷, Nicodemus Emmanuel⁸

^{1, 2, 3, 7, 8} Department of Biochemistry, Faculty of Pure and Applied Sciences. Federal University Wukari, Taraba State, Nigeria

⁴ Department of Biochemistry, Faculty Basic Medical Sciences. University of Jos, Jos, Plateau State, Nigeria

⁵ Department of Microbiology, Faculty of Pure and Applied Sciences. Federal University Wukari, Taraba State, Nigeria

⁶ Department of Biochemistry, Faculty of Life Sciences, Federal University of Technology Akure, Ondo State, Nigeria

* Corresponding Author: **Moses Adondua Abah**

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Abstract

Focus on plant based research has increased all over the world and a large body of evidences has shown immense potential of medicinal plants used in various traditional systems. These medicinal plants are considered rich sources of bioactive phytochemicals useful in the field of phytomedicine. The present study is aimed at investigating the phytochemical and proximate composition of *Phyllanthus niruri*. Fresh *Phyllanthus niruri* whole plant (roots, stems, leaves and seeds) samples were collected from Wukari, Taraba state, Nigeria. The plant samples were shade dried for 10 days. The dried samples were ground to powder with the aid of a mortar and pestle. The coarse materials were then sieved. Extraction of *P. niruri* was done by cold maceration with 100% methanol. The extract was stored in the refrigerator which was later used for phytochemical screening and proximate analysis. The results for proximate composition of *Phyllanthus niruri* extract showed that, *P. niruri* has high content of carbohydrate (52.91%). Protein content was 14.7% and crude fiber was 6.6 %, followed by total ash content (11.29%) while lipid and moisture content were found to be 9.9% and 4.6% respectively. Phytochemical screening of *P. niruri* revealed the presence of some bioactive phytochemicals like saponins, tannins and glycoside, while analysis for steroid and flavonoid proved negative. The results suggest that *P. niruri* contains several phytochemicals that could be of good therapeutic purpose in phytomedicine.

Keywords: *Phyllanthus niruri*, Phytochemicals, Bioactive compounds, Proximate composition, Extract

Introduction

Recently, synthetic drugs are gradually being replaced by drugs produced from medicinal plants. This is due to the fact that synthetic drugs with time tend to exhibit adverse effects on the users, unlike drugs produced using medicinal plants which are said to be less toxic and safer than synthetic drugs (Yakubu *et al.* 2022) ^[21]. As a result of this, focus on plant based research has increased all over the world and a large body of evidences has shown immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 15 years period (Chattopadhyay *et al.* 2006) ^[5] and over four-quarter of the world's population had been reported using herbal products as necessary medicine (Patel *et al.* 2011) ^[13]. Medicinal plants are a rich source of bioactive phytochemicals of which the most common include polyphenols, carotenoids, flavonoids, coumarins, indoles, isoflavones, lignans, organosulfures, catechins, phenolic acids, stilbenoids, isothiocyanates, saponins, procyanidins, phenylpropanoids, anthraquinones, ginsenosides, etc. It is well known that plants produce these chemicals to cover themselves, but recent exploration demonstrates that they can cover humans against disease conditions (Yakubu *et al.* 2019) ^[19, 20]. These phytochemicals are known to accumulate in different parts of plants, such as in the roots, stems,

leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues (Yakubu *et al.* 2021) [22]. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (De souza *et al.* 2002) [7]. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals. These compounds are known as secondary metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property (Santos *et al.* 2002) [16].

Phyllanthus niruri L. (Euphorbiaceae) is a small erect herbaceous medicinal plant with pale green leaves having small oblong elliptic glabrous leaves with diverse global naturopathic applications and potentials yet to be fully elucidated. It is widely distributed in the tropics, and grows up to 30-40 cm in height (Umoh *et al.* 2013) [17], with little girth. This plant is widely spread throughout the tropics and subtropics in sandy region as weed in cultivated and waste lands (Pinmai *et al.* 2008) [14], and has a high utility in ethnobotanical medicine. It is popular with rural people because of its immense medicinal properties like antidote against liver diseases, antiviral properties, antioxidant, hepatoprotective, anti inflammatory and strong inhibitory effect against neurogenic pain (Chattopadhyay *et al.* 2006) [5]. *Phyllanthus niruri* leaves contain bitter substances such as Phyllanthin, Hypophyllanthin, Niranthin, Nirtetralin, Niruretin, Nirurin, Nirurisode, Phyltetralin. Quercetin, kaempferol-4- rhamnopytanoside (Chang *et al.* 2002; Panche *et al.* 2016) [4, 12]. *P. niruri* has been reported to have antioxidant and hepatoprotective properties and antiinflammatory potential (Achi *et al.* 2017) [2]. Some of the flavonoids obtained from this plant had shown antinociceptive properties (Santos *et al.* 2002) [16], latex with oil in Ophthalmia. The stem and leaves yield a black dye which possesses antibacterial property (Egbon *et al.* 2017) [8]. The plant has also been reported to play a role in treating ulcers and urinary tract stones (Panche *et al.* 2016) [12]. However, due to lack of research, there is insufficient evidence on its safety and effectiveness.

There is presently little information available on the phytochemical and proximate compositions of aqueous extract of *P. niruri* us. The Importance of such information cannot be overemphasized since the plant's extract is taken as a tonic in many homes and many local medicinal preparations are not properly evaluated before use. This research is therefore aimed at investigating the phytochemical and proximate composition of *Phyllanthus niruri*.

Material and Methods

Study area

The study was carried out in the Central Research Laboratory located at Federal University Wukari, Taraba State, Nigeria, between November and March 2021.

Plant collection

Fresh *Phyllanthus niruri* leaves were collected from Wukari, Taraba state, Nigeria, in the month of November 2021. The plant samples were shade dried for 10 days. The dried samples were ground to powder with the aid of a mortar and

pestle. The coarse materials were sieved and packaged for further analysis.



Fig 1: *Phyllanthus niruri* plant (Achi *et al.* 2017) [2].

Preparation of methanolic extract of *Phyllanthus niruri*

Cold maceration technique was employed for the extraction. The method of Yakubu *et al.* (2022) [21] was adopted with little modification. To 250g of the powdered crude sample, 500ml of 100% methanol was added. The mixture was sealed with foil paper and allowed to stand at room temperature for 7 days with frequent shaking. The mixture was sieved, strained and the marc (damp solid material) was pressed. The solvent extract was filtered using Whatman No 1 filter paper as fine solvent extract was obtained. The methanolic extract was concentrated by evaporating the solvent (100% methanol) at 50°C using a rotary evaporator and vacuum oven to obtain the oily-sticky extract. It was dissolved in 80% for an aqueous extract of *Phyllanthus niruri* (AEPN), the AEPN was stored in the refrigerator prior to the commencement of treatment.

Carbohydrate content determination

Total carbohydrate content was determined by subtracting the sum of the percentage composition of crude fibre, moisture content, crude lipid, ash content and crude protein from 100%. The total carbohydrate of each sample was calculated using the formula below:

Carbohydrate content = 100 - (% protein + % fat + % ash + % Crude fibre + % moisture).

Determination of crude protein

Micro-Kjeldahl method was used to determine the nitrogen content of the sample. Two grams dried powdered sample was placed into a 100 cm Kjeldahl digestion flask. A Kjeldahl digestion tablet and 10 cm of concentrated tetraoxosulphate (VI) acid were added and the sample digested gently until frothing stopped. The mixture was boiled until the digest become clear. The content was filtered into a 100 cm volumetric flask and made up to 100cm with distilled water. 10 cm of the aliquot solution and 20 cm of 45% sodium hydroxide solution were put into a distillation flask and steam distilled. The ammonia liberated was collected over 50 cm 20% boric acid mixed indicator solution, cooled and titrated with standard 0.01M HCl solution. Blank determination was carried in similar manner. The percentage crude protein was calculated by $T \times 1.75\%$ where T is the titre value.

Moisture content determination

Moisture content was determined using AOAC (1990) procedure. Washed porcelain dishes were dried in a

gallenkemp oven at 100°C for about 2 hours, cooked in desiccators and reweighed. 2g of the sample was weighed into the weighted dishes and placed in the oven at 100°C for 24 hours. The dish containing the sample was cooled in desiccators, weighed and dried repeated similarly until a constant weight was obtained. The percentage moisture was calculated using formula below:

$$\text{Moisture content} = \frac{X - Y}{Y} \times \frac{100}{1}$$

Where;

X=Initial weight of sample; Y=Final weight of sample

Ash content determination

Ash content was determined by the incineration of 2g samples in a muffle furnace at 600°C for 2 hours. The percentage residue weighed was expressed as ash content. It was calculated as;

$$\frac{\text{Weight of ash}}{\text{Weight of sample}} \times \frac{100}{1}$$

Crude lipid content determination

2g of dried samples were weighed into porous thimble and its mouth plugged with cotton. The thimble was placed in an extraction chamber, which was suspended above weighed into receiving flask containing petroleum ether (B.P 40-60°C) and below a condenser. The flask was heated on heating mantle for eight hours to extract the crude lipid. After the extraction, the thimble was removed from the Soxhlet and the apparatus reassembled and heated over water bath for the solvent recovery. The flask containing the crude lipid was disconnected, cleaned with dry cloth, oven dried at 100°C for 30 minutes, cooled in a dessicator and weighed. The difference in weight is expressed as percentage crude lipid content.

Crude fibre content estimation

Crude fibre was estimated by acid-base digestion with 1.25% H₂SO₄ (w/v) and 1.25%NaOH (w/v) solutions residue after crude lipid extraction was put into a 60 cm³ beaker and 200 cm³ of boiling 1.25% H₂SO₄ added. The content was boiled for 30 minutes, cooled, filtered through a filterpaper and residue washed with three 50 cm³ portions of boiling water. The drained residue was returned to the original beaker and 200 cm³ of boiling 1.25% NaOH added. The content was boiled for 30 minutes, filtered, washed as above, residue drained. Calculation involved was as thus;

$$\% \text{ Crude fibre} = \frac{X_2 - X_3}{X_1} \times \frac{100}{1}$$

Where; X₁= Weight of sample, X₂= Weight of dried sample and, X₃= Weight of ashed sample.

Determination of total saponins

The method described by Chang *et al.* (2002) [4] was used for the determination of total saponins. The samples were ground and 20g of each were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous

layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After the evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

Determination of total flavonoid content (TFC)

The flavonoid was determined using the method described by Yakubu *et al.* (2019) [19,20]. About 10g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Determination of total tannins

Tannin determination was carried out using the method described by Chang *et al.* (2002) [4]. About 500mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 NHC1 and 0.008 M potassium Ferro-cyanide. The absorbance was measured at 720 nm within 10 min.

Statistical analysis

Data obtained from this study were subjected to basic descriptive statistics. Values of all experimental variables were given as Mean ± Standard deviation.

Results

Proximate composition of *Phyllanthus niruri*

In this present study, proximate composition of *Phyllanthus niruri* leaf extract was investigated. The result showed that *P. niruri* has high content of organic matter (75.71%) and crude fiber (76.53%). The result also revealed low total ash content, recording 24.29%. Lipid and moisture content were found to be 10.9% and 5.6% respectively (figure 2).

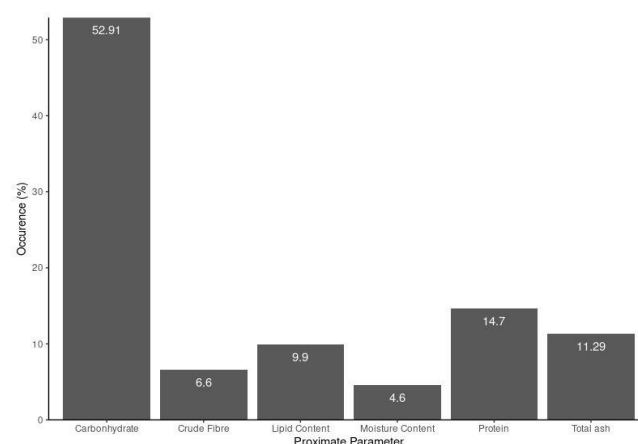


Fig 2: Proximate composition of *Phyllanthus niruri* * Values are Mean ± Standard deviation

Phytochemical screening of *Phyllanthus niruri*

Phytochemical analysis of *P. niruri* revealed the presence of saponins, tannins and glycoside, while steroid and flavonoid proved negative. Saponin was slightly present while

glycoside and tannins were significantly present (Table 1).

Table 1: Phytochemical composition of *Phyllanthus niruri*

Phytochemical	Result
Steroid	-
Saponin	+
Flavonoid	-
Glycoside	++
Tannins	++

*Key: - = absent; + = slightly present; ++ = moderately present

Discussion

In recent years, much interest has been placed on the chemical and pharmacological properties of the plant genus *Phyllanthus* which has long history in folk medicine, especially in traditional Chinese medicine (Pandie *et al.* 2016), where they have been used in the treatment and management of human diseases and hence have been the fundamentals of human medicine (Pisoschi and Negulescu 2012) [15]. Plant-derived medicines have been of great importance to millions of people in many African countries, particularly the poor living in rural and peri-urban areas where these medicinal plants are readily available and easily affordable (Oduola *et al.* 2018) [11]. The plant genus *Phyllanthus* have been used to treat kidney and urinary bladder disturbances, intestinal infections, diabetes and hepatitis B infection (Unofin *et al.* 2017) [18]. A good number of organic compounds such as; alkaloids, tannins, flavonoids, terpenoids, saponins and phenolics, generally known as antioxidants have been isolated from the plant genus *Phyllanthus* and found useful in the production of novel drugs to hinder the growth of fungal pathogens, bacterial and to annul or inhibit Reactive Oxygen Species (ROS) via a number of mechanisms to the host cell (Khan *et al.* 2012) [9]. These reactive oxygen species, which are majorly chemically reactive molecules containing oxygen are formed as natural products of cellular metabolism (Brieger *et al.* 2012) [3].

The result of the phytochemical screening of methanolic extract of *P. niruri* leaves revealed the presence of tannins, saponin, and glycoside. Flavonoid and steroid were absent. Saponin was found to be slightly present whereas glycoside and tannins were moderately present (table 1). This result does not totally agree with the findings of Egbon *et al.* (2017) [18] who reported the presence of flavonoids steroids and other bioactive phytochemicals in his investigation. However the result is in tandem with the report of Chang *et al.* (2002) [4]. The presence of these metabolites suggests great potential for the plant as a source of useful phytomedicines. For instance, the presence of tannins shows that the plant is astringent and helps in wound healing. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine. Tannins are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation (Dailey and Vuong 2015) [6].

Saponins are terpene glycosides. They are one of many secondary metabolites found in natural sources with saponins found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped in terms of phenomenology by the soap-like foaming they produce when shaken in aqueous solutions and in terms of structure, by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene

derivative (Yakubu *et al.* 2021) [22]. Saponins are effective in maintaining liver function, lowering blood cholesterol, preventing peptic ulcer, osteoporosis as well as platelet agglutination (Umoh *et al.* 2013) [17]. The beneficial effects of saponins have been applied commercially in drugs and medicines, emulsifiers, adjuvants, taste modifiers, sweeteners and precursors of hormone synthesis. However, dietary saponins are highly toxic to cold-blooded animals due to its haemolytic property, in which it ruptures erythrocytes and release haemoglobin. Saponins were found to reduce nutrient utilization and conversion efficiency as in ruminants (Egbon *et al.* 2017) [8].

The result for proximate composition of *P. niruri* revealed percentage values of moisture content, total ash, crude fibre, carbohydrate, crude protein and lipid content (figure 2). The result showed that the plant has high content of carbohydrate (52.91%). Crude protein was revealed to be 14.7% while crude fiber recorded 6.6%. Total ash content was 11.29% while lipid and moisture content recorded 9.9% and 4.6% respectively. This result is slightly different from the findings of Egbon *et al.* (2017) [8] who reported a lower value for total ash (9.0 %) and a higher value for moisture content (37.4 %). This result suggests that *P. niruri* is a rich source of carbohydrate. Carbohydrate biomolecules can serve as an interchangeable source of energy for a man. 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 (Dailey and Vuong 2015) [6]. The result also revealed some level of lipids present in the samples. Fat aids absorption of fat-soluble vitamins and are required for growth, immune function and reproduction (Kumar and Goel 2019). The crude protein value obtained signifies the healing properties of the plant which are essential for the synthesis/repair of the study tissue and enzyme.

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 (Unofin *et al.* 2017) [18] while mineral elements are the essential constituents of ash. This study revealed that the plant *P. niruri* provide limited level of moisture which is an advantage over microbial activities. The moisture level is suggestive of potent stability of crude drug against microbial degradation (Kumar and Goel 2019) which could lead to breakdown of important constituents of the drug sample, and microbial growth during the storage of the sample drug. Total ash on the other hand, indexes relative purity: freedom from foreign organic matters, sand, and soil adulterants which could alter the quality of the whole drug (Patel *et al.* 2011) [13]. The results of the present study suggest that *P. niruri* leaves possess tangible nutraceutical value.

Conclusion

The results from this study revealed that *Phyllanthus niruri* leaves contain significant nutritional components and bioactive phytochemicals that are good for human health. It can be concluded that *P. niruri* laves have a good potential for drug development in pharmaceutical industries..

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