Nigerian J. Anim. Sci. 2021, 23 (3): 108-115

Assessment of the nutritive value of *Phyllanthus niruri Linn.* (stonebreaker) leaves

Olufayo, O. O., Tayo, G. O., Olumide, M. D. and Akintunde, A. O.

Department of Agriculture and Industrial Technology, Babcock University, Ilishan-Remo, Ogun State, Nigeria

***Corresponding Author:** olufayo0455@pg.babcock.edu.ng, **Phone Number:** +234 8056246950*

***Target Audience:** Poultry farmers, Researchers and Feed millers.*

Abstract

The leaves of Phyllanthus niruri was evaluated for the proximate, minerals, vitamins and phytochemical composition. Phyllanthus niruri leaves were harvested, air-dried at room temperature for 4 days and milled to form Phyllanthus niruri leaf meal. Atomic absorption spectrometry was used in determination of the leaves for macro (Sodium Na, Calcium Ca, Phosphorus P, Potassium K, Magnesium Mg) and micro (Iron Fe, Zinc Zn, Manganese Mn, Copper Cu) minerals; and phytochemicals (total phenolics, tannin, alkaloid, saponin, phytate, oxalate, flavonoid) were also determined using appropriate techniques. Proximate analysis indicated that the leaves had high concentration of dry matter (91.06%), crude fibre (16.90%), crude protein (14.74 %), ether extract (7.55%), ash (7.33%), neutral detergent fibre (43.81%), acid detergent fibre (25.49%), metabolizable energy (7.74MJ/Kg) and in vitro organic matter digestibility (57.40%). Mineral and vitamin analysis showed that Phyllanthus niruri leaves contained macro minerals (ppm) such as Na (1947), Ca (2911), P (6978), K (18252), Mg (2914), micro minerals (ppm) such as Mn (94), Fe (665), Cu (12), Zn (37) and high content of vitamins A (812.78 µg/100g), D (34.79 µg/100g) and E (57.18 mg/100g) respectively. Phytochemical evaluation revealed that Phyllanthus niruri leaf have high contents of total phenolic (11.85mgGAE/100g), oxalate (22.69mg/g), phytate (5.40g/100g), moderate in alkaloid (3.67g/100g), but low in tannin (0.001g/100g), saponin (0.20g/100g), and flavonoid (1.40g/100g). The results showed that Phyllanthus niruri leaves are of high nutritional quality due to high crude protein, vitamin A, mineral contents especially potassium with the resultant phyto-chemicals attributes that could serve as feed additives in poultry production.

***Key words:** Phyllanthus niruri, nutritive, proximate composition, minerals, phytochemicals*

Description of Problem

Antibiotic feed additives have long been utilized in poultry nutrition as growth enhancers. However, there has been some concern regarding the emergence of antibiotic-resistant microorganisms. As a result of increased consumer demand to minimize the use of antibiotic growth promoters (AGPs) in chicken diets, the animal feed industry is scrambling to identify alternative feed additives (1, 2). Antimicrobial activity is widespread in many plants and their bioactive components (3, 4, 5). Due to their antibacterial qualities, herbs could be utilized as a replacement for AGPs in poultry diet. Herbs and plant extracts have been shown to promote the growth of good bacteria while reducing the activity of harmful bacteria in the gastrointestinal tract (6, 7). Steroids, alkaloids, flavonoids, saponins, phenols, and other bioactive compounds are abundant in herbal plants. The amount of these secondary metabolites varies by plant species, but they serve an

essential function in boosting feed intake and palatability, performance, immune system strength, digestive enzyme stimulation, and stabilizing the eubiosis of intestinal microscopic organisms (8).

Phyllanthus niruri leaf is one of the prospective herbal plants, as it contains various bioactive compounds with high pharmacological activity. *Phyllanthus niruri*'s medicinal efficacy has been widely employed in the treatment of a variety of illness conditions, including liver and kidney ailments, fever, jaundice, prostrate difficulties, and so on (9). *Phyllanthus niruri*, also known as stonebreaker, gale of the wind, or seed-under-leaf, is a 2-foot-tall weed with small leaves arranged in two rows in an alternate pattern. The leaves are membranous, thin, and glaucous beneath their surface, elliptical in form, and have a slender base with two stipules (10).

Human food and animal feed contain complex mixes of phytochemicals as well as several anti-nutritional agents that can impact phytochemical concentrations in the body. As a result, the nutritional makeup and different phytochemicals found in *Phyllanthus niruri* leaves were investigated in this study.

Materials and Methods

Experimental site

The experiment was carried out at the Animal Science Unit Teaching and Research Farm and laboratory of Babcock University Ilishan-Remo, Ogun State, Nigeria situated in the South-Western rainforest belt with annual rainfall of 1500mm and mean daily temperature 0f 28^{0C} within Latitude $6^{0}52'$N and Longitude $3^{0}43'$E .

Sample collection and processing

Phyllanthus niruri plants were obtained near Federal Polytechnic Ilaro in Ogun State. The leaves were carefully removed from the harvested plants, air-dried at room temperature (35^{0}C) for four days, milled, and stored in an airtight container for the assessment of proximate, mineral, vitamin, and phytochemical composition.

Proximate compositions and metabolizable energy of Phyllanthus niruri leaves

The proximate contents of a sample of *Phyllanthus niruri* leaf meal were determined using (11) techniques. An adiabatic bomb calorimeter was used to determine metabolisable energy. The moisture content was determined using the oven dry method, as recommended by (11).

Crude Fibre, a non-digestible part of food sample has two stages of test; acid and alkaline solution using (12) method. The crude protein was determined using the Kjeldahl Nitrogen method described by (12).

Mineral determination

Minerals were analysed by dry ashing the samples to a consistent weight at 550^{o}C and then dissolving the ash in a volumetric flask with distilled, de-ionized water and a few drops of concentrated hydrochloric acid. The concentrations of sodium, calcium, and potassium were measured using a flame photometer using NaCl and KCl as standards. Atomic absorption spectrophotometry was used to determine Fe, Zn, Cu, and Mg using buck 600 AAS. Phosphorus and Manganese were determined using calorimetric method.

Phyto-chemical analysis

Determination of total phenolic

The method given by (13) was followed in determining the total phenol content of *Phyllanthus niruri*. 1g of powdered material was weighed into a flask, followed by 25ml of 70% methanol, which was shaken violently for 10 minutes before being filtered. 0.5 ml of the filtrate was pipetted

into a 50 ml volumetric flask, along with 35 ml of water. 2.5ml of Folin and Ciocalteus reagent was added and swirled to mix and incubate for 2hrs at room temperature. 7.5ml of Na_2CO_3 was added to mark up with distil water and allowed to incubate at room temperature for 2hour. The absorbance was then measured using spectrophotometer at 765mm.

Determination of alkaloids

Alkaloids were determined according to the method of (14). In a 250 ml beaker, 5 grams of the sample were weighed, 200 ml of 10% acetic acid in ethanol was added, and the mixture was covered and left to stand for 4 hours. This was filtered, and the extract was concentrated to one quarter of its original volume in a water bath. Drop by drop, concentrated ammonium hydroxide was added to the extract until it was completely precipitated. After allowing the entire solution to settle, the precipitate was collected and washed with dilute ammonium hydroxide before being filtered. The residue is the alkaloid, which was dried and weighed.

Determination of flavonoids

A 50ml of HCl was added to 2g (W1) *Phyllanthus niruri* leaf meal and boiled for 30 minutes to measure the flavonoid content (14). After cooling, the sample was filtered. To obtain a precipitate, 5ml of the filtrate was pipetted into a new flask and 5ml of ethyl acetate was added in drops. After that, the precipitate was filtered through a pre-weighed filter paper (W2) and dried in an oven at 300^0C. The dried filtered paper was weighed (W_3) and flavonoid content was calculated using: $(W_3 – W_2)/W_1 \times 100$

Determination of tannins content

20ml of 50% methanol was added to 0.2g of powdered *Phyllanthus niruri*. The samples were covered in paraffin and heated in water bath at 800^0C for 1 hour. The samples were allowed to cool before being filtered through Whatman filter paper into a 100ml volumetric flask and then marked off to the 100ml mark. To the sample and the standard (blank), 20 ml distilled water was added, along with 2.5 ml Folin-Dennis reagent. Finally, 10 ml of 17% sodium carbonate solution was added to the sample and standard. The samples were let to sit for 20 minutes to generate a bluish-green coloration. A photospherometer was used to measure the absorbance at 760nm. Tannin was calculated using the formula: abs × average gradient × dilution factor/weight of sample ×1000

Determination of saponins

In a conical flask, 20 g of sample was placed, and 100 ml of 20% ethanol was added. At roughly 55ºC, the sample was heated for 4 hours in a hot water bath with constant stirring. After that, the mixture was filtered, and the residue was extracted using 200 ml of 20% ethyl alcohol. Over a water bath at around 90ºC, the mixed extract was reduced to 40 ml. After that, the concentrate was transferred to a 250 ml separating funnel, and 20 ml of diethyl ether was added to the extract, which is forcefully agitated. The aqueous layer was kept, while the diethyl ether layer was thrown away, and the purification procedure was repeated. 60 ml n-butanol was added, and the combined n-butanol extracts were washed twice with 10 ml sodium chloride (5%). After evaporation, the leftover solution was boiled in a water bath, and the samples were dried in the oven to a consistent weight.

Determination of oxalate

75ml of 1.5N H_2SO_4 was added into100ml conical flask and weighed 1.0g of sample (w1). Using a magnetic stirrer, it was

stirred for 1 hour. Whatman filter paper was used to filter. 25ml of the extract was pipetted into another conical flask. When the extract was heated, 25ml of the heated extract was titrated against a 0.1N $KMnO_4$ solution to a light pink colour. The titre value was recorded. Oxalate = (Titre value x 0.9004mg/g).

Results and Discussion

Plant chemical characteristics have been found to be responsible for the nutritional and therapeutic qualities of plants (15). Table 1 shows the proximate composition of the *P. niruri* leaves. The plant's leaves had greater dry matter content (91.06%). The leaves are high in glucose and energy, protein, crude fibre, moderate fat, and a little amount of fat and ash, according to proximate analysis.

Feed fibres have been demonstrated to aid in the absorption of dietary minerals while also lowering cholesterol absorption. Proteins are required for the synthesis/repair of bodily tissues and as enzymes, and the crude protein content of the leaf (14.74%) exceeded that of most therapeutic plants (16).

Table 1: Proximate composition (g/100g DM) of *Phyllanthus niruri leaf* (PNL)

Nutrients	% Dry matter
Dry matter	91.06
Crude fibre	16.90
Crude protein	14.74
Ether extract	7.55
Ash	7.33
Nitrogen free extract	44.54
Neutral detergent fibre	43.81
Acid detergent fibre	25.49
Metabolizable energy (MJ/Kg)	7.74
In vitro organic matter digestibility	57.40

For the mineral and vitamin contents, the leaf is rich in potassium (K), phosphorus (Ph) and moderate level of calcium (Ca), magnesium (Mg) and sodium (Na); but low in copper (Cu), Iron (Fe), zinc (Zn) and manganese (Mn) (Table 2). The traditional use of the plant to prevent oedema, kidney problems and oliguria (17) may be linked to the high concentrations of potassium, magnesium and phosphate ions in the leaf which may serve the purpose of the maintenance of osmotic pressure, water balance and pH in the body. The level of calcium in the leaf may be adequate for the removal of the anti-nutritional factors (oxalate) and modulate oxalate intoxification by forming complexes with oxalate. Oxalates from plant sources have been known to cause irreversible oxalate nephrosis when ingested in large doses. It is an anti- nutrient and prevents the absorption of some vital nutrients in feed, especially divalent metals (Ca, Mg etc.) and fatty acids by forming salts. Oxalate intoxification (high ingestion of oxalate) causes mal-absorption syndromes leading to steatorrhoea, in which fatty acids are not absorbed, causing formation of insoluble calcium salt of fatty acid (17). Complex formation between calcium and oxalate makes more calcium unavailable, but ensures excretion of oxalates. Presence of minerals such as zinc, iron and magnesium in the leaf may possibly contribute to immunomodulatory action, since these substances have been implicated in immune modulation (18) and may also enhance the

activities of antioxidant enzymes (19), since these elements serve as cofactors for such enzymes and ultimately modulate the immune system (20), thus be an important activity that could be attributed to its neuroprotective property.

Table 2: Mineral and vitamin composition of *Phyllanthus niruri* leaf

Parameters	Concentration
Sodium (ppm)	1947
Calcium (ppm)	2911
Phosphorus (ppm)	6978
Potassium (ppm)	18252
Magnesium (ppm)	2914
Manganese (ppm)	94
Ferric (ppm)	665
Copper (ppm)	12
Zinc (ppm)	37
Vitamin A (µg/100g)	812.78
Vitamin B6 (mg/100g)	0.234
Vitamin D (µg/100g)	34.79
Vitamin E (mg/100g)	57.18
Vitamin K (mg/100g	19.83

The phytochemical content of *Phyllanthus niruri* leaf is presented in Table 3. The plant is rich in alkaloids (3.67g/100g), oxalate (22.69mg/g), total phenolics (11.85mgGAE/100g) but moderate in phytate and flavonoid. This coincides with the report of (17) that alkaloids were the highest in terms of concentration.

Presence of alkaloids, flavonoids, phenols and triterpenes in the plant had been reported (21). The presence of high levels of alkaloids and total phenolics in the leaves of *P. niruri* suggests that the plant may have good blood glucose lowering properties by reducing dietary glucose absorption in the gastrointestinal tract, as well as marked effects on immune system modulation and oxidative stress prevention (22). According to (17), some bitter alkaloids in plants are metabolised in the liver into dimethylxanthine and finally methyl uric acid bycytochrome P450 oxygenase systems which stimulates the expression of tumor necrosis factor (in the endothelia cells of the liver by macrophages), and modulates the immune system. Saponins are expectorants, cough suppressants and administered for hemolytic activities (23). The intake of the leaf could have a positive role in cholesterol metabolism due to the presence of saponin (24). Saponin present in the leaf (0.2g/100g) could result in the permeabilization of plasma membranes due to the bipolar structure of saponins, in which the lipophilic components integrate easily and complex with the lipid fraction of plasma membranes, while the hydrophilic glycosidic portion forms complexes with transmembrane proteins, thereby causing irreversible disorder and disruption of the plasma membrane (25). Tannins, on the other hand, are well-known for their antioxidant and antibacterial characteristics, as well as for providing calming relief, skin regeneration, anti-inflammatory, and dieresis properties (26). Antiviral, antibacterial, anti-parasitic, anti-inflammatory, anti-ulcer, and antioxidant activities have been discovered in tannins (27). The leaf's phenols and flavonoids may aid in avoiding oxidative

stress by scavenging free radicals and bio-activation of carcinogens for excretion in the liver, among other things (28). This suggests that the plant could be beneficial in the treatment of disorders that cause cell harm, such as neuroinflammation caused by free radicals, as well as the prevention of aging.

Table 3: Phytochemical contents of *Phyllanthus niruri* leaf

Parameter	Concentration
Total Phenolics (mgGAE/100g)	11.85
Tannin (g/100g)	0.001
Alkaloid (g/100g)	3.67
Saponin (g/100g)	0.20
Phytate (g/100g)	5.40
Oxalate (mg/g)	22.69
Flavonoid (g/100g)	1.40

Conclusion and Applications

1. The nutritional and phytochemical analysis of the *Phyllanthus niruri* leaf indicated that it is a valuable plant that can be utilized as a food supplement and contains potent bioactive chemicals that can be employed for therapeutic purposes and as precursors for the manufacture of beneficial pharmaceuticals.
2. Furthermore, its powder was discovered to be extremely high in nutritious composition and chemical components required by feed manufacturers.

References

1. Humphrey, B.D., Huang, N. and Klasing, K.C. (2002). Rice expressing lactoferrin and lysozyme has antibiotic–like properties when fed to chicks. *Journal of Nutrition*. 132: 1214 –1218.
2. Botsoglou, N.A., Christaki, E., Florou-Paneri, P., Giannenas, I., Papageorgiou, G. and Spais, A.B. (2004). The effect of a mixture of herbal essential oils or alpha-tocopheryl acetate on performance parameters and oxidation of body lipid in broilers. *South African Journal of Animal Science*. 34: 52– 61.
3. Tucker, L.A. (2002). Plant extracts to maintain poultry performance. *Feed International*. 23 (9), 26-29.
4. Cross, D.E., Svoboda, K., McDevitt, R.M. and Acamovic, T. (2003). The performance of chickens fed diets with and without thyme oil and enzymes. *British Poultry Science* Vol. 44, pp. 18-19.
5. Lewis, K.A., Tzilivakis, J., Green, A., Warner, D.J., Stedman, A. and Naseby, D. (2013). Review of substances/agents that have direct beneficial effect on the environment: mode of action and assessment of efficacy. EFSA supporting publication, EN-440, 182 pp.
6. Langhout, P. (2000). New additives for broiler chickens. *World poultry*, 16: 22–27.
7. Wenk, C. (2000). Why all the discussion about herbs? Biotechnology in the feed industry. Proceeding of Alltech's 16th Annual Symposium. Alltech Technical Publications, Nottingham University, Pres. Nicholasville, KY, 79- 96.
8. Kroismayr, A., Sehm, J., Pfaffl, M., Plitzner, C., Foissy, H., Ettle, T., Schreiner, M., and Windisch, W. (2006). Effects of essential oils or Avilamycin on gut microbiology and blood parameters of weaned piglets. *Journal of*

Land Management Food Environment 59:111–120.
9. Nguyen, H.P., Brian, O.P. and Nguyen, Q.T. (2012). Detoxifying effects of a commercial additive and *Phyllanthus amarus* extracts in pigs fed fumonisins-contaminated feed. *Livestock Research for Rural Development*; 24(6).
10. Oktavidiati, E. (2012). Study of agronomic character of medicinal plants meniran (*Phyllanthus niruri L.* and *Phyllanthus urinaria L*.). Bogor: Bogor Agricultural University; 2012.
11. Association of Analytical Chemistry, A.O.A.C. (2010). Official Methods of Analysis, 18th Edition, Washington, DC. USA.
12. Association of Analytical Chemists, A.O.A.C. (2012). Official Methods of Analysis 19th Ed. Association of the Analytical Chemists, Washington D.C.
13. Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with Phosphomolybdic-phosphotungstic acid reagents. *Amsterdam Journal of Enology and Viticulture* 16, 144-158.
14. Harbone, I.B. (1973). Phytochemical Methods: A guide to modern techniques to plant analysis. Chapman and hall, New York, USA 2nd Edition.
15. Hasan, H.H., Habib, H.I., Gonaid, M.H. and Islam, M. (2011). Comparative phytochemical and antimicrobial investigation of some plants growing in Al Jabal AlAkhdar. *Journal of Natural Products Plant Resources*., 1(1): 15-23.
16. Vanghan, J.G. and Judd, P.A. (2003). The oxford book of health foods: A comprehensive guide to natural remedies. Oxford University Press New York.
17. Oyewo, E.B., Akanji, M.A. and Adekunle, A.S. (2012). Immunomodulation capabilities of aqueous leaf extract of *Phyllanthus amarus* in male Wistar Rats. Report and Opinion; 4(1).
18. Ravalglia, G., Forti, P. and Maioli, F. (2000). Effects of micronutrient status on natural killer cell immune function in healthy free-living subjects age>90years. *American Journal of Clinical Oncology* 3(2): 871.
19. Prasaid, A.S. (2000). Effects of zinc deficiency on Th1 and Th2 cytokinesshift. *Journal of Infectious Diseases.,* 182: 62-68.
20. De la Fuente, M., Hernanz, A. and Vallejo, M.C. (2005). The immune system in the oxidative stress conditions of aging and hypertension: favourable effects of antioxidants and physical exercise Antioxidant Redox Signal, 7: 1356-1366.
21. Saranraj, P. and Sivasakthivelan, P. (2012). Screening of antibacterial activity of the medicinal plant *Phyllanthus amarus* against urinary tract infection causing bacterial pathogens. *Applied Journal of Hygiene*; 1(3): 19-24, DOI: 10.5829/ idosi.ajh.2012.1.3.71111
22. Adelowotan, O., Aibinu, I., Adenipekun, E. and Odugbemi, T. (2008). The in-vitro antimicrobial activity of *Abrus precatorius* (L) fabaceae extract on some clinical isolates. *Nigerian Postgraduate Medical Journal.,* 15: 32-37.
23. Okwu, D.E. (2005). *Phytochemicals*, vitamins and mineral contents of two Nigerian medicinal plants. *International Journal of Molecular Medicine and Advance Sciences*, 372-381.
24. Alli Smith, Y.R. (2009). Determination of chemical composition of *Senna-siamea* (Cassia leaves). *Pakistan Journal of Nutrition*., 8: 119-121.
25. Choi, Y.E., Yang, D.C., Kusano, T. and Sano, H. (2001). Rapid and efficient Agrobacterium mediated genetic transformation by plasmolyzing pretreatment of cotyledon in Panax ginseng. Plant Cell Reports., 20: 616-621.

26. Okwu, D.E. and Okwu, M.E. (2004). Chemical composition of *Spondias mombin* lin plant parts. *Journal of Sustainable Agriculture and Environment*., 6: 140-147.
27. Ndhlala, A.R., Kasiyamhuru, A., Mupure, K., Chitindingu, M., and Benhura, A. (2007). Phenolic composition of *Flacourtia indica*, *Opuntia megacantha* and *Sclerocarya birrea*. *Food Chemistry Journal* 103: 82-87.
28. Khanna, A.K., Rizvi, F. and Chander, R. (2002). Lipid lowering activity of *Phyllanthus niruri* in hyperlipideamia rats. *Journal of Ethanopharmacology*., 82(1): 19-22.