Novelty Journals

ISSN 2394-966X

International Journal of Novel Research in Life Sciences
Vol. 11, Issue 6, pp: (26-34), Month: November - December 2024, Available at: www.noveltyjournals.com

NUTRITIONAL, MINERAL AND PHYTOCHEMICAL COMPOSITIONS OF *PENNISETUM PURPUREUM* (SCHUMACH)

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DOI: https://doi.org/10.5281/zenodo.14565328

Published Date: 28-December-2024

Abstract: **The present study was done on *Pennisetum purpureum* (Schumach) leaf extract to investigate the nutritional, phytochemical screening as mineral composition profile. The study examined the proximate and mineral content such as sodium, potassium, calcium, magnesium, manganese, copper, iron, zinc, cadmium and lead in *Pennisetum purpureum* from Orie Emene Market in Enugu Metropolis. These metals were measured using FAAS - air/acetylene flame. The moisture, ash, fat, fibre, protein and CHO revealed 86.68, 1.80, 1.70, 2.04, 0.00 and 4.78% respectively. Phytochemical screening and assay revealed the presence of bioactive substances. The results of metals showed that the average concentrations detected were 31.00, 0.59, 2.70, 39.50, 0.94, and 0.00, 0.81 mg/kg for Na, K, Ca, Mg, Mn, Cu, Fe, Zn, Cd and Pb, respectively. The abundance of these mineral elements followed the order Mg > Na > Zn > Ca > Mn > Fe > K > Cd, Pb. The levels of these minerals found in this study are compared with permissible limits of FAO/WHO.**

***Keywords: Pennisetum purpureum*, nutritional composition, mineral content, phytochemicals.**

1. INTRODUCTION

Pennisetum purpureum K. Schumach (common names: Elephant Grass, Napier Grass, Uganda Grass) is a robust perennial grass belonging to the family Poaceae (or Gramineal). Elephant Grass is a tropical C4 bunchgrass with a high growth rate and biomass production (Wang *et al.* 2002). The plant has a wide geographic distributional range in the tropics and subtropics. Elephant Grass is usually associated with ecological zones prone to recurrent annual bush fires, particularly in transitions between forest belts and the savannah ecological zones. It is called achara by the Igbo speaking people of South Eastern Nigeria. It is generally used as animal food, an ornamental and for erosion control (USDA, 2008). The dried matured shoots are used for making fences in Northern Nigeria. The matrices of the matured shoots are used for preparing the special soup called 'ofe achara' by the Ngwa and Umuahia people of Abia State in South Eastern Nigeria.

Man depends on plants as source of medicine. From a historical perspective, it is evident that the fascination for plants is as old as mankind itself. The lants are used as food, medicine fuel and fibre through the beginning of civilization of human beings (Bukhah *et al.* 2007). Medicinal plants are the richest bioresources of traditional system of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al.* 2008). About 80 % of the population of developing countries of the world depend upon medicinal plants for primary health care (Bogbam and Kocipal-Abyazan 1994). Medicinal plant are easily available and

less expensive (Catherine and Nagajan, 2011). Most of the medicinal plants are used as raw drugs and they possess beneficial properties (Mahesh and Sathish, 2008).

Pemnisetum purpureum has crude protein and fibre contents. The protein content attributed to its leafy nature (i.e. having abundant leaves as crude protein is more concentrated in the leaves. The fibre content is attributed to its stemy nature particularly at the end of growing period. The presence of calcium and sodium in high quantities has also been reported in this biomass. The high calcium, sodium and magnesium content was thought to be responsible for its high crude fibre content as calcium forms structural components of the cell was in plants. In addition to these minerals, Ziblim *et al.* (2012) reported the presence of potassium, phosphorus, and magnesium, and that seasonal change does not significantly charge the levels of calcium and magnesium.

2. MATERIALS AND METHODS

Sources of Raw Material

Samples of fresh young shoots of *Pennisetum purpureum* were purchased from retailers in Enugu, Enugu State, Nigeria. The collected samples were taken to the Chemical Analysis Laboratory of Projects Development Institute (PRODA) Enugu. The collected samples were identified at the University of Nigeria, Nsukka, Nigeria. After ridding them of dirt, their outer, hard and fibrous portions were removed and discarded, while the inner fresh, tender and edible portion was retained. These were divided into four portions; the first portion was used immediately for proximate analysis, the second portion was used for mineral determination, the third portion was for phytochemical and screening/qualitative analysis while the last portion was used for quantitative analysis of the phytochemicals. Prior to the quantitative analysis, the last portion was air dried to a considerable weight and ground into powders which was then packed into dark polyethylene bags and stored in a desiccator for subsequent uses in the phytochemical analysis (qualitative and quantitative).

Determination of nutritional composition

Proximate analysis of samples for moisture, crude protein, fat, ash, fiber and total carbohydrate contents were carried out in triplicates according to standard methods (AOAC, 2011).

Moisture Content

A Sartorius moisture analyzer MA-300000V3 (Gottingen, Germany) was used to determine the moisture content of the raw material, flours, and blends following the procedure of the instruction manual. The moisture analyzer was warmed up for at least 30 minutes; approximately 2 gram of sample was evenly spread on the tarred aluminum pan. Analysis was performed in the fully automated mode at 105^{o}C.

Total Ash

Ash determination was carried out according to AOAC (2011) procedure. Two grams of sample was placed in silica dish had been ignited for 6 h, cooled in a dessicator and weighed. The dish and sample was ignited first gently and then at 550 ^{o}C in a muffle furnace (Thermotec TIC-400) for 3 h, until a white or grey ash was obtained, the dish and content was cooled in a desiccator and weighed.

Fat content

The fat content was determined according to AOAC (2011) using Soxhlet extraction method. A 500 mL capacity round bottom flask was filled with 250 mL n-hexane and fixed to the Soxhlet extractor. Five grams of sample was placed in a labeled thimble. The extractor thimble was sealed with cotton wool. Heat was applied to reflux the apparatus for three hours. The thimble was removed with care. The n-hexane was recovered for reuse through distillation. The flask was dried at 70 ^{o}C for 15 minutes in a heating mantle, cooled in a desiccator and weighed.

Crude protein

Crude protein was determined using the Kjeldahl method (AOAC, 2011). One gram of sample was digested in a 50 mL micro Kjeldahl flask containing 10 grams of anhydrous sodium sulphate (Na_2SO_4), one gram of copper sulphate, and 20

mL of concentrated sulphuric acid in a fume chamber until a bluish green clear solution was obtained. The digested sample was allowed to cool at room temperature and then transferred into 250 mL volumetric flask and made up to volume with distilled water. The distillation apparatus was set up and 100 mL of 4% boric acid solution with few drops of methyl red indicator was introduced into a distillate collector. The conical flask was placed under the condenser. Then 5 mL of the sample digested was pipetted into the apparatus and washed down with distilled water. 40 mL of 60% NaOH solution was added to the digest. The sample was heated until 200 mL of distillate was collected in the receiving flask. The content of the flask was titrated with 0.1N H_2SO_4 to a pink colored end point. A blank with filter paper was subjected to the same procedure.

Crude fiber

Crude fiber was determined using the method of AOAC (2011). One (1) gram of the defatted sample was weighed into a 250 mL conical flask. Then 200 mL of 1.25% sulphuric acid and few drops of anti-foaming agent was added to the flask. The flask was placed on digestion apparatus with readjusted hot plate and boiled for 30 minutes, rotating flask periodically to keep solid from adhering on the sides of the flask. At the end of 30 minutes period, the mixture was allowed to stand for one minute and then filtered through a Buchner funnel. Without breaking suction, the insoluble matter was washed with boiling water until it was free of the acid. The residue was washed back into the original flask with 200 mL of 1.25% sodium hydroxide solution. It was boiled again briskly for 30 minutes with similar precautions as before. After boiling for 30 minutes, it was allowed to stand for one minute and then filtered immediately under suction. The residue was washed with boiling water, followed by 1% hydrochloric acid and finally with boiling water until it was free of acid. It was washed twice with alcohol and then with ether three times. The residue was transferred into ashing dish and dried at 100 ^oC to a constant weight. Ashing was done at 600 ^oC for 6 h, cooled in a desiccator and weighed. The difference between oven dry weight and the weight after incineration was taken as the fiber content of the sample. This was expressed as a percentage mass of the original sample taken for analysis.

Carbohydrate content

This was determined by difference method

Phytochemical Screening

Qualitative analysis of the plant extracts was carried out using standard phytochemical method as described by Harborne (1973)

Extraction of the plant material

The shoot was extracted with the following solvents: (n-hexane, butanol, acetone, water and ethanol). Exactly 50 gram of the sample was used for each solvent. 100 mL of each solvent (n-hexane, butamol, acetone, water and ethanol) was used against 50 g of the sample. The mixture was placed in a vibrator shaker at room temperature for 3hrs at 400 rpm. The volume of the extract was reduced to about 50 mL using the procedure below:

Recovery of Extracted mixture

Each extract was filtered through Whatman no. 1 filter paper into a washed, oven-dried and pre-weighed 250 cm^3 round bottom flask. This was then mounted to the rotary evaporator which is attached to a receiving flask and condenser, and carefully inserted into waterbath which is then activated and solvent distillation carried on until the volume reduce to about 50mL at the end. The extract was transferred into another washed, oven-dried and weighed basin. The remaining solvent was allowed to evaporate in the fume chamber and the extract was then cooled at room temperature and re-weighed.

Phytochemical Screening Test

The extracts were separated based on the solvent used; and tested for the presence of alkaloids, flavonoids, tannins, glycosides, steroids, polyphenol and saponin and as per the procedures outlined below.

Test for alkaloids

Exactly 1 gram of each extract was weighed and placed into two separate test tubes. To the first test tube, 2 drops of Dragendoff's reagent were added while the second tube contains 2 drops of Meyer's reagent. The development of orange-red precipitate (turbidity) in the first tube (with Dragendoff's reagent) or white precipitate (turbidity) in the second test tube (with Meyer's reagent) were indicative of the presence of alkaloids.

Test for saponins

Five gram of the extract was weighed and placed into a test tube. This was followed by the addition of distilled water. The mixture was vigorously shaken. The appearance of a persistent froth that lasted for 15 minutes was indicative of the presence of saponins.

Test for flavonoids

3mL of extract was added to 10mL of distilled water and the solution was shaken well, 1mL of 10% NaOH solution was added to the mixture. The appearance of yellow coloration was taken to indicate the presence of flavonoids.

Test for steroids

The presence of steroid was detected by the Salkowski's test. Five drops of concentrated H_2SO_4 was added to 1mL of the each extract in a separate test tube. The appearance of red coloration indicate the presence of steroids.

Test for tannins

2 (mL of each extract in a separate test tube were boiled gently for 2minutes and allowed to cool. To each tube 3 drops of ferric chloride solution were added. The appearance of orange coloration was taken to indicate the presence of tannins.

Test for glycosides

(Keller-Killiani test) 0.5gram of extract was dissolved with 5mL distilled water. Exactly 2mL of glacial acetic acid containing 1 drop of ferric chloride solution. This was undrlaid with 1 mL of concentrated sulphuric acid. A brown ring at the interface indicated the presence of glycosides.

Quantitative Analysis

Flavonoid content

Total flavonoid content was determined spectrophotometrically using aluminum chloride ($Alcl_3 6H_2O$) solution and quercetin was used as a reference to produce a standard curve. The total flavonoid content was described by Boham and Kocipai-Abyazan (1994). 5 mL of sample extract was placed in a 50mL volumetric flask containing 20mL of distilled water and 1mL of 5% sodium nitrite was added and mixed. After 5 min, 1 mL of 10 % aluminum chloride solution was added and the mixture is allowed to stand for another 6 mins, after which 5 mL of 1 M sodium hydroxide was added and properly mixed. Absorbance of the reaction mixture was read at 510nm after 15-30 mins, with spectrophotometer. Quercetin (10-750µ/mL) was used to plot a standard curve. Total flavonoid content was expressed in milligram quercetin equivalent per gram of sample mg QE/mc or (mg QE/100mL or per g sample extract). Flavonoid (mg QE/g) = C×V×/M x D.F.

Tannin content

Tannin content was described by Folin-Denis spectrophotometric method. A measured weight of each sample (1.0g) was dispersed in 50ml distilled water and agitated. This was left to stand for 30 min at room temperature, being shaken every 5 min. At the end of the 30 min, it was centrifuged and extract obtained. 2.5ml volumetric flask. Similarly, 2.5ml of standard tannic acid solution was dispersed into a separate 50ml volumetric flask. A 1.0ml Folin-Denis reagent was measured into each flask, followed by 2.5ml of saturated sodiumtrioxocarbonate solution. The mixture was diluted to mark in the flask (50ml), and incubated for 90 min at room temperature. The absorbance was measured at 250nm on a spectrophotometer. Readings were taken with the reagent blank at zero.

The tannin content was given as follows:

Tannin (mg/g) = An×C×Vf×D.F/As×M×Va×1000

Alkaloid content

Alkaloid content was determination was done by gravimetric method as described by Harborne (2001). Exactly one gram of the sample was weighed and dispersed into 50ml 10% acetic acid in ethanol. The mixture was shaken and allowed to stand for 4hours before filtering. The filtrate was exposed to one quarter (¼) of its original volume. Add dropwise conc NH4OH (50-100ml) to precipitate the alkaloids. Filter off the precipitate with a weighed filter paper and wash with 1% NH4OH solution. The filtering should be done with a weighed filter. Dry the precipitate filter paper in the oven at 80˚C for 30 min and reweigh. By weigh difference, the weight of alkaloid is determined and expressed as milligram per gram.

Alkaloids (mg/g) = $M_3 - M_2/M1 \times 1000$

Saponin content

Saponin content was carried using gravimetric method as described by Harborne and Obadoni (2001). Exactly 5gram of sample was weighed into a 250ml conical flask. The mixture was soaked in 100ml of 20% C2H5OH for few minutes, and then it was heated for a period of 3hours at mild temperature of 55˚C. Then it was extracted with another 100ml of 20% ethanol, the extract were pulled together, then the volume of the extract was concentrated to one quarter at 90˚C. The concentrate was then transferred into a 500ml separating funnel 25ml of diethylether was added and shaken vigorously. Two layers were formed and the aqueous layer (upper layer) was discarded. The purification was repeated. Then 60ml n-butanol was added, two layers were formed. The bottom layer was discarded and the upper was recovered, then the combined n-butanol extract was washed with 10ml of 5% aqueous Nacl twice. The lower layer was discarded and upper layer was recovered. The remaining solution was heated in a water bath to dryness and recorded.

Saponin (mg/g) = $M_2 - M_1/M \times 1000$

Cyanogenic glycoside

Cyanogenic glycoside was determined spectrophotometrically as described by Boham and kocipan (1994). 5 gram of the sample was weighed, soaked and agitated in 100ml distilled water for 3hours at room temperature. The mixture was filtered and 1ml of the extract was measured into a 50ml conical flask. 10ml of 10% 2,2 Dinitrosalyeic acid solution/reagent was added. The mixture was boiled in a waterbath at 60˚C for 30 min and later allowed to cool for 15 min. The mixture was then diluted in 50ml volumetric flask and absorbance was taken at 540nm.

Glycoside (mg/g) = Abs × VTe × D.F/Volume analyzed × Mass of sample ×Volume of cuvette used.

Steroid content

Steroid content of the sample was determined as described by Harborne and Obadoni. The mixture was filtered using whatman filter paper No 42. The filtrate was transferred to a separatory funnel. Equal volume of ethylacetate acid was added, mixed and allowed to separate into two layers. The ethylacetate layer (extract) was recovered while the aqueous layer was discarded. The extract was dried at 100˚C for 5 min in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture became turbid and a weighed whatman filter paper No 42 was used to filter the mixture properly. The dried extract was then cooled in a desiccator. The process was repeated two more times and average obtained. The concentration of the steroid was determined and expressed as a percentage.

Steroid (%) = M2−M1/Mass of test portion ×100/1.

Determination of Mineral Composition

A wet digestion method described by AOAC (2011) was adopted. Exactly one gram of the retained portion was digested with 15ml of 2:1 mixture of concentrated nitric and perchloric acid in a 100ml beaker and placed in a waterbath at 65˚C for 1hour. The clear digest obtained after the stipulated time was cooled at room temperature and filtered with whatman No 42. Filter paper. The mixture was made up to mark with deionized water in 100ml standard flask. It was then transferred into sterile sample bottle for elemental analysis using a BUCK 210 VGP Flame Atomic Absorption Spectrophotometer (FAAS). Analytical grade reagents and deionized water were used in preparing all solutions. Stock solutions containing 1000mg/kg of each element were prepared from salts of Cu, Zn, Mg, Cd, Ca, K, Mn, Fe, Pb, and Na. working standard solutions were

prepared by appropriate dilution of the stock solutions. Blank determination were carried out as described in the instrument manual. The concentrations of the metals in each digested samples were determined with an air acetylene flame. Metal contents were calculated by comparison with standard curves of the respective metals. Hollow cathode lamps having resonance lines at 228.9, 327.4, 279.5, 283,3 and 372 nanometers were used as radiation sources for the determination of Cd, Cu, Mn, Pd and Fe respectively. Others are 422.7, 202.6 203.0, 589.0 and 307.6 nanometers for Ca, Mg, k, Na, and Zn respectively. Lamp intensity and band pass were used according to the manufacturer's recommendation. Acetylene and air flow rates were 5 and 20 l miñ1, respectively, for all elements.

3. RESULTS

The results in Table 1 shows the proximate composition of *Pennisetum purpureum* on wet and dry basis

Table 1: Proximate composition of *Pennisetum purpureum* (%)

Parameter	Wet basis	Dry basis
Moisture	86.68	--
Total ash	1.80	19.50
Crude protein	3.00	25.78
Crude lipid	1.70	13.09
Crude fibre	2.04	10.15
Total Carbohydrate	4.78	31.48

Values are means of triplicate determinations.

The phytochemical screening/ qualitative analysis of the *Pennisetum purpureum* is shown in Table 2.

Table 2: phytochemical screening of *pennisetum purpureum* (Schumach).

Solvent					
Parameter	**Hexane**	**Butanol**	**Acetone**	**Ethanol**	**Water**
Alkaloids	+++	++	++	—	—
Flavonoids	—	++	—	—	—
Tannin	—	—	—	—	—
Cyanogenic	+	+++	+	+++	++
Steroids	—	++	—	—	—
Polyphenol	—	—	—	—	—
Saponin	+++	—	—	+	—

The phytochemical assay of *Pennisetum Purpureum* is presented in Table 3.

Table 3: Quantitative analysis of *Pennisetum Purpureum*

Flavonoid (mg QE/g)	Glycosides (mg/g)	Saponin (mg/g)	Alkaloids (mg/g)	Steroid (mg/g)
0.033	0.026	0.069	0.178	0.034

The mineral composition of *Pennisetum purpureum* is presented in Table 4.

Table 4: Mineral content of P.purpureum (mg/kg)

Na	K	Ca	Mg	Mn	Fe	Zn	Cd	Pb	Cu
31.0 ±1.41	0.59 ±0.02	2.70 ±0.27	39.50 ± 9.19	0.94 ±0.0 00	0.81 ±0.76	11.06 ±0.92	ND	ND	ND

Values are means of triplicate determinations.

4. DISCUSSION

The proximate composition of *Pennisetum Purpureum* is given in Table 1. The result showed that *Pennisetum Purpureum* has high moisture content (86.68%). The moisture content of any food is an index of its water activity (Frazier and Westoff, 1978) and is used as a measure of stability and the susceptibility to microbial contamination (Scott, 1980). This implies that *Pennisetum Purpureum* may have a short shelf-life due to its high moisture content. The high moisture content also signifies that dehydration would increase the relative concentrations of the other food nutrients and improve the shelf life or preservation of purpureum diet/meal.

The protein content of *Pennisetum Purpureum* shown in Table 1 is higher than those reported for Nypa fruiticans fruits and seeds (Osabor *et al.*, 2008), *Boerhavia diffusa and Commelina Nudiflora* (Ujomundu *et al., 2008), Trichosanthes anguina* (Ojiako and Igwe, 2008) and compares with that of *Parkia biglobosa* (Esenwah and Ikenebomeh, 2008) and those of most conventional protein sources (Pyka, 1979). The level of protein proportion can even be increased further by dehydrating the Pennisetum purpureum. Therefore, when Pennisetum purpureum is dehydrated, it can be a good source of protein, which can be used to combat protein deficiency.

The ash content values observed is relatively high composed with the reported values for protein-based foods such as meat, egg and comparable with that of wheat flour (Singh, 2004).

The relative values of the crude fibre, total fat, and total carbohydrate of the investigated *Pennisetum purpureum* can be improved by dehydration technique.

The phytochemical screening revealed that *Pennisetum purpureum* is rich in alkaloids, glycosides, saponin, flavonoids, and steroids in Table 2. Alkanoids, flavonoids and saponins are known to have antimicrobial activity, as well as other physiological activities (Sofowora, 1980; Evans, 2005). In fact, flavonoids have a wide range of biochemical and other biological systems. They possess anti-inflammatory, antioxidant, antiallergic, hepato protective, anti-thrombic, antiviral and anticarcinogenic activities (Middleton *et al.*, 2000).

The concentration of some of the phytochemicals in terms of quantity is given in Table 3. The level of flavonoid in this study is less than the reported values 1.763 mg QE/g by Ojiako and Igwe (2008) for *Trichosanthes anguina*. Flavonoid possesses level toxicity effect even at higher concentration.

The amount of glycoside values was 0.026 mg/g for *Pennisetum purpureum.* This value is lower when compared to the reported value 2.83mg/g by Okaraonye and Ikewuchi (2009). The significance of glycosides are numberous; they possess antioxidant, anti-inflammatory effect; and can be used to treat cancer by inhibiting the growth of cancer cells and inducing apoptosis (cell death).

The saponin content value reported in this study agreed slightly with the reported value (0.850 mg/g) by Okaranoye and Ikewuchi (2009). Saponin reduce the uptake of certain nutrients including glucose and cholesterol at the got through intra-lumenal physicochemical interaction. Hence, it has been reported to have hypocholesterolemic effects (Price *et al.*, 1987) and thus they may aid is lessening the metabolic burden that would have been placed on the liver.

The concentration of alkaloids was highest among the secondary metabolities. The value obtained in this study is in accordance with the reported value 0.203 mg/g by Gimba *et al.* (2019). According to answer.com (2014), alkaloids are basic organic compounds of plant origin, containing combined nitrogen. Alkaloids have physiological effects on human and other animals like cows, goats, horses, sheep etc but their functions in the plants that produce them are poorly understood (Gimba *et al.* 2019).

The steroid content was relatively lower, which is in consonance with the reported value 0.043 mg/g by Gimba *et al.* (2019). Steroids are group of naturally occurring and synthetic lipids, or fat-soluble chemicals with a great diversity of physiological activity (Gimba *et al.* 2019).

The result presented in Table 4 is the summary of the mineral content of purpureum. The table indicates the presence of eight minerals of which magnesium had the highest concentration of 39.5 mg/kg. In comparison to the required daily intake values of this minerals, the amount contained in Napier grass is in accordance with the needed daily intake. An earlier study by Mohammed *et al.* (2015) obtained the calcium content as 4.34 % and the sodium content as 0.27% respectively for the whole stem analysis. Their results are in close consonance with the findings of this present investigation except for sodium content which is significantly high. The calcium and sodium content of Bermuda and Guinea grasses have been determined from studies to be 4.2 and 4.6 mg/kg for calcium and 0.2 and 3.1 mg/kg for sodium respectively from studies by Heuze *et al.* (2015a). It has been observed that grasses in the tropics and subtropics have a deficiency of sodium mineral (Khan *et al.*, 2007). This result implies that the plant is not a recommended source for dietary minerals. Again, this result underscores the non-toxic nature of the plant.

5. CONCLUSION

The study on the nutritional, mineral and phytochemical composition of Napier (*Pennisetum Purpureum)* grass has being investigated. The results of the study revealed that the plant has low level of carbohydrate and sugar. Also, the plant could contribute significantly to the RDA of protein intake in human beings. The mineral contents of *Pennisetum purpureum* are inadequate in meeting the RDA for humans, but the phytochemical profile of the plant suggests that the plant may exhibit some pharmacological activities

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