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Original Article

Comparison of the Proximate and Some Selected Phytochemicals Composition of Fluted Pumpkin (*Telfairia occidentalis*) Leaves and Pods

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The leaves and pods of fluted pumpkin (*Telfairia occidentalis*) were analyzed for their proximate and some phytochemical compositions using standard methods. The results of proximate composition for fluted pumpkin leaves (FPL) and pods (FPP) indicated that carbohydrates, moisture, and crude fibers contents values were 51.41±0.60 and 63.91±1.02%, 85.70±0.66 and 91.21±0.3%, 15.05±0.37 and 22.05±1.47% for FPL and FPP, respectively. The crude fats, crude proteins, and ash contents were 6.67±037 and 3.57±0.81%, 22.97±0.66 and 12.47±0.66%, 10.12±0.31 and 5.74±0.5% for FPL and FPP, respectively. The crude proteins, crude fats and ash contents were higher in leaves in comparison with pods, while the pods showed higher levels of carbohydrates, crude fibers, and moisture contents. Results of phytochemical analysis showed that terpenes were the most abundant of the three determined phytochemicals (terpenes, flavonoids, and phenols) in both leaves and pods (25.19±0.16 and 21.83±0.00 mg/g) while phenols had the lowest value (0.68± 0.31 mg/g and 0.52±0.03 mg/g) for both FPL and FPP, respectively. Therefore, *Telfairia occidentalis* may be considered as a rich source of carbohydrates, crude fibers and proteins as well as terpenes.

Keywords: *Telfairia occidentalis*, fluted pumpkin**,** phytochemical composition

Fluted Pumpkin (*Telfairia occidentals)* is one of the most popular vegetables widely cultivated in Nigeria. It belongs to the genus *Cucurbita* and the family Cucurbitaceae which comprises a wide range of plants that have common characteristics of large leaves, creeping, or climbing systems usually with tendrils, fleshy fruits with many seeds and more or less fibrous root system (1).

Pumpkins represent various species which include *Cucurbita pepo, Cucurbita moshata*, and *Cucurbita maxima.* Pumpkin is a very common vegetable widely consumed by many people not only in Nigeria but all over the world (2). This vegetable is widely consumed in the eastern part of Nigeria and also in the west. Pumpkins are cooked and consumed in many ways and most of it are edible from flesh to the seeds. They are consumed in soups and juices.

Pumpkins are being used as traditional medicine in some countries like China, Argentina,

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and Brazil. In Nigeria it is widely believed that the flesh and seed are very rich in proteins and amino acids which is the reason why many traditionalists would prefer to take or consume it regularly rather than taking blood tonic supplement tablets (3). Pumpkins are also believed to contain anti-oxidant vitamins like carotenoids and tocopherols (Vitamin E) (4). Carotene is believed to reduce skin damages from the sun, and also acts as an anti-inflammatory agent. Alpha carotene is believed to be slowing down the aging process, and is also thought to prevent some forms of cancer (5).

Both the leaves and seeds are used as vegetables. The succulent and tender leaves and the immature seeds are cooked and consumed as vegetables. The pumpkin leaves may be cooked with grounded melon seeds (*Citullus lanatus*) for consumption, and could also be taken alone (6, 7). Sometimes they are mixed with locust bean *(Gnetum afvricanum*) and *Pterocarpus soyauxii.* They may also be cooked with fish, meat and tapioca, and are then eaten with pounded yam, 'eba', 'apu', amala', and etc. that are favorite throughout central and southern Nigeria (1, 8). Sometimes male flowers are picked for consumption together with the shoots and leaves. When the leaves are becoming coarse, they are often mixed with other vegetables such as waterleaf (*Talinum fruticosum*). The immature seeds are shelled and the kernels are boiled for about 30-60 min. This is then added to the soup in ground form (8). Mature seeds are first washed to remove the dye found in the cotyledon. They are less tasty, but are good sources of edible oil. Ground seeds are used for making cakes which are high in proteins, and are suitable as fortifying foods, while the oil is served as cooking oil and for making margarine (9). The oil can also be used as drying oil for paints and varnishes (1). Pregnant women and patients suffering from anemia use the leaf juice to strengthen the blood. Other uses include stems maceration to produce fibers that are used as sponge; the oily seeds of pumpkin have lactating properties and are therefore in high demand by women with young babies; the raw flour shows better water and fat adsorption properties than the oil, hence it is useful in baking and ground meat products; the rind and pulp of the fruit are used as fodder for livestock.

The sliced young leaves mixed with coconut water and salt could be stored in a bottle and used for the treatment of convulsion (10). Also, the leaf extract alone is useful in the management of hypercholesterolemia, liver problems, and impaired immune system (11) but the seeds' oil could result in hyperlipidemia if consumed excessively. Protein energy malnutrition is rarely seen among the dwellers where *Telfairia occidentalis* is consumed in large proportion daily (12). The use of *Telfairia occidentalis* in reproductive and fertility is gaining grounds because it has the potential to regenerate testicular damage and increase spermatogenesis (13). *Telfairia occidentalis* is high in anti-oxidant and free radical scavenger properties, and that may contribute to why many use the leaves' extract in oxidative damage conditions such as cancers, and liver diseases (14). In Nigeria, the fresh leaves are ground and the juice is used as tonic by women that have just given birth; its high iron content assists in the replenishment of lost blood; being used for treatment of anemia, chronic fatigue, and diabetes (15, 16). The blood schizontocidal activity of the root of *Telfairia* is comparable to that of chloroquine (17). The extract also shows inhibitory effect on growth of some bacteria (18). *Telfairia* roots are very poisonous because of their high saponins content and are used to kill rats and mice as rodenticide and ordeal poison (10, 19) the aim of this study was to determine the phytochemical composition of leaves and pods of *Telfairia occidentalis*.

Materials and Methods

Sample collection and preparation

The fluted pumpkin leaves and pods were obtained from Temipemi Street, off Ilesha Road, Ile-Ife, Osun State. The "fluted pumpkin" stems were cut at 50 cm from the tip. Both the stem, the tendrils

and leaves were sliced, and analyzed together as leaves. The "fluted pumpkin" pod was also harvested from the same plant, and sliced. Both were oven dried overnight at 70 ºC, grounded and kept in airtight polyethylene bags for further analysis.

Determination of nitrogen and crude proteins contents

The Kjeldahl method determines the total nitrogen as NH- in the food i.e. true protein N, amino nitrogen, and amide N. This is then converted into protein by multiplying its percentage of nitrogen by an appropriate conversion factor.

0.5 g of the prepared sample was accurately weighed into a kjeldahl digestion flaks; a scoop of the digestion mixture and 20 ml of concentrated sulfuric acid were added. The mixture was placed in the electro thermal digestion heater for about 2 h until a clear solution was obtained. After digestion, the flasks were removed from the digester, cooled and diluted with water and made up to 50 ml. 50 ml 2% boric acid (plus indicator) was placed in a 250 ml Erlenmeyer flask. The flask was placed under the receiving tube of the distillation unit in such a way that the end of the tube was below the level of the boric acid. 50 ml of 40% NaOH was added carefully to the 20 ml diluted digested sample, and then attached to the still. The heat was tuned on low and the flasks were swirled to mix the contents. The distillation proceeded until the total contents of the Erlenmeyer flasks were about 150 ml. The distillate was titrated with standard HCI until the blue color disappeared.

Equation for the reactions

$2CNH_3+O_2+2H_2SO_4 \rightarrow (NH_4)_2SO_4 + 2CO_2 + 2SO_2$

$(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + N_{a2}SO_4 + 2H_2O$

$NH_3 + H_3BO_3 \rightarrow NH_4H_2BO_3$

$NH4H2BO3 + HCI \rightarrow H3BO3\ NH4CI$

Determination of crude fiber content

Crude fiber is the part of the sample remaining after the removal of crude proteins, fats, and free nitrogen from the extract. Therefore it is composed of the cellulose, hemi-cellulose, and some materials that encrust the cell walls such as lignin and pectic substances. Crude fiber was obtained by hydrolyzing the fat with diluted acid and then diluted alkali solution.

Two g of the sample was weighed into the 600 ml crude fiber beaker. Then, 200 ml 1.25% sulfuric acid that has just been brought to boiling was added. This was placed on the crude fiber apparatus which has been pre-set to maintain steady boiling. It was refluxed for about 30 min. The solution was filtered on a piece of closed-texture linen. The residue was washed with boiling water until the washings were free from acid. The residue was returned as cleanly as possible (using a tin spatula) to the crude fiber beaker containing 200 ml 1.25% sodium hydroxide solution, which had previously been brought to boil. It was then brought to boil within 1 min, and then refluxed on the crude fiber apparatus for 30 min. The solution was filtered through a Whatman no.4 filter paper. The residue was washed with boiling water, then with 1% HCI, and again with boiling water until free from acid. The residue was then washed with 95% alcohol, and then three times with petroleum ether, using small quantities. The residue was allowed to drain and was transferred cleanly tot a Pyrex beaker. It was dried in the oven overnight at 70 ºC. It was then cooled and weighed. Later, it was ashed at 500 ºC for 3 h, cooled and weighed. The cross in weight was taken as the crude fiber.

Moisture content determination

A known weight of sample was dried to constant weight in an oven, and the loss in weight was equated to be the moisture content of the fluted pumpkin leaf /pod**.**

Two g of the sliced leaf and pod were weighed into the crucible of known weight. The weight of crucible plus the sample was recorded; this was dried in the oven at 70 oC overnight. It was removed and cooled in a desiccator. The weight of the crucible plus ash was recorded and the moisture content was calculated by assuming that the loss in weight of the sample on drying was due to loss of moisture only.

Crude fat determination

In the Soxhlet system of fat estimation, lipids were extracted out of the fluted pumpkin leaf and pod samples by continuous extraction with petroleum ether.

The Soxhlet extractor with reflux condenser and a distillation flask, which has been previously dried and weighed, was set up. Two g of the powdered pumpkin samples were accurately weighed into a fat-free thimble, plugged lightly with cotton wool. It was placed in the extractor, and petroleum ether was added until it siphoned over once. The petroleum ether was added more until the barrel of the extractor was half-full, the condenser was replaced and the joints were tightened and placed on a boiling water bath. The heat was adjusted so that the solvent boiled gently and extraction was then carried out for 4 h. Finally, it was watched until the ether was just short of siphoning over, then the flask was detached and its content siphoned into the stock bottle. It was well drained. The flask was detached; the extractor was cleaned, and then dried in the oven to constant weight.

Ash content determination

The total mineral content of the Ugwu /pod may be estimated as the ash content which is the inorganic residue remaining after the organic matter has been burnt away.

Two g of the sample were accurately weighed into a previously ignited, cooled and weighed crucible. It was heated gently over a Bunsen burner until the sample was charred. The crucible was transferred into a muffle furnace at about 550 ºC, and was left until a white or light grey ash resulted. It was cooled in desiccator and reweighed.

Carbohydrates content determination

Carbohydrates content was obtained by calculation, having estimated all other fractions by proximate analysis.

Total phenolics content determination

The total phenolics content of the extract was determined by the method of Singleton et al. (20).

0.2 ml of the sample extract was mixed with 2.5 ml 10% polyene ciocalteau's reagent and 2 ml 7.5% sodium carbonate (Na_2CO_3).

The reaction mixture was incubated at 45 ºC for 40 min, and the absorbance was measured at 700 nm in a spectrophotometer. Galic acid was used as standard phenol.

Total flavonoids content determination

The total flavonoids content of the sample extract was determined using a colorimetric assay developed by Bao et al. (21).

0.2 ml of the extract was added to 0.3 ml 5% $NANO_3$ at zero time. After 5 min, 0.6 ml 10% $A1C1_3$ was added and after 6 min, 2 ml 1 M NaOH was added to the mixture followed by the addition of 2.1 ml distilled water. Absorbance was read at 510 nm against the reagent blank and flavanoids content was expressed as mg/g.

Total terpenoids content determination

The procedure described by Sofowora was used (22).

0.5 g of finely grounded sample was weighed into a 50 ml conical flask, 20 ml of chloroform/methanol (2/1) was added to the mixture. The mixture was shaken thoroughly, and allowed to stand for 15 min at room temperature. The suspension was centrifuged at 3000 rpm. The supernatant was discarded and the precipitate was rewashed with 20 ml chloroform/methanol (2/1) and then recentrifuged again. The precipitate was dissolved in 40 ml 10% SDS solution.

One ml 0.01 M ferric chloride was added to the solution, and it was allowed to stand for 30 minutes at room temperature before the absorbance was taken at 510 nm. The standard terpenoid (α -terpenol) concentration ranging from zero to 5 mg/ml from the stock solution was used to calibrate the spectrophotometer.

Results

The proximate contents of dried fluted pumpkin pods (FPP) and leaves (FPL) are represented in Table 1. With more than 50% of dried contents, carbohydrates were the most prominent

Table 1. Proximate composition of dried pumpkin leaves and pods (%)

	Ash	Moisture	Crude proteins	Crude fats	Crude fibers	Carbohydrates
Leaves	10.12±0.31	8.79±0.43	22.97±0.66	6.68±0.37	15.05±0.37	51.41±0.60
Pods	5.74±0.50	14.30±0.46	12.47±0.66	3.58±0.82	22.06±1.47	63.91±1.02

Table 2. Phytochemical composition of fluted pumpkin leaves and pods (mg/g)

	Flavonoids	Terpenes	Phenols
Leaves	9.04±0.17	25.19±0.16	0.68±0.31
Pods	6.53± 0.24	21.83±0.00	0.52±0.03

components presents in both pods and leaves, followed by crude fibers (15 and 22%) and crude proteins (22 and 12%)

Table 2 shows the phytochemical compositions of fluted pumpkin leavesFPL and FPP. They both contained more than 20 mg/g terpenes and less than 0.7 mg/g phenols.

Discussion

The FPL had higher percentages of proteins, ash and fats in comparison with FPP while the pods contained higher percentages of moisture, crude fibers and carbohydrates. Therefore, the leaf is more nutritive than the pod. Carbohydrates were the most prominent in both parts, whereas proteins were the second most abundant component in the leaf and crude fibers showed the second highest percentage in the pod.

The considerably high fiber contents of FPL and FPP (15.05% and 22.055%, respectively) indicate that it could serve as a good source of dietary fiber, which may improve bowel functioning and reduce plasma cholesterol (23). The high mean carbohydrates contents (about 51 and 64% in FPL and FPP, respectively) found in the present study is comparable to of the report of Effiong et al. with 46.52± 1.5% content (5). If the recommended daily allowances value for children is 130 g, FPL can provide 36% of the requirement when 100 g dry mass of the leaves are consumed.

The ash content (10.12±0.31%), crude fibers (15.41±0.37%) and crude proteins (22.97±0.66%) were also comparable to that obtained by Effiong et al. (9.68±0.73, 10.36±2.0 and 19.4±1.29%, respectively) (5). Although the ash content is slightly lower than those of bitter leaf (V*eronia colorate)* (15.86%) and *Moringa oleifera* (15.09%), fluted pumpkin could however be a good source of mineral elements.

The leaves' crude proteins content is high and compared favorably with 24% in *Amaranthus vivid's* (24), 20.72% in *Moringa Oleifera* (25). The results indicate that these vegetables can provide more than 12% of their energy from proteins, and are therefore considered good sources of proteins (26). Assuming complete adsorption, fluted pumpkin leaves meet this requirement.

The mean crude fiber content of FPL 15.05±0.5%) was high and compared favorably with those of *Laisanthera Africana* (15.3-18.1% dry mass) (27)and *Heinsia crinata* (13-15% dry mass) and values fall within the range (8.5-20.9%) reported for leafy vegetables by Effiong et al. With the recommended daily allowance dietary fibers for children being 19-25%, FPL could meet these requirements. Intake of dietary fibers can lower the serum cholesterol level, risk of coronary heart disease, hypertension, diabetes and colon and breast cancer (28).

Crude fat contents of FPL and FPP were very low (6.675±0.5% and 3.57± 0.81%, respectively) indicating that these vegetable parts are

advantageous health wise as they could not result in obesity if regularly consumed.

Results also show high mean moisture contents of leaves 85.7± 0.66% and pod 91.21± 0.3% (wet mass). These values are within the reported range (58-93%), in some leafy vegetables, for example, *Amaranthus* (84.0%) and *Talinum* (90.8%) (29) consumed in Nigeria. These results suggest that the bulk of FPL and FPP contain water, implying that these vegetables have low storage capacity and are easily perishable highlighting the problem of conservation in warm climatic conditions. The high water content in vegetables can help enhance food digestion and peristaltic movement on consumption.

The leaf has higher contents of phytochemicals than the pod (30). Of all the phytochemicals, terpenes has the highest percentage (31) followed by the flavanoids, while phenols have the lowest percentages both in the leaves and pods.

Nutritionally, this leafy/pod vegetable may be compared favorably with, and is better than most vegetables consumed in Nigeria. The crude proteins, ash, and crude fats were higher in leaves than in pod. Also, the carbohydrates, crude fibers and moisture contents were higher in pods than in leaves. Therefore, pods should contain more crude fibers which is also responsible for the high carbohydrates content.

Both the pod and leaf contain high levels of phytochemicals although those found in leaves were higher than those of pod.

The results of the present study demonstrate that fluted pumpkin (Te*lfairia occidentalis)* may be consumed daily.

Conflict of interest

The authors declared no conflict of interest.

Reference

1. Grubben, G.J.H. and Denton O.A. Plant Resources of Tropical Africa2 vegetables. PROTA Foundation, Wageningen, Netherlands Backhuys Publishers, Leiden, Netherlands/ CTA, Wageningen Netherlands. 2004.

2. Kim M Y, Kim E J, Kim Y-N, et al. Comparison of the chemical compositions and nutritive values of various pumpkin (Cucurbitaceae) species and parts. Nutr Res Pract. 2012;6:21-7.

3. Martinez M J A, Lazaro R M, Olmo L M B, et al. Anti-infectious activity in the anthemideae tribe. In: Atta-ur- (Ed.) Studies in Natural Products Chemistry. Elsevier. 2008;35:445-516.

4. Gayatri N, Mruntyanjay S, Rajani K. Antioxidant Potential and Nutritional Values of Vegetables: A Review. Research Journal of Medicinal Plants. 2014;8:50-81.

5. Effiong G, Ogban P, Ibia T, et al. Evaluation of Nutrientsupplying Potentials of Fluted Pumpkin (Telfairia occidentalis, Hook, F.) and Okra (Abelmoschus esculentus)(L.) Moench. Academic Journal of Plant Sciences. 2009;2:209-14.

6. Jacob A, Etong D, Tijjani A. Proximate, mineral and anti-nutritional compositions of melon (Citrullus lanatus) seeds. British J Res. 2015;2:142-51.

7. Oluba O M, Ogunlowo Y R, Ojieh G C, et al. Physicochemical Properties and Fatty Acid Composition of Citrullus lanatus (Egusi Melon) Seed Oil. J Biol Sci. 2008;8:814-7.

8. Schippers, R R. African Indigenous Vegetables, an Overview of the Cultivated Species 2002. Revised edition on CD-ROM. National Resources International Limited Aylesford, United Kingdom. 2002.

9. Firn R.. Nature's Chemicals. Oxford University Press, Oxford. 2010;74-75.

10. Oyewole O A and Abalaka M E. Antimicrobial Activities of Telfairia occidentalis (fluted pumpkins) Leaf Extract against Selected Intestinal Pathogens. Journal of Health Science. 2014;2:1-4.

11. Eseyin A O, Igboasoiyi C A, Mbagwu H, et al. Studies on the effects of an alcohol extract of the leaves of telfairia occidentialis on alloxan induced diabetic rats. Global Journal of Pure and Applied Sciences. 2005;11:77-9.

12. Obinaju L C, Asa, U A. Economic Analysis of Vegetable (Telfairia Occidentalis Hook F.) Production among Farming Households In Ibiono Ibom Local Government Area Of Akwa Ibom State, Nigeria. European Journal of Agriculture and Forestry Research. 2015;3:17-24.

13. Nwangwa E, Mordi J, Ebeye O, et al. Testicular regenerative effects induced by the extract of Telfairia occidentalis in rats. Caderno de Pesquisa, sér Bio. 2007;19:27-35.

14. Puupponen-Pimia R, Nohynek L, Ammann S, et al. Enzyme-assisted processing increases antimicrobial and antioxidant

activity of bilberry. J Agric Food Chem. 2008;56:681-8.

15. Dina O A, Adedapo O A, Oyinloye A A, et al. Effect of Telfairia occidentals. Extract on experimentally induced anaemia in domestic. Afr J Biomed Res. 2006;3:181-8.

16. Alada A R A. The haematologic effect of Telfairiai occidentalis in diet preparation. Afr J Biomed Res. 2000;3:185-6.

17. Okokon J E, Ekpo A J, Eseyin O A. Antiplasmodial activity of ethanolic root extract of Telfairia occidentalis. Res.J Parasitol. 2007;2:94-8.

18. Oboh G, Nwanna E E, Elusiyan C A. Antioxidant and antmicorbial properties of Telfairia Occidentalis (fluted pumpkin) leaf extract. J Toxicol Pharmacol. 2006;1:167-75.

19. Kayode A and Kayode O. Some medicinal values of Telfairia occidentalis: A review. Am J Biochem Mol Biol. 2011;1:30-8.

20. Singleton V L, Orthofer R, Lamuela-Raventós R M. (14) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in Methods in Enzymology. Academic Press. 1999;299:152-178.

21. Bao J, Cai Y, Sun M, et al. Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (Myrica rubra) extracts and their color properties and stability. J Agric Food Chem. 2005;53:2327-32.

22. Sofowora, A. Medicinal Plants and Traditional Medicines in Africa. Chichester Sohn, Willey and Sons, New York. 1995;256.

23. Lattimer J M and Haub M D. Effects of dietary fiber and its components on metabolic health. Nutrients. 2010;2:1266-89.

24. Andini R, Yoshida S, Ohsawa R. Variation in Protein Content and Amino Acids in the Leaves of Grain, Vegetable and Weedy Types of Amaranths. Agronomy. 2013;3:391-403.

25. Lockett C T, Calvert C C, Grivetti L E. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria. Int J Food Sci Nutr. 2000;51:195-208.

26. Stephane-Joel E E, Nestor K K, Camille A K, et al. Nutritional Composition of Five Spontaneous Wild Plants Used as Human Foods in Côte d'ivoire Areas (West Africa), a Potential Role in Household Food Security. Pak J Nutr. 2018;17:171-8.

27. Levakhin G, Duskaev G, Dusaeva H. Assessment of Chemical Composition of Grain Crops Depending on Vegetative Stage for Feeding. Asian J Crop Sci. 2015;7:207.

28. Ramula P and Rao P U. Dietary fibre content of fruits and leafy vegetables. Nutr News. 2003;24:1-6.

29. Otitoju G T O, Ene-Obong H N, Otitoju O. Macro and Micro-nutrient Composition of Some Indigenous Green Leafy Vegetables in South-East Zone Nigeria. J Food Process Technol. 2014;5:389-400.

30. Ogbonna P C and Idumah M C. Phytochemical and Mineral Content in Leaves, Stem and Bark of Pterocarpus Santalinoides (Nturukpa) from Afikpo, Ebonyi State, Nigeria. J Appl Sci Environ Manage. 2018;22:1147–50.

31. Agbafor K N and Nwachukwu N. Phytochemical Analysis and Antioxidant Property of Leaf Extracts of Vitex doniana and Mucuna pruriens. Biochem Res Int. 2011;2011:459839.