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Proximate and Phytochemical Compositions of Dried Seeds of Fluted Pumpkin (*Telfairia occidentalis*)

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Authors' contributions

This work was carried out in collaboration between both authors. Author LCC designed the study, wrote the protocol and performed the statistical analysis. Author NCC wrote the first and final draft of the manuscript, managed the analyses of the study, managed the literature searches, carried out the bench work and collated data. Both authors read and approved the final manuscript.

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ABSTRACT

Proximate and phytochemical analyses were carried out on dried fruited pumpkin (*Telfaira occidentalis*) seeds. Standard experimental procedure and statistical analysis using SPSS version 20.1 were carried out after triplicate evaluation of the sample. Results of the proximate analysis indicated that the dried seeds contains moderate percent of carbohydrate (11.43±0.92%) and fibre (15.71±0.74%), high percentage protein (34.56±1.36%) and lipid (32.50±1.08%), ash (4.40±0.02%) and moisture (1.40±0.01%) contents were low statistically. The qualitative and quantitative phytochemical analysis showed that dried seeds of *Telfairia occidentalis* possessed very low (statistically insignificant, $p>0.05$) level of cyanogenic glycoside (0.001±0.01 µg/g) which makes it non toxic and consumable. Tannin (0.488 ± 0.012 µg/g) and oxalate (0.194±0.01 µg/g) where low in percentage, while phylate (3.75±0.018 µg/g) and saponin (4.00±0.02 µg/g) levels were statistically high ($p<0.05$). The analysis revealed the sample as rich in protein and lipid, with moderate amounts of carbohydrate and fibre. It also indicates absence of cynogenic glycoside and

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low oxalate level which interfere with mineral absorption in the body. Hence, the seed is a very good protein source especially for patients with protein energy malnutrition (PEM), growing children and diabetic patients due to its rich energy source for body utilization and for extraction of cooking oil due to its high lipid content.

Keywords: Phytochemical; proximate; analysis; fluted pumpkin; minerals.

1. INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis*) a vegetable of great importance in Nigeria and native of west and central Africa, belongs to the genus Telfairia of the squash family. Its seeds other words regarded as oyster nut, a name it commonly shares with related species- *Telfairia pedata* and *Telfairia batestii* (rare) which are woody, herbaceous dioecious vines bearing squash-like fruits, with rich nutritious oil seeds. Okolie and Mgbeogwu [1], reported that seeds of *Telfairia occidentalis* and *Telfairia pedata* are very rich in protein (25%) and oil (55%), making them a good source of nutrition. Okolie and Mgbeogwu, posited that fermented seed flour of *T. occidentalis* is used by food industries in making baby food, while the leaves and shoot of are cooked and consumed as vegetables. Also, the seeds high protein content confers a supplementary effect on daily protein requirement of the body. Hence, symptoms of PEM (i.e. kwashiorkor and marasmus) were uncommon among dwellers in regions with adequate fruits/seeds and leaves of plants rich in protein such as *Telfairia occidentalis* [2, 3].

Proximate analysis is a parameter used in estimating the relative amount in percentage of protein, lipid, water, fibre, ash and carbohydrate in an organism or sampled food. Parameters like protein, lipid and carbohydrate each contribute to the total energy content of an organism sample while water and ash only contribute to the mass.

Phytochemicals (antinutrients) as chemical compounds that occur naturally in plants, which can interfere with the absorption of some nutrients in humans, examples of such include; oxalate, phylate, cyanides, tannins, saponins, etc. However, some proteins such as trypsin inhibitors and lections found in legumes are partly referred to as antinutrients. These enzyme inhibitors which interfere with digestion is essential as many traditional methods of food preparation such as fermentation, looking and malting increase the nutritive quality of plant foods by reducing the available antinutrients in such foods [4].

The aim of this study is to determine the proximate and phytochemical composition of dried seeds of fluted pumpkin (*Telfairia occidentalis*), hence evaluating its nutritional benefits.

2. MATERIALS AND METHODS

2.1 Materials

All materials and reagents used for this study were of laboratory and analytical standard.

2.2 Methods

2.2.1 Sample preparation

Fresh sample of *Telfairia occidentalis* seed pod was bought from Aluu market in Choba, Port Harcourt, Rivers State, Nigeria.

Upon carefully cutting the pumpkin, the seeds were processed by extracting them from the fruits and the seed coats removed before drying at 50^oC. The dried sample was pulverized using a mechanical grinder stove in a plastic container and preserved in a refrigerator below 4^0C.

2.2.2 Proximate analysis

2.2.2.1 Determination of carbohydrate

The Cleg Anthrone method was used for this determination. The sample (0.1 g) was weighed and transferred into a volumetric flask and 1 ml of distilled water was added. Then 1.3 ml of 62% perchloric acid was added and allowed to mix for 20 minutes using an electric shaker. The content of the flask was then made up to 25 ml with distilled water and the solution was filtered using a suction funnel and filter paper. Afterwards, 1 ml of working solution was pipette into a clean test tube and 5 ml of Anthrone reagent was added. Also 1 ml of distilled water was put into a test tube and 5 ml anthrone was added. Glucose standard solution of 0.1 ml was diluted with 0.9 ml of distilled water and put in a test tube and was treated the same way as the sample. The absorbance of the 3 test tube samples were read

at 630 nm wavelength using the 1 ml distilled water as the blank.

Calculation:

% CHO as glucose = (Absorbance of sample x 25) / Standardglucose

2.2.2.2 Determination of protein

The Kjeldahl method was used for this determination which entails a three (3) stage procedure.

Digestion: The sample (0.1 g) was weighed into a clean conical flask, and 3g of the digestion catalyst (which contains copper sulphate and sodium sulphate in the ratio of 4 g: 1 g) was added. Then 20 ml of concentrated sulphuric acid was added and the sample was heated to digest. The digest upon colour change (from black to sky blue) was cooled and diluted to 100 ml with distilled water.

Distillation: The digest (20 ml) was measured into a distillation flask and the flask kept in place on an eletrothermal hot plate and connected to a condenser which was further connected to a receiver beaker containing 10 ml of 2% boric acid indicator. Then, 40 ml of sodium hydroxide (NaOH) was injected using a syringe into the digest until the digest became strongly alkali. The mixture was heated to boiling and the ammonia gas distilled passed through the condenser into the receiver beaker. The boric acid induced a colour change from purple to green as the introduced ammonia distillate interacted with the boric acid.

Titration: The distillate was then titrated with standard 0.1N hydrochloric acid solution back to purple from green colour, and the volume of hydrochloric acid added to effect this colour change was recorded as litre value.

Calculation:

$$\% \text{ nitrogen (organic)} = \frac{\text{titre value} \times 1.4 \times 100 \times 100}{1000 \times 20 \times 0.1}$$

Where;

1.4 is the nitrogen equivalent to the normality of HCl used in the titration 0.1N 100 is the total volume of digest dilution
100 is percentage factor
1000 is conversion factor from gram to milligram
20 is intergral volume of digests analyzed and distilled
0.1 = weight of the sample digested.

2.2.2.3 Determination of lipid

The soxhlet extraction method was adopted for this determination. In the procedure, 2 g of the sample was applied onto a filter paper, and then placed into a soxhlet extractor. A round bottom distillation flask was dried to a constant weight and the soxhlet extractor attached to the flask. The solvent (acetone) was introduced into the distillation flask through the attached extractor and the set up was held in place on a retort stand and clamp. Cooled water was allowed to flow into the condenser and the heated solvent was refluxed. When the lipid was observably extracted from the sample, the extractor and condenser were removed and the solution containing acetone and lipid was heated slightly to evaporate the acetone, leaving the lipid concentrate. The flask is then dried in an oven and the weight of the flask and lipid extract in it was taken.

Calculation:

% lipid = weight of empty round bottom flask & extract- weight of empty flask / Weight of sample used x 100 / 1

2.2.2.4 Determination of ash

The furnace method as described by AOAC [5] was adopted for this determination. The weight of an empty crucible was recorded and the sample (5.256 g) was weighed. The weighed sample was then placed into the crucible which was subsequently placed into a muffle furnace and set at a temperature at 630°C. The crucible was heated for 3 hours and allowed to cool. After heating, the weight of the crucible and ash were taken to determine the ash content.

Calculation:

% Ash content = weight of crucible ash sample – weight of empty crucible / Weight of sample used × 100/1

2.2.2.5 Determination of moisture

The air-oven method according to AOAC [5] was use for this determination. The fresh fluted pumpkin seed was collected and the weight of the seed was determined. After determination, the seed was put into an oven and allowed to dry

to a constant weight. After drying, the weight of the dry seed was obtained.

Calculation:

Moisture content (%) = weight of fresh sample – weight of dried sample / Weight of sample.

2.2.2.6 Determination of fibre

The mathematical method was employed for fibre determination. The fiber content is calculated by difference.

Calculation:

Fibre content (%) = 100 – (carbohydrate + protein + lipid + moisture + ash content).

2.2.3 Phytochemical analysis

2.2.3.1 Determination of tannin

The sample (0.1 g) was put in a flat bottom flask and 100 ml of distilled water added. The sample was boiled for 1 hour for the water solution to reduce by 50% (50 ml) after boiling. The reduced solution was made up to 100 ml by adding 50 ml of distilled water.

Then 0.1 ml of the sample solution was put in a test tube and 0.25 ml of the Folin Dennis Reagent was added. The blank which was distilled water (0.1ml) was put into a test tube and the Folin Dennis Reagent was added, also 1 ml of 17% Sodium Carbonate was added to the sample and the blank. The sample solution and blank was made up to 50 ml by adding distilled water. The purpose of the blank is to determine the tannic acid present. The tannic acid present changes the sample solution to bluish-green. The absorbance of the sample and the blank were read.

Calculation:

$$\text{Tannin } (\mu g/g) = \frac{\text{C (mg) 0 x Extract volume}}{10 \times 1 \times 0.1}$$

2.2.3.2 Determination of phylate and oxalate

The determination of phylate and oxalate was done according to the method of Sofowora [6] with slight modification. The sample (2.5 g) was inserted into a measuring cylinder and 5 ml acetic was added. The sample solution was made up to 100 ml by adding ethanol. The resulting solution was allowed to stand for 4 hours. The solution was then filtered using Whatman filter paper.

The filtrate was heated to dryness and ammonium solution added, the resulting solution was filtered using a previously weighed Whatman filter paper.

The residue in the filter paper was then dried and the resulting weight of the filter paper and sample was taken.

Alkaloid (µg/g) = weight of filter paper + extract – weight of empty paper / weight of sample used

2.2.3.3 Determination of saponin

The weight of an empty round bottom flask was taken after the flask was properly dried, and 2 g of the dried fluted pumpkin seed sample was inserted into a filter paper and was stapled and inserted into soxhlet extractor. The solvent was acetone just as in lipid extraction. After the lipid extraction using acetone, extraction using methanol took place. The mixture of the saponin and methanol was distilled to recover the methanol. Afterwards, the flask containing the saponin extract was put inside an oven and allowed to dry properly. The weight of the flask and the extract was then taken.

Saponin (µg/g) = weight of bottom flask & extract – weight of empty round bottom flask / weight of sample used

2.2.3.4 Determination of cyanogenic glycoside

A measured amount (5 g) of the sample was weighed into a clean distillation flask, 20 ml of distilled water was added and the sample allowed to stand overnight for hydrolysis to occur. The sample was distilled into 20 ml sodium hydroxide (NaOH). The hydrogen cyanide present was passed from the sample through the distillation tube into the mixture of water and sodium hydroxide in a beaker. Ammonium hydroxide buffer was then added into the beaker. Thereafter, 0.2 ml of 5% KI was added and titrated with 0.02 ml silver nitrate ($AgNO_3$). The presence of hydrogen cyanide turns the solution turbid. This method is called Alkaline Titrimetric Method.

Table 1. Proximate and phytochemical (qualitative and quantitative) analysis

Analysis	Dried sample	Dried sample
Carbohydrate	Positive (++)	11.429± 0.92%
Protein	Positive (+++)	34.563± 1.36%
Lipid	Positive (+++)	32.50 ± 1.08%
Ash	Positive (+)	4.40 ± 0.02%
Moisture	Positive (+)	1.40 ± 0.01%
Fibre	Positive (++)	15.708 ± 0.74%
Tannin	Positive (++)	0.488 ± 0.012 µg/g
Phylate	Positive (++)	3.75 ± 0.018 µg/g
Oxalate	Positive (+++)	0.194 ± 0.01 µg/g
Saponin	Positive (+++)	4.00 ± 0.02 µg/g
Cyanogenic glycoside	Negative (-)	Nil µg/g

Results are triplicate determination of mean ± standard deviation (SD).
Key: (+) indicates present
(-) indicates absent

3. RESULTS

The qualitative and quantitative analyses of proximate and phytochemical studies are shown in Table 1. The result of proximate composition indicated a high percentage of protein (34.563± 1.36%) and lipid (32.50 ± 1.08%), with relative amount of carbohydrate (11.429± 0.92%) and fibre (15.708 ± 0.74%). The phytochemical evaluation revealed a significant level of phylate (3.75 ± 0.018 µg/g) and saponin (4.00 ± 0.02 µg/g), with relative amount of tannin (0.488 ± 0.012 µg/g) and oxalate (0.194 ± 0.01 µg/g). There was no trace of cyanogenic glycoside in the sample analyzed as indicated (Table 1).

4. DISCUSSION

The result of proximate analysis of dried fluted pumpkin (*Telfairia occidentalis*) seeds showed that the seeds are highly rich in protein (34.563%), outweighing the value of other nutrients and can be recommended for children suffering from protein energy malnutrition (PEM) such as Kwashiorkor and Marasmus. The sample was also found to contain a high lipid value of 32.50% which can be a good source of cooking oil and soap production, its carbohydrate content of 11.429%, though low makes it a good source of energy for growing children and essential source of nutrition for diabetic patients. The seeds are also a good source of fibre as it contains a considerable amount of fibre (15.708%) which helps in bowel movement; its ash content (4.40%) also shows that it is a good source of minerals. The low level of moisture (1.40%) was as a result of air-oven drying to obtain a dried sample.

The results of the antinutrient analysis showed no value for cyanogenic glycosides, indicating it's absent in the seeds which makes the seeds non-toxic. The absence of cyanogenic glycoside means no production of the hydrogen cyanide gas after consumption, which makes the seeds consumable and non-toxic after proper blanching. This agrees with the report of Bhattacharya [7].

The sample was also found to have high contents of phylate and saponin (4.00 ± 0.02 µg/g and 3.75 ± 0.018 µg/g respectively), with oxalate and tannin having very low values (0.194 ± 0.01 µg/g and 0.488 ± 0.012 µg/g). These antinutrients are found to interfere with the absorption of minerals such as calcium, iron, magnesium, phosphorus, etc, making these minerals unavailable for utilization in metabolic processes. Saponin also found to be present with a value of 3.75 ± 0.018 µg/g serves as antifeedant [8] and to protect the plant against microbes and fungi. Most plant saponin enhances nutrient absorption and acid in animal digestion. Tannins have traditionally been considered as an antinutrient but their beneficial and antinurient properties depend on their chemical structure and dosage.

5. CONCLUSION

In conclusion, *Telfairia occidentalis* dried seeds are found to be highly proteinous with the carbohydrate and lipid contents useful to man as energy source and oil. Tannin found to be present could cause growth depression in rats [9] and in poultry [10]. Due to the high content of phylate and low oxalate, the seeds should be properly cooked before consumption.

This study has examined the primary parameters one needs to look into when considering any substance as having any sort of therapeutic, nutritious or poisonous properties.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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