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Comparative study of the physicochemical properties of male and female flutted pumpkin (*Telfairia occidentalis*)

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Abstract

Fluted pumpkin (*Telfairia occidentalis*) is a dioecious greenish leafy vegetable plant cultivated for its delicious and medicinal purposes in Nigeria. The research was aimed at comparing the phytochemical and physicochemical properties of the male and female fluted pumpkin leaves. Fresh leaves of male and female *T. occidentalis* collected from farm in Lafia, Nasarawa State, Nigeria were air dried and ground for the tests. Qualitative and further quantitative analyses of the leaves were carried out, while proximate analysis were determined using titrimetric methods. The mineral constituents of the male and female plants were determined using atomic absorption spectrophotometer. Results of the research showed that alkaloid, saponin, phenol, and tannins in the male leaf obtained 0.12 g, 0.08 g, 7.45 g, and 0.86 g, while in the female leaf, the values were 0.11 g, 0.12 g, 9.46 g, and 0.87 g respectively. Ash content, crude protein content, and crude fibre content in the female leaf plant were 33.22%, 33.33%, and 12.71% higher than values obtained in the male leaf of the plant. Mineral composition showed that calcium had the highest concentration in the two sexes examined (7.5 Mg/L for male and 8.4 Mg/L for female) followed by sodium with 4.50 Mg/L (male) and 3.25 Mg/L (female), while the least concentration of 0.001 Mg/L was obtained for both the male and female sexes. The amino acid profile of the plants for the two sexes showed that leusine obtained the highest value of 6.20 g/100g protein, while the least value of 0.81 g/100g protein was also obtained in the male leaf. Other amino acid present in the sampled leaves include lysine, valine, and histidine. Vitamin C had the highest concentration of 12.33 Mg/100g (male) and 14.20 Mg/100g (female). Vitamin thiamine, riboflavin, niacin, pyridoxine, and cobalamine obtained values ranging from 0.12-0.64 Mg/100g. Findings showed that the male and female *T. occidentalis* leaves contain an array of important components needed for body development. Differences between the sexes likely resulted from ecological variations, mineral uptake, and genetic factors imposed on the plants by the environment. While the study presented evidences that the female plant leaves contains higher values of components, leaves from both sexes could be employed to supplement feeding needs among impoverished populations.

Keywords: Dioecious, Phytochemicals, Pumpkin, Supplement, Vitamin.

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis*) is a tropical creeping vegetable vine that spread on the ground with large lobbed leaves and long twisting tendrils, cultivated in some parts of Nigeria and Africa [1]. It belongs to the family Oliffieace and the sub-family cucurbitaceae [2]. It is called ubong, ugu, ewekoro and ekumarku Ejashains in Nigeria and Cameroon [1]. It is a perennial; drought tolerant plant with young shoots and leaves that are used in cooking soups, yam and vegetables sauces, and for medicinal purposes [3]. The shrub is a dioecious plant whose sex is not known until after flowering. The female plants has very broad leaves with big stem and usually is more succulent; producing fruits which contain seeds, while the male plants produces only flowers with smaller leaves and tiny stems. The female plants have significantly higher concentrations of protein and fat, while the male plants have higher fibre, ash other anti-nutritive contents [4].

Phytochemicals are naturally occurring biologically active compounds with protective or disease preventing potentials. These vary in plants depending on their growing conditions, varietal differences, age at harvest, extraction methods, storage conditions, and age of the plant [5]. *T. occidentalis* like other leafy vegetables contain considerable levels of anti-nutrients and toxic substances which have negative effect on animal and human health at high concentrations [1]. The leafy vegetable has been widely accepted as a dietary constituent among Nigerians [6]. Akwaowo *et al.* [6] reported that, the older leaves of fluted pumpkin were higher in percentage crude protein, crude fat, ash and crude fibre, while the younger leaves were higher in moisture content and carbohydrate. The older leaves contain 39.4% crude protein in comparison to 22.4% for younger leaves.

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Adequate consumption of leafy vegetables has been reported as an important means of fighting hunger and malnutrition, ensuring food security and generating income for farmers. Fluted pumpkin has been associated with several healing properties for treating and alleviating certain diseases and illnesses. It can also be used for making soaps as well as for preparing local spices known as ogiri or ogili. The seeds, approximately between 10 and 200 are found in gourd depending on the size of the gourds [7]. The leaves are rich in proteins, oil, vitamins, and minerals that are advantageous to human health though it has low crude fibre content but very rich in folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C, and K. The leaves have both medicinal and nutritional values [4, 8, 9], while the young leaves can be sliced and stored in a bottle to which salt and coconut water are added and subsequently used for the treatment of convulsion [10]. The leaf is also useful in the management of hypercholesterolemia, liver problems, and impaired immune defence system, curing heart disease, hypertension and diabetes, and in cases of meningitidis [11]. The aim of the research is to compare the phytochemicals and physicochemical properties of male and female fluted pumpkin leaves.

Materials and Methods

a) Collection and identification plant materials

Fresh leaves of suspected male and female *Telfairia occidentalis* were collected from a farmland in Lafia, Nasarawa State, Nigeria. The plants were identified by Botany Department of Faculty of Science, Federal University Lafia, Nasarawa State, Nigeria.

b) Preparation and treatment of *Telfairia occidentalis* leaves

The leaves were separately washed with water, then rinsed with distilled water and kept for 21 days in the shade to dry, while they were turned every 3 days. The leaves were subsequently ground into powdered form and divided into 250 g portions for the different analysis to be carried out.

c) Quantitative phytochemical screening of the male and female *Telfairia occidentalis*

i) Estimation of alkaloids in leaves

A total of 200 ml 20% acetic acid was added to 5 g ground leaf and covered for 4 hours. The mixture containing the solution was filtered and the volume was reduced to the quarter using water bath. To the filtrate was added NH_4OH drop by drop until precipitate was formed. The whole solution was allowed to settle and the precipitate was collected by filtration and the weight calculated thus:

$$\text{Percentage (\%) alkaloid} = \frac{\text{Weight of residue x 100}}{\text{Weight of sample taken}}$$

ii) Estimation of saponin content in leaves

About 20 g of extract was measured into a conical flask and 200 ml of 20% ethanol added to it and the suspension was heated over hot bath at 55 °C for 12 h with continuous stirring using a magnetic stirrer. The mixture was filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. They combined extract was reduced to to 40 ml of the original size over water bath at 55 °C. The purification process was repeated two more times and 4 g NaCl was added to adjust the pH meter. The solution was taken with 60 ml and 30 ml portion of n-butanol extract and later washed twice with 10 ml 5% aqueous NaCl. The remaining solution was evaporated to dryness in oven to a constant weight. The saponin content was calculated in g/100 as;

$$\text{Percentage (\%) Saponin} = \frac{\text{Weight of residue x 100}}{\text{Weight of sample}}$$

iii) Estimation of flavonoid in leaves

Ten grams of the ground sample was extracted repeatedly and separated with 100 ml 40% aqueous methanol at room temperature then shaken and left for 4 hours. The suspension was later filtered and filtrate transferred into a crucible and evaporated to dryness over water bath, then dried on an electric oven to a constant weight.

The flavonoid content was expressed in percentage as follows.

$$\text{Percentage (\%) flavonoid} = \frac{\text{Weight of residue x 100}}{\text{Weight of sample}}$$

iv) Estimation of tannin content in leaves

About 0.5 g of the sample was weighed into 1000 ml plastic bottle and 500 ml distilled water added and shaken for 1 h on a mechanical shaker. Then, 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1 M iron (III) chloride in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide $K_4(Fe(CN)_6)$. The absorbance was measure in spectrophotometer at 720 nm wavelength within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannic acid to get 100 ppm and measured using the formula below:

$$\text{Tannin content (\%)} = An / As \times C\ 100 / W \times Vf / Vg$$

Where:

An - absorbance of the test sample

As - absorbance of the standard solution

C - Concentration of the standard solution

W - Weight of the sample used

Vf - total volume of extract

Vg - volume of extract analyzed

d) Proximate Analysis of male and female *Telfairia occidentalis*

i) Determination of crude protein content in leaves

The Microkjedahl method described by AOAC [12] for determination of total nitrogen (N) content was used from which protein content of the leaves were determined. Crude protein was estimated by multiplying nitrogen value with conversion factor 6.25 (N x 6.25). Leave samples is weighed in triplicates, and 1 g of the sample is put into Kjedahl flask. Kjedahl salts (Na_2SO_4) is added. Five (5 ml) of the concentrated sulphuric acid is added into each flask, stoppered and swirled. Each flask is placed on Kjedahl digestion rack in a fume chamber and heated for at least 1 h until the solution is turned clear. The digested sample is then allowed to cool sufficiently after which a little amount of distilled water is added down the side of the flask with a wash bottle until reaction occurs. The digested sample is made up to 100 ml with distilled water.

Ten (10) ml of mixed boric acid and methyl red indicator is put into a 50 ml conical flask and placed under the collection spigot of the distillation apparatus. Five (5) ml of 60% sodium hydroxide is added to 5 ml of the digested (100 ml) in the distillation apparatus. The solution is allowed to steam (distilled) for about 5-7 minutes or when the volume of ammonia with the boric acid in the receiver flask measured 50 ml and the solution turned green. The green solution in the conical flask is titrated with 0.01N hydroxide acid until the solution turns grey in colour. Calculation of the percentage (%) crude protein is done using the formula below:

$$\text{Percentage (\%) cruid protein} = \frac{\text{Titre value} \times \text{normality of HCl} \times 14.007 \times 6.25 \times 10}{\text{Weight of sample}}$$

ii) Determination of total lipids

The lipid contents of the leaves are estimated using the modified methods of Pearson [13]. About 2 g of the sample is weighed and put into already weighed extraction cups. The Soctec apparatus was set accordingly and sample is extracted with acetone for 3 h. The solvent free fat in the cup was dried in an air oven for 30 minutes at 80 °C. The cooled cup and content was reweighed.

$$\text{Percentage (\%) fat} = \frac{\text{Weight of extract + cup – weight of cup} \times 100}{\text{Original weight of sample}}$$

iii) Determination of total ash

Ash content is estimated by incinerating known weight of leaf samples in a muffle furnace at 550 - 600 °C using the AOAC [12] procedure. The weights of the crucibles were recorded and 2 g each of the samples were weighed in triplicates. Sample is placed in pre-heated 550 °C furnace overnight. The samples are removed and cooled in a desiccator. Weights of the ashed samples were recorded by using the formula below:-

$$\text{Percentage (\%) ash} = \frac{\text{Weight of ash + crucible – weight of crucible} \times 100}{\text{Weight of original sample}}$$

iv) Determination of crude fibre

The crude fibre contents are determined by the official methods of AOAC [12]. About 2 g of the samples is weighed into 500 ml beaker. The content is boiled for 30 minutes, then filtered through a fluted funnel and washed with boiling water until the washing is no longer acidic. The sample is boiled for 30 minutes with 200 ml sodium hydroxide solution, and filtered with hot water using muslin cloth; then rinsed with one percent (1%) HCl and methylated spirit. The residue obtained is collected and dried in an oven for 30 minutes. The contents is cooled in a dessicator and then weighed. This is taken to the furnace for ashing at 550 °C for 30 minutes. The ashed sample is removed from the furnace after the temperature returned to 200 °C and put into the dessicator and later weighed. The loss in weight between the incinerations was taken as the crude fibre content.

$$\text{Percentage (\%) cruide fibre} = \frac{\text{Weight of residue x 100}}{\text{Weight of sample}}$$

v) Determination of carbohydrate content

This was determined by the difference method. The carbohydrate content was obtained by difference. The percentage of crude protein, crude fibre, fat, ash and moisture was summed. The value obtained was deducted from 100%. The total carbohydrate of each sample represented the difference in value.

CHO = 100 – (% of protein + % of fat + % of ash + % crude fibre + % moisture)

vi) Determination of moisture content of leaf samples

This was determined using the AOAC [12] method. Two grams of each sample was weighed into crucible and put into muffle furnace at 550 °C for 3 h until ash was obtained. The weights of the dishes were recorded and 2 g of each of the samples were weighed in triplicates. They were placed in the oven at 105 °C for 6 h. The samples were removed and cooled in a dessicator. Then the dried samples were weighed.

$$\text{Percentage (\%) moisture} = \frac{\text{Weight of sample – weight of dried sample} \times 100}{\text{Weight of sample}}$$

e) Determination of the concentration of mineral

Six (6) gram of the powdered sample was weighed into a crucible and gently heated over a Bunsen burner until it charred. The charred sample with the crucible was transferred into a lento muffle furnace at about 600 °C and content ashed until grayish white ash was obtained. It was cooled first at room temperature and then in a desiccator. About 5 cm^3 concentrated HCl was added and heated for 5 minutes on a hot plate in a fume cupboard. The mixture was then transfer into a beaker and the crucible washed several times with distilled water. The mixture was made up to 40 cm^3 and boiled for 10 minutes over a bunsen burner. This mixture was then cooled, filtered and rinsed into 100 cm^3 volumetric flask and made up the volume to 100 cm^3 [14]. The solution was prepared in triplicates.

The minerals content were determined using atomic absorption spectrophotometer. Triplicate digestion of samples and blank were carried out to ensure precision. Sodium (Na) and Potassium (K) were analysed by flame atomic emission spectrophotometer. Phosphorus (P) was determined with spectrophotometer at 420 nm using vanadium phosphomolybdate (vanadate) colorimetric method with KH_2PO_4 as the standard [14].

Amino acid analysis

Determination of amino acid content of the male and female plant was according to the modified methods of Vázquez-Ortiz *et al*. [15]. Powdered samples (3 mg) were hydrolysed with HCl 6 M at 150 °C for 6 h, after which the acid was removed by rotary evaporation. Sample was re-suspended in 2 mL sodium citrate buffer pH 2.2. Sample derivation was carried out and HPLC analysis subsequently done.

Vitamins determination

VitaFast® is a microbiological microtiterplate test kit to quantitate, vitamin B1 (thiamine), vitamin B2 (ribofl avine), vitamin B6 (pyridoxine), vitamin B3 (niacin), vitamin B5 (pantothenic acid), vitamin B12 (cyanocobalamin), folic acid and vitamin B7 (biotin). The vitamins are extracted from the plant leave samples and the extract is diluted. In the case of folic acid, the diluted extract and the folic acid assay-medium are pipetted into the wells of a microtiter plate which is coated with *Lactobacillus rhamnosus* (ATCC Nr. 7469). The growth of *Lactobacillus rhamnosus* is dependent on the supply of folic acid. Following the addition of folic acid as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is carried out in the dark at 37 °C for 44 - 48 h. The intensity of metabolism or growth in relation to the extracted folic acid is measured as turbidity and compared to a standard curve. The measurement is done using an ELISA reader at 610 - 630 nm (alternatively at 540 - 550 nm).

RESULTS

Compositional values of phytochemicals in male and female pumpkin leaves

Alkaoids, flavonoid, saponin, phenol, and tannins were obtained from both the male and female *T. occidentalis* leaves in varying quantities. As shown in Table 1, alkaloid quantity in the male leaves was 0.12 g, while in the female, it was 0.11 g. Cardiac glycosides (0.02 g) obtained the least quantity in the male leaf, followed by saponin content of 0.08 g, while phenol content (9.46 g) was the highest in the female leaves followed by 3.13 g for flavonoid.

Quantitative proximate composition of male and female *Telfairia occidentalis*

Ash content in the male fluted pumpkin ranged from 6.05 to 6.00%, while the female ranged from 8.05 to 8.00% (Table 2). Crude protein content was highest in the female (16.01%), while the lowest value of 12.00% was obtained in the male pumpkin leaves. The crude fibre

mean values in the male and female leaves were 8.19% and 9.22% respectively. The Fat content mean values in male and female leaves were 5.25% and 5.20% respectively.

Mineral composition of male and female *T. occidentalis*

The minerals obtained from the male and female plant leaves were the same; calcium, sodium, manganese, zinc and a host of others. Of the eight minerals obtained as shown in Table 3, only lead had the same value of 0.001 mg/L for both the male and female sexes. Calcium, magnesium, iron, and copper all had higher compositional values in the female leave compared to the male, while the male leaves obtained higher quantities of sodium, manganese, and zinc when compared with the female leave.

Amino acid composition in male and female *T. occidentalis*

Ten amino acids were extracted from the male and female leaves of the pumpkin plants (Table 4). Leusine obtained the highest value in the male leaves 6.23 g/100 g protein, while cysteine had the least value of 0.81 g/100 g protein. Values obtained in the female leaves were generally higher when compared with the female leaves except lysine and threonine that were higher in the male plant leaves as shown in Table 4.

Male and female *T. occidentalis* vitamins compositions

Vitamin C obtained the highest values for both the male (12.35 mg/100 g) and female (14.22 mg/100 g) plant leaves respectively as shown in Table 5. Other vitamins obtained were thiamine, riboflavin, niacin, pyridoxine, and cobalamine with riboflavin obtaining the least value of 0.12 in the male pumpkin leaves.

Table 1: Quantitative composition of phytochemicals in male and female *T. occidentalis* leaves

		Male leaf		Female leaf	
		Mean	Range	Mean	Range
S/N	Constituent	Quantity (g)			
1	Alkaloid	0.12	0.11 – 0.13	0.11	0.10 – 0.12
2	Flavonoid	2.87	2.86 – 2.88	3.13	3.12 – 3.14
3	Cardiac glycosides	0.02	0.019 – 0.02	0.036	0.036 – 0.036
4	Saponin	0.08	0.07 – 0.09	0.115	0.11 – 0.12
5	Phenol	7.45	7.4 – 7.5	9.46	9.4 – 9.5
6	Tannins	0.86	0.860 – 0.861	0.871	0.870 – 0.872

Table 2: Quantified proximate composition of fluted pumpkins

S/N	Constituents	Male leaf		Female leaf	
		Mean	Range	Mean	Range
		Quantity (%)			
1	Moisture Content	12.52	12.50 - 12.55	11.74	11.73 - 11.75
2	Ash	6.02	6.05-6.00	8.02	8.05-8.00
3	Crude Protein	12.00	12.01-12.00	16.00	16.01-16.00
4	Crude Fibre	8.18	8.26-8.05	9.22	9.25-9.21
5	Fat Content	5.25	5.26-5.25	5.20	5.21-5.20

Table 3: Mineral composition ranges in male and female *T. occidentalis* leaves

S/N	Element	Male leaf	Female leaf
		Mg/L	
1	Calcium	7.593 - 7.626	8.433 - 8.462
2	Sodium	4.500 - 4.510	3.250 - 3.300
3	Magnesium	2.890 - 2.896	2.938 - 2.945
4	Manganese	2.280	2.536 - 2.550
5	Zinc	0.416 - 0.421	0.327 - 0.332
6	Iron	0.925 - 0.950	1.010 - 1.025
7	Lead	0.001	0.001
8	Copper	0.814	2.261 - 2.288

Table 4: Amino acid values in male and female *T. occidentalis* leaves

	Amino acid	Male pumpkin	Female pumpkin
S/N		g/100g protein	
1	Lysine	4.20 - 4.21	3.95 - 3.97
2	Valine	3.92 - 3.94	4.20 - 4.22
3	Leusine	6.20 - 6.23	6.41 - 6.43
4	Isoleusine	2.85 - 2.87	3.11 - 3.12
5	Tyrosine	4.10 - 4.13	4.21 - 4.22
6	Cysteine	0.81 - 0.83	0.91 - 0.92
7	Threonine	1.80 - 1.82	1.70 - 1.73
8	Methionine	1.20 - 1.21	1.33 - 1.34
9	Phenylalanine	4.31 - 4.33	4.35 - 4.36
10	Histidine	2.05 - 2.06	2.41 - 2.42

Table 5: Male and female *T. occidentalis* vitamins compositions

S/N	Vitamin	Male pumpkin	Female pumpkin
		Mg/100g	
B1	Thiamine	0.61 - 0.63	0.62 - 0.64
B2	Riboflavin	0.12 - 0.63	0.62 - 0.64
B3	Niacin	0.16 - 0.17	0.12 - 0.13
B6	Pyridoxine	0.41 - 0.42	0.38 - 0.40
B12	Cobalamine	0.52 - 0.53	0.51 - 0.52
C		12.33 - 12.35	14.20 - 14.22

DISCUSSION

Male and female *T. occidentalis* contain an array of important phytochemicals as shown in the study. The study agreed with the earlier findings by Arowosegbe *et al*. [11] that the pumpkin contain saponin, alkaloids, and phenol. Findings from literature further presented evidences that flavonoid may inhibit inflammation, tumour growth, and boost production of detoxifying enzymes in the body. In this study, terpenoid an important chemical with the capacity to protect cells from becoming cancerous, strengthen immune function, and limit the production of cancer-related hormones and fight viruses was not present in the samples used in the study. Manian *et al*. [16] reported that tannin present in the pumpkin had the potency to attract xenobiotic compounds in animal blood as they are high molecular weight compounds attracting lower weight foreign substances in the blood. Hussaine *et al*. [17] explained that most of the phytochemicals are secondary metabolites produced in plants, and do not participate in metabolic processes within the plants but are result of metabolic activities in the plant. In the result presented by Ajibade *et al*. [4], the male *T. occidentalis* had higher tannin concentration which disagreed with findings in this result where the female plant leaves had higher values though the difference in value was less than 0.2 g.

This metabolite as reported by Das *et al*. [18] have immune-stimulant, hypocholesterolaemic, and membrane-permeabilising potential in animals and humans. The author added that, it kills protozoans, and impair protein digestion and uptake of vitamins in the gut. Ayoola and Adeyeye [19] described alkaloids as the most effective phytochemical as the metabolite possesses antispasmodic, antibacterial, healing, and antimalarial effects as confirmed by studies conducted by Okwu and Okwu [20], Stray [21], Trease and Evans [22]. Cardiac glycosides is faintly present in the male and female plant leaves, while the female plant has ample content from the results obtained in the study. Cardiac glycosides are triterpenoids with capacity to regulate the contraction of the heart without increasing the demand for oxygen in the heart's muscle [19]. Phenol present in both the sexes of the plants is an important secondary metabolite with antioxidant properties [23]. Antioxidants create protective effects by neutralizing free radicals produced in the course of normal catabolic and anabolic processes within the cells. They act through hindering oxidative damage by bonding free radicals thus inactivating the radicals [24, 25]. Saponin present in the male and female *T. occidentalis* were in high quantities

Reports presented in this research on the type of phytochemical in *T. occidentalis* agreed with Verla *et al*. [26] who reported the presence of alkaloids, flavonoids, phenols, saponins, and tannin in the leaves. Variation and differences observed in phytochemical constituents of the male and female plants might be as a result of age of the leaves used in the study, growing conditions, age at harvest, extraction methods, and environmental factors; as these factors were posited by Anjorin *et al*. [27] and Cragg and David [28] to be responsible for variations that could be recorded in phytochemical composition of plants.

Moisture content of the male and female *T. occidentalis* leaf sampled were generally low when compared with the values obtained by Idris [29] who got 87%. Their respective report contrasted the values presented by Mohd *et al*. [30], Adeyeye and Omolayo [31], and Usunobun and Egharebva [32] who obtained 7.45%, 6.6%, and 10.94% respectively which were closer to values obtained in this study. The low level of moisture obtained might have been caused by the fact that the study was carried out during the dry season when samples were collected. Low levels of moisture in food crops help prevent mould infection and subsequent spoilage. Other factors that might be responsible for moisture content differences in the male and female leaves include application of organic fertilizer [33] and genetic. The ash content of the two sexes compared in the study were within the ranged reported by Mohd *et al*. [30] – 7.73%, Kajihausa *et al*. [33] – 8.19 to 10.75%, Usunobun and Egharebva [32] – 8.31%, and Arowosegbe *et al*. [11] – 7.67 to 10.17%. Adeyeye and Omolayo [31] obtained higher value (12.3%) which disagreed with findings in this study. Ash content reported for female *T. occidentalis* had higher value than those for the male plant which showed that the female plant leaves contained higher amount and content of minerals than their male counterparts [11, 29, 30].

The amount of crude protein in both the male and female pumpkin leaves were similar to results obtained by Arowosegbe *et al*. [11] – 13.70 to 23.82%. Reports from other authors presented far higher values of protein content: Mohd *et al*. [30] – 56%, Adeyeye and Omolayo [31] – 35.4%. Protein is an important part of the diet responsible for growth and replacement of worn-out tissues. Crude fibre content obtained in the study agreed with the values reported in study carried out by Kajihausa *et al*. [33]: 7.65 – 12.18%, and higher than values obtained by Adeniyi *et al*. [34]: 4.22%. Iheanacho and Udebuani [35], Ishida *et al*. [36], Rao and Netwmark [37] posited that crude fibre have the advantage in aiding digestion, reducing high cholesterol levels, reducing high blood pressure, combating diabetes, and breast cancer. Like the ash content, the crude fibre content between the male and female *T. occidentalis* were not significantly different from each other in value.

Values obtained for the fat content between the male and female *T. occidentalis* were very close. The findings in the study agreed with the values reported by Usunobun and Egharebva [32] which was 6.46% and 4.22% by Adeniyi *et al*. [34]. The male and female pumpkin leaves contained low amount of fat making them suitable for reduction of weight. Gordon and Kessel [38] explained that fat food sources reduces cholesterol in animals and human. The female plant obtained higher concentration levels in the work authored by Ajibade *et al*. [4] for protein and fat which agreed with the presented results for this study, and the male plant in the report of the same authors showed that the fiber and ash contents had higher values which disagreed with ash value being higher in the female plant leaves in the result presented in this study.

Calcium, sodium, magnesium, zinc, iron, lead and copper were the minerals extracted from the leaves (male and female). The amount of calcium in the female pumpkin leaves was higher than the values obtained for the male; and contrasted values obtained by Kajihausa *et al*. [33]: 1.74 – 2.42 mg/100g, Idris [29]: 27.48 mg/100g dry matter, while the values agreed with the report of Mohd *et al*. [30]: 75.0 mg/100g dry matter. Calcium is needed for strong skeletal build up and formation. The amount of calcium in the body is determined by the ratio of Phosphorus to Calcium as high amount of phosphorus leads to calcium loss through the urine. The amount of sodium in the female *T. occidentalis* is low when compared to the male leaves, and the findings

in the study agreed with repults presented by Idris [29] who obtained 47.81 mg/100g dry matter. The author and Verla *et al*. [26] posited that element sodium and potassium maintains osmotic balance in body fluids, and regulate the uptake and adsorption of glucose and also enhancing retention of protein. Magnesium and Manganese obtained from both sexes when compared were close.

Amount of manganese in the plant leaves met the recommended dosage prescribed by NRC of 2 -5 mg/day, while magnesium presented lower amounts compared to the 350 mg/day RDA recommended. While manganese activates some enzymes involved in digestion, magnesium on the other hand regulates DNA and RNA synthesis, participate in growth and reproduction reactions and acts as a co-factor to some enzymes [26]. Zinc as explained by Okaka *et al*. [39] and Mohd *et al*. [30] aids normal functioning of the immune system and aiding in healing process in the body. Values obtained in the male plant was slightly higher than in the female plants though far lower than the RDI of 12 -19 mg/day recommended by NRC. The values obtained agreed with studies carried out by Kajihausa *et al*. [33] of 5.41 – 6.68 mg/100 g and Verla *et al*. [26] who obtained 3.15 mg/100 g dry weight though the plant sex was not described.

The results obtained for iron and copper for the two sexes used in the study agreed with the values presented in the works of Adeyeye and Omolayo [31] (Fe: 6.4 mg/100g), and Verla *et al*. [26] with Fe: 7.64 mg/100g and Cu in female *T. occidentalis* being 8.10 mg/100 g. Iron is an important constituent of haemoglobin responsible for the transport of oxygen from one part of the body to another. Values of iron obtained in the pumpkin leaves were lower than the recommended amount of 10 -15 mg/100 g, while for copper amount obtained from the leaves exceeded the recommended daily tolerable intake level. Test obtained presented trace amounts of lead in the two sexes sampled. Findings in the study presented evidences of the abundant member of amino acid in both male and female *T. occidentalis*. Amino acid from other studies have shown to increase weight when supplemented in animal feed [40]. The obtained results showed that the amount of lysine, valine, and histidine agreed with findings in Arowora *et al*. [41] who obtained 3.96%, 4.21%, and 2.42% respectively in their study.

Differences observed between the male and female *T. occidentalis* were not significant as values only showed slight variations which can be attributed to pathological and dietary conditions of growth in the plants (male and female) which encompasses soil condition, fertilizer application [42, 43]. Report presented in the study collaborated the conclusion of Arowora *et al*. [41] that the plant is capable of providing the needed amino acid for growth, replacement, and synthesis in the body. Phenylalanine obtained from the fruit help normal functioning of the central nervous system [41, 44]. The authors also explained that tryptophan, another amino acid present in the male and female *T. occidentalis* aid in the production of serotonin – a neurotransmitter that control sleep and wakefulness. The amino acid was also reported as having the capacity to treat vascular migraine, rheumatoid arthritis and help the central nervous system to function properly.

Of the vitamins found in the leaves of the male and female plants, vitamin C recorded the highest amount. The vitamin is obtained from consumed food substances as the body cells and organs cannot produce it. The vitamin is important in the formation of strong bones and teeth, acts as a non-enzymatic antioxidant with potential to mop and scavenge free radicals in the body. Halliwell [45] and Rekha *et al*. [46] believed the vitamin has the capacity to regenerate other small molecule antioxidants such as α-tocopherol, urate from their respective radical species. Riboflavin (vitamin B2) is an important vitamin needed for freeing locked-up energy in food substances and other metabolism of nutrients. Riboflavin is found as flavin mononucleotide (FMN) and flavin adeninenucleotide (FAD) which are electron transporters within cells. Kajihausa *et al*. [33] and Olaiya and Adebisi [47] obtained 1.86 – 2.27 mg/100 g and 1.73 mg/100 g which were higher than values presented in this study. Vitamin A present in both sexes of the plant is important for the normal functioning of rhodopsin which enhances good eyesight, while thiamine (vitamin B1) values obtained agreed with the report of Kajihausa *et al*. [33] and aid in metabolising glucose. Vitamin B3 (niacin) lowers blood lipids level and provide nicotinamide in the body for normal metabolism.

The many differences noted between the sexes have been reported to be caused by ecological interactions (mutualistic and antagonistic co-evolutionary relationship with animals and microorganisms), plant defences build up, growth, seed predation, and differences in ecological optimal niches [48, 49, 50, 51, 52]. Lloyd and Webb [49], Goldman and Wilson [53], and Obeso [54] in explaining ecological interactions opined that the female plant expend more resources for reproduction, seed development, and gamete production which is likely responsible for higher phytochemicals and mineral composition in them.

CONCLUSION

The findings obtained in this study revealed that the leave (both male and female) *T. occidentalis* is a reservoir of novel compounds, minerals, and vitamins with important advantages for human health and body development. The male and female plant could be supplementary in use for particular metabolites where nutrient content is deficient and vice versa. Differences recorded in the male and female *T. occidentalis* plant leave metabolites' make up resulted from genetic built of the plant, cost of reproduction, ecological interactions, application of manure and fertilizer, soil mineral content and other factors.

Authors' contributions

Author 1 conceived and performed the experiments, while Author 2 designed the experiment, analysed the data, and prepared the manuscript.

Conflict of interest

There is no conflict of interest.

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