

GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2 Cross Ref DOI: 10.30574/gscbps Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Sex-based assessment of nutrient composition in fluted pumpkin (*Telfairia occidentalis* Hook. F)

Dorcas Olubunmi Ibitoye ^{1,*}, Adesike Oladoyin Kolawole ⁴, Olaide Ruth Aderibigbe ², Emmanuel Oluwakayode Ajayi ³ and Ojo Adewale A ¹

¹ Genetics Resources Unit, National Horticultural Research Institute, P.M.B. 5432, Dugbe, Ibadan, Oyo State, Nigeria. ² Product Development Programme, National Horticultural Research Institute, P.M.B. 5432, Dugbe, Ibadan, Oyo State, Nigeria.

³ Vegetable Research Programme, National Horticultural Research Institute, P.M.B. 5432, Dugbe, Ibadan, Oyo State, Nigeria
⁴ Department of Crop Production and Soil Science, Ladoke Akintola University of Technology, Ogbomoso, P. O. Box 22663, UI Post Office, Ibadan, Oyo state, Nigeria.

GSC Biological and Pharmaceutical Sciences, 2023, 24(03), 224–233

Publication history: Received on 03 August 2023; revised on 12 September 2023; accepted on 15 September 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.24.3.0380

Abstract

Fluted pumpkin (*Telfairia occidentalis* Hook. F), is an under-utilized leafy vegetable cultivated and consumed for its valuable nutrients and minerals playing a crucial role in combating micronutrient deficiencies. This shrub is dioecious, exhibiting distinct differences in sexual morphology. The study aims to contrast the phytochemicals, physico-chemical and mineral profiles of the female and male fluted pumpkin leaves. Seedlings of fluted pumpkin were evaluated at National Horticultural Research Institute (NIHORT), Ibadan, Nigeria using a randomized complete block design with three replications. Fresh leaves were harvested at flowering from identified male and female vines for nutrient composition analysis. Beta-carotene, sodium and fibre content were notably higher in female leaves, whereas vitamin C, minerals, and anti-nutrients were more abundant in the male leaves. The concentrations of phenolic, flavonoid, vitamin C, tannin, saponin, and certain minerals emerged as the most influential factors for distinguishing between the sexes. Hence, leaves from both sexes can contribute to an enhanced diet as both sexes harbor valuable genetic traits that could be harnessed for the nutrient improvement of fluted pumpkin varieties.

Keywords: Mineral; Physico-chemical; Phytochemicals; Sex; Ugu leaves

1. Introduction

The tropical fluted pumpkin (*Telfairia occidentalis* Hook. F) is a dioecious and perennial plant belonging to the family of Cucurbitaceae and is characterized by broad lobed greenish leaves commonly referred to as Ugu in Nigeria. The shrub grows into either a male or female plant which can only be identified at onset of flowering. Some morphological characteristics were reported by [1] to be valuable in early sex detection of ugu. While the female plant which produces big fruits containing the seeds has broad and succulent leaves; the male plant only produces flowers and smaller leaves. The stems of the female plant are usually big and strong while that of the male plants are tiny. Farmers usually harvest the male plant for leaves while the female plants are left on the field for productions of seeds for the next planting season. However, consumers prefer the female leaves for culinary uses because of its succulent texture and broadness of the leaves [2]. Majority of the studies conducted on the sex of ugu had been on morphological basis of identification for production and yield ration [1, 3, 4]. 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 [5]. Given

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^{*} Corresponding author: Dorcas Olubunmi Ibitoye; Email: bunmiajisafe@yahoo.com

that the leaves of fluted pumpkin form a significant component of the diet and traditional remedies for Nigeria's population, this research aims to contrast the phytochemicals, physico-chemical and mineral properties of male and female plants. The goal is to provide valuable insights for consumers and offer empirical backing for the deliberate selection of preferred sex characteristics in fluted pumpkin cultivation.

2. Material and methods

2.1. Plant material and evaluation

Single pod of ugu was bought from Ojoo local market in Ibadan, Nigeria. Seeds were collected from the pod and sown in nursery trays filled with sawdust in the screen house at National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. Four weeks after sowing, seedlings were transplanted to the research field in NIHORT (3° 56'E, 7° 33' N; 168 meters above sea level) in 2020 cropping season. The seedlings were planted at a spacing of 1m x 1m using a Randomized Complete Block Design (RCBD) with three replications. At flowering, vines were tagged separately with regards to the sex type expressed. Harvested fresh ugu leaf samples were collected from tagged vines and taken to the Product Development Laboratory of NIHORT for quantitative analyses of mineral composition, phytochemicals and physico-chemical properties.

2.2. Preparation of ugu leaves

The leaves were first sorted to remove any infested parts; they were then washed under running water to remove dirts and drained off in a perforated plastic basket. The leaves were dried using air food dehydrator at 50°C and later ground into powdery form and kept in an airtight container in a refrigerator for further analyses.

2.3. Determination of phytochemicals, physico-chemical, proximate and mineral composition of ugu leaves

Quantitative analysis of phytochemical properties (ferric reducing antioxidant power, phenolic acid, flavonoid, vitamin C, tannin, beta-carotene and saponin), proximate composition (Protein, fat, fibre, moisture, ash and carbohydrate) and mineral composition (sodium, iron, calcium, zinc, potassium, manganese and magnesium) of ugu leaves were investigated using standard analytical methods [6, 7, 8]. All analyses were done in triplicate for each sample at the Product Development Laboratory of NIHORT, Ibadan, Nigeria.

2.4. Data collection and analysis

All recorded data and derivatives were first subjected to analysis of variance (ANOVA) using SAS version 9.4 [9]. Means of male and female ugu leaves were computed and tested with standard error (S.E). The differences in phytochemical and mineral compositions of the male and female ugu leaves were calculated by comparing the mean for each parameter using T test. Stepwise multiple regressions were estimated with sex as the dependent variable using PROC REG in SAS. Pearson correlation coefficients were computed to determine the degree and direction of association among all quantitative traits. PROC PRINCOMP in SAS was used to determine the contribution of each measured trait to the total observed variation based on principal component analysis retaining only principal components having eigen value greater than 1.

3. Results and discussion

3.1. Variation in nutrient composition

Significant variation was observed in the nutrient composition in the male and female ugu leaves (Table 1). The differences seen in the phytochemicals, physico-chemical properties, and mineral composition of ugu leaves in this study are exclusively influenced by the plant's sex. Although, the female ugu attracts higher premium in Nigeria vegetable market because the leaves and the vines are broader and more succulent, it is intriguing to note that the quantities of iron, calcium, potassium, and the measured proximate parameters, with the exception of fiber, are alike in both male and female ugu leaves. This suggests leaves from male and female vines are healthy and nutritious for consumption. In this study, the leaves from the female plants had significantly (P<0.01) higher concentrations of ferric reducing antioxidant power, beta carotene, sodium and fibre while the male leaves had more phenolic, flavonoid, vitamin C, tannin, saponin, zinc, manganese and magnesium, an indication of higher medicinal properties in female leaves. Mineral composition was conducted to establish the nutritional value of leaves from both sexes. The seven minerals evaluated were calcium, sodium, potassium, zinc, iron, manganese and magnesium. Sodium, iron and potassium were higher in the female leaves (Table 2). Protein is important in diet as it is responsible for growth

and replacement of worn-out tissues. The amount of crude protein in both male and female leaves ranged between 18 to 21% (Figure 1) similar to previous reports of [10] and [5]. Notably, the values obtained for iron (9.5-9.9 mg/100g) for leaves from both sexes fall within the recommended dietary allowance (RDA). This further support the indigenous beliefs among many Nigerians that ugu leaves are sources of blood and extracts from the leaves are given to anemic individuals to increase their packed cell volume (PCV) and for prevention and amelioration of sickle cell disease in infants and adults [11], [13], [14] [15]. In a healthy diet, fibre helps to remove harmful cholesterol from the arteries, reducing the risk of heart disease, aid in digestion, combats diabetes [16]. The amount of crude fibre obtained in this study for both male and female ugu leaves (1.9% and 2.2% respectively) were lower to what was reported by [5]: 8.18% and 9.22%. The difference may be due to the genotype of the ugu used in the different studies. Fats is important in diet because they are a source of energy for the body, they protect the body's vital organs and they are required for the absorption of the fat-soluble vitamins A, D, E and K. Figuring out the daily amount that is allowable for maintaining healthy diet can be somewhat confusing. Over the last 50 years, many people have moved from a moderate fat to a lowfat diet following the recommendations from health organizations. The values obtained for fat content in this study is 19.5% for female leaf and 18% for male leaf. Although, the result in this study is higher than the findings of [5]: 5.25, female and 5.2, male, it still falls within the range reported by [17]. Leaves from the female plants had higher values for crude protein and fat content which is similar to the findings of [2] and [5].

Antioxidants aid in the neutralization of free radicals, the primary cause of inflammatory conditions, and thus may try to protect from diseases caused by free radicals. Vitamin C (ascorbic acid) and β -carotene are considered important antioxidants in humans. In vitro studies suggest that they protect against free radical-mediated damage by reducing free oxygen radicals and replenishing antioxidant reserves [12, 18]. The results obtained for vitamin C content in this study was higher for male (14.73%) which disagreed with the findings of [5] who reported higher vitamin C content in the female leaves. β -carotene was higher in the female leaves (20.64mg/100g) than in the male (18.96mg/100g). since there is no RDA for β -carotene, it suggests that consumption of ugu leaves irrespective of the sex will promote healthy lifestyle by providing adequate source for β -carotene.

Phytochemicals are secondary metabolites which are products of metabolic processes but do not participate in metabolic processes in the plants. Plants especially, medicinal plants with high level of important phytochemicals like saponin, phenol, oxalate, tannin, phytate and flavonoids have been reported to have inhibitory activities against inflammation, tumor growth and boost detoxifying enzymes in the body [10], [11]. Flavonoid was higher in the male leaves (210mg/100g) which contradicts the findings of [5] which reported higher flavonoid content rather in the female leaves. Flavonoids have been reported to inhibit inflammation, tumor growth and boost the production of detoxifying enzymes in the body. Tannin and saponin are higher in the male leaves (30.80mg/100g and 4.55% respectively), this agrees with findings of [2] but in in contrast with findings of [5] which reported higher contents in the female leaves. Dietary tannins and saponins have been reported to important in food plants because they lower plasma and cholesterols in many mammals as they reduce risk of coronary heart disease [19], [20]

Minerals help maintain acid-base balance, to keep the body pH neutral. Minerals help regulate body processes, such as in enzyme systems. Minerals function in nerve impulse transmission and muscle contraction. Minerals help release energy from food. Calcium plays an important role in building strong and keeping healthy bones and teeth both early and later in life. The amount of calcium in the male (33.24mg/100g) leaves was higher than the values obtained for the female (Table 2); this finding contrasted the values reported by [17] and [5]. Manganese content in the male leaves was higher (4.66mg/100g) which falls within the RDA range of 2-5mg/day and disagrees with the finding of [5] which reported higher content in the female leaves. Magnesium content was found to be higher in male leaves (10.6mg/100g). This may be linked to the high concentration of chlorophyll A/B also observed in the male leaves since magnesium is a component of chlorophyll. While manganese activates some enzymes involved in digestion, magnesium on the other hand regulates DNA and RNA synthesis, participate in growth and reproduction processes and act as co-factor to some enzymes [21], [22]. The human body needs iron for the formation of oxygen carrying protein haemoglobin and myoglobin. Iron content was higher in the female leaves (9.83mg/100g) which agrees with the findings of [5]. Zinc help to speed up the healing process after an injury. The concentration of zinc in male leaves was higher (0.83mg/100g). Comparing the finding of this study with the RDA of zinc which is 10-19 mg/day, the observed concentration of zinc measured in the leaves of ugu (either male or female) are suggested the variety to be a moderate source zinc.

Source of variation		DPPH (%)		Flavonoid (mg/100g)					Antiox (mg/100g)	Beta ((mg/100g)	C Saponin (%)	Oxalate (%)	Ch A/B
Replicates	2	10.24	8.66	32.84	0.2	8.69*	0.76	6.81	266.72	0.02	0	0	0.34
Sex	1	22.3	308.44*	3008.93**	12.18**	129.76***	136.03*	511.65*	65965.33***	0.52***	0.52***	0.01	31.22***
Error	2	20.87	15.54	27.77	0.12	0.18	2.16	16.94	21797547	0	0	0	1.04
CV		5.67	10.91	2.8	2.65	0.26	5.65	8.47	2.05	0.9	0.33	8.59	6.71
		Na (mg/100g)	-	Ca (mg/100g)	Zn (mg/100g)			Mg (mg/100g)					
Replicates	2	0.02	0.04	1.67	0	116.67	0	0.19					
Sex	1	18.83***	0.12	1.03	0.05**	2204.17	12.13**	3.60*					
Error	2	0.03	0.03	0.37	0	516.67	0	0.14					
CV		1.65	1.65	1.85	3.44	3.5	1.27	3.78					

*, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively; CV = Coefficient of variation, DPPH = 2,2-Diphenyl-1-picrylhydrazyl, Vit. C = Vitamin C, FRAP = Ferric reducing antioxidant power, Antiox = Antioxidant, Beta C = Beta Carotene, Ch A/B = Chlorophyll A/B, Na = Sodium, Fe = Iron, Ca =Calcium, Zn = Zinc, K = Potassium, Mn = Manganese, Mg = Magnesium

	Mean				
Traits	Female	Male	Minimum	Maximum	Difference
DPPH (%)	82.15±3.11	78.30±0.84	75.94	85.45	3.86
Phenolic (mg/100g)	28.96±0.41	43.30±2.81	28.45	47.32	14.34*
Flavonoid (mg/100g)	165.70±1.05	210.50±4.37	163.80	216.50	44.79**
Vit. C (%)	11.88±0.06	14.73±0.32	11.81	15.06	2.85**
FRAP (mg/100g)	168.40±1.25	159.10±1.19	156.80	170.20	9.30**
Tanin (mg/100g)	21.28±0.65	30.80±0.74	19.99	31.68	9.52***
Phytate (mg/100g)	57.81±0.70	39.334±2.72	35.08	59.20	18.47**
Antiox (mg/100g)	614.10±0.64	823.80±12.69	612.80	836.80	209.70***
Beta C (mg/100g)	20.64±0.07	18.96±0.11	18.79	20.78	1.68***
Saponin (%)	3.96±0.01	4.551±0.01	3.95	4.57	0.59***
Oxalate (%)	0.48±0.02	0.38±0.02	0.36	0.50	0.11**
Ch A/B	12.94±0.18	17.50±0.65	12.60	18.75	4.56**
Na (mg/100g)	11.76±0.07	8.22±0.11	8.01	11.88	3.54***
Fe (mg/100g)	9.83±0.13	9.55±0.06	9.47	9.97	0.29
Ca (mg/100g)	32.41±0.42	33.24±0.71	31.71	34.16	0.83
Zn (mg/100g)	0.65±0.01	0.834±0.01	0.64	0.85	0.19***
K (mg/100g)	668.30±8.82	630.00±11.55	610.00	685.00	38.33
Mn (mg/100g)	1.81±0.03	4.66±0.01	1.76	4.67	2.84***
Mg (mg/100g)	9.06±0.15	10.601±0.30	8.78	11.20	1.55**

Table 2 Mean comparison of phytochemicals and mineral compositions of female and male ugu leaves

*, **, *** Significantly different from Male Ugu at the 0.05, 0.01 and 0.001 probability levels, respectively, using t test; DPPH = 2,2-Diphenyl-1picrylhydrazyl, Vit. C = Vitamin C, FRAP = Ferric reducing antioxidant power, Antiox = Antioxidant, Beta C = Beta Carotene, Ch A/B = Chlorophyll A/B, Na = Sodium, Fe = Iron, Ca =Calcium, Zn = Zinc, K = Potassium, Mn = Manganese, Mg = Magnesium

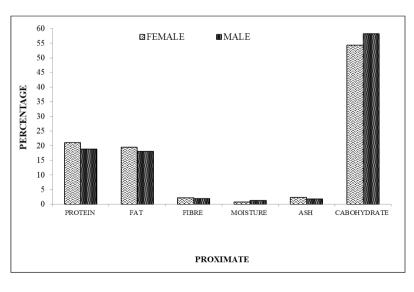


Figure 1 Comparison of percentage proximate composition of the male and female Ugu leaf

3.2. Correlation among traits

Associated traits depending on their strength of relationship can be useful as selection criteria employed in improvement programme [23]. Strong and significant positive or negative correlations were observed among the measured traits (Table 3). Positive and significant correlation (r > 0.8) were recorded between phenolic content and flavonoid, phytate, antioxidant content, manganese and magnesium content while negative and significant correlation (r > 0.8) were recorded between phenolic content and relation (r > 0.8) were recorded between phenolic content and oxalate, FRAP and potassium content (Table 3).

3.3. Regression of traits on gender

Regression analysis examining the relationship between gender and the phytochemicals and mineral compositions of ugu leaves further indicated that phenolic, flavonoid, vitamin C, tannin, saponin, zinc, manganese, and magnesium exhibited strong significance in distinguishing between the sexes (coefficient of determination, R2 > 0.9) (Table 4). Consistent with the important differentiating parameter, a regression analysis examining the relationship between sex, phytochemicals and mineral compositions of ugu leaves further indicated that phenolic, flavonoid, vitamin C, tannin, saponin, zinc, manganese, and magnesium exhibited strong significance in distinguishing between the sexes. This report is contrary to the report of [2], however, the large coefficient of determination (0.85-0.99) for these parameters indicates their importance in explaining differences between the leaves of the two sexes.

3.4. Principal component analysis (PCA)

PCA revealed the pattern of variation contributed by the measured traits contributed [24]. The first three principal axes had eigen values >1 and accounted for 96% of the total cumulative variation among the measured traits (Table 5). Character possessing highest coefficient in a PC axis suggests the relatedness of such character to that axis. The first PC had the highest contribution (84%) to the total observed variation and flavonoids, vitamin C, phenolic content, magnesium and manganese contributed substantially to this axis with positive association. The PC2 which accounted for 6% of the variation captured iron and calcium having high and positive loadings of values 0.47 and 0.69 respectively. PC3 which also accounted 6% captured DPPH and calcium with high and positive loadings (0.63 and 0.43 respectively).

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	Phenolic	Flavonoid				Phytate			Saponin			Na	Zn	К	Mn
	(mg/100g)	(mg/100g)	(%)	(mg/100g)	(mg/100g)	(mg/100g)	(mg/100g)	(mg/100g)	(%)	(%)	A/B	(mg/100g)	(mg/100g)	(mg/100g)	(mg/100g)
Flavonoid	0.88*														
Vit. C	0.89*	1.00***													
FRAP	-0.8	-0.96***	-0.95***												
Tanin	0.96***	0.94**	0.94**	-0.90***											
Phytate	-0.90**	-0.98***	-0.99***	0.91***	-0.92**										
Antiox	0.96***	0.96***	0.95***	-0.90**	0.99***	-0.94***									
Beta C	-0.92**	-0.99***	-0.99***	0.91**	-0.96***	0.98***	-0.98***								
Saponin	0.92**	0.98***	0.98***	-0.95***	0.98***	-0.95***	0.99***	-0.99***							
Oxalate	-0.83*	-0.96***	-0.96***	0.85*	-0.85*	0.97***	-0.90***	0.96***	-0.92**						
Ch A/B	0.80*	0.98***	0.98***	-0.94***	0.89*	-0.95***	0.92**	-0.97***	0.96***	-0.96***					
Na	-0.93**	-0.96***	-0.96***	0.92**	-0.98***	0.94**	-0.99***	0.98***	-1.00***	0.91**	-0.94***				
Zn	0.91**	0.99***	0.98***	-0.92**	0.94**	-0.97***	0.97***	-0.99***	0.98***	-0.97***	0.97***	-0.98			
К	-0.80*	-0.86*	-0.88*	0.8	-0.76	0.92**	-0.79	0.83***	-0.79	0.87*	-0.79	0.76	-0.84		
Mn	0.93**	0.98***	0.97***	-0.94***	0.98***	-0.95***	0.99***	-0.99***	1.00***	-0.92**	0.96***	-1.00***	0.98***	-0.8	
Mg	0.95***	0.92**	0.94**	-0.86*	0.94***	-0.95***	0.93**	-0.93**	0.92**	-0.85	0.85*	-0.91**	0.90**	-0.88*	0.92**

Table 3 Pearson's correlation coefficient between phytochemical properties and mineral composition of Ugu

*, **, *** Correlation is significant at 0.05, 0.01 and 0.001 probability levels, respectively; Vit. C = Vitamin C, FRAP = Ferric reducing antioxidant power, Antiox = Antioxidant, Beta C = Beta Carotene, Ch A/B = Chlorophyll A/B, Na = Sodium, Zn = Zinc, K = Potassium, Mn = Manganese, Mg = Magnesium

Table 4 Contributions of phytochemicals and mineral compositions to sex differences in ugu leaves based on stepwise
regression

Phytochemical and mineral composition	Mean ± SE	Coefficient of determinatio n (R ²)	Regressio n coefficien t (β)	Intercep t (α)	Significan t F level	Regression equation $y = \beta x + \alpha$
DPPH (%)	80.22±2.64	0.26	-3.86	86.01	0.297	y = -3.86x + 86.01
Phenolic (mg/100g)	36.13±2.28	0.86	14.34**	14.62	0.007	y = 14.34x + 14.62
Flavonoid (mg/100g)	188.09±3.04	0.96	44.79***	120.91	0.000	y = 44.79x + 120.91
Vit. C (%)	13.31±0.20	0.94	2.85***	9.03	0.001	y = 2.85x + 9.03
FRAP (mg/100g)	163.77±0.25	0.88	-9.30**	177.72	0.006	y = -9.30x + 177.72
Tanin (mg/100g)	26.04±0.85	0.96	9.52***	11.75	0.001	y = 9.52x + 11.75
Phytate (mg/100g)	48.57±2.38	0.92	-18.47***	76.28	0.003	y = -18.47x + 76.28
Antiox (mg/100g)	718.93±8.52	0.99	209.71***	404.37	0.000	y = 209.71x + 404.37
Beta C (mg/100g)	19.80±0.10	0.98	-1.68***	22.32	0.000	y = -1.68x + 22.32
Saponin (%)	4.26±0.01	0.99	0.59***	3.37	0.000	y = 0.59x +3.37
Oxalate (%)	0.43±0.02	0.86	-0.11**	0.59	0.008	y = -0.11x + 0.59
Ch A/B	15.22±0.59	0.92	4.56***	8.38	0.003	y = 4.56x + 8.38
Na (mg/100g)	9.99±0.09	0.99	-3.54***	15.31	0.000	y = -3.54x + 15.31
Fe (mg/100g)	9.69±0.09	0.49	-0.29	10.12	0.122	y = -0.29x +10.12
Ca (mg/100g)	32.83±0.35	0.20	0.83	31.59	0.373	y = 0.83x + 31.59
Zn (mg/100g)	0.74±0.01	0.97	0.19***	0.46	0.000	y = 0.19x + 0.46
K (mg/100g)	649.17±13.1 2	0.63	-38.33	706.67	0.057	y = -38.33x + 706.67
Mn (mg/100g)	3.23±0.02	0.99	2.84***	-1.03	0.000	y = 2.84x - 1.03
Mg (mg/100g)	9.83±0.21	0.85	1.55**	7.51	0.009	y = 1.55x + 7.51

DPPH= 2,2-Diphenyl-1-picrylhydrazyl, Vit. C = Vitamin C, FRAP = Ferric reducing antioxidant power, Antiox = Antioxidant, Beta C = Beta Carotene, Ch A/B = Chlorophyll A/B, Na = Sodium, Fe = Iron, Ca =Calcium, Zn = Zinc, K = Potassium, Mn = Manganese, Mg = Magnesium

Table 5 Eigenvalue, proportion of variability and estimate of phytochemical properties and mineral compositioncontributing to first three principal components

Phytochemical and mineral composition	PC1	PC2	PC3
DPPH (%)	-0.14	-0.36	0.63
Phenolic (mg/100g)	0.24	0.19	0.17
Flavonoid (mg/100g)	0.25	-0.09	-0.06
Vit. C (%)	0.25	-0.07	-0.05
FRAP (mg/100g)	-0.23	0.29	-0.08
Tanin (mg/100g)	0.24	0.06	0.22

Phytate (mg/100g)	-0.25	0.02	0.12
Antiox (mg/100g)	0.25	0.09	0.10
Beta C (mg/100g)	-0.25	-0.05	0.04
Saponin (%)	0.25	-0.01	0.07
Oxalate (%)	-0.24	-0.03	0.29
Ch A/B	0.24	-0.10	-0.12
Na (mg/100g)	-0.25	-0.05	-0.10
Fe (mg/100g)	-0.17	0.47	-0.33
Ca (mg/100g)	0.10	0.69	0.43
Zn (mg/100g)	0.25	0.03	-0.09
K (mg/100g)	-0.22	0.14	0.23
Mn (mg/100g)	0.25	0.00	0.07
Mg (mg/100g)	0.24	0.04	0.12
Eigenvalue	15.88	1.30	1.15
Proportion (%)	84	6	6
Cumulative (%)	84	90	96

DPPH= 2,2-Diphenyl-1-picrylhydrazyl, Vit. C = Vitamin C, FRAP = Ferric reducing antioxidant power, Antiox = Antioxidant, Beta C = Beta Carotene, Ch A/B = Chlorophyll A/B, Na = Sodium, Fe = Iron, Ca = Calcium, Zn = Zinc, K = Potassium, Mn = Manganese, Mg = Magnesium

4. Conclusion

This study revealed that both male and female ugu leaves harbor essential minerals, physico-chemical and phytochemicals crucial for promoting a healthy diet and overall well-being. The distinctions between the sexes were based on phenolic, flavonoid, vitamin C, Tannin, saponin, zinc, manganese and magnesium. The differences recorded in the nutrient, physico-chemical and mineral composition of the two sexes may be due to the environmental effect, genetic differences and genotype by environment interaction. Consequently, there is a possibility for enhancing the nutrient content of ugu across different sexes through breeding.

Compliance with ethical standards

Acknowledgments

We acknowledge the field technical assistance of Mr. Bolaji Olatubosun.

Disclosure of conflict of interest

The authors declare no conflict of interest.

Author contribution

Conceptualization: DOI, experimental layout and data collection: DOI, ORA, AOK and OAA, formal analysis: AOK, original draft preparation: ORA and EOA, Review and editing: DOI and AOK. All authors read and approve the final manuscript.

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