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Research Article

Nutritional Evaluation of the Seeds of *Corchorus olitorius*: A Neglected and Underutilized Species in Nigeria

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Abstract

Background and Objective: The seeds of *Corchorus olitorius* (*C. olitorius*), a neglected leafy vegetable in Nigeria, are usually discarded in the process of harvesting the leaves. This practice may result from a lack of knowledge regarding the nutritional content of the seeds as well as a lack of research interest. The aim of this study was therefore to evaluate the nutritional composition of the seeds of 14 accessions of *C. olitorius*. The results will help to determine the suitability of these seeds as a source of nutritious food for human consumption and of feed for the livestock industry. **Materials and Methods:** The seeds of the 14 accessions of *C. olitorius* were procured from the seed bank of the National Centre for Genetic Resources and Biotechnology, Moor Plantation, Ibadan, Nigeria. Standard food composition analysis techniques were employed to evaluate the proximate, mineral and vitamin contents of the seeds. The obtained data were analysed with Genstat and SPSS. **Results:** The proximate components were present in varying ranges, as follows: moisture (6.087-10.77 mg/100 g), ash (1.866-2.657%), fat (5.274-5.896%), protein (0.966-1.135%), fibre (0.973-1.913%) and carbohydrate (59.91-87.34%). The mineral contents were as follows: calcium (1.242-2.610%), iron (1.235-1.623 mg/100 g), potassium (0.320-1.232%), sodium (0.002-0.007%), lead (0.150-0.274 mg/100 g), tin (0.086-0.167 mg/100 g), zinc (0.090-0.942 µg/100 g), manganese (0.123-0.147%), magnesium (0.896-1.475 mg/100 g), copper (0.126-0.143 mg/100 g) and phosphorus (0.624-0.973 mg/100 g). The vitamin contents included the following: 2.155-2.838 mg/100 g of vitamin A, 0.519-0.884 mg/100 g of vitamin C and 0.357-0.394 mg/100 g of vitamin D. The Na/K ratio ranged from 0.005-0.016 and the Ca/P ratio ranged from 1.31-3.43. The highest positive correlation coefficient (<0.05) was obtained between vitamin A and vitamin C (r^2 = 0.521, 52%) and the highest negative correlation was observed between tin and manganese (r^2 = 0.581, 58%). Principal component analysis revealed that two principal components with eigenvalues $\geq$1 accounted for 90.09% of the total variability among the accessions. These two components were carbohydrates (57.12%) and the moisture composition (32.37%). **Conclusion:** Seeds of *C. olitorius* are nutritionally adequate and have acceptable limits of metal contents. Thus, they have the potential to be incorporated into the human diet and livestock feed.

Key words: Corchorus olitorius seed, Corchorus, human diet, leafy vegetables, livestock feed

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Corchorus olitorius L. (Family Sparrmaniaceae) is a neglected indigenous leafy vegetable in Nigeria. Opabode and Adebooye[1] noted that few germplasms of this species have been collected and characterized. The leaves of this species are mainly consumed by certain tribes in Nigeria. Other countries in which the leaves are consumed include Egypt, Sudan, India, Bangladesh, the Philippines, Malaysia and Japan[2]. Common names of this species include Bush Okro, Jute Mallow or Jew's Mallow. In Nigeria, the species is locally called Ahihara by the Igbo, Ewedu by the Yoruba and Malafiya by the Hausa[3].

The leaves of *C. olitorius* contain high levels of iron, folate, protein, fibre, calcium, riboflavin, carotene, vitamin C and phenol[4-6] and it has been demonstrated that anti-nutrient levels in the leaves are low, while zinc bioavailability is high[7]. Furthermore, reports of various uses of the jute fibre of this species abound, including utilization as a raw material for packaging, textiles, non-textiles, construction and the production of nets and sacks[8]. Jute is also used for the production of highly absorptive fibre for surgical dressings[9]. The fibre content has been shown to be high and is 100% environmentally friendly[8]. The contents of other proximate components and minerals in the leaves are also appreciable[10-11].

Medicinal uses of *C. olitorius* have been documented by many authors. Aqueous extracts of the seeds were reported by Zakaria *et al.*[12] to possess peripheral, anti-inflammatory and anti-pyretic activities. Antibacterial activities have also been reported by Pal *et al.*[13] from methanol extracts of the seeds. Ibrahim and Fagbohun[14] concluded from their investigations that the seed oil of *C. olitorius* can potentially be used as a food preservative as well as for medicinal purposes, due to its antimicrobial quality.

However, the seeds of this species have largely been neglected and wasted and are mainly used only for propagation[15]. Adebooye *et al.*[16] observed that wasting of *C. olitorius* seeds can arise as a result of the practice of leaving fruits on the plant for too long, such that some fully ripe inflorescences burst and shed their seeds. Ezeagu[17] reported on the scarcity of literature on the nutritional composition of tropical plant seeds. He noted that lesser-known tropical seed plant species have remained underutilized because they have received very little or no research attention. He therefore suggested that a concerted national research effort into the nutritional qualities and physical characteristics of these lesser-known plant species and processing methods for them should be undertaken. Such an effort would enhance the utility of these species as food or feed ingredients. Agatamor[18] reported that seeds are indispensable in diets, due to their nutritive and calorific value and Matsufuji *et al.*[19] were of the opinion that seeds can serve as food during famine and could provide raw materials for local industries.

There are very few reports on the proximate and mineral contents of *C. olitorius* seeds. This study was therefore initiated to investigate the nutritional composition of fourteen accessions of *C. olitorius* to ascertain the potential for these seeds to serve as sources of food as well as a source of feed for the livestock industry. The information obtained from this study will also be useful for designing plant breeding programmes for the improvement of the species.

MATERIALS AND METHODS

The fourteen accessions of *C. olitorius* examined in this study were procured from the seed bank of the National Centre for Genetic Resources and Biotechnology Moor plantation, Ibadan, Nigeria. These accessions included A_1-NG/AA/SEP/09/173 (Osun State), A_2-NG/TO/AUG/09/008 (Ogun State), A_3-NG/A0/AUG/09/003 (Lagos State), A_4 NG/0E/10/002, A_5-NG/MR/MAY/09/004 (Ogun State), A_6-NG/SA/DEC/07/0403 (Niger State), A_7-NG/SA/DEC/07/0402 (Niger State), A_8-NG/AO/MAY/09/018 (Osun State), A_9-NG/SA/07/189 (Osun State), A_{10}-NG/OA/JUN/09/001 (Oyo State), A_{11}-NG/SA/JAN/09/142 (Niger State), A_{12}-NG/OCT/09/001, A_{13}-NG/AO/MAY/09/013 and A_{14}-NG/SA/07/203 (Ondo State). Information on the states where A_4, A_{12} and A_{13} were collected was not provided to the researchers.

Proximate analysis: The proximate analysis was performed according to the standard methods of Pearson[20] and the Association of Official Analytical Chemists (AOAC)[21]. The assessed proximate components included the following:

- **Ash content:** A total of 2 g of the respective ground samples was used for this assessment. The ash content was determined using a muffle furnace at 600°C for 3 h.
- **Protein content:** Protein content was determined via the micro-Kjeldahl method. Briefly, 0.5 g of each of the ground samples was transferred to a 30 mL Kjeldahl flask. Next, 15 mL of conc. H_2SO_4, 0.1g SeO_2 and $CuSO_4$ (1 g) were added to the flask. Then, the flask was heated under a fume hood until a clear greenish solution appeared. After cooling, 10 mL of 40% NaOH was added and the mixture was then transferred to the Kjeldahl distillation apparatus. The distillate was later titrated to a pink colour with 0.01 M HCl.

- **Crude fat content:** A total of 2 g of the respective ground samples was placed inside the extractor thimble, which was then placed inside the Soxhlet extractor. Petroleum ether was used for extraction, which ran for 4 h.
- **Crude fibre:** A total of 1.5 g (w_1) of ground seeds of the respective accessions was used for crude fibre determination. Sample digestion was achieved by first adding 150 mL of pre-heated H_2SO4. After 30 min of boiling, subsequent filtering and washing three times with hot water, 150 mL of pre-heated KOH was added. Then, the mixture was again boiled for 30 min, after which it was filtered and the residue was washed three times each with hot water and acetone. Finally, the sample was dried at 103°C for 1 h, weighed (w_2), heated at 500°C and then weighed again (w_3).
- **Moisture content:** A total of 2 g of the respective ground accessions was dried to a constant weight at 60°C in a hot air-circulating oven for 24 h. The moisture content was then calculated as the difference in weight before and after drying.
- **Carbohydrate content:** The carbohydrate content was determined by subtracting the total of all of the proximate parameters from 100.

Mineral and vitamin analyses: Measurement of magnesium
A total of 10 mL of the test solution was poured into a 250 mL conical flask. Then, 25 mL of NH_3-NH_4 buffer solution was added to the prepared test solution. Next, 25 mL of water and 2-3 drops of Eriochrome Black-T indicator (Sigma-Aldrich) were added and the solution was titrated against a 0.01-M EDTA solution. The volume of EDTA used was equivalent to the calcium and magnesium in the mixture. The volume of magnesium was calculated as follows: magnesium volume = (vol. Ca and Mg.-Vol. Ca).

- **Measurement of calcium:** A total of 10 mL of the test solution was pipetted into a 250 mL conical flask. Then, 25 mL of KOH, 25 mL of water and a pinch of calcine indicator were added. An EDTA solution was titrated with this mixture to an end point. The volume of EDTA was equivalent to the volume of calcium in the solution.
- **Measurement of phosphorous:** To prepare the stock solution, 0.879 g of dried phosphorous dihydrogen orthophosphate (dried at 105°C for 1 h) was dissolved in water, to which 1 mL of conc. HCL was added. The resulting solution was diluted to 200 mL and 2 mL of toluene was added to obtain a concentration of 1 mg P mL^{-1}. The standard was prepared by measuring 0-50 mg mL^{-1} of the phosphorous stock solution at volumes of 0, 2, 4, 6, 8 and 10 mL into 6 corresponding 200 mL volumetric flasks and adding water to the mark.

To derive the standard curve, 5 mL of each sample was pipetted into a 50 mL graduated flask. Then, 10 mL of molybdate mixture was added and the solution was diluted to the mark with water. The sample was allowed to stand for 15 min for colour development. Then, the absorbance at 400 nm was measured against the blank. A graph relating absorbance to mg of phosphorous present was constructed and the sample solution was analysed as described above, using the sample solution instead of the phosphorous standard. Parts per million or mg mL^{-1} values were read from the graph; the mg of phosphorous equivalent to the absorbance of the sample was calculated and the blank was determined.

- **Measurement of tin:** The sample was transferred to a macro-Kjeldahl digestion flask containing 15-35 mL of concentrated nitric acid followed by the addition of 10-15 mL of concentrated sulphuric acid. Then, the sample was heated to oxidize organic matter and small additional quantities of nitric acid were immediately added until the liquid turned black. Next, the sample was heated until white fumes appeared. After cooling, 10 mL of water, 1 g of potassium chlorate and 15 mL of concentrated hydrochloric acid were added. Then, the mixture was heated until copious white fumes evolved. The cooled oxidized solution was washed into a 350-500 mL conical flask with successive small quantities of water. Half a gram of aluminium foil and 25 mL of concentrated hydrochloric acid were added. Then, the flask was closed with a valve filled with 10% potassium bicarbonate solution. The flask was subsequently boiled until the aluminium was dissolved, cooled and more bicarbonate solution was added to the valve, such that it was always at least half filled with liquid. The valve was removed and a few pieces of marble and a starch solution were added to the colourless liquid, which was then titrated to reduce tin as rapidly as possible with a 0.01 M iodine solution. The amount of tin was calculated in the sample in ppm.
- The sodium and potassium contents were determined by the flame photometer method, while the contents of iron, zinc, copper, lead and vitamins A and C were determined spectrophotometrically.

- **Measurement of vitamin D:** The vitamin D content was determined by mixing acetyl chloride with freshly prepared free-from-alcohol Carr-Price reagent (20% m/v of antimony trichloride in chloroform with 4% pure acetyl chloride). Nine volumes of this reagent were added to 1 volume of unsaponifiable matter in chloroform and the $E^{1\%}_{1cm}$ value at 500 nm was measured. This extinction x 1800 represents an approximate measure of vitamin D per gram.

Data analysis: The obtained nutritional composition data were analysed using one-way analysis of variance (ANOVA) with 5% level of significance. A correlation analysis was performed to determine the relationships among the nutritional compositions of the fourteen accessions. In addition, a principal component analysis (PCA) was conducted. The statistical packages used were Genstat and SPSS. Fisher's least significant difference (FLSD) was used for separating means at a 5% level of significance.

RESULTS

The results of ANOVA presented in Table 1 show that there were significant differences in the nutritional composition among the fourteen accessions studied. The compositions of proximate components, minerals and vitamins in the 14 accessions are shown in Table 2-4,

Table 1: Mean squares (MS) values and variance ratios (VR) obtained through ANOVA

Nutritional attributes		MS	VR
Ash	Accessions	1.036E-01	11891.71*
	Error	8.714E-06	
Fats	Accessions	0.10738927	7381.91*
	Error	0.00001455	
Fibre	Accessions	0.1495673	362.44*
	Error	0.0004127	
Protein	Accessions	5.912E-03	1.235.39*
	Error	4.786E-06	
Moisture	Accessions	7.818463	2661.52*
	Error	0.002938	
Carbohydrate	Accessions	113.22	1.32*
	Error	85.97	
Calcium	Accessions	0.54220844	30202.59*
	Error	0.00001795	
Copper	Accessions	8.937E-05	16.68*
	Error	5.357E-06	
Iron	Accessions	0.06114779	1623.39*
	Error	0.00003767	
Lead	Accessions	0.002612	2.21*
	Error	0.001183	
Magnesium	Accessions	1.035E-01	10424.20*
	Error	9.929E-06	
Sodium	Accessions	6.952E-06	435.80*
	Error	1.595E-08	
Manganese	Accessions	1.775E-04	27.81*
	Error	6.381E-06	
Potassium	Accessions	1.592E-01	114203.73*
	Error	1.394E-06	
Phosphorous	Accessions	2.298E-02	2942.70*
	Error	7.810E-01	
Tin	Accessions	2.841E-03	321.66*
	Error	8.833E-06	
Zinc	Accessions	0.010907	1.82*
	Error	0.005996	
Vitamin A	Accessions	0.13194799	3089.08*
	Error	0.00004271	
Vitamin C	Accessions	0.02631008	366.39*
	Error	0.00007181	
Vitamin D	Accessions	4.412E-04	63.25*
	Error	6.976E-06	

Table 2: Comparison of the proximate components of the accessions of *C. olitorius*

Accessions	Moisture (mg/100 g)	Ash (%)	Fats (%)	Proteins (%)	Fibre (%)	Carbohydrate (%)
A_1	9.512 ± 0.0019^g	2.533 ± 0.0020^k	5.415 ± 0.0025^c	0.966 ± 0.0015^a	1.044 ± 0.0015^b	80.5 ± 0.0058^b
A_2	10.536 ± 0.0032^k	2.332 ± 0.0012^d	5.633 ± 0.0020^f	1.054 ± 0.0023^c	0.973 ± 0.0009^a	79.5 ± 0.0088^b
A_3	10.771 ± 0.0012^i	2.412 ± 0.0015^i	5.883 ± 0.0020^l	1.115 ± 0.0012^h	1.112 ± 0.0012^{cd}	59.9 ± 19.306^a
A_4	9.440 ± 0.0015^g	1.866 ± 0.0017^d	5.274 ± 0.0026^a	1.066 ± 0.0035^d	1.055 ± 0.0009^b	81.3 ± 0.0033^b
A_5	8.510 ± 0.0017^f	2.145 ± 0.0012^b	5.844 ± 0.0020^k	1.033 ± 0.0006^b	1.080 ± 0.0015^{cd}	81.8 ± 0.2767^b
A_6	10.110 ± 0.0026^i	2.231 ± 0.0019^c	5.393 ± 0.0017^b	1.085 ± 0.0012^e	1.222 ± 0.0009^f	80.0 ± 0.0067^b
A_7	7.365 ± 0.0006^d	2.516 ± 0.0023^j	5.652 ± 0.0034^h	1.126 ± 0.0009^i	1.135 ± 0.0006^{de}	81.1 ± 1.1317^b
A_8	6.087 ± 0.0015^a	2.394 ± 0.0020^h	5.714 ± 0.0012^j	1.113 ± 0.0006^h	1.913 ± 0.0437^h	82.8 ± 0.0467^b
A_9	9.532 ± 0017^g	2.414 ± 0.0020^i	5.664 ± 0.0018^i	1.054 ± 0.0012^c	1.157 ± 0.0012^e	80.2 ± 0.0033^b
A_{10}	6.402 ± 0.0640^b	2.377 ± 0.00120^g	5.555 ± 0.0017^e	1.066 ± 0.0012^d	1.276 ± 0.0015^g	83.3 ± 0.0633^b
A_{11}	7.849 ± 0.0015^e	2.346 ± 0.0120^f	5.443 ± 0.0032^d	1.106 ± 0.0009^g	1.145 ± 0.0012^{de}	87.3 ± 5.207^b
A_{12}	6.742 ± 0.0009^c	2.337 ± 0.00115^e	5.713 ± 0.0021^j	1.063 ± 0.0009^d	1.163 ± 0.0017^e	83.0 ± 0.0984^b
A_{13}	9.843 ± 0.0978^h	2.415 ± 0.00186^i	5.896 ± 0.0018^m	1.096 ± 0.0009^f	1.136 ± 0.0010^{de}	79.6 ± 0.0033^b
A_{14}	10.327 ± 0.0024^g	2.637 ± 0.00208^k	5.641 ± 0.0018^g	1.135 ± 0.0069^j	1.096 ± 0.0015^c	79.2 ± 0.0033^i
LSD	0.091	0.005	0.006	0.004	0.034	15.51

Mean values were significant at $p<0.05$. Values with the same letter are not significantly different at the 5% level of probability

respectively. The evaluated nutritional properties were observed in all of the accessions and exhibited a range of low to high values. Accessions from the same state were observed to differ significantly in many of the proximate components (Table 2). The accessions from Niger State (A_6, A_7 and A_{11}) were significantly different in their mean percentage proximate composition, except for their fibre contents. For fibre content, accessions A_7 and A_{11} were not significantly different but they both differed significantly from A_6. The Ogun State accessions (A_2 and A_5) differed significantly in all proximate components, with the exception of carbohydrate content. The accessions from Osun State (A_1, A_8 and A_9) were also significantly different, except for their carbohydrate and moisture contents. These three accessions were not significantly different in terms of their carbohydrate content. Accessions A_1 and A_9 were not significantly different in terms of their moisture content but the moisture content of both accessions was significantly different from that of A_8. The accessions with the lowest and highest mean values were as follows: moisture (A_8 and A_3, respectively), ash (A_4 and A_{14}), protein (A_1 and A_{14}), fat (A_4 and A_{13}), fibre (A_2 and A_8) and carbohydrate (A_3 and A_{11}). However, there were other accessions whose mean values were not significantly different from these values at the 5% level of probability. Accession 3 was significantly different from all thirteen accessions in terms of its carbohydrate composition (LSD = 15.51, $p>0.05$).

The mean mineral compositions are shown in Table 3. It was again observed that certain accessions from the same state differed in their compositions. These accessions included the following: A_1, A_8 and A_9 (Osun State); A_2 and A_5 (Ogun State); and A_6, A_7 and A_{11} (Niger State). However, these accessions did not differ significantly in their contents of Na, Pb, Zn and Cu. A_1 was observed to be high in Ca, Fe, K and Na. A_{10} exhibited the lowest Fe, Mg and Cu contents, while A_{12} and A_5 were found to be lowest in Ca and K, respectively. Although A_{12} was low in Ca, this accession showed the highest copper content. The highest contents of Mg and Zn were observed in A_6 and $A_{2,}$ respectively. A_1 and A_3 exhibited the lowest Pb and Zn contents, respectively, while A_9 and A_2 exhibited the highest Zn content. As observed for the proximate components, there were other accession whose mean values were not significantly different from these means at the 5% level of probability. There was a significant difference in copper content between A_{12} and the other accessions (LSD = 0.005, $p<0.05$).

The vitamin compositions of the accessions are shown in Table 4. Accessions from the same state differed in their vitamin A content but vitamins C and D did not vary considerably. A_{14} was observed to have the highest contents of vitamins A, C and D (Table 4). The lowest vitamin A and vitamin C contents were observed in A_5, whereas A_6 presented the lowest content of vitamin D. There was a significant difference in vitamin A contents among all of the accessions (LSD = 0.0101, $p<0.05$). The vitamin C content was observed to be significantly different among A_5, A_6, A_8 and A_{14} (LSD = 0.014, $p<0.05$). There was no significant difference among A_1, A_3, A_4, A_5, A_9, A_{13} and A_{14} in vitamin D content. Varying degrees of non-significance in vitamin D contents were also observed among the mean values of the other accessions.

There were different degrees of correlation between certain nutritional components, as shown in Table 5. A moderate positive correlation existed between potassium and ash ($r^2 = 0.360$ (36%), $p<0.05$), vitamin D and moisture ($r^2 = 0.307$ (31%), $p<0.05$), vitamin D and sodium ($r^2 = 0.354$ (35%, $p<0.05$) and vitamin A and vitamin C ($r^2 = 0.521$ (52%), $p<0.05$). A moderate negative correlation was observed

Table 3: Comparison of the mineral compositions of the accessions of *C. olitorius*

Accessions	Ca (%)	Fe mg/100 g	K (%)	Na (%)	Pb mg/100 g	Sn mg/100 g	Zn µg/100 g	Mn (%)	Mg mg/100 g	Cu mg/100 g	P mg/100 g	Na/K Ratio	Ca/P Ratio
A_1	2.610±0.0009^{i}	1.623±0.0015^{f}	0.494±0.0002^{k}	0.007±0.0001^{c}	0.150±0.0735^{a}	0.164±0.0012^{h}	0.865±0.0022^{abc}	0.134±0.0219^{d}	1.234±0.0015^{bc}	0.126±0.0019^{a}	0.762±0.0009^{f}	0.014	3.430
A_2	1.635±0.0012^{h}	1.532±0.0006^{e}	0.355±0.0020^{c}	0.003±0.0000^{a}	0.244±0.0019^{b}	0.086±0.0021^{a}	0.942±0.0012^{c}	0.136±0.0024^{k}	1.473±0.0012^{bc}	0.126±0.0015^{a}	0.836±0.0285^{i}	0.008	1.956
A_3	1.245±0.0009^{a}	1.237±0.0015^{ab}	0.422±0.0015^{f}	0.004±0.0001^{b}	0.242±0.0015^{b}	0.107±0.0018^{cd}	0.742±0.0015^{a}	0.123±0.0015^{j}	1.387±0.0012^{a}	0.135±0.0009^{abc}	0.755±0.0017^{e}	0.009	1.649
A_4	1.341±0.0018^{a}	1.247±0.0012^{b}	0.346±0.0003^{b}	0.004±0.0001^{b}	0.249±0.0012^{b}	0.114±0.0022^{e}	0.853±0.0015^{abc}	0.147±0.0009^{h}	1.367±0.0003^{e}	0.131±0.0012^{b}	0.824±0.0009^{h}	0.010	1.627
A_5	1.376±0.0074^{d}	1.295±0.0009^{d}	0.320±0.0002^{a}	0.004±0.0001^{b}	0.220±0.0006^{b}	0.126±0.0019^{f}	0.826±0.0020^{ab}	0.126±0.0015^{g}	1.294±0.0015^{a}	0.134±0.0012^{abc}	0.823±0.0015^{h}	0.010	1.670
A_6	2.107±0.0012^{f}	1.236±0.0015^{ab}	0.425±0.0001^{g}	0.004±0.0001^{b}	0.245±0.0009^{b}	0.096±0.0015^{b}	0.897±0.0019^{bc}	0.133±0.0015^{k}	1.475±0.0024^{bc}	0.126±0.0012^{a}	0.843±0.0009^{j}	0.009	2.499
A_7	2.125±0.0015^{j}	1.297±0.0012^{d}	0.536±0.0001^{i}	0.004±0.0000^{b}	0.259±0.0006^{b}	0.094±0.0018^{b}	0.754±0.0015^{ab}	0.126±0.0022^{i}	1.374±0.0012^{a}	0.126±0.0012^{a}	0.673±0.0018^{b}	0.008	3.158
A_8	1.354±0009^{k}	1.272±0.0125^{bc}	0.455±0.0001^{h}	0.004±0.0001^{b}	0.235±0.0012^{b}	0.107±0009^{c}	0.763±0.1672^{ab}	0.132±0.0009^{d}	1.236±0.0015^{b}	0.133±0.0018^{ab}	0.873±0.0012^{i}	0.009	1.551
A_9	1.275±0.0019^{e}	1.246±0.0012^{ab}	0.378±0.0001^{e}	0.006±0.0001^{b}	0.274±0.0026^{b}	0.112±0.0012^{de}	0.875±0.0027^{abc}	0.142±0.0015^{e}	1.266±0.0019^{de}	0.137±0.0012^{d}	0.973±0.0019^{k}	0.016	1.310
A_{10}	1.258±0.0012^{c}	1.235±0.0015^{a}	0.457±0.0001^{i}	0.004±0.0001^{b}	0.252±0.0015^{b}	0.156±0.0015^{g}	0.838±0.0009^{abc}	0.136±0.0017^{a}	0.896±0.0015^{bc}	0.126±0.0009^{a}	0.752±0.0015^{e}	0.009	1.673
A_{11}	1.258±0.0009^{b}	1.533±0.0015^{e}	0.359±0.0002^{d}	0.002±0.0000^{a}	0.265±0.0015^{b}	0.165±0.0022^{h}	0.783±0.0015^{ab}	0.142±0.012^{b}	0.964±0.0012^{d}	0.134±0.0012^{abc}	0.683±0.0019^{c}	0.006	1.842
A_{12}	1.242±0.0019^{a}	1.237±0.0009^{ab}	0.463±0.0001^{j}	0.003±0.0000^{a}	0.233±0.0100^{b}	0.167±0.0009^{h}	0.827±0.0012^{abc}	0.138±0.0027^{c}	1.145±0.0009^{c}	0.143±0.0019^{e}	0.744±0.0022^{d}	0.007	1.669
A_{13}	1.743±0.0018^{i}	1.277±0.0009^{c}	0.657±0.0002^{m}	0.004±0.0001^{b}	0.233±0.0021^{b}	0.155±0.0022^{g}	0.786±0.0012^{ab}	0.145±0.0021^{b}	0.966±0.0027^{de}	0.135±0.0015^{bc}	0.792±0.0006^{g}	0.006	2.200
A_{14}	1.584±0.0026^{g}	1.535±0.0013^{i}	1.232±0.0002^{n}	0.006±0.0001^{b}	0.227±0.0012^{b}	0.166±0.0020^{h}	0.896±0.0009^{bc}	0.134±0.0012^{f}	1.272±0.0012^{a}	0.137±0.0009^{d}	0.624±0.0015^{a}	0.005	2.539
LSD	0.007	0.01	0.002	0.0002	0.056	0.004	0.13	0.004	0.005	0.005	0.005		

Mean values were significant at p<0.05. Values with the same letter are not significantly different at the 5% level of probability

between moisture and fibre (r^2 = 0.581 (58%), p<005), phosphorus and potassium (r^2 = 0.334 (33%), p<0.05) and tin and manganese (r^2 = 0.762 (58%), p<0.05).

PCA yielded 5 principal components (Table 6) that accounted for approximately ninety-nine percent of the total variance. It was observed that the carbohydrate composition was highly correlated with the first component (57.12% of the variability), moisture with the second component (32.37% of the variability), fats with the third component (3.66% of the variability), calcium with the fourth component (3.27% of the variability) and vitamin A with the fifth component (2.79% of the variability).

DISCUSSION

The finding that seeds of accessions from the same state varied in certain proximate, mineral and vitamin components in some cases can be explained when the soil conditions of these states are considered. These states were Osun, Ogun and Niger. Soil is the product of five dominant factors: climate, parent materials (rocks and their physical and chemical derivatives), relief, organisms (fauna and flora) and time. The different types and degrees of these soil-forming factors and their combinations give rise to quite a variety of soil types[22]. Each area of the world is characterized by a specific climate, rocks and vegetation; therefore, the soil of each region exhibits unique characteristics[23].

Tropical rainforests and savannah vegetation are characteristics of both Osun and Ogun States, whereas Niger State is in the Sudan savannah zone[24]. Soils of tropical rainforests belong to the orders of Ultisols and Oxisols, while soils in the savannah zone fall within the orders of Alfisols and Oxisols[22]. Hence, the differences in nutritional compositions among the accessions collected from the same state may potentially be due to the different types of soil found in the state in which the plants were grown.

Ultisols are similar to Alfisols. However, Ultisols result from extensive weathering and leaching of parent rocks, whereas Alfisols are less leached and present lower acidity than Ultisols. The major difference between these two soil types is that Ultisols have a base saturation of less than 35% at 0.75-2.0 m, while Alfisols exhibit at least 35% base saturation [25]. Soils with a high base saturation are generally more fertile because they contain greater amounts of the essential plant nutrient cations K^+, Ca^{2+} and Mg^{2+} [26]. Thus, Alfisols are more fertile than Ultisols. This phenomenon may potentially account for the nutritional differences observed among the accessions obtained from the same state.

Table 4: Comparison of the vitamin compositions of the accessions of *C. olitorius*

Accessions	Vitamin A Mg/100 g	Vitamin C Mg/100 g	Vitamin D µg/100 g
A_1	2.596±0.0173^f	0.854±0.0009^{ef}	0.3847±0.0009^{de}
A_2	2.713±0.0027^h	0.844±0.0015^{ef}	0.3750±0.0009^c
A_3	2.335±0.1258^c	0.867±0.0009^{fg}	0.3870±0.0012^e
A_4	2.773±0.00153^i	0.874±0.0017^{gh}	0.3850±0.0015^d
A_5	2.155±0.0020^a	0.519±0.0015^a	0.3820±0.0015^{de}
A_6	2.334±0.0019^c	0.745±0.0012 ^{b}	0.3570±0.0009^{ac}
A_7	2.412±0.0019^d	0.825±0.0015^d	0.3640±0.0026^b
A_8	2.567±0.0186^e	0.834±0.0019^{de}	0.3720±0.0018^c
A_9	2.274±0.0021^b	0.797±0.0019^c	0.3850±0.0027^{de}
A_{10}	2.587±0.0015^f	0.855±0.0012^{ef}	0.3660±0.0009^b
A_{11}	2.614±0.0015^g	0.853±0.0175^{def}	0.3570±0.0012^a
A_{12}	2.586±0.0012^f	0.846±0.0019^{de}	0.3640±0.0012^b
A_{13}	2.796±0.0015^j	0.876±0.0009^{gh}	0.3860±0.0009^{de}
A_{14}	2.838±0.0012^k	0.884±0.0019^i	0.3940±0.0015^d
LSD	0.0101	0.014	0.004

Mean values were significant at p<0.05. Values with the same letter are not significantly different at the 5% level of probability

Oxisols, on the other hand, result from extreme weathering and leaching; these soils are commonly found in hot, humid climates and are extremely infertile. Aluminium and manganese toxicity are also characteristic of such soils. However, these soils can be productive when supplemented with lime and fertilizers[22,27].

The moisture content of the *C. olitorius* seeds (6.087±0.002 to 10.77±0.0012) recorded in the present study was higher than that (5.32±0.4) obtained by Oloye *et al.*[15] for *C. olitorius* seed flour but comparable to the range of 7.0±0.00 to 11.0 ± 0.00 that is typical for most leguminous flour, as reported by Fagbemi (2007), who was cited by Oloye *et al.*[15]. Low moisture contents of plant seeds have been associated with a long shelf life before cultivation[28]. Therefore, accession 3, which exhibited the highest mean moisture content, may not have as long a shelf life as the other accessions and may be attacked easily by microorganisms, since a high moisture content favours microorganism growth, as noted by Emmanuel *et al.*[29]. The high moisture content of accession 3 may be due to the climatic conditions of Lagos State, where this accession was obtained. According to the Nigerian Investment Promotion Commission (NIPC)[24], Lagos State falls within the swamp forest of the coastal belt and dry lowland rainforest. The average annual relative humidity in the state is 84.0%[30]. This high humidity might have contributed to the high moisture content of accession 3.

Accession 14 (from Ondo State) presented the highest contents of ash, protein and vitamins A, C and D and exhibited high levels of the other nutrients analysed. These high levels may be due to the soil on which the plants were grown. Three vegetation zones can be found in Ondo State, including mangrove swamp, rainforest and derived savannah zones[24].

Table 5: Linear correlation matrix among the nutritional compositions of the fourteen accessions of *C. olitorius*

	Ash	Ca	CHO	Cu	Fats	Fibre	Fe	K	Pb	Mag	Mn	Na	Protein	Moisture	P	Sn	Vit C.	Vit A	Zn	Vit D
Ash	1																			
Ca	0.495	1																		
CHO	-0.081	0.0750	1																	
Cu	0.060	-0.3940	-0.059	1																
Fats	0.351	-0.0390	-0.272	0.455	1															
Fibre	0.082	0.2290	0.111	0.046	0.137	1														
Fe	0.410	0.4500	0.130	-0.206	-0.283	-0.326	1													
K	0.600*	0.1920	-0.030	0.229	0.147	-0.049	0.317	1												
Pb	-0.152	-0.4580	0.011	0.127	0.042	0.105	-0.400	-0.107	1											
Mag	0.422	-0.1890	0.298	0.100	-0.353	-0.078	-0.080	-0.326	0.177	1										
Mn	0.197	0.0096	-0.255	-0.241	-0.108	-0.166	-0.010	-0.094	-0.067	-0.390	1									
Na	0.430	0.4590	-0.069	-0.037	-0.122	-0.100	0.308	0.439	-0.323	-0.160	0.091	1								
Protein	0.203	0.2570	-0.117	0.228	0.269	0.306	-0.260	0.417	0.473	-0.240	-0.010	-0.352	1							
Moisture	-0.005	-0.0820	-0.341	-0.071	0.000	-0.611*	0.280	0.211	-0.143	-0.040	0.499	0.314	-0.085	1						
P	-0.427	-0.1250	-0.019	-0.024	0.018	0.166	-0.400	-0.578*	0.087	0.398	0.255	0.082	-0.409	0.152	1					
Sn	-0.296	-0.0050	0.196	0.383	-0.031	-0.129	0.334	0.413	-0.224	0.191	-0.762*	0.227	-0.167	-0.215	-0.489	1				
Vit C	0.333	-0.1620	-0.071	0.002	-0.223	0.011	0.204	0.337	0.034	0.283	-0.230	0.054	0.289	0.055	-0.321	0.226	1			
Vit A	0.099	0.1630	0.169	0.020	-0.254	-0.070	0.416	0.494	-0.134	0.376	-0.310	-0.037	0.152	0.065	-0.399	0.419	0.722*	1		
Zn	-0.074	-0.0580	0.095	-0.112	-0.285	-0.324	0.261	0.094	-0.121	0.148	0.221	0.194	-0.290	0.299	0.192	-0.04	-0.038	0.135	1	
Vit D	0.107	0.1340	-0.293	0.239	0.308	-0.236	0.151	0.403	-0.254	-0.090	0.167	0.595*	-0.139	0.554*	0.120	0.08	0.064	0.18	0.123	1

Level of significance p<0.05*

Table 6: Principal component analysis

Nutritional composition	First component	Second component	Third component	Fourth component	Fifth component
Moisture	-0.092	0.981	0.044	0.113	-0.117
Ash	0.012	-0.023	0.681	0.372	0.441
Fats	-0.079	-0.033	0.843	-0.213	-0.105
Protein	0.026	-0.024	0.361	-0.351	0.314
Fibre	0.029	-0.653	0.254	0.085	-0.051
Carbohydrate	0.871	-0.396	-0.203	-0.143	0.154
Calcium	-0.075	-0.198	0.168	0.956	0.089
Iron	0.178	0.297	-0.123	0.552	0.417
Potassium	-0.181	0.258	0.411	0.104	0.752
Sodium	-0.128	0.257	0.226	0.477	0.110
Lead	0.050	-0.093	0.007	-0.486	-0.054
Tin	0.156	-0.110	0.020	-0.100	0.696
Zinc	-0.125	0.309	-0.290	0.052	0.058
Magnesium	-0.196	0.402	-0.056	0.252	-0.537
Manganese	0.134	0.018	-0.558	-0.121	0.109
Copper	0.014	-0.006	0.337	-0.494	0.195
Phosphorous	-0.096	0.070	-0.111	-0.044	-0.684
Vitamin A	-0.086	0.149	-0.318	0.165	0.821
Vitamin C	-0.052	0.112	-0.147	0.162	0.627
Vitamin D	-0.181	0.528	0.282	0.157	0.097
Variability (%)	57.120	32.370	3.660	3.270	2.790

Level of significance p<0.05*

Soils of the rainforest and derived savannah zones can be productive when supplemented with lime or fertilizer. Soils of mangrove swamp fit into the soil order Entisols[31,32].

Entisols are soils of unstable environments, such as floodplains, sand dunes or steep slopes. In floodplains, flowing floodwater leaves behind clay, silt and sand when it runs off. These deposits are known as alluvium[33]. According to Thompson[34], alluvial soil is rich in minerals and nutrients. It is highly fertile and is a good crop soil. Thus, the soils of Ondo State might have contributed to the high nutritional content of accession 14.

The range of the ash contents of the accessions (1.866±0.002 to 2.637±0.002%) was similar to the values obtained by Ekop[28], Alinnor and Oze[35] and Akpabio *et al.*[36] for *Gnetum africanum* (1.20±0.01%), *Pentaclethra macrophylla* (2.95±0.02) and *Chrysophyllum albidum* (2.00%), respectively but lower than the value (5.09%) obtained by Oloye *et al.*[15] for *C. olitorius* seed flour. According to Akinhami *et al.*[37], for seed ash contents to be of value to livestock, they should fall within a range of 1.0-2.5%; thus, the *C. olitorius* seeds used in this study could potentially be conveniently incorporated into livestock feeds.

The fat and carbohydrate contents recorded in the present study were higher than those obtained by Onwordi *et al.*[38] from the leaves of *C. olitorius*. On the other hand, Mensah *et al.*[39] reported higher values of carbohydrates, protein, moisture and fibre in the leaves of *C. olitorius*. The palatability of a plant is derived from its fat content, as fats retain flavours. Fats in the diet promote the absorption of fat-soluble vitamins and provide high calorific energy[40]. Carbohydrates are also sources of energy and they reduce the wastage of proteins[41]. The values obtained in this study (59.91-87.34%) comparable favourably with those reported for *Gnetum africanum* (87.62%)[28] and *Chrysophyllum albidum* (83.38%)[36].

The protein content of the seeds was quite low, far lower than that shown by Onwordi *et al.*[38] for *C. olitorius* leaves and Oloye *et al.*[15] for *C. olitorius* seed flour. The present results suggest that the seeds of the 14 accessions are poor sources of protein.

The observed range of fibre contents of 1.28-1.91% can be compared with the values obtained by Elinge *et al.*[42] for *Cucurbita pepo*. However, the range obtained in this study was lower than the recommended daily allowances of fibre for children, adults, pregnant women and lactating mothers, which are 19-25, 21-38 and 28-29%, respectively, as reported by Hussain *et al.*[43]. The same report noted that a high fibre content can cause intestinal irritation, especially in weaning children, which lowers nutrient bioavailability. Eromosele *et al.*[44] also reported that high fibre intake can aggravate gut disturbances in young animals such as piglets and chicks.

The concentrations of the minerals calcium, iron and zinc detected in the present study were similar to the values obtained by Ndlovu and Afolayan[45] for *C. olitorius* leaves. The calcium and iron values obtained by Kamga *et al.*[46], also for *C. olitorius* leaves, compare favourably with the values obtained in this study, even though they were lower. Conversely, Idirs *et al.*[10] obtained very high calcium values from the leaves of *C. olitorius*. According to Payne (1990), cited by Elinge *et al.*[42], "Calcium helps to ease insomnia and to regulate the passage of nutrients through cell walls. In the absence of calcium, the nerves, muscles and blood clotting mechanism do not function normally and the dentition of both children and adults are affected"[47].

The iron content of the studied accessions (1.23-1.62 mg/100 g) was observed to fall within the recommended daily allowance of 0.27-27 mg g^{-1} [48]. Shahid *et al.*[49] reported that *C. olitorius* leaves have a higher iron content than eggplant and spinach leaves. Thus, Kamga *et al.*[46] concluded that vulnerable groups, such as children under five and lactating mothers, can benefit greatly from consuming this vegetable. The many vital functions of iron in the human body, according to the FAO. and WHO[50], include serving as (1) An oxygen carrier to the tissues, as iron is the primary component of haemoglobin, (2) a transport medium for electrons within cells and (3) an integrated component of cytochrome enzymes, which help to synthesize steroid hormones and bile acids, detoxify foreign substances in the liver and participate in the signal regulation of certain neurotransmitters, such as the dopamine and serotonin systems in the brain.

The levels of zinc observed in this study were higher than those reported by Ndlovu and Afolayan[45] and Onwordi *et al.*[38] for *C. olitorius* leaves. Akpabio *et al.*[36] noted that zinc is widely distributed in plant and animal tissues. According to the FAO. and WHO[50], zinc is an essential component of more than 300 enzymes that participate in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids as well as the metabolism of other micronutrients. Brar *et al.*[51], citing Welch (1986), noted that the contents of iron and zinc in crops depend on the efficiency with which minerals are translocated from the roots to the edible organs of the plant and the subsequent accumulation of the minerals in those organs. The authors further listed the factors that determine variability in iron and zinc contents in seeds, which include phloem sap loading, translocation and unloading rates within the reproductive organs.

The magnesium, phosphorus, potassium and sodium contents of the 14 accessions were lower than those observed by Nzikou *et al.*[52] and Mensah *et al.*[39]. These minerals have been reported to participate in enzymatic activity and improve the poor electrolyte balance of fluids. Elinge *et al.*[42] noted that parathyroid hormone cannot function maximally in the absence of magnesium. The authors further reported that magnesium is indispensable in tissue respiration.

The ratios of sodium to potassium (Na/K) obtained in this study were within acceptable limits. According to Yusuf *et al.*[53], a proper ratio of Na/K is absolutely necessary because it is associated with the control of high blood pressure. The authors also noted that a Na/K ratio of less than 1 is recommended; therefore, *C. olitorius* seeds, which exhibit a ratio of less than 1, should be highly desired, as they can aid in blood pressure control.

Furthermore, the ratios of calcium to phosphorus (Ca/P) found in this study were also within acceptable limits. The concept of the Ca/P ratio, according to Yusuf *et al.*[53], was brought into the limelight by Shills and Young (1988), who observed that animal proteins and phosphorus-rich foods promote the loss of calcium in urine and therefore cause a decrease in bone calcium levels. A food item is considered optimal if its Ca/P ratio is above one and poor if its ratio is below 0.5[54]. All of the accessions of *C. olitorius* assessed in this study are excellent sources of bone calcium, as they all exhibited Ca/P ratios greater than one.

Heavy metals, such as lead, are major contaminants of the environment[55]. The lead levels observed in this study (0.150-0.274 mg/100 g) were higher than those observed by Asaolu and Asaolu[56] for *C. olitorius* leaves (0.05 mg g^{-1}). However, the concentrations observed in this study were lower than the acceptable range reported by the World Health Organization[57].

Kinser[58] noted that many minerals interfere with the utilization of other minerals and, uniquely, the effect on copper appears to be specific. The levels of molybdenum, sulphur and iron in both an individual's diet and the water they consume can affect the availability of copper[58]. The authors further noted that 'research in recent times has shown that trace mineral deficiencies have a negative effect on the immune system, leading to health problems and failure of commonly accepted disease treatments.'

The 14 accessions were found to vary in vitamin A content, suggesting a strong influence of genetic and environmental factors, or both. According to the FAO. and WHO[50], vitamin A is an essential nutrient that is needed in minute quantities for healthy human vision, growth and development, maintenance of epithelial cellular integrity, immune function and reproduction. Vitamins C and D did not vary considerably among the accessions. Insufficient vitamin C leads to a human deficiency disease known as scurvy; since humans, as well as many other species, cannot synthesize vitamin C, they depend wholly on the dietary supply of this vitamin. The occurrence of scurvy can be prevented by the intake of 6.5-10 mg per day of vitamin C[50]. The accessions studied in this work, however, exhibited very low levels of vitamin C. The highest vitamin C level observed was

0.88 mg/100 g. The vitamin C content of plants can be influenced by both their genotype and environment, as noted by Walker *et al.*[59].

Vitamin D functions to (1) Maintain normal blood levels of calcium and phosphate, which are necessary for the normal mineralization of bone, muscle contraction, nerve conduction and general cellular functions in all cells of the body[50] and (2) Modulate cell growth, neuromuscular and immune function and reduce inflammation[60]. According to the National Institute of Health[60], a serum concentration of vitamin D of less than 12 ng mL^{-1} (0.012 µg) is associated with vitamin D deficiency, which leads to rickets in infants and children and osteomalacia in adults. The accessions analysed in this study were shown to be good sources of vitamin D, as their vitamin D content ranged from 0.357-0.394 µg.

A moderate correlation observed in the data indicated that 36, 31, 52 and 35% of the variation in potassium, vitamin D, vitamin A and vitamin D, respectively, could be accounted for by variations in ash, moisture, vitamin C and sodium, respectively. A moderate negative correlation also indicated that 37, 33 and 58% of the variation in moisture, phosphorous and manganese, respectively, could be accounted for by variations in fibre, potassium and manganese, respectively. Seventy-nine percent of the variation in the ash composition was shown to be accounted for by variation in the alkali content of Musa species[61]. The potassium-ash correlation could potentially explain how the ash composition affects variations in the mineral composition of plants[62]. The moisture-fibre correlation suggests a possible explanation for the variation in the fibre composition observed among the seeds of the accessions examined in this study.

PCA revealed that carbohydrates exhibited the highest variability (57.22%) in the first component, followed by moisture (32.37%) in the second component, suggesting that the carbohydrate content could be a major determinant or an indicator of nutritional variability among these 14 accessions, followed by the moisture content. Carbohydrate content, as a major determinant, explains the differences between A_3 and the other thirteen accessions.

CONCLUSION

This study demonstrated that *C. olitorius* seeds could potentially serve as a good source of food as well as a source of feed for the livestock industry due to the nutritional content of these seeds. However, nutritionists should take into consideration the antinutrients in the leaves and seeds of *C. olitorius* [63-65]. Such antinutrients include cyanogenic glycosides, oxalates, phytic acid, tannins and alkaloids. The only antinutrients that were observed at significant levels by the above mentioned authors were cyanogenic glycosides. The other antinutrients were within acceptable limits.

SIGNIFICANCE STATEMENT

This study found that seeds of *C. olitorius* have the potential to be incorporated into the human diet and livestock feed. This study also provides more comprehensive information, beyond what exists in the literature, on the nutritional compositions of the seeds of *C. olitorius*. These results may encourage researchers to evaluate seeds of other local and neglected leafy vegetables with the aim of diversifying sources of food and feed. Knowledge of the variability in nutrient contents among the accessions may be very useful to plant breeders in designing programmes for the improvement of *C. olitorius*. However, further studies are warranted to ascertain the relative contributions of genotype and environment to the observed variability. This information will be indispensable for designing breeding programmes.

Stored seeds in a seed bank were used in this study; thus, it is recommended that freshly harvested seeds of these accessions be evaluated for comparison with the results obtained here.

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