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Nutritional evaluation of some common leafy vegetables in Enugu, Nigeria

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Abstract
Telfairia occidentalis, *Amaranthus hybridus* and *Ocimum gratissimum* are commonly consumed vegetables amongst the study population, there is poor information on their nutritional content. We therefore, in this study evaluated their nutritional status, to provide information on their content and reasons to support their continued consumption. Vegetable samples were randomly collected and analysed using standard methods. On the average *A. hybridus* contains 2.47 of crude protein, 7.63 of crude fibre, 9.67 of ash and 2.09 of fat, while *Ocimum gratissimum* contains 2.226 of crude protein, 7.156 of crude fibre, 8.70 of ash and 1.92 of fat. Similarly, *O. gratissimum* contains 2.861 of crude protein, 7.806 of crude fibre, 10.619 of ash, 2.380 of fat; all values in ppm. All three were found to be high in iron, as well as copper and zinc. They also contain small amounts of both essential and non-essential amino acids. The results of this study present the nutritional contents of these leafy vegetables; and our findings support their increased cultivation, consumption and commercialization.

Keywords: *Telfairia occidentalis*, *Amaranthus hybridus Ocimum gratissimum*, leafy vegetables, Enugu.

1. Introduction
Vegetable consumption amongst many populations especially in developing countries has been attributed to its common availability, and being a cheap source of quality protein, minerals, vitamins and roughages[1,2]. The nutritive and non-nutritive contents of the vegetables are essential for normal development and healthy living if supplied in appropriate amounts, thereby playing significant roles in human nutrition [3]. Experimental reports have shown that vegetables contain a number of minerals and vitamins that are very important in the human metabolic processes [4]. The highly desired vegetables associated micronutrients, as well as the dietary fibres that are important in the prevention of chronic and lifestyle diseases, can be obtained from the consumption of many leafy vegetables [5]. Research findings have shown that some tropical vegetables contain as much as 1% dry weight of minerals and more than 90% of antioxidants; appreciable percentage of crude protein, crude lipids and carbohydrates [6-8], the constituents, which some compared significantly well to those found in lettuce and cabbage [9]. However, lack of information on their nutritional advantages have over the years been ignored, hence abandoning them for the poor and rural dwellers [10, 11]. Though, consumption of some tropical leafy vegetables is currently being encouraged globally because of their appreciable content of micronutrients and other bio-active compounds. For example, *Amaranthus* presently is seen as a promising food crop mainly due to its high nutritional value and being rich in proteins and micronutrients; also being used as medicine to heal many diseases in African communities [12].
Telfairia occidentalis, *Amaranthus hybridus* and *Ocimum gratissimum* are described as staple vegetables amongst the study population. Despite this, a definitive study has not been carried out to itemise the nutritive values of these vegetables in this area. Considering the variations in the concentrations of vitamins and minerals found in vegetables as a result of differences in the regions of cultivation [13], (for instance, Enugu seats on coal), levels of vitamins and mineral contents of vegetables vary significantly from region to region due to different nature of soil types. However, significant differences in nutrient contents could also be due to differences in cultivars [14]. As these vegetables are consumed in large amounts in this area, it is important to evaluate their nutritive constituents to appreciate how much they contribute to the diet of the populace to justify their staple consumption. The evaluation will also give proper information if need be for more viable alternatives. Also, the continued consumption of these vegetables amongst the populace should be based on experimental studies to provide scientific evidence to support their regular consumption as staple vegetables.
We, therefore, in this study aimed to determine the proximate composition, mineral and amino acid contents of the vegetables: *Telfairia occidentalis*, *Ocimum gratissimum* and *Amaranthus hybridus* to justify their high consumption among the study population.

2 Materials and methods
2.1 Collection and treatment of samples
Leaves of *Telfaira occidentalis*, *Amarathus hybridus* and *Ocimum gratissimum* were randomly bought from 7 different markets (Artisan, Coal Camp, Polo Market, Ogbete main market, New market, Abakpa market and Kenyetta), all in Enugu metropolis. The vegetables sold in the markets are sourced from Enugu metropolis and surrounding towns and villages of Enugu state. The three vegetables were bought at each location randomly for about 6 weeks during the rainy season. The vegetables were exposed to air in a laboratory condition for a day before being oven dried at 40°C, 5hrs for two days with a Gallenkamp oven. The crispy leaves were ground into fine powder using pestle and mortar and each sample kept separate for analysis.
2.2 Proximate analysis
The recommended methods [15] were used to determine the moisture content and crude fibre. Amino acid content was

estimated using amino acid analyser, while the crude protein was determined by Kjeldahl nitrogen using (N x 6.25) as the conversion factor. Minerals were determined by wet digestion and using atomic absorption spectrophotometer.

2.3. Moisture content

The moisture content was determined using the Standard Official Methods of Analysis [16]. This involved drying to a constant weight at 100-102^{0}C and calculating moisture as the loss in weight of the dried samples. The percentage moisture content was calculated as loss in weight of the original sample.

Calculation:

Moisture (%) = (B-C)-(D-C)/A x 100

Where A = weight of sample to be determined (g)

B = weight of moisture dish + sample before oven drying (g)

C = weight of moisture dish (g)

D = weight of moisture dish + Dry sample after drying (g)

2.4 Ash determination

The ash content was determined using the earlier described method [16]. An empty crucible was fire-polished in a muffle furnace and allowed to cool in a desiccator containing calcium chloride for 20 minutes and then weighed. About 2.0 g of dried sample was weighed out into the crucibles and transferred into a muffle furnace at 650^{0}C for 3 hours for complete ashing. The crucible was removed from the muffle furnace, placed in a desiccator and allowed to cool after which it was re-weighed to get the final weight.

Calculation

The percentage (%) ash content of the samples was then calculated as:

X-Y/W x 100

Where X = weight of crucible + ash

Y = weight of crucible

W = weight of sample to be determined in (g) before ashing.

2.5 Crude fat determination

The crude fat was determined using the Soxhlet Extraction Method [16]. A 250ml round bottom flask was washed and dried in an oven at 60^{0}C for 25 minutes. It was subsequently allowed to cool at room temperature before it was weighed. Approximately 10.0 g of sample was then weighed and wrapped in a thimble. This was inserted into the extraction column with the condenser connection. Two hundred (200) ml of the extracting solvent (petroleum ether, boiling point 60-80^{0}C) was poured into the round bottom flask and fitted into the extraction unit. The flask was then heated with the aid of electro thermal heater at 60^{0}C for 2 hours. Losses of solvent due to heating were prevented with the aid of the condenser so that it cooled and refluxed the evaporated solvent. After extraction, the thimble was removed and the solvent salvaged by distillation. The flask and its content were left in the oven overnight at a low temperature to completely evaporate the solvent and the residue was weighed to obtain the percentage crude fat.

Calculation:

Percentage Crude Fat = Weight of Fat (g)/Weight of Sample (g) x 100

2.6 Protein content

The crude protein content of the samples was determined using the Microkjeldahl method [16]. The summary of the whole process can be summed up as digestion, distillation and titration. Approximately 1.0 g of each dried sample was digested with 25.0 ml concentrated H_2SO4, using a mixture of sodium sulphate and copper sulphate pentahydrate as catalysts in the ratio of 10:1. These were transferred into Kjeldahl flasks, each with four antibumping chips (Protein free) added to prevent sticking of the mixture to the flask during digestion and to enhance boiling. This was heated with an electrothermal heater at a temperature of 107^{0}C in a fume cupboard. Heating was continued until frothing ceased and the colour of the mixture changed to a clear solution. The digested sample was transferred into a 50.0 ml volumetric flask and made up to mark with distilled water. About 10.0 ml of 2% boric acid was added into a 200ml beaker and 2 drops of double indicator (methylene blue / methyl red) was added. Approximately 20.0 ml of the digested sample was added into a 150 ml distillation flask. The Markham distillation unit was set up. Approximately 20.0 ml of 4% NaOH solution was introduced with the aid of the pipette into the flask. This was to allow for exhaustive distillation and to ensure that most of the ammonia liberated was trapped by boric acid. Then the green coloured ammonium borate was titrated with 0.1 N HCl. The colour change to pink marked the end of the titration and the volume of acid used (the titre value) was recorded alongside the percentage nitrogen.

Calculation:

The percentage nitrogen is calculated as follows:

Percentage Nitrogen = 14 x V/100 x 0.1 x W/100

Where V = (ml of 0.1N acid added) - (ml of 0.1 N NaOH used to neutralize the ammonia nitrogen)

W = sample weight (g)

Thus, the total crude protein calculated as: Percentage Nitrogen x 6.25

2.7 Crude fibre content

Crude fibre is the organic residue left after the defatted material has been treated with boiling dilute [H_2SO_4] solution, boiling sodium hydroxide solution, dilute hydrochloric acid, alcohol and ether. Crude fibre was determined in the sample using the standard methods of analysis [16]. A conical flask (about a litre) was washed and dried in an oven for an hour and allowed to cool at room temperature. About 3.0 g of the dried sample was weighed and wrapped in a thimble. The weighed sample was defatted using the Soxhlet extraction technique. Approximately 200 ml of boiling 0.25M [H_2SO_4] was added and the flask placed on a hot plate to heat to boiling as quickly as possible. A funnel of about 10cm diameter was placed on the mouth of the flask to lessen evaporation. The heating was controlled in order to ensure that gentle ebullition was maintained and continued for 30 minutes. Antifoam was added to reduce excessive frothing and boiling water was added to maintain the volume. A Buchner flask and funnel were connected via a trap to a vacuum pump, and a Whatman filter paper was placed in the funnel, filled with hot water. At the end of the boiling period, the flask was removed from the heat source and allowed to settle a few seconds. The content was decanted through the Buchner

funnel using gentle suction such that the funnel was not allowed to empty completely until most of the flask contents were transferred. The residue was allowed to air-dry and then the paper was removed and opened. The content was carefully transferred to a clean crucible with the aid of a spatula. The residue was dried in the oven at 500^0C for 2 hours, cooled and reweighed. The loss in weight represents the fibre content.

Calculation:

The loss in weight was calculated as

Percentage Crude Fibre = Loss in Weight from Incineration/Weight of Sample before x 100.

2.8 Total Carbohydrate content

This method involved adding up the percentage values of crude protein, crude fat, crude fibre, moisture and ash constituents of the sample, and subtracting this total from 100. The value obtained is the percentage carbohydrate constituent of the sample.

2.9 Determination of mineral elements

This analysis was carried out at the Analytical Services Laboratory, using standard protocols as outlined below.

a. Determination of calcium, sodium, magnesium and potassium by atomic absorption spectrophotometry About 1.0g of the sample was first digested with 20ml of an acid mixture (650ml conc. HNO_3, 80ml perchloric acid and 20ml H_2SO_4) by weighing the sample into a digestion flask followed by addition of 20ml of the acid mixture. The digestion flask containing the sample and the digestion acid mixture was heated until a clear digest was obtained. The digest was later diluted with distilled water to the 500ml mark. After obtaining the digest, aliquots were used for atomic absorption spectrophotometry, measuring at the specific filter for each element. The concentration of each element was determined using their calibration curve prepared with its standard solution. The percentage values were later calculated by multiplying the concentrations by 100

b. Determination of phosphorous by molybdate method

0.5ml of the mineral digest and 9.5ml of 10% trichloroacetic acid were mixed in a test tube. This was followed by agitation for 5 minutes and then filtration through a filter paper. 5ml of the filtrate was then measured into a cuvette. Also, 5ml of the trichloroacetic acid and 5ml of the working standard were each measured into cuvettes to serve as blank and the standard solution, respectively. 0.5ml of the molybdate reagent was then added to each cuvette, mixed and allowed to stand for 10 minutes. The absorbance of the test and the standard solutions were read in a spectrophotometer at 660nm against the blank.

Calculation:

$$P\ \% = \frac{A_T . C . 100}{A_s}$$

Where P = Protein

A_T = Absorbance of test

C = Conc. of standard.

A_s = Absorbance of standard

3 Results and Discussion

The results of the mineral elements determined (Table 1); the amino acid content determined (Table 2), and the values from proximate analysis of the three leafy vegetables studied (Table 3) are presented.

High values of Potassium, Calcium, Magnesium, Sodium and Zinc were reportedly found in the leaves of *O. gratissimum* [17]. However, these published concentrations (in mg/100g) are multiple folds higher than the values that we have found in this study (Table 1). Another report found lower concentrations of these elements than we have found in the leaves of *O. gratissimum* [18]. The reasons for the wide variation in these published values are not clear, though simple conversion errors could be a factor. Similarly, a multiple fold higher Potassium concentration was published for *T. occidentalis* than the value obtained in this study (Table 1) while concentrations that are multiple folds lower than the values obtained in this study were found for Sodium and Zinc [19]. Similar values to the concentrations found in this study were however published for Calcium and Magnesium [19]. It is however important to note that the mineral content of *A. hybridus* was found to vary widely depending on the harvesting stage [20]. This could also be a factor contributing to the variations in mineral content reported for *O. gratissimum* and *T. occidentalis*.

A report was only able to detect 17 amino acids in *A. hybridus*, with glutamic acid being the most abundant, and the concentration found for each of the 17 amino acids in *A. hybridus* [21] is multiple times higher than the values found in this study. Another report detected 15 standard amino acids, with leucine as the most abundant [22], while a third report detected 18 standard amino acids, with aspartic acid as the most abundant [23]. We detected all the 20 amino acids in *A. hybridus*, with isoleucine as the most abundant (table 2). Similarly, 18 amino acids were detected in a report that found glutamic acid as the most abundant amino acid in the leaves of *T. occidentalis* [24]. Another report was able to detect the 20 standard amino acids in *T. occidentalis*, with histidine as the most abundant, while demonstrating that growing *T. occidentalis* in a hydroponic medium yielded more of each amino acid than when grown in a geoponic medium [23]. We detected all the 20 standard amino acids in the leaves of *T. occidentalis*, with phenylalanine as the most abundant (Table 2).

There has been a number of studies on leafy vegetables as concerns their vitamins and minerals contents [25-29]. Fewer studies [30], considered the amino acid content of these vegetables. This may be in response to the perceived shift of interest from protein to micronutrient malnutrition in the global health programme [31]. This reduction of interest however overlooks the fact that at least 30 % of children globally have protein-energy malnutrition [32]. This study presents the mineral content of three African leafy vegetables namely *Ocimum gratissimum, Telfairia occidentalis* and *Amaranthus hybridus*, and in addition presents findings that suggest that these vegetables are low to moderate potential sources of essential and non-essential amino acids.

Deficiencies of iron and zinc cause significant public health burden globally [27]. Of the 800 million women and children diagnosed with anaemia in 2011, the proportion due to iron deficiency was found to be 42% of the children and 50% of the women of the study population [33]. The amount of iron in the leafy vegetables was 44.23 mg/kg, 45.88 mg/kg and 40.58 mg/kg (table 1) in *Ocimum gratissimum, Telfairia occidentalis* and *Amaranthus hybridus*, respectively. These values are each higher than the reference iron intake for a pre-menopausal adult female which is 14.8 mg/day [34]. Since the adult female requires more iron intake than all

other age groups, the three vegetables studied, therefore each contains enough iron in 1 kg to meet the reference iron intake of all human age groups. The highest reference copper intake is 1.2 mg /day by adult male or female while the reference zinc intake of 9.5 mg/day for the adult sexually active male is also the highest of all age groups [34]. Table 1 shows that the vegetables studied could meet the daily requirement for both copper and zinc if the appropriate amount of any of the vegetables is consumed. These vegetables are therefore potential sources of these minerals as well as other mineral nutrients including calcium, magnesium, potassium and phosphorus (table 1), which are contained in smaller amounts in (compared to their reference intake values) in these vegetables. However, these minerals can still contribute in making up the reference intake when they are taken as part of a balanced diet. Calcium, potassium and magnesium are required for repair of worn out cells, strong bones and teeth in humans, building of red blood cells and for body mechanisms [35].

The vegetables each contains above 2 mg of crude protein per kilogram of the leafy vegetable (table 3). The amount of amino acids found in the leafy vegetables is shown in table 2. *Ocimum gratissimum*, *Telfairia occidentalis* and *Amaranthus hybridus* were found to contain small amounts of both the essential and non-essential amino acids, (table 2). Essential amino acids cannot be produced in the body, and as such must be obtained from the diet. These leafy vegetables can complement other sources of essential amino acids in the diet. Also, since these vegetables are relatively easy to grow in large quantities, they can serve as cheap sources of these amino acids.

This study presents the nutritional constituents of three locally available leafy vegetables consumed by the residents of Enugu, Nigeria. The results presented here will provide information to the nutritionists on the usefulness of these vegetables for human nutrition.

Table 1: The range, Mean±SD (PPM or mg/kg) and coefficient (%) of variation of the nutritive minerals of *T.occidentalis*, *A. hybridus* and *O. gratissimum*

Mineral	*A. hybridus*			O. gratissimum			*T.occidentalis*		
	Range	**Mean±SD**	**CoV (%)**	**Range**	**Mean±SD**	**CoV (%)**	**Range**	**Mean±SD**	**CoV (%)**
Sodium	19.52-34.82	26.050±4.78	18.3	20.08-35.90	29.30±4.770	16.3	18.96-32.60	25.490±4.37	17.1
Manganese	8.30-13.20	11.010±1.37	12.4	8.83-13.84	11.11±2.020	18.2	7.38-11.67	9.800±1.20	12.2
Iron	30.07-50.92	40.580±6.69	16.5	37.65-57.18	44.23±5.860	13.2	37.57-53.43	45.880±5.22	11.4
Zinc	4.82-9.57	6.610±1.530	23.1	4.40-8.41	6.21±1.090	17.5	5.40-9.26	7.630±1.36	17.8
Copper	0.47-0.74	0.613±0.795	12.9	0.50-0.08	0.676±0.117	17.3	0.60-0.86	0.667±0.10	13.1
Calcium	0.79-1.46	1.002±0.229	22.9	0.78-1.45	1.073±0.204	18.9	0.80-1.38	1.054±0.21	20.0
Magnesium	0.44-0.76	0.578±0.093	16.1	0.49-0.61	0.547±0.043	07.9	0.47-0.73	0.621±0.08	13.6
Potassium	0.23-0.36	0.294±0.417	14.2	0.20-0.32	0.258±0.039	15.2	0.28-0.46	0.341±0.05	14.5
Phosphorus	0.48-0.69	0.573±0.077	13.6	0.43-0.60	0.538±0.051	09.5	0.39-0.51	0.452±0.44	09.8

Table 2: Range, Mean ± SD (PPM or mg/kg) and coefficient of variation (%) of the amino acid constituents of the vegetables

	A. hybridus			O. gratissimum			*T.occidentalis*		
Amino Acid	Range	Mean/SD	CoV (%)	Range	Mean/SD	CoV (%)	Range	Mean/SD	CoV (%)
Phenylalanine	3.32-7.76	5.41±1.36	25.1	4.78-8.21	6.41±1.04	16.2	5.66-8.50	6.84±0.91	13.3
Threonine	1.10-2.82	1.88±0.53	28.1	1.08-3.41	2.16±0.57	26.4	1.64-2.75	2.05±0.27	13.3
Valine	1.53-4.49	3.29±1.01	30.8	2.87-5.02	4.17±0.53	12.7	3.52-5.34	4.41±0.64	14.4
Tryptophan	2.73-5.46	4.07±0.77	18.9	2.84-5.06	3.95±0.69	17.5	1.99-4.28	3.22±0.62	19.3
Isoleucine	5.85-8.18	7.01±0.81	11.6	4.99-7.52	6.33±0.75	11.8	4.99-7.83	6.07±0.74	12.1
Methionine	1.60-5.42	3.32±1.23	3.71	2.87-8.49	4.69±2.17	46.2	2.73-4.33	3.47±0.60	17.4
Histidine	2.54-9.21	5.81±2.09	36.2	3.96-7.16	5.05±1.00	19.9	4.96-7.64	5.74±0.94	16.3
Arginine	3.23-6.09	5.24±1.61	30.7	3.76-3.73	5.97±0.99	16.6	3.46-7.09	5.13±1.00	19.5
Lysine	2.69-9.21	5.75±1.92	33.5	3.87-8.28	5.42±1.36	25.1	3.89-6.16	4.94±0.76	15.4
Leucine	3.13-5.28	5.41±1.11	20.5	4.43-7.51	5.99±0.91	15.2	5.81-7.28	6.54±0.58	08.8
Cysteine	1.16-5.28	2.34±1.02	43.8	1.77-6.15	3.82±1.68	43.9	1.80-2.18	1.96±0.16	07.9
Alanine	3.54-5.86	4.79±0.74	15.4	4.56-7.14	5.46±0.71	13.0	3.21-5.44	4.62±0.72	15.5
Tyrosine	1.40-2.33	1.89±0.32	16.7	1.25-2.44	2.04±0.32	15.8	1.82-2.18	1.99±1.14	05.7
Glycine	3.77-5.77	4.65±0.65	14.0	3.77-5.30	4.44±0.51	11.6	3.67-6.15	4.80±0.76	15.9
Serine	3.19-5.82	4.28±0.86	20.1	3.13-6.31	4.82±1.09	22.7	4.87-8.31	6.09±0.99	16.4
Aspartic	3.34-6.07	4.79±0.86	17.9	3.83-6.15	4.81±0.74	15.5	3.78-5.30	4.66±0.55	11.9
Glutamic	2.83-5.28	4.10±1.05	25.5	1.87-5.18	3.36±1.34	39.9	1.82-2.42	2.05±0.19	09.6
Asparagine	1.49-2.25	1.88±0.20	10.7	1.14-2.18	1.82±0.34	18.4	1.90-2.18	2.07±0.09	04.5
Glutamate	1.12-2.24	1.91±0.36	18.8	1.38-2.29	2.04±0.28	13.5	1.82-2.87	2.15±0.29	13.3
Proline	3.82-5.09	4.38±0.46	10.5	3.88-5.27	4.53±0.54	12.0	3.88-6.30	4.92±0.72	14.6

Table 3: Proximate composition of leaves of *Amaranthus hybridus*, Ocimum gratissimum and *Telfairia occidentalis* (Mean±SD in PPM or mg/kg, and Coefficient of variance in %).

	Amaranthus hybridus		*Ocimum gratisimum*		*Telfairia occidentalis*	
	Mean±SD	CoV (%)	Mean±SD	CoV (%)	Mean±SD	CoV (%)
Moisture	15.038±2.486	16.5	16.113±2.970	18.4	17.318±1.849	10.7
Crude protein	2.471±0.718	29.0	2.226±0.794	35.7	2.861±0.542	18.9
Crude fibre	7.638±1.300	17.0	7.516±1.174	15.6	7.806±1.298	16.6
Ash	9.670±1.641	17.0	8.700±1.491	17.1	10.619±1.282	12.1
Fat	2.019±0.485	24.0	1.920±0.223	11.6	2.380±0.535	22.5
Free Nitrogen extract	63.102±3.72	05.9	63.53±2.66	04.2	59.02±3.55	06.0
Total Carbohydrate	0.062±0.0	0.0	0.0±0.0	0.0	0.0±0.0	0.0

5 Conclusions

Leafy vegetables such as *Ocimum gratissimum*, *Telfairia occidentalis* and *Amaranthus hybridus* can act as supplementary sources of essential nutrients such as minerals and amino acids. Their consumption as part of the diet is justified by the results of this study.

6 Competing interests: The authors declare no conflict of interest.

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