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Proximate and Mineral Composition analysis of the leaves of *Gongronema latifolium* and *Piper guineense*

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Abstract
Gongronema latifolium and Piper guineense are two leafy vegetables commonly consumed in south eastern region of Nigeria. The leaves were analyzed separately using standard analytical techniques for proximate and mineral compositions. The proximate results showed high levels of protein: 26.0-24.8 g/100g and carbohydrate: 39.0-41.2 g/100g. Crude fibre was also of significant value in both samples with low levels of crude fat. The total metabolizable energy ranged from 1275-1281 kJ/100g (304-306 kCal/100g). The highest percentage energy contribution was due to carbohydrate (52.0-54.7%). The least contribution was made by fat (12.4-13.3%). The following minerals were highly abundant (mg/100g): Ca (297-304), Mg (513-548) and K (659-682). Reported in trace amounts were (mg/100g): Co (1.1 e-3-1.3 e-3), Se (1.5 e-3-2.1 e-3), Cd (8.1 e-4-1.0 e-3), Ni (3.6 e-3-4.1 e-3) and Pb (1.6 e-3-1.8 e-3). Most of the mineral ratios especially Ca/P and Na/K would promote good health. Results of the mineral safety index indicated that most of the minerals were within the normal ranges. Statistical analyses revealed low levels of coefficient of variation percent (CV%) showing high level of closeness of the samples. Correlation coefficient values were positive and significant with high indices of forecasting efficiency indicating high compatibility of the samples.

Keywords: Proximate, minerals, *Gongronema latifolium*, *Piper guineense*

INTRODUCTION
The nutritional quality, especially the mineral content of vegetables is usually a function of the soil on which they germinated. Environmental pollution was reported to have been responsible for contamination of soil; waste water irrigation had resulted in the significant mixing of heavy metal composition of agricultural lands (Mapanda *et al*., 2005). As a result, crops and various vegetables would absorb these metals from the soil and would eventually get to human tissues after consumption. Because they cannot be easily biodegraded, they get accumulated in human organs resulting in different kinds of illness. Vegetables, especially leafy vegetables have significant roles to play in the health status of human beings. Leafy vegetables could come from varieties of plants but they are similar in most of their nutritional profiles. They are very important in many homes of the developing countries

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such as Nigeria. They are essential items in many Nigerian homes and they are important sources of nutrients especially in rural areas where they contribute significantly to minerals, vitamins and other nutrients which are usually in little supply in daily diets (Mosha and Gaga, 1999). Leafy vegetables may be consumed raw or processed in so many ways before consumption: boiling, steaming, stir-frying, as well as being stewed.*Gongronema latifolium* is a rain forest plant that belongs to the family Asclepiadaceae. It is widely spread in tropical Africa. It is popularly called *utazi* around the south eastern part of Nigeria. *G. latifolium* was reported to have some medicinal values. It is used in south east Nigeria for the treatment of high blood pressure (Mensah *et al*., 2008).*Piper guineense* is a vegetable plant native to tropical regions of Central and Western Africa that is semi-cultivated mainly in southern part of Nigeria (Okwu, 2001). It is a perennial plant, characterized by heart-shaped leaves and clustered reddish brown riped fruits. The leaf is fondly called *uziza* in Igbo land and *Iyere* in Yoruba land.The leaf has peppery taste; it is pale green in colour when fresh and darker green when it is dried. *Piper guineense* leaf is usually used as flavouring agent for stews and other local delicacies.

Nigeria is blessed with various traditional vegetables and each ethnic group is comfortable with certain varieties of vegetables. It is worthy of note that imbalances in the nutritional composition of the food we eat would have significant impacts on our health and physical performance. The presence of anti-nutritional factors and accumulated toxic metals have been the major problems with vegetable consumption. The objective of this research therefore is to investigate the proximate, minerals and mineral safety index of *Gongronema latifolium* and *Piper guineense*. It will further reveal the health implications of their consumption.

MATERIALS AND METHODS

Sample preparation

The two leafy vegetables (*Gongronema latifolium* and *Piper guineense*) were obtained from Aba, south eastern Nigeria. They were separately sorted to remove the bad leaves. They were rinsed with distilled water to remove the dirt and dust. The leaves were carefully later air-dried for six days to avoid loss of essential nutrients and quality. They were dry-milled separately using electric blender and stored in dry plastic containers prior to use.

Sample Analysis

Proximate Analysis

The moisture content was analyzed using oven-drying method as described by Association of Official Analytical Chemists (A.O.A.C., 2005). 3g of the samples was put into petridishes and transferred into the oven set at a temperature of 105^{o}C, dried for three hours. The petridishes were quickly moved to the desiccator to cool and the weight was observed. The process was repeated until a constant weight was obtained. The percentage weight loss during drying was calculated as the percentage moisture content. Ash content was determined by measuring 1g of finely ground sample into clean, dried, pre-weighed crucibles with lid. The crucibles were immediately taken to the muffle furnace set at 550^{o}C . The ashing continued until a light grey ash was obtained. The crucibles were then cooled in a desiccators and weigh was noted. Continuous extraction method was used for fat determination. 1g of the sample was weighed into a weighed filter paper and folded neatly. The filter paper was put inside a pre-weighed thimble. The thimble containing the samples was weighed and put inside Soxhlet apparatus and extraction under reflux was carried out with petroleum ether for 6h. At the end of the extraction, the thimble was dried in the oven for about 30 minutes at 100°C to evaporate the solvent. The thimble was later cooled in a desiccator and then weighed. The fat extracted from the sample was then calculated (A.O.A.C., 2005). For crude fibre determination, 2.0g sample was weighed into 1L conical flask. 200 ml of boiling 1.25% H_2S0_4 was added and boiled gently for 30 minutes. The mixture was filtered through muslin cloth and rinsed well with hot distilled water. The sample was scraped back into the flask with spatula, 200ml boiling 1.25% NaOH was added and allowed to boil gently for 30 minutes, filtered and the residue washed thoroughly with hot distilled water, rinsed once with 10% HCl, twice with industrial methylated spirit and rinsed to drain dry. The residue was later scraped into a crucible, dried in an oven at 105°C, cooled in a desiccator and weighed. The residue was ashed at 550°C for 90 minutes in a muffle furnace, cooled in a desiccator and weighed (Joslyn, 1970).In the determination of crude protein, 1.0g of the sample was weighed into a digestion flask and one tablet of selenium catalyst and 15ml of concentrated H_2SO_4 were added. The mixture was allowed to digest on an electro thermal heater. The flask was made to cool and the solution was diluted with distilled water to 50ml. 10ml of this was transferred into the distillation apparatus. 50ml of 2% boric acid was pipetted into a receiver flask and four drops of screened methyl red indicator were added. 50% NaOH was continually added to the digested sample until the solution turned light yellow. As the process of distillation was still going on, the pink colour solution of the receiver flask changed to blue which indicated the presence of ammonia. The distillation was continued until the content of the round bottom flask was about 50ml. The resulting solution in the conical flask was then titrated with 0.IM HCl (Pearson, 1976).The carbohydrate content was determined as difference between 100 and the sum of the percentages of moisture, ash, fat, fibre and protein content.

% Carbohydrate = 100- (%Moisture+% Ash+% Fibre+ % Fat + % Protein)

Mineral Analysis

Mineral analysis was carried out using the solution obtained by dry-ashing the samples as described above. Ash of the samples was dissolved in 10% HCl, heated, cooled, filtered and made up to the mark in 100ml standard flask with distilled water. The metal contents of the samples were analyzed using atomic absorption spectrophotometer (Buck Scientific Instrument) (Pearson, 1976). Phosphorus was determined calorimetrically using a spectronic 20 (Gallenkamp, London, UK) instrument with KH_2PO_4 as a standard.

RESULTS AND DISCUSSION

Results

The proximate composition of *Gongronema latifolium* and *Piper guineense* leaf samples is given in Table 1. The summary of the results is given as (g/100g): *G. latifolium* - moisture (9.50) crude fat (4.60), crude protein (26.0), carbohydrate (39.0), total ash (8.50), crude fibre (12.4); *P. guineense* - moisture (9.60), crude fat (4.30), crude protein (24.8), carbohydrate (41.2), total ash (8.30), crude fibre (11.8).

Table 1. Proximate composition (%) of *Gongronema latifolium* and *Piper guineense* leaves

Parameter	*G. latifolium*	*P. guineense*	Mean	CV%
Moisture	9.50	9.60	9.55±0.071	0.740
Crude fat	4.60	4.30	4.45±0.212	4.77
Crude protein	26.0	24.8	25.4±0.849	3.34
Carbohydrate	39.0	41.2	40.1±1.56	3.88
Total ash	8.50	8.30	8.40±0.141	1.68
Crude fibre	12.4	11.8	12.1±0.424	3.51

SD = standard deviation, CV% = coefficient of variation percent

Table 2 presents the percentage energy proportions contributed by fat (PEF %), protein (PEP %) and carbohydrate (PEC %) in the samples as well as the utilizable energy due to protein (UEDP %).

Table 2. Percentage energy contributions due to fat, carbohydrate and protein

Pazameter	*G. latifolium*	*P. guineense*	Mean	CV%
Total energy	1275	1281	1278±4.24	0.332
PEF%	13.3	12.4	12.9±0.636	4.93
PEP%	34.7	32.9	33.8±1.27	3.77
PEC%	52.0	54.7	53.4±1.91	3.58
UEDP%	20.8	19.7	20.3±0.778	3.83

PEF% = percentage energy contribution due to fat, PEP% = percentage energy contribution due to protein, PEC% = percentage energy contribution due to carbohydrate, UEDP% = percentage utilizable energy contribution due to protein

The mineral composition of *Gongronema latifolium* and *Piper guineense* leaf samples is shown in Table 3. In both samples, potassium had the highest concentration among the major elements and by extension in all the minerals.

Table 3. Mineral composition (mg/100g) of *Gongronema latifolium* and *Piper guineense* leaf samples

Minerals	*G. latifolium*	*P. guineense*	Mean	CV%
Fe	13.3	9.63	11.5 ± 2.60	22.6
Cu	1.91	1.71	1.81 ± 0.141	7.81
Co	0.0011	0.0013	0.0012±0.0001	11.8
Mn	2.45	1.98	2.22±0.332	15.0
Zn	4.71	5.92	5.32±0.856	16.1
Pb	1.6e-3	1.8e-3	1.7e-3±1.0e-4	8.32
Ca	304	297	301±4.95	1.64
Mg	548	513	531±24.7	4.66
K	659	582	621±54.4	8.77
Na	44.7	35.9	40.3±6.22	15.4
P	119	132	126±9.19	7.30
Se	0.0021	0.0015	0.0018±0.0004	23.6
Cd	0.0008	0.0010	0.0009±0.0001	15.7
Ni	0.0036	0.0041	0.0039±0.0004	9.07

Table 4 presents the calculated mineral ratios of the samples. The following ratios comprising of trace elements were high: Fe/Pb (5350-8313), Ca/Pb (165000-190000), and Zn/Cd (5888-5920). The major mineral ratios were extremely low: Na/K (0.062-0.068), Ca/Mg (0.555-0.579) Na/Mg (0.070-0.082), Ca/K (0.461-0.510) and K/(Ca + Mg) (0.719-0.773).

Table 5 shows the mineral safety index (MSI) of *Gongronema latifolium* and *Piper guineense* leaf samples. Difference (D) was obtained by subtracting the calculated value from the table value and percent difference was obtained by dividing the difference by table value and multiplying by 100.

Table 4. Calculate mineral ratio of *Gongronema latifolium* and *Piper guineense* leaf samples

Minerals	*G. latifolium*	*P. guineense*	Mean	CV%
Ca /P	2.55	2.25	2.40±0.212	8.84
Na/K	0.068	0.062	0.065±0.004	6.53
Na/Mg	0.082	0.070	0.076±0.008	11.2
Ca/Mg	0.555	0.579	0.567±0.017	2.99
Ca/K	0.461	0.510	0.486±0.035	7.13
K/(Ca + Mg)	0.773	0.719	0.746±0.038	5.12
Fe / Cu	6.96	5.63	6.30±0.940	14.9
Fe /Pb	8313	5350	6832±2095	30.7
Ca/Pb	190000	165000	177500±17678	9.96
Zn/Cd	5888	5920	5904±22.6	0.383

Table 5. Mineral safety index (MSI) of *Gongronema latifolium* and *Piper guineense* leaf samples

Mineral	TVof MSI	*G. latifolium* *P. guineense*					
		CV	D	%D	CV	D	%D
Calcium	10.0	2.53	7.47	74.7	2.48	7.53	75.3
Magnesium	15.0	20.6	-5.55	-37.0	19.2	-1.36	-9.07
Sodium	4.80	0.429	4.371	91.1	0.345	4.46	92.8
Copper	33.0	21.0	12.0	36.4	18.8	14.2	43.0
Zinc	33.0	10.4	22.6	68.5	13.0	20.0	60.6
Phosphorus	10.0	0.992	9.01	90.1	1.10	8.90	89.0
Iron	6.70	6.03	0.67	10.0	4.30	2.40	35.8
Seleniun	14.0	0.42	13.6	97.0	0.30	13.7	97.9

TV = table value, CV = calculated value, D = difference, %D = percent difference

Statistical analysis of Tables 1-4 is summarized in Table 6. Parameters determined were linear correlation coefficient, coefficient of determination, linear regression coefficient, coefficient of alienation and index of forecasting efficiency.

Table 6. Statistical analysis of the results (*G. latifolium/P. guineense*)

Statistics	Table 1	Table 2	Table 3	Table 4
r_{xy}	0.9977	0.9973	0.9986	0.9999
r_{xy}^2	0.9954	0.9946	0.9973	0.9998
R_{xy}	1.05	1.09	0.9112	ND
C_A	0.0678	0.0735	0.0520	0.0141
IFE	0.9322	0.9265	0.9480	0.9859
Remark	*	*	*	*

r_{xy} = correlation coefficient, r_{xy}^2 = coefficient of determination, R_{xy} = regression coefficient, C_A = coefficient of alienation, IFE = index of forecasting efficiency, * = results significantly different at n – 2 and $r_{=0.05}$, ND = not determined

DISCUSSION

The results of proximate analysis showed that the crude fat of both samples were relatively low. The fat levels in this study were comparable to the following literature values (g/100g): *Cucurbita maxima* (4.65) *Amaranthusviridis* (4.60) (Adesina and Olaleye, 2017); *Lablab purpureus* (4.57) (Adesina and Olaleye, 2017). The values were however lower than 37.4-43.8 (g/100g) reported for three varieties of melon seeds flour (Adeyeye and Olaleye, 2015) and 19.7 g/100g in *Crysophyllumalbidun* (Adubiaro *et al*., 2017). The protein content levels of the pressent study were found to be high, even higher than what was obtained in most leguminuous samples such as *Vigna subterranean* (18.4 g/100g) (Olaleye *et al*., 2013) and *Prosopisafricana* (23.6 g/100g) (Aremu*et al*., 2006). The protein content is enough to meet the daily protein requirement of human (NRC, 1989). The carbohydrate contents of the samples, though were high but lower than those in most samples of plant source (g/100g): *Triticum durum* (65.7) (Adeyeye and Ajewole, 1992), *Vigna subterranean* (60.8) and *Lablab purpureus* seed flour (61.2) (Olaleye and Olatoye, 2017). The concentrations of crude fibre in this study were higher compared to most literature values (g/100g): *Chrysophyllumalbidum* (3.00) (Adubiaro *et al*., 2017) *Vigna subterranea* (4.90) (Olaleye *et al*., 2013), five different insect samples (3.48-5.31) (Adeyeye and Olaleye, 2016). High levels of crude fibre in this study will help the consumers in facilitating faecal elimination. Low levels of coefficient of variation percent, CV% (0.740-4.77) showed that the results of the analysis were very close.

In the energy contritions, the total energy obtained for the samples, 1275 kg/100g (*Gongronema latifolium*) and 1281 kg/100g (*Piper guineense*) were lower than the following (kJ/100g): African star apple (1613) (Adubiaro *et al*., 2017); adult bee (1541), winged termite (1505), maize weevil (1522) and mopane worm (1545) (Adeyeye and Olaleye, 2016). The percentage energy contributed by fat was least in both samples. This could be due to low concentrations of fat, which is the major contributor of energy in food samples. However, highest contribution was due to carbohydrate. Both PEP% and UEDP% were higher in *Gongronema latifolium* than *Piper guineense* sample.

Mineral analysis showed that sodium contents were low in both samples. However, the concentration of K and Na in the samples were higher than 65.5 mg/100g (K) and 32.2 mg/100g (Na) reported for *Chrysophyllumalbidum* (Adubiaro *et al*., 2017). Both K and Na are needed in the body to regulate the body pH, control glucose absorption and assist protein retention during growth (NRC, 1989). Iron content of this report was 13.3 mg/100g (*G. latifolium*) and 9.63 mg/100g (*P. guineense*). The Fe levels were higher than 4.60-9.30 mg/100g in West Africa edible snails (Adeyeye, 1996), *Zonocerusvariegatus* (3.70 mg/100g) (Olaofe *et al*., 1998) and 0.2 – 0.5 mg/100g in fresh water fishes (Adeyeye, 1994). The human requirement of iron is: 10-15 mg (children); 18 mg (women); 12mg (men) (Fleck, 1976). Iron was reported to facilitate the oxidation of carbohydrates, protein and fats (Adeyeye *et al*., 2014). Zn concentrations in this study were lower than the zinc recommended dietary allowance (RDA) of 15-20 mg per day (Fleck, 1976). Low levels of Zn in this report corroborates the argument of Pew Initiative on Food and Biotechnology (2007) that Zn is one of the trace elements that are deficient in diets. Also low Zn concentration is typical of plant sources; this is because, zinc in plant sources is not as available as animal sources (NAS, 1971). The concentrations of lead (Pb) and cadmium (Cd) in this study were very low at 1.6 e-3–1.8 e-3 mg/100g (Pb) and 8.0 e-4 – 1.0 e-3 mg/100g (Cd). These two toxic elements are not needed at all by the body for any biochemical function and their presence is an indication of pollution. The coefficient of variation percent levels were not too high, indicating the closeness of the results.

The levels of Na/K ratio in the report (Table 4) were far below the critical level of 0.60 and would therefore prevent the development of high blood pressure and by extension prevent hypertension. Ca/P ratios in this study were nutritionally good, far above the recommended minimum of 0.5 for proper absorption of Ca and bone formation (Nieman *et al*., 1992). High levels of toxic mineral ratios (Fe/Pb, Ca/Pb and Zn/Cd) were due to very low concentrations of Pb and Cd.

In the mineral safety index calculation, the standard MSI denoted as TV was obtained from recommended adult intake (RAI) and minimum toxic dose (MTD). Using Ca as an example, RAI value is 1,200 mg whereas MTD is 12,000mg. The standard MSI for Ca therefore equal to MTD/RAI, i.e. 12000/1200 = 10 The same procedure goes for other elements determined. Aside from Mg, all other minerals had their standard values (Table values, TV) greater than the calculated values (CV) in both samples. This means that the concentrations of the minerals would not pose any health risk for the body. This is a welcome development especially for Zn as excess Zn reduces the level of high density lipoprotein (HDL) cholesterol circulating in the blood and this is capable of enhancing the risk of heart diseases.

The statistical interpretations of the results is given in Table 6. The correlation coefficients were high, positive and significant. The values reported for regression coefficient showed that for a unit change in *G. latifolium*, there is corresponding change in *P. guineense*. The coefficients of alienation or the indices of non-relationship (C_A) were relatively low with corresponding high values of index of forecasting efficiency (IFE). This means, the two samples could be exchanged for each other in terms of biochemical performance.

CONCLUSION

The results of this finding revealed that *Gongronema latifolium* and *Piper guineense* leaves are rich in crude protein, crude fibre and carbohydrate. They can be good sources of major minerals especially K, Mg and Ca with trace amounts of Pb and Cd. Virtually most the mineral ratios would promote good health and make the samples safe for consumption. Apart from Mg, all other minerals considered for mineral safety index fell within the United States recommended dietary allowance (USRDA). On a comparative basis, most of the minerals are more concentrated in *Gongronema latifolium* than *Piper guineense*. The statistical interpretations of the results showed high, positive correlation coefficients with high levels of index of forecasting efficiency indicating insignificant errors of prediction.

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