



Research Article

Proximate, Mineral and Phytochemical Composition of *Piper guineense* Seeds and Leaves

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Abstract

Background and Objective: *Piper guineense* seeds and leaves are among the plant parts commonly used as spices and also in herbal medicine for the treatment and management of different conditions. The proximate, mineral and phytochemical composition of *Piper guineense* seeds and leaves were evaluated in this study. **Materials and Methods:** *Piper guineense* seeds and leaves were purchased at the new market in Wukari, Taraba State, Nigeria. The healthy parts were selected, air-dried and pulverized. The proximate parameters were evaluated, while AAS and GC-MS were used for the mineral and phytochemical analysis, respectively. **Results:** Results of the study show that *Piper guineense* seeds was high in percentage dry matter (94.03 ± 0.21), crude lipid (4.06 ± 0.12) and carbohydrates (65.46 ± 0.85) compare to *Piper guineense* leaves, while *Piper guineense* leaves was high in percentage moisture content (6.11 ± 0.01), crude protein (15.17 ± 0.39), crude fibre (20.99 ± 0.16) and ash (11.98 ± 0.03) compared to *Piper guineense* seeds. The differences in the moisture content and dry matter were statistically non-significant, while the differences in the percentage crude protein, crude fiber, crude lipid, ash and carbohydrates were statistically significant. *Piper guineense* leaves has a high amount of magnesium, calcium, manganese and copper than *Piper guineense* seeds, while *Piper guineense* seeds has a high amount of chromium, zinc, iron, potassium, sodium and phosphorus than *Piper guineense* leaves. A wide range of phytochemicals which possess different biochemical and physiological functions were detected in *Piper guineense* seeds and leaves. **Conclusion:** The results showed that *Piper guineense* seeds and leaves possess nutritional and pharmacological properties. However, some of the phytochemicals detected could possess abortive properties.

Key words: Enzymatic reactions, homeostasis, macronutrient, phytochemical, *Piper guineense*, spices

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INTRODUCTION

Different plant materials such as seeds, leaves and fruits are used as food spices, while many of them are believed to possess different medicinal values. Plant materials abound in nutrient, vitamins and minerals which are required by humans on daily basis for proper metabolic and biochemical functions¹. Spices contain several essential compounds like oil and complex mixtures of organic compounds. Many of these plant parts are also used in herbal medicine in the treatment and management of different conditions. *Piper guineense* leaves and seeds belong to this class of plants as the use is already on the increase. Most medicinal plants have been reported to play a vital role in the discovery of drugs and are essential for human to treat different ailments².

Piper guineense is a West African tropical plant³. It belongs to the family *Piperaceae*. The plant is a climbing vine that can grow up to 20 m in length and bears pepperish berry fruits which are usually dried to prolong its shelf life. It is commonly known as African black pepper and popularly called Uziza by the South-Eastern Nigerians and lyre by the Yorubas. The leaves and seeds are consumed widely as spice and used in preparation of different dishes. The leaves and seeds are considered to be medicinal as have been reported in literatures. In some parts of Nigeria, the seeds and leaves are used in preparation of a popular dish consumed by women after childbirth, to enhance the contraction of uterine and also to enhance the expulsion of placenta and other remains from the womb⁴. They are used for treating rheumatic pains and for weight control.

Ngane *et al.*⁵ and Ekanem *et al.*⁶ have reported the antimicrobial, antiparasitic and antifungal activities of *P. guineense* leaves and seeds. The effect of aqueous extract of *Piper guineense* seeds on some liver enzymes, antioxidant enzymes and some haematological parameters in albino rats has been reported by Uhegbu *et al.*³. Mbongue *et al.*⁷ reported that the leaves of *Piper guineense* have been used by traditional medical practitioners for treatment of respiratory diseases and correction of female infertility problems, while the seeds are used as an aphrodisiac. Hence, this study was conducted to investigate the proximate, mineral and phytochemical composition of *Piper guineense* leaves and seeds.

MATERIALS AND METHODS

Duration and year of study: This study was carried out from the month of March to May, 2017 in Wukari, Nigeria.

Plant material used: *Piper guineense* leaves and seeds were purchased at the new Market in Wukari, Taraba State, Nigeria. The seeds and leaves (Fig. 1, 2) were sorted for healthy parts, air-dried and milled with manual blender.

Preparation of plant extract: The plant powder was macerated in 70% ethanol for 48 h with occasional shaking, thereafter filtered and the filtrate concentrated to eliminate the ethanol. The crude plant extract was used for phytochemical analysis.

Determination of mineral and proximate composition of *Piper guineense* leaves and seeds: Proximate composition of plant materials gives information of the level of acceptance of the materials in general nutrition⁸. The percentage dry matter, protein, fibre, moisture, lipid, carbohydrates and ash of



Fig. 1: Photograph of *Piper guineense* seeds



Fig. 2: Photograph of *Piper guineense* leaves

Piper guineense leaves and seeds were determined using the method of AOAC⁹, while the concentrations of Ca, P, Mn, Mg, Cr, Zn, Fe, Cu, Na, and K were carried out using Atomic Absorption Spectrophotometer (model AA280FS, Agilent Technologies, USA) following the conditions recommended by the manufacturer. All chemicals used were of analytical grade and as recommended by the manufacturer.

Determination of phytochemical constituents of ethanolic extract of *Piper guineense* leaves and seeds:

The GC (model No. 7890B) and MS detector (model 5977A) were used for the phytochemical analysis. It was equipped with column: Agilent HP 5MS ultra Inert (350°C) 30 m × 250 µm × 0.25 µm. Helium (He) was used with flow: 0.7 mL min⁻¹, pressure: 4.4867 psi and average velocity: 30.641 cm sec⁻¹. The injection volume was 1 mL, inlet temperature 250°C, split ratio 20:1 and split flow 14 mL min⁻¹. The temperature of the oven used was 60°C with equilibrating time of 1 min, maximum oven temperature 350°C and total run time 35.857 min. Identification of the phytochemical constituent was done by matching the spectra of the phytochemical to be identified with the mass spectra of reference compounds contained in the database of National Institute of Standards and Technology (NIST 14). The amounts of phytochemicals suggested were then expressed as area percent which is comparable to the total peak area.

Statistical analysis: After the proximate analysis, the results were analyzed statistically using Paired-Samples T Test with the use of Statistical Package for Social Sciences (SPSS) version 21. Means for each parameter were compared for significance at $p \leq 0.05$ and result presented as Mean ± SD.

RESULTS AND DISCUSSION

Proximate analysis shows that *Piper guineense* seeds was high in percentage dry matter, crude lipid and carbohydrates compare to *Piper guineense* leaves, while *Piper guineense* leaves was high in percentage moisture content, crude protein, crude fibre and ash compared to *Piper guineense* seeds. The differences in the moisture content and dry matter were statistically non-significant, while the differences in the percentage crude protein, crude fiber, crude lipid, ash and carbohydrates were statistically significant.

The proximate composition of *Piper guineense* leaves and seeds (Table 1) showed that the seeds may last longer than the leaves when stored in the form purchased at the market (as commonly sold). Although the difference in the percentage moisture of the leaves and seeds is not statistically significant, it is possible that the high moisture content of the leaves may permit certain microbial activities than the seeds.

Table 1: Proximate composition of *Piper guineense* seeds and leaves (%)

Proximate composition	<i>Piper guineense</i> seeds	<i>Piper guineense</i> leaves
Moisture content	5.98 ± 0.21 ^a	6.11 ± 0.01 ^a
Dry matter	94.03 ± 0.21 ^a	93.90 ± 0.01 ^a
Crude protein	12.99 ± 0.15 ^b	15.17 ± 0.39 ^c
Crude fibre	6.95 ± 0.27 ^b	20.99 ± 0.16 ^c
Crude lipid	4.06 ± 0.12 ^b	1.91 ± 0.18 ^c
Ash	4.55 ± 0.11 ^b	11.98 ± 0.03 ^c
Carbohydrates	65.46 ± 0.85 ^b	43.86 ± 0.08 ^c

Values are mean ± standard deviation of triplicate determination (n = 3). Mean in the same row, with different letters of the alphabet as superscript are statistically significant ($p < 0.05$)

The percentage dry matter content of the leaves and seeds supports the result of moisture content. The non-significant high percentage dry matter in the seeds compared to the leaves shows that the seeds contains less moisture than the leaves.

The leaves of *Piper guineense* contain significant high fibre and ash compared to *Piper guineense* seeds. Fibre is known to increase the bulk of diet content and also enhance the frequent release of bowel content⁸. This has shown positive impact in human health since it can reduce certain conditions such as constipation. However, the frequent removal of bowel content can cause indigestibility in animal such as human. Adequate caution should be taken in consumption of food materials that are very high in fibre since indigestibility will cause loss of nutrients and utilization of the undigested food materials. The high percentage ash in the leaves show that there will be a corresponding high mineral content in the leaves compared to the seeds.

Carbohydrates and fat/lipid are known to be good sources of high energy generation compound^{8,10}. The human body depends on carbohydrates and lipid/fat largely to drive its regular activities. The significant high differences in the lipid and carbohydrates content of the seeds compared to the leaf show the seed could be a better source of carbohydrates and lipids than the leaf. This also shows that the use of the seeds in preparation of special dishes such as the soup for nursing mothers could contribute to the energy gained after consumption of such food. The human body may also use the lipid and carbohydrates contained in the seeds as well as in the leaves for the generation of some necessary intermediates required for proper functioning of the human body system. Certain hormones of lipid origin are required for regulation of certain human bodily functions. *Piper guineense* seeds may provide the required lipids needed for the biosynthesis of such hormones. This may be the reason why its use in food prepared for nursing mothers help in the relaxation of muscles and resumption of the normal menstrual cycle. The lipid and carbohydrates contents of *Piper guineense* seeds and leaves shows that apart from their use as spices, they can contribute positively to general nutrition.

Piper guineense leaves was significantly high in protein content than *Piper guineense* seeds, however, the seed has appreciable protein content. This shows that apart from carbohydrates, the seeds and leaves of *Piper guineense* is a good source of proteins. This means the use of the leaves and seeds in general nutrition contributes to the provision of proteins which the human body requires for growth, replenishing worn-out tissues, provision of amino acids and hormones required for certain biochemical processes, provision of energy and enzyme catalysis.

Although mineral elements do not yield energy, they are very necessary for many biochemical and physiochemical processes. The human body requires the minerals for metabolic functions¹¹, activations of certain enzymes, immune boosting and regulation of homeostasis. The result of mineral analysis (Table 2) show *Piper guineense* leaves has a high amount of magnesium, calcium, manganese and copper than *Piper guineense* seeds, while *Piper guineense* seeds has a high amount of chromium, zinc, iron, potassium, sodium and phosphorus than *Piper guineense* leaves.

Calcium was the mineral with highest concentration in both the leaves and seeds. This shows the leaves and seeds will play significant supportive role in bone, teeth and muscle functions and even as cofactor in enzyme catalysis. The high calcium content in the leaves show it can aid the reduction of the risk of osteoporosis and diseases associated with calcium deficiency. Consumption of foods rich in calcium such as *Piper guineense* leaves may aid the prevention of certain conditions such as spasms and muscle twitching mostly in the arms and face. However, it is possible that high level of calcium may cause agitation or gritty eyes. Hays and Swenson¹² and Murray *et al.*¹³ reported that irritability of nerve tissues may be increased by a reduced extracellular blood calcium, while the spontaneous discharges of the nerve impulses which leads to convulsions and tetany may be caused by low level of calcium. The slightly low levels of

phosphorus in the leaves and seeds could complement calcium functions since it can aid proper functions of nerves, teeth, bone and muscles. Excess phosphorus and calcium are excreted by the kidney. The P and Ca excreted in faeces are mostly the unabsorbed dietary P and Ca. Phosphorus is important in many metabolic processes and is located in cells of animal body¹². Toxicity associated with diseases of phosphorus may result to bone loss¹³. Phosphorus is also a component of nucleic acid¹¹. The low level of zinc in *Piper guineense* leaves and seeds show it will support the functions of calcium when the plant parts are consumed, since zinc plays an important role in the absorption of calcium in the bones. Zinc also play vital role during physiological growth in animal. Some enzymes involved in cell replication and metabolism of macronutrient are dependent on Zn^{12,14}.

The levels of potassium and sodium in both the leaves and seeds of *Piper guineense* show that the use of the plant parts in general nutrition could aid in maintaining homeostasis and in regulating the osmotic balance between interstitial fluid and bodily cells of animals. Sodium concentration largely influence changes in osmotic pressure^{12,13}. The metabolism of sodium is regulated by aldosterone. Hyponatraemia has been reported to occur in intestinal obstruction, vomiting, acute Addison's disease and diarrhea. Toxicity disease of sodium may cause hypertension in susceptible individuals. Potassium deficiency disease may result to impaired neuromuscular functions of some muscles (such as skeletal and cardiac), mental confusion, paralysis and muscular weakness^{12,13}.

Iron was high in the seeds compared to the leaves. This show the seeds will supply more iron which is required to carry oxygen in the haemoglobin of red blood cells. Fe is required for making Hb and it is a prooxidant which is also needed by microorganisms for proliferation¹⁵. Transportation of oxygen will be favoured by consumption of *Piper guineense* seeds and leaves in nutrition. This is believed to be one of the importance of the use of *Piper guineense* seeds in preparation of pepper soup usually given to women immediately after childbirth.

Magnesium level was high in the leaves compared to the seeds. The appreciable level of magnesium implies that consumption of *Piper guineense* leaves and seeds will support biochemical reactions, especially those enzymatic reactions implicated in the metabolism of food components and in regulation of cholesterol. Magnesium is an active enzyme cofactor (kinases, etc.) and also a constituent of teeth and bones¹³.

Table 2: Mineral composition of *Piper guineense* seeds and leaves (ppm)

Mineral composition	<i>Piper guineense</i> seeds	<i>Piper guineense</i> leaves
Magnesium	6.723±0.0008	13.311±0.0010
Calcium	11.195±0.0008	47.127±0.0020
Manganese	0.159±0.0004	0.284±0.0002
Chromium	0.195±0.0004	0.109±0.0009
Copper	0.069±0.0004	0.074±0.0002
Zinc	0.649±0.0003	0.568±0.0005
Iron	3.786±0.0006	2.646±0.0014
Potassium	8.870±0.1200	8.570±0.0600
Sodium	5.370±0.0600	5.270±0.1500
Phosphorus	1.560±0.0600	1.290±0.0800

Values are mean±standard deviation (n = 3)

Table 3: Phytochemical constituents of ethanolic extract of *Piper guineense* seeds

Name of compounds	RT (min)	Area peak (%)
Linalool	12.263	0.51
Caryophyllene	21.874	0.77
cis-beta-Farnesene	22.682	0.49
Humulene	22.808	0.29
Germacrene D	23.533	0.83
alpha-Guaiene	23.696	0.24
trans-alpha-Bergamotene	23.779	0.27
Bicyclogermacrene	23.924	0.32
beta-Bisabolene	24.130	1.49
Dodecanoic acid, methyl ester	24.425	0.27
Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,	24.548	1.66
Bicyclo[7.2.0]undec-4-ene, 4,11,11 trimethyl-8-methylene-aromadendrene	25.527	0.60
4,5-dimethoxy-6-(2-propenyl)- Apiol	27.125	0.30
Methyl tetradecanoate	29.292	0.26
Hexadecanoic acid, methyl ester	33.688	0.58
11-Octadecenoic acid, methyl ester	37.219	0.69
Palmitoleic acid	37.338	0.34
Methyl stearate	37.693	0.34
15-Hydroxy pentadecanoic acid	40.847	0.25
Piperidin-2-one-5-carboxylic acid	41.699	0.46
7-butyl -(R,R)-2,3-dimethyl- Octanoic acid,	42.049	1.34
Glycerol tricaprilate	42.132	2.74
2-methylpentyl ester Pyrrolidine,	42.221	0.95
4,5-dimethyl-2-propyl-Octanoic acid,	42.256	0.65
Caprylic anhydride	42.333	2.21
2,2,6,6- tetramethyl-Propanoic acid,	42.441	1.63
Diethylmalonic acid	42.482	3.04
3-phenyl-5-nitro-Propylphosphonic acid	42.553	1.64
Pyridazine	42.655	2.81
2-(Decanoyloxy)propane-1,3-diyl dioctanoate	42.720	2.71
3,5-Heptanedione, 2,2,6,6-tetramethyl-Fumaric acid	42.778	1.18
3,5-Heptanedione, 2,2,6,6-tetramethyl-	42.822	1.50
3-(Octanoyloxy)propane-1,2-diyl bis(decanoate)	42.860	1.16
Thiazole, 4-ethyl-2-propyl-	42.885	1.86
5-Benzylidene-3-(4-methoxybenzoyl) rhodanine	43.050	11.00
Oleic Acid	43.939	0.47
Octadecanoic acid	44.027	0.43
Fumaric acid, 4-heptyl tridecyl ester	44.147	0.21
Diglycolic acid, 3-methylbutyl nonyl ester	44.335	0.69
3-Phenyl-1,4,2-dithiazole-5-thione	45.108	0.71
1-{3,3-Diphenyl-3-[(trimethylsilyl)oxy]propyl}piperidine	45.468	3.59
Myristin	45.629	0.98
2,4-Thiophenedicarboxylic acid	45.749	2.43
1,2-Cyclohexanedicarboxylic acid	45.809	2.38
(Z)(Benzo[d][1,3]dioxol-5-yl)- (piperidin-1-yl)pent-3-en-1-one	46.063	2.87
m-Anisic acid	46.345	6.43
4-Methoxybenzoic acid, 2,6-dimethyl-4-(2,2,2-trifluoro-1-hydroxy-1-z rifluoromethylethyl)phenyl ester	46.729	13.72
Cyclooctane acetic acid	46.939	0.51
Piperlonguminine	47.103	1.26
Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	48.189	0.24
Piperidine,1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-,(Z,Z)-	48.436	1.56
1-(4Nitrophenyl)piperazine	48.926	0.32
Benzylalcohol, bromomethyl dimethylsilyl ether	49.300	1.16
11,11,13,13-tetradecamethyl-Octasiloxane	49.427	0.38
4H-Pyran-3-carboxylic acid, 2-amino-5-cyano-6-ethyl-4-(3-pyridinyl)-methyl ester	50.116	0.18
2-Propenoic acid, 2-cyano-3-phenyl,ethyl ester	50.438	6.19
Piperine	50.922	5.91

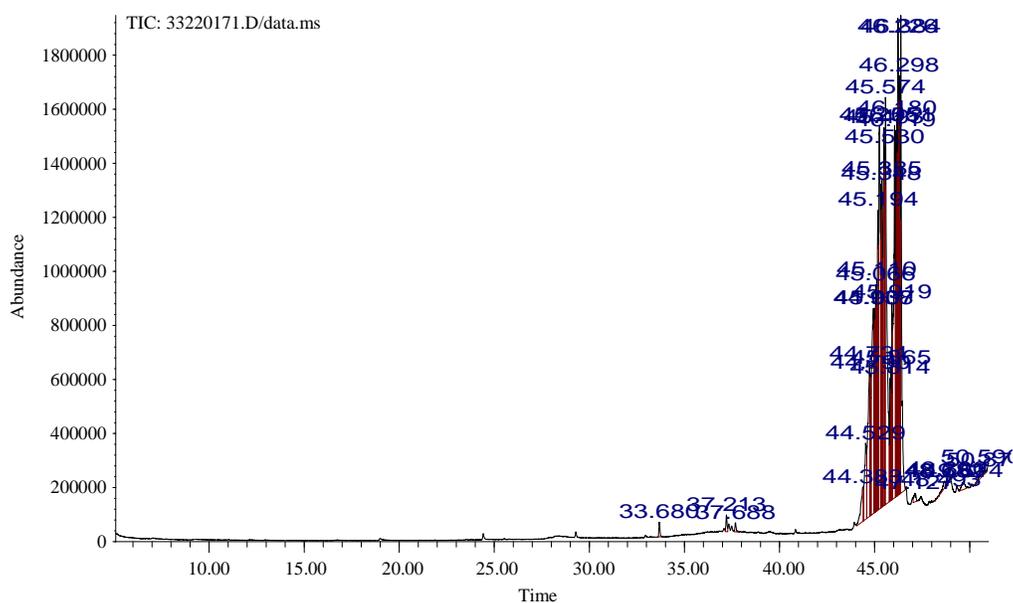


Fig. 4: GC-MS chromatogram of ethanolic extract of *Piper guineense* leaves

Table 4: Phytochemical constituents of ethanolic extract of *Piper guineense* leaves

Name of compounds	RT (min)	Area peak (%)
Hexadecanoic acid, methyl ester	33.680	0.20
Octadecenoic acid, methyl ester, (Z)-	37.213	0.25
Methyl stearate	37.688	0.14
1-Naphthalene carboxamide, N-butyl-	44.383	1.08
3-Dibenzofuranamine	44.731	6.48
Sarcosine, N-(naphthoyl)-octyl ester	44.790	1.22
2-(Octanoyloxy)propane-1,3-diyl bis(decanoate)	44.938	5.45
1-Naphthalene carboxamide, N-(1-methylpropyl)	45.007	2.59
Decanoic acid, 1,2,3-propanetriyl ester	45.066	5.49
2-(Octanoyloxy)propane-1,3-diyl bis(decanoate)	45.194	6.71
3-(Octanoyloxy)propane-1,2-diyl bis(decanoate)	45.255	12.92
1-Naphthamide, N-butyl-N-octyl-	45.348	3.12
2-Ethyl-4-methylthiazole	45.385	3.81
1-Naphthamide, N-butyl-N-hexyl	45.574	9.49
Lauric anhydride	45.814	1.41
Fumaric acid, hexyl 2,3,5-trichlorophenyl ester	45.865	1.14
Fumaric acid, 2-chlorophenyl, isohexyl-ester	45.919	2.22
Fumaric acid, 3,5-difluorophenyl isohexyl ester	46.051	7.62
Fumaric acid, hexyl pent-4-en-2-yl ester	46.119	3.68
Fumaric acid, 2-formylphenyl isohexyl ester	46.226	7.88
Fumaric acid, isohexyl 3-nitrophenyl ester	46.298	5.56
Fumaric acid, 4-cyanophenyl isohexyl ester	46.384	10.25
5-(3,4-Dimethoxyphenyl)-1,3-dimethyl-6H-pyrrolo[3,4-d]pyrimidine-2,4-dione	47.127	0.23
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	48.493	0.05
Oxirane, tetradecyl-	48.682	0.19
2,3-Dihydroxypropyl elaidate	48.751	0.04
6-Octadecenoic acid, (Z)-	48.800	0.05
9-Octadecenoic acid	49.674	0.33
1-Tricosene	50.590	0.30
2-(n-Propyl)oxybenzylidene acetophenone	50.873	0.09

acid methyl ester has been reported to exhibit anti-proliferative properties against tumor cells and induced apoptosis in the tumour cells²⁸. Some of these chemicals present in the seeds and leaves *Piper guineense* may

contribute these effects when these plant parts are used in nutrition. Some of these chemical properties supports the used of *Piper guineense* seeds and leaves in traditional medicine.

CONCLUSION

Piper guineense seeds and leaves possess high amount of carbohydrates and appreciable level of protein and fibre. Mineral elements which play vital roles in human metabolic systems and for activating certain enzyme catalysis were detected in appreciable amount in the leaves and seeds. This shows that consumption of the plant parts may aid improving human immune system. Different phytochemicals which possess important physiological and biochemical functions are present in *Piper guineense* seeds and leaves. This study revealed the nutritional, possible medicinal and pharmacological properties of the leaves and seeds. These results showed that *Piper guineense* seeds and leaves are important in general nutritional and could be used for certain pharmacological needs. The use of *Piper guineense* seeds and leaves is recommended in general nutrition, especially for women after child birth.

SIGNIFICANCE STATEMENT

This study is significant because investigation into the investigation of the phytochemical composition of *Piper guineense* leaves and seeds will reveal the possible medicinal and pharmacological properties of the leaves and seeds, while investigation of the proximate composition will give information of the overall level of acceptance of the plant materials in general nutrition. This therefore, warrants study into the proximate, mineral and phytochemical composition of *Piper guineense* leaves and seeds.

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