



Phytochemicals, Elemental and Proximate analyses of *Piper Guineense* Leaves

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ABSTRACT: *Piper guineense* leaves were studied for their phytochemical, proximate, and mineral content. Standard methods, such as elemental determination with an atomic absorption spectrophotometer and Flame Photometry, were used for the analyses. The carbohydrate content, crude fat, crude protein, crude fiber, ash content, and moisture content were 40.60%, 2.00%, 17.55%, 9.55%, 14.00%, and 12.00%, respectively, according to the experimental results. Phytochemical analysis revealed that the leaf samples included a high quantity of alkaloids, flavonoids, reducing sugar, phenols, tannins, quinones, and cardiac glycosides. The mineral compositions of the investigated leaf show the presence of Fe (20.49 ± 0.25 Mg/100g), Zn (5.67 ± 0.10 Mg/100g), Mg (23.03 ± 5.10 Mg/100g), K (12.06 ± 0.10 Mg/100g) and Ca (120.98 ± 10.00 Mg/100g). The presence of essential minerals, phytochemicals such as alkaloids, flavonoids, tannins, and cardiac glycosides, as well as carbohydrate and crude protein, suggests that it might function as a spice, food, preservative, insecticide, herbal medicine, fragrance in the cosmetic industry, and alternative source of medicine.

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Plant components such as seeds, leaves, and fruits are utilized as food spices (Ifijen et al., 2023), and many of them are thought to have therapeutic properties (Ifijen et al., 2020; Ifijen et al., 2019a). Plant materials are rich in nutrients, vitamins, and minerals that people need on a regular basis for appropriate metabolic and biochemical functioning (Ifijen et al., 2019a). Spices include a variety of important chemicals, including oil and complex combinations of organic molecules. Many of these plant parts are also utilized in herbal medicine to treat and manage various ailments (Uzoekwe and Mohammed, 2015). *Piper guineense* leaves and seeds fall under this category, and their use is growing. Most medicinal plants have been reported to have an important part in medication discovery and are necessary for humans to treat various disorders. *Piper guineense* is a tropical plant found in West Africa (Imo et al., 2018). It's a member of the

Piperaceae family. The plant is a climbing vine that may reach a height of 20 meters and yields peppery berry fruits that are commonly preserved to extend their shelf life. It is usually known as African black pepper, and the South-Eastern Nigerians and Yorubas name it Uziza and Iyre, respectively. The leaves and seeds are extensively used as a spice and are used in the creation of various cuisines. According to literature, the leaves and seeds are considered medicinal (Imo et al., 2018). The seeds and leaves are used in the making of a famous dish ingested by women after childbirth in some regions of Nigeria to increase uterine contraction and also to increase the ejection of placenta and other leftovers from the womb (Udoh et al., 1999). They are used to alleviate rheumatic discomfort and to help people lose weight. *Piper guineense* seeds are taken by women in some parts of Nigeria after childbirth to promote uterine

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contractions for the evacuation of the placenta and other residues from the womb. They are also used as an adjuvant in the treatment of rheumatic symptoms, as an antiasthmatic, as well as for weight control (Jabeen et al., 2010). *Piper guineense* has considerable nutritional and medicinal qualities (Ozcan, 2003). As there are not many studies in the literature that explicitly analyze the nutritional value and phytochemical content of *Piper Guineense* leaves, this study was carried out to investigate the nutritional and phytochemical composition of *Piper guineense* leaves.

MATERIALS AND METHODS

The leaves of *Piper guineense* were collected from an open field in Ramat Market in Benin City, Edo State, Nigeria. Identification and authentication were performed at the University of Benin's Herbarium Division, Department of Plant Biology and Biotechnology. The *Piper guineense* leaves were carefully chosen, cleaned in water, and air dried for two weeks. Prior to the extract preparation, the dried leaves were pounded into powder using a wooden mortar and pestle and packed into airtight containers. Analytical grade chemicals were employed in this investigation, including acetic acid, iron (III) chloride (FeCl_3), hydrogen tetraoxosulphate (IV) acid (H_2SO_4), acetic anhydride, ethanol, chloroform, ammonia, sodium hydroxide, n-hexane, and others.

Preparation of the Plant Extract: The airtight container containing the powdery sample of *piper guineense* leaves was brought out and 2000g was measured. The powdery sample was macerated in 3.5 litres of distilled water at room temperature for 72 hours with constant stirring. After 72 hours, it was filtered with Mushin cloth. A concentrated extract was obtained by using a rotary evaporator at 45 °C.

Qualitative Analysis of Phytochemicals: Phytochemicals are bioactive elements of medicinal plants that are not nutrients however are extremely beneficial to the plants. Several bioactive elements of ethanolic extract were qualitatively examined for flavonoids, tannins, Cardiac glycosides, Saponin, Steroids, terpenoids, anthraquinones, alkaloids, and reducing sugars. Phytochemical screening was performed on the extracted material using standard procedures to identify secondary metabolites (Das et al., 2018; Senguttuvan et al., 2014).

Determination of Flavonoids Content: A previously recognized method was used to determine the presence of flavonoids in the plant sample (Warsi and Sholichah, 2017). One drop of ethanolic extract and one drop of ethyl acetate fraction were placed on filter paper and allowed to dry. The dry droplets of the

samples on filter paper were steamed with ammonia. When the color changed from faintly yellow to vivid yellow, it indicated that the samples contained flavonoids.

Determination of Tannins Content: The tannin content was determined using the method given by Gul et al. (2017). In a test tube, the stock solution (3 mL) was diluted with chloroform and acetic anhydride was added (1 mL). Finally, 1 mL of sulphuric acid was carefully added to the solution at the side of the test tube. The presence of tannins resulted in the formation of a green color.

Determination of Cardiac glycosides Content (Keller-Killani test): Cardiac glycosides content determination was carried out as described by Gul et al. (2017). A 4.0 mL solution of glacial acetic acid with 1 drop of 2.0% FeCl_3 mixture was combined with 10 mL aqueous plant extract and 1 mL concentrated H_2SO_4 . A brown ring formed between the layers, revealing the presence of cardiac steroidal glycosides.

Determination of Saponins Content: The extract and plant fractions were placed separately in 1 mL test tubes and mixed with 2 mL of hot water before being cooled and shaken for 10 seconds. If the fume produced stabilized in less than 10 minutes, it was considered saponin positive (Gul et al., 2017).

Determination of Steroids Content: The steroids content of the studied plant was determined using a procedure detailed by Auwal et al. (2014). 2ml acetic anhydride was added to 0.5g of ethanolic extract from each sample, along with 2ml H_2SO_4 . The hue of several samples changed from violet to blue or green, indicating the presence of steroids.

Determination of Terpenoids Content (Salkowski test): Terpenoids content determination was carried out as described by Gul et al. (2017). In a typical determination, 2.0 mL chloroform was mixed with 5 mL aqueous plant extract and evaporated on the water bath before being heated with 3 mL concentrated H_2SO_4 . Terpenoids appeared as a grey color.

Determination of Alkaloid Content: The flavonoid test employed was described by Ezeonu et al. (2016). The components were extracted from 2 grams of each wood powder sample using 5% tetraoxosulphate (VI) acid (H_2SO_4) (20 cm³) in 50% ethanol by boiling for 2 minutes and filtering through Whatman filter paper number 42. (125 mm). In a separating funnel, 5 cm³ of 28% ammonia solution (NH_3) was used to make the filtrate alkaline. In a subsequent solution extraction, an equal volume of chloroform (5.0 cm³) was utilized,

and the chloroform solution was extracted with two 5 cm³ volumes of 1.0 M dilute tetraoxosulphate (VI) acid. This final acid extract was then used to perform the following test: Dragendorff's reagent (Bismuth potassium iodide solution) was mixed with 2 cm³ of acid extract, and the precipitated orange color indicates the presence of alkaloid.

Determination of Anthraquinones: Anthraquinones were determined using the method outlined in a previous work (Auwal et al. 2014). In a dry test tube, one gram (1 g) of powdered seed was inserted, and 20 mL of chloroform was added. This was steamed for 5 minutes in a steam bath. The extract was filtered and allowed to cool while still hot. An equal volume of 10% ammonia solution was added to the filtrate. After shaking, the upper aqueous layer was examined for brilliant pink coloration, which indicates the presence of Anthraquinones. In a test tube, 10 mL of 10% ammonia solution was mixed with 5 mL of chloroform.

Determination of mineral and proximate composition of Piper guineense leaves: The moisture, lipid, protein, ash, crude fiber, and carbohydrate contents of the *Piper guineense* plant were determined using standard protocols established by the Association of Official Analytical Chemists (AOAC, 1984; 1990), while the concentrations of K, Ca, Mg, Zn, and Fe were determined using an Atomic Absorption Spectrophotometer under manufacturer-recommended conditions.

Determination of Moisture Content: In a crucible, 5g of fresh samples were weighed. The crucible was weighed and placed in a 105°C oven for 3 hours to achieve a steady weight for the samples. The percentage of moisture was then computed as follows:

$$\% \text{ moisture content} = \frac{\text{initial weight} - \text{final weight}}{\text{weight of sample used}} \times 100$$

Determination of Crude Fat Content: Weighing was done on the round bottom flask (initial weight). A soxhlet extractor was used to extract 10g of the material using n-Hexane as the solvent. The round bottom flask was dried in an oven following the extraction procedure, and the final weight was determined using a digital weighing balance. The calculation for the crude fat percentage is presented below:

$$\% \text{ crude fat} = \frac{\text{initial weight} - \text{final weight}}{\text{weight of sample used}} \times 100$$

Determination of Ash Content: The crucible was weighed, and 5g of sample was deposited in it before

being placed in a muffle furnace at 600°C for 6 hours. The resulting ash was allowed to cool before being weighed. The ash content was then determined as follows:

$$\% \text{ ash} = \frac{\text{initial weight} - \text{final weight}}{\text{weight of sample used}} \times 100$$

Determination of Crude Protein Content: The crude protein content of the samples was evaluated using the AOAC (1984) Microkjeldahl method, which involves protein digestion and distillation. The percentage crude protein was computed using the %nitrogen as follows:

$$\% \text{ crude protein} = \% \text{ N} \times \text{F}$$

Where; F (conversion factor) is equivalent to 6.25.

Determination of Crude Fibre Content: The crude fiber was calculated using the method (AOAC, 1990). The percentage crude fiber was estimated using the following formula

$$\% \text{ crude fibre} = \frac{\text{weight after drying}}{\text{weight of sample}} \times 100$$

Determination of Mineral Content (AOAC, 1990): The estimation of mineral elements reflects the quantity of inorganic elements contained in the sample. The determination was made in accordance with regular processes. During the analysis, the sample was ashed and dissolved in a solvent before being inhaled into an air-acetylene flame. Iron (Fe), zinc (Zn), calcium (Ca), magnesium (Mg), and potassium (K) were determined using spectrophotometric methods, with sodium (Na) and potassium (K) determined using flame emission spectrophotometers and the others determined using atomic absorption.

Table 1: Result Mineral composition

S/N	Minerals	Concentrations (Mg/100g)
1.	Potassium (K)	12.06 ± 0.00 mg/100g
2.	Calcium(Ca)	120.98 ± 0.00mg/100g
3.	Magnesium(Mg)	23.03 ± 0.02 mg/100g
4.	Zinc (Zn)	5.67 ± 0.00 mg/100g
5.	Iron (Fe)	20.49 ± 0.00 mg/100g

Mineral elements not only serve as nutritional sources for both plants and animals, but they also play vital functions in the ecosystem. Inorganic chemical elements have been found to be necessary in feeding and to be structural components in biological functions. Table 1 shows the mineral composition of the *Piper guineense* plant. The findings of the elemental composition analysis revealed the presence of calcium, magnesium, iron, potassium, and zinc in the studied *Piper guineense* plant. The mineral having the highest concentration in the *Piper guineense* plant

was calcium ($120.98 \pm 0.00\text{mg}/100\text{g}$). This indicates that the leaves will have an important supportive role in bone, tooth, and muscular functioning, as well as a cofactor in enzyme catalysis (Imo *et al.*, 2018). Because of the high calcium content in the leaves, it can help reduce the risk of osteoporosis and disorders connected with calcium insufficiency. Calcium-rich foods, such as *Piper guineense* leaves, may aid in the prevention of certain disorders, such as spasms and muscle twitching, particularly in the arms and face ((Uzoekwe, et al., 2021). A high calcium level, on the other hand, may induce irritation or gritty eyes. According to Hays and Swenson (1985) and Murray et al. (2000), decreased extracellular blood calcium may enhance irritability of neural tissues, whereas low calcium levels may produce spontaneous discharges of nerve impulses, resulting in convulsions and tetany. Magnesium content in the tested plant ranks second, at $23.03 \pm 0.02\text{mg}/100\text{g}$. Because of the high magnesium content, eating *Piper guineense leaves* may supports biological activities, particularly those enzymatic responses involved in food component metabolism and cholesterol management (Uzoekwe and John-Okpabi, 2016). Magnesium is an active enzyme cofactor (kinases, for example), as well as a component of teeth and bones. When compared to the other minerals studied, iron was abundant in the *Piper guineense* plant. This indicates that the seeds will provide more iron, which is essential to transport oxygen in red blood cells' haemoglobin (Hb) (Ozcan, 2003). Fe is necessary for the formation of Hb and is a prooxidant required by microbes for proliferation. The consumption of the *Piper guineense* plant in nutrition will aid in the transportation of oxygen. This is thought to be one of the benefits of using *Piper guineense* plants in the creation of pepper soup (a Nigerian delicacy) which is usually given to women immediately after childbirth. Other minerals like potassium, and zinc were also found in the leaf extract, with values of $12.06 \pm 0.00 \text{ mg}/100\text{g}$, and $5.67 \pm 0.00 \text{ mg}/100\text{g}$, respectively. The elements detected are known to influence various physiological functions based on their concentrations, as they play key roles in remedying human/animal health and diseases. This demonstrates that the eating of *Piper guineense* leaves may provide mineral components needed for several enzymatic and other biochemical processes. The phytochemicals and qualitative screening of the *Piper guineense* plant are shown in Table 2. Phytochemical analysis of the aqueous extract of the *Piper guineense* revealed the presence of flavonoids, alkaloids, cardiac glycosides, reducing sugar, phenols compound, tannins and quinones. The extract's chemical ingredients have been claimed to have a wide range of medicinal properties. Flavonoids have received a lot of interest due to their possible health advantages

(Hossain et al., 2013). Many experimental studies in recent years have established the biological and pharmacological capabilities of various flavonoids, including their antibacterial activity, anti-inflammatory, antioxidant, and anti-tumor actions, which are connected with free radical-scavenging action (Auwal et al., 2014).

Table 2: Phytochemicals Qualitative screening of *Piper guineense*

Key: + = Present, ++ = largely present

Phytochemicals	Distilled
Flavonoids	++
Alkaloids	++
Cardiac glycosides	++
Reducing Sugar	++
Phenols compound	+
Tannins	+
Quinones	+

Flavonoids have also been linked to hypoglycemia and anti-diabetic properties. They also have antioxidant action, which protects cells from oxidative damage and lowers the risk of developing some types of cancer (Auwal et al., 2014). The presence of alkaloids in many plants is responsible for their anti-malaria action (Ifijen *et al.*, 2020). Cardiac glycosides are steroids that can have a specific and significant effect on the heart muscle (Kumavath *et al.*, 2021). A very small amount can have a positive simulation on a sick heart. These chemicals are most beneficial in the treatment of congestive heart failure (Kumavath *et al.*, 2021). They raise the force of heart contraction without increasing the amount of oxygen consumed. As a result, the myocardium becomes a more efficient pump capable of meeting the needs of the circulatory system. Reducing sugar has not been shown to have any therapeutic impact, although it may boost the effectiveness of the therapeutically essential components (Auwal *et al.*, 2014). As a result, the mixture of active components in each plant may provide a better therapeutic impact than a single isolated chemical (Unuofin *et al.*, 2020). Reducing sugar was recently used in the production of a polysaccharide immunomodulator with therapeutic and vaccine applications (Auwal *et al.*, 2014). Tannins have an antidiarrheal action, and these chemicals may precipitate proteins on enterocytes, limiting peristaltic movement and intestinal output. Phenolic compounds contain redox potentials and can donate hydrogen atoms to free radicals, allowing them to function as antioxidants (Udoh *et al.*, 1999). Phenolic compounds have a direct impact on free radical reduction. The high phenolic content of the *Piper guineense* plant suggests that it will have a high level of antioxidant activity. Because phenolic chemicals are free radical terminators, they directly contribute to antioxidant action". Tannins are the most abundant secondary metabolites generated by plants, accounting for 5 to

10% of the dry weight of tree leaves (Katzner and Gernot, 2015). The presence of tannins in *Piper guineense* plants suggests that it could be effective in the fight against insect herbivores through discouragement and/or toxicity. The presence of quinone in *Piper guineense* plants indicates that it offers a wide range of medicinal qualities. Quinones are electron carriers that participate in photosynthesis. As vitamins, they are a type of chemical that can help prevent and treat ailments including osteoporosis and cardiovascular disease. Quinones boost overall health due to their antioxidant activity (Edeoga et al., 2005). Several of the cancer treatments that have been authorized or are now in clinical development are quinone-related chemicals. Quinones have toxicological consequences as a result of their presence as photo-products of air pollution. Quinones are redox cycling compounds that can connect to thiol, amine, and hydroxyl groups.

Table 3: Proximate Analysis

Proximate	<i>Piper guineense</i> Leaves
Ash	14.0%
Moisture	12.0%
Crude Protein	17.5%
Lipid	2.0%
Crude fibre	13.0%
Carbohydrate	40.6%

The proximate composition of *Piper guineense* leaves is depicted by Table 3. *Piper guineense* leaves appreciable percentage of ash (14.0%), moisture (12.0%), Lipid (2.0%), crude protein (17.5%), crude fiber (13.0%), and Carbohydrate (40.6%), according to proximate analysis. The high percentage of ash in the leaves indicates that there will be a high mineral content. The high moisture level of the leaves may allow for some microbial activities. The crude protein content of 17.5% found in the investigated plant was slightly greater than that reported by Imo et al. (2018). Because of the high crude protein content, the *Piper guineense* leaves may be necessary for the numerous types of livestock that rely on them for nourishment. When the crude protein content is inadequate, the bacteria responsible for digestion are unable to maintain appropriate levels to process the leaves. The human body relies heavily on carbs and lipids/fat to power its daily operations. The large percentage of carbs in the leaves suggests that the seed may be a better source of carbohydrates. This also suggests that using the leaves in the production of particular foods, such as soup for nursing women, may contribute to the amount of energy obtained after eating such food (Hanson, 2010). The human body may also use the lipids and carbohydrates found in the leaves to produce several intermediates required for healthy human body system functioning. Some lipid-derived

hormones are essential to regulate various human body systems (Oliver, 2006). *Piper guineense* may supply the lipids required for the production of such hormones. This could be why its inclusion in nursing mother's meals aids in muscle relaxation and the resumption of the normal menstrual cycle. The lipid and carbohydrate composition of *Piper guineense* leaves indicates that, in addition to their usage as spices, they can benefit general nutrition. The leaves of *Piper guineense* have a high fibre content (da Fonsêca et al., 2019). Fibre is known to increase the bulk of diet content as well as the frequency with which stool stuff is released. This has a good impact on human health because it can alleviate diseases such as constipation. Yet, regular removal of intestinal content might result in indigestibility un animals such as humans. Consumption of high-fiber foods should be approached with caution, as indigestibility results in nutrient loss and use of the undigested food items. Carbohydrates and fat/lipid are well-known sources of high energy-generating compounds.

Conclusion: The phytochemical composition, elemental analysis, and proximate analyses of *Piper guineense* leaves were investigated in this work. A number of essential phytochemicals were found in the studied leaves, according to the results of the experimental tests, indicating that they may have pharmacological and therapeutic properties. The outcomes also indicate that the plant will receive a sufficient level of acceptability in terms of general nutrition. To fully understand the *piper guineense* leaves' potential medical benefits, more research on the plant's leaves is required.

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