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Proximate Composition Analysis of *Paullinia pinnata* L Leaves

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Abstract
Despite wide consumption of *Paullinia pinnata* leaves for both nutritional and therapeutic purposes, little is known about its proximate composition. Proximate analysis of *Paullinia pinnata* leaf sample is important for the plant's quality and safety, its product development and labelling. Moisture content, crude fibre, protein, carbohydrate, lipid and ash content were determined in this work by standard methods. Results obtained revealed that protein has 13.13±1.22% contents, lipid (1.79±0.05%), ash (7.61±0.90%), moisture (8.69±1.10%), crude fibre (15.49±2.30%) and carbohydrate (53.29±4.90%). Furthermore, the proximate composition showed that carbohydrate (53.29±4.90%) and lipid (1.79±0.05%) have the highest and lowest values respectively. The result suggests that *Paullinia pinnata* L leaves is not just a very good source of carbohydrate for our daily diet but that the plant poses no health risk to both the young and also the elderly whom consumption of high-fat diet may predispose to cardiovascular diseases.
Key Words: *Paullinia pinnata*, Proximate Analysis

Introduction

The plant can be described as a climbing shrub that presents with compound leaves and winged rachis; its inflorescences stand axillary on long stalks bearing paired tendrils with white flowers [1]. The apex of the leaf is acuminate (tapering to a slender point), having an irregularly crenate margin, a symmetrical base. The leaflets are about 0.114 to 0.138 m in length and 0.056 to 0.066 m in width with 0.056 to 0.063 m long petioles. Each stalk of *Paullinia pinnata* bears five leaflets, one lone terminal and other two pairs of leaflets. The lone terminal leaflet of the imparipinnate leaf appears to be larger than the other paired leaflets (Figure 1). Taxonomy of *Paullinia pinnata* L is as shown in Table 1

Table 1: Taxonomy of *Paullinia pinnata*

Rank	Scientific Name and Common Name
Kingdom	*Plantae* -plants
Subkingdom	*Tracheobionta* - vascular plant
Superdivision	*Spermatophyta* - seed plant
Division	*Magnoliophyta* - flowering plant
Class	*Magnoliopsida* – dicotyledons
Subclass	*Rosidae*
Order	*Sapindales*
Family	*Sapindaceae* - soapberry family
Genus	*Paullinia* - bread and cheese
Species	*Paullinia pinnata* L - bread and cheese [2]

Fig. 1: *Paullinia pinnata* (Bread and Cheese) [2]

In Brazil, it is used in the treatment of eye infections (ophthalmic purposes). The white aril part of the seed is consumed in some West African countries as part of their diet [3]. *Paullinia pinnata* is a key component of the traditional pharmacopoeia for cardiovascular disease. Its hypotensive, antioxidant and circulatory properties make it a valuable plant for cardiac well-being [4].

In West Africa, the slender woody shoot growing out from the stem of *Paullinia pinnata* is boiled and the decoction is used for the treatment of jaundice, heart irregularities and yellow fever while the decoction from the leaf serves as a remedy for diarrhea and infant's intestinal obstruction (colic) [5, 6]. *Paullinia pinnata* is also used for the treatment of gonorrhea [7]. *P. pinnata* has been shown to possess erectile function [8], antioxidative activity [9], antimicrobial property [10] and anxiolytic activities [11].

The leaf powder of *Paullinia pinnata* was screened for anti-insecticidal activity against bean weevil (*Acanthscelides obtectus*) [12]. The highest total percentage mortality was observed with *Paullinia pinnata* (93%). The observed percentage mortality is an indication that *Paullinia pinnata* caused significant mortality on the target insects. The result revealed that *P. pinnata* possessed insecticidal potential and may be very useful in the control of *Acanthscelides obtectus* [12]. It puts *P. pinnata* forward as a low cost and natural alterative insecticide from plant [12]. The insecticidal activity of essential oil of *Paullinia pinnata* was also evaluated [13]. The essential oil of *Paullinia pinnata* (constituting pentadecanoic acid, isoaromadendrene and wine lactone) displayed 100% mortality (*fumigant* toxicity) against *S. zeamais* adults (test concentration 150 mg/ml and LC_{50} of 51.87mg/ml) suggesting *Paullinia pinnata* as a potential natural herbal plant for the control of insect pest [13].

Despite these wide consumptions of *Paullinia pinnata* leaves for both nutritional and therapeutic purposes, there is paucity of information about the components of the plant including its proximate composition. Proximate analysis of *Paullinia pinnata* leaf sample is important for the plant's quality and safety, its product development and labelling. In this work, moisture content, crude fibre, protein, carbohydrate, lipid and ash content were determined by standard methods.

Materials and Methods

Plant Material

Paullinia pinnata L leaves were obtained from a farm in Okpaligbo-Ogu, Nsukka Local Government Area, Enugu State, Nigeria. The plant was identified and authenticated by Mr Felix I Nwafor, a plant taxonomist, and the Voucher Specimens (Voucher No PCG/UNN/0401) deposited at the Herbarium of the Department of Pharmacognosy and Environmental Medicine, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka

Determination of Moisture Content

A quantity, 5 g of the powdered sample (W_2) was weighed into crucible (W_1) and was placed in a hot drying oven at 105°C for 3 hrs according to Association of Official Analytical Chemists standard methods as described by [14] and [15]. The crucible was removed, allowed to cool in desiccators and weighed. The process of drying, cooling and weighing was repeated until a constant weight (W_3) was obtained. The weight loss due to moisture was obtained thus:

$$\text{Moisture (\%)} = \frac{(W_1+W_2)-W_3}{W_2} \times 100 \quad (1)$$

Determination of Ash Contents

A quantity, 5 g of the dried powdered sample was weighed (W_2) into an empty crucible (W_1) and placed in

a muffle furnace at 550°C for 5 hrs. The ash was allowed to cool in a desiccator and weighed (W_3). The weight of the ash was determined by the difference between the dry powdered sample, pre-weighed crucible and the ash in the crucible according to [16] and [15]. Percentage ash was obtained by the equation:

$$\text{Ash (\%)} = \frac{W_3 - W_1}{W_2} \times 100 \quad (2)$$

Determination of Crude Fat Content

The crude fat content was determined according to the procedure described by [14] and [15]. A quantity, 3 g of powdered sample (W_2) was weighed into a porous extraction thimble and covered with a clean white cotton wool. Petroleum ether (200 ml) was poured into a 250 ml extraction flask which has been previously dried in the oven at 105°C and weighed (W_3). The porous thimble was placed in the Soxhlet and the rest of the apparatus were assembled with cold water circulation in the condenser. Extraction was done for 8 hrs. After extraction was over, the extraction thimble was removed carefully and the extraction flask placed in a water bath so as to evaporate the petroleum ether and then dried in the oven at a temperature of 1000°C for 1 hr to completely free the solvent and moisture. After that, the flask was removed and allowed to cool in a desiccator and weighed (W_1). The percentage crude lipid was calculated using the equation below:

$$\text{Crude lipid (\%)} = \frac{W_3 - W_1}{W_2} \times 100 \quad (3)$$

Determination of Crude Fibre Content

A quantity, 3 g of powdered samples was digested with 150 ml of 0.1275 M H_2SO_4 for 30 minutes. The content was filtered over Buchner funnel using filter paper and rinsed with hot water to remove acid. Further, the residue was boiled with 150 ml of 0.313 M KOH for 30 minutes, then rinsed with boiling water and acetone. The residue was dried in an oven at 105°C for 12 hrs and weighed, then transferred in muffle furnace at 520°C for 3 hrs. The loss of weight represented the crude fibre. The crude fibre was calculated as follows.

$$\text{Crude fibre (\%)} = \frac{W_3 - W_1}{W_2} \times 100 \quad (4)$$

Where W_1 = weight of ash, W_2 = weight of sample and W_3 = weight of insoluble matter

Determination of Crude Protein Content

The crude protein of the sample was determined by using ThemicroKjedahl method as described by [15]. The sample (1 g) was weighed along with 20 ml of distilled water into a microKjedahl digestion flask, shaken and allowed to stand for 15 mins. One tablet of selenium catalyst was added, followed by the addition of 20 ml concentrated sulphuric acid. The flask was heated on the digestion block at 100°C for 4 hrs until a clear light green colour is formed. The flask was removed from the block and allowed to cool. The content was transferred into 50 ml volumetric flask and diluted to the mark with water. An aliquot of the digest (10 ml) was transferred into another microKjedahi flask along with 20 ml of distilled water and was placed in the distilling outlet of the microKjedahi distillation unit. A conical flask containing 20 ml of boric acid indicator was placed under the condenser outlet. Then, 40% sodium hydroxide solution (20 ml) was added to the content in the micoKjedahi flask by opening the funnel stopcock. The distillation process was started and heat was supplied and regulated to avoid sucking back. Then all the available distillate was collected in 20 ml of boric acid, the distillate was stopped. The nitrogen in the distillate was determined by titrating with 0.01M H_2SO_4. The end point was obtained when the colour of the distillate changes from green to pink. Protein content was then calculated by multiplying the total nitrogen content by a constant, 6.60, which is based on the assumption that protein contains about 16% nitrogen (N) which include both true protein and non-protein and does not make a distinction between available or unavailable protein. The crude protein was calculated using the formula:

Crude protein (%) = % N X 6.60 (5)

The nitrogen content of the sample is given by the formula below

$$\text{N (\%)} = \left[\frac{T_v \times N_a \times V_1}{GV_2}\right] \times 100 \quad (6)$$

Where T_v = titre value of acid, N_a = normality of acid, V_1 = volume of distilled water used for distilling the digest, V_2 = volume of aliquot used for distillation and G = original weight of sample used.

Determination of Carbohydrate

The total proportion of carbohydrate in the sample was obtained by calculation using the percentage dry method [15, 16] ie by subtracting the percentage sum of other food nutrients from 100% as follows:

Carbohydrate (%) = 100 - (crude protein + crude lipid + crude fibre + ash + moisture) %

Result and Discussion

Table 2 shows the results of proximate composition of *Paullinia pinnata* L leaves

Table 2: Proximate Composition of *Paullinia pinnata* L leaves

S/N	Content	Percentage (%)
1	Crude protein	13.13±1.22
2	Lipid	1.79±0.05
3	Ash	7.61±0.90
4	Moisture	8.69±1.10
5	Crude fibre	15.49±2.30
6	Carbohydrate	53.29±4.90

Results are expressed as Mean ± MD (n = 3)

Proximate composition analysis of *Paullinia pinnata* L leaves revealed the following contents in decreasing order: crude carbohydrate (53.29±4.90%) > crude fibre (15.49±2.30%) > crude protein (13.13±1.22%) > moisture (8.69±1.10%) > ash (7.61±0.90%) > lipid (1.79±0.05%) (Table 2). The analysis results show that *Paullinia pinnata* L leaves possess high carbohydrate content (53.29±4.90%) which is appreciably higher than that of *Parquetin nigrescen* (36.03%) and *Magnifera indica* (40.23%) [17] as well as *Chenopodium ambrosiodes* (43.76%) [17] but lower than that of *C. dolichopentalum* (63.35%) [18]. [19] reported 12.23% carbohydrate for *J. curcas* stem and 31.85% carbohydrate for *Ceiba pentandra* stem. The recommended dietary allowance for carbohydrate is set at 130 grams per day [20]. Food or herbs rich is carbohydrates are an important part of a healthy diet. Carbohydrates provide the body with glucose, which is converted to energy used to support bodily function and

physical activities. The high carbohydrate content (53.29%) is beneficial since carbohydrate constitutes a major class of naturally occurring organic compounds that are essential for the maintenance of plant and animal life and also provide raw material for industries [21]. Carbohydrates, along with fat and protein are the macro components of the diet – the principal dietary sources of energy [22]. However, because of the desirability of limiting the intake of fat and perhaps protein, it is recommended that 45 to 65 percent of our daily calories' intake should come from carbohydrates [22]. High carbohydrate content in *Paullinia pinnata* L leaves also explains why the plant is used in the management of snake venom victims. Drugs or extract rich in carbohydrates is very important in snakebite management because it has been reported that hypoglycemia is associated with snakebite [23]. It is thus assumed that while other components of this plant may neutralize the venom, the carbohydrate would serve as adjuvant - providing energy for a hypoglycemic snakebite victim. This result suggests that *Paullinia pinnata* L leaves is not just a very good source of carbohydrate in our daily diet but can be used to support people suffering hypoglycemia, more importantly snake venom-induced hyopglycemia.

The crude protein proportion, shown to be 13.13±1.22%, is close to 12.66% reported of average crude protein content of leafy vegetables consumed in Nigeria [24]. The crude protein value was less than that of *Oscium gratissimum* (29.01%), *Parquetin nigrescen* (25.06%) [17], but has fairly good values than *Carica papaya* leaves extracts (6.50 ± 0.30%) [25], and *C dolichopentalum* (7.63%.) [18]. This value is also almost nearer to the one reported by [26] from the leaves of *Carallia brachiate* (13.59%). Differences in crude protein value among plants have been reported by [27]. Envenomation is associated with protein denaturation in the snakebite victim [28]. Morbidity by snake venom is caused by toxins that directly or indirectly destroy cells and degrade the extracellular matrix and proteins [29]. There is therefore the need for protein supplement in the management of snake envenomation. Consumption of the protein-rich *Paullinia pinnata* L leaves by snakebite victims is highly encouraged because the most common laboratory findings associated with severity of envenomation were thrombocytopenia (abnormally low levels of platelets), hypoproteinemia (abnormally low level of protein in the blood) and hyperlactatemia (an elevated blood lactate concentration) [30].

The crude fat content (1.79±0.05%) of this plant is lower than that of *Carica papaya* (2.01%) [25] and *C. dolichopentalum* (12.14%) [18]. This value is lower than the value (4.60% and 4.30) acknowledged by [31] for *Gongronema latifolium* and *Piper guineense* leaves which in turn is lower than that documented for *Jatropha curcas* stem (16.70%) [19]. Availability of energy in food and its ability to regulate blood pressure can be estimated by its fat contents. However, fat consumption must be less than 30 calories per day [32]. A foodstuff having crude fat value of 1 – 2% is sufficient to maintain good health by reducing risk of diseases such as obesity, atherosclerosis, cancer, and aging caused by its excess consumption [32]. The low crude fat value of *Paullinia pinnata* L suggests that the plant poses no health risk to both the young and also the elderly whom consumption of high-fat diet may predispose to cardiovascular diseases [33, 34].

The moisture content (8.68±1.10%) is within the acceptable limit of about 8% for most vegetable drugs [35] and within acceptable limit for long term storage [36]. This value was higher than 3.67% reported of tender leaves of *Psidium guajava* [37]; lower that 10.76% reported of *Abyssinian purple* wheat [36]. The amount of moisture in plant material determines its absorption and assimilation rate within an organism [35]. Moisture content determines storability and plant quality since high moisture content is associated with lower storage stability. Low moisture content reduces errors in the estimation of the actual weight of drug material [37]. It reduces hydrolysis of components by reducing the activities of hydrolytic enzymes which may destroy the active component. It also reduces the proliferation of microbial colonies and therefore minimize the chance of spoilage due to microbial attack [37]. If the moisture of a plant is low, it has great potential in slowing the growth and development of microorganism and inhibiting hydrolysis of component present in plant material, so that the material can be stockpiled for a long period of time with no risk of microbial attack [37]. The low content of this *Paullinia pinnata* L leaves would enhance its storage stability by avoiding mould growth, biochemical reactions [36] and extend the shelf life of the plant.

The crude fiber content (15.49±2.30%) is higher than 4.32% reported of *Litchi chinensis* seeds [35] but lower than 33.58% reported of *Alchornes cordifolia* leaves [38]. It is also higher than that of *C. dolichopentalum* (7.34%) but lower than 50.53% crude *fibre* for *J.* curcas stem [18]. [39] reported 19.75% crude fibre for *Ceiba pendandra* stem. Though plants with high amount of fibre is advised for the treatment of obesity, diabetes, cancer, gastrointestinal disorders; management of coronary heart disease, hypertension, constipation, diabetes etc [40, 41], high proportion of crude fibre is associated with bowel movement which can cause abortion [42]. The low crude fibre value of this plant shows that it is not contraindicated in pregnancy as it poses no risk of bowel movement that can lead to abortion [42]. This implies that both pregnant and non-pregnant snakebite victims can be administered with *Paullinia pinnata* L leaves without any risk of abortion.

The ash content (7.61±0.90%) is lower than 15.3% reported of *Bombax buonopozense* [43] and also lower than 14.2% and 11.83% reported of *Costus afer* (Bush cane) stem and leaf respectively [44]. Ash content is a measure of the non-volatile inorganic constituents such as calcium, sodium, potassium and chlorine remaining after ashing ie it is the residue remaining after ignition at 500 – 600◦C [45]. Determining the ash content of a plant is part of proximate analysis for nutritional evaluation, and it is important quality attribute for some food ingredients [45]. High ash content of a plant is an indication of its high mineral content [46]. *Paullinia pinnata* L leaves can be a good source of minerals because of its high ash contents

Conclusion

The proximate analysis conducted in this research revealed the presence of carbohydrates, proteins, lipids, crude fibre, moisture and ash contents in *Paullinia pinnata* leaves at different proportions. The result suggests that the plant part, within

acceptable limit for long term storage, is a good source of energy for our daily diets, has no potential to cause cardiovascular diseases and poses no risk of abortions in pregnancies.

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