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Studies on Proximate, Phytochemicals, Minerals and Anti-Nutritional Factors on the Leaves and Stems of *Pterocarpus milbraedii* (White Campwood)

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ABSTRACT

Pterocarpus milbraedii (Family-Leguminosae) leaves and stems were analyzed for their proximate, mineral element, phytochemicals and anti- nutritional factors using analytical methods. Some tribes in Eastern and Southern Nigeria use the leave extracts in the treatment of headaches, fever, convulsions, and respiratory disorders and as antimicrobial agents. In Ghana, the trees are utilized in cocoa plantations to provide shade. Also, in Tanzania, the wood is used in carving cutlass handles and mortar making. The result of the proximate composition indicates the presence of moisture, crude fat, crude protein, ash, crude fibre and carbohydrate respectively in the leaves and stems. The mineral elements composition showed that the leaves has abundant micro mineral with iron having the highest value. The phytochemical screening showed the presence of Cardiac glycosides and polyphenols in the water and petroleum extracts of the leaves while alkaloids and saponins were present in the water and petroleum extract of the stems. Quantitative analysis of the phytochemicals revealed that polyphenols have the highest value occurring in both water and petroleum extract of the leaves. From the results obtained, it shows that *Pterocarpus milbraedii* is a good source of food and therapeutic agent which will be of use to both food and pharmaceutical industries.

Keywords: Anti-nutritional, medicinal plants, minerals, phytochemicals, proximate, *pterocarpus milbraedii*

INTRODUCTION

In Africa, studies have shown that a vast number of indigenous plants play a significant role in the diet of the populace. [1] Nigeria is blessed with many medicinal plants whose roots, barks, seeds and leaves are used for the treatment of different diseases. The leaves of *Pterocarpus milbraedii* locally known as "oha" in the Eastern Nigeria and "Mkpafere" in the southern Nigeria are consumed widely in Nigeria. Vegetables are the fresh and edible portions of herbaceous plants which can be eaten raw or cooked. [2] They are important class of food substances and highly beneficial for the maintenance of health and prevention of diseases.

They contain valuable food ingredients which can be successfully utilized to build up and repair the body. They are different kinds of vegetables; they may be edible roots, stems, leaves, fruits or seeds. Each group contributes to diet in its own way.

Green leafy vegetables despite their nutritional value are to be consumed with caution because of the presence in them of anti-nutrients. Anti-nutrients are natural compounds that interfere with the absorption of nutrients, hence are known to reduce nutrients availability to animals and humans. The higher the concentrations, the greater the risk posed on consumption.

Pterocarpus milbraedii (White Campwood leaf) is an evergreen or semidecidious tree with the small, rounded crown, it can grow up to 15-36 metres tall, with reports of trees up to 36 metres in east Africa. The long straight bole can be up to 60cm in diameter. The edible leaves are often harvested from the wild for local use and are also often sold in local market. The leaves of *Pterocarpus milbraedii* are used for soup making in Nigeria. In Ghana, the trees have been extensively utilized in cocoa plantations to provide shade. [3] *Pterocarpus milbraedii* is widely exploited for its timber e.g. in Tanzania, the wood which is whitish are widely used for carving cutlass handles, tie – rod and mortar making. The leaves which are stiff are green in colour. Some tribes in Eastern and Southern Nigeria use the leave extracts of *Pterocarpus milbraedii* in the treatment of headaches, pains, fever, convulsions, and respiratory disorders and as antimicrobial agents as similarly reported for *Sanservieriatrifasciata.* [4]

Figure 1: *Pterocapus milbraedii* leaves

Figure 2: *Pterocapus milbraedii* stems

MATERIALS AND METHODS

Sample Preparation

Fresh leaves and stems of *Pterocarpus milbraedii* were collected from Atim Asam in Akpabuyo Local Government Area of Cross River State. The samples were taken to Botany department of the University of Calabar, where it was authenticated. The leaves and stems were packed in a large polythene bag and taken to the laboratory where the leaves were separated from the stem and washed with clean water to remove traces of dust and unwanted materials. Both samples of the leaves and stems were weighed separately and placed in an oven until it was dried to a constant weight. Then they were allowed to cool and grinded into powered form and packaged separately in a fume cupboard until it was ready for analysis.

Solvents used for extraction were distilled water and petroleum ether. For water extraction, 20g of the grind samples of the leaves and stems were weighed separately and packed into extraction thimble and fitted into Soxhletsets. The Soxhlet apparatus was heated under reflux using heating mantle for 6h until extraction was complete. The sample process was repeated for the samples using petroleum ether, and the extracts obtained was transferred into reagent bottles after cooling and kept safely in the laboratory for use. [5]

Proximate analysis

Proximate analysis (moisture, protein, fat, ash and crude fibre) was carried out on *Pterocarpus milbraedii* according to the methods described by AOAC (2000), while carbohydrates contents were calculated by differences using the expression

% CHO=100 – (% Ash + Crude Protein + Crude Fibre + % Crude fat)

Phytochemical screening (extract preparation)

The extract of the sample was prepared by macerating a known weight of the plant, 5g of the ground leaves and stems were separately refluxed in a soxhlet extractor using 200cm^3 of petroleum ether until all the soluble compounds are extracted as judged by loss of colour on the filtrate. The same process was repeated using water as the solvent. The extract was used to test for plant constituents such as alkaloids, cardiac glycosides, *saponins*, *tannins*, flavonoids, anthranoids, polyphenols, phlobatanins, reducing compounds and anthraquinones. The analysis determines the

biologically active non-nutritive compound which contributes to the colour, flavor and characteristics of plant parts.

Quantitative estimation of some phytochemicals

Quantitative estimation of saponins, alkaloids, polyphenols, cardiac glycosides and reducing sugar were estimated using the method of. [6] The analyses determine the appropriate concentration and quantity of each of the phytochemicals in the sample of *Pterocarpusmilbraedii.*

Sample digestion

Mineral element analysis

Mineral analysis was determined using the method of. [6] The Na and K was determined using flame emission photometer (FES). The principle of this procedure lies on the fact that the excitation of a metal in a flame gives rise to the emission of characteristics colour. The intensity of the colour emitted by the excitation of any given atom is a property of the specific element and the concentration of that element while other (Ca, P, Mg, Zn, Pb, As, Cd, and Cu, Fe) were analyzed by atomic absorption spectrometer (AAS). The principle of this procedure relies on the absorption property of the elements. The vapour of the elements containing free atoms absorbs light having wavelength as that which the atoms of the elements are capable of emitting**.**

Antinutrients

Hydrocyanide was determined by alkaline titration method of [6], oxalate was estimated by the method of [7], phytic acid was extracted using the method of. [8]

Method of statistical analysis

The data obtained from their research were reported as mean ± standard deviation of mean. The significant difference between the values were estimated using f-test and t-test analysis at a confidence level of 95% given of. [9]

Table 1: Results of proximate composition of the leaves and stems of *Pterocarpusmilbraedii*(%)

Nutrients	Leaves	Stems
Moisture	20± 0.36	22± 0.38
Ash content	8± 0.27	2± 0.11
Crude fat	4 ± 0.16	6±0.22
Crude fibre	6± 0.22	2±0.11
Carbohydrate	77.1±0.67	68.9±0.62
Crude Protein	14.91±0.46	21.11±0.52

Mean ± standard deviation of triplicate determination

RESULTS AND DISCUSSION

The result obtained shows that the stems have more moisture of 22±0.38% than the leaves (20±0.36%). The results are lower than the moisture contents of 81.00±0.12% obtained by [10] for *Diplazuim summattii* leaves but higher than those reported for gourd seed (3.46%) [11,12] and calabash seed (5.27%). [13] It is however comparable with that reported for unripe pulp of *Carica papaya* (18.65%) by. [14] Moisture content of food is usually used as a measure of stability and susceptibility of microbial contamination. [15] The result shows that the leaves contain higher levels of 8±0.27% ash than the stems (2±0.11%). These values are higher than the value 1.94±0.01 obtained for the leaves of *Penthaclathra macrophylla* and some legumes such as cowpeas, lima beans, bambara groundnut [16], Oyenuga 1968), Kersting's groundnut (3.2%) and Bambara groundnut (4.30%) as reported by. [12] This suggests that the sample could be a better source of essential valuable and useful minerals needed for good body development.

The crude protein composition shows that the stems contain a higher level of 21.11±0.52% than the leaves which contains 14.91±0.46%. The sample is relatively high in protein content compared to that reported for vegetable like *Amaranthuscruentus* 4.0±6.0% [17]. However, the concentration is relatively lower compared to the 35.9% reported by Ekuagbere (2007) for calabash seed, 43.1% for luffa cylindrical kernel [18] and the 23.7 ± 30.8% for gourd seed. [12] Proteins are essential components of the diet needed for survival of animals and humans, their basic functions in nutrition is to supply adequate amounts of required amino acids. [19] Protein deficiency causes growth retardation, muscle washing, abnormal swelling of the belly and collection of fluids in the body. [20] The daily protein requirements for children and adult are 23-25g and 45-56g respectively. [21] This means that the sample is a good source of protein especially in an area where the majority depends largely on starchy foods.

The result of the carbohydrate content shows that the leaves are higher in carbohydrate content (77.1±0.67%) than the stems which contain

68.9±0.62%. This is higher compared to 24.60±0.32% reported by [22] for Phymatodes *scolopendria* leaves, (6.93%) in pumpkin [12] 31.5% in groundnut flour [23]. When carbohydrate is sufficient in food, it prevents the unnecessary usage of protein and allows it to be used for body building process. [24] The recommended daily allowance for carbohydrate for adults is 39-45% (NRC, 1989). The leaves of *Pterocarpus milbraedii* offer a good source of energy.

The result shows that the leaves have more fibre content with value of 6±0.22% than the stems with value of 2±0.11%. This is lower compared to 8.80% reported by [22] for *Phymatodes scolopendria* but higher compared to the 2.8% in gourd seed [11], 4.28% of Soyabean [25] and 2.53% calabash seed [13]. Crude fibre provides roughages that aid digestion and reduce the accumulation of carcinogen in the body. [26] Biologically, fibre helps in the maintenance of human health and has been proven to reduce cholesterol level in the body. However, emphasis has been placed on the importance of keeping fibre intakes low in the nutrition of infant and pre-school children. [27] Medically, fibre content in plant samples has been reported to reduce constipation and enhance smooth intestinal digestion. In like manner, it suggests that *Pterocarpus milbraedii* leaves would provide high dietary fibre in the diet. The result of the crude fat shows that the stems value is higher with 6±0.22% than the leaves with 4±0.16%.

This is very low compared to those of calabash seed (43%) [27] and groundnut (43%). [17] The stems of *Pterocarpus milbraedii* could still be grouped under oil rich plant food and therefore could be used as source of oil for industrial and domestic purposes.

Table 2: Results of elemental analysis of the leaves and stems of *Pterocarpus milbraedii* (mg/ 100g)

Nutrients	Leaves	Stems
Calcium	0.764±0.15	3.234±0.22
Copper	0.016±0.07	0.261±0.11
Cobalt	BDL	BDL
Cadmium	BDL	BDL
Iron	29.33±0.46	53.66±0.63
Potassium	0.036±0.09	0.441±0.28
Lead	BDL	BDL
Magnessium	5.44±0.41	10.610±0.34
Phosphorus	0.36±0.13	0.57±0.16
Manganese	0.39±0.14	0.56±0.15
Sodium	4.56±0.38	8.63±0.31
Mercury	BDL	BDL

Mean ± standard deviation of triplicate determinations

BDL = Below Detection Limit

The result of the calcium content shows that the stems have more calcium of 3.234± 0.22 mg/100g than the leaves with 0.764+0.15 mg/100 g. These values are low compared with the value of 58. 76 mg/100g for curry leaf tree by [28] and 14.53 mg/100 for *Phymatodesscolopendria* by. [12] This clearly shows that the concentration of calcium is below the required daily calcium allowance of 360 – 400g/day for both children and adult. However, this amount can be complemented with other food stuff by co-nutrition physiologically, calcium is required in the body for normal growth of bones and teeth. [26] Besides this, calcium plays a good role during blood clotting in the normal functioning of calcitonin and calciferole. The result of magnesium contents shows that the stems contain 10. 610± 0.34 mg/100g than the leaves (5.44± 0.41 mg/100 g). This is lower compared to 109.12ppm reported by [29] for *Afzeliaafricana* seed cap, (61.25 mg/l) reported by [28] for curry leaf tree. Magnesium is required for various body processes, notably as an activator of various enzymes. The recommended daily allowance for women and men is 300 – 400 mg/day (NRC, 1989).

The result of sodium shows higher levels in the stems (8.63± 0.31 mg/100g) than in the leaves (4.56±0.38). The values can be compared with 4.59± 2.31 mg/100g obtained by [30] for *Afzeliaafriana* seed cap but lower than the value of 480.02± 0.11 reported by [10] for *Diplaziumsummattii* leaves. Sodium is the principal electrolyte in extracellular fluids for the maintenance

of normal osmotic pressure and water balance. Any disturbance in the concentration becomes a risk to the biological system. The recommended dietary intake of sodium is 250 mg/day for man. [21]

The result of the potassium content for *Pterocarpusmilbraedii* samples is presented in Table 2. The potassium content of the stems were found to be 0.441± 0.28 mg/100g higher than the leaves (0.036± 0.09 mg/100 g). The values are low compared to 515.43± 2.66 mg/100 g reported by [30] for domesticated elephant grass shoot. The recommended dietary intake of potassium is 1875-5625 mg/100g for adults.

The result of the phosphorus content of *Pterocarpus milbraedii* sample is presented in Table 2. The result obtained shows that the stems were found to be 0.57 ± 0.16 mg/100g higher than the leaves (0.36± 0.13 mg/100g). Phosphorus is essential to the body being a major constituent of body fluids. Phosphorus in phosphate form combines with calcium ion to form calcium phosphate, which is one of the basic constituents of the bone.

The result of the copper content for *Pterocarpus milbraedii* sample is presented in Table 2. The result of copper content shows that the stems contain 0.261±0.11 mg/100g and the leaves (0.016± 0.07 mg/100 g). These values are lower than 6.56+0.10 mg/100g reported by [31] and 9.78± 0.61 mg/100 g reported by [32] obtained for the seeds of similar plants. A daily allowance of 2g copper is considered to be satisfactory for the adult and 0.08 mg/kg is sufficient for infants. [26] Copper is required for diverse functions, including melanin pigment formation, maturation of collagen etc.

The result of the iron content of *Pterocarpus milbraedii* samples is presented in Table 2. The result shows that the stems have 53.66±0.63 mg/100g and the leaves 29.33±0.46 mg/100 g. These values are higher than (1.14±3.62) mg/100 g reported by [28] for curry leave tree. This is higher than 25.56±0.32 mg/100g reported by [22], 3.12± 1.22 mg/100g for *Phymatodesscolopendria.*

Protein involving iron helps in oxygen transport, ingestion of large quantity of iron in man can cause iron accumulation which is known as heamochromatosis, it is also poisonous to children when administered in excess drugs.

The result shows that the stem contains 0.56± 0.15 mg/100g and the leaves 0.39± 0.14 mg/100g of Iron. Studies on experimental animals have shown that manganese is required for normal bone growth and development, normal lipid metabolism, reproduction and regulation of nervous irritability. [33] In many countries, plant extracts which are good sources of manganese have been used as home remedies in diabetes mellitus. [34]

Phytochemical Screening and Quantification

Table 3 and 4 shows the result of phytochemicals screening and quantification.

The result obtained showed that cardiac glycosides were present in both petroleum and water extract of both samples. However, quantitative analysis showed that cardiac glycoside content was 9.29± 0.38 mg/100g for the pet ether of the stems and 15.7+0.42 mg/100g for water extract of the stems. Most cardiac glycosides are toxic and may have pharmacological activity, especially in the heart. [35] It has been used as arrow poisons of drugs and therapeutically to strengthen a weakened heart, and make it work effectively. [36]

Table 3: Results of phytochemical screening of the leaves and stems of *Pterocarpus milbraedii*

Parameters	Water Extract of the leaves	Petroleum ether extract of the leaves	Water extract of the stems	Petroleum ether extract of the stems
Cardiac glycosides	+	+	+	+
Alkaloids	-	-	+	-
Saponins	-	-	+	-
Tannins	-	-	-	-
Flavonoids	-	-	-	-
Polyphenols	++	++	++	+
Reducing sugar	-	-	-	-
Phlobatanins	-	-	-	-
Anthranoids	-	-	-	-
Anthraquinones	-	-	-`	-

Keys

+= present in moderate quantity, ++ = present in large quantity, - = Absent

Table 4: Results of quantitative phytochemical analysis of the leaves and stems of *Pterocarpus milbraedii* (*mg/100* g)

Parameters	Water Extract of the leaves	Petroleum ether extract of the leaves	Water extract of the stems	Petroleum ether extract of the stems
Cardiac glycosides	5.0±0.21	1.143±0.03	15.7±0.42	9.29±0.38
Alkaloids	_	_	6.8+0.26	_
Polyphenols	60.7±0.67	54.3±0.58	2.27±0.16	1.92±0.07
Saponins	_	_	5.4±0.24	_

Mean ±standard deviation of triplicate determinations

It was observed that alkaloid was present in the water extract of the stems; the quantitative analysis showed that alkaloids concentration was 6.8±0.26 mg/100g. Some alkaloids stimulate the central nervous systems; act as pain reliever and tranquilizers while others exhibit antimicrobial potency. [37]

The phytochemical screening showed that polyphenols were found to be present in large quantity of both extract. The quantitative estimation showed that polyphenol content was 1.92 ± 0.07 mg/100g for pet ether of the stems, 2.27+0.16 mg/100g for water extract of the stems. Polyphenols protects plants against chemical damage and perform same function in human, help in contracting the blood capillaries and prevent hemorrhage, also protect against stomach cancer. [38]

The result also showed that saponins were present only in the water extract of the stems. The quantitative estimation showed that saponin content was 5.4 +0.24 mg/100g, in the water extract of the stems. Saponins cause haemolysis of red blood cells and are of economic interest because of their occasioned toxicity to cattle and are mild laxatives, diuretics and explorants. There are two types of compounds which aid absorption of nutrients, i.e. steroidal saponins which have marked hormonal activity and triterpenoids. [39]

Anti-nutrients

Table 5 shows the result of anti-nutrient. The levels of hydrocyanide are 2.83±0.22 and 1.5± 0.08mg/100g in the leaves and stems respectively. The values are higher than 0.43± 0.02 and 0.17± 0.02% reported by Abasiekong *et al*. (2014) [40] for Lasenthra Africana and also higher than 0.54± 0.01 and 0.09 ± 0.12 for endocarp and exocarp of yellow monkey kola (cola lepidota) by [10] but competes with 1.80± 1.12 mg/100g recorded for *D. summattii* by. [10]

However, the low concentration of hydrocyanic acid in Pterocarpusmilbraedii would have little effects on metabolic enzymes, although the concentration in Pterocarpusmilbraedii may not have effect on nutrition.

The phytate levels are $8.106x10^{-3}$± 0.34 and $3.99x10^{-3}$ ± 0.27 mg/100g for the leaves and stems respectively. In comparison, the amount of phytic acid in an unprocessed *Treculiaafricana* was found to be 0.78±0.01 mg/100g which is higher. This concentration makes *Treculiaafricana* more toxic than *Pterocarpusmilbraedii.* High concentration of phytate cause adverse effect on digestibility. [41]

Table 5: Result of toxicant analysis of the leaves and stems of *Pterocarpus milbraedii*in mg/100 g

Toxicants	Leaves	Stems
Hydrocyanide	2.83+0.22	1.5 + 0.08
Phytate	8.1106×10^{-3} ±0.34	$3.99x10^{-3}$±0.27
Oxalate	5.5 + 0.67	28.6+0.48

Mean ± standard deviation of triplicate determinations

The oxalate levels showed 5.5 ± 0.6 and 28.6± 0.48 mg/100g for the leaves and stems respectively. These values compete with 28.20 ± 0.11 mg/100 g soluble oxalate obtained by Osabor *et al.* (2010) for *Diplaziumsummattii* leaves but higher than 9.97± 0.03 and 2.03± 0.25 mg/100g for *Hensiacrinata* reported by Abasiekong *et al*. (2014). The higher concentration of oxalic acid would actually reduce the amount of major and minor metallic nutrients in the biological system, by forming a complex. [42]

CONCLUSION

From the results obtained, the following conclusions were drawn:

- The leaves of *Pterocarpus milbraedii* is a valuable source of nutrients and it is comparable to many proteins-rich crops hence, it could be used as a protein source for human consumption especially where protein sources from animal products are very expensive.
- The leaves are also a good potential industrial raw material for food formulation and drug development.
- The mineral composition revealed high content of minerals such as Fe, Mg and Na indicating it as a viable source for minerals in nutrition.

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