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Chemical profile of leaves and seeds of *Pentaclethra macrophylla* Benth.

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ABSTRACT

The chemical profile of *Pentaclethra macrophylla* Benth. (African oil bean) seeds and leaves were analyzed using standard analytical methods. The result of the proximate composition indicates the presence of crude protein, crude fat, crude fibre, moisture and carbohydrate in the leaves and seeds. The macro-mineral composition showed that calcium has the highest value and magnesium the least in the leaves and seeds. Other minerals present include sodium, potassium and phosphorus. The micro-minerals showed the highest value for iron and lowest value for cobalt. Other micro-minerals determined are copper, zinc, manganese, cadmium for the leaves and seeds. The phytochemical screening showed the presence of saponins, flavonoids, alkaloids, cardiac glycosides, polyphenols and reducing sugar in both water and petroleum ether extract of the plant. Quantitative analysis of the phytochemicals revealed that polyphenols have the highest value and the least value was obtained for saponins occurring in the water extract and petroleum extract of the leaves and seeds. Other phytochemicals found include flavonoids, alkaloids, cardiac glycoside and reducing sugar in the leaves and seeds. The anti-nutrients composition consists of phytate, tannin, hydrocyanide, oxalate for the leaves and seeds. The results obtained show that *P. macrophylla* Benth. is a good source of food and therapeutic agent which will be of use for food and pharmaceutical industries.

INTRODUCTION

Studies have indicated that vast number of indigenous plants plays a vital role in the diet of man. Some of these plants serve as food and sources of nutrients because they provide minerals that are essential for body building and regulation of body functions. Many of such plants have been identified, but lack of analytical data on their chemical composition has limited the prospects of their utilization. They contain chemical substances that produce a definite physiological action on human beings. About 25% of all prescribed medicines today are substances derived from plants (Ngaski, 2006).

Medicinal plants play major role in healthcare delivery since time immemorial. The global market value of medicinal plant products exceeds $100 billion per annum (Sofowora et al., 2013).

Pentaclethra macrophylla Benth. has continued to be the major source of raw materials for nutrition and pharmaceutical industries which could be effectively utilized for nutrition and medicinal purposes. The outer bark is greyish to reddish brown, thin, flaking irregularly, inner bark fibrous, yellow to orange; twigs brown stellate-hairy, seeds are elliptical in outline, flattened and smooth, purplish brown, seedling with hypogeal germination (Ladipo and Boland, 1995). The African oil bean is a delicacy around the country Nigeria supplying the body with essential amino acids and fatty acids which is a good food source. Also, has anti-microbial and wound healing agent and the leaf extract could be used to cure diarrhea (Alinnor and Oze, 2011).

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However, the analytical data of this plant commonly found in Nkitaku village in Agulu in Anaocha Local Government Area of Anambra state has not been reported. It is found mostly in the southern and middle belt region of Nigeria and in some parts of West Africa. The present investigation is qualitative and quantitative analyses of the leaves and seeds of *P. macrophylla* Benth. The nutritive and phytochemical content were also investigated to predict their nutritional viability and medicinal potentials (Enujiugha and Akanbi, 2005).

MATERIALS AND METHODS

Sample preparation

Fresh leaves and seeds of *P. macrophylla* Benth. were collected from forest in Nkitaku village in Agulu in Anaocha Local Government Area of Anambra State. The samples were taken to Botany department of the University of Calabar, Calabar where it was authenticated. The samples of the leaves and seeds were packed in a large polythene bag and taken to the laboratory where the leaves were separated from the branch and washed with clean water to remove traces of dust and unwanted materials. The seeds of the sample was washed properly and boiled at 100°C in a clean metallic pot for about 3 h, the hard brown testa (shell) was removed and the cotyledon was shredded into pieces. It was weighed and placed in an oven until it dried to constant weight. Both samples of the seeds and leaves were weighed separately and placed in an oven until it was dried to a constant weight. Then they were allowed to cool and ground into powdered 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, 20 g of the ground samples of the leaves and seeds were weighed separately and packed into extraction thimble and fitted into soxhlet sets. The soxhlet apparatus was heated under reflux using heated mantle for 6 h until extraction was complete. The same 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 AOAC (2000).

Proximate analysis

Proximate analysis was determined using the method described by AOAC (2000) for the sample of the leaves and seeds. The method was used to estimate moisture, ash, crude fat, protein, crude fibre and carbohydrate contents of the sample. The percentage carbohydrate was estimated using the expression:

%CHO = 100 - (%Ash + %Crude protein + Crude fibre + %Crude fat)

Phytochemical screening

Phytochemical screening procedure was adopted from previous work on plant analysis (Sofowora, 2008). The extracts were screened for cardiac glycosides, alkaloids, saponins, tanins, flavonoids, polyphenols, reducing compounds, phlobatanins, anthranoids and anthraquinones. The analysis determines the biologically active non-nutritive compound which contributes to the colour, flavour, and characteristics of plant parts.

Quantitative estimation of some phytochemicals

Quantitative estimation of saponins, flavonoids, alkaloids, polyphenols, cardiac glycosides and reducing sugar were estimated using the method by Sofowora (2008). The analysis determines the appropriate concentration and quantity of each of the phytochemicals in the sample of *P. macrophylla* Benth.

Mineral elements analysis

Mineral analysis was determined using the method of AOAC (1990). The Na and K was determined using flame emission photometer (FES). The principle of this procedure relies on the fact that 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 others (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 (AOAC, 1990), oxalate was estimated by the method of (Dye, 1956), phytic acid was extracted using the method by (Mecance and Widdowson, 1953) while tannins were estimated by the method of (Burn, 1971).

Method of statistical analysis

The data obtained from this research, were reported as

Table 1. Proximate composition of the leaves and seeds *of P. macrophylla* Benth. (%).

Nutrients	Leaves	Seeds
Moisture	28.04 ± 0.15	30.50 ± 0.22
Crude fat	10.54 ± 0.20	25.54 ± 0.10
Crude fiber	22.12 ± 0.12	12.00 ± 0.55
Ash content	2.00 ± 0.20	4.00 ± 0.11
Carbohydrate	51.59 ± 0.24	34.42 ± 0.76
Crude protein	15.75 ± 0.12	24.04 ± 0.67

Mean ± standard deviation of triplicate determinations.

mean ± standard deviation of mean. The significant difference between the values was estimated, using F-test and T-test analysis at a confidence level of 95% given by ($p \leq 0.05$) Sachs (1984) and Brooks (1976).

RESULTS AND DISCUSSION

Proximate composition

Table 1 shows the result of proximate composition. The result obtained shows that the seeds have more moisture of 30.50 ± 0.22% than the leaves of 28.04 ± 0.15%. These results are lower than the moisture content of 49.77 ± 0.02 for fermented *P. macrophylla* Benth. by Chimezie and Olasupo (2013), the high moisture content of seeds to the leaves of *P. macrophylla* Benth. indicates that the seeds cannot be stored favourably for a long period of time because of deterioration. The stability and susceptibility of microbial contamination of food is determined by its moisture content (Scott, 1980).

The result also shows that the leaves 4.00 ± 0.20% contain higher levels of ash than the seeds 2.00 ± 0.11%. These values are higher than the values 1.94 ± 0.01 obtained for the seeds of *P. macrophylla* Benth. by Onyeleke et al. (2014). The ash content is a reflection of its mineral content. The high ash content of the leaves compared to the seeds presents that the minerals are more concentrated in the leaves than in the seeds (Oyoyede, 2005).

The crude protein composition shows that the seeds contain a higher protein level of 24.04 ± 0.67% than the leaves which contains 15.75 ± 0.12%. The result is in conformity with the report by Umoh et al. (1998) that seeds are usually higher in nitrogenous component than other parts of the fruit. The basic function of protein in nutrition is to supply adequate amounts of required amino acids (Pugalenthi et al., 2004). Protein deficiency causes growth retardation, muscle wasting, and abnormal swelling of the belly and collection of fluids in the body (Zarkada and Voldeng, 1997).

The result of the carbohydrate content shows that the leaves are higher in carbohydrate content 51.59 ± 0.24% than the seeds which contain 34.42 ± 0.76%. These values compare with 24.60 ± 0.32% reported by Osabor et al. (2011) for *Phymatodes scalopendria* and higher than 10.58 ± 0.41% (Eze and Okonkwo, 2012). When carbohydrate is sufficient in food it prevents the unnecessary usage of protein and allows it to be used for body building process (Davidson et al., 1975).

The result shows that the leaves have more fibre content with value of 22.12 ± 0.12% than the seeds with value of 12.00 ± 0.55%. Crude fibre provides roughages that aids digestion and reduces the accumulation of carcinogen in the body (Robinson, 1978). It is also used in the analysis of various food products to detect adulteration and also in the determination of conformity to the existing standard of quality and identity in processed food products.

The result of the crude fat shows that the seeds value is higher with 25.54 ± 0.10% than the leaves with 10.54 ± 0.20% but lower than 46.95% reported by Alinnor and Oze (2011) for *P. macrophylla* Benth. seeds. The result shows that African oil bean seed is a better source of oil than the leaves hence it could be grouped under oil rich plant foods (Robinson, 1978). It aids in storing energy in the body and in excess is associated with cardiovascular disorder.

Mineral composition

Table 2 and 3 shows the result of mineral composition. The result of the calcium content shows that the leaves have more calcium of 329.12 ± 0.35 mg/100g than the seeds with 314.12 ± 0.44 mg/100g. These values are quite high compared with the values of 110.05 ± 0.02 mg/100g reported by Ogueke et al. (2010) for fermented *P. macrophylla*. Calcium is required in the body for normal growth of bones and teeth (Robinson, 1978). It is an important mineral required for bone formation and neurological function of the body. The recommended daily calcium allowance for children and adult is 360–1200 mg/100g (NRC, 1989).

Table 2. Macro-mineral analysis of the leaves and seeds of *P. macrophylla* Benth. (mg/100g).

Element	Leaves	Seeds
Ca	329.12 ± 0.35	314.12 ± 0.44
Mg	126.14 ± 0.04	104.33 ± 0 16
Na	265.68 ± 0.72	156.40 ± 0.54
K	160.00 ± 0.04	127.19 ± 0.24
P	221.2 ± 0.17	172.0 ± 0.54

Mean ± standard deviation of triplicate determinations.

Table 3. Results of Micro-mineral analysis of the leaves and seeds of *P. macrophylla* Benth. (mg/100g).

Element	Leaves	Seeds
Cu	16.33 ± 0.01	13.60 ± 0.22
Zn	12.0 ± 0.25	10.0 ± 0.22
Mn	54.20 ± 0.17	27.40 ± 0.54
Fe	55.28 ± 0.11	52.55 ± 0.12
Co	8.5 ± 0.25	0.05 ± 0.01
Hg	0.25 ± 0.05	TRACE
Pb	5.5 ± 0.22	TRACE

Mean ± standard deviation of triplicate determinations.

The result of the magnessium shows that the leaves contain 126.14 ± 0.04 mg/100g which is higher than the seeds with 104.33 ± 0.16 mg/100g which are lower than the RDA standard. 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, 1974).

The result of sodium shows high levels of 265.68 ± 0.72 mg/100g for the leaves and 156.40 ± 0.54 mg/100g for the seeds. Sodium is the principal electrolyte in extracellular fluids for the maintenance of normal osmotic pressure and water balance. The recommended dietary intake of sodium is 2500 mg/day for man (NRC, 1989).

The potassium content of the leaves were found to be 160.00 ± 0.04 mg/100g higher than the seeds 127.19 ± 0.24 mg/100g which were lower than the recommended dietary intake of potassium of (1875 – 5625 mg/100g) for adults.

The result obtained shows that the leaves contain 221 ± 0.17 mg/100g phosphorous than the seeds of 172.0 ± 0.54 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 copper content shows that the leaves contain 16.33 ± 0.01 mg/100g and the seeds 13.60 ± 0.22 mg/100g. Similar research work by Onyeleke et al. (2014) has a value of 5.25 ± 0.05 mg/100g for *P. macrophylla* Benth. seeds. These values are high when compared to the body daily requirement of 2.0 mg/day (NRC, 1989).

The leaves of *P. macrphylla* Benth. shows a Zn levels of 12.0 ± 0.25 mg/100g and the seeds 10.0 ± 0.22 mg/100g. These values are higher than 6.56 ± 0.10 mg/100g reported by Onyeleke et al. (2014) and 9.78 ± 0.61 mg/100g reported by Enujiugha and Akanbi (2005) obtained from the seeds of similar plant. Zinc is essential for all living things. The body daily requirement for zinc is 15 mg/day. The zinc present in pancreas may aid in the storage of insulin. Zinc in the plants could mean that the plants play essential roles in the management of diabetes, which result from insulin malfunction (Okaka et al., 2002).

The result of iron content shows that the leaves of *P. macrophylla* Benth. have 55.28 ± 0.11 mg/100g and the seeds have 52.55 ± 0.12 mg/100g of iron. These values compared favourably well with the values 56.28 ± 5.42 reported by Enujiugha and Akanbi (2005), higher than 8.71 ± 0.02 by Onyeleke et al. (2014) of the seeds of similar plants. The recommended daily requirement of iron is 10–18 mg/day for both children and adult (NRC, 1974). Iron is required for the formation of blood and its deficiency causes anaemia (Turan et al., 2003).

Table 4. Results of Phytochemical Screening of the Leaves and Seeds of *P. macrophylla* Benth.

Parameters	Water extract of the leaves	Petroleum extract of the leaves	Water extract of the seeds	Petroleum extract of the seeds
Alkaloids	_	+	_	+
Saponins	++	_	_	+
Tannins	_	_	_	_
Flavonoids	_	++	_	+
Polyphenols	+	+	++	++
Reducing sugar	+	_	++	_
Phylobatanins	_	_	_	_
Anthranoids	_	+	_	_
Anthraquinones	_	_	_	_
Cardiac Glycosides	++	_	_	+

+, Present in moderate quantity; **++,** present in large quantity; **_,** absent.

Table 5. Results of quantitative phytochemical analysis of leaves and seeds of *P. macrophylla* (mg/100g).

Parameter	Water extract of the leaves	Petroleum ether extract of the leaves	Water extract of the seeds	Petroleum ether extract of the seeds
Saponins	0.5 ± 0.22		--	0.3 ± 0.42
Flavonoids	--	1.5 ± 0.13	--	0.5 ± 0.06
Alkaloids	--	0.4 ± 0.11	--	0.52 ± 0.68
Polyphenols	0.9 ± 0.12	1.5 ± 0.78	2.2 ± 0.26	3.5 ± 0.01
Cardiac glycosides	2.5 ± 0.54	--	--	0.2 ± 0.26
Reducing sugar	0.32 ± 0.15	--	4.45 ± 0.51	--

Mean ± standard deviation of triplicate determinations.

The result of magnesium shows that the leaves contain 54.20 ± 0.17 mg/100g and the seed contains 27.40 ± 0.54 mg/100g. Magnesium is required for various biophysiological processes, notably as an activator of various enzymes (Maynard and Loosle, 1969).

Phytochemical screening and quantification

Table 4 and 5 shows the result of phytochemical screening and quantification. The results of the phyto-chemical screening shows that higher concentration of saponins was obtained in water extract than in petroleum ether extract. However, quantitative analysis carried out on this sample, shows that the sample contains 0.5 ± 0.22 mg/100g for the water extract of the leaves and 0.3 ± 0.42 for the petroleum ether extract of the seeds. Saponins have numerous medical uses, they cause haemolysis of red blood cells and are of economic interest because of their occasional toxicity to the cattle and are mild laxatives, diuretics and explorants (Trease and Evan, 1996). They are of two types, saponins triterpenoids which aid in absorption of nutrient and steroidal saponins which have marked hormonal activity (Rahaman, 2010).

It was observed that the flavonoids occurred only in the petroleum ether extract of the leaves and seeds, the quantitative estimation shows that the samples contains 1.5 ± 0.13 mg/100g for the petroleum ether extract of the leaves and 0.5 ± 0.06 mg/110g for the petroleum ether extract of the seeds. Flavonoids strengthen the blood capillaries and prevent the small cutaneous hemorrhage; it relieves cramps of the smooth muscles while others improve circulations in the coronary arteries. They are known for their anti-inflammatory and anti-allergic effect for inhibition of tumor production and its projection for gastric mucosa while some have anti-bacterial and anti-fungal properties. They are useful to man due to their physiological activities and most important classes are the coloured anthocyanin pigment (Harbone, 1973).

The analysis of the phytochemical showed that alkaloids was obtained in the petroleum ether extract of the leaves and seeds, the quantitative estimation shows that the samples contains 0.4 ± 0.11 mg/100g for the

Table 6. Toxicant analysis of seeds and leaves of *P. macrophylla* Benth. in mg/100g.

Toxicants	Leaves	Seeds
Hydrocyanide	1.8 ± 0.01	1.2 ± 0.02
Tannins	1.2 ± 0.32	0.8 ± 0.22
Phytate	0.6 ± 0.32	0.42 ± 0.12
Oxalate	13.64 ± 0.71	53.24 ± 0.54

Mean ± standard deviation of triplicate determinations.

petroleum ether extract of the leaves and 0.52 ± 0.68 mg/100g for the petroleum ether extract of the seeds. Alkaloids are nitrogen containing organic compounds. They act as pain reliever, tranquilizers, while others act against infectious micro-organism (Solomon and Craig, 1998).

The screening result shows that the petroleum ether extract contains higher levels of polyphenols than the water extract. Quantitative analysis carried out reveals that the samples contains 0..9 ± 0.12 mg/100g for the water extract of the leaves, 1.5 ± 78 mg/100g for the petroleum extract of the leaves, 2.2 ± 0.26 mg/100g for water extract of the seeds and 3.5 ± 0.01 mg/100g for the petroleum extract of the seeds. Phenols and polyphenols protect plants against chemical damage and perform same function in human, help in contracting the blood capillaries and prevent hemorrhage, also protects against stomach cancer (Brown and Arthur, 2001).

Phytochemial analysis shows that the water extract contains higher level than the petroleum ether extract for cardiac glycosides while quantification shows 2.5 ± 0.54 mg/100g for water extract of the leaves and 0.2 ± 0.26 mg/100g for the petroleum ether extract of the seeds. Cardiac glycosides are organic compound containing glycosides that act on the contractile force of cardiac muscle, used in the treatment of cardiac failure. Most cardiac glycosides are toxic and may have pharmacological activity especially in the heart (Harbone, 1973). Cardenolides is a special type of cardiac glycosides which appear to be abundance in Asclepieadeceae and apycynaceae, their action is anti-inflammatory, antiseptic, analgesic and used in the treatment of rheumatism (Trease and Evans, 2002).

Screening result shows that the reducing sugar occurred in the water extract of the leaves and seeds. Quantitative analysis shows that the extract contains 0.32 ± 0.15 mg/100g for water extract of the leaves and 4.45 ± 0.01 mg/100g for water extract of the seeds. Anthranoid was present in the water extract of the leaves. Also, tannins, phylobatanins and anthraquinone were absent in both extracts.

Anti-nutrient

Table 6 shows the result of anti-nutrient. The levels of hydrocyanide are 1.8 ± 0.01 and 1.2 ± 0.02 mg/100g in the leaves and seeds respectively. The values are higher than 0.43 ± 0.02 and 0.17 ± 0.02% reported by Abasiekong et al. (2014) for *Lasenthra Africana*. The low level concentration of hydrocyanic acid in the species signifies it would have little effects on metabolic activities. Hydrocyanide inhibit cytochrome omadase, cellular omadase and activity of vitamin K. The recommended lethal dose of hydrocyanide is 35 mg/100g (Oke, 1969).

The level of tannin showed 1.2 ± 0.22 and 0.8 ± 0.32 for the leaves and seeds respectively. The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also as pesticides, and in plant growth regulation. The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit or red wine or tea, low dosage as (0.15–0.2%) in the diet may be beneficial.

The phytate levels are 0.6 ± 0.32 and 0.42 ± 0.12 4 mg/100g for the leaves and seeds respectively. Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc, although the binding of calcium with phytic acid is pH dependent. The binding of phytic acid with iron is more complex, although there certainly is a strong binding affinity, molecules like phenols and tannins also influence the binding. When iron and zinc bind to phytic acid they form insoluble precipitate and are far less absorbable in the intestines. This process can therefore contribute to iron and zinc deficiencies in people whose diets rely on these foods for their minerals. The recommended lethal dose of phytic acid is 2.78 mg/100g (Haros et al., 2009).

The oxalate levels showed 53.24 ± 0.54 and 13.64 ± 0.01 for the leaves and seed respectively. The higher concentration of oxalic acid in *P. macrophylla* would actually reduce the amount of major and minor metallic nutrients in the biological system, by forming a complex (Ugwu and Onaye, 2006).

Conclusion

From the results obtained, the following conclusions have been drawn.

- ❖The leaves and seeds of *P. macrophylla* Benth. are rich in carbohydrate, protein, fat and fibre with low level of ash.
- ❖The mineral element analysis revealed the presence of macro and mineral elements.
- ❖The phytochemical screening of the samples revealed the presence of saponins, alkaloids tannins, polyphenols, cardiac glycosides and reducing sugars.
- ❖The toxicant content analysis of *P. macrophylla* Benth. samples revealed high levels of oxalates and low levels of phytic acid, hydrocyanic acid and tannin.
- ❖The high concentration of bio-active compounds such as polyphenols, cardiac glycosides and reducing sugar etc. and also the high levels of carbohydrate, protein and fat in the sample, indicates that it is a good source food and therapeutic agent.

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