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PROXIMATE ANALYSIS AND PHYTOCHEMICAL INVESTIGATION OF *PLUKENETIA CONOPHORUM* STEM BARK.

[1]P. B. Ayoola and [2*]O. O. E. Onawumi

[1]Department of Science Laboratory Technology, Ladoke Akintola University of Technology, P.M.B.4000, Ogbomoso, Oyo State, Nigeria.
[2]Department of Chemistry, Ladoke Akintola University of Technology, P.M.B.4000,Ogbomoso,Oyo State, Nigeria.

***Author for Correspondence: Prof. O.O.E. Onawumi**.
Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso., Oyo State, Nigeria.

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ABSTRACT
This research work aim at investigating the chemical constituents present in the *Plukenetia conophora* stem bark in order to ascertain its usefulness as a medicament and food supplement. The proximate analysis results: 51 % moisture, 3.24 % fat, 27.42 % fibre, 6.29 % protein, 4.26 % Ash and 7.79 % carbohydrate. The mineral analysis revealed the following: K, 5750 mg/kg, Na, 5040 mg/kg, Ca, 21750 mg/kg, Mg, 1658.75 mg/kg, Fe, 9460 mg/kg, Zn, 55.30 mg/kg, Mn, 68.50 mg/kg, Cu, 20.65 mg/kg. Vitamin composition results showed: Thiamine (B_1) 0.21 µg/100g, riboflavin (B_2) 0.15 µg/100g, niacin (B_3), 0.27 µg/100g, cyanocobalamin (B_{12}) , 0.14 µg/100g, ascorbic acid (C) 0.003 µg/100g, tocopherol (E), 0.79 µg/100g. The phytochemical screening and analysis shows; alkaloids, 2.890 mg/kg, saponins, 1.260 mg/kg, tannins, 0.340 mg/kg, and anthraquinones, 0.064 mg/kg. The study shows that *Plukenetia conophorum* stem barks could be a potential food supplement and useful source of drug formulation.

KEYWORDS: *Plukenetia conophora*, Stem bark, Proximate, Phytochemical.

1.0 INTRODUCTION
Plukenetia conophorum (formerly called *Tetracarpidium conophorum*) is known in the southern Nigeria as *ukpa* (igbo), Western Nigeria as *awusa* or *asala* (Yoruba). It is found in Uyo, Akamkpa, Akpabuyo, Lagos, Kogi ,Ajaawa-Ogbomoso and Ibadan. This plant (Plate 1) is cultivated principally for the nuts which are cooked and consumed as snacks (Oke,1995). A bitter taste is usually observed upon drinking water immediately after eating the nuts. This could be attributed to the presence of chemical substances such as alkaloids. Ayodele (2003) reported the presence of oxalate, phylates and tannin in the raw *Tetracarpidium conophorum* nuts. Edem *et al*., (2009) reported on the proximate composition, ascorbic acid and heavy metal contents of the nut. Oyenuga (1997) reported on the amino acid and fatty acid components of the nut and on the use of its leaf juice for the treatment of prolonged and constant hiccups. Though the stem barks have been in use many years ago by the traditional medicine practitioners in Nigeria, yet much work has not been reported on the proximate and phytochemical investigation of the stem bark within the locality and state covered in this report. Therefore, the objective of this work is to evaluate the proximate, mineral, vitamin and phytochemical compositions of *Plukenetia conophorum* stem bark in order to ascertain its possible usefulness as food supplements and in formulation of drugs.

2.0 MATERIALS AND METHODS
2.1 Collection of plant sample
The stem bark used for this study was collected from a farmland at Osupa ile village in Ajaawa, Ogo-oluwa Local Government Area of Ogbomoso in Oyo State, Nigeria.

2.1.1 Sample Identification
The plant sample (stem bark) was authenticated and identified at the herbarium of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State. Specimens (Voucher No: F.H.I 109504) was deposited at FRIN.

2.1.2 Preparation of sample
The sample (stem bark) (Plate 1) was peeled with a stainless knife to remove the bark and was cut into pieces to facilitate drying and was thoroughly washed, spread on white cardboard papers and air dried at room temperature (25 ^{o}C) in the laboratory for 30 days before milling to a powdery form and stored in an air tight bottle prior to analysis.

3.1 Proximate analysis: Moisture content was determined by drying to constant weight at 105 ^{o}C in an oven, ash content by ignition at 550 ^{o}C in a muffle furnace for 4 h, oil content by soxhlet extraction with hexane as solvent, protein by the kjeldahl method, and crude fibre by the acid and alkaline digestive methods all described by Lees (1975). The carbohydrate content was estimated by difference, subtracting the sum of water, protein, fat, crude fibre and ash percentages from one hundred.

3.1.1 Mineral analysis: The AOAC, (1984) method was used for the determination of minerals in the test sample. Calcium, sodium, potassium, magnesium were determined by flame photometric method while iron, zinc, manganese, copper and chromium were determined by atomic absorption spectrophotometric method.

3.1.2 Vitamin analysis: The composition of the water-soluble vitamins such as thiamine (B_1), riboflavin (B_2), niacin (B_3), cyanocobalamin (B_{12}) and ascorbic acid (vitamin C) were all determined by the method of Scalar (2000).While tocopherol (vitamin E) content was determined by the method of AOAC (1984).

3.1.3 Phytochemical analysis: The phytochemical screening was done on the sample using methods as described by (Sofowara, 1993). Alkaloids were extracted using a slightly modified method of Maxwell *et al* (1995). Here, the dried sample was homogenized and the alkaloid extracted from 10 g of the sample for 4 h using 20 % v/v acetic acid in ethanol. The extract was filtered to remove cellulose debris and then concentrated to about one quarter of the original volume. One percent NH_4 OH was added drop wise until a precipitate occurred. The crude alkaloids was dried to constant weight in an oven and the percentage alkaloids calculated. The Bohmand Kocipai-Abyazan method (1994) was used for the determination of tannins, while saponin was analyzed using that of Peng and Kobayasli (1995)

4.0 RESULT AND DISCUSSION

Based on the results of the proximate composition of the stem barks as shown in Table 1, the stem bark contained all the nutrients that the human system needs for survival and sound health, therefore, stem bark of *P. conophora* could serve as food supplement.

The mineral contents (Table 2) present shows that calcium is the highest concentration and considering the importance of calcium in human body for both therapeutic and prophylactic purposes, one would say, it is a good source of calcium. *P. conophora* stem bark is also a good source of manganese and copper, two elements that are very useful to mankind. Manganese is used in the management of diabetes (Edem *et al.*, 2009).

The vitamin compositions of the stem bark are shown in table 3. As a result of the presence of vitamins $B_{1,2,3,12}$ in the stem bark, the plant can be used in herbal medicine for the treatment of some diseases such as pellagra (Okwu and Okeke, 2003). Other vitamins such as C and E, though in trace amount are essential for body metabolism. There is also an interesting ability of ascorbic acid as an antioxidant, to prevent or at least minimize the formation of carcinogenic substances from dietary material (Hunt *et al*., 1980).

The phytonutrients present in the stem bark sample analyzed are shown in table 4 while the amounts are displayed in table 5. The stem bark contained more alkaloids and saponnins, while tannins and anthraquinones are present in minute quantity. Alkaloids are the most efficient plant substances used therapeutically. Pure isolated alkaloids and the synthetic derivatives are used as the basic medicinal agent because of their analgesic, antispasmodic and bacterial properties. The presence of tannins (Table 4) in the stem bark of *P. conophora* can support its strong use for healing of haemorrhoids, frost bite and varicose ulcers in herbal medicine (Igboko, 1983; Maduiyi, 1983). The result of mineral compositions clearly shows that *P. conophora* stem contains rich source of mineral elements.

This result become so important when the usefulness of such minerals like Ca, Mg, Na, K in the body are considered, However, the Na and K content of *P. conophorum* is an added advantage because of the direct relationship of Na intake with hypertension in human (Dahl, 1972). This may be the reason why the plant is used to prevent and control high blood pressure (James, 2009). The presence of copper may be responsible for the absorption of iron, it is therefore often seen with iron naturally. Copper is important for cellular defence and protection of the mucous membrane, antianaemic and essential for the formation of haemoglobin from iron (Claude and Paule, 1979). The presence of manganese shows that the plant can be used to protect bone disease (James, 2009). The activity of this element is noticed in the metabolism of food incorporated into the bone. According to Claude and Paule (1979), manganese is necessary for the functioning of the pituitary gland, the pineal gland and the brain, it promotes hepatorenal function, combat anaemia and also essential for growth. The presence of zinc is an indication that the stem bark may have some effect on the nerve function and male fertility. It is important for normal sexual development, especially for the development of testes and ovaries, it is also essential for reproduction. Zinc stimulates the activity of vitamins, formation of red and white corpuscles (Claude and Paule, 1979), healthy functioning of the heart and normal growth (Elizabeth, 1994).

Fugure 1: Stem bark of *Plukenetia conophora*

RESULTS AND DISCUSSION

Table 1: Proximate analysis of the *Plukenetia conophorum* stem bark

Parameter	Composition (%)
Moisture content	51±0.68
Crude Fat content	3.24±0.10
Crude Protein content	6.29±0.02
Crude Fibre content	27.42±0.03
Ash content	4.26±0.08
Carbohydrate content	7.79±0.02

Results are mean of three determinations

Table 2: Mineral composition of the stem bark on a dry weight basis

Mineral	Concentration (mg/kg)
Potassium	5750.00±0.01
Sodium	5040.00±0.20
Calcium	21750±0.03
Magnesium	1658.75±0.2
Iron	9460.00±0.03
Zinc	55.30±0.50
Manganese	68.50±0.10
Copper	20.65±0.02

Results are mean of three determinations

Table 3: Vitamin composition of the stem bark on a dry weight basis

Vitamin	Amount
Tocopherol (E) µg/100g	0.79±0.08
Ascorbic acid (C) µg/100g	0.003±0.002
Thiamine (B_1) µg/100g	0.21±0.3
Riboflavin(B_2) µg/100g	0.15±0.01
Niacin (B_3) µg/100g	0.27±0.02
Pantothenic acid (B_5) µg/100g	ND
Pyridoxine(B_6) µg/100g	ND
Folic acid (B_9) µg/100g	ND
Cyanocobalamin (B_{12}) µg/100g	0.14±0.2

Results are mean of three determinations
ND= Not detected

Table 4: Phytochemical screening of the stem bark

Constituent	Bioassay
Alkaloids	+
Flavonoids	-
Cardiac glycosides	-
Saponins	+
Tannins	+
Anthraquinones	+

Key:
+ = present in a minute amount
-ve = absent

Table 5: Quantitative estimates of phytochemicals of the stem bark

Constituent	Quantity w/w (mg/kg)
Alkaloids	2.890±0.01
Flavonoids	ND
Cardiac glycosides	ND
Saponins	1.260±0.02
Tannins	0.340±0.1
Anthraquinones	0.064±0.1

Results are mean of three determinations
ND= Not detected

5.0 CONCLUSION

The present study has shown the proximate, vitamins, minerals and phytochemical compositions of *P. conophora* (African walnut) stem bark. This partly supports the use of this stem bark in herbal medicine. As a rich source of alkaloids, coupled with the presence of essential vitamins and minerals, *P. conophora* can be seen as a potential source of useful food supplements and drugs. The presence of tannin supports its anti-inflammatory property. This also proves that the stem may be helpful in asthma, rheumatoid and arthritis. Further studies could be carried out to isolate, characterize and elucidate the structure of the bioactive compounds from the stem bark for industrial drug formulation.

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