Original Research Article

Evaluation of Proximate, Minerals, Vitamins and Antinutrient Composition of *Combretum platypterum* (Welw.) Hutch. & Dalziel. (Combretaceae) Leaves.

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Abstract

Background: *Combretum platypterum* (Welw.) Hutch. & Dalziel. (Combretaceae) leaves are used in treatment of jaundice and hepatitis in South-Eastern Nigeria. Evaluations of the nutritional composition of medicinal plants can enhance their profile as sources of food and medicines. This research aims to determine the proximate composition, minerals, vitamins, and anti-nutrients contents of *Combretum platypterum* (Welw.) Hutch. & Dalziel *(Combretaceae)* leaves.

Methods: Proximate, minerals, and vitamins analysis are done according to the standard methods described by AOAC. Spectrophotometric methods and AAS were used to determined minerals. For the ANFs, oxalate concentration was determined using the permanganate titration method, phytate determination was based on the analysis of phosphorus in a ferric phytate complex, assuming a constant 4Fe: 6P molecular ratio in the complex. Tannins, trypsin inhibitors, and hemagglutinins were evaluated using other standard methods.

Results: Mineral content: Cal 1.14 mg/100g, Mg*1*.58 mg/100g, Mn *0*.268 mg/100g, Cu 0.176 mg/100g, Zn 5.9 mg/100g, Se 0.957 mg/100g, Fe 3.596 mg/100g, K 1.537 mg/100g, Na 0.6 mg/100g, phosphorus 0.58mg/100g. Proximate analysis*:* protein 3.327 %, moisture 4.45 %, ash 2.65%, fibre 4.3%, fat 2.20 %, carbohydrates 83.07 %. Anti-nutrients content*:* oxalate:

0.577 (mg/100g). phytate 0.567 (mg/100g), hemagglutinin 0.428 (HU/mg), tannins 123.53 mg/100g), trypsin inhibitor 0.647±.003 HIU/mg. Vitamin analysis: Vitamin C 0.328 mg/100g, vitamin D 0.227 mg/100g, vitamin E 0.59 mg/100g, vitamin K 0.137 mg/100g, and vitamin A (retinol) 4.507 µg/g

Conclusion*:* *Combretum platypterum* (Welw.) Hutch. & Dalziel *(Combretaceae)* leaves are rich in proximate elements, vitamins and essential minerals and low levels of antinutrients.

Keywords: *Combretum platypterum*, Proximate Analysis, Vitamins, Nutrients.

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Introduction

Proximate composition

Proximate analysis is estimation and determination of how much of the major food components, which are Moisture, Carbohydrates, Lipids, Proteins, Ash, Crude Fiber, exist in a given food. Vitamins are a heterogenous group of organic compounds, distinct from major food nutrients (proteins, lipids, and carbohydrates) essential for growth and maintenance of animal life. They are required by the animal in trace amounts. Vitamins are classified based on their solubility into two broad groups namely (1) the water soluble vitamins and (2) the fat soluble vitamins.[1]

According to the Food and Agriculture Organization (FAO), there are about 20 inorganic minerals essential to animal life. They are classified according to their concentration into macroelements and microelements. There include principal cations (sodium, potassium, magnesium and calcium)., principal anions (phosphorus, chlorine, and sulphur)., and trace or microelements (selenium, iron, zinc, manganese, copper, iodine, etc.). They serve as essential constituents of skeletal structures and soft tissues, and enzyme systems. They also maintain osmotic pressure and pH in the body.[2]

Several classes of compounds have been shown to have the potential of acting against optimal nutrition in humans and animals. They inhibit or retard efficient utilization of nutrients contained in food. Among these are tannins, phytates, oxalates, alkaloids, hemagglutinins, protease inhibitors, trypsin inhibitors, and saponins. ANFs frustrate efficient and proper nutrition by inhibiting enzymes, barricading or diverting nutrients from absorption sites or interfering with nutrient availability through complexation and/or chelation

Phytate (Phytic acid, Hexaphosphate Inositol**,** Inositol Hexaphosphate, IP6)

The salt form of phytic acid found in plants and animals is known as phytate. It exists mainly as the salt of mono- and divalent cations of potassium, magnesium, and calcium. It has a great potential to chelate free iron.[3] Phytate (also known as isonitol hexakisphosphate (InsP6)) is ubiquitous among plant seeds and grain.[4] Phytate is primarily present as the salt of mono- and divalent cations K^+, Mg^{2+}, and Ca^{2+} and accumulates in seeds during the ripening period. It serves a storage form for both phosphate, high-energy phosphoryl groups, and isonitol in plant seeds and grains.[3] Anti-nutrient activity of phytic acid is ascribed to its highly negatively charged structure which makes it attract positively charged ions such as zinc and calcium, and proteins. [5] This imparts antinutrient property to the compound. Structural analysis of a prepared calcium-phytate complex shows that five calcium atoms combine with one phytate molecule.[6]

Phytates and oxalates have the ability to form chelates with di- and trivalent metallic ions such as Cd, Mg, Zn, and Fe to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract, thus reducing their bioavailability.[7] Oxalates that bind with calcium are practically insoluble. These crystals solidify in the kidneys to form kidney stones, or in the urinary tract causing pain and irritation.[8]

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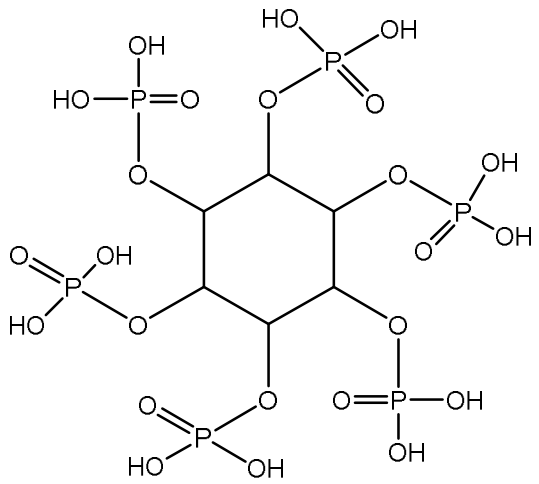


Phytate (PubChem CID 890).

Hemagglutinin and lecitins

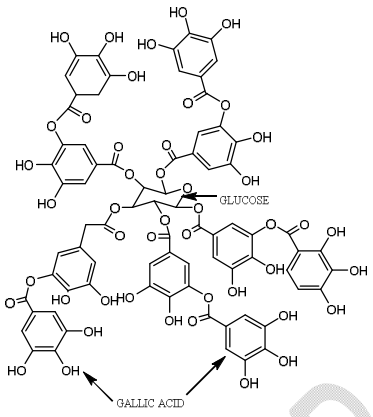
Interaction of red blood cells with some proteins/glycoproteins results in agglutination. Some proteins combine with specific sugars while the sugars involved in other associations are not known. Those that bind to specific sugars are called lecitins.[9] while those whose sugar specificity is not known are referred to as hemagglutinins.[10] Hemagglutinin refers to any group of glycoproteins that cause red blood cells to clump together (agglutinate).[11]

They interfere with the digestion and absorption of nutrients in the gastrointestinal tract, thereby, depressing animal growth,[12] lecitins and hemagglutinins are proteins/glycoproteins which have at least one non-catalytic domain that exhibits reversible binding to specific monosaccharide or oligosaccharides. They can bind to the carbohydrate moiety on the surface of erythrocytes and agglutinate them without altering the properties of the carbohydrate.[10]

Tannins

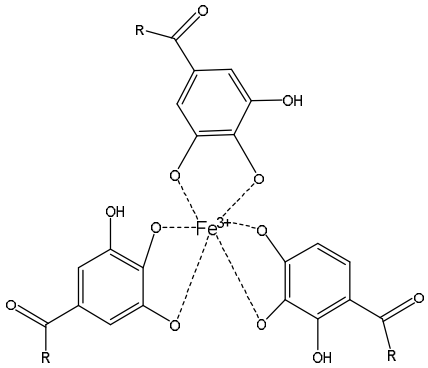
The ability to form complexes and precipitate proteins, alkaloids and some metals like iron, is a basic and re-occurring property in the definition and classification of the class of secondary metabolites called tannins. Some compounds, although sharing some properties with others

are not regarded as tannins because of their inability to do this. Such compounds are referred to as “false or peudotannins”. According to one definition, ‘Tannins are water-soluble phenolic compounds with molecular weight between 500 and 5000 showing the usual phenolic reactions and distinguished by the ability to precipitate proteins, alkaloids and gelatin”.[13] There are also some simple low molecular weight phenolics, such as gallic acid, catechins, and chlorogenic acid, which show the reactions of phenolics but fail the goldbeater test. These are referred to as pseudotannins.[14] The ability to precipitate proteins and metals makes their presence in materials intended for use as human food or animal feed a cause of concern because proteins and some mineral elements are essential nutritive elements.



Tannic acid

Tannins are polyphenolic compounds with a potential to exert several physiological and chemical effects. Most of these result from the presence of many hydroxyl groups in the molecule. Their antinutrient effect is by enzyme inhibition or complex formation with food elements making them unavailable or chelation of metal such as iron.



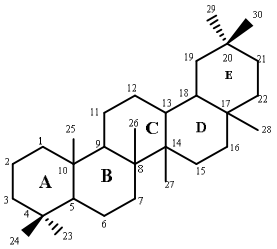
Fe^{3+}-Tannic acid complex

Trypsin inhibitors

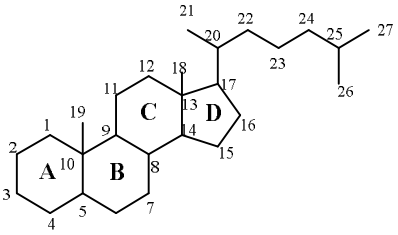
Trypsin inhibitors that inhibit the activity of the enzymes trypsin and chymotrypsin in the gut, thus preventing protein digestion, are found in many plant species mainly in different grain-legumes. Trypsin inhibitors are a unique class of proteins found in raw soybeans that inhibit protease enzymes in the digestive tract by forming indigestible complexes with dietary protein. These complexes are indigestible even in the presence of high amounts of digestive enzymes. Protease inhibitors reduce trypsin activity and to a lesser extent chymotrypsin, therefore impairing protein digestion by monogastric animals and some young ruminant animals.[15]

Saponins

Saponins are a diverse group of natural compounds characterized by their surfactant property and hemolytic activity. Structurally their outstanding features are the triterpenoid 30 carbon fiver ring structure or the 27 carbon four ring structure. They are the aglycones of glycosides. The tetracyclic steroidal saponins and a pentacyclic terpenoids have a glycosidic linkage at C-3 and have a common biogenic origin through mevalonic acid and isoprenoid unit.[15,16]



pentacyclic triterpenoid



tetracyclic ring system

Steroidal saponins are found in agavaceae, dioscoreseae, and similaceae, while triterpenoids are common in leguminoceae, euphobiaceae, verbenaceae, araliaceae and ranuculaceae plant families. The aglycones of steroidal saponins are found in tomatoes seed, alliums, asparagus, yam, fenugreek oats, pepper, and ginseng.[17]

Advances in science and technology have made it possible to obtain information on the chemical and nutritional composition of items used as food for humans and animal feed. Such information will be obtained from proximate, mineral, vitamin and anti-nutrient composition evaluations/studies**.** This will assist in decisions concerning the value, safety, need, and appropriateness of food items.

Materials and methods

Collection of Plant Samples

Combretum platypterum (Welw.) Hutch. &Dalziel leaves were collected from Obukpa, Nsukka LGA in Enugu State, South East Nigeria, between April and May 2021, and identified by Mr. Felix Nwafor, a taxonomist in the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. Herbarium specimens were deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. (Voucher number: PCG/UNN/0096)

Preparation of Plant Material: Fresh leaves were collected, foreign materials were removed and dried at room temperature for seven days and powdered mechanically.

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Anti-nutrients content evaluation

Determination of Oxalates content:

The oxalate concentration was determined using the permanganate titration method. A mixture containing 1g of powdered plant in 75 ml of 1.5 m H_2SO_4 was shaken on a mechanical shaker for an hour, and filtered with Whattman filter paper No.1. A 25 ml of the filtrate was titrated with 0.1M $KMnO_4$ solution. The endpoint was the appearance of a faint pink colour that persisted for 3o seconds. The volume used (titre) was noted.[19]

The percentage oxalate was calculated thus:

$$\% \text{ Oxalate} = \frac{\text{Titre mol } KMnO_4 \text{ x } .0045 \text{ x DF x} 100}{\text{Weight of sample}}$$

Where DF is the dilution factor. 1 ml of 0.1M $KMnO_4$ = 0.00450g oxalic acid

Determination of Trypsin Inhibitors

Trypsin inhibitor activity was determined according to.[20]

Determination of Phytic acid (Phytate)

The quantitative determination of phytic acid may be based on the analysis of phosphorus or iron in the isolated ferric phytate,[21] or indirectly, or the determination of residual iron in solution after precipitation of ferric phytate from a known concentration of ferric salt in acid solution[22]. Quantification by analysis of phosphorus or iron in the isolated ferric phytate is based on the principle that ferric chloride forms a stable complex with phytate in dilute acid solution and is the only phosphate compound, at least in significant concentration in nature, with this property. [23]

Procedure

The phytate was extracted with TCA and precipitated as the ferric salt.

A 2g of the sample was extracted with 3% TCA with occasional swilling by hand for one hour. It was centrifuged and the supernatant was separated. A 4ml of $FeCl_3$ (containing 2 mg of ferric iron per ml) in TCA was added to 10 ml of the supernatant. The test tube was heated

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in a boiling water bath for 45 minutes, allowed to cool, and centrifuged for 15 minutes. The supernatant was decanted. The residue was washed twice by being dispersed in 3% TCA, and the preceding step repeated. The washing was repeated with water.

The iron content of the precipitate is determined colorimetrically, reading absorbance at 480nm. A standard curve was constructed with portions containing 25 µg, 50 µg, 75 µg 100 µg and 150 µg. of P/ml was prepared by making up to 10ml, 0.25,0.50, 1.0 and 2.0 ml. of a standard solution containing 0.1mg/ml using a blank solution prepared with 1ml H_2SO_4 made neutral with 40% NaOH and made up to 100ml

The phytate phosphorus content was calculated from the value obtained above, assuming a constant 4Fe: 6P molecular ratio in the complex[21,24], and multiplication by a factor of 0.19. (phosphorus constitute 186g of phytic acid 660g molar mass , this gives 0.19 when applied to the ratio 4Fe: 6P) .

Quantitative estimation of tannin

Estimation of Tannin

Five hundred milligrams (500 mg) of the powdered leaves was weighed and put into 100ml plastic bottle. 50ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50ml conical flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a tube and mixed with 3ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferricyanide. The absorbance was measured in a spectrophotometer at 530nm wave length, within 10 minutes. The tannin content was calculated from the regression equation of calibration curve of gallic acid standard and the results were expressed as gallic acid equivalent (mg/g).

Determination of hemaglutinin

The method used was that of Armtfield et al. (1985).[25] A 1g of the sample was extracted (mixed) with 100 ml of phosphate bufferred solution pH 6.4. It was centrifuged and filtered after one hour to obtain the extract for the analysis. Three test tubes were labelled test while another three were labelled control. 1ml of the extract was placed in those labelled test and 9ml of trypsinated blood was added to the test tubes (test and control) and allowed to stand

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for 10 minutes at room temperature. The absorbance of both sets of test tubes were read at 510nm. The result was expressed as hemagglutinin units per milligram of the sample using the equation

Hemagglutinin unit/mg $= A_s - A_c x \frac{1}{w} x \frac{Vf}{Va}$

Where A_S = absorbance of test sample solution, A_C = absorbance of the blank control, w = weight of sample, V_f = total volume of extract, and V_a = volume of extract used in the assay.

Mineral-content analysis

Sodium and potassium were analyzed on samples digested with hydrochloric acid, using Flame Photometry according to (WHO, 1994) [26], Calcium was estimated using EDTA titration methods (Mohawk, 2008) [27]. Spectrophotometric methods were used to determine copper, and phosphorus (AOAC (1990) [28]. Selenium, mercury, arsenic, magnesium, manganese, zinc, iron, and phosphorus were analyzed using atomic absorption spectrometry. These metals were determined by atomic absorption spectrophotometry using Shimadzu AAS Machine Model AA-7000 *(*Shimadzu, Kyoto 604-8511, Japan) with standards, wavelengths and other parameters adjusted for each element according to the manufacturer's specifications.

Vitamin content analysis

Vitamin content was determined by AOAC 2019 methods[29].

Vitamin A official method 43.002, Vit E official method 948.26, vit C, official method 967.21, Vit D official method 975.42 Vitamin K was analyzed by the method described by Sethi (1956).[30]

Proximate Analysis

The proximate analysis was done according to the standard methods described in (AOAC 21st ed., 2019).[29] Moisture (AOAC.2019 official method 966.02) lipid (AOAC.2019 official

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method 954.02), protein (AOAC.2019 official method 955.04.21), fibre (AOAC.2019 official method 993.21), and ash content (AOAC.2019 official method 923.03). Total carbohydrates content was calculated by difference using the following formula:

100 - (weight in grams [protein + fat + water + ash + fiber] in 100 g of food) [31]

Results

Table 1: Metal Analysis result

Metal	Ca	Mg	Mn	Cu	Na	K	Fe	Se	Zn	As (ppm)	Hg (ppm)
Conc. (mg/100g)	0.14	12.09	1.78	0.18	1.34	4.5	5.01	0.36	5.9	0.9	2.00

Table 2: Vitamins result

VITAMIN	Vit. A	Vit. D	Vit. E	Vit. K	Vi. t C
Conc.	4.507 µg/g	0.227 mg/g	0.59 mg/g	0.437 mg/g	0.328 mg/g

Table 3: Proximate analysis result

Parameter	Qty
Protein	3.327 %
Moisture	4.45 %
Ash	2.65%
Fiber	4.3%
Fat	2.20 %
Carbohydrates	83.07 %

Table 4: Antinutrient analysis result

Parameter	Quantity
Oxalate (mg/100g)	.593±0.36
Phytate (mg/100g)	1.895±.209
Heaglutinin (HIU/mg)	1.895±.209

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Tannin (mg/100g)	171.58 ±.625
Trypsin Inhibitor (HIU/mg)	.673 ±.008

Data Expressed as Mean ± SEM

Metal Analysis

The major trace metals are present in the plant including the essential minerals selenium, zinc, manganese, magnesium and iron. Calcium, sodium, potassium and copper are also present in *Combretum platypterum* leaf (Table 1).

Vitamins

The selected vitamins whose presence were evaluated are found to be present and the concentrations are reported in table 2. Vitamin A is much higher than other fat soluble vitamins D,E, and K.

Proximate Analysis

The proximate analysis showed a reasonably high amount of carbohydrates and moderate quantities of protein, moisture, and fatinTable 3).

Antinutrients analysis

The plant material contains low levels of anti-nutrient agents, Heaglutinin (HIU/mg) 1.895±.209, Trypsin Inhibitor (HIU/mg) .673 ±.008, Tannin (mg/100g) 171.58 ±.625, Phytate (mg/100g), 1.895±.209, Oxalate (mg/100g) .593±0.36. Apart from tannins which was estimated to be present at 171 mg, all the other recognized anti-nutrient factors evaluated were in low concentrations. (Table 4).

Discussion

Of equal importance to the quantity of food available is the quality of the food. Climate change and environmental degradation have affected the chemical and nutrient composition

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of our food. The world is becoming increasingly concerned with the quality of our food. Besides natural reduction in the chemical and nutrient content of our natural sources plants and animals), due to changes in climate and soil conditions, human activities through intentional compromise of quality are worsening the situation. Knowing the quality of crude food items will greatly guide in determining the extent to which a processed item has been adulterated (it quality) or the level of pollution of the region it originated from. Food adulteration deterrence is complex because of its unpredictable nature. Significant efforts have been made recently in many aspects by food companies, authorities and academics.[32] Although sophisticated lab techniques are accurate, precise, and reliable, yet they are costly and time consuming. It is essential to develop reliable "quick screening tests" which a common person can perform. [33]

Food adulteration affects both the individual's health and finance. A 2016 study conducted on food fraud in Canada showed that more than 75 percent of respondents reported that they would pay an extra one to five percent more for zero food fraud certified products; 24 percent perceived food fraud as a high risk to their health.[34]

Estimation of ash value during proximate analysis exposes any adulteration with phosphate and silicate containing agents. Oxidative stress has been implicated in many diseases conditions and a number of natural products are known to have ameliorative effects in such conditions. These include vitamins A, C and E, and minerals like selenium and zinc, that are known to have antioxidant activities and impart beneficial health benefits.

The activities of antinutrient agent can lead to malnutrition with its health outcome. There may also be involved in food-drug interactions which may cause treatment failure and/or health emergencies. For example iron, chelation by tannins and other polyphenolic compounds may cause anaemia. Also, phytates adversely affect the intestinal level of calcium ions through complex formation. One molecule of phytate can bind five calcium ions.[35]

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It is not only fear and anxiety when some of the classes of compounds such as saponins and tannins mentioned in this research as antinutrients are mentioned or discussed. They are also known to have some beneficial health effects on humans. Saponins have been shown by clinical studies to have health-promoting activities such as boosting immunity, lowering glucose and cholesterol levels, and protecting against cancer.[36] Glycyrrhizin is effective in viral hepatitis. [37] It is a triperpenoid saponins with several pharmacological activities such as antiulcer, antiviral, anticancer, and antispasmodic [38]. Ginsenosides, the active components in ginseng are triterpenoid saponins and are shown to involve in several physiological activities.[39] Phytates have been demonstrated to protect against cancer, diabetes, and cardiovascular disease. The effect is thought to be related to phytic acid's ability to bind to zinc, thus lowering the ratio of plasma zinc to copper which is known to dispose humans to cardiovascular diease.[40] Phytic acid is also known to cause an improvement of blood glucose control. There are also suggestions of amylase inhibition thereby controlling blood sugar. Tannins exert anti-diabetic activity by reducing postprandial glucose level through α-amylase inhibition. [41]

Human activity is affecting the quality of our environment and contaminating our food chain. The search and investigation of local food items is a worthwhile undertaking. The low levels of mercury (Hg 2.0 ppm*)*, and arsenic (0.9032 ppm), are indications that the site where it was collected is minimally contaminated by heavy metals and the plant may be considered not to be accumulating heavy metals.

The presence of trace essential minerals in plants is beneficial to health when the plant is used by humans. The levels of magnesium (12.093 ppm*)*, manganese (1.782 ppm), selenium (0.36 ppm*)* and zinc (0.511 ppm*)*, are hints of antioxidant activity. The plant material contains low levels of antinutrient agents. Except tannins which was estimated to be present at 171 mg, all

the other recognized anti-nutrient factors evaluated were in low concentrations. (Table 4). A low level of ANF is positively correlated with nutritional potentials.

Conclusion

Combretum platypterum (Welw.) Hutch. & Dalziel (Combretaceae) leaf is a good source of nutrient factors, vitamins and essential minerals with low levels of anti-nutrient factors.

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