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Proximate Analysis and Phytochemical Composition of *Uvaria chamae* Root

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Abstract
Uvaria chamae in Nigeria has a wide spread reputation as a medicinal plant, and is valued for its hypoglycaemic properties as it provides an alternative treatment for diabetes. This study was undertaken to assess the phytochemical, mineral constituents and the proximate composition of the root of Uvaria chamae. Standard analytical procedures were used for the determinations. Proximate analysis of Uvaria chamae root indicated the presence of ash (3.88 ± 0.17%), moisture (6.25 ± 0.20%), fat and oil (7.00 ± 0.30%), crude fibre (47.27 ± 0.40%), protein (1.25 ± 0.10%), carbohydrate (34.35 ± 0.60%), and the elemental constituents are Ca (40.50 ± 0.80 mg/100g), Mg (12.76 ± 0.40 mg/100g), K (1.40 ± 0.10 mg/100g), P (48.3 ± 0.50 mg/100g), Fe (51.00 ± 0.60 mg/100g), Mn (1.70 ± 0.10), Cu (0.10 ± 0.01 mg/100g) and Pb (< detection limit). Phytochemical screening showed the presence of alkaloids (2.25 ± 0.10%), tannins (0.06 ± 0.00%), flavonoids (10.47 ± 0.50%), oxalates (0.27 ± 0.01%), saponins (2.87 ± 0.10%), and cyanogenic glycosides (0.02 ± 0.00 %). The demonstration of the richness of Uvaria chamae in phytochemicals and minerals in this study is a pointer to the medicinal and nutritive value of this plant.
Keywords: *Uvaria chamae*, Proximate, Phytochemical, Root

Introduction
Medicinal properties of plants are normally dependent on the presence of certain phytochemical compounds, such as tannins, flavonoids, alkaloids and saponins which are bioactive bases [1]. Many of these indigenous plants are used as spices and food plants. Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include fruits, seeds, herbs and vegetables [2]. Herbs and spices are accessible sources for obtaining natural antioxidants [3]. Phytochemicals are chemical compounds formed during plants normal metabolic processes. These chemicals are often referred to as ―secondary metabolities of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids [3]. Flavonoids and glycosides have a wide range of biological activities and the protective role in living systems is mostly due to their antioxidant potential, which is related to the transfer of reactive oxygen species (ROS), chelation of metal catalysts, activation of antioxidant enzymes and inhibition of certain type of oxidases and colon cancer [4, 5]. *Uvaria chamae* is a medicinal plant used throughout its range to treat fevers and has antibiotic properties [6]. The plant is revered for its hypoglycaemic properties [7, 8] as this provides an alternative treatment for diabetes. *Uvaria chamae* (*P.Beauv*) belongs to the family of *Anonacaea*. It is a climbing large shrub or small tree native to the tropical rain forest of West and Central Africa where it grows in wet and coastal shrub lands [9]. It is also known as finger root or bush banana. This common name refers to the fruit growing in its small branches and the fruit carpel are in finger-like clusters, the shape giving rise to the many native names translated as bush banana, implying wildness. It is an evergreen plant that grows to about 3.6 to 4.5 m high, cultivated as well as wild [10]. The plant is extensively branched with sweet, aromatic and alternate leaves commonly used to cure diseases and heal injuries [10].

The drug benzyl benzoate used as antifungal preparation has a mutagenic compound, chamuveritin, a benzyldihydrochalcone that was isolated from *Uvaria chamae* [11]. Recently, uvaranol, and cytotoxic tri-benzylated flavanon compounds have been isolated from *U. chamae* [12]. *Uvaria chamae* is commonly called by the Igala people of the eastern part of Kogi State, Nigeria as *Awuloko* or *Ayiloko* by others, *Kas Kaifi* by the Hausas, *Mmimi Ohia* by the Igbos, *Oko oja* or *eruju* by the Yorubas, *Akotom*po by the Fula-Fante people of Ghana, *Boelemimbo* by the Fula-Pwaar people of Guinea Bissau, *Liasa* by the Yoruba- Ife people of Togo [10].

Uvaria chamae in Nigeria has a wide spread reputation as a medicinal plant. The root-decoction is used as a purgative and also as a lotion [10]. Sap from the root and stem is applied to wounds and sores and the root is made into a drink and a body wash for oedematous condition [13]. The root bark yields an oleo-resin that is taken internally for catarrhal inflammation of mucous membranes, respiratory catarrh and gonorrhoea. The root extract is used in phytomedicine for the treatment of piles, menorrhagia, epistaxis, haematuria and haemolysis [10].

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In folk medicine, extract of the root, bark and leaves are used to treat gastroenteritis, malaria fever, vomiting, diarrhoea, dysentery, wounds, sore throats, inflamed gums and a number of other ailments [14]. Furthermore, it is a common ingredient for *Agbo* in Lagos, Nigeria chiefly for febrile conditions in children. All parts of the plant are fragrant. It is used to make pomade in Ghana and also for severe abdominal pain, dysentery, piles. In Sierra Leone, it is used for yellow fever, jaundice, and cough. In Senegal, it is used as medicine for renal and coastal pain, healing sores and as a concoction called *n'bata* in the Bayot dialect, which is reported to cure infantile rickets [10]. This study was undertaking to determine the phytochemicals, proximate and mineral composition of this plant, so as to evaluate its potential health benefits.

Materials and Methods

Sample Collection

Fresh root pieces of wild *Uvaria chamae* grown in Igwo, Oyo state of Nigeria and harvested in 2012 were purchased from the herbal market, at Oyingbo, in Lagos, Nigeria. The root pieces were identified and authenticated by a taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria as root of *Uvaria chamae*. A voucher specimen of the plant with reference number UBH 266 has been deposited at the University's Plant Biology and Biotechnology Department Herbarium. The plant roots were thoroughly washed, cut into small pieces and sun dried. A mechanical grinder was used to grind the roots into a uniform powder (pulverization). Proximate and phytochemical analysis and selected mineral contents were determined.

Proximate Composition

Determination of Moisture Content

Moisture content was determined by standard official methods of analysis [15].

Determination of Ash Content

The ash content was estimated using the methods as described [15, 16].

Determination of Fat Content

The crude fat was determined using Soxhlet extraction method [15].

Determination of Crude Fibre Content

The crude fibre was determined as described [16].

Determination of Protein Content

The protein content was determined from the organic nitrogen content as described [16]. **Determination of** *Carbohydrate Content*

The total percentage of carbohydrate content was determined by the method described [17].

Elemental Analysis

Determination of Wood Phosphorus

The determination of phosphorus involves the digestion of sample with a strong acid [18].

Determination of Sodium, Potassium, Calcium, Magnesium and Phosphorus.

A 5.0 g of the dried plant material was put in an oven at 150 ^{0}C for 1 hr and 2.0 g of the material was put in 50 ml pyrex beaker. The beaker was placed in a muffler furnace set at 550 ^{0}C, and the material was allowed to ash for 3 hr. The ash was dissolved in 10 ml 10% HNO_3 and heated gently on a hot plate for 20 min, cooled and filtered into a 100 ml flask and diluted to mark with distilled water. The Na, K, Ca, Mg and phosphorus in the filtrate were determined using the atomic absorption spectroscopy (Bulk scientific VGP210 atomic absorption spectroscopy)

Determination of Heavy Metals.

To a weighed 0.25 g of the pulverized plant material, 5 ml of Nitric- perchloric acid mixture was added and allowed to soak overnight. A small glass funnel was inserted to act as a reflux condenser and heat was applied for 1 hr at 150 ^{0}C. The temperature was gradually raised to 233 ^{0}C until white dense fumes appeared, and the heating was continued for another 2 hr. It was removed from the block, cooled to about 100 ^{0}C and to it was added 1 ml of 1:1 HCl. It was heated until white fumes appeared and a colourless solution obtained. The colourless solution was poured into a 100 ml flask and washed 5 times with distilled water each time. The washing was added to the flask and made up to the mark with distilled water. The heavy metals in the sample were determined using the atomic absorption spectrophotometer (Bulk scientific VGP210 atomic absorption spectroscopy), procedure outlined for each of the metals (Cu, Fe, Pb, Mn).

Phytochemical Determination

Determination of Alkaloids

The methods described [19, 20] were used..

Tannin Determination

This was by the method as described [21].

Determination of Saponins

The method used is as described [22].

Determination of Flavonoids

The method used is as described [23].

Determination of Cyanogenic Glycosides

A 1.0 g of sample was weighed into 250 ml round bottom flask and 200 ml of distilled water added and allowed to stand for 2 hr (for autolysis to occur). Full distillation was carried out and 150-170 ml of distillate was collected in a 250 ml conical flask containing 20 ml of 2.5%NaOH. An antifoaming agent (silicon oil or tannic acid) was added before distillation. To 100 ml of distillate containing glycoside, 8 ml of 6N NH_4OH and 2 ml of 5% KI were added, mixed and titrated with 0.02 N silver nitrate ($AgNO_3$) using micro-burette against a black background. Permanent turbidity indicated end point. Triplicate measurements were performed and the mean computed.

Determination of Oxalates

To 2.0 g portion of powdered sample was added 10 ml of distilled water followed by 10 ml of 6M HCl in a 250 ml volumetric flask and the mixture was heated to 90 ^{0}C for 10 min. The mixture was allowed to cool and made up to 250 ml mark with distilled water and subsequently filtered. From the filtrate 120 ml was measured into a beaker and few drops of methyl red indicator added followed by few drops of aqueous ammonia until the test solution changed to yellow. The mixture was filtered and the filtrate heated to 90 ^{0}C before adding 1 ml of 5 % $CaCl_2$ solution. Subsequently the mixture was centrifuged after it has stood overnight. The supernatant decanted and the precipitate collected was dissolved in 100 ml of 20 % (v/v) H_2SO_4 solution. The mixture was heated to near boiling and titrated against 0.05 M $KMnO_4$ solution to a faint pink colour. Triplicate titrations were carried out and the average titre computed.

Results and Discussion

Proximate analysis of *Uvaria chamae* root indicated the presence of ash, moisture, fat and oil, fibre, protein, carbohydrate and reducing sugars. The proximate composition of *Uvaria chamae* as assessed is contained on Table 1. The data showed that *Uvaria chamae* is richest in crude fibre as its major constituent of dry matter. The protein content is least while carbohydrate level is high compared with the other components of diet.

Table 1: Proximate Composition of *Uvaria chamae*

Content	**% Composition**
Ash	3.88 ± 0.17
Moisture	6.25 ± 0.21
Fat and Oil	7.00 ± 0.30
Crude Fibre	47.27 ± 0.42
Protein	1.25 ± 0.11
Carbohydrate	34.35 ± 0.60
Glucose (Reducing sugar)	4.65 ± 0.22

Values are expressed as means ± SEM, (n=3)

The elemental analysis of *Uvaria chamae* root showed the presence of calcium, magnesium, potassium, phosphorus, Iron and manganese. The elemental composition of *Uvaria chamae* as assessed is contained on Table 2, which showed that *Uvaria chamae* root is high in Fe, P and Ca, while lead was undetected.

Table 2: Elemental Constituents of *Uvaria chamae* root

Elements	**Conc (mg/100g)**
Ca	40.50 ±0.80
Mg	12.76 ±0.40
K	1.40 ± 0.12
P	48.33 ± 0.51
Fe	51.00 ± 0.60
Mn	1.70 ± 0.12
Cu	0.10 ± 0.01
Pb	< Detection limit

Values are expressed as Means ± SEM, (n=3)

The result of phytochemical determination of *Uvaria chamae* in water and ethanol is shown on Table 3. It showed that tannin, alkaloid and reducing sugar were richest in water and ethanol, while saponin and flavonoid were present only in the aqueous medium.

Table 3 . Phytochemical Screening of *Uvaria chamaeroot* root

Constituents	Qualitative (Water)	Qualitative (Ethanol)
Flavonoid	++	– –
Tannin	++	++
Alkaloid	++	++
Saponin	++	– –
Phabatanin	– –	– –
Cardiac glycoside	– –	– –
Steroid	– –	– –
Reducing Sugar	++	++

The phytochemical screening of *Uvaria chamae* revealed the presence of alkaloids, tannins, flavonoid, oxalate, saponins and cyanogenic glycosides. The phytochemical determination of *Uvaria chamae* as assessed is contained on Table 4. The data showed that *Uvaria chamae* is richest in flavonoid as its major constituent of dry matter. The cyanogenic glycosides content is the least, while saponins and alkaloids levels are high compared to the other components of the diet.

Table 4: Phytochemical composition of *Uvaria chamae* root

Content	**Composition (%)**
Alkaloids	2.25 ± 0.10
Tannins	0.06 ± 0.00
Flavonoid	10.47 ± 0.50
Oxalate	0.27 ± 0.01
Saponins	2.87 ± 0.10
Cyanogenic glycosides	0.023 ± 0.00

Values are expressed as Means ± SEM, (n=3)

The medicinal and nutritional potentials of the root of *Uvaria chamae* were assessed in this study through quantitative determination of the relative distribution of phytochemicals, proximate composition and mineral content. Plants are known to play prominent roles in the treatment of diseases as some species especially the *Euphorbia* have been reported to possess antitumour and anticancer activities [24, 25]. *Uvaria chamae* (*P.Beauv*) belongs to the family of *Anonacaea* and has strong potentials for use both medicinally and nutritionally. The results obtained from this study showed that there is a relative distribution of phytochemicals. For example, alkaloids (2.25 ± 0.10 %), tannins (0.06 ± 0.00 %), flavonoid (10.47 ± 0.50 %), oxalate (0.27 ± 0.01 %), saponins (2.87 ± 0.10 %), cyanogenic glycosides (0.023 ± 0.00 %).

Polyphenols and flavonoids are known to exercise anti-oxidative activities, protection against allergies, inflammation, platelet aggregation, microbes, ulcers, hepatic toxicity, viruses and tumour [26,27,28]. Saponin presence in appreciable but safe amounts in the root of Uvaria chamae means that it has great potentials. Levels <10% are considered safe and non-toxic as high saponin levels have been associated with gastroenteritis, manifested by diarrhoea and dysentery [29]. There is a significant amount of alkaloids in the root of *Uvaria chamae.* Alkaloids are one of the most efficient therapeutically significant bioactive substances in plants. The pure isolated alkaloids and synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bactericidal properties [30]. It has been reported that [31], tannin usually forms insoluble complexes with proteins, thereby interfering with their bioavailability. Poor palatability is generally attributed to high tannin diets [32], however the value of tannins (0.06 ± 0.00 %) in the root of *Uvaria chamae* is negligible. The results of the phytochemicals showed that the root of *Uvaria chamae* possessed strong potentials for medicinal use and could serve as agents for the treatment of a wide range of diseases and infections.

The results obtained from proximate analysis of *Uvaria chamae* root established the fact that they can be used as rich carbohydrate sources. Carbohydrates serve as a source of energy and aid digestion and assimilation of other nutrients. The proximate analysis revealed moisture contents for the root of *Uvaria chamae* (6.25 ± 0.21 %), which fell within the acceptable limits of about 6%–15% for most vegetable drugs [33].

Total ash value recorded for the root of *Uvaria chamae* (3.88 ± 0.17 %) is within the range given for some official drugs such as *Citrus* leaf (7.0%), *neem* leaf (11.6%) and *Atropa* leaf (16%) [33]. The total ash value is a diagnostic purity index. It represents both physiological and nonphysiological ash. Physiological ash is the ash inherent in the plant due to biochemical processes and the non-physiological ash contaminants from the environment. These may be carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals which were taken up from the soil [33]. The non-physiological ash component of the total ash could be reduced by rinsing the fresh plant material several times in clean water before drying and processing for medicinal uses [34].

The crude fibre content (47.27 ± 0.42 %) of the root of *Uvaria chamae* was high. This has nutritional implications in the sense that fibre prevents diverticulosis and aids absorption of trace elements in the gut as well as helps in the elimination of undigested food materials through the bowel [35,36].

There was presence of Fe, Ca, Mg, Mn, Cu, P in the root of *Uvaria chamae*. Fe is important in immune function, cognitive development, temperature regulation and energy metabolism [37]. It is also required for the synthesis of haemoglobin and myoglobin while its deficiency causes anaemia. Calcium along with P is required for formation and maintenance of bones and teeth. It is also required in blood clotting and muscle contraction [37]. Magnesium which is needed in several enzymes utilizes adenosine triphosphate. It contributes to the synthesis of DNA and RNA during cell proliferation. It is also important for nerve and heart function as well as enhance the release of insulin from the beta- cells of pancreas. It also has a positive correlation with blood pressure by dilating arteries and preventing abnormal heart rhythm [37]. When potassium elicits electrical potential, nerve impulses are conducted and the contraction of muscles is enabled. It participates in facilitating the absorption of nutrients such as glucose and amino acids in the small intestine. The presence of Ca, Mg and K collectively are known to reduce hypertension and high blood pressure as well as in the prevention and treatment of high blood pressure [37]. Hence, their presence in the root of *Uvaria chamae* gives credence to the potential nutritional benefit of the plant. High levels of anti-nutritional agents such as oxalates, tend to render Ca unavailable by binding to plasma Ca ion to form complexes [38, 39], however the level in the root of *Uvaria chamae* was insignificant. Cobalt and Cadmium were absent, but the concentration of Pb (< Detection limit) in *Uvaria chamae* cannot lead to any health hazard in consumers since it is lower than the maximum permissible limit of 3mg/100g lead for vegetables [40] and this is within the safe limits for consumption of the plant as a herbal medicine

Conclusion: Plant medicine is highly considered safe and better than synthetic drugs for human health. The present study has shown the phytochemical, proximate and mineral compositions of the root of *Uvaria chamae*. The rich array of secondary plant metabolites of *Uvaria chamae* demonstrates its very good potential for medicinal use.

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