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Proximate and anti-nutrient profiles of tubers of *Dioscorea bulbifera* from Rivers State, Nigeria: Nutritional and health implications of consumption

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ABSTRACT

The proximate and anti-nutrient profiles of *Dioscorea bulbifera* tubers were determined. The proximate profile included high crude fiber (18.17±6.28% per wet weight and 20.34±6.67% per dry weight) and total carbohydrate (60.83±3.82% per wet weight and 68.41±5.78% per dry weight). The anti-nutrients composition included tannins, cyanogenic glycosides, oxalates, phytates and saponins. These results support the medicinal uses of *Dioscorea bulbifera* tubers, and suggest its potentials as food, after proper processing.

Introduction

Dioscorea bulbifera belongs to the family Dioscoraceae (alt. Yam), and is commonly called air potato, potato yam, bitter yam or air yam. Its synonyms include *Dioscorea anthropophagum* Chev, D. Hoffa Cordemy, *D. sativa* Thunb, and *D. sylvestris* de Wild. It is often confused with *D. alata* and is characterized by its aggressively high-climbing annual twining stems, large heart-shaped leaves with prominent veins, and potato-like aerial tubers in the leaf axils, with small or no underground tubers [1]. It is not winged (as in *D. alata*), and its stem grows to about 20 m or more in length, freely branching above, with internodes round or slightly angled in cross-sections [2]. It is cultivated in West Africa, Southeast Asia, and South and Central America. The wild form also occurs in both Asia and Africa [3].

These tubers are edible and cultivated as food crop, especially in West Africa, and often have a bitter taste, which can be removed by boiling [4]. The Zulus use the raw form of this yam as bait for monkeys, and hunters in Malaysia use it to poison tigers [5]. Phytochemical constituents include bafoudiosbulbins A-G, tetracosanoic acid, 1-(tetracosanoyl)-glycerol, β-sitosterol, 3-O-β-D-glucopyranosyl-β-sitosterol, trans-tetracosanylferulate, kaempferol and 3,5,4′-trihydroxy-3′-methoxybibenzyl [4, 6]. It has been reported have anti-diabetic property [7]. The present study was designed to assess the tubers' nutrients and anti-nutrients composition, in order to adduce possible health and safety implications of its consumption.

Materials and Method

Collection of plant samples

Samples of fresh *Dioscorea bulbifera* were collected from within the "Choba" and "Abuja" Campuses of University of Port Harcourt, Port Harcourt, Nigeria. They were identified at the University of Port Harcourt Herbarium, Port Harcourt, Nigeria. After ridding them of dirt, they were divided into two portions.

Determination of the proximate composition

A portion was used for proximate analysis, to determine (in triplicate) the moisture, crude protein, fat, ash, fibre and total carbohydrates were determined according to standard methods. The moisture content was determined according to AOAC Official Method 967.03 [8]. Two grams of the sample was heated to a constant weight in a crucible placed in an oven (Plus II Sanyo, Gallenkamp PLC, England) maintained at 105 °C for 3.5 h. The ash was determined according to AOAC Official Method 942.05 [8]. One gram of the sample was incinerated in a muffle furnace (LMF4 from Carbolite, Bamford, Sheffield UK) maintained at 550 °C for 5 h. The crude protein (% total nitrogen x 6.25) was determined by Kjeldhal method [9], using 1.0 g of samples. Crude fat was determined according to AOAC Official Method 920.39 [8]. This involved exhaustive extraction of 5.0 g of sample in a Soxhlet apparatus using petroleum ether (boiling range 40-60 °C) as the extractant. The total carbohydrate content was determined by the anthrone method [10]. The ground sample (0.1 g) was mixed with 1.0 mL of distilled water and hydrolyzed with 1.3 mL of 52% perchloric acid, and the hydrolysate was quantified at 630 nm after developing its colour with 0.2% Anthrone Reagent. The crude fibre content was determined by subtracting the sum of all the other components from 100 g. The energy value was calculated with the Atwater factors 4, 9 and 4 for protein, fat and carbohydrate respectively [11].

Determination of per cent daily value

Per cent daily values were calculated by comparison to the appropriate daily values [12]. It was calculated using the following formula.

$$\textit{Per cent daily values}\ (\%) = \frac{\textit{weight of a given nutrient in}\ 100\ g\ \textit{of sample}}{\textit{daily value of the nutrient}} \times 100$$

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Determination of the anti-nutrient composition

The other portion was homogenized and used in the quantitative determination of cyanogenic glycosides, oxalates, phytates, tannins and saponins, using standard methods. Assays were carried out in triplicates.

Assay of the cyanogenic glycoside content

The determination was as previously described by Mbatchou and Kosoono [13]. Two grams of the homogenized sample was soaked in a mixture of 40 mL of distilled water and 2 mL of orthophosphoric acid. The mixture was left for 12 h to release all bound hydrocyanic acid. Anti-bumping agents were added and the solution distilled until 30 mL of the distillate was collected. An aliquot of 4 mL of the distillate was taken into a conical flask and diluted with 8 mL of distilled water, before adding 1.6 mL of 6.0 mol/L ammonium hydroxide and 0.4 mL of 5% (w/v) potassium iodide. The mixture was then titrated with 0.02 mol/L silver nitrate until a faint but permanent turbidity was obtained. The cyanogenic glycoside content (mg HCN equivalent/100g sample) was calculated as follows:

$$\boldsymbol{Cyanogenic\ glycoside\ content}\ (\boldsymbol{mg/100g}) = \frac{\boldsymbol{titre \times 1.08 \times 42}}{\boldsymbol{4 \times 2}} \times \boldsymbol{100}$$

Where 1 mL of 0.02 mol/L $AgNO_3 \equiv 1.08$ mg HCN; 42 = total volume of the hydrolysate (mL); 4 = volume of distillate used (mL); 2 = weight of sample used (g).

Assay of the oxalate content

The oxalate was determined according to AOAC Official Method 974.24 [8], with modifications. An aliquot of 10 mL of 6 mol/L HCl was added to 2 g of homogenized sample in a 250 mL volumetric flask, which was then placed in a water bath at 80 ℃ for one hour. The resultant mixture was further diluted with 50 mL of deionized water and filtered through Whatman #1 filter paper. The pH of the filtrate was adjusted with concentrated NH_4OH solution until the colour of solution changed from salmon pink colour to a faint yellow colour. Thereafter, the filtrate was treated with 10 mL of 5% $CaCl_2$ solution to precipitate the oxalate. The suspension was centrifuged at 2500 rpm for 20 min, after which the supernatant was decanted, and the precipitate was completely dissolved in 10 mL of 20% (v/v) H_2SO_4. The resultant solution was made up to 300 mL. An aliquot of 125 mL of the this solution was heated until near boiling point and then titrated against 0.05 mol/L $KMnO_4$ solution, to a faint pink colour which persisted for about 30 s. The oxalate content (mg/100g sample) was computed from the titre value, with the following formula.

$$\boldsymbol{Oxalate\ content}\ (\boldsymbol{mg/100g}) = \frac{\boldsymbol{titre\ (mL) \times 0.05 \times 2 \times 300}}{\boldsymbol{5 \times 125 \times 2}} \times \boldsymbol{100}$$

Where 0.05 = molar concentration (mol/L) of standardized $KMnO_4$ solution; 2 and 5 are the stoichiometric ratio of permanganate and oxalate from the balanced equation; 125 = volume of aliquot used (mL); 300 = total volume of filtrate (mL); 2 = weight of sample used (g).

Assay of the phytates content

The determination was as previously described by Mbatchou and Kosoono [13]. A portion (0.8 g) of the homogenized sample was soaked in 20 mL of 2% hydrochloric acid for 5 h and was filtered. Then, 5 mL of the filtrate was taken into a conical flask and 1.0 mL of 3% ammonium thiocyanate solution was added. The resultant solution was titrated with iron (III) chloride TS until a brownish-yellow colour persisted for 5 min. Standard phytate solution was prepared by dissolving sodium phytate in distilled water and subjected to the same treatment as the sample. The phytate content (mg/100g sample) was determined with the following formula.

$$\boldsymbol{Phytate\ content}\ (\boldsymbol{mg/100g}) = \frac{\boldsymbol{C \times titre_{sample} \times 20}}{\boldsymbol{titre_{standard} \times 0.8}} \times \boldsymbol{100}$$

Where C = concentration of standard (mg/mL); 20 = total volume of homogenate solution (mL); 0.8 = weight of sample used (g).

Determination of saponins content

The determination was as previously described by Mbatchou and Kosoono [13]. Four grams of the homogenized sample was added to 20 mL of 20% aqueous ethanol and kept in a shaker for 30 min, before heating on a water bath for 4 h at 55 °C. The mixture was then filtered and the residue re-extracted with another 40 mL of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 mL over water bath at 90 °C. The concentrate was transferred into a separating funnel, and extracted twice with 4 mL of diethyl ether. The diethyl ether layer was discarded, while aqueous layer was retained, and 12 mL of n-butanol was added to it. The n-butanol extract was washed twice with 2 mL of 5% aqueous sodium chloride. The remaining solution was heated on a water bath, and after evaporation the residue was dried in an oven (40 °C) to a constant weight. The saponin content (mg/100g sample) was calculated using the following formula.

$$\boldsymbol{Saponin\ content}\ (\boldsymbol{mg/100g}) = \frac{\boldsymbol{weight\ of\ residue\ (mg)}}{\boldsymbol{4}} \times \boldsymbol{100}$$

Where 4 = weight of sample used (g).

Assay of tannins content

The determination was as previously described by Mbatchou and Kosoono [13]. Distilled water (25 mL) was added to 250 mg of the sample in a 250 mL flask and kept in a shaker for one hour. The resultant mixture was filtered into a 25 mL volumetric flask and made up to the mark. The filtrate (2.5 mL) was pipetted into a test tube and mixed with 1 mL of 0.1 mol/L $FeCl_3$ in 0.1 mol/L HCl and 0.008 mol/L potassium ferrocyanide. The absorbance was measured at 605 nm within 10 minutes. Tannic acid was used to prepare the standard. The tannin content was determined as tannic acid equivalent in mg per 100 g sample, using the following formula.

$$\boldsymbol{Tannin\ content}\ (\boldsymbol{mg/100g}) = \frac{\boldsymbol{C \times A_{filtrate} \times 25 \times 1000}}{\boldsymbol{A_{standard} \times 250}} \times \boldsymbol{100}$$

Where *C* = concentration of standard (mg/L); 25 = total volume of filtrate (mL); 1000 = conversion factor from mg to g; 250 = weight of sample used (mg); A = absorbance.

Derivation of composition per dry weight from composition per wet weight

The compositions per dry weight of the determined parameters were derived from the compositions per wet weight and vice-versa, using the following formula, adopted from Health Canada Official Methods [14].

$$\textbf{Composition per dry weight}\ (\%) = \frac{\boldsymbol{composition\ per\ wet\ weight}\ (\%)}{\%\ \boldsymbol{dry\ matter\ content}} \times \boldsymbol{100}$$

Statistical analysis of data

All values are reported as the mean ± SD (standard deviation).

Results and Discussion

Table 1 shows the proximate composition of *Dioscorea bulbifera* tubers. The present result indicates that *D. bulbifera* has lower moisture content than African yam bean and fluted pumpkin [15]. The protein content of *D. bulbifera* tubers (Table 1) was higher than those reported for potato and other

yam species [5], but was less than those reported for *Chromolaena odorata* [16], African yam bean and fluted pumpkin [15]. Dehydration can improve the relative protein content of *D. bulbifera* tubers. Although, a 100 g dry sample of *D. bulbifera* will not be able to meet the daily protein requirement of 23-56 g [11, 12, 17]; since it can provide only about 5-7% of the daily value (Table 2) [12]. The ash value observed in *D. bulbifera* tubers was relatively high compared with those of *C. odorata* [16], egg and meat; and comparable with that of wheat flour [18], African yam bean and fluted pumpkin [15]. The very high total carbohydrate content recorded in the tubers in this study, confirms the earlier report by Food and Agricultural Organization [5] that the dry matter of root crops is made up mainly of carbohydrate, usually in the range of 60 to 90 per cent. It was higher than those of African yam bean, fluted pumpkin [15] and *C. odorata* leaves [16]. A 100 g serving of the tubers can provide about 21-23% of the daily value (Table 2) [12]. The level of crude lipid observed in the tubers was higher than that of *C. odorata* [16], but less than those of African yam bean and fluted pumpkin [15]. A 100 g serving of the tubers can provide about 2-3% of the daily value [12].

Table 1. Proximate composition (/100g) of *Dioscorea bulbifera* tubers

Parameter	/Wet weight	/Dry weight
Moisture (g)	10.97±1.89	-
Dry matter (g)	89.03±5.34	100.00±0.00
Total ash (g)	5.38±0.30	6.04±0.34
Crude protein (g)	2.77±0.67	3.11±0.72
Crude lipid (g)	1.87±0.28	2.10±0.28
Total carbohydrate (g)	60.83±2.82	68.41±3.78
Crude fibre (g)	18.17±1.28	20.34±1.67
Caloric value (kcal)	271.29±2.40	305.01±6.56

Values are means ± SD of triplicate determinations

High crude fibre content was recorded in the tubers. This was higher than the 5% wet weight reported for sweet potato, although equal to the 20% dry weight in the same report [18]. A 100 g serving of the tubers can provide about 71-81% of the daily value [12]. Studies suggest that increased fibre consumption ensures a faster rate of intestinal transit and may contribute to a reduction in the incidence of coronary heart disease, stroke, hypertension, diabetes, obesity, gallstones, cancer, and certain gastrointestinal diseases [19, 20, 21, 22]. Increasing fibre intake lowers serum cholesterol levels [22].

Table 2. Per cent daily values (per cent daily value* (%)/100 g) of the proximate nutrients in *Dioscorea bulbifera* tubers

Parameter	/Wet weight	/Dry weight
Crude protein	5.55±1.34	6.23±1.44
Crude lipid	2.90±0.43	3.26±0.43
Total carbohydrate	20.54±1.29	23.07±1.95
Crude fibre	71.83±4.83	80.68±6.46
Caloric value	13.42±0.61	15.07±0.82

Values are means ± SD of triplicate determinations.
*Comparison to daily values from Food and Drug Administration (2013)

The anti-nutrients composition of *D. bulbifera* tubers is given in Table 3. The level of oxalates observed here was less than that reported for *Gnetum africanum* seeds [23]. Oxalates depress calcium and magnesium absorption by promoting the formation of non-absorbable complexes [24]. They also cause muscular weakness and paralysis, gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi, hypocalcaemia and nephrotic lesions [25]. The toxic effects of oxalate could be avoided, by cooking the tubers before consumption [25].

Table 3. Anti-nutrients composition (mg/100 g) of *Dioscorea bulbifera* tubers

Anti-nutrient	/Wet weight	/Dry weight
Saponins	7.150±0.304	8.031±0.341
Tannins	4.114±0.059	4.621±0.066
Oxalates	4.837±0.491	5.433±0.551
Phytates	9.213±0.135	10.348±0.152
Cyanogenic glycosides	5.040±0.312	5.661±0.350

Values are means ± SD of triplicate determinations

The phytate level observed is less than the reported values for *C. odorata* leaves [16] and seeds of *G. africanum* [23]. Phytate forms complexes with dietary minerals, especially calcium, magnesium, iron, zinc, selenium and copper, thereby reducing their availability in the body and causing mineral-related deficiency in humans [24, 26]. It also negatively impacts starch, protein and lipid utilization [24]. The phytate content of the tubers can be reduced by various processing methods such as thermal processing, mechanical processing, soaking, germination, malting and fermentation of phytate-containing foods in order to release endogenous phytases which begin the phytate breakdown prior to consumption [26]. It is also possible to include specific probiotics such as *Lactobacillus plantarum*, which produces low-level phytase activity [26]. Apart from being an anti-nutrient, dietary phytate exhibits beneficial health effects, such as protection against a variety of cancer and heart-related diseases, diabetes mellitus, dystrophic calcification, renal stones, dental caries and dyslipidemia [24, 27]. The antioxidant properties of phytate have also been found useful in the preservation of a variety of oxygen-sensitive biological food materials [24].

Very high saponin content was observed in the tubers. It was less than that of *C. odorata* leaves [16]. Saponins have been observed to kill protozoans, impair the protein digestion and the uptake of vitamins and minerals in the gut and to act as antimicrobial, immune-stimulant, hypoglycemic, hypocholesterolemic and anticancer agent [28, 29].

High tannin content was recorded in the tubers. Although, it was lower than those of *C. odorata* leaves [16] and *G. africanum* seeds [23]. Processing methods such as cooking or autoclaving, soaking and fermentation can help decrease the tannins content [25]. Tannins have anthelmintic, anti-cancer, anti-inflammatory, antimicrobial, antioxidant, cardio-protective, hypoglycemic, hypocholesterolemic and immune-modulatory activities [28, 30]. The level of cyanogenic glycosides observed in the tubers was less than those of *C. odorata* leaves [16] and *G. africanum* seeds [23]. Though, the level observed here may be a likely source of worry, especially if the tuber is consumed without proper processing. Processing methods such as cooking and fermentation can help reduce the cyanide levels [25].

Finally, this study supported the medicinal uses *Dioscorea bulbifera*, and corroborated earlier reports of its high nutritional potential. However, our candid advice is that before consumption, the tubers should be subjected to established processing techniques that will reduce the levels of the anti-nutrients.

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