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PRELIMINARY STUDIES ON *CANAVALIA ENSIFORMIS* (JACKBEAN) DC. SEEDS: PROXIMATE ANALYSIS AND PHYTOCHEMICAL SCREENING

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ABSTRACT
Nutritional and chemical composition of *Canavalia ensiformis* DC seeds, an underexploited crop seed in Nigeria was investigated using standard methods. Proximate analysis in g% dry weight revealed the seeds to be rich in protein (34.47 ± 0.18) and ash content (4.92 ± 0.42) with moderate carbohydrate content of (42.13 ± 0.21), crude fat (4.09 ± 0.13), crude fibre (5.86 ± 0.46), while moisture was 14.39 ± 0.12. Phytochemical screening of the seeds showed that tannins, flavonoids, alkaloids, saponins and cardiac glycosides were present. Anthraquinones were not detected. The results support the medicinal uses of the seeds and suggest it could serve as a cheap source of protein food/supplement for man and livestock. The moderate level of carbohydrate in the seeds should be beneficial to weight watchers.

Keywords: *Canavalia ensiformis*, proximate composition, phytochemical screening.

INTRODUCTION

Canavalia ensiformis DC (C.ensiformis) commonly known as Jackbean, is a leguminous plant belonging to the family Leguminosae. Other names include ikpakpa (Auchi, Nigeria), ikpakpa no khua (Benin, Nigeria), cat eye, horse bean, one eye bean and overlook (West Indies) The plant is a climber usually growing on a support. Though a native of Central America and West Indies, it has been widely cultivated in the humid tropics of Africa and Asia. The seeds which have a bitter taste are arranged in a pod. Their useful potential is limited by growth inhibiting compounds which must be detoxified before seeds are edible (Rachie, 1979). The growth inhibiting proteins are canavalin, concanavalin A and B, the enzyme urease and the amino acid canavanine (Leslie, 1965; Bailey & Boulter, 1971). In the Auchi area of Edo State, Nigeria, the seeds are boiled three times (with two changes of water) within two days before being eaten, due to their unacceptable flavor and texture. They can also provide a plentiful green manure and forage though livestock eat it with reluctance (Leslie, 1965). Cattle consuming more than 30% of their diet as seed or meal of *C. ensiformis* are at risk (Skerman *et al*., 1988). In Angola, the plant has not been used as food, but the seeds were at one time used as a trivial form of currency. *C. ensiformis* seeds have been reported to possess antihypercholesterolaemic (Marfo *et al*., 1990), convulsant (Carlini & Guimaraes, 1981), agglutinin and mitogenic (Krupe *et al*., 1968), and haemagglutinin (Raychaudhuri & Singh, 1986) activities. The aqueous extract is commonly employed in the treatment of patients suffering from diabetes (Keay, 1989). Using an animal model Nimenibo-Uadia & Osagie, (1999) justified the use of an aqueous extract of the seeds in treating diabetic persons. Despite these therapeutic activities, no information pertaining to the phytochemical components of this seed was found, though Leslie (1965) had reported the proximate composition of the immature and ripe fruits, and beans. In order to fill this gap in scientific knowledge, this work was undertaken to screen for phytochemical components in the aqueous extract of *C. ensiformis* seeds since traditional herbal remedies are mainly aqueous and to also evaluate its proximate composition.

MATERIALS AND METHOD

Chemicals
All chemicals/reagents used were of analytical grade.

Plant Material
The seeds of *C. ensiformis* were purchased from Oba market, Benin City with a fair degree of quality assurance. After identification at the Department of Plant and Biotechnology Herbarium of the University of Benin, stone and any infested seeds were carefully removed. The purchased sun-dried seeds were further oven-dried at 40 ^{0}C for 6 h and milled to powder (Corona, Landers Y CIA, SA) to pass through a 0.8mm sieve and stored in air-tight containers until needed for analysis.

Phytochemical Screening
The milled crude seed of *C. ensiformis* was screened for alkaloids, saponins, tannins, anthraquinones, cardiac glycosides and flavonoids using standard phytochemical procedures (Harborne, 1973; Odebiyi & Sofowora, 1978; Sofowora, 1993).

Preparation of Aqueous Extract
The aqueous extract of the seed sample was prepared by placing 25.0 g of the dry powder plant sample in 500 ml distilled water and boiled for 10 min. The suspension was suction filtered and the filtrate evaporated to dryness in vacuo. The residue was further dried at 45 ^{0}C to a constant weight in a hot air oven (Gallenkamp,UK). The procedure was repeated several times and the dried extracts pooled. Portions of the extract were weighed and screened for phytochemicals.

Tests for Alkaloids
0.5 g of the extract was mixed with 5 ml 1 % HCl, warmed and filtered. 1.0 ml each of the filtrate was treated with *Mayer's* reagent and *Dragendorff's* reagent in separate test tubes. Turbidity or precipitate indicated the presence of alkaloids.

Test for Saponins
0.5 g of the extract was mixed with water and shaken vigorously to frothing. The mixture was warmed. The formation of a stable persistent frothing indicated the presence of saponins.

Test for Tannins
0.5 g of the extract was added to 10 ml distilled water, stirred and filtered. A few drops 0.1 % $FeCl_3$ solution was added to 2.0 ml of filtrate. The appearance of blue-black green or blue-green precipitate indicated the presence of tannins.

Test for Anthraquinones
0.5 g extract was added to 10 ml benzene, shaken well and filtered. The filtrate was shaken with 5 ml of 10 % ammoniacal solution (NH_4OH). Presence of pink, red or violet color in ammoniacal solution (lower phase) indicated the presence of anthraquinones.

Tests for Cardiac Glycosides

(i) *Salkowski Test*: 0.5 g extract was mixed with 2.0 ml chloroform, followed by the careful addition of 2.0 ml concentrated H_2SO_4 to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring (i.e. aglycone portion of the cardiac glycoside).

(ii) *Keller-Kiliani Test*: 0.5 g extract was dissolved in 2.0 ml glacial acetic acid containing 1 drop of 10 % $FeCl_3$ solution. This was underplayed with 1.0 ml concentrated H_2SO_4. A brown ring at the interphase indicated presence of deoxysugar of cardenolides; or a violet ring may appear below the brown ring; or a greenish ring may form just above the brown ring (i.e. in the acetic acid layer) and gradually spread throughout the layer.

Tests for Flavonoids

(i) *Lead Acetate Test*: 0.5 g extract was mixed with 1.0 ml of 10 % lead acetate. A redish-brown colouration (or precipitate) indicated the presence of flavonoids.
(ii) *Ferric Chloride Test*: 1.0 g extract was mixed with 1.0 ml of 10 % $FeCl_3$. A dark brown (dirty brown) precipitate indicated the presence of flavonoids.
(iii) *Sodium Hydroxide Test*: 1.0 g extract was mixed with 1.0 ml dilute NaOH. A golden yellow precipitate indicated the presence of flavonoids.

Proximate Analysis
The proximate analysis to determine moisture, crude lipid, ash, crude fibre contents of the seeds were carried out in triplicates based on the methods of the Association of Official Analytical Chemists (AOAC, 2000). Crude protein was estimated by the semi-micro Kjeldahl method of Markham (1942) as reported by Pearson (1973). Carbohydrate was determined by "difference" (FAO, 1998). The energy value (kcal/100g) of the seeds was calculated using the Atwater factors of 4, 9 and 4 for protein, lipid and carbohydrate respectively (Atwater & Bryant, 1900).

Statistical Analysis
Means and standard error of means were calculated for three independent determinations of each proximate component except for total carbohydrate which was by 'difference'.

RESULTS AND DISCUSSION
Phytochemical screening of *C. ensiformis* seeds (Table 1) revealed the presence of tannins, flavonoids, alkaloids, saponins and cardiac glycosides while the Bontrager's test for anthraquinones was negative. These chemical compounds have been reported to have pharmacological activities (Oliver-Bever, 1980). Some of them like tannins, alkaloids, saponins are referred to as antinutritional factors because under certain conditions like high doses, they inactivate some nutrients causing them to be bio-unavailable. The mechanism through which the antinutritional and beneficial health effects of food are exerted is the same (Gemede & Ratta, 2014).
For example, the poor palatability of high tannin diets has been ascribed to its astringent property. This same property gives them their medicinal value. In the free state and in large doses, they irritate the mucosa. In small doses they precipitate small amounts of proteins in the cells of the mucosa which are thus rendered impermeable, thereby preventing other irritants from penetrating to the deeper layers of damaged mucosa, hence aiding healing (Okuda *et al*., 1982). Tannins from some plants are reported to have hypoglycaemic activity (Oliver-Bever, 1980), hence the presence of tannins in the seeds of *C. ensiformis* (Table 1) lends credence to its use as an antidiabetic remedy.
Alkaloids, a heterogeneous group with diverse chemical structures comprises the largest single class of secondary plant substances. Often they are toxic to man and livestock but many have significant pharmacological activity, hence their wide use in medicine (Paterson, 1993). Some alkaloids from *Trigonella foenum-graecum* (Fenugreek) seed are reported to lower blood sugar, cholesterol and triacylglycerols (Shani *et al*., 1974), metabolites which are usually elevated in diabetes mellitus. The presence of alkaloids (Table 1) therefore in *C. ensiformis* might explain its use in diabetic treatment. Plant families such as Leguminosae to which *C. ensiformis* belongs, Amaryllidaceae and Compositae are noted for high levels of alkaloids (Paterson, 1993). Some of the toxicological manifestations of potato glycoalkaloids involve gastrointestinal upsets and neurological disorders, especially in doses in excess of 20mg/100g sample (Osagie, 1998). The level of alkaloids in *C. ensiformis* therefore needs to be ascertained.

Table 1: Phytochemical screening of aqeous extract of *C. ensiformis* seeds

Component	Test	Result
Tannins	Ferric chloride	+
Flavonoids	Sodium hydroxide	+
	Ferric chloride	+
	Lead acetate	+
Alkaloids	Dragendorff's	+
	Mayer's	+
Saponins	Frothing	+
Anthraquinones	Bontrager's	-
Cardiac glycosides	Salkowski's	+
	Keller-Kiliani's	+

Key: + = Present - = Not detected

In the past, saponins were recognized as antinutrients due to their adverse effects such as growth impairment, reduction in food intake due to the bitterness and throat irritating activity, reduction in the bioavailability of nutrients and its effects on protein digestibility by inhibiting various digestive enzymes such as trypsin and chymotrypsin (Liener, 2003). Recently though, saponins are attracting considerable interest as a result of their beneficial biological effects in humans. The nutritional significance of saponins stems largely from their hypocholesterolaemic action, suggesting that they may prove useful in the control of human cardiovascular disease (Oakenful & Sidhu, 1983). The presence of saponins in *C. ensiformis* seeds (Table 1) thus supports its use as an antidiabetic agent. Nimenibo–Uadia (2003) had reported the antihypercholesterol and antihypertriacylglycerol effects of the aqueous extracts of *C. ensiformis* in diabetic rats. The hypocholesterolaemic activity of dietary saponins may be due to the formation of some complexes with dietary cholesterol or their bile salt precursors which are then made unavailable for absorption (Gee & Johnson, 1988). Besides lowering serum cholesterol, dietary saponins possess immunostimulatory and anticarcinogenic properties (Gemede & Ratta, 2014).

The presence of flavonoids (Table 1) suggests *C. ensiformis* seeds to be a source of antioxidants possessing free-radical scavenging abilities (Torel *et al.,* 1986; Tiwari & Rao, 2002).. Flavonoids have been reported to exhibit numerous pharmacological activities including anti-inflammatory effects, anti-fungal, anti-bacterial, anti-viral and anti-toxic activities (Leung, 1980; Cook & Samman, 1996).

Cardiac glycosides also revealed to be present in *C. ensiformis* seeds (Table 1) are a group of triterpenoids. Most are toxic but many have pharmacological activity. They are the active constituents of the major group of cardiovascular drugs (Trease & Evans, 1999).

Whether these chemical components in *C. ensiformis* act as antinutritional factors or therapeutic agents depends on the levels present. The deleterious effects can be minimized or eliminated by various processing techniques (Elegbede, 1998). In Auchi, Edo State, Nigeria, the seeds are heat treated (wet) and then eaten as food or the liquid used to treat diabetic persons (Nimenibo-Uadia & Osagie, 1999).

Table 2: Proximate composition of *C. ensiformis* seeds (g % dry matter)

Component	Value (g %) Composition
Moisture content	14.39 ± 0.12
Crude protein	34.47 ± 0.18
Crude lipid	4.09 ± 0.13
Crude fibre	5.86 ± 0.46
Total ash	4.92 ± 0.42
Total carbohydrate *	42.13 ± 0.21
Caloric value	343.21 kcal/100g

Values are expressed as % of dry matter and are means ± SEM of triplicate determinations
* Calculated by 'difference'

The proximate compositions of *C. ensiformis* seeds presented in Table 2 are comparable to those earlier reported by Leslie (1965), except for higher crude protein and fat contents but lower carbohydrate level reported in the present study. Variability in research findings may be due to research methodology, age of plant, geographical location in terms of soil components, season and time of collection of sample. The highest nutrient component in the present study was carbohydrate followed by crude protein, (Fig. 1), consistent with the pattern reported by Leslie (1965). Yet, the carbohydrate content is low when compared to 59.9 % of cowpea (Elegbede, 1998), or bambara nut with 66 % carbohydrate content (Apata and Ologhobo, 1994). This makes *C. ensiformis* a good choice of carbohydrate for dieters.

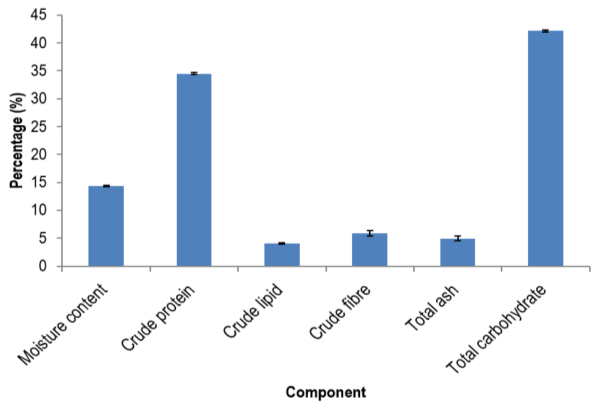

Figure 1: Proximate composition (%) of *C. ensiformis* seeds

Protein content of most legume seeds range from 20 – 28 % (Apata & Ologhobo, 1994) but that of *C. ensiformis* reported in this study is higher, making it a good source of protein food or supplement after due processing to leach out its antinutrients.

Quiescent seeds generally have low (5 - 15 %) moisture content (Bowley & Black, 1994). That of *C. ensiformis* seeds falls within this range. The lower the moisture content, the better the shelf-life of the plant.

The lipid content of *C. ensiformis* falls within the 1 – 5% range given for legumes not classified as oil legumes. Lipid content range from about 18 % in soyabean, to as high as 43% in groundnut have been reported for oil seeds (Apata & Ologhobo, 1994). Obviously *C. ensiformis* cannot be classified as an oil seed, but its lipid component can contribute significantly to the overall energy content.

Crude fibre recorded for *C. ensiformis* in this study (Table 2) is higher than the 4.28 % for soyabean reported by Temple *et al*. (1991) and the 4.2 % for kidney beans reported by Apata and Ologhobo (1994).

The ash content for this seed falls within the 3.0 to 4.8 % range reported for legumes (Apata & Ologhobo, 1994; Elegbede, 1998). The ash content is a reflection of its mineral composition.

Conclusion

Phytochemical screening in this study has revealed the presence of active pharmacological compounds in *C. ensiformis* seeds, thus supporting its antidiabetic and other medicinal uses. The rich content of nutrients and moderate levels of carbohydrate suggest that *C. ensiformis* seeds could be used as a cheap source of protein and low calorie food, with particular benefits to the obese population, if the levels of the antinutrients are ascertained and appropriate procedures applied for their reduction/elimination.

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