CHEMICAL ANALYSIS OF THE SEED OIL OF CANAVALIA ENSIFORMIS LINN. FOR NUTRITIONAL AND INDUSTRIAL QUALITIES

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ABSTRACT

The extensive demand for oils by man for consumption and industrial applications has led to an increasing search for oils from non-conventional sources to augment the available ones. Thus, the seed oil of *Canavalia ensiformis* Linn., a leguminous plant abundantly found in South-western Nigeria was extracted and analysed for its nutritional and industrial applications. Phytochemical screening and proximate analysis of the seeds along with physicochemical parameters of the oil using standard procedures were done. Different weights of the powdered seeds were extracted using a soxh let extractor with four solvents- n-hexane, chloroform, petroleum ether and ethanol. The fatty acid composition of the oil was characterized by Gas chromatography-Mass spectrometry (GC-MS).
Canavalia ensiformis seed contained saponins, anthracene derivatives, phenols, tannins, sapogenin and reducing sugar. The proximate analysis gave 1.50±0.84, 25.51±0.21, 2.10±0.23, 3.05±0.92, 56.94±1.96, 10.90±1.11 for moisture content, protein, ash content, crude fibre, carbohydrate, and oil extracts respectively. The physicochemical parameters of the oil were: pH-4.69, specific gravity-0.89±0.01, acid value-6.2, saponification value-203.3 mg KOH/g, free fatty acid-3.1, peroxide value-18.50 and iodine value-61.0 g of I_2/100 g oil, while the oil is miscible with petroleum ether. The fatty acid compositions were 11-octadecenoic acid (43.86%) 9-octadecenoic acid (27.49%), hexadecanoic acid (17.07%), cis-11-eicosenoic acid (4.32%), ethyl cis-9-octadecenoate (3.80%) and methyl octadecanoate (3.46%). The presence of 75.67% monoenoic acids in its composition, high saponification value, good foaming and emulsion properties makes the oil useful as an emollient, excipient in pharmaceuticals and solubilizing agents in aerosol products and also of great importance in soap and cosmetics industries.

Keywords: oilseeds, *Canavalia ensiformis,* saponification value, iodine value, peroxide value

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INTRODUCTION

Oilseeds are leading suppliers of superior quality and specialty vegetable oils for nutritional products, natural food and premium snack food worldwide (Sarwar, 2013). Numerous researches have been done on the analysis of oilseeds because of the extensive demands for oils both for human consumption and industrial applications; consequently there is an increasing need to search for oils from non-conventional sources to augment the available ones and also to meet some specific applications (Kyari, 2008;Abitogun and Olumayede, 2008; Adepoju and Abiodun, 2013). Oil seeds add important nutritional value to the diet due to the presence of high quality protein and also to vegetable oil, together with oil soluble vitamins like vitamin A (Abitogun and Oshodi, 2010).
Canavalia ensiformis Linn., belongs to the Leguminosae family. It is called Jack bean, horse bean, in English, 'Sesenla' in Yoruba and as 'Popondo′ in Ijebu. It is the most common shrub in the North coast of Columbia (Sanchez-Vioque *et al*., 1998). It is rich in most essential amino acids, including those deficient in wheat (Lawal and Adebowale, 2005). It is usually planted in Nigeria as an ornamental plant grown near houses and allowed to trail on walls and trees and in some places as “snake repellant” (Udedibe, 1990).Young

pods and beans of C. ensiformis are eaten as vegetables but only after much preparation and cooking as they contain mild poison in the form of anti-nutritional factors such as protease inhibitors, lectins, saponins and tannins (Doyle *et al.,* 2000; Kajita *et al.,*2001; Wojciechowski *et al.,* 2004). *Canavalia ensiformis* has been investigated as a potential source of the urease enzyme which is also the source of concanavalin A, a lectin used in biotechnology applications, such as lectin affinity chromatography (Lewis *et al.,* 2005).As with other legume seeds, a major drawback to the use of *Canavalia ensiformis* seeds in animal feeding is the presence of several endogenous toxic anti-nutritional factors (Carlini and Gumaraes, 1981), which include thermo-stable factors (canavanine, concanavalin, canavalin, canatoxin) and thermo-labile factors (protease inhibitors, lectins, phytic acid). The aim of present study is to provide basic data on the chemical composition of *C. ensiformis* oil seed in Nigeria, evaluate the phytochemical potential and proximate analysis of the seed, physicochemical properties of *C. ensiformis* oil seed, and to assess the oilseed for both industrial and nutritional values.

Materials and Methods

Plant materials

Fresh seeds of *Canavalia ensiformis* were harvested from Ago-Iwoye, Ogun State, Nigeria (N 06 571 4.111 and E 03 571 57.911). Identification and authentication were done at the Herbarium of the Forestry Research Institute of Nigeria (FRIN), Jericho Ibadan, where voucher specimen with FHI number 110444 was deposited.

Seed preparation

Canavalia ensiformis seed shell was manually opened to remove the seeds from the pods after which they were oven dried at 40^{O}C until a constant weight is gotten. The seeds were pulverized using an electric blender, stored in labeled plastic bags until ready for analysis.

Phytochemical screening

The preliminary phytochemical analysis of the powdered sample were carried out to determine the presence of anthracene, saponin, tannins, phenols, flavonoids, alkaloids and cardiac glycosides using standard methods (AOAC, 1990).

Proximate Analysis of Oil seed

This refers to the determination of the major constituents of the powdered sample and it is used to assess if a sample is within its normal compositional parameters or has somehow been adulterated. This method grouped nutrients in samples into five components: moisture content, ash content, crude protein, crude fibre and ether extract. They were determined using the methods of AOAC (1990). The carbohydrate content of the seed was then determined by difference.

Extraction Process

A soxhlet extractor was used for extracting the oil using different solvents (petroleum ether, ethanol, chloroform and n-hexane). The solvents used were then recovered by simple distillation. Each solvent (300 mL) was poured into a round bottom flask while 100 g of the powdered sample was placed in the thimble. The soxhlet was heated for 1 h to 50^{O}C, 60^{O}C, 70^{O}C and 80^{O}C when using petroleum ether, chloroform, n-hexane and ethanol as solvent respectively. The oil extracted and solvent were then removed from the round bottom flask, distilled to remove the solvent, dried in the desiccator and weighed to determine the amount of oil extracted. The whole process was repeated and the weight of extracted oil was found to be the same in each case. Physicochemical parameters of the oil were then determined.

Determination of the percentage of oil seed extracted

Different weights of the powdered sample (10, 30, 50, 70 and 100 g) were placed in the thimble and 150 mL of each solvent (petroleum ether, chloroform, n-hexane, and ethanol) was poured into the round bottom flask respectively. The soxhlet apparatus was heated for 1 h at 50^{O}C, 60^{O}C, 70^{O}C and 80^{O}C for petroleum ether, chloroform, n-hexane, and ethanol respectively. At the end, each solvent and extracted oil was distilled and the percentage of oil extracted was determined (AOAC, 1990).

Determination of Physicochemical Parameters

The following physicochemical parameters were determined using standard procedures approved by BPC (1988) and AOAC (1990): acid value, peroxide value, saponification value, solvent miscibility, pH value, density and specific gravity.

Determination of Acid Value

5 g of extracted oil was weighed accurately into 200 mL flask and 25 mL of a mixture of equal volume of 96% ethanol and ether were added which had been neutralized with 0.5 mL dilute phenolphthalein solution. This solution was titrated with 0.1 M KOH, shaking constantly until a pink colour which persisted for about 15 s was obtained. The volume of KOH required was noted (BPC, 1988).

Peroxide value

To 1 g of the oil sample, 1 g of potassium iodide and 20 mL of solvent mixture (glacial acetic acid/chloroform, 2/1 v/v) were added and the mixture was boiled for one min. The hot solution was poured into a flask containing 20 mL of 5% potassium iodide. A few drops of starch solution were added to the mixture and the latter was titrated with 0.025 N sodium thiosulphate and the peroxide value was determined (AOAC, 1990)as follows:

$PV = S \times N \times 10^{3} / W.$

Where S is volume of $Na_2S_2O_3$ in mL, N is normality of $Na_2S_2O_3$, W is weight of oil sample (g)

Saponification value (SV)

2 g of the sample was weighed into a 200 mL flask. 25 mL of an ethanolic solution of KOH (prepared by dissolving 40 g KOH in 20 mL of water and sufficient alcohol (98%) was added to make 1000 mL. The solution was allowed to stand overnight and the clear liquid was poured off. While the solution is still hot, 1 mL phenolphthalein indicator was added and titrated with excess of alkali using 0.5 N hydrochloric acid. The number of mL required (a) is noted. The operation was repeated without the sample being tested. The number of mL required (b) is noted (BPC, 1988).

SV= {(a-b) x 0.02805 x 100}/ weight (g) of sample

Solvent miscibility

This was determined by physical observation of the uniform blending of oil samples in petroleum ether and water at room temperature.

pH value, density and specific gravity

The pH value of the oil was obtained using a pH meter, a density bottle was used to measure the density at 25^{O}C while the specific gravity determined by calculation.

Gas chromatography-mass spectrometry analysis (GC-MS)

GC-MS analyses were performed on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; Polaris Q S/N 210729). HP-5MS non polar fused silica capillary column (50 m x 0.32 mm, 1.25 µm film thickness) was used under the following conditions: oven temperature program from 40^{O}C (2 min) to 280^{O}C at 5^{O}C/min, and the final temperature kept for 10 min; injector temperature 250^{O}C; carrier gas He, flow rate 1m L/min; the volume of injected sample was 1.5 µL of diluted oil in hexane; split less injection technique; ionization energy 70 eV, in the electronic ionization (EI) mode; ion source temperature 200^{O}C; scan mass range of m/z 40-650 and interface line temperature 300^{O}C. The constituents of the oils were identified based on their Kovat Index calculated in relation to the retention time of a series of alkanes (C4- C28) as reference products, in comparison with those of the chemical compounds gathered by Adams table (Adams, 2001), and the similarity of their mass spectra with those gathered in the NIST-MS library and or reported in the literature (Woerdenbag *et al.,* 1993).

RESULTS

The results of the phytochemical screening of the powdered seed of *Canavalia ensiformis* is shown in Table 1. The seed was found to contain tannin, saponins, emulsifying property, reducing sugar, phenol, steroids and alkaloids.

Proximate analysis of *C. ensiformis* seed showed that it contained 1.50% moisture, 10.90% crude oil, 25.51% crude protein, 56.94% carbohydrate (by difference), 3.05 % crude fibre and 2.10% ash as shown in Table 2.

The extracted oil was bright yellow in colour and a liquid at room temperature. The oil content of the seeds for the four solvent sutilized is shown in

Table3 with the oil extracted using petroleum ether giving the highest yield of 10.9% on average.
Physicochemical parameters are shown in Table 4 for *Canavalia ensiformis* seed oil. The specific gravity for *Canavalia ensiformis* oil seed was found to be 0.85, the free fatty acid and the acid value were determined to be 3.10% and 6.20 % oleic acid respectively, saponification value (SV) is 203.3 mgKOH/g, and iodine value is 61.0 mg/100g while the peroxide value was determined to be 23.50 mL/g.
The fatty acid composition of the oilseed showed that vaccenic acid, oleic acid, and palmitic acid are abundantly present; other fatty acid present were stearic acid, ethyl oleate, methyl stearate, and gondoic acid as shown in Table 5.

Table 1: Phytochemical screening of powdered seed of *Canavalia ensiformis*

Secondary metabolites	Results
Phenol	++
Flavonoids	+
Alkaloids	+
Tannins	++
Steroids	+
Emulsifying Property	+++
Saponins	+++
Anthracene derivatives	
-anthraquinone	– –
-anthraquinone aglycone	– –
-anthracene derivatives	+++
Sapogenin and reducing sugar	+++

Key += Present in trace amount, ++ = moderately present, +++= abundantly present
- = absent

Table 2: Proximate analysis of *Canavalia ensiformis* seed powder

Parameters	Results
Moisture content	1.50 ±0.84
Protein	25.51±0.21
Ash content	2.10±0.23
Crude fibre	3.05±0.92
Carbohydrate	56.94±1.96
Oil extracts	10.90±1.11

Table 3: Yield of *Canavalia ensiformis* Oil seed extracted using different solvents

Sample (g)	Hexane (g)	(%)	Chloroform (g)	(%)	Pet-ether (g)	(%)	Ethanol (g)	(%)
10	0.38	3.8	0.33	3.3	1.23	12.3	0.12	1.2
30	1.10	3.6	1.00	3.3	3.27	10.9	0.35	1.2
50	1.70	3.4	1.63	3.3	5.50	11.0	0.60	1.2
70	2.50	3.5	2.25	3.2	7.63	10.9	0.85	1.2
100	3.50	3.5	3.30	3.3	10.90	10.9	1.30	1.2

Table 4: Physicochemical parameters of crude *Canavalia ensiformis* Crude Oil

Parameters	Results
pH	4.69
Specific gravity (27^0C)	0.89±0.01
Density (g/cm^3)	0.89 ±0.01
Solubility (ether)	miscible
Acid value	6.2
Saponification value (mgKOH/g)	203.3
Free fatty acid(% oleic acid)	3.1
Peroxide value	18.50
Iodine value (g of I_2/100g oil)	61.0

Table 5: Fatty acid compositions of Crude *Canavalia ensiformis* Oil

Fatty acids	Old name	Results (%)
11-Octadecenoic acid	Vaccenic acid	43.864
9-Octadecenoic acid	Oleic acid	27.492
Hexadecanoic acid	Palmitic acid	17.072
Cis-11-Eicosenoic acid	Gondoic acid	4.318
Ethyl cis-9-octadecenoate	Ethyl Oleate	3.798
Methyl octadecanoate	Methyl stearate	3.456

Discussion and Conclusion

The phytochemicals present in the seed sample of *C. ensiformis* is an indication that the plant is of pharmacological importance. The observed phytochemicals are of great biological and biochemical importance in the human system. Tannins when taken in excess are found to be harmful to man (Ferreira *et al.,* 2008) due to its ability to chelate with metal ions rendering them unavailable to the human system. The oil extracted with petroleum ether had the highest yield of 10.9% on average; this is due to the increased ability of the solvent to overcome forces that bind lipids within the sample matrix (Lumley *et al*., 1991) and also the polarity of the solvent and the oil. The low percentage of oil makes this seed not to be a potential raw material for the oil industry. According to Egbekun and Ehieze, (1997), variation in oil yield may be due to the differences in variety of plant, cultivation climate, ripening stage, the harvesting time of the seeds, the extraction method and solvent used.
The low pH of 4.69 suggests the presence of a reasonable amount of fatty acids in the oil, which is a good indicator of the advantageous utilization of the oil. The preferential solubility in petroleum ether shows that the oil has a high level of unsaturated fatty acids (bearing in mind that oils rich in unsaturated fractions are readily soluble in ether),implying that the oil can undergo polymerization and this imparts on the oil a level of industrial utility (SLTC, 1963).The acid value in the oil of *C. ensiformis* being 6.20 % Oleic is comparable to 6.4 % given by Abitogun and Olaseheinde, (2012). The free fatty acid value was determined to be 3.10 % oleic acid thus; there are corresponding high levels of free fatty acids in the oil, which suggest high level of hydrolytic and lipolytic activities in the oil. The value is an indication that the oil can be refined to edible vegetable oil. The saponification value (SV) of 203.3mgKOH/gis comparable to those reported by Kyari, (2008) and also for common oil such as palm oil, groundnut oil and coconut oil (SV is 200,193 and 257 mg KOH/g respectively)thus, this oil may be used in soap making. The iodine value of 61.0

mg/100 g was comparable to that obtained for *C. ensiformis* 67.2 mg/100 g by Abitogun and Olaseheinde, (2012).The lower iodine value of *C. ensiformis* when compared with the iodine values of *Bligiasapida* 89.6 mg/100 g, castor oil 87.72 mg/100 g and olive oil 81.0 mg/100 gall of which are non-drying oils (Akpan *et al*., 2006; Kyari, 2008) suggests that this oil could be better as anon-drying oil and this could be significant during cargo handling and tank cleaning. The peroxide value was determined to be 18.50 mL/g; which is low and indicative of a low level of oxidative rancidity and of antioxidants. The combination of low iodine value and low peroxide value suggests that the oil could also be stored for a long period without deterioration. These also show that the oil possesses the desirable qualities of edible oils and could therefore be used for food purposes and as a feedstock in the industries. The specific gravity for *C. ensiformis* oil seed was found to be 0.85 which was similar to 0.87 reported for crude *Laffa cylindrical* oil by Abitogun and Olumayede, (2008) but lower to crude soya bean oil, sunflower oil reported by Abitogun and Oshodi, (2010).

Oleic acid (in triglyceride form) is included in the normal human diet as a part of animal fats and vegetable oils. Oleic acid as its sodium salt is a major component of soap as an emulsifying agent, and can also be used as an emollient (Carrasco, 2009). Small amounts of oleic acid are used as excipients in pharmaceuticals, and as an emulsifying or solubilizing agent in aerosol products (Smolinske, 1992).Vaccenic acid, also known as (E)-Octadec-11-enoic acid is a naturally occurring trans-fatty acid found in the fat of ruminants and in dairy products such as milk, butter, and yogurt. It is also the predominant fatty acid comprising trans-fat in human milk (Precht and Molkentin, 1999).

In conclusion, the phytochemicals present in the sample is an indication that the plant is useful pharmacologically. The presence of more than 70% monoenoic acid in its composition, high saponification value, good foaming and emulsifying properties makes the oil useful not only as a solubilizing agent in aerosol products, an emollient and excipients in pharmaceuticals, but also of great importance in soap and cosmetics industries. It could be used in food systems as a functional ingredient after modification to improve functional properties. Overall, Canavalia ensiformis has good nutritional quality and can be used as functional foods.

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