

Proximate, Dietary Element and Gcms Analysis of *Brachystegia Eurycoma*, *Detarium Microcarpum* and *Mucuna Sloanei* as Soup Thickeners

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Abstract: *Brachystegia eurycoma*, *Detarium microcarpum* and *Mucuna sloanei* are commonly consumed in Eastern and Southern Nigeria for their thickening and flavouring properties in soup. Chemical analyses reveals they are rich sources of dietary protein, fat, fibre, energy and dietary elements. However, they are poor bioaccumulator of 'heavy' metals and as such may not be suitable for bioremediation of 'heavy' metal pollution as shown in their respective 'heavy' metal analysis. GCMS analyses of their respective soxhlet petroleum ether extracts shows that they are rich in dietary significant nutrient such as gamma tocopherol, beta tocopherol and linoleic acid and several secondary metabolites. An understanding of the nutrient composition of these legumes is needful to guide toward informed nutritional decision making to enhance healthy nutrition.

Keywords: GCMS, AAS, Soup Thickeners, Heavy Metals, Dietary Elements.

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I. Introduction

The rich endowment of Vegetation provides varied plant resources that enhance food variations in West Africa. Such vegetation endowment characteristically varies across the region and seems to undertone certain dietary differentiations observable across its various parts and people group. For instance, Ene-obong and Carnoalue (1982) observed that cereals dominate in the northern parts of Nigeria, while legumes, nuts, seeds and starchy roots or tubers are the main food components in the South. Many a time the legumes are consumed alongside starchy roots or tubers and animal proteins traditionally in Southern Nigeria

Among the legumes commonly consumed as soup thickeners in Southern Nigeria are *Brachystegia eurycoma*, *Detarium microcarpum* and *Mucuna sloanei* (Nwosu, 2011; Ezueh, 1997). *B. eurycoma* is locally known as 'achi' by the Igbo's, 'alkolodo' by Yoruba's, 'okweri' by the people of Benin; 'eku' by the Isharis; 'ukung' by the Efiks; 'akpakpo' by the Ijaws and 'oyam' by the Kwales. Also, *D. microcarpum* is locally called 'ofor' by the Igbos, 'ogbogbo' by Yorubas and 'taura' by Hausas. *M. sloanei* (commonly called horse-eye bean, velvet bean and devil bead), a legume of the fabaceae family, is referred to as 'Ukpo' by Ibos; 'karasuu' by Hausas and 'Yerepe' by Yorubas (Adewale and Mozie, 2010; Ezeoke, 2010) and has other species such *M. urensi*, *M. argyrophylla*, *M. bennettii*, *M. pruries*, and *M. Veracrua* among the lot that are also found within the tropical vegetation (Nwosu, 2011; Armstrong, 2010; Nkpa, 2004).

Adebowale and Lawal (1986) emphasized that the soup thickening properties of soup thickeners are due to the presence of the simple, yet not true, food and seed gum termed hydrocholloids. The type and proportion of these hydrocholloides present in each of these thickening agents are closely related to their gelation properties, swelling capacity and overall thickening capacity. Pulverization increases the extractability of these hydrocholloides, ability to swell in water and therefore, the viscosity of these soup thickeners as asserted by Ajayi and colleagues (2006). Nwosu (2012) observed that not only the proximate composition but also the rheological properties of *B. eurycoma*, *D. microcarpum* and *M. sloanei* are affected by processing methods.

In light of the increasing need for the ever growing African population to expand the knowledge base of the foods in their environment as a panacea for healthy nutrition, this study attempted investigating the mineral

nutrient and lipid composition of *B. eurycoma*, *D. microcarpum* and *M. sloanei* as soup thickeners using spectrometric and chromatographic techniques.

II. Materials And Methods

Collection of Materials

All the soup thickeners (*B. eurycoma*, *D. microcarpum* and *M. sloanei*) were obtained from Oyigbo Market, Lagos, Nigeria. The analyses reported herein were carried out the Department of Biochemistry, Babcock University, Ilisan-Remo, Ogun State, Nigeria; except the GCMS analysis.

Sample Preparation

The samples were respectively sorted to remove dead seed and other organic debris, after which each sample washed and boiled in water at 120°C before dehulling. Each dehulled sample was dried in a SearchTech Electrothermal moisture extraction oven (DHG model) at 60°C for 6 hours. Each sample was then milled into finely particulate texture, using a laboratory hammer mill, packed and preserved for analyses.

Determination of Crude Protein

Plant samples for nitrogen determination were digested in sulphuric acid at temperature ranging between 360 to 410°C. The rate of digestion is accelerated by using mixture of copper sulphate and sodium sulphate/potassium sulphate in the ratio of 1:9 respectively as catalyst. On completion of digestion, the samples were cooled and distilled in the presence of NaOH. The distilled ammonia was collected in boric acid and titrated against standard acid (Horneck and Miller, 1998). Then, crude protein was estimated as the nitrogen content multiplied by the legume protein factor (AOAC, 2005).

Determination of Crude Fat

Crude fat was determined as prescribed by Method 954.02 of Association of Analytical Chemists (AOAC, 1990). The sample was placed in the oven overnight at 105°C and cool in desiccator to completely remove water from it. 5g of dehydrated sample was weighed into a completely dried thimble and uprightly positioned in Soxhlet apparatus whose flask was filled to a third of quarter with ether. Cooling water was set to run through the condensing compartment while heating the flask. The system was adjusted to condense at 5-6 drops per second and left refluxing for four hours. The thimble was removed from the Soxhlet apparatus and kept at room temperature for ether to evaporate, and then kept overnight in the oven at 105°C. The thimble was removed from the oven, cooled in desiccator and weighed. Amount of crude fat was estimated as percentage of the weight of sample taken:

$$\% \text{ Crude Fat} = \frac{\text{weight of fat}}{\text{Weight of sample (g)}} \times 100$$

Determination of Crude Fibre

Crude fibre was determined according to standard procedure (AOAC, 1980). Four grams of each moisture-free sample was weighed into a 250ml beaker, and 50ml 4% H₂SO₄ added followed by distilled water to a volume of 200ml. This was then heated to boiling and kept boiling for exactly 30 minutes, with constant stirring using a rubber tipped glass rod to remove all particles from sides of the containing beaker. The volume was kept constant by addition of hot distilled water. After 30 minutes of boiling, the content was poured into a Buchner funnel fitted with an ashless Whatman filter paper No. 40 and connected to a vacuum pump. Beaker was washed several times with hot distilled water and then transferred quantitatively with a jet of hot water. Washing continued on the funnel until the filtrate was acid-free as indicated by litmus paper. The acid-free residue was transferred quantitatively from the filter paper into the same beaker, while removing the last traces of sample with 5% NaOH solution and hot water to a volume of 200ml. Again the mixture boiled for 30 minutes with constant stirring as earlier described, keeping the volume constant with hot water. The mixture was then filtered and washed as earlier described until it was alkaline free. Finally, the resultant residue was washed with two portions of 2ml 95% alcohol. Residues on filter paper were transferred to a pre-weighed porcelain crucible. The content of the crucible was then dried in an oven maintained at 110°C to constant weight. Crucible content was then ignited in a muffle furnace at 550°C for 8 hours, cooled and weighed. Each sample was analyzed in triplicate. Crude fibre was calculated as loss in weight due to ignition as mathematically represented below:

$$\% \text{ Crude fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

Where:

W_1 = Weight of sample

W_2 = Weight of sample after extraction

W_3 = Weight of sample after ashing in muffle furnace

Determination of Moisture Content

The standard method of AOAC (1990) was adopted to determine the moisture contents of the samples. For each sample, a set of three evaporating dishes was cleaned, labeled and dried in hot air Cabolite oven set to 105°C for 40 minutes. They were then cooled in a dessicator containing dried silica gel as desiccant, and their weight was noted. 2g each of the milled samples was accurately weighed into the labeled evaporating dishes. The gross weight of each samples and its containing dish was read using a previously balanced Adams top loading balance. The sample was then dried in the oven at 105°C for 5hours, cooled in the dessicator, and weighed. Drying and weighing of samples continued, at same temperature but at 1hour time interval, until constant weight was attained by each sample. The moisture content of each sample was calculated as the difference in weights before and after drying to constant weights and expressed in percentage.

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where:

W_1 = Weight of evaporating dish

W_2 = Weight of sample and evaporating dish before drying in the oven

W_3 = Weight of sample and evaporating dish after drying in the oven

Determination of Ash Content

Ash content of the samples was determined as prescribed by standard procedure (AOAC, 1990). Empty lidded porcelain crucibles were ignited for 15 minutes in a muffle furnace at 550°C, cooled in a dessicator and weighed. 2g of each sample was accurately weighed into labeled crucible and the gross weight of the crucible and sample read. Crucibles containing their respective samples were ignited in the muffle furnace at 550°C for 6 hours to light gray coloured ash; then they were removed by means of tong and placed immediately in dessicator containing silica gel dessicant to cool. The weight of the crucible and ash was noted. Ash content determination was carried out in triplicate. Weight loss by samples due to ignition in the furnace was calculated as organic matter content of each food sample. Whereas, the difference between the gross weight of crucibles and the sample on one hand, and that of the crucible containing the ash was read as the weight of ash of each sample. Ash content of each sample was calculated and expressed in percentage.

$$\% \text{ Ash content} = \frac{W_2 - W_0}{W_1 - W_0} \times 100$$

Where:

W_0 = Weight of empty crucible

W_1 = Weight of sample and crucible before igniting in furnace

W_2 = Weight of sample after igniting in furnace

Estimation of Total Carbohydrate

Carbohydrate content of each sample was estimated using the *Carbohydrate by Difference Theory* as prescribed by standard procedure (AOAC, 1980). The theory suggests that proximate carbohydrate composition of a food sample could be estimated by the difference between the sum of the samples' proximate protein, fat, fibre, ash, and moisture and the percentage value of 100

Calculation of Caloric Value of the Food samples

The factors 4, 9 and 4 represent the approximate amount of energy available to the body per gram of carbohydrate, fat and protein respectively (physiological fuel value). These were used to compute the caloric values of the food samples analyzed as follows:

Total caloric value = Sum (gram of each nutrient in diet x factor) (Solomon, 2005)

Details of the mathematical relation, according to Elinge *et. al.* (2012) is presented below:

Energy (kcal) = [(%CHO x 4) + (%CP x 4) + (CL x 9)]

Where CHO, CP and CL stand for carbohydrate, crude protein and crude lipid respectively.

Dietary Elements Analyses

2g of each powdered sample was weighed in a 250ml pyrex conical flask into which 5ml of concentrated H₂SO₄ was added. The acid-sample mixture was heated at 100°C for 30 minutes during which frothing ceased, and allowed to cool. 5ml of the tri-acid digestion mixture, a mixture of concentrated nitric, perchloric, and sulphuric acids in the ratio 9:2:1 (Elinge *et al.*, 2012), was then added to the flask content. The entire reaction system was heated at 200°C until dense white fume evolved and transparent white residue is left behind in the flask. The reaction system was allowed to cool and 50ml of double distilled deionized water was added to dissolve the residual paste. The solution was filtered into a 100ml pyrex volumetric flask with 4 to 5 washing and made up to the volume. This filtrate was used as stock solution for the various mineral analyses. Fe, Zn, Co, Mg, Ca and Mn were determined by Atomic Absorption Spectrophotometry (Alpha 4 model, Buck Scientific Ltd USA). Na and K were determined using atomic emission spectrometer (200-A model, Buck Scientific Ltd UK), while colorimetric method was adopted determining the amount of phosphorus in the different samples (Elinge *et al.*, 2012).

Gas Chromatography Mass Spectrometry (GCMS)

The GCMS analysis was carried out at the Specialized Instrument Laboratory, Department of Chemistry, University of Lagos, Nigeria using the Agilent Technology model 7890A Gas Chromatograph/Mass Spectrometer, coupled to an MSD 5975C mass detector with Injector 7683B series. The initial and final temperatures were 100°C and 270°C respectively at the rate of 10°C per minute. 1µl of concentrated Soxhlet (petroleum ether) extract was aspirated. The heater temperature was 250°C at 3.2652 psi (pressure), slit less mode type, with column dimension 30m X 320µm X 0.25µm (HP5MS column type). Helium gas (99.9999% purity) was the mobile phase; the flow rate was 1.4963ml/min at average velocity of 45.618cm/s. Electron impact mode with ionization voltage of 70eV was adopted. Identification of the Phytoconstituents of the extract was carried out by comparing their respective retention times and mass weights with those of the authenticated samples obtained from the database of National Institute Standard and Technology (NIST), Version 2.0 MS, AOCs Lipid Library.

III. Results

Proximate compositions of Samples

Table 1 shows the proximate composition of *B. eurycoma* (achi), *D. microcarpum* (ofor) and *M. sloanei* (okpo). The empirical data obtained indicate that crude protein is highest in *M. sloanei* (21.73%) and lowest in *B. microcarpum* (10.11), whereas crude fat is highest in *D. microcarpum* (13.02%) and lowest in *B. eurycoma* (2.58%); meanwhile crude fibre is in the range 12.31% (*D. microcarpum*) - 1.19% (*M. sloanei*). *M. sloanei* has the highest moisture content (10.74) and energy value (3320.446 Kcal/Kg) whereas the lowest moisture content is observed in *D. microcarpum* as *B. eurycoma* shows the lowest energy level. The proximate ash composition of *B. eurycoma* (achi), *M. sloanei* (okpo) and *D. microcarpum* (ofor) are 2.58%, 3.15% and 5.12% respectively.

Dietary Elements Analysis

Table 2 detailed the dietary element composition of *B. eurycoma* (achi), *D. microcarpum* (ofor) and *M. sloanei* (okpo). *B. eurycoma* show highest level of both calcium (1.260%) and sodium (215.104mg/g) while *M. sloanei* is least in both calcium (0.023%) and sodium (109.239mg/g) contents respectively. Proximate magnesium compositions is highest in *D. microcarpum* (0.321%) and least *M. sloanei* (0.236%); whereas phosphorus is highest in *D. microcarpum* (0.288%) but least in *B. eurycoma* (0.181%). *M. sloanei* (1.511%) is highest in potassium, followed by *D. microcarpum*, (0.441%) and then *B. eurycoma* (0.183%). Manganese (mg/g) levels of *M. sloanei*, *B. eurycoma* and *D. microcarpum* are 16.304, 256.250 and 83.673; and 139.130, 193.750 and 169.898 for iron (mg/g). Levels of copper and zinc are respectively highest in *M. sloanei* (33.152mg/g and 25.543mg/g). Cobalt was not detected in any of the three soup thickeners studied.

Heavy Metal Analysis

Among the 'heavy' metals chromium, cadmium, lead and nickel is investigated, only chromium (mg/g) was detected; 41.145, 35.715 and 32.609 for *B. eurycoma*, *D. macrocarpum* and *M. sloanei*. The foregoing suggests that either *B. eurycoma*, *D. macrocarpum* and *M. sloanei* are bad bioaccumulators cadmium, lead and nickel or the samples where grown in environment not exposed to such elemental pollution assault. Therefore, further study is recommended to establish whether these plants are effective for phytoremediation of 'heavy' metal pollution.

GCMS Analysis

The Gas Chromatograms of Soxhlet petroleum ether extract of *B. eurycoma*, *D. macrocarpum* and *M. sloanei* are shown presented in Figures 1, 2 and 3 respectively. The mass spectra of all major peaks shown in the respective gas chromatograms were analyzed and some physiologically important secondary metabolites were identified as shown in Tables 4, 5 and 6.

Table 4 presents that *B. eurycoma* contains 1,2,3-trimethyl benzene (2.04%); 2,4-decadienal (2.16%); 9,12-octadecadienoic acid (linoleic acid) (8.79%); Methyl 9,12-heptadecadienoate (11.92%); 9,17-octadecadienal (8.72%); 1,2-benzenedicarboxylic acid, diisooctyl ester (9.68%); and gamma tocopherol (8.16%). The phytochemicals in *D. macrocarpum* hot petroleum ether extract are 1,2,4-Trimethyl benzene (13.82%); 2,4-decadienal (41.21%); 2-Undecanal (2.51%); Benzene, (1-butylheptyl)- (1.79%); Benzene, (1-pentylheptyl)- (2.30%); Benzene (1-butylheptyl)- (1.91%); Benzene (1-pentylheptyl)- (2.28%) and Benzene (butylheptyl)- (1.51%) as detailed on Table 5. On Table 6, 1,2,3-Trimethylbenzene (7.95%); 1,2,4-Trimethylbenzene (6.49%); Dodecyl acrylate (4.84%); 1-Pentylheptyl benzene (1.90%); Butylheptyl benzene (1.70%); Pentylheptyl benzene (2.22%); 2,6,10,14,18,22-Tetracosahexaene (34.23%) and 1,2-benzene dicarboxylic acid, diisooctyl ester (9.60%) were identified in soxhlet petroleum ether extract of *M. sloanei*.

Table 1: Proximate composition of *Mucuna sloanei* (okpo), *Brachystegia eurycoma* (achi) and *Detarium microcarpum* (ofor)

	<i>M. sloanei</i>	<i>B. eurycoma</i>	<i>D. microcarpum</i>
Crude Protein (%)	21.73	10.11	12.15
Crude fat (%)	6.25	2.58	13.02
Crude fibre (%)	1.19	1.59	12.31
Moisture (%)	10.74	8.57	7.80
Ash (%)	3.16	2.58	5.12
Carbohydrate (%)	56.93	74.57	49.60
Energy (Kcal/Kg)	3320.446	3195.064	3250.586

Table 2: Mineral Nutrient Profile of *Brachystegia eurycoma* (Achi), *Detarium microcarpum* (Ofor) and *Mucuna sloanei* (Okpo)

	<i>M. sloanei</i>	<i>B. eurycoma</i>	<i>D. microcarpum</i>
Ca (%)	0.023	1.260	0.144
Mg (%)	0.236	0.286	0.321
K (%)	1.511	0.183	0.441
P (%)	0.273	0.181	0.288
Na (mg/g)	109.239	215.104	165.306
Mn (mg/g)	16.304	256.250	83.673
Fe (mg/g)	139.130	193.750	169.898
Cu (mg/g)	33.152	7.292	8.163
Zn (mg/g)	25.543	14.826	5.510
Co (mg/g)	ND	ND	ND

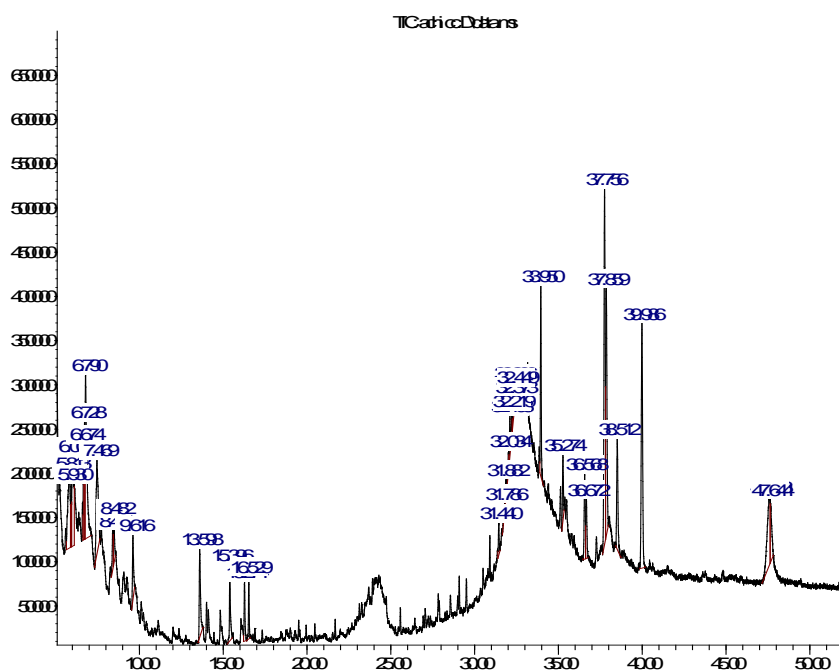
Note: ND means 'Not Detected', implying the quantity present was below detectable limit

Table 3: HEAVY METAL ANALYSIS Of *Brachystegia eurycoma* (Achi), *Detarium microcarpum* (Ofor) and *Mucuna sloanei* (OKPO) (2014)

	<i>M. sloanei</i>	<i>B. eurycoma</i>	<i>D. microcarpum</i>
Cr (mg/g)	32.609	41.145	35.715
Cd (mg/g)	ND	ND	ND
Pb (mg/g)	ND	ND	ND
Ni (mg/g)	ND	ND	ND

Note: ND means 'Not Detected', implying the quantity present was below detectable limit

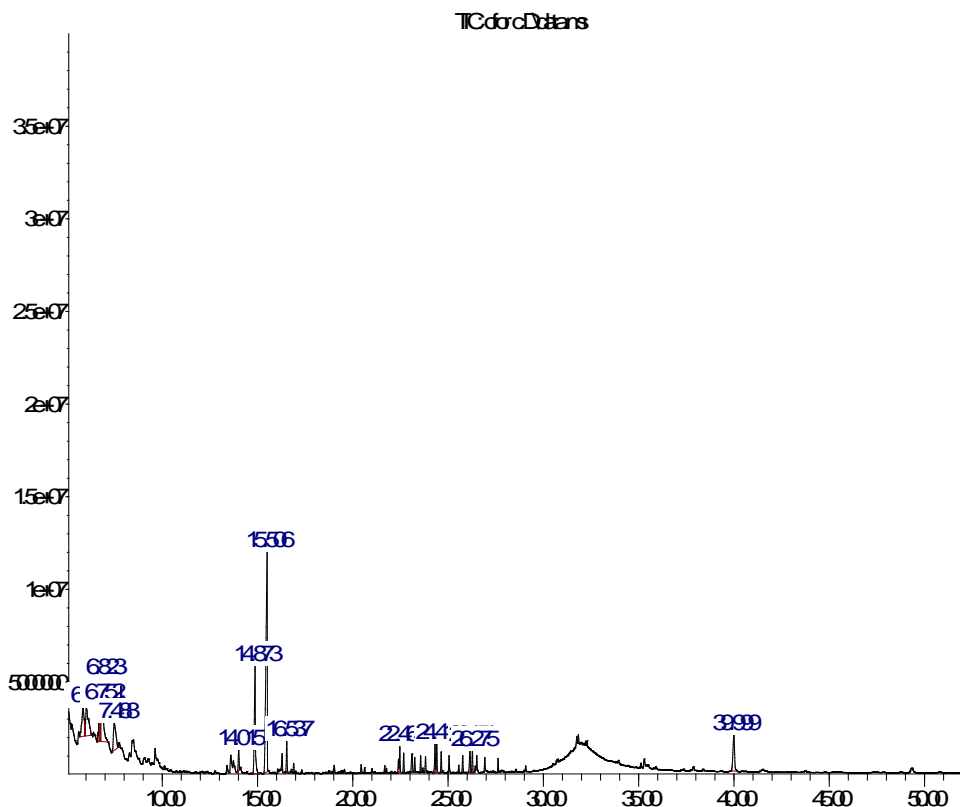
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Figure 1: Gas Chromatogram of hot petroleum ether extract of *Brachystegia eurycoma* (achi)

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Figure 2: Gas chromatogram of soxhlet petroleum ether extract of *Detarium microcarpum* (ofor)

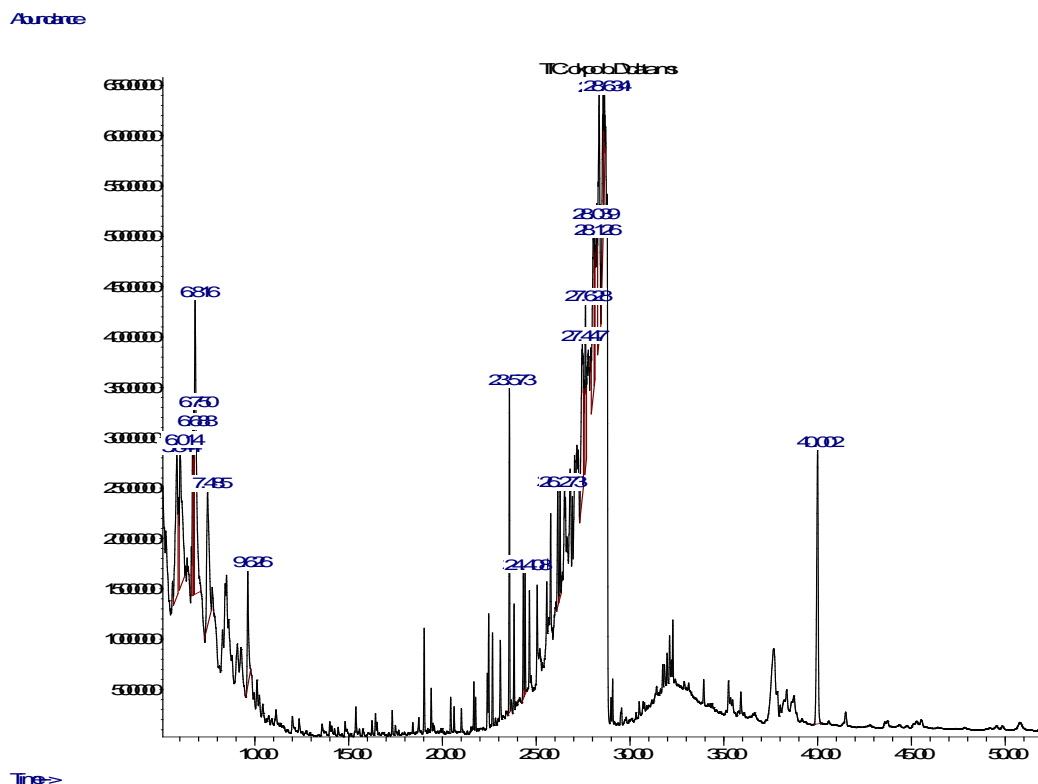


Figure 3: Gas chromatogram of hot petroleum ether extract of *Mucuna sloanei* (okpo)

TABLE 4: Mass Spectra Analysis Of Soxhlet Petroleum Ether Extract Of *B. Eurycoma* (Achi) Using Gcms

S/No.	Compound	Amount
1.	1,2,3-trimethyl benzene	2.04
2.	2,4-decadienal	2.16
3.	9,12-octadecadienoic acid (linoleic acid)	8.79
4.	Methyl 9,12-heptadecadienoate	11.92
5.	9,17-octadecadienal	8.72
6.	1,2-benzenedicarboxylic acid, diisooctyl ester	9.68
7.	Gamma tocopherol	8.16

‘Amount’ represents the percentage of total for each metabolite detected at minimum quality level of 90.

TABLE 5: Mass Spectra Analysis Of Soxhlet Petroleum Ether Extract Of *D. Microcapum* (Ofor) Using Gcms

S/No.	Compound	Amount
1.	1,2,4-Trimethyl benzene	13.82
2.	2,4-decadienal	41.21
3.	2-Undecanal	2.51
4.	Benzene, (1-butylheptyl)-	1.79
5.	Benzene, (1-pentylheptyl)-	2.30
6.	Benzene (1-butylloctyl)-	1.91
7.	Benzene (1-pentylloctyl)-	2.28
8.	Benzene (butylnonyl)-	1.51

‘Amount’ represents the percentage of total for each metabolite detected at minimum quality level of 90.

TABLE 6: Mass Spectra Analysis Of Soxhlet Petroleum Ether Extract Of *M. Sloanei* (Okpo) Using Gcms

S/No.	Compound	Amount
1.	1,2,3-Trimethylbenzene	7.95
2.	1,2,4-Trimethylbenzene	6.49
3.	Dodecyl acrylate	4.84
4.	1-Pentylheptyl benzene	1.90
5.	Butylloctyl benzene	1.70

6.	Pentyloctyl benzene	2.22
7.	2,6,10,14,18,22-Tetracosahexaene	34.23
8.	1,2-benzene dicarboxylic acid, diis octyl ester	9.60

‘Amount’ represents the percentage of total for each metabolite detected at minimum quality level of 90.

IV. Discussion

Mucuna species have been noted for their high protein value and as such have been suggested for dietary inclusion to enhance healthy protein nutrition (Nwosu, 2012; Arinathan *et al.*, 2003; Jambunathan and Singh 1980). The proximate values of achi, ofor and ukpo are consistent with those obtained from related studies (Igwenyi & Azoro 2014; Nwosu, 2012; Nwosu, 2011). The proximate levels of fat and carbohydrate suggest that these legumes do not only serve emulsifying and gelation role, but also prove to be a good energy source in diets when incorporated. Fibre has been heralded for several physiological roles including serving as combustible energy source, improving bowel movement, preventing colon cancer and improving digestibility of food (Salvin *et al.*, 1997; Anderson *et al.*, 1995).

The dietary elements contained in the three legumes studied indicated they could be nutritionally adequate sources of calcium, sodium, potassium, phosphorus, iron, zinc, manganese, cobalt and copper. In a review by Sridhar and Bhat (2007), these dietary elements are needful for proper muscle contraction, proper immune response, strong bone, healthy growth and development, optimal blood formation and healthy neuronal activity among the lot. *B. eurycoma*, *D. macrocarpum* and *M. sloanei* seem to be very poor at bioaccumulation of heavy metals, suggesting are not likely to contribute to heavy metal toxicity in the food chain and may be poor in bioremediation of heavy metal polluted soil.

B. eurycoma could be a good source of gamma tocopherol (important in vitamin nutrition) and linoleic acid, which supports healthy cardiovascular functioning.

V. Conclusion

The results obtained in this study suggest that *B. eurycoma* (achi), *D. macrocarpum* (ofor) and *M. sloanei* (ukpo) are good sources of dietary macronutrients and mineral nutrients. Appropriately combined, these legumes could support nutrient adequacy of the traditional West African diets in which they are incorporated. The secondary metabolites contained in them suggest they could possess certain antimicrobial properties.

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