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Mucuna pruriens (L.) Dc. (Var. Okeagbaloko) Can Bridge the Protein Gap in the Menu of the Rural Poor in South Eastern Nigeria

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Abstract: Acute shortage and high costs of protein-rich foods of animal origin in south Eastern Nigeria have resulted to the search for indigenous inexpensive and sustainable alternative sources of protein of plant origin. A usually avoided high yielding legume that grows wild in south eastern Nigeria is a variety of *Mucuna pruriens,* called; with highly itching pods. Proximate, amino acid, nutrient and anti-nutrient analyses, of both its seeds and leaves were carried out following standard analytical protocols. The results showed that both the leaves and seeds are rich in minerals such as, calcium, copper, zinc, iron and phosphorus; vitamins like A, B_1, B_2, B_6 and B_{12}. The amino acid profile and protein content of the seed were significantly higher than those of the leaf and compares favourably with that of high protein crops like soya beans. The lysine content of the seed was higher than the FAO/WHO 1991 requirement pattern. It also contains anti-nutrients substances like tannins, phytic acid and hydrogen cyanide. The findings suggest that apart from the general use of the leaf extracts as blood tonic, the seed could bridge the yawning protein gap in the menu of the people of the region.

Key words: *Mucuna pruriens* · Proximate Analysis · Amino Acid Profile · Nutrient and Anti-Nutrient · High Protein Crop

INTRODUCTION

Large segments of the human population and animals in developing countries suffer from protein malnutrition. About 800 million people are consuming less than 2000 calories a day and are living under conditions of permanent or intermittent hunger so that they are chronically undernourished. Most of the hungry are women and young children [1]. The populations of most developing countries are increasing rapidly; by the year 2020 there will be an additional 1.5 billion mouths to feed, mostly in the developing world. The search for alternative food and feed ingredients for man and livestock continues to attract the attention of researchers all over the world [2]

Legume seeds including *Mucuna pruriens* are important sources of plant proteins, constituting more than 80% of available protein in human diets in developing countries [3]. The primary nutritional importance of proteins is that they are sources of amino acids. High quality proteins contain essential amino acids in quantities corresponding to human requirements and are readily digestible. Humans require certain minimal quantities of essential amino acids from a biologically available source as part of a larger protein/nitrogen intake. It is important to determine the relative efficiency with which individual protein sources meet these requirements. The underutilized plants which have tremendous potential for commercial exploitation and which remain ignored, offer a good scope in this context [4]. Accounts of important underutilized plant species which await exploration for food, fodder, energy and industrial purposes have been given by Siddhuraju *et al*. [5] and Arinthan *et al*. [6].

Among the various under-utilized wild legumes, the velvet bean *Mucuna pruriens* which is widespread in tropical and sub-tropical regions of the world is regarded as a good source of food [7]. It is considered a viable source of dietary proteins [2, 8] due to its high protein

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concentration (23–35%). In addition, its digestibility compares favourably with other pulses such as soybean, rice bean and lima bean [9].

Although the velvet beans are currently exploited as a protein source in the diets of fish, poultry, pig and cattle after subjection to appropriate processing methods, in different parts of the world, because they contain high levels of protein and carbohydrate; their utilization is limited due to the presence of a number of antinutritional/antiphysiological compounds, phenolics, tannins, L-Dopa, lectins, protease inhibitors, etc., which may reduce the nutrient utilization [8].

The fact not withstanding that many studies have been carried out on the medicinal properties of *M. pruriens,* in some parts of the world [10-14] yet the full potentials of this wonder plant to both human and animal health care delivery is yet to be unraveled.

In south Eastern Nigeria, the utilization of the plant for food is very much limited and its utilization as a medicinal plant is essentially at the level of ethno-medicine, with the leaves receiving much more research attention as shown by the reports of [15-17]. The essence of the present study is to evaluate the proximate mineral composition, some anti-nutritive factors and metals, as well as the amino acid profile of a very high yielding, potential elite, *M. pruriens* cultivar "Okeagbaloko", from the region; with a view to exploring its inclusion into the food menu of the people. This will help in narrowing the protein-calorie gap in the region; which is currently widening due to the general economic down turn in the country, with the concomitant effect that the conventional food proteins are almost out of the reach of the poor rural dwellers.

MATERIALS AND METHODS

Collection of Sample Seeds: The seeds and dry pods of the local itching beans (*Mucuna pruriens* (*L.*) *DC. var.* ("Okeagbaloko") (Figs. 1-2) used for the study were provided and identified by Mr, Alfred Ozioko, of the herbarium section of Biodiversity and Conservation Programme/International Centre for Ethno-medicine and Drug Development, No. 110 Aku, Road Nsukka.

Collection of Fresh Plant Leaves: Seeds of the sample were planted in polythene bags in the Botanic garden as well as in the field in the New Departmental Garden, University of Nigeria, Nsukka, during the month of June 2014, to grow plants for close observation of the habit of the plant (Figs. 3-5) and to provide fresh leaves for proximate analysis of the leaves.

Treatment of Fresh Leaves for Analysis: The harvested fresh *Mucuna pruriens* leaves (Figs. 4-5) were dried in the screen house at the Botanic Garden, university of Nigeria, Nsukka at an average temperature of 38.5°C and 45.6 % relative humidity for 14 day. After drying, the samples were weighed and ground into a uniform powder using Thomas Willey Laboratory Mill Model 4 and stored in an airtight container until required for analysis.

Experimental Site (Location): The proximate analysis was conducted at the Department of Crop Science Analytical Laboratory Faculty of Agriculture, University of Nigeria, Nsukka located at (6°51'E, 7°29' Altitude 400m).

Proximate Analysis of the Leaves and Seeds of *Mucuna pruriens*: Proximate composition of *Mucuna pruriens* leaves and seeds was carried out to determine the ash content, crude protein content, crude fat content, crude fibre, moisture content, CHO, vitamin (Vitamin B, B_1, B_2, B_6 and B_{12}) and anti-nutritional content, tannin, phytate, hydrogen cyanide and minerals; copper, zinc, calcium, phosphorus and iron. The proximate analysis was carried out using modified standard method of the Association of Official Analytical Chemists [AOAC] [18].

Determination of Total Crude Protein: The protein content was estimated by the use of micro Kjeldahl method. The principle behind this is that the nitrogen in the sample is converted to ammonium–nitrogen by digestion with tetraoxosulphate (vi) acid using a catalyst mixture of selenium oxide and copper (ii) tetraoxosulphate (vi) acid. The ammonia liberated when this digest is reacted with sodium hydroxide is removed by steam distillation and collected with 1% boric acid indicator mixture. This is then titrated with 0.01N HCl to give percentage nitrogen in the sample.

Digestion Procedure: Dried milled sample of plant material (0.5g) was transferred into 50 ml Kjeldahl flask. One gram of catalyst was added using a spatula. The catalyst is a mixture of selenium oxide and copper (ii) tetraoxosulphate (vi) acid, ($CuSO_4$) was also added, 15 ml of conc. H_2SO_4 was carefully added. The flask was swirled gently and then taken to the heater. Heating was done gently at first until frothing stopped. This was heated continuously and strongly on digestion rack under fume hood until a clear greenish solution resulted. The mixture was heated for another 30 minutes and was allowed to cool and digest transferred into a volumetric flask.

Fig. 1: Dry fruits of *Mucuna pruriens var.*

Fig. 2: Dried seeds of *Mucuna pruriens var.*

Fig. 3: *Mucuna pruriens var.* seedling (11 days after planting)

Fig. 4: *Mucuna pruriens* var. seedlings growing in plastic bags in the Botanic garden, University of Nigeria, Nsukka

Fig. 5: Established *Mucuna pruriens* var. plants twining on a stake in the Botanic garden, University of Nigeria, Nsukka

Distillation Procedure: The distillation apparatus was steamed for ten minutes. The volume of digest was made up to 50 ml with distilled water to avoid caking. The flask was shaken properly then 10 ml of digested sample was pipetted and transferred into the unit (Kjeldahl apparatus). A receiver flask (50 ml) was placed under condenser of distillation apparatus. Also 10 ml 40% sodium hydroxide (NaOH) solution was added into the sample chamber through a funnel stop cork and distillation commenced. The distillate (35 ml) was collected on the condenser tip and rinsed with distilled water. Liberated ammonia, collected with 10ml of boric acid-indicator mixture, turned green after which distillation went on for about five minutes.

Titration procedure The content of the conical flask (Distillate) was titrated with 0.01 M HCl to a colour change (Until the original colour of the boric acid was restored). The concentration of protein was calculated using the formula shown below:

$$\%Nitrogen = \frac{Titre\ value\ x\ 14.1\ x\ 0.01\ x\ 100\ x\ 50}{1000\ x\ 0.5g\ x\ 10ml} x1000$$

% Protein = Nitrogen × 6.25 (Where 6.25 is a constant)

Determination of Crude Fat Content: Soxhlet extractor was used. An extraction flask was thoroughly washed and dried in hot oven for 30 minutes. It was placed in a desiccator to cool. Two grams (2 g) of the ground sample was transferred into a rolled ashes filter paper and then placed inside the extractor thimble, which was put inside the Soxhlet extractor. Petroleum ether (200 ml) was added. The apparatus was set up and then heated and allowed to run for 4 hours. The ether was recovered at the end of the extraction before the thimble was removed. The oil collected in the flask was dried at 100°C in an oven and then weighed.

The percentage (%) fat content was calculated using the formula;

$$\%Fat = \frac{C - A}{A} x \frac{100}{1}$$

A = weight of empty flask
B = weight of sample
C = weight of flask oil after drying.

Determination of Crude Fibre: Weende analytical method was employed in the determination of the crude fibre content. To determine this, the protein, starch and other digestible carbohydrate had to be hydrolyzed out of the sample. The organic constituent of the insoluble matter remaining after the sample had been treated with sulphuric acid and sodium hydroxide under controlled experimental condition is the fibre.

To 150 ml of 0.128 M pre-heated and weighed (W_1)H_2SO_4 was added 5 g of the ground sample and then heated to boiling for 30 minutes and then filtered. The residues were washed three times with hot water. To this was added 150 ml of 0.128 M pre- heated KOH and heated to boiling. Five drops of antifoaming agent was added which was allowed to boil slowly for 30 minutes and then filtered using Buchner funnel in cold extraction unit. The residue was washed three times with hot water and another three times with acetone. It was dried in the oven at 103°C for 1 h and put in the desiccator for cooling and weighing (W_2). This was placed in a cool muffle furnace and the temperature was increased up to 500°C until ashing was completed. Then the crucible containing the ash was removed from the furnace, cooled in the desiccator and weighed W_3).

Percentage fibre was calculated using the formula below:

$$\%Crude\ Fiber\ = \frac{W_2 - W_3}{W_1} x \frac{100}{1}$$

(W_1) = Initial weight of the ground sample used
(W_2) = weight of residue after drying at 103°C
(W_3) = weight of ash

Determination of Ash Content: The residue remaining after destruction of the organic matter of the sample is referred to as ash.

Silica dish was heated to 100°C for 1 h, cooled in a desiccator and weighed. Two grams of the sample was put into the silica dish. The silica dish and sample was placed in a muffle furnace and ashed (heated) at 600°C for 3 hours and allowed to cool in a desiccator and weighed. Percentage of ash content was calculated using the formula below:

$$\%Ash = \frac{Weight\ of\ Ash}{Fresh\ Weight\ of\ Sample} x100$$

Determination of Moisture Content: Two grams (2g) of the ground sample was dried to a constant weight at 60°C in a hot air circulating oven for 24 hours. The moisture content was calculated as the difference in weight after drying as shown below:

$$\frac{W_2 - W_3}{W_2 - W_1} x \frac{100}{1}$$

W_1 = initial weight of empty crucible sample
W_2 = weight of crucible sample before drying
W_3 = final weight of crucible after drying.

Determination of Dry Matter Content: This was determined by weighing 1g of the milled sample into a dish of known weight (W_1). This was then dried in the oven for 24 hours at 100°C to a constant weight, cooled in the desiccator and weighed (W^2).

$$\%\ Dry\ matter = \frac{100 - (W_1 - W_2 \times 100)}{Fresh\ weight}$$

W_1 = weight of sample before oven drying
W_2 = weight of sample after oven drying.

Determination of Total Carbohydrates:
This was estimated by calculation
Total carbohydrate (%) = 100 – (% fibre + % moisture + % fat + protein + % ash).

Determination of the Mineral Content of Samples
Mineral Analysis: Determination of mineral elements followed the method of AOAC [18]. Two grams of the sample was reduced to ashes in a furnace at 550°C for 18hrs and the ash was dissolved in 10 ml of 0.1M HCl. This was filtered into a 100 ml volumetric flask and made up to mark with double distilled water. This was used in the determination of the mineral content of the sample using Atomic Absorption Spectrophotometer (AAS) as well as prepared standards of Cu, Zn, Ca, P and Fe. The estimates were done using the atomic absorption spectrophotometer method.

Vitamin Determination of the Samples
Vitamin A: (Lycopene): One gram of the sample was dissolved with 10 ml of acetone in 50ml conical flask. This was allowed to stand for 20 minutes and followed by shaking gently at 4 minutes interval to extract the colour substance in the sample. After agitation it was allowed to settle and decant to obtain a clear solution in a test tube. Five ml of benzene was added with gentle/shaking. Two distinct layers were observed. The upper layer was obtained by separation with a separating funnel and collected in a glass cuvette. The absorbance was read at 487nm following the method of AOAC [18].

Calculation - $\frac{Abs \times 10 \times 1/5 \times 10^4}{2570}$
Abs = absorbance

Vitamin B_1 (Thiamine): One gram of the sample was dissolve with 10 ml of cyclohexane in 50 ml conical flask. Allowed to stand for 20 minutes and shook gently at 4 minutes interval to extract the colour substance in the sample. After agitation it was allowed to settle and decant to obtain a clear solution in a test tube. 5 ml of benzene was added and shook gently. Two distinct layers were observed. The upper layer was obtained by separation with separating funnel and collected in a glass covet and read off in the absorbance at 325 nm. The same method was used in other vitamin B series; B_2 but with the wavelength of 428nm, vitamin B_6 300nm and vitamin B_{12} 360nm following the method of AOAC [18].

Anti-Nutrient Determination of the Samples
Tannin: Two grams of the sample was accurately weighed into a 50 ml conical flask and 10 ml of 2M HCl was added followed by shaking. Twenty (20 ml) of water was added before shaking. The content was filtered into 50 ml of volumetric flask and made up to 50 ml. Five ml of the solution was pipetted into a test tube and 5 ml of water into another test tube as a control. Three ml of $FeCl_3$ was added to each test tube, 1ml HCl and 3 ml of $(K_3F(CN)_6)$ respectively. It was allowed to stand for 30 seconds. The absorbance was read at 760nm following the method of AOAC [18].

Phytate: One half gram of the sample was weighed into a 500 ml round bottomed flask and extracted with 100 ml of 2.4 % HCl for one hour at room temperature. This was decanted and filtered. 1 M of the filtrates was pipetted and diluted to 25ml with water. Ten millilitres of the diluted sample was pipetted into a test tube through amberlet resin grade 200 - 400 mesh to elute inorganic phosphates and 15 ml of 0.7 sodium chloride was added. The absorbance was read at 520nm following the method of AOAC [18].

Hydrogen Cyanide: To 1 L of distilled water, 1.015g anhydrous KCN was added and dissolved. Twenty ml of the solution was pipette d into 250 ml flask. The solution was made alkaline by addition 3ml 6M ammonium solution. To this was added 0.2g of potassium iodide. The solution was agitated gently to dissolve the salt. It was titrated with 0.02N silver nitrate and calculated the equivalent in the solutions to obtain 0.40. One ml of the above stock solution = 0.4mg HCN. Aliquots of 0, 5, 10, 15, 20, 25 and 30 ml were taken into seven 100ml volumetric flask and 20 ml of H_20 and 10 ml of 0.03M picric acid was added. The flask was capped and incubated for 5 minutes at 94°C in a water bath. The flask was cooled at room temperature and the optical density (Absorbance) was measured at 540 nm following the method of AOAC [18].

Quantitative Amino Acid Analysis: The quantitative amino acid analysis was carried out at the Nigerian Institute of Science Laboratory Technology Laboratory, Ibadan Nigeria. The greenhouse dried seeds and leaves of *M. pruriens* were ground and powdered using an Uddy Cyclone Mill with a 1.00mm mesh screen. For both the seeds and leaves, 0.5g of each of the ground sample was hydrolyzed with HCl in the presence of Phenol for 22 h at

a temperature of 108°C. Each of the samples was dissolved in water (HPLC) grade and placed in HPLC for derivation, separation and quantification of amino acids at 254 nm wavelength following the method of AOAC [18].

Data Analysis: The Statistical Package for Social Sciences (SPSS) version 17 was used to analyze the data collected. The mean and the standard deviation were calculated and analysis of variance was used to separate the mean.

RESULTS

The result obtained from proximate composition of *Mucuna pruriens* var. *pruriens* leaves and seeds (Table 1) showed that the moisture content of the leaves (40.6 %) was significantly higher than that of the seed (3.33 %). The seeds had higher protein content (30.43 %) than that of the leaves (9.7 %) and it was significant at P =.05. The seed had a higher fat content (3.56 %) than that of the leaves (1.5 %). The ash content of the seeds was (6.9 %) which was significantly higher than that of the leaves (2.6 %) at P =.05. For fibre content of the seeds (5.96 %) was significantly higher than that of the leaves (3.6 %). The carbohydrate content of the seeds was (48.8 %) which was higher than that of the leaves (40.3 %) at P = .05.

Table 2 shows the mineral composition of *Mucuna pruriens* leaves and seeds. The copper content of the leaves (23.3 mg/kg) was significantly higher than that of the seeds (11.99 mg/kg) at P =.05. The zinc content of the leaves (42.00 mg/kg) was significantly higher than that of the seeds (30.33 mg/kg) at P =.05. The seeds had a significantly higher calcium composition (14.33mg) than that of the leaves (7.83 mg) at P =.05. The phosphorus content of the seeds (6.16 mg/100g) was significantly lower than that of the leaves (9.66 mg/100g) at P =.05. The iron content of the leaves (1.07 mg/100g) was significantly higher when compared with that of the seeds (0.36 mg/100g) at P =.05.

Table 3 shows the vitamin composition of *Mucuna pruriens* leaves and seeds. The vitamin A content of the leaves (14.23 Ng/g) was significantly higher than that of the seeds (5.40 Ng/g) at P = .05. The vitamin B_1 content of the seeds (0.78 mg/100g) was significantly higher than that of the leaves (0.17 mg/100g). The vitamin B_2 content of the seeds (0.13 mg/100g) was significantly higher than that of he leaves (0.12 mg/100g).

Table 1: Proximate composition of the leaves and seeds of *Mucuna pruriens*

Nutrients (%)	Leaves	Seeds
Moisture	40.6±0.80[a]	3.33±0.16[b]
Crude protein	9.7±0.35[a]	30.43±0.21[b]
Fat	1.5±0.33[a]	3.56±0.03[b]
Ash	2.6±0.13[a]	6.9±0.03[b]
Fibre	3.6±0.03[a]	5.96±0.03[b]
Carbohydrate	40.3±0.66[a]	48.8±0.41[b]

Mean ± SE of triplicate determination

[a b] Mean values with different superscripts on the same row are statistically different at P =.05.

Table 2: Mineral composition of the leaves and seeds of *Mucuna pruriens* var.

Nutrients	Leaves	Seeds
Copper (mg/kg)	23.3±0.66[a]	11.99±0.00[b]
Zinc (mg/kg)	42.00±1.00[a]	30.33±0.33[b]
Calcium (mg/100g)	7.83±0.16[a]	14.33±0.33[b]
Phosphorus (mg/100g)	9.66±0.33[a]	6.16±0.16[b]
Iron (mg/100g)	1.07±0.00[a]	0.36±0.00[b]

Mean ± SE of triplicate determination.

[a b] Mean values of different superscripts in the same row are statistically different at P = .05.

Table 3: Vitamin composition of the leaves and seeds of *Mucuna pruriens* var.

Nutrients (units)	Leaves	Seeds
Vitamin A (Ng/g)	14.23±0.61[a]	5.40±0.01[b]
Vitamin B_1 (mg/100g)	0.17±0.00[a]	0.78±0.00[b]
Vitamin B_2 (mg/100g)	0.12±0.00[a]	0.13±0.00[b]
Vitamin B_6 (mg/100g)	0.15±0.00[a]	0.85±0.00[b]
Vitamin B_{12} (mg/100g)	0.21±0.00[a]	0.37±0.00[b]

Mean ± SE of triplicate determination

[a b] Mean values of different superscripts in the same row are statistically different at P = .05

Table 4: Anti-nutrient composition of the leaves and seeds of *Mucuna pruriens* var.

Nutrients	Leaves	Seeds
Tannin (mg/100g)	15.10±0.05[a]	48.18±0.91[b]
Phytate (mg/100g)	3.66±0.23[a]	2.33±0.00[b]
Hydrogen cyanide (mg/100g)	0.96±0.03[a]	0.31±0.00[b]

Mean ± SE of triplicate determination

[a b] Mean values of different superscripts in the same row are statistically different at P = 0.05

The vitamin B_6 content was significantly lower in the leaves (0.15 mg/100g) than in the seeds (0.85 mg/100g). The vitamin B_{12} content of the seed (0.37mg/100g) was significantly higher than that of the leaves (0.21mg/100g).

Table 4 shows the anti-nutrient composition of *Mucuna pruriens* leaves and seeds. The tannin content of the seeds (48.18mg/100g) was significantly higher than that of the leaves (15.10mg/100g) at P = .05.

Table 5: Amino Acid Profile (g/l00g Protein) of *Mucuna pruriens* var.

Amino acid	Leaf	Seed	FAO/WHO (1991) Requirement Pattern
Lysine	4.62±.02[a]	6.71±.05[b]	5.8
Histidine	2.48±.04[a]	4.44±.03[b]	1.9
Arginine	4.02±.03[a]	5.06±.03[b]	
Threonine	2.46±.01[a]	3.27±.02[b]	3.4
Methionine	0.40±.03 [a]	1.24±.01[b]	2.5
Valine	1.42±.04 [a]	2.98±.03[b]	3.5
Leucine	4.33±.02 [a]	5.24±.01[b]	6.6
Isoleucine	-nd[a]	6.94±.02[b]	2.8
Phenylalanine	2.30±.03[a]	3.98±.01[b]	6.3
Tyrosine	0.86±.02[a]	4.94±.01[b]	
Proline	-nd[a]	2.98±.04[b]	
Glycine	1.06±.02[a]	5.95±.03[b]	
Alanine	-nd[a]	4.24±.01[b]	
Aspartic	-nd[a]	10.98±.02[b]	
Glutamine	2.23±.03[a]	16.11±.05[b]	
Serine	-nd[a]	4.40±.04[b]	
Cystine	0.50±.04[a]	0.54±.01[a]	
Tryptophan	0.44±.03[a]	0.58±.03[b]	1.1
Crude protein	16.26±1.16[a]	36.48±1.18[b]	

nd = not detected

Mean ± SE of triplicate determination

[a b] Mean values with different superscripts on the same row are statistically different at $P = .05$

Unlike the tannin, the phytate content of the leaves (3.66 mg/100g) was significantly higher than that of the seeds (2.33 mg/100g) at $P = .05$. This was also similar for the hydrogen cyanide content of the leaves (0.96 mg/g) which was significantly higher than that of the seeds (0.31 mg / g) at $P = .05$.

Table 5 shows the amino acid profile and crude protein content of the seeds and leaves of *M. pruriens.* A total of 18 amino acids were detected on the seed sample while only 14 were detected on the leaves. Isoleucine, Proline, Alanine, Aspartic acid and Serine could not be were detected on the leaf. With the exception of cysteine, the amino acid profile and the crude protein content of the seeds differed significantly from those of the leaves for the amino acids detected.

DISCUSSION

The results of the proximate composition of the itching bean (*M. pruriens* var. leaves and seeds showed that the leaves moisture content (40.6 %) was higher than the seeds (3.33 %). This was because the leaves were used directly without prior drying leaves at room temperature. The crude protein content of the seed (30.43 %) was higher than that of leaves, apparently because the seed need to store essential nutrients needed for germination and initial establishment of the seedling. The protein content of *Mucuna pruriens* seeds was lower than that of soy bean seeds (37.08%) [19] but is similar to the protein content of seeds of *Mucuna monosperma* and *Mucuna pruriens* var. *utilis* [20]. The seed contain higher crude protein when compared with a commonly consumed pulse like pigeon pea (22.4%) [21]. The seed protein was quite higher than that obtained from the seeds of *Moringa olerifera* seeds (26.71%) [22]. The crude protein content of the leaves (9.7 %) was lower than that of the leaves of *Zanthoxylum acanthopodium* (28.06%) and *Clerodendrum colebrookianum* (27.67%) as reported by Seal [23]. This shows that the seeds can be conveniently used as another rich source of protein.

The fat content of the seed (3.56%) was significantly higher than that of leaves (1.5%) but was lower than the fat content of the soy bean seeds (18. 38 %) [19]. It was also lower than the fat content of the seeds of *Arachis hypogea* (46.10%) [24]. The ash content is a measure of a plants mineral content. The ash content of *M. pruriens* was higher in the seeds (6.9 %) than in the leaves (2.66%) indicating that the seeds contain appreciable amount of mineral elements like copper, zinc, calcium, phosphorus and iron. This again can be explained by the fact that seed stores enough nutrients essential for the continuity of the species through germination and seedling growth.

This value was equally higher than (4.16%) reported for seeds of *Mucuna flagellipes* [25]. The crude fibre content of *M. pruriens* seeds (5.96%) is higher than the fibre content of soy bean seeds (5.12%) reported by [26].

This value was also higher than the 3.65% reported for seeds of 12 *Mucuna* accessions in Nigeria [25] and lower than 22.36% reported by Hassan and Ngaski [27] but the fibre content (3.65% for the leaf) is lower than (8.61%) reported for leaves of *Amaranthus hybridus* [28] and lower than 18.00% reported by Hassan and Umar [29] for the leaves of *Momordica balsamina* and 16.4% for the leaves of *Myrianthus arboreus* [30]. Adequate intake of the leaves of *M. pruriens* as dietary fibre among other high dietary fibre vegetables can lower serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer [31, 32].

The carbohydrate (CHO) content of the seeds (48.8%) was higher than the carbohydrate content of *Arachis hypogaea* seeds (17.41%) [33]. The CHO content of the *M. pruriens* leaves (40.3%) was found to be higher than the (22. 36%) reported by [27] for the leaves of *Cassia siamea* and 38.24% reported by Iniaghe *et al*. [34] for leaves of some *Acalypha* species.

Copper is an essential trace element in the human body where it exists as an integral part of copper proteins (Ceruloplasmin) which is concerned with the release of iron from the cells into the plasma and is involved in energy metabolism [35]. The copper content of *Mucuna pruriens* leaves (23.3 mg/100g) was significantly higher than that of the seeds (11.99 mg/100) and was also higher than the copper content of *Vernonia amygdalina* leaves (2.32mg/ 100g) [36]. Copper equally plays a role in the production of key proteins in our body such as collagen and haemoglobin, which transport oxygen [37].

Zinc is an essential element in the nutrition of man where it functions as an integral part of numerous enzymes or as a stabilizer of the molecular structure of sub-cellular constituents and membrane [38]. Zinc participates in the synthesis and degradation of carbohydrate, lipids, proteins and nucleic acids and has been shown to play an essential role in polynucleotide transcription and translation and thus is involved the process of genetic expression [38]. The Zn content of *Mucuna pruriens* leaves (42.0 mg/100g) was higher than that of the seeds (30.33mg/100g) and also higher than 3.18 mg/100 g reported by Hassan and Umar [29] for *Mormordica balsamina* leaves. All these indicate that the leaves are therefore good sources of vegetables for human diet.

The calcium content of the seeds (14.33 mg/100g) was significantly higher than that of the leaves (7.83 mg/100g) as well as higher than the (2.62 mg/100g) calcium content of soy bean [26] and (1.18 mg/100g) reported for the seeds of *Arachis hypogaea* [33]. This shows that the seeds are a better source of calcium than the leaves. Calcium is good for growth and maintenance of bones, teeth and muscles [39, 40]. Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrates, proteins and fats [41]. Iron in *Mucuna pruriens* could help in boosting of the blood level in anaemic conditions. Rural dwellers who use the leaves to treat such conditions have claimed huge success and percentage iron content (1.07 mg/100g) tends to support their claim. Based on the above analysis, itching bean can be considered as a potential source of such minerals like Ca, Mg, Zn, Cu and P; because any diet that contains 2/3 of the RDA values is considered to be adequate [42].

The vitamin A content of the leaves was higher than that of the seeds .This is because leafy vegetables are mostly good sources of vitamin A. Vitamin A has multiple functions: it is important for growth and development, for the maintenance of the immune system and good vision [43]. This was different for other vitamins. Vit B_1 content of the seeds was higher than that of the leaves. Vit B_1 is involved in energy production for the electron transport chain, the citric acid cycle, as well as the catabolism of fatty acids [44]. Its deficiency causes ariboflavinosis and abnormal digestion. Vit B_2 is usually stored in the body as pyridoxal 5'-phosphate (PLP), which is the co-enzyme form of vitamin B_6. Pyridoxine is involved in the metabolism of amino acids and lipids) its deficiency may lead to microcytic anemia [45].

For vitamins B_6 and B_{12}, the content of the seeds was higher than those of the leaves. Vitamin B_6 is involved in the metabolism of amino acids and lipids. Deficiency of vitamin B_6 alone is uncommon because it usually occurs in association with a deficit in other B-complex vitamins. It results to a decreased level of plasma pyridoxal 5-phosphate (PLP) and urinary 4-pyridoxic acid. Infants are especially susceptible to insufficient intakes, which can lead to epileptic form of convulsion [46]. Cobalamin (Vitamin B_{12}) in the seeds of *Mucuna pruriens* was higher than that of the leaves showing the seeds are better source of the plant nutrients. Vitamin B_{12} is involved in the cellular metabolism of carbohydrates, proteins and lipids. It is essential in the production of blood cells in bone marrow, nerve sheaths and proteins. Deficiency results in a macrocytic anemia, elevated homocysteine, peripheral neuropathy, memory loss and other cognitive deficits [47].

The tannin content of the seeds (46.18mg/100g) was significantly higher than the leaves (15.10 mg/100g) tannin inhibits the activities of some digestive enzymes like

lipase, amylase and trpsin [22]. The phytate content of *Mucuna pruriens* leaves (3.66mg/100g) as shown in Table 4, is lower when compared to 8.2 mg/100 g reported by Hassan and Ngaski [27] for *Cassia siamea* leaves. The negative effect of phytate in nutrition is the chelating of certain essential elements such as Ca, Fe and Zn. This contributes to mineral deficiencies in people whose diets rely on some food rich in phytate for their mineral intake [48]. On the other hand, phytates are considered as phytonutrients providing an antioxidant effect and their mineral binding properties prevents colon cancer by reducing oxidative stress in the lumen of the intestinal tract [49].

Researchers believe that phytate found in legumes and grains is the major ingredient responsible for prevention of colon cancer and other cancers [50]. It has been shown that phytate is protective against Parkinson's disease *in vitro* [51]. The compound significantly decreased apoptotic cell death induced by 1-methyl-4-phenylpyridinim in a cell culture model. The hydrogen cyanide content of leaves (0.96mg/100g) was significantly higher than the seeds (0.31mg/g). The level of hydrogen cyanide in *M. pruriens* var. *pruriens* seeds (0.31mg/100) is negligible when compared with the lethal level of HCN (36mg/100g) in cassava [52], but similar to safe level of HCN present in *Phaseolus lunatus* [53].

The presence of certain anti-nutrients might not be an impediment in the safe use of this legume, as proper processing methods like (Soaking the bean followed by cooking so as to reduce /eliminate most of the anti-nutritional factor without affecting the protein and digestibility of the legume proteins) have been reported to detoxify the legume seeds to make them edible [54].

Soaking prior to boiling could reduce the concentrations of the tannins, hydrogen cyanide and phytate. Consequently, therefore soaking in water or periodic changing of the water during soaking, would give a more appreciable reduction in the phytochemical concentrations. This simply implies that the presence of anti-nutritional factors identified in the analysis should not pose a problem for utilization of the seeds in the fight against in malnutrition in the sub- Saharan Africa.

The finding that with the exception of cysteine; that the amino acid profile as well as the crude protein content of the seed was significantly higher than those of the leaves is quite interesting. This is because in the ethnobotanical use of the plant in the region the leaves are commonly utilized as haemoglobin booster for anemic patients. The seed is not accorded the same common usage apparently due to ignorance of its enormous potentials. A closer study of the amino acid profile of the seeds reveals that it contains the 9 indispensable amino acids- lysine, methionine, threonine, tryptophan, isoleucine, leucine, valine and phenylalanine as designated by FAO [55]. The indispensable amino acid profile of the seed proteins not only compared favourably with the pattern established by FAO/WHO [56] but surpassed this pattern with respect to three amino acids- isoleucine, lysine and histidine. Additionally the high lysine content of the seed implies that the seed can be used in the place of soya beans for the fortification of cereals, especially wheat, that are known to have lysine as their most limiting amino acid [57] and which constitutes a major part of daily food intake of people in the region.

The 36.48±1.18 crude protein content of the seed surpassed the crude protein range of 27.5-31.2% reported for 5 accessions of *M. pruriens* by Kalidass and Mohan [58] in Tamil Nadu, India. Also the amino acid profile for the 9 indispensable amino acids showed that surpassed these 5 accessions in India, with respect to Methionine, Isoleucine, Lysine and Histidine, but not for the other 5 indispensable amino acids. These are indicators of the existence of wide variations both in the crude protein and amino acid profile of this neglected crop, which can be harnessed for global improvement of the crop. The observation of numerous root nodules on the root hairs of this plant (not part of this report) is an indication of its ability to habour nitrogen fixing bacteria. This could constitute a major reason for its richness in crude protein, amino acids and other nutrients; which endows it with the potentials to play multiple beneficial roles for the well being of man and animal in the region. This is in line with the reports of Fathima and Mohan [59], Franche *et al.* [60], Ejelonu *et al.* [61], Leelambika *et al.* [62], Maobe *et al.* [63] and Lukong *et al.* [64].

Addressing the anti-nutritional constituents of this pulse needs urgent research attention for better exploitation of the potentials of this crop. Although traditional methods of removing these factors from the seeds had been developed by the people, it is time consuming and often highly fuel inefficient. According to Kalidass and Mohan [58] to make this less-known legume palatable, tribal people in Western Ghats, Tamil Nadu, India, follow a special processing method of continuous boiling and draining for about eight times until the boiled water changes from black to milky white. On the short term, this method could be adopted by the people of south Eastern Nigeria for the incorporation of into the menu of the people. On the long term, an intensive

collection and screening of local germplasm is needed with a view to identifying cultivars with low anti-nutritional factors that could be used in the improvement of this highly nutritive cultivar. Introduction of improved germplasm from other *M. pruriens* growing parts of the world, could accelerate the speed of incorporation of low or zero anti-nutritive genes into the local varieties in south Eastern Nigeria to produce elite *Mucuna pruriens* cultivars.

CONCLUSION

The results of this present study has shown that both the leaves and seeds of *Mucuna pruriens* var are very rich in minerals nutrients, vitamins, amino acids and proteins, with the nutrient content of the seeds being significantly higher than that of the leaves. With the lysine content of the seeds being higher than the FAO/WHO 1991 requirement pattern, the seeds should be promptly included into the menu of the people, through intensive education. The anti-nutritive factors could be easily removed on the short term following the methods adopted by the Indians. On the long run, screening of local germplasm and introduction of cultivars with low anti-nutritive factors could assist in developing elite local cultivars that can adequately play a crucial role in bridging the protein gap in the menu of the people.

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