



Original Research Article

Nutritional and toxicological studies on wild cowpea (dekindtiana var. Pubescen)

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The nutritional values of the wild cowpea (*Dekindtiana* var. *pubescen*) collected from three areas in Sokoto, Nigeria, were determined by standard procedures as follows, $3.3 \pm 0.08\%$ ash, $4.7 \pm 0.08\%$ moisture, $55.8 \pm 0.08\%$ crude protein, $3.2 \pm 0.11\%$ crude lipid and 23.0% carbohydrate. Others include $10.0 \pm 0.06\%$ fibre and 344Kcal gross energy respectively. The anti-nutritional factors composition was found to be $9.55 \pm 0.01\text{mg}$ tannin, $70.5 \pm 0.08\text{mg}$ phytate, $188.0 \pm 0.83\text{mg}$ total oxalate and $99.5 \pm 0.11\text{mg}$ soluble oxalate. The toxicity effects of the wild cowpea reflected on the experimental animals via weights declined from 226.74g to 118.40g for group A and from 257.98g to 99.40g respectively. More ever, the enzymes activities measured both in the serum and liver homogenate were also declined. In this study, the wild cowpea was identified as a good source of dietary protein despite of its anti – nutritional toxic agents which might likely cause unacceptable health problems.

Key words: Wild cowpea flour, proximate analysis, anti – nutritional factors, liver homogenate, serum

INTRODUCTION

The wild cowpea samples that belong to *vigna unguiculata* subsp. *Dekindtiana* var. *dekindtiana* are almost have growth habit and leaf shape to local cowpea varieties grown by the farmers, except the pod shape in the wild type which is tiny, black at maturity and held in upright position with faint leaf marks (Padulosi, 1990)

The perennial wild *vigna unguiculata* has 2.5m long creeping stems, distinctive hair, large stipules and deep purple flowers. The wild type is typical to *vigna unguiculata* subsp. *Dekindtiana* var. *pubescens*. It also has long and narrow leaves, similar to those of *vigna unguiculata* subsp. *Stenophylla*, available in southern Africa.

The wild cowpea *V. unguiculata* var *spontanea* is not for human consumption neither for animals feeds. In Malalani an area of wild population in coastal Kenya, after each mechanical clearing of the roadsides, the wild population increased. During annual seed supplementation trial in Muhaka field station, Kenya, the ploughed plots had wild cowpea plants more than the undisturbed plots (OECD working group, 2015 cited R.S. Pasquet, personal

communication). The wild cowpeas are available in natural ecosystems from eastern Cameroon, Uganda, and western Ethiopian lowlands and found only in disturbed places (fields, field margins, roadsides and fallows). Others include Burkina Faso, western Niger and northern Ghana.

Wild cowpea and its relatives are not for addressing significant weed problem in sub – Saharan Africa (OECD working group, 2015 cited Huesing et al., 2011).

The cultivated cowpea and wild type, locally known as “Waken Gizo” are both in the same genus, *vigna*, species *unguiculata* and subsp *Dekindtiana*. They differ from each other only in variety. The wild type is var. *pubescen* while the cultivated cowpea derived from var. *Dekindtiana* (Padulosi and Ng, 1990).

Cowpea is an annual herbaceous warm – season plant that has similar appearance to common bean except that the leaves are all darker green, shinier and less pubescent. Generally, cowpea are more robust in appearance compare to common beans and also has better developed root system, thicker stems and branches. The growth habit of

the cowpea can be erect, semi erect, prostrate (trailing), or climbing depending mostly on genotype but photoperiod and growing conditions can also affect plant stature (Michael et al., 2007).

Cowpea (*Vigna unguiculata* L. Walp) ($2n = 2x = 22$) is a phaseoleae tribe member of the Leguminosae family. Phaseoleae members mostly are economically important warm season grain and oil seed legumes, such as soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vigna radiata*).

In tropical Africa, Asia, North and South America, cowpea is grown mainly as a grain but few as a vegetable and fodder crop. It is an important food source being the major dietary protein source for over 200 million people in sub-Saharan Africa and one of the top ten fresh vegetables in Republic of China.

In English-speaking countries of Africa known as cowpea whereas Francophone regions of Africa named it as "niebe. Other local names include "seub" and "niao" in Senegal, "luba hilu" in Sudan and "wake" or bean in Nigeria, the giant of Africa. In United States,, referred as black eye beans, black eye peas, crowder peas and southern peas. In Indian subcontinent known as "lobia" and in Brazil called "caupi." In China, known as "long bean" or asparagus bean.

Cultivated cowpeas broadly classified into five cultivar groups based mainly on pod, seed and ovule characteristic (OECD working group, 2015 cited Pasquet, 1999, 1998). The *Vigna unguiculata* species are recently classified into eleven subspecies but ten of them are perennial and one "cowpea" is annual (OECD working group, 2015 cited Padulosi, 1993, Padulosi and Ng, 1997, Pasquet, 1997, 1993_a, 1993_b).

The cowpea dry grain is highly nutritious with about 15.06 – 38.5% dietary protein (Carlos et al., 2017 cited Ravelombola et al., 2016). But a study was done on five varieties of cowpea consumed in Samaru, Nigeria, reported crude protein content of $19.84 \pm 0.18\%$, crude lipid content of $3.46 \pm 0.05\%$, carbohydrate content of 60.57%, crude fibre content of 14.17% and moisture content of 5.08% respectively (Inobeme et al., 2014).

The cowpea grain nutritional profile is similar to that of other pulses. It has low fat content and a total protein content of two to four times greater than cereal and tuber crops. Its protein is rich in essential amino acids such as lysine and tryptophan compared to cereal grains but deficient in methionine and cysteine when compared animal proteins. The critical role of the cowpea in the lives of people in Africa and some parts of the developing world, as a major source of dietary protein that nutritionally complements staple low protein cereal and tuber crops, is over emphasized. In addition, it is valuable and dependable commodity, especially to farmers and traders because they earned much from it (Michael et al., 2007 cited Singh, 2002, Langyintuo et al., 2003).

About 4.5 million metric tonnes of cowpea produced worldwide on 12 to 14 million hectares of land (Carlos et al., 2017 cited Boukar et al., 2016). Globally, Nigeria and Niger are the cowpea leading producers while countries

like the United States, India, Brazil and Sri Lanka produced appreciable amount of the crop. Others include Myanmar, Australia, and Bosnia as well as Herzegovina respectively (Carlos et al., 2017 cited Pereira et al., 2001).

In Ghana, farmers produced cowpea purposely for livelihood. In rural areas cowpea is a source of employment for farmers while in urban areas cowpea traders (Carlos et al., 2017 cited Langyintuo et al., 2003). The cowpea fresh leaves, fresh pods and dry seeds are edible via various dietary combinations in Ghana. Likewise, gari and fried ripened plantain are eaten with boiled dry grains of cowpea. On the other hand, its flour (cowpea) can also be into dough and fried in cooking oil to form a cake (kose), an edible food in Ghana.

Wild species had higher protein content but lower protein digestibility, while cowpea varieties had lower protein content but higher protein digestibility (Markoni et al., 1990). Nevertheless, the available protein in wild species was slightly higher than that of the cultivated species. The differences in digestibility may be attributed either to factors that are intrinsic to the protein themselves or to other substances present such as tannins, phytates, fibre or protease inhibitors, that may interact with proteins (Markoni et al., 1990). Wild species are having higher inhibitors than cultivated cowpea (Carnovale, 1990).

MATERIAL AND METHODS

Chemicals and Reagents

Chemicals used in this study were analytical grade reagents

- British Drug House (BDH) Ltd, Poole, Dorset. U.K.: Ammonium fluoride, Ammonium hydroxide, Ammonium molybdate – vanadate, Calcium chloride, Concentrated ammonium solution, Concentrated hydrochloric acid, Concentrated sulphuric acid, Dithiocarbamate, Dithizone, Ethylene diamine tetra acetic acid (EDTA), Ferric ammonium citrate, Methyl red indicator, Murexide powder, Perchloric acid, 1, 10 – Phenanthroline-stannous chloride, Sodium hydroxide pellets, Sodium cyanate, Sodium diethyl reagent.
- Mayer and Baker Ltd, Dagenham, England: DL – Alanine, DL – Aspartate, Diethanol amine buffer, 2, 4, - dinitrophenyl – hydrazine, Ferric chloride, Crystals magnesium chloride, P – nitro phenyl phosphate, Oxoglutarate, Petroleum ether, Phosphate buffer 7.4, Tannic acid.
- Hopkins and William, England: Boric acid, Disodium hydrogen orthophosphate, Potassium dihydrogen orthophosphate.
- Ben Meadows Company, England: Hydroquinone, Sodium acetate.
- Shermud Sussex, England: Anhydrous sodium carbonate.
- East Angalia Chemicals: Potassium permanganate.

Equipments

Kjeldahl flask (Pyrex U. S. A), Soxhlet (Pyrex U. S. A), Weighing balance (Mettlet pm 16 -K and Brain weigh), pH meter (Pierron), Water bath (Gallen Kamp), Muffle furnace (Gallen Kamp), Spectronic 21(Bausch and Lump), Centrifuge (Shermond), Heating mantle (B and T Germany), Condenser (Pyrex U. S. A), Oven electric (unspecified source), Test - tube racks (unspecified source), what man No 42 filter paper, Crucible, Spatula and Mortar and Pestle

Preparations of Reagents

Kjeldahl Catalyst

Potassium sulphate 100g, 20g of copper sulphate and 2g of selenium oxide were finally ground together until a homogenous powder was obtained. The mixture was kept in a bottle container. (Use for crude protein determination).

Mixed indicator solution

Bromocressol green 0.1g and 0.1g of methyl red were weighed into separate 100ml volumetric flasks and dissolved into 100ml of ethanol. About 75ml of the bromocressol green solution was added to 25ml of methyl red solution in a 250ml volumetric flask, the resulting mixture was thoroughly shaken. Boric acid 8g was dissolved in minimum amount of boiling water and on cooling, 25ml of the indicator solution was added to the boric acid solution in a litre volumetric flask and then diluted to the mark with distilled water. (Use for crude protein determination).

Iron Reagent

Iron (iii) ammonium citrate 1g was dissolved into 80ml of distilled water in 100ml volumetric flask and made up to the volume with distilled water. (Used for tannin determination).

Phosphate buffer (pH 8.5)

Sodium hydrogen orthophosphate 9.5g was placed into 1000ml volumetric flask and dissolved with 500ml of distilled water. The pH was adjusted to 8.5 using potassium dihydrogen orthophosphate buffer pH8.5 and made up to one litre. (Use for tannin determination).

Tannin solution (stock)

Tannin acid 50mg was placed into one litre volumetric flask and dissolved with 500ml of distilled water, then marked to the volume. (Use for tannin determination)

Boric acid (2%)

Boric acid 2g was dissolved in 50ml of distilled water in

100ml volumetric flask and made up to 100ml with distilled water. (Use for crude protein determination)

Calcium chloride (5%)

Calcium chloride 50g was dissolved in 80ml of distilled water in 100ml volumetric flask and the volume was made up to 100ml with distilled water. (Use for oxalates determination)

Sulphuric acid (25%)

Concentrated sulphuric acid 13ml was diluted to 52ml with distilled water (Use for oxalates determination)

Hydrochloric acid

Hydrochloric acid 12.9ml was diluted to 25ml with distilled water (Use for oxalates determination)

Potassium permanganate (0.25M)

Potassium permanganate 39.5g was dissolved into 800ml of distilled water in one litre volumetric flask and made up to the volume with distilled water. (Use for oxalates determination)

Ammonium Molybdate - Vanadate reagent

Ammonium molybdate 45g was dissolved into 500ml of distilled water then made up to 800ml with distilled water. Ammonium vanadate 2.5g was dissolved into 400ml of boiling distilled water and then made up to 600ml with boiling distilled water, left to be cooled and then mixed to solution (i). (Use for phytic acid determination)

Hydrochloric acid (0.5N)

Hydrochloric acid 4.3ml was diluted to 100ml with distilled water (Use for phytic acid determination)

Sodium hydroxide (1.0N)

Sodium hydroxide 4.0g was dissolved into 50ml of distilled water in 100ml volumetric flask and made up to the volume with distilled water (Use for phytic acid determination)

Hydrochloric acid (0.17N)

Hydrochloric acid 1.4ml was diluted to 10ml with distilled water (Use for phytic acid determination)

Sodium hydroxide (0.5N)

Sodium hydroxide 2.0g was dissolved into 50ml of distilled water in 100ml volumetric flask and made up to 100ml with distilled water (Use for phytic acid determination)

Dilute dithizone solution

Dithizone solution one volume was diluted with four volume of tetra chloromethane (Use for zinc determination)

Carbamate solution

Na diethyl dithiocarbamate 1.25g was placed into one litre volumetric flask and dissolved into 50ml of distilled water and then the volume was made up to one litre with distilled water (Use for zinc determination).

Dilute ammonium hydroxide

1N NH₂OH 20ml was diluted into two litres with distilled water (Use for zinc determination).

Substrate

One vial of substrate was reconstituted with the appropriate volume of buffer:- 10ml of the 10x 10ml kit (AP 307). It was stable for 30 days at +2 to +8°C (Use for alanine transaminase, aspartate transaminase and alkaline phosphatase determinations)

Biuret reagent

- Copper sulphate 17.3g was dissolved into 100ml of hot distilled water.
- Sodium citrate 17.3g and anhydrous sodium carbonate 100g were dissolved into 800ml of distilled water by heating. After cooling, then a and b mixed while stirring and then diluted to 1000ml (Use for total protein and albumin determinations).

Protein standard solution

Ready for use (Use for total protein determination)

Citrate buffer

Citric acid 12.4g and trisodium citrate 12.0g were dissolved into 1000ml of distilled water (Use for albumin determination)

Working bromocresol green solution

One volume of stock bromocresol green solution was mixed with three volume of citrate buffer (Use for albumin determination)

Albumin standard solution

Ready for use (Use for albumin determination).

Sample preparation and analysis

Wild cowpea (*Dekindtiana* var. *Pubescen*) samples were

collected from the residential gardens around the Federal Housing Authority (FHA) Estate, Runjin Sambo and the backyard bushes around residential quarters of Mabera and Mainanata, all within the Sokoto city around the month of November, 1998. While the experimental guinea pigs were procured from Gusau central market, Zamfara state and acclimatized for three weeks. They were fed with chicken feed bought from Sokoto central market.

The wild cowpea seeds were processed into flour using mortar and pestle to mill and stored for further utilization.

Proximate Analyses (AOAC, 1990)

The proximate analysis on the sample was conducted based on the procedures of Association of Official Analytical Chemist (AOAC, 1990).

Ash Determinations (AOAC, 1990)**Procedure**

A crucible was washed with distilled water then dried in an oven at 180°C for 30 minutes and cooled in desiccator and weighed (W_1). Three gram (3g) of the sample was then added to the crucible and weighed (W_2). After weighing, the crucible then heated at 600°C in a muffle furnace for 5 hours, cooled in desiccator and weighed (W_3).

Calculation:

Ash (%) = Weight of ash/Weight of sample x 100

$$\frac{W_3 - W_1}{W_2 - W_1} \times 100$$

Moisture Determinations (AOAC, 1990)**Procedure**

A crucible was washed with distilled water then dried in an oven at 180°C for 30 minutes and cooled in desiccator and weighed (W_1). Three gram (3g) of the sample was then added to the crucible and weighed (W_2). After weighing, the crucible then heated at 105°C in a muffle furnace for 5 hours, cooled in desiccator and weighed (W_3). The process was repeated until a constant weight was reached

Calculation:

Moisture (%) = Loss in weight / weight of sample x 100

$$\frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Lipid Determinations (AOAC, 1990)**Procedure**

Five gram (5g) of sample(wild cowpea flour) wrapped in what man No. 42 filter paper then placed into a barrel of the extractor. Secondly, the round bottom flask of the extractor was filled with petroleum ether up to $\frac{3}{4}$ of its volume, and

then heated by using heating mantle at 5°C for 6 hours. During the heating the petroleum ether containing the extracted fat was evaporated but the residue (crude lipid) was left in the flask and weighed.

Calculation:

Lipid (%) = Weight gain in flask / weight of sample x 100

Nitrogen and Crude protein Determinations (AOAC, 1990)

The method has been widely used in food and feeding stuff analyses and several modifications have accumulated.

The nitrogen content of proteins varied from 15 - 18%, if an average value of 16% is assumed, crude protein is estimated by nitrogen x 6.25. This value, 6.25, is the common factor used to convert the measured nitrogen to crude protein as many proteins contain 16% nitrogen.

The procedure involves three stages as follows:

- A. Digestion
- B. Distillation
- C. Titration

A. Digestion

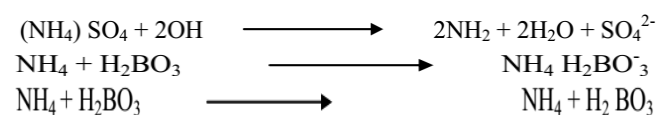
Two gram (2.0g) of sample was placed into the kjeldahl flask then added a tablet of kjeldahl catalyst and 2g of copper sulphate (CuSO₄. 5H₂O) and 20ml of concentrated sulphuric acid. The flask was placed on heating mantle in fume chamber. Heating was started gently until black fumes cease, then vigorous heating continued until a light green liquid was observed. So, to ensure complete digestion further heating continue for 2 hours then the flask allowed cooling at room temperature. After cooling, the content and several washing were transferred into 50ml volumetric flask and marked up the volume, then shaken thoroughly.

Organic nitrogen + concentrated H₂SO₄ $\xrightarrow{\text{reduction}}$ (NH₄)₂SO₄

Carbon $\xrightarrow{\text{oxidation}}$ CO₂

B. Distillation

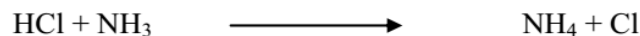
The round bottom flask was heated into the micro kjeldahl apparatus for 10 minutes to allow the steam in. 20ml of 2% boric acid and mixed indicator were added at the receiving end of the condenser, in such a way that the tip was below the boric acid and mixed indicator. Then 20ml of 40% NaOH solution was added to the digest (20ml) which was steam distilled into the conical flask until the boric acid solution turned colourless.



C. Titration

The conical flask was removed and cooled, and then the

content was titrated with 0.1ml of HCl to colour.



Calculation:

1 mole of HCl neutralizes 1mole of NH₃

Nitrogen (%) = TV x N x 0.014 x vol / weight of sample x aliquot x 100

Where TV = Titre value

N = Normality

Carbohydrates Determinations (AOAC, 1990)

Procedure

Carbohydrates were determined "by difference" i.e. by deducting the sum of all other constituents from the total weight.

Therefore, Ash + moisture + crude protein + lipid + crude fibre (3.3% + 4.7% + 55.8% + 3.2% + 10.0% = 77.0%)

Carbohydrate = 100% - 77% = 23%

Fibre Determinations (AOAC, 1990)

Procedure

Two gram (2.0g) of sample was defatted with diethyl ether for 8 hours and boiled under reflux for 30 minutes with 200ml of 1.25% of H₂SO₄. Then filtered and washed with boiling water until washings were no longer acidic. The residue was boiled in a round bottom flask with 200ml of 1.25% NaOH for 30 minutes and filtered through previous weighed crucible, dried in an oven at 100°C and cooled in a desiccator, weighed, (C₂) and incinerated in a muffle furnace at 600°C for 3 hours, cooled in a desiccator and weighed (C₃)

Calculation:

Weight of fibre = C₂ - C₃

% fibre = C₂ - C₃ / weight of sample x 100

Calculation of Gross Energy (AOAC, 1990)

The energy contents of the seeds were determined by multiplying the crude protein, lipid, and total carbohydrates by the factors 4kcal, 9kcal and 4kcal respectively.

Iron Determinations (AOAC, 1990)

Two gram (2g) of sample was digested with 20ml of concentrated H₂SO₄ and then pipetted 5ml of aliquot into a 50ml volumetric flask. 1ml of 2M sodium acetate, 4ml of hydroquinone and 4ml of 1, 10 - phenanthroline were all added and marked (50ml) with distilled water, then mixed well and allowed to stand for one hour to complete the reduction of the iron.

The standard was also determined by the use of similar aliquot containing a few drops of bromophenol blue, 1ml of 2M sodium acetate was added and turned pH to 3.5 ± 1.0.

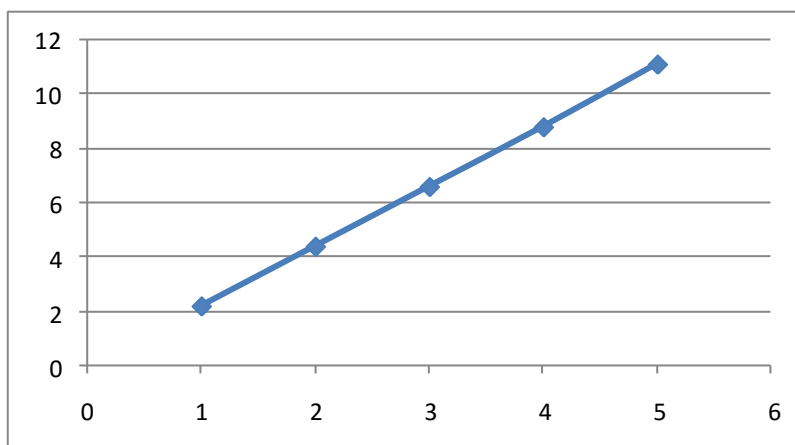


Figure 1: A graph of absorbance versus iron standard

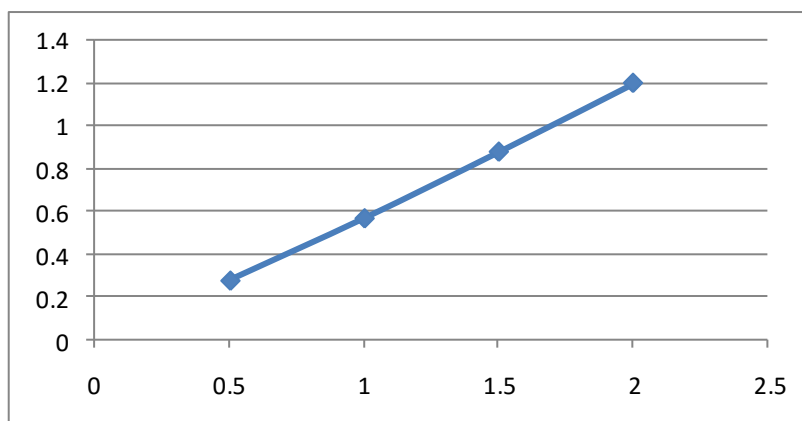


Figure 2: A graph of absorbance versus zinc standard

The colour intensities of these solutions were allowed to be developed and exactly 6 minutes after that, the concentrations of iron in the solution were determined spectrophotometrically at 515nm from 5ml of each of the solution (Figure 1).

Calculation:

Iron content = Absorbance x dilution factor

Zinc Determinations (AOAC, 1990)

Preparation of standard curve

Into 200ml volumetric flask placed 0, 2, 4, 6, 8, 10, 12, and 14ml of zinc working standard solution. To each flask added 2 drops of methyl solution, neutralized with 1N NH_4OH , followed by 3.2ml of HCl, and diluted up to mark with distilled water. Then pipetted 0.5 to 2.0ml aliquot of each of these solutions into amber glass separators. Ammonium citrate solution 40ml was added then 5ml of carbamate solution, 35ml working solution and dithizone reagent through burette with vigorous shaking for one minute. Left to stand and layers separated. The aqueous

layers were drawn through fine tip glass tube connected to aspirator with rubber tubing. 50ml of 0.01N NH_4OH was added and shaken for 30 second to remove the excess dithizone from CCl_4 . The absorbance of each solution was measured at 535nm and values plotted against concentration.

Procedure

Dried funnel stem with pipe stem cleaner was flashed out with 2ml of zinc dithizone solution. Adequate portion of remaining solution was added in 25ml Erlenmeyer flask. Absorbance was measured against CCl_4 spectrophotometrically at 535nm.(Figure 2)

Calcium Determinations (AOAC, 1990)

Procedure

Two gram (2.0g) of sample was digested with 50ml of concentrated HCl and 25ml of the aliquot was pipetted into 100ml volumetric flask, and then diluted to 50ml with

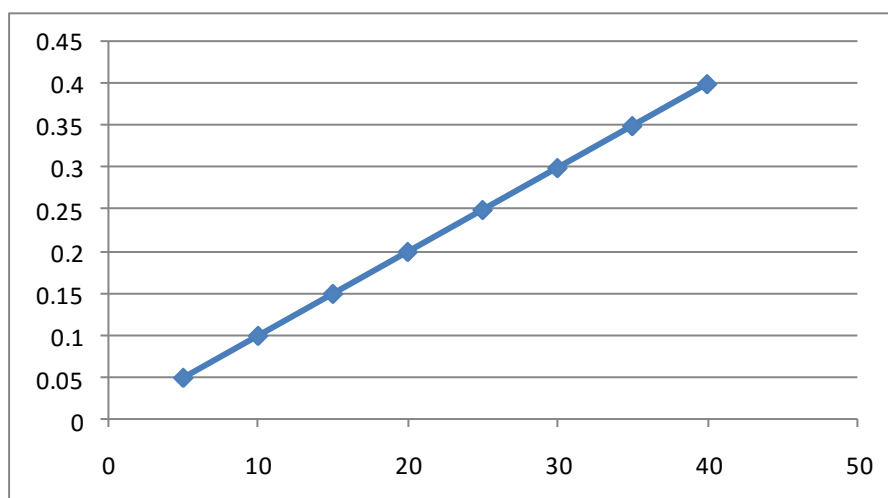


Figure 3: A graph of absorbance versus tannin acid standard

distilled water. 1ml of 4N NaOH was added, 1ml of NaCN, and few murexide powder indicator was added too. The mixture was titrated against EDTA.

Calculation:

Calcium = $TV \times N \text{ EDTA} \times 500 / \text{Initial weight of sample}$

Where: TV = Titre value

N = Normality of EDTA

Tannin Determination

Preparation of standard curve

In 100ml Erlenmeyer flasks 5 to 14ml of aliquots of tannin standard solution were added and each flask made up to 50ml with distilled water then followed by 5ml of buffer and 5ml of iron reagent within 10 minutes. Mixed and left to stand for 30 minutes. The absorbance of each flask was measured at 550nm.

For blank, in 100ml volumetric flask 25ml standard solution, 25ml distilled water and 5ml buffer were added and used for zeroing absorbance (Figure 3).

Procedure

50ml of supernatant was pipetted into 100ml Erlenmeyer flask, followed by 5ml of buffer and 5ml of iron reagent. The mixture was left to stand for 30 minutes and absorbance was read at 550nm (Agunbiade and Olanlokun, 2006 cited Czernianwski method, 1958)

Phytic acids Determination

Preparation of standard curve

A stock solution of potassium dihydrogen phosphate was prepared by dissolving 0.2197g of its salt into 800ml of

distilled water and added 10ml of ammonium molybdate – vanadate reagent then made up the volume up to 1 litre with distilled water. Serial dilutions were made to give concentrations of 0 – 1ppm and the absorbances were read at 470nm (Figure 4).

Procedure

5ml of aliquot was pipetted and mixed with 10ml of ammonium molybdate – vanadate reagent. Then used the phosphorus determination procedure. The phosphate content represented the phytic phosphate (Agunbiade and Olanlokun, 2006 cited McCance and Widdonson, 1975).

Oxalate Determination

Procedure

- In a beaker 2.5g of sample was placed
- 190ml of distilled water and 10ml of 6N HCl were added and then the mixture was digested in a water bath for 4 hours at 70°C.
- The digested sample was filtered and diluted with 250ml of distilled water using 250ml volumetric flask.
- 50ml of aliquot was placed into a beaker and evaporated to about 25ml then filtered off the brown precipitate and washed with hot distilled water.
- The filtrate was mixed with washings then 2 drops of methyl red indicator added and treated with concentrated ammonia until the pink colour of methyl red change to faint yellow.
- The solution was heated in a water bath at 90°C, and then 10ml of 5% calcium chloride solution was added and left to stand overnight, then centrifuged.

In a beaker the precipitate was washed with hot 25% sulphuric acid and diluted to 25ml with distilled

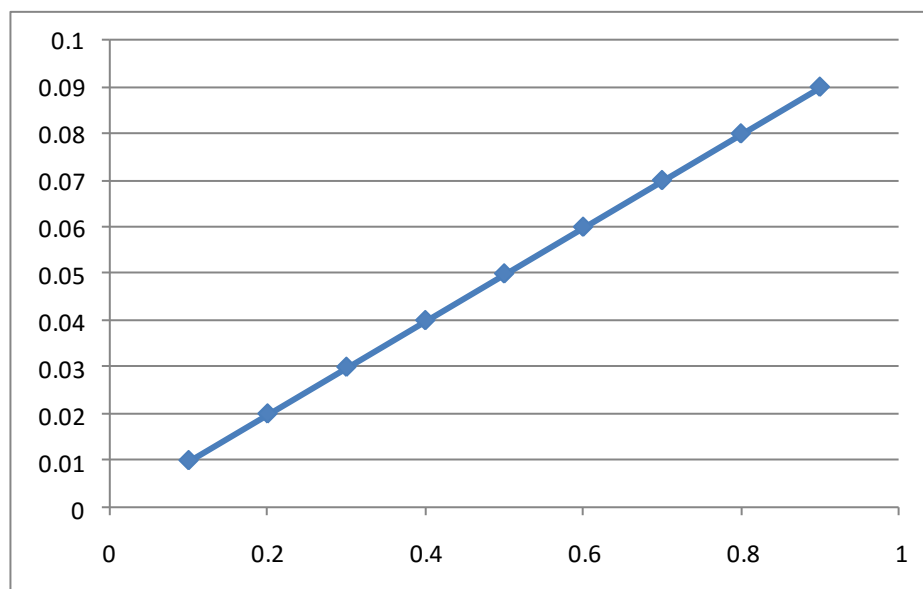


Figure 4: A graph of absorbance versus phytic acid standard

water, warmed in a water bath at 90°C and titrated with 0.25M KMnO_4 (Musa and Ogbadoyi, 2014 cited Oke, 1966)

Soluble Oxalate Determination

Procedure

- In 500ml volumetric flask 2.0g of sample was placed then 200ml of distilled water added and digested in a water bath at 90°C for 4 hours.
- The digested sample was filtered and diluted with 250ml of distilled water using 250ml volumetric flask.
- 50ml of aliquot was placed into a beaker and evaporated to about 25ml then filtered off the brown precipitate and washed with hot distilled water.
- The filtrate was mixed with washings then 2 drops of methyl red indicator added and treated with concentrated ammonia until the pink colour of methyl red change to faint yellow.
- The solution was heated in a water bath at 90°C, and then 10ml of 5% calcium chloride solution was added and left to stand overnight, then centrifuged.
- In a beaker the precipitate was washed with hot 25% sulphuric acid and diluted to 25ml with distilled water, warmed in a water bath at 90°C and titrated with 0.25M KMnO_4 . (Musa and Ogbadoyi, 2014 cited Oke, 1966)

Treatments of Animals

Fifteen (15) guinea pigs weighing between 222.26 to 257.98g were separated into 3 groups of 5 guinea pigs each (control, group A and group B). The animals were acclimatized for three weeks inside the laboratory. Control

group was supplied with chicken pea while guinea pigs in other groups (A and B) were fed with 15% of crushed wild cowpea and 85% of chicken pea (mixed) and group B were fed with 30% crushed cowpea and 70% of chicken pea (mixed). The treatment lasted for three weeks.

After three weeks of the treatment, the guinea pigs were slaughtered and their blood collected into a separate centrifuged test tubes and serum separated by centrifuging at 4000RPM.

Alanine Transaminase Determinations in Serum (Reitman and Frankel Method)

Preparation of Reagent blank

In a test tube 1.0ml of buffer was pipetted and added 0.2ml of distilled water, mixed, then incubated at 37°C for 30 minutes. After incubation, 1.0ml of 2, 4 - Dinitrophenyl - hydrazine added then mixed and allowed to stand at 25°C for 20 minutes then followed by addition of 10.0ml of sodium hydroxide.

Procedure

In three separated test tubes 0.2ml of serum from each three serum test tube was pipetted then added 1.0ml of buffer to each separated test tube mixed and incubated at 37°C for 30 minutes. After incubation, 1.0ml of 2, 4 - dinitrophenyl hydrazine was pipetted into each separated test tube, mixed, then allowed to stand at 25°C for 20 minutes and pipetted 10.0ml of sodium hydroxide to each separated test tube and then mixed. The absorbances were measured against the reagent blank after 5 minutes at

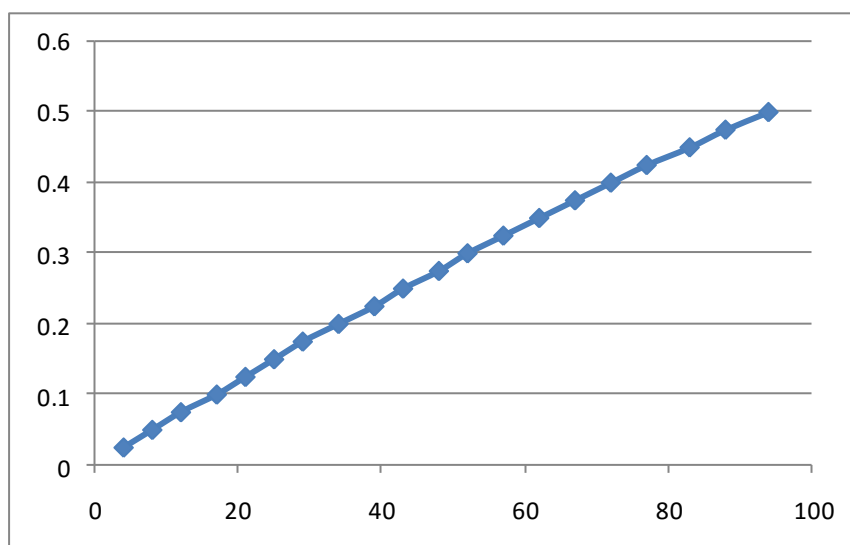


Figure 5: Absorbance versus enzyme standard

540nm.(Figure 5)

Aspartate Transaminase Determinations in Serum (Reitman and Frankel Method)

Preparation of Reagent blank

0.5ml of buffer was pipetted into a test tube and added 0.1ml of distilled water, mixed, then incubated at 37°C for minutes. After incubation, 0.5ml of 2, 4 - dinitrophenyl hydrazine was added, mixed, then allowed to stand at 25°C for 20 minutes and added 5ml of sodium hydroxide then mixed.

Procedure

In each three separated test tube 0.1ml of serum was pipetted into each three separated test tube and added 0.5ml of buffer to each test tube, mixed, then incubated at 37°C for 30 minutes. After incubation, 0.5ml of 2, 4 - dinitrophenyl hydrazine added to each test tube, mixed, then allowed to stand at 25°C for 30 minutes and added 5ml of sodium hydroxide to each test tube, and mixed. The absorbances were measured against the reagent blank after 5 minutes at 540nm.

Alkaline Phosphatase Determinations in Serum (Rec. CscC (DCKC Method)

In each three separated test tube 0.02ml of serum was pipetted into each of the three separated test tube and added 1.0ml of reagent at room temperature to each test tube and mixed. The absorbances were read at 405nm and started timer simultaneously. The absorbances were read against the reagent blank after 1, 2, and 3 minutes.

Total Protein Determination in Serum (Biuret method)

Preparation of control

In a test tube 6.0ml of working biuret solution was pipetted and added 0.1ml of distilled water, mixed and incubated at 37°C for 10 minutes. The absorbance was read at 540nm using distilled water as blank.

Preparation of standard

6.0ml of working biuret solution was pipetted into test tube and added 0.1ml of standard solution, mixed and incubated at 37°C for 20 minutes. The absorbance was read at 540nm using distilled water to zeroing the spectrophotometry.

Procedure

In each three separated test tube 6.0ml of working biuret solution was pipetted and added 0.1ml of serum from each separated test tube to each three separated test tube, mixed and incubated at 37°C for 20 minutes. The absorbances were read at 540nm using distilled water as blank.

Calculation:

Total protein (g/dl) = T/S x conc. of standard

Where T = Test

S = Standard

Albumin Determination in Serum (Bromocressol green method)

Preparation of blank

8.0ml of working bromocressol green was pipetted into test

Table 1. Proximate chemical composition of wild cowpea

Component	Wild cowpea %/100g	Cowpea beans (Bosso) % / 100g	Non – defatted flour of brebra seed % / 100g
Moisture	4.7 ± 0.07	5.20 ± 0.95	4.24 ± 0.04
Ash	3.3 ± 0.08	3.80 ± 0.36	3.24 ± 0.0
Crude Protein	55.8 ± 0.08	23.30 ± 0.50	29.7 ± 0.23
Crude Lipid	3.3 ± 0.11	3.90 ± 0.35	48.5 ± 0.99
Carbohydrate (by difference)	23.0 ± 0.13	61.97 ± 0.95	14.32 ± 0.2
Crude Fibre	10.0 ± 0.06	2.80 ± 0.26	2.41 ± 0.12
Gross energy	344.0 Kcal/g	376.18Kcal/g	612.58 ± 0.01

tube and added 0.05ml of distilled water, mixed and incubated at 37°C for 10 minutes.

Preparation of standard

In a test tube 8.0ml of working bromocressol green was pipetted then followed by 0.01ml of standard solution, mixed and incubated at 37°C for 10 minutes. The absorbances were read at 628nm using blank to zeroing the spectrophotometry.

Procedure

Working bromocressol green 8.0ml was pipetted into each of the three separated test tube and added 0.05ml of serum from each of the three separated test tube, mixed and incubated at 37°C for 10 minutes. The absorbances were read at 628nm against blank.

Calculation:

Albumin (g/dl) = T/S x conc. of standard

Where T = Test

S = Standard

Preparation of Liver Homogenate

All the guinea pigs belonged to each group were dissected and their livers removed. The removed livers were placed into three separated beakers then washed them with normal saline and weighed each. After weighing each, a small portion from each beaker was removed and homogenized by using mortar and pestle. The supernatant was collected from beaker after centrifugation.

RESULTS AND DISCUSSIONS

DISCUSSIONS

Moisture content

In this study, the wild cowpea had lower moisture value of 4.7% when compared with that of cowpea beans called Bosso (5.20%) (Inobeme et al., 2014) and other legumes moisture value ranging between 5.0% and 11% respectively (Berhanu et al., 2014, Aremu et al., 2006, Lge et

al., 1984). The presence of moisture content of a sample quantifies the water content of that sample, but varies from small percentage in dry sample to a large percentage in the moist sample (Table 1).

Ash content

The ash content of the wild cowpea under study was 3.3%, lower when compared to that of Bosso cowpea beans which had the ash value of 3.80 ± 0.36 (Inobeme et al., 2014). But its content was within the recommendation of Pomeranz and Clifto, 1981, which stated that the ash contents of seeds and tuber should be in the range between 1.5 – 3.5%, to be suitable for animal's feeds. Because the ash content of the investigated cowpea falls within this range, it can be recommended for human consumption and animal feeds. (Berhanu and Amare, 2014). On the other hand, the ash content of non-defatted flour of brebra seed (3.24%) when compared had lower percentage to the investigated wild cowpea. The presence of low percentage of ash content in a sample may probably suggested low minerals content of that sample. (Brosio et al., 1990).

Protein content

The under study wild cowpea investigated found to have high amount of crude protein (55.8%) when compared to Bosso cowpea beans (23.30%) and non – defatted flour of brebra seed (29.7%) (Inobeme et al., 2014; Berhanu and Amare, 2014). The protein quantity may likely make it to serve as a major source of dietary protein that nutritionally complements stable low protein food. Markoni et al., 1990, stated that the wild species had higher protein content but lower protein digestibility while cowpea varieties had lower protein content but higher protein digestibility. (Markoni et al., 1990). The differences in digestibility may be attributed either to factors that are intrinsic to the proteins themselves or other substances present, such as tannins, phatates, fibre or protease inhibitors that may interact with protein.

Lipid content

The lipid content of the wild cowpea (3.2%) when compared to that of Bosso cowpea beans (3.90%) and non –

Table 2: Minerals composition of wild cowpea

Mineral	Wild cowpea mg/100g	Ripened beans mg/100g	Cashew nut Kerrel mg/100g	Conophor nut mg/100g
Iron	4.0 ± 0.07	1.2 ± 0.1	0.6 ± 0.1	155 ± 0.08
Calcium	2.0 ± 0.04	140.0 ± 7.8	21.5 ± 0.0	41.6 ± 2.01
Zinc	1.5 ± 0.08	10.7 ± 0.7	0.8 ± 0.1	6.84 ± 0.02

Table 3. Anti – nutritional components of the wild cowpea

Component	Wild cowpea mg/100g	Non – defatted flour of brebra seed mg/ 100g
Tannin	9.55 ± 0.01	84.3 ± 0.89
Phytic acid	70.5 ± 0.08	291.62 ± 0.87
Oxalate	188.0 ± 0.83	20.97 ± 0.36

defatted flour of brebra seed was relatively low (Inobeme et al., 2014; Berhanu and Amare, 2014).

Carbohydrate content

The carbohydrate value (23%) of the investigated wild cowpea as found in this study was relatively lower than that of Bosso cowpea beans (61.97%), but within the acceptable range of legumes value 20 – 60% of the dry weight (Berhanu and Amare, 2014 cited Arkroyed and Doughty, 1964). On the other hand, the wild cowpea had almost similar carbohydrate content with Conophor nut (Berhanu and Amare, 2014 cited Enujiugha, 2003) but higher than cashew nut (Berhanu and Amare, 2014 cited Akinhanmi et al., 2008).

Thus, this indicates that wild cowpea is a poor energy source.

Fibre content

The fibre content (10%) of wild cowpea found in this study when compared to that of Bosso cowpea beans and non – defatted flour of brebra seed (2.80% and 2.41%) had greater percentage (Inobeme et al., 2014, Berhanu and Amare, 2014). The role of fibre in the evaluation of nutritional value is significantly important, especially for its chelating effects, where the availability of mineral and protein are declined respectively, and main reason for being an anti – nutritional factors. The positive fibre functions include regulation of both lipid and glucose metabolism as well.

Gross Energy content

The calculated energy value (344Kcal/g) of the wild cowpea when compared to that of Bosso cowpea beans (376.18Kcal/g) and non – defatted flour of brebra seed (612.58Kcal/g) was relatively low. Thus, the sample may be considered as a poor source of energy.

Tannin content

From the results shown in Table 3, the wild cowpea tannin content (9.55mg) was incomparable with that of non – defatted flour of brebra seed (84.3mg) (Berhanu and Amare, 2014) and that of some dry bean seed varieties (930mg)(Berhuna and Amare, 2014 cited Deshpande et al., 1986). The tannin contents found in this study may likely not be as harmful as expected for consumption. Large quantity of tannins combines with proteins to form a complex compound which turn the proteins less susceptible to proteolytic attack (Berhanu and Amare, 2014 cited Carbonaro et al., 1996).

Phytic acid content

From Table 3, it was observed that wild cowpea had lower content of phytic acid, almost four times than that of non – defatted flour of brebra seed (Berhanu and Amare, 2014). Phytic acid affects calcium, protein and zinc nutrition by formation of an insoluble non – nutritional complex which reduces their bioavailability and turns them insufficient to herbivores.

Total Oxalate content

In this study, it seems that the total oxalate content (188mg) of the investigated wild cowpea had higher value of total oxalate when compared to non – defatted flour of brebra seed (20.97mg) (Berhanu and Amare, 2014). But for spinach (978mg) and soybean (497mg), its total oxalate was relatively low almost over forty times respectively. (Muhammad et al., 2011). In terms of carrot (49mg), beet root (67mg), red bean (158mg) and white bean (113mg), their total oxalate contents were relatively low compared to that of the wild cowpea. On the other hand, the soluble oxalate of the wild cowpea (99.5mg) was higher than that of the French bean (88mg) (Jacek et al., 2011) when compared too.

Table 4: The Effects of Wild cowpea on Experimental Animals

Group N = 5	Treatment	Food intake (g)		Body weight (g)		
		14 days	21 days	0 day	14 days	21 days
Control	None	970.52	1260.70	222.26 ± 0.01	249.61 ± 0.01	277.40 ± 0.01
Group A	15 % cowpea	872.90	1394.45	226.79 ± 0.02	195.74 ± 0.01	118.40 ± 0.01
Group B	30% cowpea	473.86	1053.66	257.98 ± 0.01	136.74 ± 0.01	99.40 ± 0.01

Table 5: Activities of Alanine transaminase (ALT), Aspartate transaminase (AST), alkalinephosphatase (ALP), Total protein (TP) and Albumin (AL) in serum and liver homogenate of Experimental Animals.

Group	Liver Homogenate					Serum				
	ALT (U/L)	AST (U/L)	ALP (U/L)	T/P (g/dl)	AL (g/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	T/P (g/dl)	AL (g/dl)
Control	63.5 ± 0.07	124 ± 0.71	234.6 ± 0.06	6.85 ± 0.01	4.25 ± 0.01	20.0 ± 0.83	23.0 ± 0.71	151.8 ± 0.07	3.45 ± 0.00	2.3 ± 0.08
Group A	51.0 ± 0.05	103.5 ± 0.07	179.4 ± 0.08	5.6 ± 0.11	3.4 ± 0.04	9.0 ± 0.71	15.0 ± 0.83	100.1 ± 0.07	2.8 ± 0.08	2.05 ± 0.00
Group B	46.5 ± 0.13	97.0 ± 0.08	124.5 ± 0.12	4.9 ± 0.07	2.8 ± 0.06	7.0 ± 0.83	13.0 ± 0.54	86.7 ± 0.08	2.0 ± 0.07	1.8 ± 0.08

As chelating compound, it's combining with dietary calcium and forms an insoluble chelating complex which accumulates in the renal glomeruli then lead to the formation of kidney stones and other renal disorders

Mineral elements

The mineral elements content shown in Table 2, indicated that the minerals investigated such as phosphorus, calcium, zinc and iron were in low amounts as compared with that of domesticated cowpea. The presence of anti – nutritional factors may probably affects the availability of such minerals. Therefore, wild cowpea may be considered as poor source of these minerals.

The effects of wild cowpea on experimental Animals

From the data observed in Table 4, the weights of experimental guinea pigs (group A and B) were significantly decreased due to the consumption of crushed wild cowpea. Though the wild cowpea had higher protein content its lower protein digestibility may be among the main factors that led to the weights reduction drastically.

Total Protein, Albumin and activities of Alanine transaminase, Aspartate transaminase and Alkaline phosphatase in serum and liver homogenate of experimental Animals

In Table 5, information gathered revealed that the total protein, albumin and enzymes activities were affected in dose dependent manner. The decreased of total protein and albumin could be a consequences of inhibition of protein synthesis which may likely due to the presence of determined anti nutritional factors.

On the other hand, the decreased in the enzymes activities ruled out the possibility of toxic effects on the liver which also may likely due to the presence of chelating compounds such as tannin, phytate and oxalate.

Conclusion

The nutritional quality of food depends not only on nutrient content and nutrient bioavailability. First requirement is safety, that is, absence of toxic or undesirable compounds (Brosio et al., 1990). So, though wild cowpea contents significant level of dietary protein and abundant in some part of Nigeria, yet not consumed by humans and animals in form of feed, may likely due to the presence of some chelating compounds, higher inhibitors and low protein digestibility, which might cause unacceptable health conditions.

Based on these findings, it is a challenge to food beverages and other food related industries to seek for affordable food processing techniques that could be employed to render this potential dietary protein food resource acceptable for human consumptions and animal in form of feed.

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