

## DETERMINATION OF PROXIMATE AND PHYTOCHEMICAL COMPOSITION OF THREE SPECIES OF BEANS SOLD IN ULI

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### ABSTRACT

Beans which play significant role in the diets of Africans. It serves as a major source of protein in the absence of sufficient animal protein for the population. Three beans varieties sold in Uli (Potiskum, Aloka and Iron beans) were analyzed for their proximate and phytochemical constituents. Phytochemical screening of the plants was conducted using different standard methods and the result revealed the presence of phytochemicals at different concentrations. The three species contained carbohydrate, protein, fibers and minerals such as calcium, magnesium, potassium, sodium, iron, zinc, manganese and copper. The crude protein was found to be 15.62 and 17.91% with the brown seeds having the higher amount. The carbohydrate content analyzed was found to be 56.80 and 60.57% with the white seeds having the higher value. The crude lipid gave the least range which is 2.13 to 2.42%. The other parameters, moisture content, crude fiber and total ash contents were 3.56 to 5.08, 13.54 to 14.15 and 4.07 to 4.27%, respectively. Potassium and copper had the highest and lowest concentration in cowpea varieties ranging from 741 to 768 and 0.58 to 0.60 mg/100 g, respectively. There were significant ( $p < 0.05$ ) differences between the potassium, calcium, sodium, magnesium, manganese and zinc concentration of the cowpea varieties, except between iron and copper concentration. From the results, it can be deduced that there is relative difference in the nutritional composition of the three beans as the results demonstrated that Iron beans is nutritionally superior to Aloka and Potiskum.

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*Keywords: [Proximate, phytochemical, composition, Beans, Uli]*

### 1.0 INTRODUCTION

Legumes refer to the seeds of leguminosae including peas, beans and pulses, “it is considered as poor man meat” due to their high protein content and low cost compared to meat and meat products (Adebiyi, *et al.*, 2015). Legumes are food stuffs of great significance to people in tropical developing countries, a large number of species and varieties of legumes are consumed by the teeming population, as they are inexpensive and important source of protein (20-40%), carbohydrate (50-60%) along with other nutrients which have beneficial effects on human health and well being (Lakshmi *et al.*, 2010).

Beans (*Vigna unguiculata*) is a legume plant that is regarded as a prominent food crop in the third world countries. It is an excellent source of thiamine, folic acid, niacin, riboflavin and biotin (Minussi *et al.*, 2003). It plays a great role in alleviating poverty and malnutrition in developing countries in many parts of West Africa including Nigeria, cowpea seeds are consumed as boiled seeds or in combination with food such as maize, rice, and plantain among others. They are also processed into paste for the preparation of various traditional foods, such as Akara (fried cowpea paste) and moimoi, steamed cowpea (Henshaw and Sanni, 2005). As the population of Africa continues to increase at its present annual rate and the low income groups are to be provided with sufficient food to meet their protein requirement, efforts have to be made in several directions to improve the quality and quantity of food supplies (Agbogidi, 2001).

Proximate analysis, also known as Weende analysis is a chemical method of assessing and expressing the nutritional value of a feed, which reports the moisture, ash (minerals), crude fibre, crude fat and crude protein present in a food as a percentage of dry weight. Carbohydrate (nitrogen free extract) is determined by difference. The proximate analyses gives the overall nutritional composition of the sample in question, this is briefly complemented by Antinutrient and Mineral composition of the sample (Bradley, 1998). From an industry standard proximates include five constituents: Ash, Moisture, Proteins, Fat and Carbohydrates (Henneberg and Stohmann, 2004).

Proximate analysis provide information on moisture content, ash content, volatile matter content, ash, fixed carbon etc. Ash is the inorganic residue remaining after water and organic matter have been removed by heating, which provides a measure of total amount of minerals within the food. The nutritional content of cowpea grain is important because it is eaten by millions of people who otherwise have diets lacking in protein, minerals and vitamins.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plants produce these chemicals to protect itself but research work demonstrates that many phytochemicals can protect humans against disease (Tsuchiya, *et al.*, 2016). Knowledge of chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Several workers have discussed the use of phytochemical screening. Dahanukar *et al.* (2020) reported that phytochemical analysis are the study of organic substances that accumulate in plants due to their chemical structure, natural and biological functions.

## 1.2 AIM OF THE STUDY

This study was conducted to compare the nutritional values of brown and white seeds of *Vigna unguiculata* and to evaluate any net nutritional benefit for some individuals preferentially consuming one and abstaining from the other, these can be achieved by carrying out proximate analysis (moisture, fiber, lipid, ash, carbohydrate and protein content) and elemental analysis.

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## 1.2 Statement of Problem

In recent times, there is a global increase in the cost of living especially in most developing nations like Nigeria. The implication of this development will obviously affect the type of food and food nutrient availability among the general populace most especially the poor. A typical African diet is usually high in carbohydrate content and low in animal protein. Access to adequate food and health are among the universally adopted human rights that are necessary input for human development. There are a variety of different beans consumed in Nigeria but majority are not consumed as a result of poor and scanty literature on its nutritional compositions. Lack of

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information on the specific nutrients in a large number of the foods with which Nigeria is richly endowed is partly responsible for their under-exploitation especially in areas beyond the traditional localities where they are found and consumed. Among the plants in which their proximate composition has not been extensively studied are mostly consumed beans varieties in Anambra state.

### 1.3 Aims and Objective of the Study

The aim of this study is the determination of proximate and phytochemical composition of three species of beans sold in Uli

#### Specific objectives are to determine the:

- proximate composition of three species of beans sold in Uli,
- qualitative phytochemical constituents of extracts from three species of beans sold in Uli and
- quantitative phytochemical constituents of three species of beans sold in Uli.

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### 1.4 Scope of Study

This work will determine and compare the proximate nutritional and phytochemical composition of three species of beans: Potiskum (black eyed pea), Ife-brown (brown beans), and Aloka sold in Uli using AOAC, (2005) method.

### 1.5 Significance of Study

The results from this work will help determine the nutrient and phytochemical composition of the widely consumed Potiskum (black eyed pea), Ife-brown (brown beans), and Aloka beans species; their nutritional benefits which will assist in combating malnutrition, and other food related diseases as well as contributing to the food security system of rural areas. It will also serve as a guide to recommend the best beans varieties with better nutritional compositions among the three species analysed.

The results will also enhance the scanty database on nutritional compositions of some locally cultivated beans varieties.

## 2.0 MATERIAL AND METHODS

### 2.1 Equipment / Apparatus

Weighing balance, testube, pipette, heating mantle, separation funnel, beakers, distillation apparatus, soxlet apparatus, colorimeter, Whatman filter paper, electric blender, reagents used includes: Sodium hydroxide, hydrochloric acid, ammonia solution, sulphuric acid, glacial acetic acid, chloroform, acetic anhydride, copper sulphate and sodium sulphate.

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### 2.2 Sample Collection

A total of three species of beans sold in Uli will be obtained from the main market. These varieties are: potiskum (black eyed pea), Ife-brown (brown beans), and Aloka beans.

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### 2.3 Sample Treatment

Prior to analysis, the five hundred grams of each sample will be cleaned by hand picking to remove stones, dirt and damaged seeds. The residual moisture will be evaporated at room temperature until properly dried. The

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dried samples will be then grinded into powder using a food blender, sieved through 2mm mesh sieve and stored in polythene bag. The powdered sample will be used for the proximate analysis.

## **2.4 PROXIMATE ANALYSIS**

### **2.4.1 Moisture content determination**

The AOAC (2005) method no. 945.38 will be used. 2g of the sample will be weighed into clean, dry and pre-weighed crucibles. The crucibles and their contents will be dried in the moisture extraction oven at 110°C for 4 hours. The samples will be cooled in desiccators and reweighed. The samples will be dried in the oven until a constant weight is obtained.

$$\% \text{ Moisture content} = \frac{\text{Initial weight} - \text{weight of oven sample}}{\text{Initial weight of sample}} \times 100$$

### **2.4.2 Crude fat determination**

Method no. 920.39A (AOAC, 2005) will be used. 5g of the air-dried ground sample will be weighed into a filter paper, wrapped carefully and put in the sample holder of the Soxhlet extraction apparatus. A clean, dry and weighed Soxhlet extraction flask will be half-filled with N-hexane and the whole apparatus will be assembled together, and the flask placed on the heating mantle and heated at 60°C.

The fat will be extracted for three hours. Then, the sample holder will be disconnected and the extraction flask removed. The percentage fat contained will be determined thus:

$$\% \text{ Crude fat} = \frac{\text{weight of flask + oil} - \text{weight of empty flask}}{\text{Initial weight of sample}} \times 100$$

### **2.4.3 Crude fiber determination**

Method No. 942.05 (AOAC, 2005) will be used. 2g of defatted sample will be weighed into a 250 ml beaker containing 200 ml of 0.125M tetraoxosulphate (iv) acid (Sulphuric acid). The mixture will be heated in a steam bath at 70°C for 2 hours, and then allowed to cool. The cooled mixture will be filtered using a muslin cloth over a Buckner funnel. The residue will be washed three times with hot water to remove the acid and then put in a beaker containing 200 ml of potassium hydroxide. The mixture will be heated as before over a steam bath for 2 hours. The solution will be filtered and the residue will be washed three times with hot water. The final residue obtained will be put in a clean pre-weighed crucible and dried at 120°C to a constant weight. The crucible with the dry sample will be put in a muffle furnace and ashed at 550°C for 30 minutes such that the sample became ash white. Percentage fibre will be calculated as follows:

$$\% \text{ Crude fibre} = \frac{\text{weight of oven dried sample} - \text{weight of ash}}{\text{Initial weight of sample}} \times 100$$

### **2.4.4 Crude protein determination**

Method no. 955.04C called the Kjeldahl method will be used (AOAC, 2005). This method will be divided into three namely, digestion, distillation and titration.

**2.4.5 Digestion:** Approximately 0.1g of ground sample will be weighed into a clean, dried Kjeldahl flask for digestion, and 0.1g copper tetraoxosulphate (iv) crystals, 0.5g sodium tetraoxosulphate (iv) crystal and 25ml of concentrated H<sub>2</sub>SO<sub>4</sub> acid will be added into the flask and some glass beads will be added into the flask content as anti-bumping agents. The Kjeldahl flask and its content will be transferred to the digesting chamber in a fume cupboard and digested. Digestion continued with constant rotation of the digestion flask until the sample changed colour (that is from black to light blue). The digestion flask will be removed from the digesting chamber and allowed to cool. The digest will be made up to 100ml using distilled water and shaken vigorously to a homogeneous solution.

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**2.4.6 Distillation:** Out of the homogenous solution of the digest, 20ml will be transferred into a distillation flask using a pipette. Then 20ml of 40% sodium hydroxide solution will be added carefully down the side of the flask through a funnel.

Then 50ml of 2% boric acid solution will be pipetted into a receiving flask and two drops of methyl red indicator added. The distillation unit will be fitted such that the condenser is connected to the receiving flask with a glass tube, and the condenser cooled with constant supply of cold water from tap. Also, the tip of the glass tube will be immersed in the boric acid. The distillation unit is heated on a heating mantle for 35 minutes until the pink solution of the boric acid turned blue and the volume increased to about 100ml by the distillate.

**2.4.7 Titration:** Ten millilitres of the distillate will be titrated against 0.1N hydrochloric acid to a colourless end point. A blank solution will also be titrated to get any trace of nitrogen in the blank. All the titre volumes will be recorded. The percentage crude protein will be calculated as follows:

%Crude protein = % Nitrogen X 6.25

#### **2.4.8 Ash content determination**

The AOAC (2005) method No 942.05 will be used. Clean dried crucibles will be weighed on an electronic balance and 5g of sample weighed into the crucibles. The samples will be dry in the oven until constant weights are obtained.

Then, the samples will be transferred into the muffle furnace with a pair of tongs and ashed at 550°C 4 hours until a white ash will be obtained. The sample will be removed from the furnace and cooled in desiccators, and reweighed. The percentage ash will be calculated as followed:

% Ash Content =  $\frac{\text{Weight of Ash} \times 100}{\text{Weight of sample (after oven drying)}}$

Weight of sample (after oven drying)

#### **2.4.9 Carbohydrate content determination**

The carbohydrate content of the sample will be obtained by difference, that is, as the difference between the total summations of percentage moisture, fat, fibre, protein, ash and 100

% Carbohydrate= 100 – (% moisture + % fat + % protein + % fibre + % ash)

## **2.5 Phytochemical analysis**

### **2.5.1 Extraction of plant material**

The aqueous and ethanol extract of the plant was prepared by soaking 100 g of the ground sample of leaf in 500 ml of water. The experimental set-up was left for 24 h at room temperature and thereafter filtered using Whatman No 1 filter paper. The extract was then concentrated to 50 ml of the original volume of the extract and stored in an airtight container in a refrigerator at 4°C until when needed.

### **2.5.2 QUALITATIVE PHYTOCHEMICAL SCREENING**

Phytochemical screening of the extract will be carried out by a procedure that was based on those earlier reports by Banso and Adeyemo, (2016 and Stankovic, 2011) so as to detect the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, coumarin, saponins, reducing sugars in the selected plant extracts.

#### **2.5.3 Test for tannins**

1g of each powdered sample was separately boiled with 20 ml distilled water for five minutes in a water bath and was filtered while hot. 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride was added and observed for any formation of precipitates and any colour change. The

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reaction mixture was observed for a brownish green or blue-black colouration for the confirmation of the presence of tannins.

#### **2.5.4 Test for saponins**

1g of each powdered sample was separately boiled with 10 ml of distilled water in a bottle bath for 10 mins. The mixture was filtered while hot and allowed to cool. The following tests were then carried out.

**2.5.4.1 Demonstration of frothing:** 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 mins, formation of froth which is stable for some minutes indicate the presence of saponin in the filtrate.

**2.5.4.2 Demonstration of emulsifying properties:** 2 drops of olive oil was added to the solution obtained from diluting 2.5 ml filtrate to 10 ml with distilled water (above), shaken vigorously for a few minutes, formation of a fairly stable emulsion indicated the presence of saponins.

#### **2.5.5 Test for steroids**

**2.5.5.1** About 0.2 g of each portion of the powdered sample was dissolved in 2 ml of chloroform. 0.2 ml of concentrated  $H_2SO_4$  was carefully added to form a layer. A reddish-brown colour at the interface between the layer indicates the deoxy-sugar characteristics of cardenolides which indicates the presence of steroid

**2.5.5.2** 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2 ml of concentrated  $H_2SO_4$ . The colour change from violet to blue or green in some samples is an indication of the presence of steroids.

#### **2.5.6 Test for alkaloids**

1 g of each powdered sample was separately boiled with water and acidified with 5 ml of 1 % HCl on a steam bath. The solution obtained was filtered and 2 ml of the filtrate was treated with few drops of the following reagents separately in different test tubes and observed.

**2.5.6.1 Mayer's Test:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide). Formation of a creamy white precipitate indicated the presence of alkaloids in the extract.

**2.5.6.2 Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown or reddish-brown precipitate was regarded as evidence for the presence of alkaloids in the extract.

**2.5.6.3 Dragendorff's Test:** Filtrates were treated with dragendorff's reagent (solution of potassium bismuth iodide), Formation of orange-brown precipitate was regarded as evidence for the presence of alkaloids in the extract.

**2.5.6.4 Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution), Formation of yellow coloured precipitate was regarded as evidence for the presence of alkaloids in the extract.

#### **2.5.7 Test for cardiac glycosides**

**2.5.7.1** 5 ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may be formed.

**2.5.7.2** 10 ml of 50 %  $H_2SO_4$  was added to 1 ml of the filtrate in separate test tubes and the mixtures heated for 15 mins followed by addition of 10 ml of Fehling's solution and boiled. A brick red precipitate indicated presence of glycosides.

#### **2.5.8 Test for free anthraquinones**

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5 ml of chloroform was added to 0.5 g of the powdered dry seeds of each sample. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

#### **2.5.9 Test for combined anthraquinones**

1 g of powdered sample of each sample was boiled with 2 ml of 10 % hydrochloric acid for 5 mins. The mixture was filtered while hot and filtrate was allowed to cool. The cooled filtrate was partitioned against equal volume of chloroform and the chloroform layer was transferred into a clean dry test tube using a clean pipette. Equal volume of 10 % ammonia solution was added into the chloroform layer, shaken and allowed to separate. The separated aqueous layer was observed for any colour change; delicate rose pink colour showed the presence of an anthraquinone.

#### **2.6.0 Test for flavonoids**

**2.6.0.1** 1 g of powdered sample of each sample was separately boiled in 20 ml of water and then filtered. 5 ml of dilute ammonia solution was added to a portion of the filtrate, followed by the addition of concentrated  $H_2SO_4$ . A yellow coloration was indicative of the presence of flavonoids.

**2.6.0.2** 1 g of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of the cooled filtrate. A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids.

#### **2.6.1 Test for terpenoids**

5 ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated  $H_2SO_4$  was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

#### **2.6.2 Test for phlobatannins**

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the phlobatannins.

#### **2.6.3 Test for carotenoids**

1 g of each sample was extracted with 10 ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85 % sulphuric acid was added. A blue colour at the interface showed the presence of carotenoids.

#### **2.6.4 Phenolics**

0.5 g of the powdered dried seeds of each sample was boiled with 10 ml of distilled water for 5 mins and filtered while hot. Then 1ml of ferric chloride solution was added. Formation of blue-black or brown colouration indicated the presence of phenol.

#### **2.6.5 Test for reducing sugars**

To about 1 g of each sample in the test tube was added 10 ml distilled water and the mixture boiled for 5 mins. The mixture was filtered while hot and the cooled; 5 ml of mixture of equal volume of Fehling's solution (A and B) was added to 2 ml of the filtrate in a test tube and the resultant mixture was boiled for 2 mins. Appearance of brick red precipitate at the bottom of the test tube indicated the presence of reducing sugar.

### **2.7 QUANTITATIVE PHYTOCHEMICAL**

#### **2.7.1 Determination of Alkaloids (Harbone method, 2010)**

5g of the sample was weighed into a 250ml beaker and 20ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at room temperature. This was filtered with filter paper and the filtrate

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was heated to one quarter of the original volume. 5ml of Concentrated ammonium hydroxide was added drop wise until the precipitate was complete. Then, filter with the pre weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven at 80°C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed. Then was calculated using the formula.

$$\% \text{ weight of alkaloid} = \frac{(\text{Weight of filter paper with residue}) - (\text{Weight of filter paper}) \times 100}{\text{Weight of the sample analyzed}}$$

#### **2.7.2 Determination of Tannins by Titration (Person method, 2004)**

10g of sample was weighed in a conical flask and 100mls of n hexane or petroleum ether was added and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes for the solvent to evaporate. It was then re-extracted by soaking 100mls of 1% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected.

25 ml of ammonium hydroxide were added to the filtrate to precipitate the alkaloids. The alkaloid was heated with electric hot plate to remove some of ammonium hydroxide still in solution. The remaining volume was measured and 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1M NaOH using 1ml of phenolphthalyne as indicator until a pink end point is reached. Tannin content was calculated in percentage ( $C^1V^1 = C^2V^2$ ) molarity

#### **2.7.3 Determination of Saponins (AOAC MEHOD, 2005)**

10grams of the ground sample was weighed into a thimble and transferred into the soxhlet extractor chamber fitted with a condenser and flask. 250 ml of methanol was put into the flask. Extraction continued for 1hr, The saponin was exhaustively extracted by heating the flask on a heating mantle. After the thimble and its content was removed and the methanol recovered leaving the saponin and little quantity of methanol in the flask. It was then taken to an oven and kept at slanting position at a temperature of 70°C to evaporate the residual methanol. The flask and content was weighed and the difference between the flask plus saponin and flask alone was the mass of saponin extracted.

The saponin content was weighed and calculated in percentage.

$$\% \text{ Saponin} = \frac{(\text{Weight of beaker + sample}) - (\text{Weight of empty beaker}) \times 100}{\text{Weight of sample analyzed}}$$

#### **2.7.4 Determination of Flavonoids (AOAC, 2005)**

10g of the plant sample was put in a beaker with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

$$\% \text{ Flavonoids} = \frac{(\text{Weight of crucible + residue}) - (\text{Weight of crucible}) \times 100}{\text{Weight of sample analyzed}}$$

#### **2.7.5 Determination of phytate (AOAC METHOD, 2005)**

20g of the sample was weighed into 250ml conical flasks. The sample was soaked in 100ml of 2% concentrated HCL for 3 hours, the sample was then filtered. 50ml of the filtrate was placed in 250ml beaker and 100ml

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distilled water added to the sample. 10ml of 0.3% ammonium thiocyanate solution was added as indicator and titrated with standard iron (III) chloride solution.

$$\% \text{ Phytic acid} = \frac{\text{Titre Value} \times 0.00195 \times 1.19}{\text{Sample analyzed}} \times 100$$

## 2.8 MINERAL ELEMENT ANALYSIS

### DIGESTION OF SAMPLE

The mineral contents of the test samples will be determined by the dry ash extraction method following each specific mineral element as described by AOAC (2010). Twenty (20) grams of the samples will be burnt to ash (as in ash determination and the resulting ash will be dissolved in 100ml of dilute hydrochloric acid (1MHCL) and then diluted to 100ml volumetric flask using distilled water. The solution will be used for the various analysis of mineral.

**Determination of Magnesium** Exactly 10ml of the sample filtrate will be pipetted into 250ml conical flask after which 25ml of ammonia buffer solution will be added into the conical flask and will be properly mixed. Then a pinch of Erichrome black T indicator will be added and titrated with 0.02N of EDTA until the colour of the solution changed from wine-red to blue colour.

$$\text{Magnesium (mg/100g)} = \frac{(\text{Tv} \times 0.2432 \times 1000)}{\text{Vol of sample used}}$$

**Determination of Iron** Exactly 5ml of the sample will be pipette into a test tube and 1ml of 2.5% hydroquinol and 1.5ml of acetate buffer will be added to the sample, after which 1ml of 0.1% pyridine will be also added and shaken properly to mix. The volume of solution will be made up with dilute water and will be properly mixed. The colour will be allowed a maximum of 24hours for it to develop and the absorbance will be read at 530nm using spectrophotometer and the concentration in mg/100 g will be calculated using the following equation:

$$\text{Iron (mg/100g)} = \frac{\text{Concentration (ppm)} \times \text{Dilution factor} \times 1000}{\text{Wt. of Sample}}$$

**Calcium Determination:** Calcium contents of the test sample will be determined by the EDTA complex isometric titration. Twenty (20) ml of each extract will be dispersed into a conical flask and panels of the masking agents, hydroxytannin, hydrochlorate, and potassium cyanide will be added followed by 20ml of ammonia buffer (pH 10.0). A pinch of the indicator-Ferrochrome black will be added and the mixture will be shaken very well. It will be titrated against 0.02N EDTA solution. A permanent blue colour will be observed and the reading taken. The calcium contents will be calculated using the formulae below.

$$\text{Calcium (mg/100g)} = \frac{(\text{Tv} \times 0.4008 \times 1000)}{\text{Vol of sample used}}$$

### Potassium (K)

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The concentrations of potassium (ppm) will be analysed using UV- spectrophotometer at a wavelength of 766.5 nm, and the concentration in mg/100 g will be calculated using the following equation:

$$\text{Potassium (mg/100g)} = \frac{\text{Concentration (ppm)} \times \text{Dilution factor} \times 1000}{\text{Wt.of Sample}}$$

#### **Sodium (Na)**

The concentrations of sodium (ppm) will be analysed using atomic absorption spectrophotometer at a wavelength of 243nm and the concentration in mg/100 g will be calculated using the following equation:

$$\text{Sodium (mg/100g)} = \frac{\text{Concentration (ppm)} \times \text{Dilution factor} \times 1000}{\text{Wt.of Sample}}$$

#### **Phosphorus (P)**

A 10 ml sample solution will be put in a 100 ml volumetric flask. The solution will be neutralized with ammonia and nitric acid solution (1:2). Twenty (20) ml of vanadate molybdate reagent will be added and diluted to the mark. It will be allowed to stand for ten minutes and absorbance read at 470nm in the ultra violet region and the mineral concentration in mg/100 g will be calculated using the following equation:

$$\text{Phosphorus (mg/100g)} = \frac{\text{Concentration (ppm)} \times \text{Dilution factor} \times 100}{\text{Wt.of Sample}}$$

### **3.0 RESULTS AND DISCUSSION**

#### **3.1 QUALITATIVE PHYTOCHEMICALS**

The result of the qualitative phytochemical composition of the ethanolic extracts of the different bean species sold in Uli is shown in table 1.

**Table 1: Qualitative phytochemical composition of bean species**

<b>PHYTOCHEMICALS</b>	<b>Potiskum beans</b>	<b>Aloka beans</b>	<b>Iron beans</b>
<b>SAPONIN</b>	+++	+++	-
<b>FLAVONOID</b>	+++	++	+
<b>ALKALOID</b>	-	-	-
<b>TANNIN</b>	+	+	-
<b>STEROIDS</b>	-	-	+

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<b>TERPENIODES</b>	+++	+++	-
<b>GLYCOSIDES</b>	++	+	-
<b>PHENOL</b>	-	-	+

#### Key

- +++ = Present in high concentration
- + = Present in moderate concentration
- ++ = Slightly or sparingly present
- = Absent.

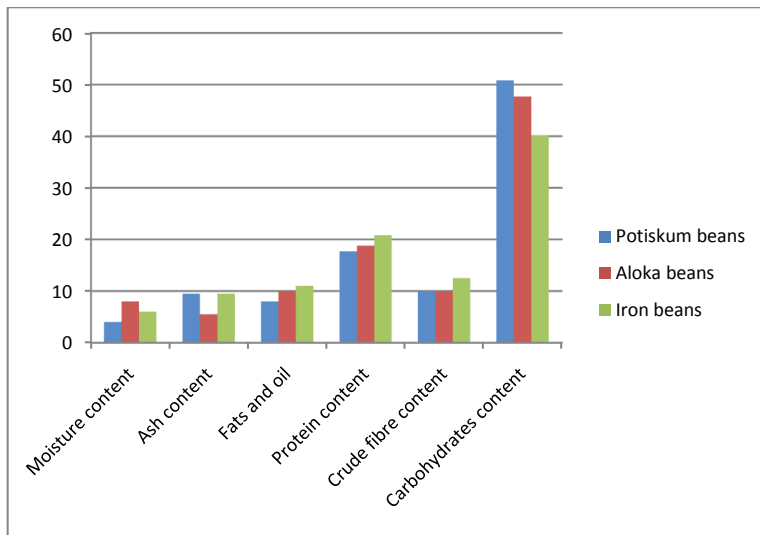
### 3.2 PROXIMATE COMPOSITION

The results of the proximate analysis of three varieties of beans sold in Uli are presented in Table 2. The beans varieties had a large amount of protein and large amount of carbohydrate. The crude protein was found to be 17.68 and 20.83% with the iron beans seeds having the higher amount. The ash and moisture content was found to range from 4.0 to 8.00 and 5.50 to 9.50%, respectively, with the iron beans also having the higher amount. The fiber and carbohydrate content was found to range from 7.50 to 12.50% and 40.17 to 51.35%, respectively, with the iron bean seeds having the higher amount of fiber and lowest amount of carbohydrate.

**Table 2: MEAN NUTRITIONAL CONTENT OF BEANS IN PERCENTAGE (%)**

Parameters (%)	Potiskum beans	Aloka beans	Iron beans
<b>Moisture content</b>	<b>4.00</b>	<b>8.00</b>	<b>6.00</b>
<b>Ash content</b>	<b>9.50</b>	<b>5.50</b>	<b>9.50</b>
<b>Fats and oil</b>	<b>8.00</b>	<b>10.00</b>	<b>11.00</b>
<b>Protein content</b>	<b>17.68</b>	<b>18.80</b>	<b>20.83</b>
<b>Crude fibre content</b>	<b>10.00</b>	<b>10.00</b>	<b>12.50</b>
<b>Carbohydrates content</b>	<b>50.82</b>	<b>47.69</b>	<b>40.17</b>

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**Fig 1: MEAN NUTRITONAL CONTENT OF BEANS IN PERCENTAGE (%)**

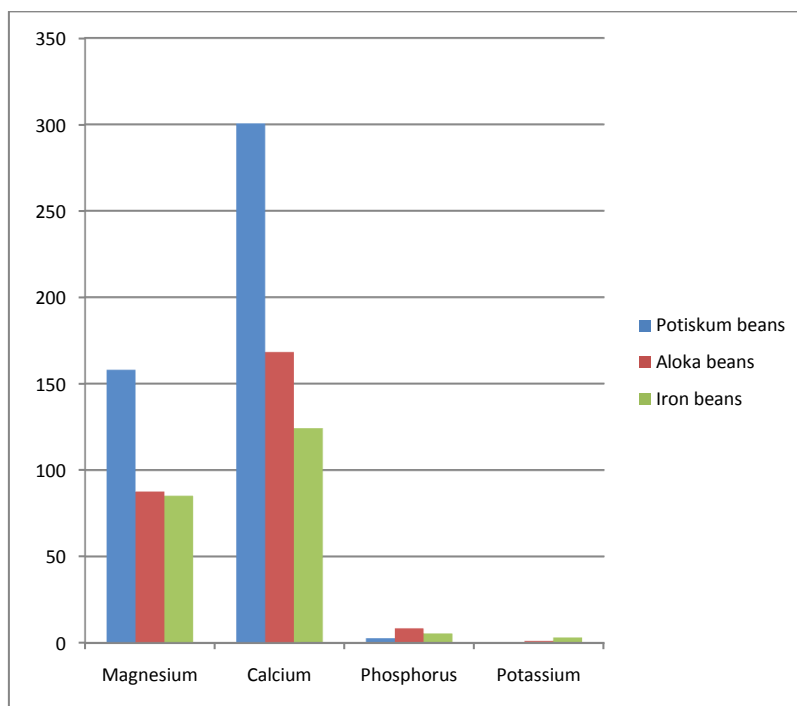
### 3.3 Mineral composition

The concentrations (mg/100 g) of the elements determined in the seeds are as shown in Table 3. Calcium was the most abundant element in the seeds. It was found to be 124.25 to 300.60 mg/100 g. The least was found to be potassium which was 0.25 to 5.20 mg/100 g. Other elements analyzed were magnesium 85.12 to 158 mg/100 g and phosphorus 2.70 to 8.50 mg/100 g respectively

**Table 3: MEAN MINERAL COMPOSITION OF BEANS (mg/100g)**

Parameters (mg/100g)	Potiskum beans	Aloka beans	Iron beans
<b>Magnesium</b>	<b>158.08</b>	<b>87.55</b>	<b>85.12</b>
<b>Calcium</b>	<b>300.60</b>	<b>168.34</b>	<b>124.25</b>
<b>Phosphorus</b>	<b>2.70</b>	<b>8.40</b>	<b>5.48</b>
<b>Potassium</b>	<b>0.25</b>	<b>1.15</b>	<b>3.10</b>

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**Fig 2: MEAN MINERAL COMPOSITION OF BEANS (mg/100g)**

#### 4.0 DISCUSSION

The present investigation revealed the presence of phytochemicals such as alkaloids, glycoside, saponins, flavonoids, phenols, tannins, and terpenoids in extracts of three beans species (potiskum, Aloka and Iron beans) sold within Uli and a similar observation was reported by Rajendran and Sundararajan (2011). The presence of alkaloid (++) in beans seed is also reported by Eleazu *et al.*, (2012). The presence of these phytoconstituents in the seed extract of *C. lanatus* indicated that, the plant possess antioxidant and antimicrobial properties. Saponin was found to also be present in beans seed according to the result obtained from this study. Saponins have the property of precipitating and coagulating red blood. These plants are used to stop bleeding and in treating wounds (Okwu, 2011). They exhibit foaming properties and cell membrane- permeabilizing properties. Their soapy character is due to their surfactant properties. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membrane (Njoku and Akumefula, 2007).

Tannin was highly present from the study and when compared with the result from similar study by Hassan *et al.* (2008), tannin was also highly present. High concentration of tannin in both studies showed they possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis,

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cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Egba *et al.*, 2012). Flavonoid was also moderately present (++) according to result obtained from this study and when compared with other studies such as Sakarkar and Deshmukh, 2011 and Yaro *et al.*, (2007) showed contradicting outcome. This variation could be attributed to the species of beans used in this study. Flavonoids have protective effects including anti-inflammatory, anti-oxidant, antiviral, and anti-carcinogenic properties.

The proximate composition of the seeds analyzed is shown in Table 2. The moisture content of the four varieties of beans analyzed was found to be 4.00 and 8.00% with the potiskum beans having the higher value. The white seeds having lower moisture content can be stored for a longer period of time than the brown seeds. High water/moisture content in seeds, predispose them to bacterial and fungal attack. As a result of the value of the moisture content of brown seeds not being so high, it can also be stored for a long period of time. The value for brown seeds falls within the range given by Yagodin (2014) which is 5 to 8%.

The crude protein content of the cowpea varieties was 16 and 18.00% with the iron beans seeds having the higher value. This range of values falls within the range given by Tobin and Carpenter (2018) which is 15 to 30%. Cowpeas are rich sources of protein. Dietary proteins are needed for the synthesis of new cell, repair of worn out tissues, enzymes, hormones, antibodies and other substances required for healthy functioning and development of the body and its protection (Cheesebrough, 2017) and for the treatment of protein energy malnutrition (Omoruyi *et al.*, 2014).

The crude fiber content of the seeds analyzed was found to be 13.54 and 14.15% with the iron beans seeds having the higher value. The fiber content in relation to the diet is adequate, exerting a major influence on the metabolism of the gastrointestinal tract (GIT) and its deficiency is linked to appendicitis, diverticular disease and hemorrhoids (Gibney, 1989). Fiber also slows down the release of glucose into the blood and decreases intercolonic pressure, hence, reduces the risk of colon cancer (Gibney, 1989). This range of values obtained falls within the range reported by Gibney (1989) which is 13 to 19%.

The lipid content obtained from the analysis of the cowpea varieties was 2.13 and 2.42% with the brown seeds having the higher value. Cowpeas have been shown to be low in their lipid content (Davidson *et al.*, 1975). Lipids provide strong energy and transports fat soluble vitamins like vitamins A, D, E and K (Ologhobo, 1988). The range of values obtained falls within the range 2.01 to 2.88% reported by Ologhobo and Fetuga (1988).

The ash contents analyzed was found to be 4.50 and 9.50% with iron beans seeds having the higher value. This range of values obtained falls within the ranges given by Ologhobo and Fetuga (1988) and Yeshajahu (1991) as 4.1 to 4.77 and 2.8 to 4.9%, respectively. With these ranges of values, it means that small amount of inorganic compounds are present in the cowpea varieties.

The carbohydrate content analyzed was found to be 40.00 and 51.07% with the Ife brown beans seeds having the higher value. Carbohydrates are good sources of energy, they are stored as glycogen which is the reservoir for glucose (Freedland and Briggs, 1977).

The elemental composition of the seeds is as shown in Table 2. Calcium and magnesium were the most abundant and was significantly higher than other elements analyzed and potassium was the least. Potassium is essential for the maintenance of normal muscle functioning of the heart, proper nerve stimulation, regulation of water balance and osmotic pressure. It also helps to maintain acid-base balance. Deficiency results in muscle weakness, loss of appetite, nausea. Cowpeas are rich sources of potassium.

Calcium ion regulates a number of physiologic and biochemical processes which includes neuromuscular excitability, blood coagulation, secretory processes, membrane integrity, plasma membrane transport neurotransmitters, bone mineralization and maintenance of healthy teeth (Dutcher and Fiela, 1967; Cheesebrough, 1987). The concentration of sodium was found to be 78.15 and 84.65 mg per 100 g with the white beans having the higher value. At  $p < 0.05$ , there was significant difference between the two varieties of cowpeas.

The concentration of magnesium was found to be 189.91 and 195.33 mg/100 g with white beans having the higher value. This range of value falls within the range reported by Ologhobo (1986) which is 148 to 220 mg per 100 g of sample. Cowpeas are good sources of magnesium (Thelma and Klein, 1966). Magnesium forms a

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part of enzyme activator and also a constituent of bones and teeth (Laestch, 1979; Murray *et al.*, 1990). It also participates in growth metabolism of protein, lipid, carbohydrate and nucleic acid (Harrison and Hoare, 1980; Guthrie, 1989).

The concentration of phosphorus which was found to be the least among the other elements was 0.2 and 0.60 mg/100 g in the cowpea varieties with the brown seeds having the higher value. At  $p > 0.05$ , there was no significant difference, which is similar to the report of Holland *et al.* (2011).

## 5.0 CONCLUSION

The results from current study reveal that beans seed sold in Uli is nutritionally rich in fats, fiber, ash and protein. The iron beans was nutritionally superior to Aloka and Potiskum in terms of proximate composition content. From the analysis, there were significant differences between the protein, fiber and carbohydrate content of the beans varieties but no significant difference between the lipid and ash contents. From the elemental analysis, there were significant differences between the potassium, calcium, magnesium, and phosphorus concentration of the cowpea varieties. In conclusion, iron beans contain higher protein, calcium, potassium and zinc than white cowpeas, while Brown contains higher carbohydrate and fiber content, magnesium. Findings of current study are significant and have potential to offer tangible contribution towards food shortage alleviation by promoting the production and consumption of common bean and pigeon pea in Sub-Saharan Africa

## RECOMMENDATIONS

Based on the above facts, it is recommended that the four varieties should be considered in the diet. Those who consume one more than the other should try to consume the two so as to supplement some of the nutrients that are reduced in one. People who consume mostly white cowpeas because they are less expensive than brown cowpeas, should try to consume brown cowpeas once in a while, because they contain more protein which is necessary for growth.

Comment [K8]: Four ?

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