PROXIMATE COMPOSITION, ANTINUTRIENT PROFILE, MINERAL CONTENT AND PHYTOCHEMICAL PROPERTIES OF PIGEON PEA (*CAJANUS CAJAN*), BLACK TURTLE BEAN (*PHASEOLUS VULGARIS*), RED KIDNEY BEAN (*PHASEOLUS VULGARIS* L.) AND AFRICAN BLACK BEAN (*VIGNA UNGUICULATA*) SEEDS

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ABSTRACT

Legumes are inexpensive sources of protein, vitamins, fiber, complex carbohydrates and essential minerals, and occupy a special place in human diet, particularly in developing countries. However, their nutritional quality is adversely affected by the presence of antinutrients which limit the proper metabolism of valuable nutrients. This study evaluated the proximate composition, antinutrient profile, mineral content and phytochemical properties of pigeon pea, black turtle bean, red kidney bean and African black bean seeds. Milled seed samples were analysed for proximate composition using standard AOAC methods, antinutrients by colorimetric and titrimetric methods, minerals by atomic absorption spectrophotometry and phytochemicals by standard qualitative and quantitative methods. Data were expressed as mean ± SD and compared by one-way ANOVA at $p<0.05$. The result revealed that Crude protein content was highest in red kidney beans (18.87%) and African black beans (18.00%), while black turtle beans had the lowest (12.05%). Ash content was highest in African black beans (8.88%) and lowest in pigeon peas (6.88%). Alkaloid content was highest in African black beans (2.84%) and lowest in pigeon peas (1.58%). Flavonoid content was highest in red kidney beans (4.17%) and least in black turtle beans (2.47%). Iron level was highest in black turtle beans (2.67 mg/kg) and lowest in African black beans (1.06 mg/kg). Copper was highest in pigeon peas (1.03 mg/kg). Manganese and selenium were highest in black turtle beans (1.50 mg/kg and 7.19 mg/kg respectively). In conclusion, the four legumes are rich in protein, minerals and phytochemicals, but their antinutrient contents suggest that processing methods such as soaking and boiling should be employed to reduce antinutrient levels before consumption.

Keywords: Proximate composition, antinutrients, minerals, phytochemicals, legumes, pigeon pea, kidney bean, cowpea.

1.0 INTRODUCTION

According to the United Nations Department of Economics and Social Affairs, the Nigerian population in 2022 was 216 million, and the global population has reached an estimate of 8 billion (Sami and Lara, 2022; United Nations, 2022). The population of Nigeria is expected to increase from 216 million in 2022 to 263 million in 2030 and 401 million in 2050. The danger of this rapid population growth is that the economy is not growing at the same rate, leading to increased unemployment, poverty, hunger, malnutrition and diseases. The unemployed and low-income earners suffer the pangs of hunger and malnutrition most because they cannot afford to prepare adequate diets (Sami and Lara, 2022). In order to have a quality population that can

contribute to the growth and development of Nigeria, and reduce the number of Nigerians battling malnutrition, cheaper but quality food crops enriched with nutrients and minerals should be sourced.

Legumes are plants belonging to the family Leguminosae that produce seeds within pods (Kouris-Blazos and Belski, 2016; Staniak *et al.*, 2014). They are inexpensive sources of protein, vitamins, fiber, complex carbohydrates, antioxidants, unsaturated fats and essential minerals (Annor *et al.*, 2014; Bouchenak and Lami, 2013), and are cheaper alternatives to meat due to their protein content. After cereals, legumes are considered the most important food source (Kouris-Blazos and Belski, 2016; Philips, 1993). Legumes also have medicinal properties since they possess beneficial bioactive compounds (Philips, 1993) and are called multipurpose plants because they are used as food for humans, animal feed, increase soil fertility and reduce production costs for farmers (Nedumaran *et al.*, 2015). However, the nutritional quality of legumes is adversely affected by the presence of antinutrients such as tannins, alkaloids, saponins, oxalate and phytate, which limit the proper metabolism of the valuable nutrients present in legumes (Sanchez *et al.*, 2015; Bora, 2014). Most of these antinutrients are destroyed by heat (Sanchez *et al.*, 2015). The consumption of legumes has positive effects on health; when consumed as supplements for diabetics, they can prevent cardiovascular risks, prevent obesity and improve bone disorders (Ndidi *et al.*, 2014; Olagunji, 2018).

Pigeon pea (*Cajanus cajan*) is one of the most common tropical and subtropical legumes, originating from India (Van der Maesen, 1995). Seeds of pigeon pea are known to be rich sources of proteins, carbohydrates and minerals, with protein content generally varying from 18–25% and as high as 32% (Aggarwal *et al.*, 2015). In Nigeria, pigeon peas are called *fio-fio* in Igbo and are grown in many parts of the country for human and animal consumption (Egbe and Kalu, 2006). Black turtle bean (*Phaseolus vulgaris*) is native to South America but has been introduced worldwide. They serve as food for many people of tropical origin and constitute a very important source of dietary protein in West African countries including Nigeria (Oboh, 2006). Red kidney bean (*Phaseolus vulgaris* L.) originated in Central and South America and is now cultivated in many parts of the world, including Nigeria. It is a nutritious type of legume that is loaded with health benefits, but contains a high amount of toxins which are only destroyed by soaking and boiling (Roadhouse *et al.*, 1990; Kouris-Blazos and Belski, 2016). African black bean (*Vigna unguiculata*), commonly known as *akidi* among the Igbo-speaking tribe in South-East Nigeria (Onwuka, 2002), is an annual plant native to West Africa and is one of the oldest crops to be farmed. It serves as a major source of energy, protein and a wide range of micronutrients for many, especially in developing countries (FAOSTAT, 2015; Gomez, 2004).

Despite the nutritional importance of these four legumes, people lack adequate nutritional information about them, coupled with the fact that they take longer time to prepare, leading to neglect and failure to reap their benefits. There is need to compare the proximate composition, antinutrient profile, mineral content and phytochemical properties of these selected local beans to ascertain their level of viability as affordable alternatives to meet nutritional needs and fight several diseases in developing countries. Therefore, this study evaluated the proximate composition, antinutrient profile, mineral content and phytochemical properties of *Cajanus cajan*, *Phaseolus vulgaris*, *Phaseolus vulgaris* L. and *Vigna unguiculata* seeds.

1.1 Aim and Objectives

The aim of this study was to evaluate the proximate composition, antinutrient profile, mineral content and phytochemical properties of the four legume seeds.

The specific objectives were:

i. to determine the proximate composition of pigeon pea, black turtle bean, red kidney bean and African black bean;

ii. to determine the antinutrient composition (alkaloids, flavonoids, saponins, tannins, phenols, oxalate and phytate) of the four bean species;
iii. to determine the mineral composition (iron, zinc, copper, manganese and selenium) of the four bean species; and (iv) to determine the phytochemical properties of the four bean species.

2.0 MATERIALS AND METHODS

2.1 Materials

The experiments were carried out with the following instruments:

i. Perfect Adjustable micropipette (USA),
ii. Gallenkamp automatic chemical balance (England),
iii. Pye Unicam 293 pH meter (England)
iv. Refrigerator (Kelvinator, Germany),
v. Gallenkamp Water bath (England),
vi. UNICO 2100UV Spectrophotometer (England),
vii. Centrifuge 80-2 (Jenalab Medical, England), and
viii. Atomic Absorption Spectrophotometer (GBC Avanta Ver 2.20 equipped with lamp).

All chemicals and reagents used were of analytical grade and were supplied by British Drug House (BDH) and Sigma.

2.2 Sample Collection and Processing

Red kidney bean, black turtle bean, African black bean and pigeon pea seeds (Figure 1) were purchased from Eke Awka market, Anambra State, Nigeria. Approximately 200 g of each sample was air-dried, ground into a fine powder with a grinder and stored in airtight containers before analysis. Part of the samples were used for nutritional composition, while the rest were extracted and used for phytochemical and antioxidant compositions.

Figure 1. Seeds of the four legume species used in this study: (A) pigeon pea (*Cajanus cajan*); (B) black turtle bean (*Phaseolus vulgaris*); (C) red kidney bean (*Phaseolus vulgaris* L.); (D) African black bean (*Vigna unguiculata*) (Photographed and sourced by Ofomata Jacinta, 2023).

2.3 Proximate Analysis

Proximate analysis of food is the determination of the major components of food, which include moisture content, crude fats, ash content, crude proteins, carbohydrates and crude fiber. The moisture, ash, crude fats, proteins and carbohydrates of all the samples were carried out using standard AOAC methods (AOAC, 1990).

2.3.1 Moisture Content

Moisture content was determined using the gravimetric method (air oven drying) as described by AOAC (1990) and FAO (1980). An accurate 5.0 g of sample was measured and transferred into a pre-weighed drying dish. The dish and contents were weighed using a weighing balance. The samples were dried to constant weight at 95–100 °C under pressure not exceeding 5 hours. After drying the samples completely, the dish was placed in a desiccator to cool. The samples were reweighed, and the loss in weight as moisture was reported. Total solid (dry matter) was calculated as 100 – moisture (FAO, 1980).

2.3.2 Crude Protein

Crude protein was determined by the Kjeldahl method with slight modification (AOAC, 1990). The determination involved three steps: digestion, distillation and titration. Using the method described by AOAC (1990), about 2.0 g of each sample was weighed into a Kjeldahl flask. A small quantity of Kjeldahl catalyst (selenium powder) and 20 mL of concentrated sulphuric acid were added to the samples. The digest was heated until a clear, colourless solution was observed. The digested samples were transferred into a 100 mL volumetric flask and diluted to 100 mL with distilled water. Accurately, 5 mL of the digested samples were pipetted into the Kjeldahl apparatus, and 5 mL of 40% NaOH was added. The mixture was steam distilled, and the liberated ammonia was collected into a 50 mL conical flask containing 10 mL of 2% boric acid plus mixed indicator solution (5 drops of bromocresol and 1 drop of methyl red). The ammonium released was titrated with 0.01 M HCl. The blue colour turns to wine pink at the endpoint. The crude protein was calculated as N × 6.25, where atomic mass of nitrogen = 14 and normality of HCl used = 0.01 (AOAC, 1990).

2.3.3 Crude Fiber

Crude fiber was determined based on the method described by AOAC (1990). About 2.0 g of the digested sample was weighed into a 200 mL Pyrex flask, and 1.25% H2SO4 was added. The beaker was covered with a watch glass and gently boiled on a hot plate for 30 minutes. The residue was decanted through a sintered glass crucible. The beaker was covered with a watch glass and gently heated with 200 mL of a solution containing 1.25 g of carbonate-free NaOH per 100 mL on a hot plate for 30 minutes. The alkali was removed and the samples washed twice with 50 mL boiling water. The content of the beaker was washed into a sintered glass crucible, dried and incinerated. The difference divided by the sample weight expressed in percentage gives the fiber content (AOAC, 1990).

2.3.4 Crude Fat

Crude fat was determined using the Soxhlet extraction method (AOAC, 1990). Five grams (5.0 g) of each sample was measured into a thimble and inserted into an extraction tube of a Soxhlet apparatus. Exactly 300 mL of hexane was measured into the 500 mL bottom flask of the apparatus, which was connected to the Soxhlet apparatus at 60 °C. The fat was extracted by running hexane over the samples at a rate of 3-4 drops per second for about 10 hours. After extraction, a rotary evaporator was used for 10 minutes to remove the hexane that was mixed with the oil. All measurements were replicated three times, and the fat percentage was calculated accordingly (AOAC, 1990).

2.3.5 Ash Content

Ash content was determined using the muffle furnace method as described by AOAC (1990). Two grams (2.0 g) each of the oven-dried samples were weighed into previously dried and weighed porcelain crucibles. The crucibles and their contents were placed in a muffle furnace, and the temperature was allowed to rise slowly to 550 °C. This temperature was maintained for 3 hours. The crucibles were then transferred into desiccators, cooled to room temperature and weighed. The percentage ash was calculated from the weight loss during combustion (AOAC, 1990).

2.3.6 Carbohydrate Content

The carbohydrate content was determined by difference using the method described by Salma *et al.* (2007). Available carbohydrate was calculated as: 100 – (moisture + protein + lipid + fiber + ash).

2.3.7 Energy Value

Energy value was determined using the calculation method as stated by Onwuka (2005). The calorific value of a food is given by: Energy value (kcal/100 g) = (Available carbohydrate × 4) + (Protein × 4) + (Fat × 9).

2.4 Determination of Phytochemical Composition

The confirmatory qualitative tests and quantitative phytochemical analyses were done according to the standard methods of Yadav and Agarwala (2011) and Harborne (1998).

2.4.1 Alkaloids

Alkaloids were determined using the method described by Harborne (1998). Five grams (5.0 g) of each sample was introduced into a 250 mL beaker with 200 mL of 20% acetic acid in ethanol, covered and allowed to stand at 25 °C for 4 hours. It was filtered and concentrated to one-quarter of the original volume by means of a water bath. Concentrated ammonium hydroxide was carefully added until precipitation was complete. The precipitate was collected, washed with 1 N NH4OH, filtered through pre-weighed filter paper and dried in the oven at 80 °C. The alkaloid content was calculated as a percentage of the sample weight analysed (Harborne, 1998).

2.4.2 Saponin

Saponin content was determined according to Harborne (1998). Five grams (5.0 g) of each sample was weighed into a 250 mL conical flask, and 100 mL of acetic acid in 20% ethanol was added. The flask was allowed to stand for 24 hours in a water bath at 50 °C. The sample was filtered and the extract concentrated to a quarter of the original volume. Concentrated NH4OH was carefully added until precipitation was complete. The precipitate was verified and calculated for saponin content (Harborne, 1998).

2.4.3 Tannin

Tannin content was determined using the method described by Harborne (1998). Twenty grams (20 g) of each crushed sample was soaked in 100 mL of petroleum ether and covered for 24 hours. The samples were filtered and the petroleum ether evaporated for 15 minutes. It was re-extracted for 4 hours by soaking in 100 mL of 10% acetic acid in ethanol. The sample was filtered and the filtrate collected. To precipitate the alkaloids, 25 mL of ammonium hydroxide was added. The alkaloids were heated on an electric hot plate to remove any ammonia still in solution. The volume was made up to 33 mL. Five millilitres (5 mL) was taken and added to

20 mL of ethanol. The titrant was 0.1 M NaOH and the indicator was phenolphthalein. The pink endpoint was observed and the tannin content was calculated (Harborne, 1998).

2.4.4 Phytate

Phytate content was determined using the method of Young and Greaves (1940) as adopted by Markakes (1975). Two grams (2.0 g) of each sample was weighed into a 250 mL conical flask. The samples were soaked in 100 mL of 2% concentrated HCl for 3 hours and then filtered. About 50 mL of each filtrate was placed in a 250 mL beaker and 100 mL distilled water was added. Ten millilitres (10 mL) of 0.3% ammonium thiocyanate solution was added as an indicator. Standard iron (III) chloride solution (0.00195 g iron per 1 mL) was used for the titration. The readings were noted and phytic acid content calculated (Young and Greaves, 1940; Markakes, 1975).

2.4.5 Flavonoid

Flavonoid content was determined according to Harborne (1998). Ten grams (10 g) of each sample was weighed and extracted using 100 mL of 80% aqueous methanol at room temperature. The solution was filtered using filter paper. The filtrate was transferred into a crucible and evaporated to dryness over a water bath, after which it was weighed at constant weight. Flavonoid content was calculated as a percentage of the sample weight (Harborne, 1998).

2.4.6 Total Phenol

Total phenol content was determined using the method described by Edeofa *et al.* (2005) and Harborne (1998). The fat-free sample was boiled with 50 mL of diethyl ether. 0.5 mL of the boiled extract was pipetted into a 50 mL flask, then 10 mL of distilled water was added. After the addition of distilled water, 2 mL of NH4OH solution and 5 mL of concentrated methanol were also added. The sample was made up to the mark and left to react for 30 minutes for colour development. This was measured at 505 nm (Edeofa *et al.*, 2005; Harborne, 1998).

2.5 Analysis of Minerals and Micronutrients

The mineral content of the samples was determined as described by Adinu *et al.* (2016). One gram (1.0 g) of each sample was digested in 15 mL aqua regia reagent (perchloric:nitric acid in the ratio of 1:2) for 1 hour. Thereafter, the digest was cooled, filtered and made up to 50 mL in a volumetric flask, and then analysed using the Buck Scientific Flame Atomic Absorption Spectrometer (AAS) (Adinu *et al.*, 2016).

2.6 Statistical Analysis

Data were analysed with the Statistical Package for Social Sciences (SPSS) version 18. The analysis of variance (ANOVA) method was used to compare any significant differences between the samples. Values were expressed as means ± standard deviations of replicate determinations. Mean differences were considered significant at $p < 0.05$.

3.0 RESULTS

3.1 Proximate composition

The proximate composition of the four legume seeds is presented in Table 1. All parameters differed significantly ($p < 0.05$) among the species. Moisture content was highest in black turtle bean (1.02%) and

lowest in African black bean (0.24%). Crude protein was highest in red kidney bean (18.87%) and lowest in black turtle bean (12.05%). Crude fat ranged from 0.67% (pigeon pea) to 0.88% (African black bean). Ash content was highest in African black bean (8.88%) and lowest in pigeon pea (6.88%). Crude fiber was highest in African black bean (5.12%) and lowest in pigeon pea (4.25%). Carbohydrate was highest in black turtle bean (73.82%) and lowest in red kidney bean (66.26%). Energy value was highest in pigeon pea (356.93 kcal/100 g).

Table 1. Proximate composition of the four legume seeds (mean ± SD, n = 3)

Parameter	Pigeon pea	Black turtle	Red kidney	African black
Moisture (%)	0.28±0.01c	1.02±0.01a	0.34±0.01b	0.24±0.01d
Crude protein (%)	14.61±0.60c	12.05±0.05d	18.87±0.03a	18.00±0.02b
Crude fat (%)	0.67±0.01c	0.83±0.04b	0.85±0.01b	0.88±0.01a
Ash (%)	6.88±0.02c	7.23±0.02b	8.68±0.02a	8.88±0.02a
Crude fibre (%)	4.25±0.02d	5.01±0.01c	5.06±0.01b	5.12±0.01a
Carbohydrate (%)	73.23±0.10b	73.82±0.02a	66.26±0.18d	67.45±0.49c
Energy (kcal/100 g)	356.93±0.82a	350.95±0.83b	347.76±0.58c	348.46±0.76c

Values in the same row with different superscript letters differ significantly ($p<0.05$).

3.2 Mineral content

The mineral contents of the four seeds are shown in Table 2. Black turtle bean had significantly higher Fe (2.67 mg/kg), Zn (11.71 mg/kg), Mn (1.50 mg/kg) and Se (7.19 mg/kg) than the other species, while pigeon pea had the highest Cu (1.03 mg/kg). African black bean recorded the lowest Fe (1.06 mg/kg) and Zn (3.70 mg/kg).

Table 2. Mineral content of the four legume seeds (mg/kg, mean ± SD, n = 3)

Mineral	Pigeon pea	Black turtle	Red kidney	African black
Fe	2.25±0.01b	2.67±0.03a	1.88±0.02c	1.06±0.06d
Zn	6.82±0.02b	11.71±0.26a	3.84±0.17c	3.70±0.06c
Cu	1.03±0.03a	0.56±0.03b	0.42±0.01c	0.45±0.01c
Mn	0.63±0.02b	1.50±0.01a	0.26±0.02d	0.38±0.01c
Se	5.89±0.02d	7.19±0.08a	6.54±0.42c	6.86±0.01b

Values in the same row with different superscript letters differ significantly ($p<0.05$).

3.3 Antinutrient Composition

The antinutrient composition is presented in Table 3. African black bean had the highest alkaloid (2.84%), tannin (2.50%), and phenol (1.62%) and oxalate (0.41%) contents. Red kidney bean had the highest flavonoid content (4.17%). Pigeon pea had the highest saponin content (4.24%), and black turtle bean had the highest phytate content (0.24%). Flavonoids and saponins were the most abundant antinutrients overall.

Table 3. Antinutrient composition of the four legume seeds (%, mean ± SD, n = 3)

Antinutrient	Pigeon pea	Black turtle	Red kidney	African black
Alkaloid	1.58±0.02c	2.31±0.19b	2.46±0.11b	2.84±0.03a
Flavonoid	2.82±0.02c	2.47±0.02d	4.17±0.05a	4.02±0.05b
Saponin	4.24±0.03a	2.34±0.04d	2.48±0.01c	3.23±0.13b
Tannin	1.77±0.02d	1.86±0.03c	2.06±0.04b	2.50±0.06a
Phenol	1.08±0.01d	1.22±0.02c	1.28±0.01b	1.62±0.02a
Oxalate	0.18±0.01d	0.22±0.01c	0.33±0.01b	0.41±0.01a
Phytate	0.21±0.01b	0.24±0.01a	0.18±0.01c	0.21±0.05b

Values in the same row with different superscript letters differ significantly ($p<0.05$).

4.0 DISCUSSION

The results obtained from the proximate analysis carried out on pigeon pea, red kidney beans, black turtle beans and African black beans seeds showed moisture contents as follows: pigeon pea 0.28%, black turtle beans 1.02%, red kidney beans 0.34% and African black beans 0.24%. At a moisture level of <10%, respiration in most food grains almost stops, increasing the grain storage life (Sujeetha, 2014). The moisture content of all four bean species studied was less than 10%; therefore, they fall within the optimum moisture content range for safe storage. Red kidney beans had the highest moisture content among the four bean species studied. Abu (2001) reported 1.2–1.9% and Mustapha and Magdi (2003) reported 8.33–8.58% for the moisture content of kidney beans. The crude protein content was highest in red kidney beans (18.87%) and African black beans (18.00%), while black turtle beans had the lowest (12.05%). These values are comparable to literature reports for legumes. The ash content was highest in African black beans (8.88%), indicating a higher mineral content compared to other samples. This agrees with the result recorded by Ifemeje *et al.* (2021), where African black beans had high ash content.

The antinutrient analysis showed that all four bean species contain alkaloids, flavonoids, saponins, tannins, phenols, oxalate and phytate, but at levels that are not toxic. Alkaloid content was highest in African black beans (2.84%) and lowest in pigeon pea (1.58%). Flavonoid content was highest in red kidney beans (4.17%); flavonoids are widely reported in the literature to possess antioxidant potential, but antioxidant activity was not assessed in the present study, and no such correlation can be inferred from the present data. The saponin content of pigeon pea (4.24%) was the highest among the samples. Tannin content was higher in African black beans (2.50%) and red kidney beans (2.06%) compared to black turtle beans and pigeon pea. These antinutrients can limit the bioavailability of nutrients, but most are destroyed by heat during processing (Sanchez *et al.*, 2015; Bora, 2014).

The mineral analysis showed that black turtle beans had the highest iron (2.67 mg/kg) and manganese (1.50 mg/kg) content, while pigeon pea had the highest copper (1.03 mg/kg). These results agree with the findings of Ifemeje *et al.* (2021), who reported similar trends in mineral distribution among these legumes. Iron plays a role in the transportation of oxygen to tissues (Holland *et al.*, 1991), and black turtle beans had the highest content when compared to other samples. Copper is essential because it is a component in several proteins indispensable for life. Copper deficiency leads to bone malformation during development (Allen *et*

al., 1982) and contributes to osteoporosis in adults (Colan *et al.*, 1990). It has also been associated with altered immune responses and increased frequency of infection (Bonham *et al.*, 2002).

5.0 CONCLUSION

This study showed that pigeon pea, red kidney beans, black turtle beans and African black bean seeds are rich in protein, minerals and phytochemicals. The four bean species have moisture contents below 10%, which makes them suitable for safe storage. However, they contain antinutrients such as alkaloids, flavonoids, saponins, tannins, phenols, oxalate and phytate, which can limit nutrient bioavailability. Processing methods such as soaking, boiling and fermentation are recommended to reduce antinutrient levels before consumption. The results from this study will serve as baseline data for nutritionists, food industries and nutraceutical companies for the formulation and production of food for healthy living.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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