Assessment of Chemical Potentials in Three Different Types of Legumes for Managing Diabetics

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DOI: https://doi.org/10.51584/IJRIAS.2025.10040095

Received: 11 April 2025; Accepted: 19 April 2025; Published: 23 May 2025

ABSTRACT

Legumes provide basic nutrition, rich sources of bioactive phytochemicals which are beneficial in various health conditions. The aim of the research work was to assess three legume species for their chemical composition. The samples of Urad bean (*Vigna mungo*), Yard bean (*Phaselous vulgaris*) and Black bean (*Phaselous vulgaris*) were brought from Abuja, prepared and analyzed for their phytochemicals, antioxidants and glycemic index compositions using standard methods. The results of the chemical composition of the samples, Urad bean, Yard bean and Black bean respectively for. phytochemical screening for both water and ethanol extract showed the presence of cardiac glycoside, protein and saponin in the three samples. The antioxidant properties were total phenol (42.24, 6.64 and 11.75) mg/g, ferric reducing antioxidant (28.18, 31.04 and 37.41) mg/g, iron chelating (63.80, 67.26 and 75.30) mg/g and DPPH (2, 2 –diphenyl-1-picryhydrazyl) (47.28, 53.42 and 58.46 %). The anti-nutritional composition were oxalate (0.00 mg/g, 0.00 mg/g and 0.00 mg/g) phytate (5.01, 4.88 and 5.96 %), alkaloid (8.61, 5.21 and 11.20) % and saponin (6.02, 5.57 and 6.78 %). The starch, amylopectin, amylose and glycemic index of yard bean, black bean and urad bean respectively were (9.20, 10.52 and 17.44 %), (41.63, 43,37 and 49.79 %), (50.83, 53-89 and 67.13 %) and (34.17, 24.68 and 36.38 %). In conclusion the three seeds contained good and adequate antioxidant properties that could help in fighting various cardiovascular and other diseases. The low glycemic index in the seeds proved them to be good sources of foods required by diabetic patients to manage diabetic disease and other related diseases.

INTRODUCTION

Black beans (*Phaselous vulgaris*), belongs to the family Fabacea (*Leguminosae*) and is genus of phaselous. The seed are called “maa ki” in india,” “kala ghevada” in Maharashtrian cuisine and in Spanish called “poroto negros”, they are also known as turtle bean and Tampioco bean respectively (Flipse, 2010). The black bean is a native of Americas; it has a dense, meaty texture, which makes it one of the vegetarian foods. The black bean is also used as soup ingredient in Cuba, usually served with white rice. It was first widely grown in the present-day United States after the Mexican-American War. However, initially, the variety was primarily grown as a snap pea (for the edible seed pod). Black beans (plate 1) are annual herbaceous plants with a climbing habit, growing best in soil with a pH of 6.0-6.5. They flourish in warm weather, germinate in 10-14 days, and reach maturity in about 100 days. Black beans can be harvested when the pods become dried and yellow (Lauren, 2019). The leaves can grow 6 to15 cm long and 3 to11 cm wide. The black bean produces white, pink, lilac or purple flowers which are approximately 1 cm (0.4) in diameter, and bean pods 8 to 20 cm long and 1 to 1.5 cm wide which can range in colour from green to yellow or black to purple.

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

Plate 1: Dried seeds of black beans

Urad bean is a grain legume, it belongs to family *legumiloseae*, sub-family *papilionaceae* originated in south and southeast Asia (Indian sub-continent) but widely grown in India, Pakistan, Bangladesh, Myanmar, Thailand, Philippines, China and Indonesia (Naga *et al*., 2006). Urad bean is famous as black gram, minapa pappu, mungo bean or black matpe bean and botanically called urad bean. Black lentil is a split black gram and after removing black skin it is sold as white lentil. Urad bean is mainly a day neutral warm season crop commonly grown in semi-arid to sub-humid low land tropics and sub-tropics. It's grown in cropping systems as a mixed crop, cash crop, sequential crop besides growing as sole crop under residual moisture conditions after the harvest of rice and also before and after the harvest of other summer crops (Parveen *et al*., 2011). The crop is resistant to adverse climatic conditions and improves the soil fertility by fixing atmospheric nitrogen in the soil (Parveen *et al.*, 2011). Most suitable climate to cultivate Urad bean is 27ºC to 30ºC with heavy rainfall. This annual crop prefers loamy soil which has high water preservation capability. Urad bean grows normally in 90 to120 days. *Urad bean* is an erect, fast-growing annual, herbaceous legume reaching 30-100cm in height. It has a well-developed taproot and its stems are diffusely branched from the base. Occasionally, it has a twining habit and it is generally pubescent. The leaves are trifoliate with ovate leaflets, 4 to 10cm long and 2 to7cm wide. The inflorescence is borne at the extremity of a long (up to 18cm) peduncle and bears yellow, small *papilionaceous* flowers. The fruit is a cylindrical, erect pod, 4 to7cm long and 0.5cm broad. The pod is hairy and has a short hooked beak (Jansen, 2006). It is a nitrogen-fixing legume that improves soil fertility and soil physical properties (Parashar, 2006). Its cultivation does not require N-fertilization but N fixation is improved by inoculation with local rhizobium strains (Sharma et al., 2011). Harvesting of Urad bean seeds can be done by picking the pods and by uprooting or cutting the whole plants. The crop residues (stems, leaves and empty pods) are used for fodder (Fuller, 2004). It serves as soil improver, intercropping spices and weed control (Ferrero, 2005).

Plate 2: Dried seed of *urad* beans

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

Yard long bean is a type of *Phaselous vulgaris* seed that belongs to *leguminosae* family, usually considered as a sub species of *Vigna unguiculata* of genus *Vigna.* Yard long bean is phenotypically different from cowpea and other species of *Vigna*. Its physiological and morphological traits make them differ (Newman, 2006). In different countries, it is known by various name such as asparagus bean, barboti, bora, string bean, podded bean, pole sitao, kusasagemae, chori, dawgauk (Ano, 2008). Growth habit notified in yard long bean is dwarf, bush and climbing, pole. (Ofori and Klogo, 2005). Various colour of flower are found in yard long bean plant such as light purple, dark purple, white and pink. Pod shows light green and dark green colour while some show purple with green tip and light green with purple tip (Rambabu *et al*., 2016). Seeds are kidney shaped and from red colour to deep red, off white, red with white tip, brown, buff and black colour. The seed eyed pattern varied from small black ring, dark red ring, large mottled brown ring, brown holstien and red holstien pattern. (Rambabu *et al*., 2016). Yard long bean is a dry season tropical crop, which favours hot temperatures. It really needs a tunnel or a glasshouse to get any sort of worthwhile crop, although it has been known to produce beans outside in hot summers. Sow into small pots inside to get the plants going. Transplant into the final position once all risk of frost has passed. They are used to growing under dry conditions. Yard long beans are not troubled by too many pests and diseases, but may develop low levels of red spider mite if conditions are very hot and dry.

Plate 3: Dried seed of Yard beans.

Antioxidants are substances which can delay or inhibit oxidation processes (Lobo *et al*., 2015). According to Satish (2015), antioxidants are naturally occurring plant substances that protect the body from damage caused by harmful molecules. They are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent (Olusegun *et al*. 2019). Oxidation reactions are known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts (Janka *et al*., 2012). Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves. Though oxidation reactions are crucial for life, they can also be damaging (Lobo *et a*l., 2015). Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and aging (Ilaria *et al* ,.2011).

Phytochemicals are chemicals of plant origin, bioactive non-nutritive plant chemicals that have protective or disease preventive properties such as anti-carcinogenic, anti-mutagenic, anti-inflammatory, and anti-oxidant properties. They protect plants from disease and contribute to the plant's colour, aroma and flavour (Shnko *et al*., 2015). Plant chemicals also protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Vibha *et al*., 2016). In wide-ranging dietary, phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Ibrahim & Fagbohun, 2012). Phytochemicals accumulate in different parts of the plants such as roots, stems, leaves, flowers, fruits or seeds (Arbuntari *et al*., 2018). Many phytochemicals, particularly the pigment molecules are often concentrated in the outer layers of the various plant tissues (May *et al*., 2012). Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (Onyeka and Nwambekwe, 2007).

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Glycemic index is a ranking of foods from 0 to 100 based on how quickly and how much they raise blood sugar levels after being eaten. It's a tool that helps in making better choices of foods before consumption (Atkinson *et al*., 2008). This is related to how quickly a carbohydrate containing food is broken down into glucose. In other words, the GI is a measure of the effects of carbohydrates on blood sugar levels (Barclay *et al*., 2010). Low GI foods produce a slower, lower rise in blood sugar levels. High GI foods produce a faster, higher rise in blood sugar levels. Low GI foods have a GI of less than 55 Medium GI foods have a GI between 55 and 70 High GI foods have a GI greater than 70 (Brand *et al*., 2009).

The glycemic load (GL) of food is a number that accounts for how much carbohydrate is in the food and how much each gram of carbohydrate in the food raises blood glucose levels. The GL combines both the quality and the quantity of carbohydrate into one value (Shyam *et al*., 2012). GL is a more accurate way to predict the impact on blood glucose of different types and amounts of food. Glycemic load is based on the glycemic index (GI) and is calculated by multiplying the grams of available carbohydrate in the food by the food's glycemic index, and then dividing by 100. Foods with a GL ≤10 have been classified as low GL and those with a value ≥20 as high GL (Sobolewskiet *et al*., 2010). In healthy individuals, stepwise increases in GL have been shown to predict stepwise elevations in postprandial blood glucose and insulin levels (Solomon *et al*., 2010).

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (Dickinfabian and Huynh, 2010). Insulin is the hormone that regulates blood sugar. Hyperglycaemia, or raised blood sugar is a common effect of uncontrolled diabetes and over time, leads to serious damage to many of the body's systems especially the nerves and blood vessels (Furnary *et al*., 2001). Type 1 diabetes (previously known as insulin-dependent, juvenile or childhood-onset) is characterized by deficient insulin production and requires daily administration of insulin. Symptoms include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger, weight loss, vision changes, and fatigue. These symptoms may occur suddenly (Golden*et al*., 2000). Type 2 diabetes (formerly called non-insulin-dependent, or adult-onset) results from the body's ineffective use of insulin. It is the majority of people with diabetes around the world, and is largely the result of excess body weight and physical inactivity (Pomposelli *et al*., 2001). Symptoms may be similar to those of type 1 diabetes but are often less marked. As a result, the disease may be diagnosed several years after onset once complications have already arisen. Until recently, this type of diabetes was seen only in adults but it is now also occurring increasingly frequently in children (Furnary *et al*., 2001). Gestational diabetes is hyperglycaemia with blood glucose values above normal but below those diagnostic of diabetes, occurring during pregnancy (Golden and Peart, 2000). Women with gestational diabetes are at an increased risk of complications during pregnancy and at delivery. They and their children are also at increased risk of type 2 diabetes in the future. Gestational diabetes is diagnosed through prenatal screening rather than through reported symptoms.

MATERIALS AND METHODS

Materials

The three varieties of the seeds (black bean, yard bean and urad bean.**)** were purchased from Karmo market Abuja. They were identified and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Ondo State**.** The undesirable impurities were removed by hand picking and the seeds were prepared for analysis by blending them into powdery, using high speed blender. The powdered samples were sieved, packed in air tight containers and kept in the refrigerator at 4^0C prior to laboratory analysis.

Methods

Qualitative Phytochemical Determination

Test for Tannins

About 5.0g of sample from each species was boiled with 10ml of distilled water and filtered. Two drops of 5% ferric chloride ($Fecl_3$) were added to 1ml of each extract. A dirty green precipitates indicates the presence of tannins (Sofowora, 2005)

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Tests for Flavonoids

About 5ml of dilute ammonia solution was added to a portion of aqueous filtrate of each sample followed by addition of concentrated sulphuric acid. A yellow colouration was observed, indicating the presence of flavonoids (Karunyadevi *et al*., 2009).

Test for Saponins

About 2g of the sample was boiled in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for staple persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and then observed for the formation of emulsion. Formation of a stable emulsion indicated the presence of saponins (Karunyadevi *et al.*, 2009).

Test for Alkaloids

The method used was prescribed by (Karunyadevi *et al*., 2009). About 0.5g of dried sample was boiled in 20ml of water in the test tube and then filtered. A few drops of 0.1% of $FeCl_2$ was added and observed for brownish colouration.

Test for Terpenoids

5ml of the sample extract was mixed in 2ml of chloroform and Conc. H_2SO_4 (3ml), it was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids (Sofowora, 2005).

Test for Cardiac Glycosides

About 1ml of the extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution 1ml of Conc. H_2SO_4 was added and brown colour was obtained at the interface indicating the presence of a deoxy sugar. A violent ring may appear below the brown ring while in the acetic acid layer, a green ring may form just above the brown ring and gradually spread throughout this layer. (A.O.A.C, 2005)

Quantitative Determination of Anti–Nutrients

Determination of Oxalate

Total oxalates were determined according to the procedure of (Ologbo *et al*., 2000). 1.0g of the sample was weighed and 75ml of 0.75 M H_2SO_4 solution was added. The mixture was carefully stirred intermediately with magnetic stirrer for one hour and then tiltrated using whatman No1 filter paper. 25 ml of the filtrate was collected and then filtered hot (80-90^oC) against 0.05M $KMNO_4$ solution till the end point of a faint pink colour appeared that persisted for at least 30 minutes. Then the amount of oxalate in each sample was then calculated by:

$$\text{Oxalate (mg/g)} = \frac{V_t \times 0.9004}{W}$$

Where:

V_t = volume of 0.05M $KMnO_4$ used for titration;

W = weight of sample.

Determination of Phytate

Exactly 4.0g of sample was soaked in 100ml of 2% HCl solution for three hours and filtered through Whatman No 2 filter paper. 25 ml of the filtrates was placed in a conical flask and 5ml of 0.3% ammonium thiocyanate (NH_4SCN) solution was added, after which 53.5 ml of distilled water was added. The solution was titrated

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against a standard iron (III) chloride solution containing 0.00195 g/ml until brownish yellow colour persisted for five minutes. The phytate content was expressed as percentage phytate in the sample (Ola and Oboh, 2000).

$$\text{Phytate (\%)} = \frac{T \times 0.00195 \times 1.19}{2} \times 100$$

Where: T- Volume of standard iron (iii) chloride solution used for titration.

Determination of Saponin

Saponin was determined using the procedure of Oloyede (2005). To 2.0g each of the samples in a conical flask was added 100ml of 20% (v/v) aqueous ethanol. The samples were heated over a hot water bath for 4 hours at 55ºC with constant shaking. The mixtures were filtered and the residues were re-extracted using 100ml of 20% aqueous ethanol. The combined extracts of each sample was reduced to 40ml over water bath at about 90ºC. The concentrate was transferred in a 250ml separatory funnel and 250ml of diethyl ether was added, shaken vigorously and allow to separate. The aqueous layer (lower portion) was recovered while the ether layer was discarded. The purification process was repeated. The combined aqueous was extracted using 60 ml of n -butanol. The combined n -buthanol extracts (upper portion) was washed twice with 10ml of 5% aqueous extract NaCl. Finally, the remaining solution was heated over hot water bath to evaporate to dryness and then dried in the oven at 105^{O}C to constant weight. The saponin constant was calculated as percentage as shown.

$$\text{Saponin (\%)} = \frac{W_r}{W} \times 100$$

Where:

Wr = Weight of saponin residue;

W = Weight of the sample.

Determination of Alkaloids. (REF)

5.0g of each sample was placed in a 250ml beaker and 200 ml of 10 % (v/v) ethanoic acid (CH_3COOH) in ethanol (C_2H_5OH) was added. The mixture was covered and allowed to stand for 4 hours at 25ºC. It was then filtered with filter paper and the filtrate was concentrated on a water bath until it reached a quarter of its original volume. Concentrated NH_4OH was added drop-wise until precipitation was completed. The mixture was allowed to settle and the precipitate collected on a previously dried and weighed filter paper. The residue was washed with dilute NH_4OH. The precipitate, alkaloid, was dried and weighed. The percentage alkaliod was calculated by difference from:

$$\text{Alkaloids (\%)} = \frac{W_2 - W_1}{Weight\ of\ sample} \times 100$$

Where:

W_1-Weight of dried empty filter paper;

W_2- Weight of filter + alkaloids residue.

Evaluation of Antioxidant Activity

Preparation of the Extract

The sample was homogenized using blender and the homogenate was then stored at 4^{o} C in a refrigerator. Distilled water was used for extraction of phytonutrients using soxhlet extraction method. The extraction was carried out for 6h. The extracts were concentrated at 55ºC using rotary evaporator and resultant residues were then made-up to 50ml and stored under refrigerated at 4^{o} C prior to analysis.

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Determination of Total Phenol Content

The phenolic contents were determined using Follin-Ciocalteu reagent and expressed as Gallic Acid Equivalents (GAE) (Singleton *et al*., 1999). The extracts were diluted with methanol by taking 3ml of methanol and 1ml of crude extract solution. To this sample solution, 1ml of 5-fold diluted Folin Ciocalteu's reagent was added. The contents were mixed well, kept for 5 minutes at room temperature followed by the addition of 1ml of 10 % aqueous sodium carbonate. After incubation at room temperature for one and half hour, the absorbance of the developed blue colour was read at 760nm (Shimadzu UV-1650 PC Shimadzu Corporation, Kyoto, Japan) against blank. Gallic acid (100-1000 mg/mL) was used to construct the calibration curve. Results were calculated as garlic acid equivalent (mg/g) of samples. The determination was done in triplicates and concentrations of phenolic compounds were calculated from obtained standard garllic acid graph.

DPPH Radical Scavenging Activity

Free radical scavenging activities of the extracts were determined using a stable 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) (Brand-Williams *et al*., 2003). DPPH is a free radical of violent colour. The antioxidants in the sample scavenge the free radicals and turn it into yellow colour from violet which was proportional to the radical scavenging activity. The assay contained 1ml of 0.1mM DPPH in methanol and varying concentrations of extracts (50-1000 ug/ml) methanol and standards in the same solvent and made up to 3.5ml with methanol. The contents were mixed immediately and then incubated for 30min at 30^{o}C in water bath. The degree of reduction of absorbance was recorded in UV-Vis spectrophotometer at 517nm. The percentage of scavenging activity was calculated as:

$A\% = (Ac - As)/Ac \times 100$

Where:

Ac = Absorbance of control (without sample);

As = Absorbance of sample.

Iron Reducing Power Assay

The reducing power of the sample was determined according to the method described by Oyaizu *et al*, (1986). 1ml of the plant extracts were mixed with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The reaction mixture was incubated at 50°C for 20minutes. After incubation period, 2.5ml of 10% trichloroacetic acid (TCA) was added and the reaction mixture was centrifuged at 1000rpm for 10min. The upper 2.5ml layer was mixed with 2.5ml of deionized water and 0.5ml of ferric chloride and then thoroughly mixed. The absorbance was measured spectrophotometrically at 700nm. A higher absorbance indicates a higher reducing power.

Determination of Sugars

These were determined by the method of Dubois *et al*., (2000). 100mg of each of the samples was weighed into a 50ml centrifuge tube and 1.0ml 80% ethanol was added. 2ml distilled water was added and mixed thoroughly. Then, 10ml hot 80% ethanol was added and mixed thoroughly. The samples were centrifuged at 1400rpm for 5 minutes. Then, the supernatant was carefully decanted into 100ml volumetric flask, followed by addition of 10ml hot 80% ethanol to the residue. The mixture was shaken thoroughly and centrifuged at 1400rpm for 5 minutes, and the supernatant decanted into the same flask. The extraction with hot ethanol was repeated and the flask was made up to volume with distilled water while the residue was kept for starch determination. An aliquot of 1.0ml of the supernatant was pipetted into a test tube and diluted to 2.0ml with distilled water. Thereafter 5% phenol was added and mixed thoroughly. Then, 5.0ml concentrated sulphuric acid was directly added to the liquid surface and not to the sides of the tube in order to obtain good mixing. The tubes were allowed to stand for 10 minutes and shaken thoroughly for proper mixing. The test tube was placed in water bath for 20 minutes at 30^{o}C and the absorbance was measured thereafter at 490nm. The blank was prepared by substituting distilled water for the sugar extract solution while standard glucose curve was prepared from a 100 mg/ml glucose solution.

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Determination of Amylose Content

A 100mg sample of each sample was weighed into a 100ml volumetric flask. Then 1ml of 95% (v/v) ethanol and 9mL of 1 M NaOH were carefully added and samples were heated for 10 minutes in a boiling water bath to gelatinize the starch; the mixture was cooled and made up to volume with water. A 5ml portion of the starch solution was pipetted into a 100ml volumetric flask, 1ml of 1 M ethanoic acid (to acidify the solution) and 2ml of iodine solution (0.20%) were added. This was then made up to volume with distilled water. Thereafter, the mixture was shaken and absorbance was read at 620nm using spectrophotometer after 20 minutes. A calibration curve was prepared from a standard amylose solution containing 100mg/ml. Amylose content of the sample was determined from the standard curve and expressed on percentage basis.

Determination of Amylopectin Content

Amylopectin in samples was calculated by difference using following formula:

Amylopectin (%) = % Total starch – Amylose (%)

***In - vitro* starch hydrolysis and estimation of glycemic index**

The *in vitro* method of Goni *et al*., (1997) as modified by Oboh *et al*., (2015) was used. The aim of the *in vitro* starch hydrolysis was to simulate the gastrointestinal tract (GIT) starch digestion. Theoral phase was simulated by means of mechanical disaggregation of 50 mg of food portions. The gastric phase was developed for 1h at 37 ^{o}C with 10 ml of HCl - KCl buffer (pH =l.5) and pepsin. The intestinal phase was carried out in sodium potassium phosphate buffer 0.05M pH 6.9 containing crude pancreatic amylase extracted from swine gut.

50mg of each sample was incubated with 1 mg of pepsin in 10 ml HCl-KCl buffer (pH 1.5) at 40^{o}C for 60 minutes in a shaking water bath. The digest was diluted with 7.5ml of phosphate buffer (0.05M, pH 6.9) and then, 2.5ml of alpha amylase solution containing 0.005g/10 ml was added. Samples were then incubated at 37^{o}C in a shaking water bath for 60 minutes. On expiration of the time, 0.2 ml aliquot was taken from each tube at 0, 30, 60, 90, 120, 150 and 180 minutes intervals and boil in a water bath at 100^{o}C for 5 minutes to inactivate the enzyme. Then 0.5ml sodium acetate buffer (0.4M, pH 4.7) was added and the residual starch digested to glucose by adding 7 ml of alpha- glucosidase solution extracted from swine gut in phosphate buffer (1:4). The mixture was incubated for 45 minutes at 60^{o}C after which 0.2ml of 3, 5- dinitrosalicylic acid (DNSA) was added. The enzyme reaction was terminated by boiling the mixture at 100^{o}C in a water bath for 5 minutes. About 2ml of distilled water was added and the mixture was centrifuged at 2000 rpm for 10 minutes. Then, the absorbance of the supernatant was taken at 450nm using a spectrophotometer. Standard white bread was also analyzed as reference product. The rate of starch digestion was expressed as the percentage of starch hydrolyzed per time using glucose standard curve. A nonlinear model established by Goni *et al*. (1997) was applied to describe the kinetics of starch hydrolysis. Values for the area under the curve (AUC) were

obtained for each of the starch hydrolysis curves and standard from which the glycemic index (GI) was calculated using the equation:

$$\text{GI (\%)} = \frac{AUC\ of\ sample}{Average\ AUC\ of\ std} \times 100$$

RESULTS AND DISCUSSION

Results

Table 1: Phytochemical Screening of Yard bean, Black bean, and Urad bean seeds using aqueous extract

Phytochemicals	Yard bean	Black bean	Urad bean
Saponin	++	++	++

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Tannin	-	-	-
Steriod	-	-	-
Flavoniod	-	-	-
Terpenoid	-	+	-
Cardiac glycoside	-	+	+
Anthraquinone	-	-	-
Protein	+	+	+
Alkaliods	-	+	-

+ = Present;

++ = Abundantly present;

- = Absent.

Table 2*:* Phytochemical Screening of Yard bean, Black bean, and Urad bean seeds using ethanol extract**.**

Phytochemical	Yard bean	Black bean	Urad bean
Saponin	-	-	-
Tannins	-	-	-
Steriods	-	+	-
Flavioniods	-	-	+
Terpeniod	+	+	+
Cardic glycoside	+	+	+
Anthraguinone	-	-	-
Protein	+	+	+
Alkaniod	-	+	+

+ = Present;

- = Absent.

Table 3: Antioxidant properties of Yard bean, Black bean and Uradbean seeds**.**

Sample	Yard bean	Black bean	Urad bean
Total phenol(mg/g)	4.24±0.09	4.98±0.09	8.81±0.23
FRAP(mg/g)	28.17±0.61	31.03±0.28	37.40±0.61

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Fe chelating (mg/g)	62.47±0.46	67.26±0.72	75.30±0.60
DPPH (%)	47.27±0.62	53.42±0.56	58.40±0.58

FRAP- Ferric reducing antioxidant properties;

DPPH- 1, 1-Diphenyl-2-picrylhydrazyl

Antioxidant Properties

Table 4: Anti-nutritional composition of yard bean, black bean and urad bean seeds

Anti – nutrient	Yard bean	Black bean	Urad bean
Oxalate (mg/g)	0.00±0.00	0.00±0.00	0.00±0.00
Phytate (%)	5.01±0.01	4.88±0.00	5.96±0.01
Alkaloid (%)	8.61±0.01	5.21±0.02	11.20±0.03
Saponin (%)	6.02±0.12	5.57±0.00	6.78±0.11

Table 5: Sarch, Amylose, Amylopectin and Glycemic index of Yard bean, Black bean and Urad bean seed

Sample	**Yard bean**	**Black bean**	**Urad bean**
Starch (%)	9.20±0.22	10.52±0.27	17.44±0.19
Amylopectin (%)	41.63±0.54	43.37±0.32	49.79±0.36
Amylose(%)	50.83±0.55	53.87±0.49	67.13±0.22
Glycemic index(%)	34.17±0.95	24.69±0.95	36.38±0.14

Note:

Low glycemic index: 0-54

Middle glycemic index: 55-56

High glgcemic index: 70 above

(Foster-powell et al., 2002)

Phytochemical Screening

Phytochemical contents in both aqueous and ethanol extracts of the samples are depicted in Tables 1 and 2 respectively. The phytochemical screening conducted on the seeds of yard bean, black bean and urad bean seeds with aqueous extract reveal the presence of saponin and protein. According to (Amira et *al.*,2017*)* saponins have potential therapeutic benefits and are theorized as an alternative medication in decreasing serum blood glucose level in patients suffering from diabetes. Black bean reveal the presence of terpeniod. Terpeniod is used for antimicrobial activities because of the increase in antibiotic resistant bacteria, which is occurring globally and at

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

an alarming rate. Addition of terpenoids into livestock feed may replace conventional antibiotic addition, which in turn would slow the rate of antibiotic resistance in bacteria (Sam and Chhandak 2008). Black bean and Urad bean showed the presence of cardiac glycoside. Alkaliods display antimicrobial and anti-parasitic properties, act as narcotics, can alter DNA, have an important role in the immune systems, and treat cardiovascular and metabolic disorders, inflammation, infectious diseases, and miscellaneous problems (Aniszewski, 2015). Black bean reveal the presence of akaliods. When ethanol was used as extract on the three samples, Saponin, tannins and anthraguinone are absent in yard bean, black bean and urad bean seeds. Steriod was present only in black bean while flavonoids were present in urad bean seed. Cardiac glycoside, terpeniods and protein were present in the three samples. Alkanoid was present in black bean and urad bean seeds.

Antioxidant Properties

The result of antioxidant properties of the seeds are presented in Table 3. Antioxidants decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans (Dejian *et al.*, 2005). The antioxidant activities of legumes are directly related on total phenol content (Balwinder *et al*., 2017). Total phenol in yard bean, black bean and urad bean seeds were 4.24mg/g, 4.98mg/g and 8.81mg/g respectively. Phenols are secondary metabolite; they carry out a number of protective functions in human body. It can boost the immune system, protect the body from radicals, kill pathogenic germ and much more keep the body fit (Nwokeji *et al*., 2005). Xu *et al*., 2007 reported that yellow pea contain total phenol content of 0.85mg/g to 1.14 mg/g which were lower to total phenol content in yard bean, black bean and urad bean seeds. Balwinder *et al*., 2017 reported that total phenol content in lentils were within the range of 4.86 mg/g-9.60 mg/g. Ferric reducing antioxidant properties measure the reducing ability which has been investigated from Fe^{3+} - Fe^{2+} transformation. Fe^{3+} reduction is often used as an indicator of electron donating activities, which is an important mechanism of phenolic antioxidant action and can be strongly correlated with other antioxidant properties (Ayoade *et al*.,2015) Ferric reducing antioxidant properties of yard bean, black bean and urad bean seeds were 28.17mg/g, 31.03mg/g and 37.40mg/g respectively. Urad bean has highest (75.30mg/g) iron chelating properties follow by black bean (67.26mg/g) and then yard bean (63.80mg/g). Iron chelate is used to avoid complications of iron overload which can result to death or diseases such as cardiac and hepatic dysfunction (Mason, 2016). DPPH (1,1-Diphenyl-2-picrylhydrazyl) is a stable free radicals, which has been widely accepted as a tool for estimating free radicals scavenging activities of antioxidant (Sancehz-Moremon,2000). The DPPH (%) of yard bean, black bean and urad bean seeds respectively were 47.27,53.42 and 58.40. The value were higher than 17.28 reported for aqueous extract of canarium fruit reported by

Anti-Nutritional Compositions

Compounds or substances which act to reduce nutrient intake, digestion, absorption and utilization and may produce other adverse effects are referred to as anti-nutrients or anti-nutritional factors (Akande *et al*., 2010). Legumes are known to contain a lot of anti-nutritionals which lower the digestibility of legume protein and decrease the absorption of divalent metal ions in the intestine (Ologho *et al* 2011). Oxalate was absent in yard bean, black bean and urad bean seeds. According to Savage 2011, oxalate have deleterious effects on human nutrition and health, mainly by decreasing calcium absorption and aiding in the formation of kidney stones. Yard bean, black bean and urad bean seeds cannot contribute to formation of kidney stone and they will not alter the function of calcium present in the seeds. Phytate present in yard bean, black bean and urad bean seeds were 5.10, 4.88 and 5.98 respectively. Phytate is a neutral component in food plant, serve has storage for phosphorus and account for mineral in seeds. According to Wenche, 2010, legume seeds phytate predominately occurs in the protein bodies of the endosperm, ranging from 0.2 -2.9%. Studies has shown that phytate has anti-oxidative effect, preventing pathological calcification, like kidney stones and calcification in the heart vessels, cholesterol lowering effects and anticancer activity (Lisbeth *et al.*,2015). Alkaloids are large group of compounds with biological, pharmacological and chemical activities. The pharmaceutical industry has succeeded in the use of natural plant alkaloids for the development of anti-malaria agents, anti-cancer agents and agents promoting blood circulation in the brain (Tadeusz, 2007). Alkaloid present in the three seeds were 8.61, 5.21 and 11.20 %. The saponins (%) contents of yard bean, black bean and urad bean seeds are 8.61, 5.21 and 11.20 respectively. These values were higher than 5.60 reported for chickpea seeds and 1.5 reported for faba bean by Kareem et.al (2005) and Sharma and Sehgal (2004) respectively

ISSN No. 2454-6194 | DOI: 10.51584/IJRIAS |Volume X Issue IV April 2025

Sugar, Amylose, Amylopectin and Glycemic index of Yard bean, Black bean and Urad bean seed

Legume-derived resistant starch and slowly digestible starch are also associated with improved glycemic response and lower postprandial glucose concentrations which can improve glycemic control among individuals with insulin resistance and type 2 diabetes (Donna *et al*., 2017). According to Foster-powell *et al*., (2002), low glycemic index ranges from 0% to 54%, middle glycemic index ranges from 55% to 69% while high glycemic index ranges from 70% above. The results from Table 5 show that yard, black and urad bean seeds have low glycemic index of 34.17%, 24.67% and 36.38% respectively. These values are lower than 50.86% reported for glycemic index of Lablab purpures by Amoo et. Al (2023).

CONCLUSION

In conclusion phytochemicals have been confirmed to lower blood glucose level as reported by Oboh *et al*., (2015). Researchers have also suggested that low glycemic index diets improve glycemic control of individuals with impaired glucose tolerance and type -2 diabetes by lowering blood glucose and improving insulin sensitivity (Oboh *et al.,* 2015). This could mean that yard bean, black bean and urad bean seeds can be incorporated into diet meant for glycemic control of diabetes mellitus such as utilization of whole flour in making bread, snacks, bean cake and biscuit.

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