CHEMICAL COMPOSITION, PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT POTENTIALS OF LIMA BEAN SEEDS COAT

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Abstract
Lima bean (Phaseolus lunatus) seeds coat was evaluated for its chemical composition, phytochemical constituents and in vitro antioxidant activity. Antioxidant activity of seeds coat flour was investigated by measuring its DPPH (1,1-diphenyl-2-picryl hydrazyl) and ABTS (2, 2'-azinobis-3-ethyl-benzothiozoline-6-sulphonic acid) radicals scavenging ability as well as its ferric reducing property. The chemical analysis indicates that the coat have moisture (4.46%), protein (15.75%), fat (0.65%), crude fibre (33.56%), ash (2.57%), carbohydrate (47.52%) on dry weight basis; Zinc (5.6 mg/100 g), Calcium (17.56 mg/100 g), Potassium (398.41 mg/100 g), Sodium (82 20 mg/100 g), Magnesium (87.1mg/100g) and Iron (11.61 mg/100 g). The sample exhibited higher amounts of threonine, valine, isoleucine, tryptophan, leucine, lysine and histidine with total essential amino acid (TEAA) of 51.07 g/100 g protein. Phytochemical screening showed that flavonoids, alkaloids, saponins, tannins and phenolic compound are present and may be responsible for the activity. High performance liquid chromatography with diode detector (HPLC-DAD) analysis showed the presence of phenolics (gallic acid, caffeic acid, ellagic acid, rutin, quercetin and kaempferol) and tocopherol. The seeds coat flour exhibited significant radical scavenging activity against DPPH (IC_{50} value 0.37 mg/ml) and ABTS Trolox Equivalent Antioxidant Capacity (TEAC value = 0.36) and considerable ferric reducing property (56.37± mg ascorbic acid equivalent/g seed coat powder). The seeds coat possesses both nutritional and health benefits due to its antioxidative property, as such a potential source of natural antioxidants.

Keywords
Phaseolus lunatus, coat, composition, antioxidants, polyphenols,

Submitted: 08.09.2014 Reviewed: 11.11.2014 Accepted: 26.11.2014

1. INTRODUCTION

Lima bean (*Phaseolus lunatus* L Walp) belongs to the family Fabacea and genus of Phaseolus. The seeds are called "kapala" (among the Yorubas), "ukpa" (among the Igbos) in South-western and South-eastern Nigeria respectively; where the seeds are commonly consumed among the rural dwellers. *P. lunatus* seeds powder is largely prescribed in traditional medicine for promoting suppuration on application to small cuts on tumours and abscesses (Ezueh, 1977).

The medicinal values of plants lie in their phytochemical components, which produce definite physiological results on the human body (Akinmoladun *et al.*, 2007). Polyphenolics appear to play a significant role as antioxidants in the protective effect of plant-derived foods and medicine (Saxena *et al.*, 2007) and have become the focus of current nutritional and therapeutic interest in recent years. Antioxidants have been of interest to health professionals due to the protective effect against degenerative diseases caused by reactive oxygen species (ROS), reactive nitrogen species (RNS) (Shahidi and Naczk, 2004). Natural antioxidants provide diverse multitude and magnitude of activities and enormous scope in correcting oxidative imbalance in biological system (Komolafe *et al.*, 2013). Epidemiological studies have demonstrated that there is a positive relationship between intake of antioxidant rich diets and lower incidence of degenerative diseases such as cancer, heart disease, inflammation, arthritis, immune system decline (Gordon, 1996). Recently, more attention has

been focused on the potential utilization of agricultural by-products in the development of new functional ingredients for food enrichment to provide an economic alternative for industries and sustainability of the environment (Salgado *et al.*, 2011). Often, agricultural by-products are sources of bioactive compounds with functional properties, such as fibre and phenolics which has antioxidative defense system against some degenerative diseases or disorders in biological system. The proportion of coat (>10% by weight) of the lima bean seeds, is quite high and as such constitutes a kind of environmental nuisance. There is dearth of information on the phytochemical constituents and antioxidant capacity of *P.lunatus* seeds coat hence the present study was undertaken to fill this lacuna.

2. MATERIALS AND METHODS

2.1 Sample collection

Lima bean (*Phaseolus lunatus* L.) with accession number NSWP96 was obtained from the Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria. The cleaned lima beans were cracked with hammer mill, soaked in water for 3 h, dehulled, hull was dried at (60±2 ^{o}C) for 24 h milled and sieved to give 40 mm mesh size flour.

2.2 Proximate analysis

The proximate composition (moisture, crude protein, crude fibre, fat, ash and carbohydrate) was determined by the standard methods described in the AOAC (2005).

2.3 Minerals

The phosphorus content of the flour was determined by the phosphovanado-molybdate (yellow) method (AOAC, 2005). The other elemental concentrations were determined, after wet digestion of sample ash with a mixture of nitric and perchloric acids (1:1 v/v), using Atomic Absorption Spectrophotometer (AAS, Buck Model 20A, Buck Scientific, East Norwalk, CT06855, USA)

2.4 Amino acid composition Analysis

An HPLC system was used to determine the amino acid profiles after sample was hydrolysed for 24 h with 6 M HCl according to the method previously described by Bidlingmeyer *et al*. (1984). The cysteine and methionine contents were determined after performic acid oxidation and tryptophan content was determined after alkaline hydrolysis.

2.5 Phytochemical screening

The sample was screened for the presence of some secondary metabolites according to the methods described by Sofowora (1993).

2.6 Determination of alkaloid

Alkaloid content was determined by the method of Harbone (1973). Five (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 min. This was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle; the precipitate was collected, washed with dilute ammonium hydroxide and filtered. The residue was dried and weighed. The weight difference was taken as the percentage alkaloid present in the sample.

2.7 Determination of total phenol content

The total phenol content was determined by mixing 0.5 ml aliquot of the sample extract with equal volume of water, 0.5 ml Folin–Cioalteu's reagent and 2.5 ml of saturated solution of sodium carbonate and the absorbance was measured after 40 min at 760 nm (Singleton *et al*., 1999). Gallic acid was used as the standard; the total phenol was expressed as mg/g gallic acid equivalent.

2.8 Determination of flavonoid

The flavonoid content was determined using a colorimetric method described by Dewanto *et al*. (2002). Approximately 0.25 ml of the

sample was dissolved in distilled water, 75 µl of 5% $NaNO_2$ solution, 0.150 ml of freshly prepared 10% $AlCl_3$ solution and 0.5 ml of 1 M NaOH solution were added. The mixture was allowed to stand for 5 min and the absorption measured at 510 nm. The amount of flavonoid was expressed as quercetin equivalents.

2.9 Determination of saponin

The spectrophotometric method of Brunner (1984) was used for saponin determination. About 2 g of the sample was weighed into a beaker and 100 ml of isobutyl alcohol (But-2-ol) was added. The mixture was filtered with No 1 Whatman filter paper into a beaker containing 20 ml of 40% saturated solution of magnesium carbonate. The mixture obtained was filtered the second time to obtain a clean colourless solution. Approximately 1 ml of the colourless solution was transferred into a volumetric flask; 2 ml of 5% iron (III) chloride solution was added. The absorbance was read against the blank at 380 nm.

2.10 Determination of tannin

Tannin content was determined according to the method described by Makkar and Goodchild (1996). About 0.2 g of the sample was weighed into a sample bottle, 10 ml of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 h at 30 ^{o}C. The solution was centrifuged and the supernatant stored in ice. Approximately 0.2 ml was pipette into a test tube and 0.8 ml of distilled water was added. Standard tannic acid solution was prepared from 0.5 mg/ml of the stock and the solution made up to 1 ml with distilled water. 0.5 ml of Folin Ciocalteu's reagent was added to the sample and standard followed by 2.5 ml of 20% Na_2CO_3. The solution was vortexed and incubated for 40 min at room temperature, its absorbance was read at 725 nm. The concentration of tannin in the sample was calculated from a standard tannic acid curve.

2.11 Determination of phytate

Phytate was determined according to the method of Wheeler and Ferrel (1971). Sample (4.0 g) was soaked in 100 ml of 2% HCl for 3 h and filtered through Whatman No. 2 filter paper. After which 25 ml of the filtrate was placed in conical flask and 5 ml of 0.3% ammonium thiocyanate solution was added, after which 53.5% of distilled water was added and this was titrated against a standard iron (III) chloride solution until a brownish yellow color persisted for 5 min. The phytate content was expressed as the percentage (%) phytate in the sample.

2.12 Determination of total cyanide

In cyanide determination, AOAC (2005) method was used, about 4 g of sample was soaked in 40 ml of distilled water and 2 ml of orthorphosphoric acid, it was mixed thoroughly and allow to stay overnight at room temperature to set free all bound hydrocyanic acid. The mixture was transferred into flask and a drop of paraffin (antifoaming agent) was added and distilled. About 45 ml of the distillate was collected in a receiving flask that contain 4 ml distilled water containing 0.1 g of sodium hydroxide pellet, the distillate was transferred into 50 ml volumetric flask and made up to mark with distilled water, 1.6 ml of 5% of potassium iodide solution was added to the distillate and titrated against 0.01 M silver nitrate ($AgNO_3$) solution. The blank was also titrated until the end point was indicated by faint pink but permanent turbidity was observed.

$$\text{Calculation (mg/kg)} = \frac{\text{Titre value} \times 0.0217 \times 1000}{M}$$

M = mass of the sample

2.13 HPLC-DAD quantification of phenolics

Reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 15 mm) packed with 5µm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), the composition gradient was: 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 80 min, respectively (Sabir *et al.*, 2012) with slight modifications. Samples were

analyzed at a concentration of 20 mg/mL. The presence of nine antioxidants compounds was investigated, namely: gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, rutin, quercitrin, quercetin and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.6 mL/min, injection volume 50 µl and the wavelength were 254 nm for gallic acid, 280 nm for catechin, 327 nm for caffeic, ellagic and chlorogenic acids, and 365 nm for quercetin, quercitrin, rutin and kaempferol. The samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025 – 0.300 mg/ml for gallic acid, chlorogenic acid, caffeic acid, ellagic acid, catechin, rutin, quercitrin, quercetin and kaempferol. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). All chromatography operations were carried out at ambient temperature and in triplicate.

2.14 HPLC-DAD quantification of β-carotene and tocopherol

Tocopherol and β-carotene analysis were carried out at reverse phase chromatographic under gradient conditions using C_{18} column (4.6 mm × 150 mm) packed with 5 µm diameter particles. The mobile phase consisted of mixtures of ACN: H_2O (9:1, v/v) with 0.25% triethylamine (A) and EtAc with 0.25% triethylamine (B). The gradient started with 90% A at 0 min to 50% A at 10 min. The percentage of A decreased from 50% at 10 min to 10% A at 20 min. The flow-rate was 0.8 ml/min and the injection volume was 40µl. Signals were detected at 450 nm, following the method described by Janovik *et al.* (2012) with slight modifications. Solutions of standards references (tocopherol and β-carotene) were prepared in HPLC mobile phase at a concentration range of 0.035 - 0.350 mg/ml. Sample was analysed at a concentration of 10 mg/mL, carotenoid was identified and quantified in the samples by comparison of retention times and UV spectra with the standard solution. All chromatography operations were carried out at ambient temperature and in triplicate.

2.15 ABTS radical scavenging activity

Total antioxidant activity was determined by the ABTS test described by Re *et al.* (1999). 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot+}$) decolourization The procedure involved pre-generation of $ABTS^{\cdot+}$ radical cation by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate and incubated for 12–16 h in the dark at room temperature until the reaction was completed and the absorbance was stable. The absorbance of the $ABTS^{\cdot+}$ solution was equilibrated to 0.70 (± 0.02) by diluting with water at room temperature. The predetermined volume of $ABTS^{\cdot+}$ solution was mixed with known volume of the test sample. The absorbance was measured at 734 nm after 6 min. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the trolox equivalent antioxidant concentration (TEAC).

ABTS radical scavenging activity (%) = 100-[Ac/As]×100

Ac – absorbance of sample, As- absorbance of control

2.16 DPPH radical scavenging activity

DPPH radical scavenging activity of the sample was determined as described by Amarowicz *et al.* (2008). The procedure involved taking known volume of sample extract or reference compound, ascorbic acid and was added to a methanolic solution of DPPH (0.03 mM). Both solutions were kept in a dark chamber for 30 min before measuring the absorbance at 517 nm. Free radical scavenging ability was calculated as percentage of $DPPH^{\cdot}$ discolouration as follows:

DPPH radical scavenging activity (%) = [(As – Ao)/A_s]×100

Where As = absorbance of the standard and Ao = absorbance of the sample.

2.17 Reducing property

The Fe^{3+}- reducing power of the sample was determined by the method described by Jayaprakash *et al.* (2001) as modified by Olaleye *et al.* (2006). An appropriate dilution of the sample extracts (0.5 ml) was mixed with 1.25 ml of 0.2 M of sodium phosphate buffer (pH 6.6) and 1.25 ml of 1% potassium ferrocyanide. The mixture was incubated at 50°C for 20 min after which 1.25 ml of 10% trichloroacetic acid (TCA) was added. The mixture was centrifuged at 650 rpm for 10 min. The upper layer was mixed with equal volume of distilled water and 0.25 ml of 1% ferric chloride and the absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power. Ascorbic acid was used as a positive control.

2.18 Statistical Analysis

All results are expressed as mean ± standard deviation. Analysis of variance (ANOVA) was performed using Statistical Software (SPSS version 16). Differences in means were determined using Duncan's multiple range tests.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition

The proximate composition of *Phaseolus lunatus* seeds coat is presented in Table 1. The moisture content was 4.46%; which is lower than 6.45% reported for bambara groundnut (Martin *et al.*, 2011), 10.39% for cowpea and 8.30% for mung bean (Masood and Rizwana, 2010). The sample showed crude protein content (15.75%) higher than what is obtainable in most cereal crops. The high protein contents of the seeds coat underline its potential as protein supplements in cereals to improve protein quality. The crude fibre was (33.56%), well above what is obtainable in most plant foods such as 4.61% found in mung bean (Masood and Rizwana, 2010); 5.35% reported for bambara groundnut (Martin *et al.*, 2011) and 10.89% in okra (Adetuyi *et al.*, 2011). Tosh and Yada (2010) have reported the enhancement of nutritional, biological and physicochemical properties of legumes fibre by decreasing the transition time through the small intestine. The ash and fat contents were 2.56 and 0.62% respectively, while carbohydrate was 47.52% in agreement with the findings of Tiwari *et al.* (2011).

Table 1: **Proximate composition of seeds coat (% dry weight)**

Parameters	Composition
Moisture	4.46 ±0.23
Protein	15.75 ±0.13
Fat	0.62 ±0.01
Crude fibre	33.56 ±0.41
Ash	2.56 ±0.10
Carbohydrate	47.52 ±0.11

Values are means of triplicate determinations ± Standard deviation

3.2 Mineral Composition

The mineral content of the sample in mg/100 g is depicted in Table 2. The most abundant minerals were potassium 398.41 mg/100 g) and magnesium (87.09 mg/100 g). In accordance to the observation of Olaofe and Sanni (1988) who reported potassium as the most predominant mineral element in Nigerian agricultural products. There have been similar observations reported for fluted pumpkin (Fagbemi, 2007) and gingerbread plum (Amza *et al.*, 2011) but contrary to the report on mucuna beans (Adebowale *et al.*, 2005). The sample also showed high sodium content (Table 2). Calcium has been known to help in bone formation and blood coagulation; the high calcium content in the seeds coat makes it an incredible source of calcium supplementation for pregnant and lactating women, as well as for children and the elderly people. Phosphorus on the other hand was 13.25 mg/100 g, iron (11.61 mg/100 g) was the most concentrated of all the micro/trace elements (iron, zinc and copper) detected. It is an essential constituent of haem in the circulating haemoglobin during

respiration (Antia *et al.*, 2006). The Na/K ratio in the body is of great concern for the prevention and management of high blood pressure, Na/K ratio less than one is recommended (Aremu *et al.*, 2006). Hence, with sodium/potassium ratio (Na/K) of 0.21 the seeds coat could probably reduce high blood pressure.

Table 2: Mineral composition of seeds coat (mg/100 g)

Elements	Composition
Zinc	5.60 ±0.20
Manganese	0.13 ±0.02
Calcium	17.56±0.17
Potassium	398.41±0.54
Sodium	82.20±0.25
Magnesium	87.09 ±0.40
Iron	11.61±0.17
Phosphorus	13.25±0.11
Na/K	0.21±0.02b

Values are means of triplicate determinations ± Standard deviation

3.3 Amino Acid Composition

All needed amino acids cannot be synthesized by human and animal as such some amino acids must be supplied through dietary intake (Sheng *et al.*, 2010). The composition of amino acids expressed as g/100 g protein for the sample is reported in Table 3, the FAO/WHO (2007) recommended mode of the essential amino acid for child and adult are also given in the table for reference purpose. The result showed high levels of essential amino acids such as lysine, leucine, arginine and phenylalanine. The total essential amino acid (TEAA) was 51.07 g/100 g protein, the value is well above 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults (FAO/WHO/UNU, 1985). In terms of the essential amino acid, the sample exhibited higher amounts of threonine, valine, isoleucine, tryptophan, leucine, lysine and histidine compared to FAO/WHO (2007) requirements for (2–5 years old) child. Similar observations have been reported by Du *et al.* (2012) and Oyetayo and Ojo (2012). Lysine, a major limiting amino acid in most cereals was 7.26 g/100 g in this study, above 4.34 g/100 g groundnut reported by Adeyeye (2010) and 3.17 g/100 g marama bean (Maruatona *et al.*, 2010).

Table 3: Amino acid composition of seeds coat (g/100 g protein)

Amino acid	Composition	FAO/WHO/UNU**	
		Child	Adult
ASX	11.77±0.12	-	-
THR*	4.57±0.10	3.40	0.90
SER	7.09±0.21	-	-
GLX	11.55±0.51	-	-
PRO	5.24±0.01	-	-
GLY	8.13±0.21	-	-
ALA	5.19±0.11	-	-
CYS*	0.21±0.01	-	-
VAL*	5.34±0.13	3.50	1.50
MET*	0.96±0.15	2.70	1.70
ILE*	4.05±0.10	2.80	1.30
LEU*	7.80±0.10	6.60	1.90
TYR*	3.18±0.01	-	-
PHE*	5.22±0.10	6.30	1.90
HIS*	4.35±0.01	1.90	1.60
LYS*	7.26±0.14	5.80	1.60
ARG*	7.02±0.20	-	-
TRP*	1.11±0.01	1.10	0.50
TEAA	51.07±0.02		

Values are means of triplicate determinations ± Standard deviation

Key

ASX = aspartic acid + asparagine; GLX = glutamic acid + glutamine, TEAA- total essential amino acids *= Essential amino acids, **= FAO, 2007.

However, the high lysine content of the *P. lunatus* seeds coat underline its potentials supplementary protein to cereal based diets which are known to be deficient in lysine. Glutamic (11.55 g/100 g) and aspartic (11.77 g/100 g) acids were the most abundant non essential amino acids present, similar to the observations of Adeyeye (2010). Dietary glutamine and asparagines end up in tissues and serve as important reservoirs of amino groups for the body (Vasconcelos *et al.*,

2010).The sample showed a good amino acid profile and potential source of protein for feeding infants and children.

3.4 Phytochemical Composition

Phytochemical screening showed the presence of anthraquinone, alkaloid, cardiac glycosides, flavonoid, phlobatannin, saponin, steroid, tannin and terpenoid (Table 4).

Table 4: Qualitative analysis of phytochemicals

Phytochemicals	
Alkaloid	present
Anthraquinone	present
Cardiac glycoside	present
Flavonoid	present
Phlobatannin	absent
Saponin	present
Steroid	present
Tannin	present
Terpenoid	present

The phytochemical contents of *P. lunatus* seeds coat is shown in Table 5. Generally, alkaloids 27.75% and total phenols 37.67 mg GAE/g were observed in high quantity in the sample. Plant alkaloids and the synthetic derivatives are used as a basic medicinal agent due to their analgesic, antispasmodic and antibacterial properties (Okwu, 2004). Saponin was 8.01 mg/g, has properties of precipitating and coagulating red blood cells, cholesterol binding properties and formation of foams in aqueous solutions (Kim *et al.*, 2003). The total phenol (37.67 mg GAE/g) is higher than (0.3-1.0 mg/g) value recorded for various species of *V. unguiculata* (Oboh, 2006). Phenolics have found to inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidised low-density lipoprotein (LDL), which is considered to induce cardiovascular disease (Amic *et al.*, 2003). Flavonoids content was 0.31 mg QE/g extract, have been implicated in the prevention of allergies and ulcers (Okwu and Omodamiro, 2005). The cyanide content was 2.05 mg/kg and below the limit of 10 mg HCN Eq/kg dry weight recommended for cassava products by FAO/WHO (1992) thereby making the sample safe for consumption. The result showed phytate content of 11.12 mg/100 g. Phytate possess the ability to chelate divalent minerals and has also been shown to have anticancer and antioxidant activity (Oboh *et al.*, 2003).

Table 5: Phytochemical composition of seeds coat

Parameters	Composition
Alkaloid (%)	27.75±0.15
Cyanide (mg/kg)	2.05±0.02
Flavonoid (mg/g)	0.31±0.01
Total phenol (mg/g)	37.67±0.47
Phytate (mg/100g)	11.12±0.41
Saponin (mg/g)	8.01±0.42
Tannin (mg/g)	5.84±0.10

Values are means of triplicate determinations ± Standard deviation

3.5 Phenolics Contents

Many of the antioxidants and therapeutic actions of phytochemicals are associated with the biologically active polyphenol components, such as flavonoids and phenolic acids, which possess powerful antioxidant activities (Pandey and Rizvi, 2009). HPLC-DAD analysis is advantageous over Folin-Cioalteu's method of total phenolics estimation, because it provides more precise information of individual compounds (Komolafe *et al.*, 2013). As revealed by the HPLC-DAD analysis, *P. lunatus* seeds coat showed positive results for phenolic acids (gallic, ellagic and caffeic acids); flavonoids (quercetin, rutin and kaempferol); β-carotene and tocopherol (Tables 6). It showed caffeic and ellagic acids as the most abundant portion of phenolics in the sample. Phenolics behave as antioxidants, due to the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring). caffeic and ellagic acids composition were 3.13 - 3.05 g/100 g. The concentration of tocopherol and β-carotene were 1.58 g/100 g and 0.94 g/100 g respectively. Another class of phenolics that occurs widely in plant tissues is tocopherol, also known as monophenolic and lipophylic compounds (Shahidi and Naczk, 2006); are powerful lipid-soluble antioxidant, which acts synergistically with selenium to prevent the

oxidation of fatty acids (Islam *et al.*, 2000) and have neuroprotective activity (Cuppini *et al.*, 2002).

3.6. *In-vitro* antioxidant activity

It has been accepted that dietary antioxidants may combat reactive oxygen species and free radicals generated during cellular metabolism or peroxidation of lipids and other biological molecules resulting in reducing the risk of chronic diseases (Gamel and Abdel-Aal, 2012). In the present study, free radical scavenging capacity against ABTS, DPPH and ferric reducing activity were evaluated. The results showed radical scavenging ability of the seeds coat against ABTS (Figure 1) and DPPH (Figure 2) free radicals in a concentration-dependent manner. The effect of antioxidants on the stable DPPH radical scavenging could be due to the hydrogen-donating ability since phenolic groups of flavonoids (Table 6) serve as a source of readily available H atoms such that the subsequent radicals produced can be delocalised over the flavonoid structure (Sandhya *et al.*, 2010).

Table 6: Phenolics composition of *P. lunatus* seeds coat (g/100 g)

Compounds	Composition
Gallic acid	0.97±0.08
Chlorogenic acid	ND
Caffeic acid	3.13±0.11
Ellagic acid	3.05±0.02
Rutin	1.54±0.03
Quercetin	1.97±0.01
Kaempferol	1.52±0.01
Tocopherol	1.58±0.02
β-Carotene	0.94±0.02

Values are means of triplicate determinations ± Standard deviation
ND-not detected

The free radical scavenging ability against DPPH at 0.5-5 mg/ml seeds coat ranges between 70.85- 96.95% with an IC_{50} value of 0.37 mg/ml an indication of higher activity.
Similar trend against ABTS was also observed, the Trolox Equivalent Antioxidant Capacity (TEAC) value of 0. 36 demonstrated that *P. lunatus* seeds coats exhibits considerable antioxidant activity by the scavenging of the cation radical. The antioxidant activity of the coats by this assay implies that action may either be by inhibiting or scavenging the ABTS radicals since both inhibition and scavenging properties of antioxidants towards this radical have been reported (Rice-Evans, 1997).

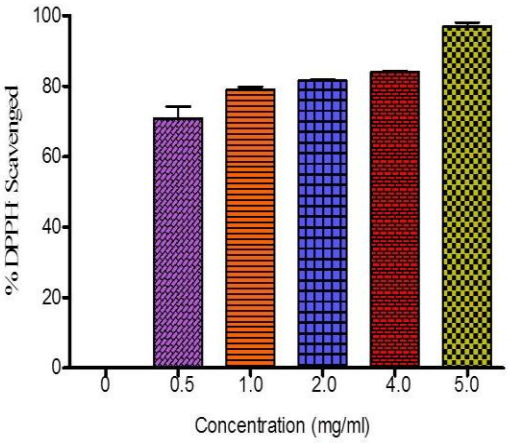

Figure 1: % DPPH scavenged of the seeds coat

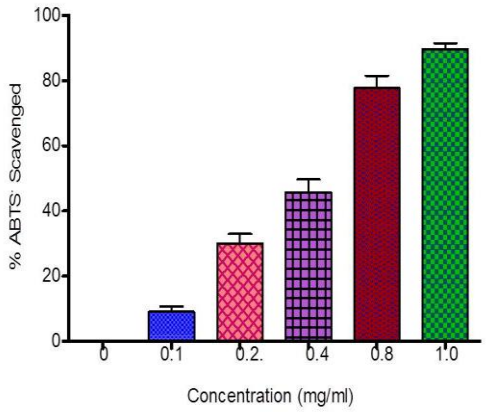

Figure 2: % ABTS scavenged of the seeds coat

The sample showed considerable ferric reducing property (56.37± mg ascorbic acid equivalent/g seed coat powder) suggesting that the phenolics present in the coats could act as reducing agents by donating electrons to free radicals and terminating the free radical mediated chain reactions (Hazra *et al.*, 2008).

The antioxidant capacity of the seeds coat was considerably high, indicating its potential as natural antioxidant sources.

4. CONCLUSIONS

The *P. lunatus* seeds coat investigated were found to be a good source of phytochemicals and radical scavenging activities. Therefore, it becomes important to promote maximal use of agro by-products such as seeds coat in the development of new functional ingredients for food and environmental sustainability.

Acknowledgements
The authors are grateful to the Departments of Food Science & Technology and Biochemistry, Federal University of Technology Akure, Nigeria, for the provision of facilities. We acknowledge with thanks the support of Federal Government of Nigeria Post-Graduate Scholarship Award.

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