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Research Article
Comparative Analysis of Phytochemical and Proximate Contents of Three Orphan Legume Species

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Abstract
Background and Objective: Orphan legume species can provide cheap and quality nutrients, serve as sources of herbal medicine, forage crop, biomass feedstock and green manure but largely unexploited. The present study investigated phytochemical and proximate contents of three orphan legumes comprising three accessions of African Yam Bean (AYB), four of Kersting's Groundnut (KG) and six of Winged Beans (WB). **Materials and Methods:** The seeds were collected from the Genebank of the International Institute of Tropical Agriculture via the Standard Material Transfer Agreement (SMTA). The samples were oven-dried, grounded into a fine powder and analyzed for their biochemical profiles using standard procedures. **Results:** Carbohydrate, betacyanin, quinone and terpenes were found in the samples, while saponins were found only in AYB and KG accessions. Alkaloids and coumarin were only detected in samples of WB and KG. The crude protein was quite high among all the samples ranged from 18.4% in TSs-33 of AYB-52.7% in TPt-126 of WB with a mean of 30.16±3.01. **Conclusion:** The study indicates that the species and the accessions are rich in proteins and essential proximate contents that can be explored as sources of food and feeds for both humans and animals.

Key words: Biochemical profile, protein contents, under-exploited legumes, herbal medicine, forage crop, breeding, Fabaceae

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INTRODUCTION

The orphan legume species are members of the Fabaceae family that have been abandoned, less exploited and tagged as minor crops, based on the level of their utilization[1,2]. They are known to exhibit diverse agronomic and economically significant traits that can serve as a potential breeding solution to the issue of food insecurity, renewable energy and sustainable development if inexorably exploited[3,4]. Most orphan legume species are endemic to the developing countries of the tropics, in Asia and Africa, where protein and food security challenges are predominant[3]. The species can provide cheap and quality nutrients, serve as sources of herbal medicine, forage crop, biomass feedstock, green manure, amongst others[3,5]. Orphan legumes are strictly cultivated by subsistence farmers in rural areas, who still possess indigenous knowledge on how to grow them[1,4,6].

Moreover, the species are resilient in a harsh climate, withstand pest, disease and drought and are capable of surviving in marginal lands, without compromising the quality of nutrients contained[3]. Thus, orphan legumes can be adopted into the cropping system to mitigate the effect of climate change on agricultural productivity. Determining the phytochemical and proximate components of these orphan legume species is critical and will serve as a reference base to confirm their quality nutritional profile. It will also help in identifying elite germplasms among and within the various species, that require crop improvement efforts. Studies have shown that the rich nutritional and phytochemical contents as well as genetic diversity of these crops, have not been fully explored and utilized in active breeding programmes[6-8].

To the best of our knowledge, there still exists, a good number of accessions/landraces whose nutritional composition has not been adequately characterized for effective utilization in breeding programs and cultivation. The successful integration of some of these species and their valuable traits will have a notable impact on the global food basket and making available cheaper and quality proteins. Thus, this study aims to comparatively determining the phytochemical and proximate contents of three orphan legume crops. The species consisted of three accessions of African Yam Bean (AYB) (*Sphenostylis stenocarpa*), four of Kersting's Groundnut (KG) (*Macrotyloma geocarpum*) and six of Winged Bean (WG) (*Psophocarpus tetragonolobus*). Data from this study will be useful in developing breeding and utilization strategies for the species via a selection of better traits.

MATERIALS AND METHODS

Study area: The study was carried out at the Biochemistry Laboratory, Department of Biochemistry, Covenant University, Ota, Ogun State, Nigeria from January-November, 2019.

Acquisition of seed samples: A total of thirteen samples of orphan legumes comprising three accessions of African Yam Bean (AYB)-*Sphenostylis stenocarpa*, four of Kersting's Groundnut (KG)-*Macrotyloma geocarpum* and six of winged Beans (WB)-*Psophocarpus tetragonolobus* were collected from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The collection followed the Standard Material Transfer Agreement (SMTA) of the International Treaty on Plant Genetic Resources with an SMTA number SMTA-00AF05-00AK77-180209. Table 1 shows the species, accessions codes and seed traits of the orphan legumes used for this study.

Sample preparation: The seeds of each sample were oven-dried at 70 for 24 hrs, milled into a fine powder using a VTCL heavy-duty solitaire blender grinder-750 Watts and then transferred into properly labelled ziplock bags. The samples were stored at room temperature and analyzed using standard procedures[9]. The proximate analysis consists of the moisture, ash, crude fat, crude fibre, nitrogen and carbohydrate contents, while phytochemicals such as tannins, saponins, alkaloids, anthocyanin and betacyanins amongst others were screened from each of the accessions.

Phytochemical screening: The 13 samples were screened for the presence of different phytochemicals using standard procedures[8]. The finely powdered samples were mixed with 100 mL of distilled H_2O, properly stirred and stored in the refrigerator for 24 hrs at the appropriate temperature, before the qualitative phytochemical tests were carried out. Such tests include:

- **Carbohydrate test:** A 2 mL of the sample extract was mixed with 1 mL of Molisch's reagent (α-naphthol in ethanol) and few drops of conc. H_2SO_4. A purple coloration indicated the presence of carbohydrates
- **Tannins test:** A 2 mL of 5% ferric chloride was added to 1 mL of the sample extract. A greenish-black coloration indicated the presence of tannins
- **Saponins test:** About 2 mL of distilled water was added to 2 mL of the sample extract, transferred into a calibrated test tube and then shaken lengthwise for approximately 15 min. The formation of a 1 cm layer of foam indicated the presence of saponins

Table 1: Passport data of the samples used for this study

Species	Code	Seed shape	Seed coat color	Seed texture
Sphenostylis stenocarpa	AYB-TSs-24	Oblong	Dark brown	Smooth
Sphenostylis stenocarpa	AYB-TSs-2	Oblong	Dark brown	Smooth
Sphenostylis stenocarpa	AYB-TSs-33	Oblong	Dark brown	Smooth
Macrotyloma geocarpum	KG-TKg-12	Rhomboid	Dark brown	Smooth
Macrotyloma geocarpum	KG-TKg-11	Rhomboid	Brown	Smooth
Macrotyloma geocarpum	KG-TKg-8	Rhomboid	Black	Smooth
Macrotyloma geocarpum	KG-TKg-9	Rhomboid	Dark brown	Smooth
Psophocarpus tetragonolobus	WB-Tpt-32	Globular	Dark brown	Smooth
Psophocarpus tetragonolobus	WB-TPt-48	Globular	Dark brown	Smooth
Psophocarpus tetragonolobus	WB-TPt-126	Globular	Dark brown	Smooth
Psophocarpus tetragonolobus	WB-TPt-153	Globular	Dark brown	Smooth
Psophocarpus tetragonolobus	WB-TPt-125	Globular	Dark brown	Smooth
Psophocarpus tetragonolobus	WB-TPt-154	Globular	Dark brown	Smooth

- **Alkaloids:** About 2 mL of the sample extract was subjected to 2 mL of conc. HCl and few drops of Mayer's reagent. The presence of green colour indicated the presence of alkaloids
- **Anthocyanin and betacyanin test:** To 2 mL of the sample extract, 1 mL of 2N NaOH was added and heated for approximately 5min at 100°C. The presence of yellow coloration indicated the presence of betacyanin, while no colour change indicated the presence of anthocyanin
- **Quinones:** To 1 mL of sample extract, 1 mL of conc. H_2SO_4 was added and the presence of red colour indicated the presence of quinones
- **Glycoside:** About 3 mL of chloroform and 10% ammonia solution was added to 2 mL of the sample extract. Glycosides were identified by the presence of pink coloration in the solution
- **Cardiac glycoside:** To 0.5 mL of the sample extract, 2 mL of glacial acetic acid and few drops of 5% ferric chloride was added, under layered with 1 mL of conc. H_2SO_4. A brown ring at the interface indicated the presence of cardiac glycoside
- **Terpenoids:** About 0.5 mL of the sample extract was mixed with 2 mL of chloroform and conc. H_2SO_4 was carefully added. The formation of red-brown colour indicated the presence of terpenoids
- **Phenols:** About 2 mL of distilled water was added to 1 mL of the sample extract, followed by few drops of 10% of ferric chloride. The green colour formation indicated the presence of phenols
- **Coumarins:** 1 mL of the extract and 1 mL of sodium hydroxide were mixed, the formation of yellow coloration indicated the presence of coumarins
- **Steroids:** Total 2 mL of the sample extract was mixed with 5 mL of chloroform and then filtered using a filter paper. A 2 mL of acetic anhydride was added to 2 mL of the filtrate with 2 mL of H_2SO_4. The colour change from violet to blue or green indicated the presence of steroids
- **Acids:** 1 mL of the sample extract was treated with a sodium bicarbonate solution. The formation of effervescence indicates the presence of acids

Proximate analysis:

- **Moisture content:** This was determined using the air oven method. Crucibles were washed, dried, labelled and cool in the desiccator and weight (W_1) was taken, using an analytical weighing balance. Approximately 0.5 g (W_2) of the samples were transferred into the crucibles and kept in a preheated incubator for 24 hrs at 100°C. The dry samples were cooled in a desiccator and the new weight (W_3) was noted and stored back into the desiccator to avoid ambiguity of weight values due to atmospheric humidity. Moisture content was evaluated using the formula as described by James *et al.*[10]:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight before drying } (W_2) - \text{Final weight after drying } (W_3)}{\text{Weight of crucible and sample } (W_2) - \text{Weight of crucible } (W_1)} \times 100$$

- Ash content was determined by subjecting the previous crucibles containing the samples to further heating in a muffle furnace at 600°C for 4 hrs. The samples were left to cool in the desiccator and then weighed to determine the new weight (W_4). The ash content for the seeds was determined with the formula:

$$\text{Ash content (\%)} = \frac{\text{IWBD } (W_2) - \text{NFWD } (W_4)}{\text{WCS } (W_2) - \text{WC } (W_1)} \times 100$$

Where:

IWBD = Initial weight before drying
NFWAD = New final weight after drying

WCS = Weight of crucible and sample (W_2)
WC = Weight of crucible

- Crude fat was determined using the Soxhlet apparatus. The boiling flasks were initially weighed (W_1) before use. About 5 g of the samples were weighed into a filter paper, folded neatly and tied with a rope. This was inserted into the thimble of the Soxhlet apparatus, the 250 mL boiling flask was filled with petroleum ether (40-60°C boiling range) and extraction under reflux was carried out for 3 hrs. At the end of the extraction process, the boiling flask was connected to the rotary evaporator to extract the petroleum ether for 30 min, before drying the flask in the preheated oven for 5 min. The flask was cooled in the desiccator for 3 min and weighed (W_2) on the analytical weighing balance. The fat extracted from 5 g of the sample was then calculated:

$$\text{Crude fat (\%)} = \frac{\text{FFWA extraction } (W_2) - \text{IFWB extraction } (W_1)}{\text{Weight of sample}} \times 100$$

Where:
FFWA = Final flask weight after extraction
IFWB = Initial flask weight before extraction

- Crude fibre content was done by serially diluting the fat-free extract gotten from the crude fat extraction process, with acid (H_2SO_4) and alkaline (NaOH). The weight of the crucibles (W_1) was measured, the weight of crucibles with the diluted fat-free extracts was also measured before ashing them in the muffle furnace for 4 hrs at 600°C and later left to cool in the desiccator. The weight of the dried crucibles and samples (W_3) was taken and the crude fibre was determined by:

$$\text{Crude fiber (\%)} = \frac{\text{Wt of C} + \text{SAP } (W_3) - \text{Wt of C } (W_1)}{\text{Weight of sample}} \times 100$$

Where:
Wt of C = Weight of crucible
SAP = Sample after ashing

- Nitrogen content was determined using Kjeldhal's method. 0.5 g of each sample was, respectively digested in 10 mL of conc. sulphuric acid, in the presence of 0.5 g activated catalysts ($CuSO_4$ and Na_2SO_4) and heated in Kjeldhal's digestion chamber for 45 min at 450°C. The samples were left to cool off in the chamber before diluting 10 mL of the digested sample with 40 mL of distilled water. For the distillation process was carried out with Kjeldhal's apparatus. About 20 mL of the diluted solvent was mixed with 20 mLs of 40% NaOH in the digestion tube, while boric acid (H_3BO_4) was used as the ammonia trapping agent, under the control of a mixed indicator. The distilled solvents were titrated against a strong acid (conc. HCL). Thus, the nitrogen/protein content was determined by:

$$P\ (\%) = \frac{V_2 - V_1 \times C \times 0.0140 \times K \times V}{W \times V^{\circ}} \times 100$$

Where:
V_2 = Titre value of sample
V_1 = Titre value of the blank
C = Molarity of acid used (0.05m)
V = Total volume of digest solution (50 Ml)
V = Volume of digested solution required for distillation (20 mL)
K = Factor converting the weight of nitrogen to weight of protein (6.25)
W = Weight of sample used (0.5 g)

- Carbohydrate content was calculated by the addition of moisture, ash, crude fat, crude fibre nitrogen and subtracting the resultant value from 100%. The difference in value was taken as the percentage total carbohydrate content of seed[11]

Data analysis: The statistical analysis on the proximate data was carried out using the Minitab® 19 Statistical Software software (https://www.minitab.com/en-us/products/minitab/) to derive the mean, standard error and p-value of each proximate. The principal component analysis and dendrogram were also derived, to determine the comparative relationships among the variables and the species. The dendrogram was generated using the Average Linkage Euclidean Distance (ALED) method.

RESULTS

Phytochemical screening: Carbohydrate and betacyanin were present in all the accessions of the three orphan legume species investigated (Table 2). Saponins were found in all the accessions of Kersting's Groundnut (KG) and African Yam Bean (AYB) but were absent among Winged Bean (WB) accessions. Only the accessions of winged beans contained alkaloids. Quinones and terpenoids were generally obtained in all the

Table 2: Phytochemical screening of the samples used for this study

Samples	CAH	TAN	SAP	ALK	ANT	BET	QUI	GLY	CAR	TER	PHE	COU	STE	ACI
KG-TKg-12	1	0	1	0	0	1	1	0	0	1	0	1	0	0
KG-TKg-9	1	0	1	0	0	1	1	0	0	1	0	1	0	0
KG-Tkg-8	1	0	1	0	0	1	1	0	0	1	0	1	0	0
KG-TKg-11	1	0	1	0	0	1	1	0	0	1	0	1	0	0
WB-TPt-153	1	0	0	1	0	1	0	0	0	0	0	0	0	0
WB-TPt-154	1	0	0	1	0	1	1	0	0	0	0	0	0	0
WB-TPt-48	1	0	0	1	0	1	1	0	0	1	0	0	0	0
WB-TPt-125	1	0	0	1	0	1	1	0	0	1	0	0	0	0
WB-TPt-32	1	0	0	1	0	1	0	0	0	0	0	0	0	0
WB-TPt-126	1	0	0	1	0	1	1	0	0	0	0	0	0	0
AYB-TSs-33	1	0	1	0	0	1	1	0	0	1	0	0	0	0
AYB-TSs-24	1	0	1	0	0	1	1	0	0	1	0	0	0	0
AYB-TSs-2	1	0	1	0	0	1	1	0	0	1	0	0	0	0

0: Absence, 1: Presence, CAR: Carbohydrate, TAN: Tannins, SAP: Saponins, ALK: Alkaloids, ANT: Anthocyanins, BET: Betacyanin, QUI: Quinones, GLY: Glycoside, CAR: Cardiac glycoside, TER: Terpenoids, PHE: Phenols, COU: Coumarins, STE: Steroids and ACI: Acid

Table 3: Proximate contents of the samples used for this study

Samples	MC (%)	AC (%)	CP (%)	CF (%)	CFB (%)	CAB (%)
AYB-TSs-33	11.0	6.0	18.4	3.10	0.25	61.25
AYB-TSs-24	4.4	10.2	20.6	2.90	0.64	61.25
AYB-TSs-2	16.6	4.0	22.1	2.40	0.04	54.86
KG-TKg-12	15.6	7.0	21.2	2.00	0.96	53.24
KG-TKg-9	2.2	4.6	22.8	2.00	0.54	67.86
KG-Tkg-8	10.4	4.8	19.5	1.80	0.90	62.6
KG-TKg-11	5.0	8.6	28.9	1.30	0.52	55.68
WB-TPt-153	2.8	5.2	44.2	3.40	0.70	43.7
WB-TPt-154	2.6	1.4	36.8	18.60	0.68	39.92
WB-TPt-48	2.6	11.4	32.8	0.70	2.67	49.83
WB-TPt-125	4.0	4.8	40.9	12.50	0.60	37.2
WB-TPt-32	8.2	5.6	31.2	18.90	0.45	43.65
WB-TPt-126	4.0	2.2	52.7	12.70	0.53	27.87
G. Mean	6.88	5.83	30.16	6.33	0.73	50.69
SE Mean	1.39	0.80	3.01	1.88	0.18	3.23
p-values	*	*	*	*	*	*

p-values: Significant at 0.05%: Percentage, MC: Moisture content, AC: Ash content, CP: Crude protein, CF: Crude fat, CFB: Crude fibre and CAB: Carbohydrate

accessions of KG and AYB while two accessions of WB lacked quinones (TPt-153 and TPt-32) and three accessions (TPt-153, TPt-154 and TPt-32) lacked terpenoids. Coumarins were only identified in the crude extract of all KG accessions (Table 2). Phytochemicals such as tannins, anthocyanin, glycoside, cardiac glycoside, phenols, steroids and acids were not detected in all the samples investigated.

Proximate analysis: The proximate contents of the samples of each of the three species are shown in Table 3. The data showed that moisture content (MC) ranged from 2.2% in TKg-9 of KG-16.6% in TSs-2 of AYB with a mean of 6.88±1.39. Among the AYB accessions, TSs-2 had the highest MC (16.6%), followed by TSs-33 with 11 % while TSs-24 had the least (4.4%). Among the KG accessions, TKg-12 had 15.6% MC values, while TKg-8 and TKg-11 had 10.4 and 5.0%, respectively. The WB accessions showed similar MC values except for TPt-32 which has 8.2%. The Ash Content (AC) ranged from 1.4% in TPt-154 of WB to 11.4%, in TPt-48 also of WB with a mean of 5.83±0.80. The Crude Protein (CP) contents of all the sample accessions were relatively high which ranged from 18.4% in TSs-33 of AYB to 52.7% in TPt-126 of WB (Table 3). The accessions of AYB and WB showed proximity in the range of crude protein contents (18.4-28.9%). Among the samples of KG, TKg-11 showed fairly high CP values of 28.9% compared to others (TKg-8 (19.5%), TKg-9 (22.8%) and TKg-12 (21.2%). A similar trend was obtained among the WB accessions with the TPt-126 having the highest CP content (52.70%). The Crude Fat (CF) ranged from 0.70 in TPt-48-18.90% in TPt-32 of WB with a mean of 30.16±3.01. The Crude Fibre (CFB) was low in TSs-2 (0.04%) of AYB and high (2.67%) in TPt-48 of WB with a mean of 0.73±0.18. All the three species and their accessions showed a high percentage of carbohydrate which ranged from 27.87% in TPt-126 of WB to 67.86% in TKg-9 of KG with a mean of 50.69±3.23. The p values derived at a confidence level of 95% showed that the variations in the mean values of each of the proximate parameters were significantly different (Table 3).

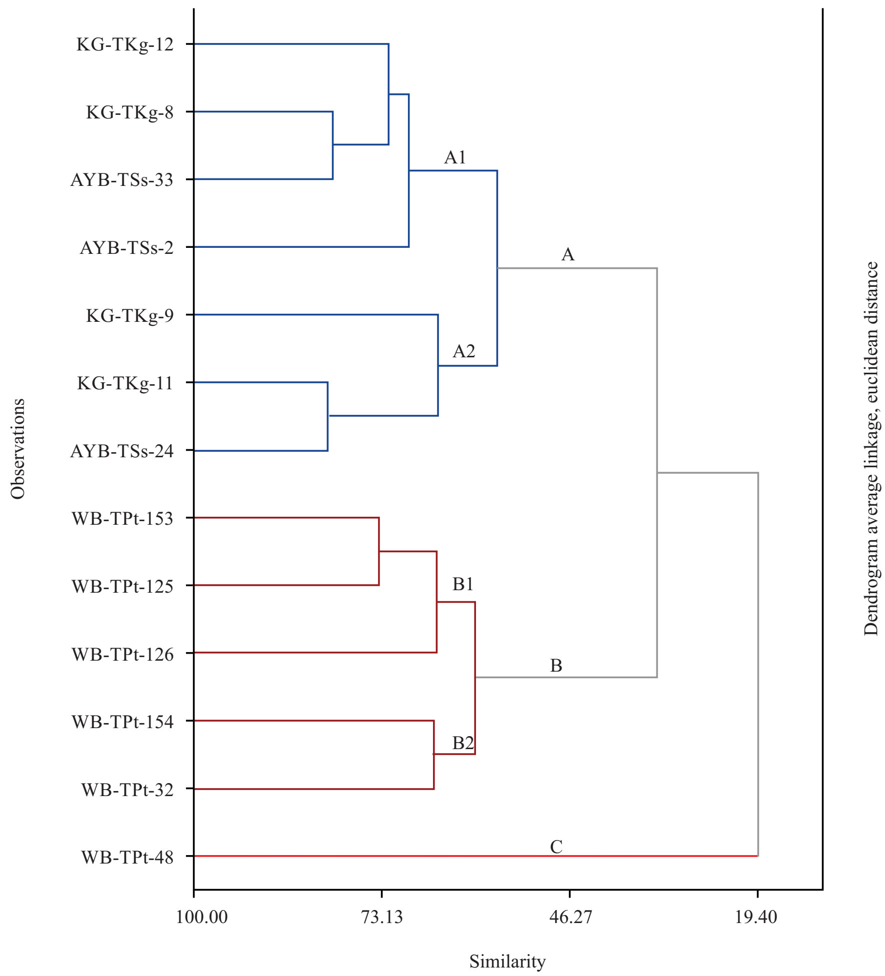

Fig. 1: Cluster analysis based on the proximate data

Table 4: Principal components, Eigenvalues and proportion of proximate variables

Variables	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6
MC	0.265	0.49	0.784	-0.067	-0.034	0.263
AC	0.369	-0.483	0.131	0.222	-0.745	0.096
CP	-0.518	-0.239	0.061	-0.481	-0.151	0.645
CF	-0.485	0.147	0.053	0.819	-0.049	0.259
CFB	0.089	-0.662	0.406	0.164	0.6	0.051
CAB	0.53	0.103	-0.443	0.13	0.242	0.66
Eigenvalue	2.98	1.75	0.60	0.3859	0.2854	0.0068
Proportion	0.496	0.291	0.10	0.064	0.048	0.001

MC: Moisture content, AC: Ash content, CP: Crude protein, CF: Crude fat, CFB: Crude fibre, CAB: Carbohydrate and PC: Principal component

Principal component analysis of the proximate contents: The data in Table 4 shows the proximate variables, principal components (PC_1-PC_6), Eigenvalues and percentage proportions. The PC_1 generated 49.60% with Eigenvalue of 2.98 and influenced by MC (0.265), AC (0.369) and CAB (0.53). PC_2 and PC_3 had 29.10 and 10%, with an Eigenvalue of 1.75 and 0.60, respectively. The variations are mostly influenced by both MC and CF.

Cluster analysis: The average linkage Euclidean distance segregated the thirteen accessions into three major cluster groups A, B and C, respectively (Fig. 1). Cluster group A comprised of seven accessions which are subdivided into two subcluster groups. The subcluster group A_1 consisted of two accessions each of KG (TKg-12 and TKg-8) and AYB (TSs-33 and TSs-2) while A_2 consisted of three accessions (TKg-9, TKg-11 and TSs-24). Cluster groups B and C are mainly Wing Beans

(WB) accessions (Fig. 1). The cluster group B consisted of two subclusters B_1 (Tpt-153, Tpt-125, Tpt-126) and B_2 (Tpt-154 and Tpt-32). The cluster group C has only one accession (Tpt-48).

DISCUSSION

The present study investigated the phytochemical and proximate contents of three orphan legume species (African Yam Bean (AYB)-*Sphenostylis stenocarpa*), Kersting's Groundnut (KG) (*Macrotyloma geocarpum*) and Winged Bean (WG) (*Psophocarpus tetragonolobus*). The study suggests that the three species can contribute to health care delivery and food security in tropical areas. The high carbohydrate and protein contents in all the samples of the three species indicate a high nutritional value of the seeds which can be exploited for food, animal feeds and breeding purposes. The presence of carbohydrates showed that the seeds are a veritable source of useable energy while protein contents have been variously linked to physical and mental growth and development[12]. Generally, legume carbohydrate is rich in starch (resistant starch), sugars such as fructooligosaccharides, raffinose and stachyose[12,13]. The biological role of these compounds as substrates to facilitate microbial ecology of the gastrointestinal tract and gut metabolism have been highlighted[12,13]. The seeds are considered as a cheap source of not only protein but also starch, which has the advantage of being resistant starch compared with root, cereal and tuber starch. Legumes are reportedly rich in polyphenols, saponins and alkaloids, betacyanins and anthocyanins amongst others. In this study, the crude solvent extracts indicated the presence of carbohydrates, saponins, betacyanin, terpenoids, quinones and coumarin obtained from the species investigated. In contrast, tannins, anthocyanins, glycosides, cardiac glycosides, phenols and steroids were not detected in all the samples. Terpenoids were found only in accessions of Kersting's groundnut (*Macrotyloma geocarpum*) while alkaloids were found in all the accessions of winged bean (*Psophocarpus tetragonolobus*).

The presence of betacyanin, quinones and saponins in most of the accessions of the three species is an indication of the potential role the species can play in the medicine and pharmaceutical industry as sources of anti-cancer and anti-inflammatory agents[12]. Betacyanin is one of the pigment subclasses of flavonoids that give colour to plant parts. Quinones are strong oxidizing agents that exhibit antioxidant, antifungal, antibacterial, antiplasmodial and antitumor properties[14-16]. The conferment of red, blue and purple colours to fruits including legume seeds, vegetables and beverages (e.g., strawberry, cherry, cranberry, blueberry, red cabbage, eggplant, red wine) was made by the water-soluble pigments called anthocyanins[17,18]. Anthocyanin and betacyanin constitute two different types of natural pigments that possess diverse health benefits which include antioxidant, anti-cancer, anti-inflammatory, antimicrobial and neuro-protective properties[17-19]. A wide range of biological activities has been reported for anthocyanins, such as antioxidant, anti-allergic, anti-inflammatory, anti-viral, antiproliferative, anti-microbial, antimutagenic, anti-tumour activities, microcirculation improvement and peripheral capillary-fragility prevention[18]. Anthocyanins are associated with low prevalence or alleviation of some disorders that include cancer, cardiovascular diseases, diabetes, obesity and cognitive decline[20,21]. Shreds of evidence have shown a lower risk of suffering Cardiovascular Diseases (CVD) is associated with high consumption of legumes[22,23]. Differential health-promoting biological activities have been attributed to betanin and betalain-rich foods which include scavenging of free radicals/ reactive oxygen species, inhibition of lipid peroxidation and LDL oxidation, prevention of DNA damage, induction of antioxidant and phase II detoxifying enzymes, gene regulatory activity, anti-inflammatory, antiproliferative and antimicrobial properties[24-26].

As earlier stated, legumes are rich in secondary metabolites, such as polyphenols, alkaloids and saponins, which are important defence compounds to protect against herbivores and pathogens and act as signalling molecules between the plant and its biotic environment[27,28]. In this study, alkaloids were detected only among the accessions of winged beans (*Psophocarpus tetragonolobus*). Alkaloids have innumerable applications in the pharmaceutical industry and their presence in all accessions of winged bean nominates the species as a potential source of anti-cancer drugs. The presence of terpenoids in Kersting's groundnut is remarkable even though not detected among the two other species. Terpenoids in Kersting's Groundnut (KG) (*Macrotyloma geocarpum*) can facilitate the rate of insect pollination to the species and hence, increased rate of cross-pollination and outbreeding variants. This has implications to enlarge the narrow genetic base of the species.

The p-values derived at a confidence level of 95%, showed that the variations in the resulting mean values of all the proximate were significantly different. The accession TPt-126 of winged bean is identified as a parental line for heterosis breeding for higher protein contents among the three species studied. Accession TPt-32 of winged bean had the highest crude fat extract (18.9%), which makes it a potential oilseed. Generally, the moisture contents were low among the species which indicate their better shelf-life and suitability for long term storage. The percentage of crude proteins was higher (52.70%) in accession TPt-126 of winged bean compared to other species (18.40% in TSs-33 of AYB and 44.20% in TPt-153 of WB) indicates the rich protein contents available in the species. The low crude protein content in accessions of AYB and that of Bambara groundnut (18-22%) are comparable to the findings of Ndidi *et al.*[29] on AYB, James *et al.*[10] on selected lesser-known legumes indigenous to Nigeria and on the whole to that of *Vigna unguiculata* and *Glycine max*[23,30,31]. The seeds of these legumes can serve as a source of animal and poultry feeds[32]. The proximate analysis results of the winged bean accessions are relatively consistent with that of unprocessed winged bean seeds as reported by Adegboyega *et al.*[33], while that of Kersting's groundnut correlates with the reports of Ayenan *et al.*[34]. The African yam bean accessions used in this study had a higher carbohydrate content and lower crude fibre content compared to that of reported values by Baiyeri *et al.*[35].

The resultant cluster analysis of mixed accessions and a mono cluster of winged beans demonstrates relatedness in phytochemical and proximate contents of the three species. The cluster analysis segregated the 13 accessions of the three species into three major clusters of one mixed cluster consisting of AYB and KG accessions and two clusters of mainly winged beans. This indicates the extremities of proximate values and the need for further research. On the whole, the findings of this study indicate that the three orphan legumes are potential sources of nutrition, food, animal feeds which can also be exploited for nutritional, medicinal and breeding purposes.

CONCLUSION

The species are rich in carbohydrates, proteins, betacyanins, saponins, alkaloids, terpenoids, quinones and coumarin comparable to the mainstream legumes. Bringing these crops into the limelight and mainstream market via sustainable cultivation, utilization and incorporation into food and farming systems will not only promote nutrition and food security but can suit current agricultural systems. Traits such as high carbohydrate, protein and fat as well as low moisture contents can be selected for heterosis breeding to create better phenotypes among the orphan legumes.

SIGNIFICANCE STATEMENT

The rich phytochemical and proximate contents of the three orphan legumes, African Yam Bean (AYB) (*Sphenostylis stenocarpa*), Kersting's Groundnut (KG) (*Macrotyloma geocarpum*) and Winged Bean (WG) (*Psophocarpus tetragonolobus*) are valuable for food, nutrition and health purposes. The study indicates that the species and the accessions are rich in proteins and essential proximate contents that can be explored as sources of food and feeds for both humans and animals.

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