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PHYTOCHEMICAL COMPOSITION OF TUBERS OF SELECTED ACCESSIONS OF AFRICAN YAM BEAN, *SPHENOSTYLIS STENOCARPA* (HOCHST.EX A. RICHMOND) HARMS (FAMILY FABACEAE)

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ABSTRACT

This study is on the quantitative determination of phytochemical composition in tubers of 17 accessions of the African Yam Bean (AYB), Sphenostylis stenocarpa (Hochst.ex A. Richmond) Harms. Standard laboratory methods were followed to ascertain the presence and quantity of some phytochemicals in the tubers of the 17 AYB accessions. The result showed that variations existed among the studied accessions for the eleven phytochemical constituents. The ranges were: total alkaloid (22.195-183g\100g), Glycosides (4.338- 14.733g\100), Flavonoids (7.732-14.037g/100), Phenols (16.448-32.287g/100), Oxalate (2.519-8.938ppm), Tannin (1.22-4.340ppm), Saponin (1.475-5.232ppm), Hydrogen Cyanide (0.261-0.928ppm), Phytate (1.532-5.435ppm), Trypsin Inhibitor (1.088-3.858ppm), and Organic Acid (11.537-23.904ppm). A high significant correlation was observed among the first three principal component axes which accounted for 90.4% of the total variation among the accessions. The cluster analysis showed the existence of two significant divergent groups. The accessions in cluster II recorded the least values for oxalate, tannins, saponins, hydrogen cyanide, phytate, trypsin inhibitor, glycoside, flavonoid and phenols while cluster I had higher values for them. The identified phytochemicals with the significant intra-specific variations seem to provide clues which underscores the possibilities of selection and improvement of these tubers for food and medicine for humans.

Keywords: African Yam Bean, tubers, underexploited organ**,** nutritional, anti-nutritional factor, diversity, phytochemicals.

INTRODUCTION

The rapid explosion of human and animal population and the fast depletion of natural resources has made it imperative to diversify the present day agriculture in order to meet the various human and animal's needs (Janardhanan *et al*., 2003). Among the possible strategies to doing this can include unravelling the potentials in many crops and crop products; a renewed focus on the neglected and underutilized crop species is enjoined (Popoola *et al*., 2020).

The medicinal properties of plants lie in its bioactive phytochemical constituents (Akinmoladin *et al*., 2007). Through phytochemical screening of different plant species or cultivars within the same species, important compounds with prophylactic and curative constituents against diseases can be detected (Sheikh *et al*., 2013) Searching to see intra-specific variability for bioactive phytochemicals is a worthwhile investigation for selection of the right genotype(s) and hence the continual production of same for exploitation of its utility (Adewale *et al.*, 2012).

Roots and tubers have been very important in the food culture of the various tribes in the tropics and sub tropics (Behera *et al*., 2009). However, there are sets of unique crops which combine duality production of both pulses and tubers; such are called tuberous legume. Mexican yam bean (*Pachyrhizus* sp.), a tuberous legume from South America has more prominence globally (Adewale, 2011). However, AYB., a tuberous legume indigenous to Africa leads in prominence in Africa (Saxon,1981; Dakora, 1996; Adewale and Odoh, 2013). *Pachyrhizus* and *Sphenostylis* are both in the family Fabaceae (Adewale *et al*., 2010). AYB., the most economically important of the seven species in the genus *Sphenostylis* E. Mey (Potter, 1992) is relished for the seeds/pulses in Nigeria, Ghana, Cote d'Ivoire, Togo and Cameroon, while it is deliberately cultivated for the tubers as a food organ in Gabon, Democratic Republic of Congo, Ethiopia, Malawi, Zimbabwe and some parts of East and Central Africa (NRC, 2006; Adewale, 2011). The utility of the tubers are, however, grossly unknown; most growers are not even aware that the crop produces tubers. The tubers which develop in clusters below the ground, spindle-shape, externally similar to sweet potatoes but non-sugary in taste, rich in protein and starch, edible and possessing additional industrial and pharmaceutical values (NRC, 1979, Saxon, 1981, Dakora, 1996). The tuber has remained as an underexploited organ.

The African Yam Bean (AYB), *Sphenostylis stenocarpa* Hochst. ex A. Richmond) Harms, (Family: Fabaceae) is a tuberous, leguminous annual. This plant, though previously underutilized, is gradually being appreciated in Sub Saharan Africa for its numerous benefits (Adewale and Odoh, 2013). Tubers are one of the major groups of cultivated crops capable of providing food security, nutrition security and eradicating poverty. Sadly, there is a dearth of information on the utilization of the tubers of AYB in Nigeria and other West African countries.

The present study was undertaken to prove the acclaimed presence of and usefulness of the phytochemical properties in this neglected organ of this plant especially for the identification of inherent phytochemicals and the assessment of their magnitude in the tubers of the different accessions. This is with a view to providing a possible template for nutritional, anti-nutritional and general phytochemical trait base selection for eventual genetic improvement of the crop for food for humans, feed for other animals and industrial applications.

MATERIALS AND METHODS

Seed of Seventeen (17) accessions of African yam bean obtained from the Genetic Resource Centre (GRC), International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria were planted in March 2019, at the Centre for Ecological Studies, Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria. The marked land area was cleared, ploughed and harrowed after which mini mounds were made at a spacing of 1m by 1m. Two seeds were sown at a depth of 2cm on the mounds. At two weeks after planting, thinning was done to one plant per stand and then staked. A row plot contained 4 plants which were the sampling unit for all data collected. Weeds was manually controlled as at when due during the experiment. The experiment was terminated in November 2019. Tubers

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were harvested from them at physiological maturity (when the vine detached from the tuber). The harvested tubers were washed thoroughly to remove soil particles. The same were peeled, cut into tiny pieces and dried in the oven at 65^{o}c for 48 hours as earlier reported by Konyeme *et al*., (2020). The dried sample were then ground into powder. Various phytochemical analysis were carried out on the grounded dried tuber samples.

Phytate determination

The determination of phytate followed the method of Oberleas (1973). The tuber flour was extracted with 0.2 N HCl such that 3-30 µg/ml phytate solution. The extract (0.5 ml) was pipetted into a test tube fitted with a ground-glass stopper and 1 ml of ferric solution (0.2 g Ammonium Iron (III) Sulphate $12H_2O$ in 100ml 2 N HCl and made up to 1000 ml with distilled water) was added. The test tube was heated in a boiling water bath for 30 minutes. Sample was cooled in ice water for 15 minutes and allowed to adjust to room temperature. The content of the tube was mixed and centrifuged for 30 minutes at 3000 rpm. The supernatant (1.00ml) was transferred to another and 1.5 ml of 2, 2-Bipyridine solution (10g 2, 2-bipyridine and 10ml thioglycollic acid in distilled water and made up to 1000ml). Absorbance of the solution measured at 519 nm against distilled water. The method was calibrated with the reference solutions as a substitute for the sample solution with each set of analyses. Preparations of the calibration curve was carried out by plotting the concentrations of the reference solutions against their corresponding absorbance. Then the absorbance of the test sample was used to obtain the concentration from the calibration curve.

Flavonoids determination

Tuber flour (1.5g) was weighed into a set of extraction tube(s) and 20ml of boiled ultra-pure water dispensed into each extraction tubes. The setup was allowed to stand for 1.5 hours and voltexed for 5 minutes. The solution was transferred to a set of centrifuge tubes, shaken for 15 minutes and centrifuged for 5 minutes at 3000rpm. Thereafter, a set of vials were used to collect the supernatants for determination on water 616/626 HPLC. The conditions for the analysis of flavonoids were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 60ml/min. (vii) Temperature: Detector- 147^{o}C; Injector port- 166^{o}C and Column: 115^{o}C (viii) Computer facilities for storing data. (ix) Printer for results reporting.

Alkaloids determination

Ten grams (10g) of tuber flour was de-fatted, out of which 5g was weighed into a flask and 100ml of 12% alcohol added, shaken, filtered and washed with industrial alcohol. The extracted residue was washed into a flask with 50ml of Ammonia water (ultrapure water) and heated in boiling water for 20 minutes and allowed to cool. Then, 0.1g of diastase (+ water) added and maintain at 50-55^{0}C for 2 hrs. It was cooled and made up to 250ml with ultrapure water, swirled and filtered. The filtrate (200ml) was mixed with 20ml hydrochloric acid (sp.g. 1.125) and heated in boiling water for

3 hours. Thereafter, it was allowed to cool, neutralized with sodium hydroxide solution and made up to 250ml. The sample was shaken centrifuged and supernatant decanted for determination using water 616/626 HPLC. The conditions of HPLC (Water 616/626) for the analysis of alkaloids were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 40ml/mins. (vii) Temperature: Detector- 170^{o}C; Injector port- 190^{o}C and Column- 125^{o}C (viii) Computer facilities for storing data. (ix) Printer for results reporting

Oxalates determination

This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

Digestion: Tuber flour (2g) was suspended in 190 ml of distilled water in a 250 ml volumetric flask. To this, 10 ml of 6 M HCl was added and the suspension digested at 100˚C for 1 hour, cooled and made up to 250 ml mark before filtration.

Oxalate Precipitation: Duplicate portions of 125 ml of the filtrate were measured into beakers and four drops of methyl red indicator was added. This was followed by the addition of conc. NH4OH solution (drop wise) until the test solution changed from salmon pink colour to a faint yellow colour (pH 4 – 4.5). Each portion was then heated to 90˚C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90˚C and 10 ml of 5% $CaCl_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5˚C. The solution was then centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

Permanganate Titration: The total filtrate resulting from digestion of 2 g of test sample was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near-boiling and then titrated against 0.05 M standardized KMnO4 solution to a faint pink colour which persists for 30 seconds. The calcium oxalate content was calculated using the formula.

Oxalate (mg/100g) =T x (Vme) (Df) x 10^5 /ME x (Mf)

Where T is the titre of $KMnO_4$ (ml), Vme is the volume – mass equivalent (1cm^3 of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), Df is the dilution of factor V_T/A (2.4 where V_T is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of the test sample used.

Tannin determination

The Folin-Denis spectrophotometric method was used to determine tannin in the flour samples of each accessions. The method was described by Pearson (1976). A measured weight of each sample (1.0 g) was dispersed in 10 ml distilled water and agitated. This was left to stand for 30min at room temperature, being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the extract gotten. A quantity (2.5 ml) of the supernatant (extract) was dispersed into a 50 ml volumetric flask.

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Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50ml flask. A 1.0 ml Folin-Denis reagent was measured into each flask, followed by 2.5 ml of saturated $NaCO_3$ solution. The mixture was diluted to mark in the flask (50 ml), and incubated for 90min at room temperature. The absorbance was measured at 250 nm in a Genway model 6000 electronic spectrophotometer. Readings were taken with the reagent blanket zero. The tannin content was given as follows:

% Tannin-An/As x C x 100/w x V_F/V_A

Where, An = absorbance of test sample; As = absorbance of standard solution; C = concentration of standard solution; W= weight of sample used; Vf= total volume of extract; Va= volume of extract analysed

Determination of Trypsin inhibitor activity

The determination of trypsin inhibitor activity (TIA) assay via spectrophotometric method as described by Arnfield *et al*. (1985) was employed. Test sample weight of 1.0 g was dispersed in 50ml of 0.5 M NaCl solution, the mixture was stirred for 30 minutes at room temperature and centrifuged. The supernatant was filtered through filter paper (Whatman No. 41) and the filtrate used for the assay. To 10ml of the substrate in a test tube, 2ml of the standard trypsin solution (N - α –Bensoyl – DL arginine – P- nitroanilide [BAPA]) was added and a blank of 10 ml of the same substrate in a test tube with no extract added. The content of the test tubes were allowed to stand for at least 5 minutes and then measured spectrophotometrically at 410 nm wavelength. One trypsin unit inhibited is given by an increase of 0.01 absorbance units at 410 nm, given a 100 ml of the mixture. That is one trypsin unit inhibited (TUI) is equal to an increase of 0.01 in absorbance unit at 410 nm. The trypsin inhibitor activity is expressed as the number of trypsin units inhibited (TUI) per unit weight (g) of the sample analysed.

TUI/mg = $Absorbance of Sample/Absorbance of Standard$x0.01F

TUI/mg = $b-a$ /0.01 X F.

Where: b = absorbance of the test sample solution; a = absorbance of the blank (control); F =experimental factor, given by F =1/wX Vf/Va X D

Where w = weight of the sample; Vf = total volume of extract; Va= volume of extract used in the assay; D = dilution factor (if any)

Hydrogen cyanide determination

Tuber flour (5 g) was made into a paste. The paste was dissolved in 50 ml distilled in a corked conical flask and allowed to stay overnight for cyanide extraction. The extract was filtered and the filtrate used for cyanide determination. To 1 ml of the filtrate, 4ml alkaline picrate (1 g of picrate and 5 g sodium carbonate dissolved in a volume of minimally warm water and the volume made up to 200 ml with distilled water) was added and incubated in a water bath for 5 minutes. After colour development (reddish brown colour), the absorbance of the corked test tube read in spectrophotometer at 490 nm. Also, the absorbance of the blank containing only 1ml distilled water and 4ml alkaline picrate solution. Then, the cyanide content was extrapolated from a cyanide standard curve (different concentration of KCN solution containing 5-50 µg cyanide in 500 ml conical flask received 25 ml of 1 N HCl each).

Glycosides determination

Tuber flour (0.5g) sample was weighed into a set of digestive tubes, 5ml of 0.1M HCL was added, warmed gently for 15minutes at 105^0c and transferred into a 50ml volumetric flask. The procedure was repeated twice. Rinsed with two to three aliquot, allowed to filter completely and the filtrate volume was made up to 100ml with the extractant solution and mixed thoroughly. A quantity (5ml) of extract solution was taken from the 100ml flask and ran through a 20cm layer (resin is packed on a macro pipette tip) cation exchange resin (CEC). Glycosides compounds was eluted with 10ml of absolute ethanol was washed from the column with ultrapure water (10ml) and the supernatant was transferred to a sample vial and ran on water 616/626 HPLC. The conditions for the analysis of glycosides were as follow: i) An autosampler ii) An automated gradient controller iii) Gradient elution HPLC pump iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room v) Detector by fluorescence vi) Carrier gas: Nitrogen gas at flow rate of 38ml\min vii) Temperature: Detector-167^{o}C ; injector port-183^{o}C and column-130^{o}C viii) Computer facilities for storing data ix) Printer for results reporting

Phenolic determination

Two grams of the African yam bean tuber flour was weighed into a set of test tubes, 3ml of 70% acetone in water was added and the tube placed in an ultrasonic water bath at 10^{o}c for 5minute and stirred occasionally with a glass rod. Filtered through a 50-60µ Gooch crucible into a 50ml Erlenmeyer flask. The extraction was repeated three times by adding 3ml of the 70% acetone in water and allowing it stand in the water bath at 10^{o}c for 5minutes. The test tubes was rinsed with final 3ml portion of 70% acetone in water and emptied into the test tubes. Then 2ml of the 0.1Myb- acetate and 15ml of 0.1M TEA reagent were added into the filtrate. The flask was closed with rubber stopper, swirled and shaken for 20minutes after transferring the sample solutions to a set of plastic volumetric tubes. Allowed to settle for 4hours and the supernatant was collected for analysis using HPLC. The conditions for the analysis of phenolics were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Argon gas at flow rate of 60ml\mins. (vii) Temperature: Detector-120^{o}C; Injector port-155^{o}C and column-117^{o}C (viii) Computer facilities for storing data. (ix) Printer for result reporting.

Organic acids determination

Five grams of the ground African yam bean tuber flour was weighed into an extraction bottle, 100ml of the ultra pure water was dispensed into the sample and placed in a cool environment at 4^{o}C for 6days. Then the sample was squeezed from the extraction bottle through layers of cheese cloth (or glass wool in a funnel) or centrifuge the liquid at 200rpm for 5minutes. The sample was placed in a corked bottle and stored in cool environment. Sample solution (1.5ml) and 1ml working standard were pipette into a 10ml centrifuge tube, mixed and allowed to stand for 30minutes. After the content was centrifuged at 300rpm for 10mins. The supernatant liquid 91.5ml was injected directly on the HPLC column (4% CW-20M 30/120 carbg pack-BDA) fitted with a

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flame ionization detector. The conditions for the analysis of organic acids were as follows: i) An autosampler ii) An automated gradient controller iii) Gradient elution HPLC pump iv) Reverse-phase HPLC column, thermostatically heated in a temperature controlled room v) Detector by flame ionization detector vi) Carrier gas: Nitrogen gas at flow rate of 65ml/ mins vii) Temperature Detector-201^{o}c; Injector Port-195^{o}c; column-150^{o}c viii) Computer facilities for storing data ix) Printer for results reporting

Saponin determination

This was determined in accordance with the procedure of AOAC (1984). Tuber flour were ground into slurry and 0.5g weighed into a conical flask and 10ml of distilled water was added. This was shaken and allowed to stand for 1hr. Observation was made for the formation of a stable foaming froth. 1ml of the mixture was pipetted into a test tube followed by the addition of about 5m of distilled water to the extract. A drop of olive oil was added and the mixture shaken till it became cloudy. A portion of the mixture was transferred into a cuvette and the absorbance measured at 620nm using a spectrophotometer. The concentration of saponin present in each sample was estimated from the standard saponin curve obtained from plotting the concentration of the standard against the absorbance. The amount of saponin was calculated as follows:

Ps= Ab x S x Df x100(mg/kg saponin).

Where Ps is the percentage of saponin, Ab the1 absorbance, S the slope, Df the dilution Factor.

Data Analysis

Descriptive statistics were employed for the analysis of the results for the 11 phytochemical components for the 17 African yam bean accessions using SAS (version 9.4, 2011). Pearson correlation analysis was used to determine the relationship between variables. Furthermore, from the 17 x 11 mean matrix data, Gower genetic distance for each pair of accessions was generated. The Gower genetic distance was further subjected to Principal Component and Clustering Analyses to enhance the understanding of the diversity among the 17 African yam bean accessions.

RESULTS

The results are as presented in Tables 1 – 5. Table 1 has the descriptive statistics of the eleven phytochemical properties for the 17 AYB accessions. Among the 11 phytochemicals studied, Alkaloid had the highest value followed by phenol. The phytochemical component with the least mean value was Hydrogen cyanide (Table 1). The five principal components shown in Table 2, explained 99.2% of the total variation among the seventeen African yam bean accessions. The highest eigenvalue (8.12) was from the first PC, with variance component contribution of 73.9% (Table 2). Within PC1, the contribution of oxalate, tannin, saponin, hydrogen cyanide, phytate, trypsin inhibitor and flavonoids were higher than 0.30 eigenvectors. Other important phytochemicals with significant contribution to the total variance were: Alkaloid, Phenol and organic acid (PC2), glycoside and organic acid in PC3 (Table 2). The first three PC axes accounted for 91% of the total variability among the 17 accessions (Table 2).

Table 3 presents the relationship among the eleven phytochemical components by Pearson correlation coefficient analysis. Highly significant ($P<0.001$) and very strong ($r = 1.00$) and positive correlation existed among oxalate, tannin, saponin, hydrogen cyanide, phytates and trypsin inhibitor (Table 3). The relationship of alkaloid with the six parameters above was mild ($r = 0.50$, $P = 0.05$) and negative. However, within Table 3, the relationship of the six parameters with flavonoid ($r = 0.89$) and phenol ($r = 0.55$) was positive and significant ($P\leq0.05$). The correlation of organic acid with oxalate, tannin, saponin, hydrogen cyanide, phytate, trypsin inhibitor, flavonoid and phenol was negative but significant at $P\leq0.01$ (Table 3).The gower genetic distance, showing the level of similarities among the 17 African yam bean accessions is presented in Table 4. The mean genetic similarity among the 17 accessions was 0.72. The most similar accessions were TSs84A with TSs98 and TSs109 with similarity of 0.91 (Table 4). Moreover, the two accessions with the least (0.18) similarity were TSs158 and TSs49 (Table 4).The ward clustering system grouped the 17 African yam bean accessions into two major groups at the similarity point of 0.2 (Figure 1). There were eight and nine accessions in clusters I and II respectively. The closest accessions (TSs84A, TSs98 and TSs109) were in cluster II. The dendograph could not identify duplicate accessions; every accession was unique at 0.00 similarity point (Figure 1). Moreover, the 17 accession became a single entity at 0.55 inflection point of similarity. Means and coefficient of variation were generated for the eleven phytochemical properties of the eight and nine African yam bean accessions which grouped in clusters I and II respectively. Higher means were observed in cluster I for oxalate, tannin, saponin, hydrogen cyanide, phytate, trypsin inhibitor, glycoside, flavonoid and phenol, while the accessions in cluster II had the highest value from alkaloids and organic acid (Table 5). For the eleven phytochemical properties in the two clusters, the coefficient of variation were generally low (< 20%), except for alkaloid and glycoside in the two clusters (Table 5).

DISCUSSION

Occurrence of significant variation in quantitative parameters within a species is a known axiom. The result on the presence of different phytochemicals in significant varying proportions for the different accessions corroborated earlier reports on different aspects of AYB; especially on the molecular characterization (Moyib *et al*., 2008, Ikhajiagbe and Mensah, 2012; Adewale *et al*., 2015; Shitta *et al.*, 2016), morpho-agronomic level (Adewale, 2011; Adewale *et al*., 2012; Abdulkareem *et al*., 2015; Ojuederie *et al*., 2015), biochemical/phytochemical (Uguru and Madukaife, 2001; Adewale and Aremu, 2013; Adesoye and Oluyede, 2015; Ojuederi and Balogun 2017; Soetan, 2017). This observation of clear and unambiguous genotypic variation for various phytochemical contents in the tubers of AYB as revealed in this study seems to re-emphasize the formidable foundation upon which crop improvement is entrenched.

Anti- Nutritional Factors (ANF) (Tannins, saponins, oxalate etc) when present in food causes adverse physiological responses to the consumer (Adewale and Aremu, 2013) especially when their content is above the optimum. There are reports (Popova and

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Mihaylova, 2019) that synthetic or natural ANF do interfere with the absorption of nutrients. Agricultural products meant for human consumption are preferably choicest when the content of ANF is bearably low. However, most positively too, the presence of some phytochemicals e.g. lectin in AYB seeds have been significantly reported (Omitogun *et al.*, 1999; Okeola and Machuka, 2001; Okeola *et al.*, 2002) to build resistance against bruchids (*Callosobruchus maculatus*). However, some of the phytochemicals which are toxic to destructive pests loses their potency during processing such as cooking, soaking and drying, thus making consumption of agricultural produces safe (Wokoma and Aziagba, 2001).

In the tubers of the 17 AYB accessions studied, the array of phytochemicals observed varied in composition of: flavonoid, saponin, glycoside, organic acid, phytate, hydrogen cyanide, alkaloids, phenols, tannin and trypsin inhibitor. The clustering system significantly demarcated the 17 AYB accessions based on the content of each phytochemicals; the eight accessions in cluster I had higher values but the nine in cluster II had lower values for most of the 11 phytochemicals; thus setting a stage for meaningful selection of genotypes. TSs 6A had the highest phenol content. This (32.29g/100g) observed in this study was however, higher than the phenol content (1.11, 1.22 and 1.64 mg) recorded by Princewill-Ogbonna and Ibegi (2005) for three cultivars of *Disocorea bulbifera*. The high phenol and flavonoid content in the tubers of African yam bean indicates that the tuber of the crop holds promising quantity of antioxidants activities. The presence of saponins indicated the ability of the tubers of African Yam Bean to resist microbes, making them a good candidate for treating fungal and yeast infection (Sheikh *et al*., 2013). This information could lead to genetic resource development for crop resistance to pathogens.

Food with high oxalate content causes adverse health challenges to the consumers; as high oxalate (the insoluble constituent) can result in kidney stones resulting from increased absorption of calcium by the kidney (Chai and Liebman, 2004). It is noteworthy however, that total oxalate is a sum of soluble and insoluble oxalate. Further investigation would be necessary to separate total oxalate in the tuber of African yam bean to the soluble and insoluble constituents.

Tannins are responsible for the astringent taste in foods and drinks (Padhan and Panda, 2020). High concentration of it affects the quality of protein in food (Raes *et al*., 2014). On the positive aspect, plants rich in tannin content have been reported (Eleazu *et al*., 2013) to be very effective for the treatment of diseases like Leycorrhea, Rhinorhea healing of wounds and diarrhoea. In this study, TSs49 had the highest (4.34ppm) tannin content but TSs 158 had the least (1.223 ppm). This indicates that TSs49 can be exploited and developed for tannin production. While food processing techniques can reduce tannin content for safe human consumption of AYB tubers, treatment of human wounds can significantly benefit from the selection of genotypes with high tannin content for increased production.

CONCLUSION

The phytochemicals present in the tubers of AYB can seem to offer great and important pharmacological utilities. Improved

utilization of AYB tubers is therefore encouraged.

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Table 1: Descriptive statistics of the phytochemical components in the tubers of African am bean

Phytochemicals	Mean+SE	Minimum	Maximum
Oxalate	5.17±0.40	2.52	8.94
Tannin	2.51±0.19	1.22	4.34
Saponin	3.02±0.23	1.48	5.23
Hydrogen Cyanide	0.53±0.04	0.26	0.93
Phytate	3.14±0.24	1.53	5.44
Trypsin inhibitor	2.23±0.17	1.09	3.86
Alkaloid	37.07±2.23	22.19	52.18
Glycosides	10.02±0.81	4.34	14.73
Flavonoids	10.00±0.51	6.99	14.04
Phenolics	23.52±1.14	16.45	32.29
Organic acid	17.25±0.90	11.54	23.90

Table 2: Principal component analysis showing the variance components and the eigenvectors of the eleven phytochemical components used for the diversity study

	PC1	PC2	PC3	PC4	PC5
Eigenvalues	8.12	1.04	0.82	0.57	0.35
Proportion	0.74	0.09	0.07	0.05	0.03
Cumulative variance	0.74	0.83	0.91	0.96	0.99
Phytochemical characters			Eigenvectors		
Oxalate	0.346	0.006	0.125	0.136	0.070
Tannin	0.346	0.006	0.125	0.136	0.070
Saponin	0.346	0.006	0.125	0.136	0.069
Hydrogen Cyanide	0.346	0.006	0.124	0.137	0.069
Phytate	0.346	0.006	0.125	0.136	0.070
Trypsin inhibitor	0.346	0.006	0.125	0.136	0.070
Alkaloid	-0.198	0.650	0.047	0.631	0.063
Glycosides	0.176	-0.287	-0.844	0.307	0.244
Flavonoids	0.322	-0.163	0.103	-0.196	-0.320
Phenolics	0.214	0.543	-0.189	-0.591	0.513
Organic acids	-0.248	-0.415	0.381	0.093	0.736

†Eigenvector ≥0.2 are significant

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Table 3: Correlations among the eleven phytochemical components

	Oxalate	Tannin	Saponin	HCN	Phytate	Trypsin inhibitor	Alkaloid	Glycosides	Flavonoids	Phenolics
Tannin	1.00***									
Saponin	1.00***	1.00***								
HCN	1.00***	1.00***	1.00***							
Phytate	1.00***	1.00***	1.00***	1.00***						
Trypsin inhibitor	1.00***	1.00***	1.00***	1.00***	1.00***					
Alkaloid	-0.50*	-0.50*	-0.50*	-0.50*	-0.50*	-0.50*				
Glucosides	0.43	0.43	0.43	0.44	0.43	0.43	-0.39			
Flavonoids	0.89***	0.89***	0.89***	0.89***	0.89***	0.89***	-0.67**	0.39		
Phenolics	0.55*	0.55*	0.55*	0.55*	0.55*	0.55*	-0.18	0.22	0.46	
Organic acid	-0.64**	-0.64**	-0.64**	-0.64**	-0.64**	-0.64**	0.19	-0.41	-0.62**	-0.62**

†**, ** and *** - indicates significance level at 0.05, 0.01 and 0.001 respectively

Table 4: Gower Genetic Distance showing the similarities among the seventeen accessions based on the eleven phytochemical properties

Genotypes	TSs49	TSs57	TSs119A	TSs49A	TSs2015_06	TSs58	AYB119A	TSs10	TSs66	TSs6A	AYB44C	TSs98	TSs84A	TSs109	TSs101	TSs158
TSs57	0.82															
TSs119A	0.69	0.80														
TSs49A	0.60	0.69	0.87													
TSs2015_06	0.66	0.78	0.79	0.84												
TSs58	0.59	0.72	0.82	0.81	0.88											
AYB119A	0.54	0.61	0.76	0.76	0.63	0.71										
TSs10	0.43	0.54	0.67	0.75	0.64	0.63	0.83									
TSs66	0.60	0.75	0.80	0.86	0.87	0.81	0.66	0.70								
TSs6A	0.51	0.65	0.81	0.88	0.76	0.76	0.74	0.74	0.86							
AYB44C	0.44	0.52	0.66	0.70	0.58	0.67	0.88	0.82	0.61	0.69						
TSs98	0.34	0.43	0.59	0.71	0.61	0.63	0.76	0.83	0.65	0.68	0.86					
TSs84A	0.41	0.49	0.65	0.78	0.67	0.70	0.84	0.88	0.71	0.75	0.86	0.91				
TSs109	0.33	0.42	0.58	0.70	0.62	0.66	0.77	0.81	0.63	0.67	0.87	0.90	0.91			
TSs101	0.20	0.29	0.44	0.57	0.51	0.57	0.64	0.66	0.49	0.54	0.76	0.82	0.77	0.84		
TSs158	0.18	0.26	0.42	0.54	0.48	0.53	0.61	0.63	0.46	0.52	0.71	0.79	0.74	0.76	0.90	
AYB30B	0.32	0.46	0.60	0.68	0.65	0.72	0.76	0.80	0.64	0.72	0.83	0.85	0.86	0.88	0.81	0.78

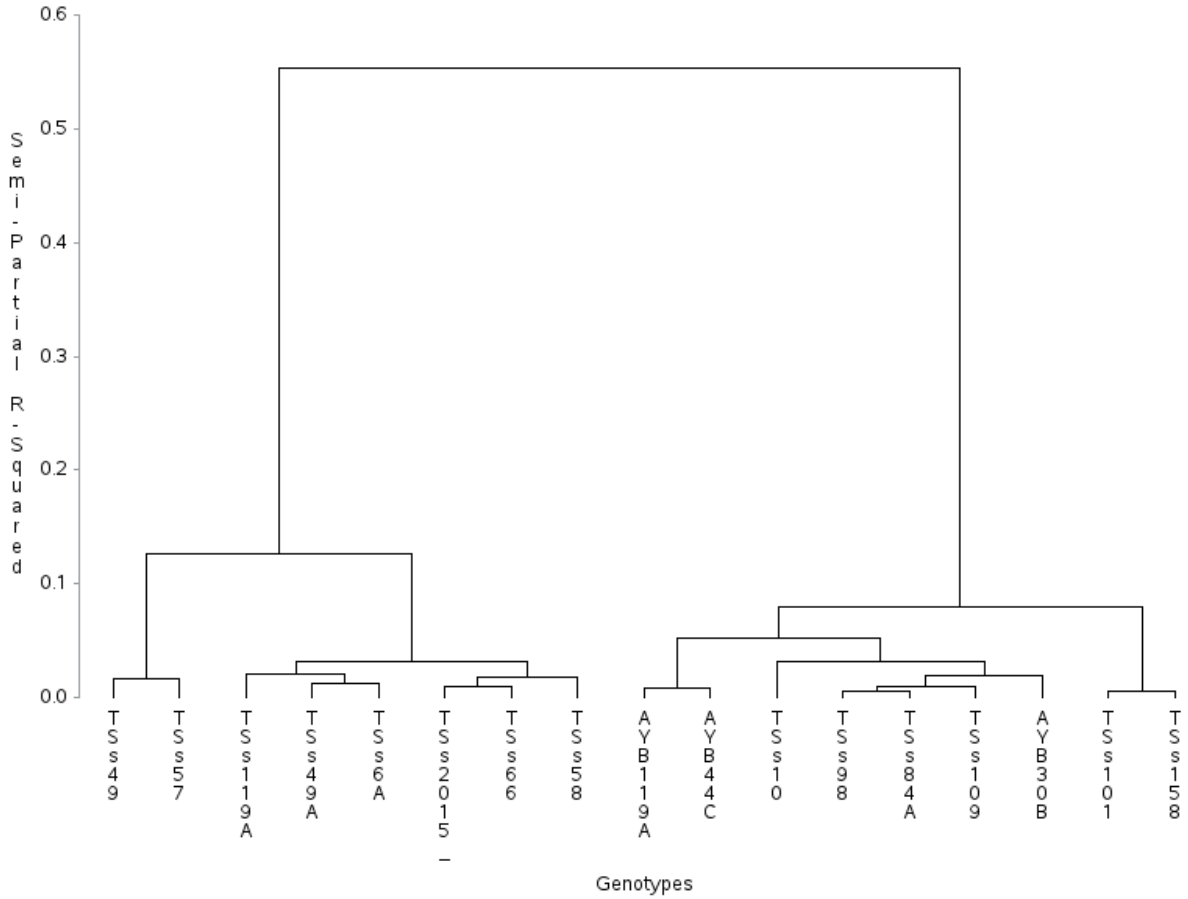

Figure 1: The grouping pattern of the seventeen African yam bean accessions based on eleven phytochemical components

Table 5: Intra-cluster variability for the eleven phytochemical components

	Cluster I		Cluster II	
Phytochemicals	Mean	CV(%)	Mean	CV(%)
Oxalate	6.75	17.40	3.89	17.63
Tannin	3.28	17.49	1.89	17.60
Saponin	3.95	17.42	2.28	17.64
Hydrogen Cyanide	0.70	17.46	0.40	17.58
Phytate	4.10	17.49	2.37	17.60
Trypsin inhibitor	2.91	17.45	1.68	17.63
Alkaloid	32.32	26.86	40.37	22.08
Glycosides	10.46	28.83	9.58	40.81
Flavonoids	11.93	13.78	8.37	9.00
Phenolics	27.05	11.96	20.78	20.09
Organic acids	14.58	18.57	19.84	12.19