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Phytochemical, natural ingredients and proximate composition analysis of two accessions of Vigna Subterranea (L.) verdc: Tvsu25 and tvsu2113 cultivated in high humid rainforest agro-ecology of South-South, Nigeria

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Abstract:

Bambara groundnut is an indigenous legume crop, cultivated throughout sub-Saharan countries by subsistence farmers. As a nutraceuticals this crop has great nutritional and agronomic potential, but it remains scientifically neglected and underutilized. The main reasons why Bambara groundnut have been underutilized include the knowledge gap in improved seed system, agronomic practices, processing, and nutritional values. The study investigated the proximate composition, amino acid, mineral, vitamin and phytochemical composition of two accessions of Bambara groundnut (*Vigna subterranea* (L.) Verdc) Tvsu 25 and Tvsu 2113, to protect and promote its cultivation for food (nutrients) and medicines, especially among rural communities in high humid rainforest agro – ecology of south – south, Nigeria. The results of the Pearson`s correlation performed on all parameters studied showed a significant positive association between both accessions with a very high correlation coefficient (r) in all parameters analysed. The correlations were observed to be significant as all P-value observed in all parameters analysed were found to be lower than the specified significance level of 0.05. The phytochemical studies revealed the presence of eleven bioactive compounds in both accessions of *V. subterranea* studied. The proximate result showed that crude fat, ash, fibre and protein are higher in Tvsu 2113. The analysis for minerals showed 19 minerals in the accessions. The total percentage vitamins showed that Tvsu 25 had a higher total percentage of 71.306% than Tvsu 2113 which showed 49.268%. 20 amino acids were present in the two accessions given a total of 91.23% for Tvsu25 and 75.88% for Tvsu 2113. These results showed that the two accessions are highly related with positive correlations in all. Bambara groundnut is endowed with many active chemical compounds that can be found to be very important in medical and therapeutic studies and also have useful application in pharmacological and nutritional research.

Keywords:

Phytochemical, Mineral, Amino Acid, Bambara Groundnut, (*Vigna subterranea* (L.) Verdc).

1. Introduction:

Plants are essential resources for human well-being, such as food, clothing, medicine, rubber, timber, and shelter. In addition, plants produce chemical compounds for self-defense (Atanasova, 2008). Depending upon species, different plants have varied chemical compositions. Several plants have been identified to have both medicinal and economic value. Plants that possess medicinal value are frequently utilized as herbal remedy to rebuild and maintain good health (Gayathri and Kiruba, 2014). Some herbal plants have been used as drugs and consequently generally harmless and effective (Uboh *et al*., 2010). Phytochemicals as bioactive non-nutrient plant compounds found in fruits have also been recognized to play a role in the reduction of the the risk of most severe diseases (Blessy *et al*., 2012). Basically, phytochemicals are grouped into primary and secondary metabolities (Kumar *et al*., 2009; Parekh and Sumitra, 2007). Carbohydrates, chlorophylls, amino acids and proteins, are the main components of phytochemical, while alkaloids, saponins, steroids, flavonoids and tannins are the plants secondary metabolites (Kumar *et al*., 2009). Phytochemical components play an essential part in the identification of crude drugs as well as in the assessment of drugs obtained from the plant sources (Moses *et al*., 2013).

Vigna subterranea (L.) Verdc) is a member of the family Leguminosae, subfamily Papilionoideae, and is commonly found in the wild from central Nigeria eastwards to southern Sudan (Goli, 1997; Brink and Belay, 2006). The common name is derived from the Bambara tribe, who now lives mainly in Mali (Doku and Karikari, 1971). This underutilized legume is a drought-tolerant crop with nutritive and medicinal values. Therefore, this crop is referred to as nutraceuticals (Udeh *et al*., 2020). Bambara plays a vital role in the diet and culture of the people of sub Saharan africa. The leaves, used for livestock feed are rich in phosphorus. Both pigs and poultry feeds on the seeds, while leafy stems are used as livestock fodder. Mainly cultivated for its underground seeds, it can be eaten fresh, dry or after cooking (Brink *et al*., 2006). The crop does not require fertile soil to grow, and there is no need for fertilizer application except when there is a serious deficiency of potassium and phosphorus (Ogazie, *et al*., 2018). Based on this, there exists a need to review the beneficial potential values of two accessions of this neglected and underutilized crop to protect and promote its cultivation for food (nutrients) and medicines, especially among rural communities in Nigeria.

The investigation into the proximate and mineral composition as well as the phytochemical screening will serve as a prelude to the mechanism of action and other possible applications of the material.

2. Materials and Methods:

The experimental design used in this research work is a Correlational study. The experiment was conducted in July 2020. The day and night greenhouse temperatures were 25^0C and 15^0C respectively. While humidity was kept at 70to 80%. Seeds of *Vigna subterranea* accessions Tvsu 25 and Tvsu 2113 were used as plant materials for this research work. The seeds were obtained from IITA Ibadan in Nigeria and planted in the Greenhouse of University of Port Harcourt, Rivers state. Harvested seeds were sent to IITA Ibadan for phytochemical analyses.

2.1. Proximate Analysis:

The proximate composition of the seeds of two *Vigna subterranea* accessions Tvsu 25 and Tvsu 2113; moisture, ash, fibre, fat and protein were determined by the standard method of AOAC (1980). Crude protein was determined by multiplying the value obtained from Kjeidahl's nitrogen by a protein factor of 6.25. Energy value was also carried out by the standard method of AOAC (1984). Carbohydrate was calculated by difference according to the method of Merrill and Watt, (1973).

2.2. Phytochemistry:

Quantification of phytochemicals involves the use of various methods in determining the amount of bioactive compounds in the plant material (Kabir, 2005). The phytochemical constituents of the sample such as saponins was determined by the standard method of (Harborne, 1973 and Obadoni and Ochuko, 2001), tannins by (Van – Burden and Robinson, 1981), while the rest; steroids, phlobatannin, phytate, oxalate, flavonoid, alkaloids and cardiac glycosides were determined by method of Ceirwyn (1995).

2.3. Mineral analysis:

Various photometric and titrimetric methods were used in the quantification of these essential nutrients. Mineral analyses shall be carried out using Buck Scientific Atomic Absorption/Emission Spectrophotometry (AAS) and Molybdemun blue method for phosphorus. The mineral contents of the sample were analyzed by dry-ashing the sample at 550^oC and dissolving the ash in 10% (v/v) HCl. It was filtered and made up to 100 ml in a volumetric flask by using distilled de-ionized water. Sodium and potassium were determined by flame photometric method using Maizeing flame photometer (Maizeing, UK, model 1405). Phosphorus was determined with Jenway 6100 spectrophotometer at 420 nm using vanadium phosphomolybdate (Vanadate) colorimetric method with KH2PO4 as the standard method of

Ceirwyn (1995). The rest of the metals Mn, Cu, Cr, Sn, Pb, Hg, N, Co, Zn, Fe, Mg and Ca were determined by the standard method of AOAC (1990).

2.4. Statistical analysis:

Microsoft excel 2010 was used for data entry while SPSS in the analyses of simple charts, correlation coefficient, T-test and P-value.

3. Results:

Quantitative comparative analyses of phytochemicals, proximate, minerals, vitamins and amino acids screening of *Vigna subterranea* (L) Verdc accessions Tvsu 25 and Tvsu2113 are presented in figures 1 - 6. Results of the Pearson correlation performed to test the linear relationship and the significance of the correlation are presented in Table 1-7

3.1. Phytochemical analysis:

Table. 1: Results of phytochemical contents used as variables in the comparative study with statistical values

Phytochemicals	Tvsu 25	Tvsu2113
Alkaloid	8.861	10.98
Saponins	0.870	0.971
Oxalate	5.565	6.275
Phenol	79.231	42.510
Glucoside	4.976	3.951
Tannins	3.823	6.758
Correlation coefficient (r)	0.98	
T statistics	16.25512412	
DF	9	
P-value	0.000	

Results of the correlation analysis showed that there was a very high positive correlation (0.98) between the two accessions that was significant at (P > 0.05) (Table 1).

3.2. Proximate analysis:

Table. 2: Showing the Pearson`s Correlation analysis of the Proximate analysis between the two accessions (Tvsu 25 and Tvsu 2113

Proximate	Tvsu 25	Tvsu2113
Crude protein	27.515%	24.524%
Carbohydrate	35.492%	42.069%
Moisture contents	11.560%.	11.853%
Fiber content	10.663%	9.673%
Fat	5.555%	3.332%
Ash	9.214%	8.545%
Correlation coefficient (r)	0.98	
T statistics	10.38394655	
DF	4	
P-value	0.001	

Correlation coefficient (r) between the two accessions was 0.98 (table 2) a very high correlation which was also found to be significant at (P > 0.05) since is lower (0.001) than the specified significance level of 0.05.

3.3. Minerals:

Table. 3: Showing the Pearson`s Correlation analysis of the Mineral analysis between the two accessions (Tvsu 25 and Tvsu 2113

Mineral	TVSU 25	TVSU 2113
% Nitrogen	4.402	3.924
% Calcium	1.483	1.541

% Magnesium	0.718	0.754
% Potassium	0.254	0.274
% Phosphorus	1.956	1.298
ppm Na	0.591	0.536
ppm Mn	184.914	165.203
ppm Fe	164.045	116.638
ppm Zn	97.219	88.302
ppm Cu	9.499	7.545
ppm Sn	0.006	0.005
ppm Pb	0.006	0.005
ppm Cd	0.003	0.003
ppm Se	0.001	0.001
ppm Cr	0.172	0.15
ppm Co	0.016	0.015
ppm Ni	0.002	0.001
ppm As	0.015	0.015
ppm Hg	0.048	0.042
Correlation coefficient (r)	0.99	
T statistics	34.52685868	
DF	17	
P-value	0.000	

The two accessions were positively correlated with a very high correlation coefficient (r) of 0.99 significant at P-value of 0.000 (Table 3) found to be lower than the specified significance level of 0.05.

3.4. Amino acid:

Table. 4: Showing Pearson`s Correlation analysis of the Amino acid contents between the two accessions (Tvsu 25 and 2113)

Amino Acids	TVSU25	TVSU2113
Threonine	9.52	7.78
Leucine	4.03	4.38
Isoleucine	0.88	0.9
Lysine	1.101	0.937
Methionine	4.103	3.109
Phenylamine	24.252	19.816
Tyrosine	0.379	0.51
Valine	4.022	3.462
Argine	0.489	0.401
Histidine	11.229	10.188
Alanine	1.385	1.027
Asparagine	4.048	3.054
Aspartic acid	6.388	5.21
Glutamic acid	2.772	1.77
Glutamine	5.435	6.376
Glycine	1.842	2.000
Proline	2.617	1.587
Serine	0.928	0.626

Tryptophane	3.574	2.498
Cystine	2.229	0.246
Total Amino acid	91.223	75.877
Correlation coefficient (r)	0.99	
T Statistics	27.69316107	
DF	18	
P-value	0.000	

The two accessions were positively correlated with a very high correlation coefficient (r) of 0.99 significant at P-value of 0.000 (Table 4) found to be lower than the specified significance level of 0.05 (P > 0.05).

3.5. Vitamins:

Table. 5: Showing Pearson`s Correlation analysis of the Vitamin contents between the two accessions (Tvsu 25 and 2113)

VITAMINS	TVSU25	TVSU 2113
Vit.A	47.587	35.211
Vit.B1	2.163	1.146
Vit.B2	6.973	3.694
Vit.B3	0.572	0.303
Vit.B6	2.685	2.893

Vit.B12	2.882	1.527
Vit.C	8.387	4.444
Vit.E	0.057	0.05
Total Vitamins	71.306	49.268
Correlation coefficient (r)	1.00	
T Statistics	33.84512247	
DF	6	
P-value	0.000	

The two accessions were positively correlated with a very high correlation coefficient (r) of 0.99 significant at P-value of 0.000 (Table 5) found to be lower than the specified significance level of 0.05 ($P > 0.05$).

3.6. Glucosides:

Table. 6: Showing Pearson`s Correlation analysis of the Glucosides contents between the two accessions (Tvsu 25 and 2113)

GLUCOSIDES	TVSU 25	TVSU 2113
Glycyrhizic acid	1.324	0.959
Glycyrrhetinic acid	0.003	0.003
18-beta-glycyrrhetinic acid	0.014	0.012

E-strophanthin acid	0.076	0.066
Digitoxin acid	0.055	0.048
Digoxin acid	0.006	0.005
Oleandrin acid	0.004	0.004
Varapamil acid	0.107	0.093
Nifedipine acid	0.287	0.249
Ameodipine acid	1.490	1.296
Lisinopril acid	0.841	0.731
Enalaprilacid	0.034	0.029
Hydrochlorathiazide acid	0.008	0.007
Captopril acid	0.026	0.022
furosemide acid	0.038	0.033
propranolol acid	0.619	0.367
Atenonol acid	0.025	0.015
metoprolol acid	0.019	0.011
Total Glucosides	4.976	3.95
Correlation coefficient (r)	0.99	
T Statistics	30.81	

DF	16
P-value	0.000

The results of the pearson`s correlation between both accessions performed on the glycosides content showed that there was a significant positive association between both accessions with a very high correlation coefficient (r) of 0.99 with a P-value of 0.000 (P > 0.05) (Table 6).

3.7. Alkaloids:

Table. 7: Showing Pearson`s Correlation analysis of the Alkaloid contents between the two accessions (Tvsu 25 and 2113)

Alkaloid	TVSU 25	TVSU 2113
Caffeine	0.376	0.711
Colchicine	0.047	0.31
Cinhonine	0.018	0.013
Rauwolfia	0.057	0.071
Morphine	0.004	0.005
Apomorphine	0.042	0.052
Atropine	0.032	0.03
Apoatropine	0.08	0.1
Quinine	0.216	0.269
Nicotine	1.122	1.398
Codine	0.633	0.789
Papaverine	0.025	0.032
Nicotine	0.019	0.024
Conine	0.006	0.008

Piperine	0.029	0.036
Ricinine	1.004	1.163
Strychine	0.002	0.002
Vincristine	0.008	0.009
Eserine	0.042	0.048
Pilocarpine	0.003	0.004
Ephedrine	0.03	0.035
Lobelline	0.002	0.003
Tubocurarine	0.059	0.068
Reserpine	0.158	0.183
Vinblastine	0.82	0.95
Piperidine	0.463	0.536
Heroin	0.019	0.021
Emstine	0.014	0.016
Quinindine	0.005	0.005
Peletrevine	0.021	0.024
Pyridine	1.054	1.359
Quinoline	0.002	0.002
Acridine	0.008	0.011
Cocaine	0.044	0.056
Ergotamine	0.003	0.004
Norpsuedoephedrine	0.0332	0.041
Nornicotine	0.002	0.003

Cinhonidine	0.062	0.079
Hyoscine	0.166	0.214
Berberine	0.861	1.111
Psychotrine	0.486	0.627
Theobromine	0.019	0.025
Thoephilline	0.015	0.019
Cephaline	0.005	0.006
B-carboline	0.022	0.028
Phenylethylamine	0.730	0.758
Total Alkaloid	8.8662	11.258
Correlation coefficient (r)	0.99	
T Statistics	44.05	
DF	44	
P-value	0.000	

The results of the pearson`s correlation between both accessions performed on the alkaloids content showed that there was a significant positive association between both accessions with a very high correlation coefficient (r) of 0.99 with a P-value of 0.000 ($P > 0.05$) (Table 7).

4. Discussion:

The results of the Pearson`s correlation performed on the proximate composition, amino acid, mineral, vitamin and phytochemical composition of two accessions of Bambara groundnut (*Vigna subterranea* (L) Verdc. (Tvsu 25 and Tvsu 2113) showed that there was a significant positive association between both accessions with a very high correlation coefficient (r) in all parameters analysed. The correlations were observed to be significant as all P-value observed in all parameters analysed were found to be lower than the specified significance level of

0.05.The results of the correlation coefficient range from 1.00 to 0.98 while that of the p-value is from 0.001 to 0.000.

The results from the proximate analysis are in conformity with reports of Onimawo *et al*., 1998; Adu-dapah and Sangwan, 2004 and Okonkwo and Okpara, 2010. This implies that it can add to the staple food in Rivers state, Nigeria and the continent of African and as well helps in requirements of achieving the sustainable development goals. This plant is also known for its nitrogen fixation property that improves the soil and sustains the environment.

The relatively low fat content of the seeds (Tvsu 25 with 5.555% and Tvsu 2113 with 3.332%) suggests that oil from the seed should not be considered for commercial purposes. But low fat values also suggest that oil from the low fat would be less prone to lipids-related forms of deterioration.

The presence of alkaloid in *Vigna subterranean* indicate a natural agent for antibacterial and antifungal purpose. The high oxalate diet can increase the risk of renal calcium absorption (Osagie and Eka, 1998). Saponin have been shown to be highly toxic under experimental conditions and acute poisoning is relatively rare both in animals and man (Osagie and Eka, 1998). Similar to flavonoids, tannins compounds have also antiviral (Lu *et al*., 2004) and anti-parasitic effect (Kolodziej and Kiderlen, 2005). McGee (2004) stated that their destruction or modification in turn, plays an important role in ripening of fruit. The high value of flavonoid in the both accessions studied showed that it has potent antioxidant effect which tells that the seeds are very useful in medicinal purposes (Alan and Miller, 1996).

5. Conclusion:

The correlations between the two accessions showed that they are highly related with positive correlations in all. This study have shown that TVSU25 and TVSU2113 accessions of *Vigna subterrane L.*Verdc can be seen as a potential source of mineral, natural products or secondary metabolites. The proximate composition, mineral content, phytochemical analysis results and the low level of anti-nutrients in the two accessions have provided a wealth of data for continuous cultivation of Bambara nut and their utilization in the ethno-botany as well as in pharmaceutical industries. Consequently, the consumption and cultivation of Bambara nut should be encouraged in Rivers State, Nigeria and Africa for human and environmental substance which is one of the millennium development goals.

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