Evaluation of proximate and anti-nutritional composition of six different accessions of *vigna subterranea* (l.) verdc.

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**Abstract.** *Vigna subterranea* L. Verdc is an annual seed crop widely favoured by resource-limited rural farmers in sub-Saharan Africa. The fresh seeds can be consumed raw or cooked or utilized to make different delicacies. The study evaluated the nutritional and anti-nutritional content of seeds of six different accessions of *V. subterranea* to obtain information on their potential suitability as an alternative source of protein for humans and livestock. Seeds of each accession were ground to fine powder, sieved and labelled separately. Three replicates of each were then analysed for both proximate and anti-nutritional factors using standard methods. The results obtained were subjected to statistical analysis using one-way analysis of variance (ANOVA) and the results were expressed as mean ± standard deviation. The result showed that the six accessions are rich in protein (19.43±0.07%), crude fibre (4.38±0.23%) and carbohydrate (55.59±2.52%) but also contained a substantial amount of anti-nutritional factors such as oxalate (4.63±0.14 mg/g) and phytic acid (4.51±0.03 mg/g). TVSu 352, an accession originating from the USA, had the highest protein (20.11%), high nitrogen-free extract (53.24%) and lowest fat (4.60%) content making it the most nutritious among the accessions studied, though it also contained the highest tannin (4.60 mg/g) level. TVSu 277 exhibited the lowest crude protein (18.91%), lowest crude fibre (3.28%), highest carbohydrate (57.43%) and highest nitrogen-free extract (54.15%). These showed that the accessions are quite rich, and exhibited diversity, in both proximate and anti-nutritional characteristics. It can be concluded that TVSu 352 is the most-nutritious among the accessions studied and can, therefore, be recommended for consumption or industrial utilization and widespread cultivation but further study is required to unravel its yield stability and resistance status. It can also be inferred that with adequate processing, the seeds of *V. subterranea* will be a good substitute for cowpea as an alternative source of protein in both humans and livestock because they are very rich in nutritional compounds.

**Keywords:** *Vigna subterranea; Seeds; Nutritional factors; Anti-nutritional factors; Accessions.*

1. **INTRODUCTION**

Bambara groundnut (*Vigna subterranea* L. Verdc) is an annual seed crop belonging to the family Fabaceae. It is a self-pollinating legume widely favoured by resource-limited rural farmers [1]. It is an indigenous African crop widely cultivated for its highly nutritious seeds and its ability to grow and produce on marginal lands which are unsuitable for the cultivation of other favoured species [2]. The seeds are consumed in diverse forms. The fresh seeds are sometimes consumed raw, boiled, or grilled [3]. The dry seeds may also be roasted and ground into a powder to make bean cakes and different delicacies [4]. Despite its high nutritional content and ability to grow in highly degraded soil, it remains an underutilized or neglected crop. This is probably due to its storage-induced defects, hard-to-cook phenomenon, or the presence of anti-nutritional factors...
present in the testa [5]. It has been reported that these anti-nutritional factors may not only reduce the bioavailability of nutrients present in food but may also be harmful to human health [6]. Although some anti-nutritional factors (e.g. phytic acid and phenol) may be beneficial to humans by helping to reduce the risk of coronary heart diseases and diabetes [7]. Hence, this study aims to determine nutritional and anti-nutritional content of the seeds of different varieties of V. subterranea to obtain information and subsequently exploiting them as alternative sources of protein, not only for human consumption but also its potential as a source of nutrient supplement in the formulation of animal feeds.

2. Materials and Methods

2.1 Sources of V. subterranea Germplasms

The seeds of six (6) accessions of V. subterranea utilized in this study were sourced from the Genetic Resources Centre, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (Table 1).

Table 1: Passport data of the accessions studied

| Varieties | Accession | Seed Colour | Geographical region | Cultivar name |
|-----------|-----------|-------------|----------------------|--------------|
| V1        | TVSu 277  | Cream       | USA (North America)  | 188-2/HILLx  |
| V2        | TVSu 733  | Cream       | Tanzania (East Africa) | Kumashi    |
| V3        | TVSu 178  | Cream       | USA (North America)  | 211-18-M-M  |
| V4        | TVSu 352  | Cream       | USA (North America)  | D66-8666X   |
| V5        | TVSu 11   | Purple      | Nigeria              | FA78-194    |
| V6        | TVSu 254  | Dark Brown  | Plateau, Nigeria      | 3H/9-2      |

2.2 Sample preparation

Seeds of each accession were ground to fine powder, sieved and labelled separately. Three replicates of each were then used for both proximate and anti-nutritional analyses.

2.3 Proximate Analysis

The moisture content of the seeds was determined at 105\(^\circ\)C while the percentage of ash was evaluated at 550\(^\circ\)C. The total nitrogen and crude protein value was determined using the micro-Kjeldahl method according to AOAC [8]. Crude fat was determined by extraction with petroleum ether using a Soxhlet apparatus. The crude fibre was determined according to Chinedu and Nwinyi [9]. Total carbohydrate was determined by subtracting the value of moisture content, ash, crude protein, crude fibre and crude fat (ether extract) from 100 according to AOAC [10].

Nitrogen Free Extract (NFE) was determined as:

\[ \text{NFE} = \text{Total Carbohydrate} - \text{Crude Fibre} \]

Gross energy per 100g dry matter was calculated using the method of Eknayake et al., [11]:

\[ \text{Gross Energy (kJ)} = (\text{crude protein} \times 16.7) + (\text{crude lipid} \times 37.7) + (\text{crude carbohydrates} \times 16.7) \]

2.4 Anti-nutritional Analysis

Total oxalate was determined, using the method of Umar et al., [12] and expressed as g/ 100g of dry seed weight. The total alkaloid in the sample was determined using the method of Shamsa et
al., [13] and expressed as g/ 100g of seed dry weight. Total flavonoid content was determined, using the method of Mistrello et al., [14] and expressed as mg of Catechin equivalents per 100g of seed dry weight. The phytic acid level was evaluated using an indirect colorimetric method of Wheeler and Ferrel [15] and expressed as mg/ g of dry seed weight. Tannin content was determined using themethod of Makkar and Goodchild [16] and expressed as mg/ g of dry seed weight.

2.5 Data Analysis
The proximate composition and anti-nutritional analysis were carried out in triplicates and the results obtained were analysed using one-way analysis of variance (ANOVA) with Statistical Tool for Agricultural Research (STAR) and the results were expressed as mean ± standard deviation. Significant differences within treatments were determined at 5% significance level.

3. RESULTS AND DISCUSSION
The result of the proximate analysis is presented in Table 2. Crude protein value was high, like most legumes, and ranged from 18.91% to 20.11%. Statistical analysis showed asignificant difference in crude protein values among the accessions. This value was lower than the 32.40% crude protein observed in white black-eyed Bambara groundnut by Chinedu and Nwinyi [9]. It was also lower than the 28.63-30.43% observed in African yam bean seeds by Abioye et al., [17]. This high level of protein would, therefore, make it an ideal food for supplementing carbohydrate-rich diet consumed by both adults and children in developing countries. It also meant it could be used as an ingredient in compounding animal feed. Mean fibre ranged from 3.28% to 5.61%. Statistical analysis showed asignificant difference in mean FIBER levels among the accessions. These values were lower than the 9.29% fibre observed in raw *V. subterranea* seeds by Ndidi et al., [18]. High fibre in this seed is advantageous because fibre not only aids peristaltic movement in the intestinal tract to ease the passage of waste, but also helps to reduce risk of intestinal cancer and lower the level of cholesterol in the blood [19]. FAT ranged from 4.60% to 6.67%. Statistical analysis showed asignificant difference in mean FAT levels among the accessions. These values were higher than the 2.84% crude lipids observed in *S. stenocarpa* by Ndidi et al., [18], but was significantly lower than the 22.25% crude lipids observed in whole seeds of *G. max* by El-Shemy et al., [20]. The low-fat content of the seed is advantageous because it can be utilized as an integral part of a weight-reducing diet. ASH ranged from 4.16% to 6.97%. Statistical analysis showed asignificant difference in ASH among the accessions. These values were slightly higher than the 3.6% ASH observed in *V. subterranea* by Mahala and Mohammed [21]. This shows that appreciable level of minerals exists in the seeds hence their consumption would help to enhance growth and development in children. The fact that the ASH is above 1.5-3.5% range, proposed as suitable for use in animal feeds by Pomeranz and Clift [22], meant they could serve as anapotenital source of animal feeds. MOC ranged from 8.88% to 9.55%. Statistical analysis showed asignificant difference in MOC among the accessions. This level was higher than the 6.51% observed in *G. max* but lower than the 10.44% observed in *Vicia faba* by El-Shemy et al., [20]. This high level of moisture in the seeds would make them more susceptible to microbial attack thus reducing their shelf-life. CHO content ranged from
53.70% to 57.43%. Statistical analysis showed no significant difference in mean CHO values among the accessions. These values were slightly lower than the 60.8% CHO observed in *V. unguiculata* by Olaleye *et al.*, [23], as well as the 64.9 g/100g observed in *V. subterranea* by Enwere and Hung [24]. This high level of CHO meant the seeds could be considered as a good source of energy since carbohydrates are energy giving food.

NFE ranged from 49.08% to 54.15%. Statistical analysis showed a significant difference in mean NFE levels among the accessions. The NFE was similar to the 54.22% observed in *S. stenocarpa* by Ndidi *et al.*, [18]. Mean GE value ranged from 1445.68 kJ to 1489.43 kJ. Statistical analysis showed a significant difference in mean GE values among the accessions. These levels were much higher than those observed in *V. subterranea* by Olaleye *et al.*, [23]. This high GE value indicates that the seeds can contribute about 20% of the 6691 kJ recommended daily intake. The generally low CV (%) observed among the accessions revealed there is little variation among the results observed.

The result of the anti-nutritional analysis is presented in Table 3. Oxalate value ranged from 3.29 mg/g to 6.23 mg/g. Statistical analysis showed a significant difference in mean oxalate content among the accessions. This value was comparable to the 5.02 mg/g oxalate observed in raw *V. subterranea* seeds by Olaleye *et al.*, [23]. Alkaloids value ranged from 0.12 to 0.43 g/100g. Statistical analysis showed a significant difference in mean alkaloid levels among the accessions. This value was comparable to the 0.14-0.39 g/100g alkaloid content observed in raw *V. subterranea* seeds by Olaleye *et al.*, [23].

Mean flavonoids value ranged from 0.28 g/100g to 0.40 g/100g. Statistical analysis showed a significant difference in mean flavonoid levels among the accessions. This value contrasts with the 2.21-2.24 g/100g flavonoid values observed in seeds of six species of *S. stenocarpa* by Onuoha *et al.* [25]. The presence of flavonoids in the seeds, albeit at a low level, indicates that its consumption may result in a decrease in the absorption of non-heme iron in the intestine and may also inhibit cellular uptake of vitamin C. Phytic acid content ranged from 3.29 mg/g to 5.54 mg/g. Statistical analysis showed a significant difference in mean Phytic acid among the accessions. This value was similar to 1.35-4.93 mg/g observed in seeds of 30 accessions of *V. subterranea* by Unigwe *et al.*, [26]. The presence of phytic acid in the seeds, albeit at a very low level, may reduce the nutritional quality of the accessions by making the nutrients unavailable through inhibition of activities of gastrointestinal enzymes such as tyrosinase, pepsin, trypsin, lipase, and amylase. Mean tannin content ranged from 0.20 mg/g to 5.26 mg/g. Statistical analysis showed a significant difference in mean tannin levels among the accessions. This value contrasts with the 0.10-0.14 mg/g tannin observed in seeds of six species of *S. stenocarpa* by Onuoha *et al.* [25]. The high tannin content may result in a reduction in the nutritional quality of the accessions by making the protein unavailable through inhibition of the activities of digestive enzymes (chymotrypsin, trypsin, amylase, and lipase) present in the seeds while also interfering with dietary iron absorption [27].
Table 2: Proximate composition of seeds of six accessions of *V. subterranea*

| Varieties | Accession   | PROTEIN (%) | FIBER (%) | FAT (%) | ASH (%) | MOC (%) | CHO (%) | NFE (%) | GE (kJ) |
|-----------|-------------|-------------|-----------|---------|---------|---------|---------|---------|---------|
| V1        | TVSu 277    | 18.91<sup>d</sup> | 3.28<sup>d</sup> | 4.74<sup>e</sup> | 6.48<sup>e</sup> | 9.16<sup>b</sup> | 57.43<sup>a</sup> | 54.15<sup>a</sup> | 1453.58<sup>d</sup> |
| V2        | TVSu 733    | 19.27<sup>c</sup> | 4.00<sup>c</sup> | 5.35<sup>d</sup> | 6.79<sup>b</sup> | 9.36<sup>ab</sup> | 55.23<sup>a</sup> | 51.23<sup>a</sup> | 1445.68<sup>c</sup> |
| V3        | TVSu 178    | 19.45<sup>b</sup> | 4.39<sup>b</sup> | 6.36<sup>b</sup> | 6.97<sup>a</sup> | 9.13<sup>b</sup> | 53.70<sup>a</sup> | 49.31<sup>a</sup> | 1461.39<sup>c</sup> |
| V4        | TVSu 352    | 20.11<sup>a</sup> | 3.68<sup>cd</sup> | 4.60<sup>e</sup> | 5.81<sup>d</sup> | 8.88<sup>c</sup> | 56.92<sup>a</sup> | 53.24<sup>a</sup> | 1459.82<sup>c</sup> |
| V5        | TVSu 11     | 19.39<sup>bc</sup> | 5.32<sup>a</sup> | 5.87<sup>c</sup> | 4.28<sup>c</sup> | 9.55<sup>a</sup> | 55.59<sup>a</sup> | 50.27<sup>a</sup> | 1473.46<sup>b</sup> |
| V6        | TVSu 254    | 19.44<sup>b</sup> | 5.61<sup>a</sup> | 6.67<sup>a</sup> | 4.16<sup>c</sup> | 9.43<sup>a</sup> | 54.69<sup>a</sup> | 49.08<sup>a</sup> | 1489.43<sup>a</sup> |
| Mean±SD   |             | 19.43±0.07  | 4.38±0.23  | 6.00±0.12 | 5.75±0.07 | 9.25±0.13 | 55.59±2.52 | 51.21±2.58 | 1463.89±1.76 |
| LSD       |             | 0.13        | 0.41       | 0.20      | 0.12      | 0.23      | 4.48      | 4.58      | 3.25     |
| CV (%)    |             | 0.38        | 5.20       | 2.07      | 1.18      | 1.38      | 4.53      | 5.03      | 0.12     |

*Values are expressed as mean of three replicates; Values with different superscripts within the same column are significantly different from each other at p<0.05. PROTEIN: Crude protein; FIBER: Crude Fibre; FAT: Crude Fat; ASH: Ash Content; MOC: Moisture Content; CHO: Total Carbohydrate; NFE: Nitrogen Free Extract; GE: Gross Energy.*
Table 3: Anti-nutritional content of seeds of six accessions of *V. subterranea*

| Varieties | ACCESSION | OXALATE (mg/g) | ALKALOIDS (g/100g) | FLAVONOID (g/100g) | PHYTIC ACID (mg/g) | TANNIN (mg/g) |
|-----------|-----------|----------------|---------------------|---------------------|-------------------|---------------|
| V1        | TVSu 277  | 4.21<sup>c</sup> | 0.40<sup>b</sup>    | 0.29<sup>de</sup>   | 5.54<sup>a</sup>  | 0.74<sup>e</sup> |
| V2        | TVSu 733  | 3.35<sup>d</sup> | 0.33<sup>c</sup>    | 0.32<sup>cd</sup>   | 4.60<sup>c</sup>  | 1.45<sup>c</sup> |
| V3        | TVSu 178  | 5.35<sup>b</sup> | 0.43<sup>a</sup>    | 0.36<sup>b</sup>    | 4.33<sup>d</sup>  | 1.15<sup>d</sup> |
| V4        | TVSu 352  | 3.29<sup>d</sup> | 0.40<sup>b</sup>    | 0.28<sup>e</sup>    | 4.95<sup>b</sup>  | 4.60<sup>b</sup> |
| V5        | TVSu 11   | 6.23<sup>a</sup> | 0.14<sup>d</sup>    | 0.34<sup>bc</sup>   | 3.29<sup>e</sup>  | 5.26<sup>a</sup> |
| V6        | TVSu 254  | 5.35<sup>b</sup> | 0.12<sup>d</sup>    | 0.40<sup>a</sup>    | 4.37<sup>d</sup>  | 0.20<sup>f</sup> |
| Mean±SD   |           | 4.63±0.14     | 0.30±0.02           | 0.33±0.02           | 4.51±0.03        | 2.23±0.11     |
| LSD       |           | 0.25           | 2.81                | 3.11                | 6.11              | 0.19          |
| CV (%)    |           | 3.03           | 5.21                | 5.28                | 0.76              | 4.78          |
| Significance (p) |   | **      | **                | **                | **              | **           |

Values are expressed as mean of three replicates; Values with different superscripts within the same column are significantly different from each other at p<0.05.

It can be observed from these results that the six accessions exhibited diversity, in both proximate and anti-nutritional characteristics. They are quite rich in nutritional characteristics, though they also contained appreciable quantities of anti-nutritional factors but these could be reduced or eliminated through adequate processing.

### 4. CONCLUSION

From the results presented above, it can be concluded that the seeds of *V. subterranea* are quite rich in nutritional and anti-nutritional compounds. They also exhibited diversity in these characteristics. It can also be concluded that TVSu 352 is the most-nutritious among the accessions studied and can, therefore, be recommended for human consumption, though with adequate processing, or industrial utilization in the formulation of animal feed and ultimately widespread cultivation and possible development as a cultivar but further study is required to unravel its yield stability and pest/disease resistance status.

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