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Research Paper

Phytochemical, Nutritional Profiles and Antioxidant Potential of *Vigna subterranean* (L) Verdc. Seed Oil Extract

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ABSTRACT

Background:

Bambara groundnut Vigna subterranea (L) Verdc., The Bambara groundnut is a drought-tolerant legume that was traditionally eaten in sub-Saharan Africa. Although its seeds have been identified to be rich in nutrients, there is an insufficient investigation of the seed oil as a rich reservoir of bioactive products and antioxidants.

Objective:

The paper examined phytochemical components, nutritional profile, mineral, and antioxidant property of Vigna subterranea seed oil extract to determine its suitability as a functional food ingredient and nutraceutical.

Methods:

Soxhlet extraction of the seed oil was done in n-hexane as the solvent. Proximate analysis was done (moisture, ash, protein, fat, fibre, carbohydrate and energy value using standard AOAC methods). Atomic absorption spectrophotometry analysis was done on mineral content. The phytochemical screening was done in a qualitative manner whereas antioxidant potential was done by DPPH radical scavenging method and ferric reducing antioxidant power (FRAP) method.

Results:

The proximate composition of the seed sample revealed moisture content (0.10±0.02%), ash (46.33±2.59%), crude lipid (4.82±0.05%), crude protein (23.25±0.15%), crude fiber (3.35±0.04%), carbohydrate (22.07%), and a calorific value of 224.72 kcal/100 g. Mineral analysis showed calcium (14.4±0.5 mg/100 g) was the most abundant, followed by potassium (9.5±0.14 mg/100 g), sodium (7.5±0.42 mg/100 g), magnesium (2.9±0.004 mg/100 g), and trace levels of iron, zinc, copper, manganese, and cadmium. Phytochemical screening confirmed the presence of flavonoids, alkaloids, saponins, tannins, and phenolic compounds. The oil extract demonstrated significant antioxidant activity, with a dose-dependent inhibition of DPPH radicals and strong ferric reducing power, indicating its potential to neutralize oxidative stress.

Conclusion:

The Vigna subterranea seed oil harbour the essential life-enabling phytochemicals, nutrients, and mineral elements, besides its commendable anti-oxidant feature. These results are of interest that the seed oil holds a lot of potential in formulation of functional food, dietary supplement feed and natural antioxidants to promote health and prevent diseases. It is suggested to conduct research on bioavailability and toxicological safety of the oil to contribute to its industrial and medical application.

KEYWORDS: *Vigna subterranean*, Nutritional, Phytochemicals, Minerals, Antioxidant

INTRODUCTION

Vigna subterranea, commonly known as Bambara Groundnut, is an indigenous legume primarily grown in sub-Saharan Africa[1]. It belongs to the Fabaceae family and is botanically classified under the genus Vigna[2]. The plant is recognized for its resilience to harsh climatic conditions, making it a vital crop for food security, particularly in regions prone to drought. The species name "subterranea" refers to its unique growth habit, where the pods develop underground, similar to peanuts[3].

Vigna subterranean is predominantly cultivated in Africa, with countries like Nigeria, Ghana, and Cameroon being key producers. It is also grown in parts of Asia and South America, though at a smaller scale. The crop thrives in marginal soils and is highly resistant to pests and diseases. Its ability to grow with minimal agricultural inputs makes it a promising crop for sustainable agriculture, especially in areas with limited resources. *Vigna subterranean* is considered a complete food due to its balanced nutritional composition[4]. It is rich in proteins (18-25%), carbohydrates (50-60%), and fats (5-8%), making it an excellent source of nutrients for both humans and animals.

Vigna subterranean contains various phytochemicals, including phenolic compounds, flavonoids[5],and tannins[6]. These compounds have been shown to exhibit antioxidant activity, which helps in scavenging free radicals in the body, potentially reducing the risk of chronic diseases such as cardiovascular diseases and cancer. The high antioxidant potential of *Vigna subterranean* contributes to its status as a functional food with potential health benefits beyond basic nutrition.

MATERIALS AND METHODS

Collection and Identification of *Vigna subterranean*

The mature and dry seed of *V. subterranean* were purchased at Oja Tuntun Market, Ilorin, Kwara, Nigeria, and taxonomically identified and authenticated at the Department of Plant and Environmental Biology, Kwara State University, Malete, Nigeria.

Proximate Composition

A 2 g of *V. subterranean* seeds was put into clean-dried crucible and weighed. The crucible and its content were oven dried at 102-105^{o}C for an interval of two hours, cooled in a desiccator, and weighed to constant weight. The difference in weight before and after drying was recorded as the moisture content of the seed[7]. Ash content was quantified according to the method described by Ceirwyn (1998), which involved dry ashing2.0 g of the sample and incinerated in muffle furnace at 550^{o}C for 4 hours until greyish white ash was obtained. Crude lipid content was determined where 50g of the sample was macerated in n-hexane (95%) at room temperature (27±2^{o}C) for 72 hours. The extract was decanted, filtered, and concentrated under reduced pressure by rotary evaporator at 40 °C[7].Crude protein of the sample was determined by multiplying the value obtained from Kjeldahl's nitrogen analysis by a protein factor of 6.25[7]. The total carbohydrate contents of the sample were obtained using the equation;

% Total carbohydrate contents = 100 – (% moisture + % protein + lipids + ash + crude fibre contents).

Mineral Quantification

Oven-dried powdered sample (5 g) was put into a dry crucible and ignited in a muffle furnace at 550 ^{O}C until greyish white ash was obtained. The sample was removed and cooled in a desiccator. A 2.0 g of the sample was weighed into a clean digestion flask 15 mL of concentrated HNO_3 and 5 mL concentrated HCl (ratio 3:1) was added into the sample in the digestion flask. The whole sample was heated in a hot plate until all the brownish fumes were expelled out (Nitrogenous compound) which confirmed that the sample was digested, and the sample was allowed to cool at and a few mLs of distilled water was added and filtered into a 50mLs volumetric flask using a Whatman no. 42 filter and it was then transferred into plastic reagent bottle for AAS analysis. The concentrations of Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb and Zn were determined using Atomic Absorption Spectrophotometer (AAS Model SP9) while Na and K were evaluated using flame photometer.

Spectroscopic Analysis

The crude extract was analyzed using SHIMADZU GC-MS QP2010 Ultra coupled with MS-5973-634071 Series, at column oven temperature of 60.0°C (increasing to 270ºC in 7 min at flow rate of 10 ml/min). Injection temperature of

250.0°C with split flow injection and linear velocity flow control modes. The velocity pressure was maintained at 100.0 kPa with total flow rate of 102.6 ml/min, column flow rate of 2.16 ml/min and linear velocity of 37.9 cm/sec. A purge flow rate of 3.0 ml/min and a split ratio of 45.1 were used. The ion source temperature was 230.0°C, interface temperature of 250.0°C, solvent cut time of 4.50 min. The MS start time was 6.0 min; end time was 26.0 min, scan event time of 0.30 sec, scan speed of 1666. The start m/z of 35.00 and end m/z of 450.00.

RESULTS AND DISCUSSION

Proximate Composition

Table 1 shows the results of the proximate composition of *Vigna subterranea* seeds, and showed distinctly low moisture content of 0.16%. This is much lower than 3.467% recorded on *V. subterranean* seeds by Chelanga*et al.* (2023), and this shows that the seeds were highly dried up in this research[8]. This moisture content is beneficial since the food can be stored and has a long shelf-life because of lesser chance of spoilage and microbial contamination.

The proportion of carbohydrates was found to be 22.07% and is less than the proportion of carbohydrates reported by Dewole*et al.*, (2013) of Cola acuminata (58.09%) and Cola nitida (66.45%)[9]. Carbohydrates are needed as dietary macronutrients, and usually, provide most of the calorie energy in a standard diet.

The energy content of the seeds of *V. subterranea* estimated in this experiment was 224.72 kcal/100g,which exceeded the 155.04 1.60 kcal/100g recorded by Mathew *et al*. (2014), further supporting the notion that the seeds have a high energy value[10].The quantity of proteins was fairly high (23.25 0.15%), which confirms the reputation of the legume as a source of proteinous food. That favors its utility as a superior vegetable source of protein, particularly in this age of scarce animal protein because of cost and availability in some places. The measured protein level is however graciously lower than that which was considered by Chinedu & Nwinyi, (2012),to be of *V. subterranea*, i.e., 32.40 + 0.02%.Its fat content was moderately high of 4.82% and this is good within the nutritional range[11]. It is said that a diet containing 1 % or 2 % of its caloric energy as fat is sufficient for human health, but a high intake amount of fat can raise the possibility of developing cardiovascular diseases[12].Mineral element in the seed was found to be considerable as the total ash attributed to 46.33%. Another significant parameter is ash content which indicates overall mineral content of food. Calcium, potassium, magnesium as well as iron are minerals that are often related to V. subterranea and are related to metabolic processes, bone formation and cardiovascular health respectively.

All values excerpted are the means of triplicate determination with standard deviations.

Table 1: Proximate Composition of *Vigna* subterranea (% Dry weight)

PARAMETER	CONTENTS
Moisture	0.16 ± 0.02
Ash	46.33 ± 2.59
Crude Protein	23.25 ± 015
Crude Fiber	3.35 ± 0.04
Crude Lipid	4.82 ± 0.05
Drying Matter	99.84
Carbohydrate	22.07
Calorie Value (kcal/100g)	224.72

Table 2: Mineral Composition of *Vigna subterranea*

MINERAL	CONCENTRATION (mg/100g)
Calcium	14.4 ±0.5
Cadmium	0.1±0
Copper	0.9±0.001
Iron	0.2±0.002
Potassium	9.5±0.141
Magnesium	2.9±0.004
Manganese	0.6±0.004
Sodium	7.5±0.424
Nickel	0±0
Lead	0.1±0.001
Zinc	0.2±0.003

MINERAL COMPOSITION

Table 2 presents the result of mineral composition of *Vigna subterranea* seeds. The findings suggest that the mineral levels that were the highest were potassium and calcium. This observation is in line with that of Musah *et al.* (2021), in which they also devoted the main mineral in the *V. subterranea* seeds to potassium[13]. Potassium is quite an important element associated with the maintenance of acid-alkaline balance in the body[14]. Mathew *et al*. (2017) in a related investigation also found that the content of potassium was high at 122.91 1.02 mg/100 g in the seeds of V. subterranea collected in Kure Market[10].

In comparison, manganese and lead were found in a comparatively lower rate with 0.6 + 0.004, mg/100 g and 0.1+0.001, mg/100 g respectively. These levels are rather minimal compared to those noted by Musah *et al.* (2021),who determined higher levels of the elements in *V. subterranea* seeds. All the reported values are in the form of mean of triplicate determinations with standard deviations[13].

In as far as sodium intake is concerned, the body should be taken in a quantity of 1500 mg a day by both genders aged 9 -50 years and 1300 mg a day amongst those aged 59 years and above the recommendation by Westrick *et al.,*(2014)[15]. The not very high content of sodium may be observed during this research alongside the low iron concentration, giving the assumption that seeds of *V. subterranea* could be useful to people with high blood pressure. This is affirmed by the results of the studies by Tiong *et al*., (2018), which insisted on applying such a nutrient profile in dietary measures of controlling high blood pressure[16].

GC-MS ANALYSIS

Table 3: Result of GCMS Analysis of *Vigna Subterranean* Oil Extract

S/N	Compound Name	Molecular Formula	Molecular weight	Retention time	% Area
1	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	270	21.205	21.78
2	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	23.580	53.91
3	Phytol	$C_{20}H_{40}O$	296	23.750	1.66
4	Methyl stearate	$C1_9H_{38}O_2$	298	23.855	12.27
5	trans-Geranylgeraniol	$C_{20}H_{34}O$	290	24.980	3.17
6	Squalene	$C_{30}H_{50}$	410	26.435	4.41
7	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326	26.740	2.80

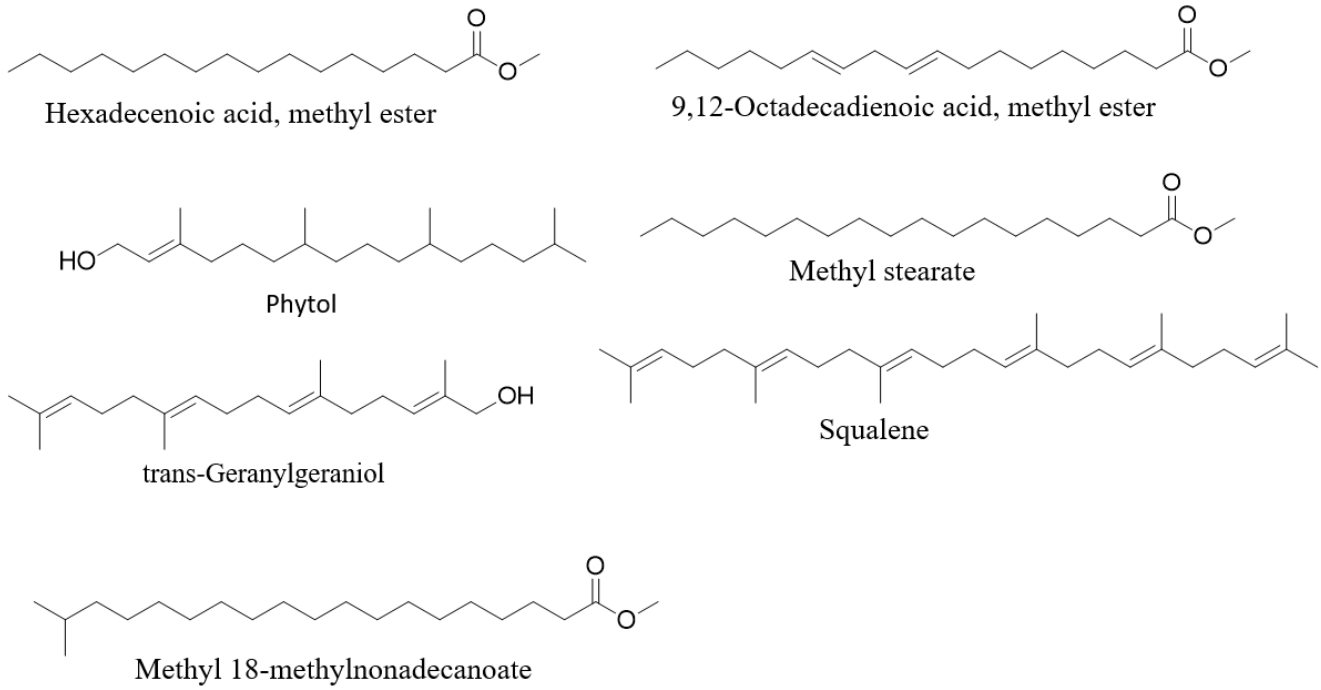

Table 4: Biological Properties of The Bioactive Compounds

S/N	COMPOUND	BIOLOGICAL ACTIVITIES	REFERENCE
1	Hexadecenoic acid methyl ester	Antioxidant, Hypocholesterolemic, Nematicide and Pesticide	Siswadi*et al.,* 2021
2	9,12-Octadecadienoic acid, methyl	Anti-inflammatory properties in addition to analgesic and ulcerogenic properties	ImadHadi Hameed *et al.,*
3	Phytol	Anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy, apoptosis -induing, anti -inflammatory, anti -nocicentive, immune -modulating and anti-microbial effect.	Muhammad Torequl Islam *et al.,* 2018
4	Methyl stearate	Anti-inflammatory, Antioxidant, Antifungal, Nematocidal	Kalpana*et al.,* 2012
5	trans-Geranylgeraniol	Prevent inhibition of the osteoclast formation and bone resorption in vitro	PratimaAnputa*et al.,* 2021
6	Squalene	Emollient for skin, anti -oxidant and for hydration and it's anti-tumor activities	ZIih-Rou Huang *et al.,* 2021
7	Methyl 18 -methylnonadecanoate	Anti-oxidant, anti-microbial and cytotoxic activities	ZIih-Rou Huang et al 2021

Antioxidant Assay Result

Sample	DPPH	ABTS	FRAP Reducing effect (mmolFe^{2+}/g)
VS	55.84±0.36	35.37±0.60	1.45±0.03
BHT	18.45±0.40	7.62±0.08	1.38±0.04

The assay of antioxidant showed that the oil extract of *Vigna subterranea* seeds had moderate to high antioxidant activity. Such was its ability to scavenge free radicals in both the DPPH and ABTS tests and the ability to reduce iron ions in the form FRAP test. IN the DPPH and ABTS radical scavenging assays, the extract had lower results of radical scavenging ability as compared to the standard antioxidant butylated hydroxytoluene (BHT), whereas the results were comparable or relatively higher in the FRAP radical scavenging test. The above-derived results imply that the seed oil extract possesses bioactive compounds with high electron-releasing capacity, which play a critical part in the prevention of oxidative agents. It also has the potential to be of antioxidant due to the presence of phytochemicals which include phenolics, flavonoids, and saponins. In support of this finding, a study conducted by Ijarotimi *et al.* (2022) claimed that Vigna subterranea flour possessed high FRAP values, an attribute that makes it a substance that can alleviate oxidative stress[17;18]. These findings substantiate the usefulness of the Vigna subterranea seed oil extract as a potential source of high-quality natural antioxidants that have prospective use in food preservation and control of diseases that arise as a result of oxidative stress.

CONCLUSION

This research papers point out the phytochemical composition, nutritive value, and antioxidant power of the oil extract in the seeds of *Vigna subterranea* (L.) Verdc. The oil extract was discovered to have high amounts of bioactive compounds such as in flavonoids, saponins, alkaloids and phenolics, related to multiple health promoting factor. Nutritional study revealed that the seed oil is a rich source of essential fatty acids, proteins, vitamins, and minerals, and it makes it an ideal constituent of functional foods.

There was also a significant antioxidant action observed in the extract, implying its possible usage in reducing oxidative stress and inhibiting resulting degenerative disorders. Besides, the low moisture content that is seen increases the shelf life and stability, making both the nutritive and phytochemical integrity persist even through transportation and storage. Therefore, the results justify the future use of Vigna subterranea seed oil in food and pharmaceutical and cosmetic sectors as a natural compound with health effects and food functionality points.

CONFLICT OF INTEREST: None

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