

Original Research Article

Comparative Phytochemical, Nutritional and Antimicrobial Screening of the Seed, Leaf and Root of *Vigna Subterranea*.

Adaora Stellamaris Ogbuagu¹, Chinwe Chioma Maduka¹, Isaac Obi Okerulu¹, Chukwuebuka Thankgod Onyema¹, Chidi Onyeizugbe² and Anthonia Uzoamaka Emezie²

¹Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University Awka, Anambra state, Nigeria.

²Medical Microbiology unit, Department of Medical Laboratory Science, Iyenu Hospital Ogidi, Anambra state, Nigeria.

ARTICLE INFO

Article history

Submitted: 2021-11-09

Revised: 2021-12-27

Accepted: 2022-01-06

Available online: 2022-05-09

Manuscript ID: PCBR-2111-1201

DOI: 10.22034/pcbr.2022.314226.1201

KEYWORDS

Phytochemical,
Antimicrobial,
Amino acids,
MIC,
MBC

ABSTRACT

Vigna subterranea, a legume regarded as a 'super food' in Nigeria has been grossly understudied and underutilised and this study investigated the constituents of the various parts of this plant for possible utilization. The seed, leaf and root of the plant were analyzed to assay the phytochemical constituents using standard gravimetric methods, nutritional and amino acid constituents using both gravimetric and spectroscopic as well as the antimicrobial activities using Agar well diffusion method. The quantitative phytochemical analysis showed high percentage content of alkaloids (0.34 ± 0.02), flavonoids (0.373 ± 0.01) and tannins (0.327 ± 0.013) on the leaf followed by the root with the highest saponins content (0.433 ± 0.02). Steroids were found in moderate quantities on all tested parts. The nutritional analysis showed high protein content on all the parts. The leaf had the highest vitamin C content ($6.453 \text{ mg}/100 \text{ g}$) while vitamin B3 was found to be highest in the seeds ($2.123 \text{ mg}/100 \text{ g}$). The total essential amino acids ($\text{g}/100 \text{ g}$) in seed, leaf and root were 42.44, 39.85, and 30.82 respectively, while total non-essential amino acids ($\text{g}/100 \text{ g}$) were 50.05, 46.84, and 38.25 respectively. The antimicrobial analysis showed n-hexane extract of the root having the highest activity against *Pseudomonas aeruginosa* ($24 \pm 1.41 \text{ mm}$ zone of inhibition). The Minimum Inhibitory Concentration (MIC) for n-hexane extract was 100 mg/L while the Minimum Bacterial Concentration (MBC) was 200 mg/L. indicates a good antimicrobial agent. The leaf and root of *Vigna subterranea* can no longer be regarded as post-harvest wastes but potential sources of drugs and nutrients.

HIGHLIGHT

- Cultivation and harvesting of *Vigna Subterranea* plant
- Analysis of leaf, seed and root of *Vigna Subterranea*
- Phyto-chemical analysis was conducted on the leaf, seed and root of the *Vigna Subterranea* plant
- Elemental and Vitamin B & C constituents of the *Vigna Subterranea* plant was also analyzed
- Essential and non-essential amino acid constituents of the *Vigna Subterranea* plant were determined
- Antimicrobial activities of different solvent extracts of the various parts of the *Vigna Subterranea* plants were studied

* Corresponding author: Adaora Stellamaris Ogbuagu

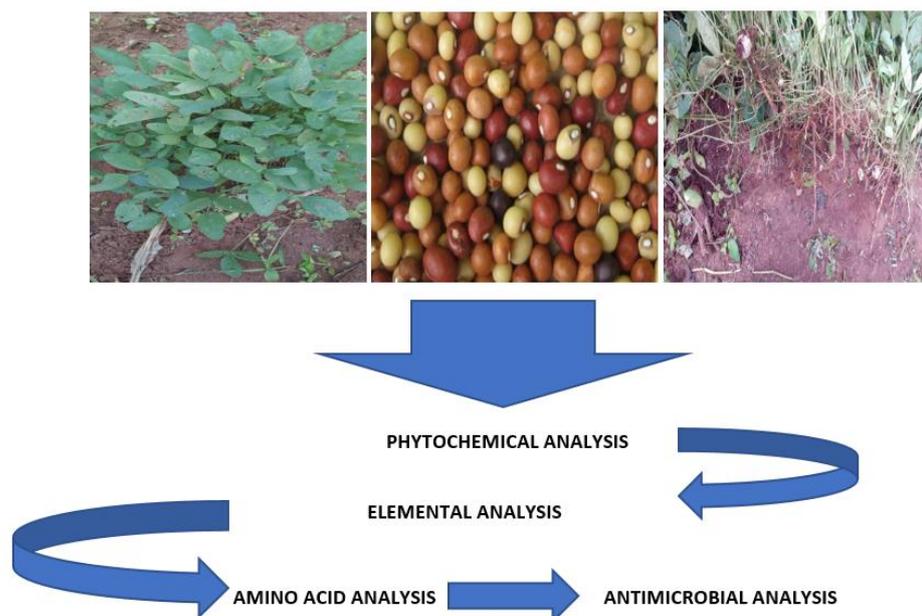
✉ E-mail: as.ogbuagu@unizik.edu.ng

☎ Tel number: +2347069111945

© 2020 by SPC (Sami Publishing Company)



GRAPHICAL ABSTRACT



Introduction

Legumes are known as important cheap sources of protein to poor resource people in Nigeria; they are the next important crops after cereal [1, 2]. Their seeds have an average of twice as much protein as cereals [3, 4]. They are good sources of essential amino acids and fats. The industrial applications of legumes depend on the knowledge of their nutritional importance and functional properties.

One of such legumes is the *Vigna subterranea* (Bambara Groundnut), its name is derived from the name of a tribe in Mali called "Bambara" [5]. *V. subterranea* is an indigenous Africa plant cultivated principally by farmers as a 'famine crop' because it has several agronomic advantages including high nutritional value, drought tolerance, and the ability to be produced in soil considered insufficiently fertile for cultivation.

V. subterranea is a small leafy, annual herbaceous legume intermediate plant with creeping stems at ground level. Various studies have reported the biochemical and the nutritive properties of the seeds of *V. subterranea* [6, 7]. The effect of soaking and/or cooking of the *V. subterranea* seeds on chemical composition, total energy, antinutritional factors, mineral contents and amino acid composition and the proximate, minerals, qualitative and quantitative composition of the seeds of *V. subterranea* have

also been conducted [8, 9]. The functional properties of the seed flour have also been conducted [10] In Senegal, leaf preparations are applied to abscesses and infected wounds, leaf sap is applied to the eyes to treat epilepsy, and the roots are sometimes taken as an aphrodisiac [11].

V. subterranea is an orphan crop and represent a neglected and under researched plant [12]. Most of the researches carried out on *V. subterranea* were on the seed, scanty or no literature was found on other parts of the plant especially the roots and the leaves. The leaves and the roots are often regarded as post-harvest waste and hence discarded as there are no known uses of these parts.

This research aims to find the phytochemical, nutritional, amino acid constituents as well as the anti-microbial activities of the leaves and roots of *V. subterranea* and these will be compared with that of the most studied part (the seed). This will provide a base line data on the various parts of the plant as well establish other areas of application of the leaves and the roots.

Experimental

Collection and Identification of Plant materials

The seed was planted in a local farm at Nnewi, Anambra State, Nigeria and harvested after three (3) months of maturity. It was identified in

the Department of Botany, Nnamdi Azikiwe University Awka as *Vigna subterranea* with herbarium number, 175^a.

Preparation of samples

The fresh leaves, seeds and roots of *V. subterranea* were harvested, sorted, washed to remove debris and dust particles and then dried for three days with a dry air oven at a temperature of 40°C, to avoid loss of active compounds. However, the roots were dried further for more seven days. They were ground to powder using a hand milling machine (mechanical grinder) and the powdered samples were stored in an air-tight container from which samples were taken for analysis.

Phytochemical analysis

An aqueous extract of each sample was obtained by dispersing 5 g of each sample in distilled water (50 mL). The mixture was allowed to stand for 30 minutes at room temperature with continuous shaking, and then filtered using Whatman No 42-filter paper. The filtrate was used as the aqueous extract for the following tests.

Qualitative phytochemical analysis was carried out to determine the presence of tannins, saponins, flavonoids, alkaloids, phenols, steroids using methods as described by Harborne [15].

Quantitative phytochemical analysis:

Alkaline precipitation gravimetric method was used to determine alkaloids. [13- 15].

Alkaloids was determined in each sample by the alkaline precipitation gravimetric method. Each sample (5 g) was dispersed in ethanol (100mL). The mixture was shaken and allowed to stand at room temperature for 4hours. It was filtered. The filtrate was concentrated to $\frac{1}{4}$ of the original volume by evaporation over a steam bath. Alkaloid in the extract was precipitated by addition of NH₄OH dropwise until full turbidity was obtained. The alkaloid precipitate was recovered by filtration using a weighed filter paper and washed with ammonia solution, dried in the oven at 80°C for an hour. It was cooled in a desiccator and re-weighed. By weight difference, the weight of alkaloid was determined and expressed as a percentage of the sample analysed.

Flavonoids

Flavonoid content was determined by spectroscopic methods. Exactly 10.0g of samples were weighed into a 250mL beaker containing 70mL of water. This was continuously shaken for 30minutes after which activated charcoal (6.0g) was added and allowed to stand for 30minutes before filtration using a 60mL fritted glass funnel containing asbestos pad. 2 drops of HCl were added to the filtrate and the filtrate was evaporated on a steam bath to about 40mL. This was quantitatively transferred into a 50mL volumetric flask and made up with water. The absorbance was read at 233nm and the concentration calculated [16].

$$\text{mg of flavonoid} = \text{Abs} \times 50/1000 \quad (1)$$

Saponins

The samples (0.1g) were weighed into a test tube, 5 mL of water added to the test tube. The content of the test tube was heated to boil for 5minutes and then filtered. 1.0 mL of the filtrate was transferred into a bigger test tube and to this, 10.0mL of distilled water was added. The absorbance was measured at 620nm and calibration curve was obtained using a standard and the concentration of the saponin was calculated [16].

$$\text{mg of Saponin}(\text{mg}/100) = \text{Reading from the curve} \times \text{Dilution factor} \times 100/1000 \quad (2)$$

Steroids

The samples (0.5g) was weighed into a test tube and 10mL of ethyl acetate added. The test tube placed in a boiling water bath for 3minutes, allowed to cool and filtered. The extract was mixed with equal volume of chloroform to form two layers. The chloroform layer(2.0mL) was pipetted into a test tube containing 5mL of distilled water and the pH adjusted to 7 using 0.1N NH₄OH. This was eluted in a column with sephadex X 100. The absorbance of the eluate was measured at 240nm and the concentration calculated [17, 18].

Proximate Composition.

Crude protein content of each sample was determined using the Kjeldahl method [19]. The moisture content was determined gravimetrically using the method described by Sadler and Murphy [21]. The ash content was determined using the furnace incineration gravimetric method as described by A.O.A.C [17,

18] The fat content was determined using continuous solvent extraction method with Soxhlet extractor as described by Pearson [21] and James [22]. The crude fibre content was assessed using Wende method [22]. Riboflavin, niacin, thiamine and ascorbic acid in each of the samples were determined spectrophotometrically [17].

Determination of Amino acid

The Amino acids determination was conducted by defatting one gramme of each sample using chloroform/ methanol in the ratio 2:1. The defatted sample (100 mg) was hydrolysed at $105 \pm 5^\circ\text{C}$ for 22 hours, hydrolysed sample (about 60 microlitre) was analysed for amino acids by loading into the Applied Biosystem PTH Amino Acid Analyser Model: 120A [23, 24].

Determination of antimicrobial activities

The n-hexane, ethanol and aqueous extracts of *V. subterranea* plant materials used for the antimicrobial analysis were prepared by dissolving 10 g each of the seed, leaf and root samples separately in 100 mL of n-hexane, ethanol and distilled water respectively. They were kept at room temperature for 48 hours with regular agitation. The extracts were filtered using muslin cloth, and the filtrates were evaporated to dryness using hot air oven at 40°C . The extracts were then re-constituted in Dimethyl sulphur oxide (DMSO) [25, 26]. Antimicrobial activities were determined using Agar-well diffusion method [27]. The following microbial isolates were used: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella specie*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. The isolates were confirmed morphologically and biochemically [28]. The MIC and MBC were set up and incubated at the same conditions [28]. All data were expressed as Mean \pm SD for triplicate (n=3) determination.

Results and Discussion

Qualitative Phytochemical Analysis.

The result obtained from the qualitative phytochemical analysis conducted on the leaf seed and roots of *V. subterranea* are reported as shown on **Table 1**.

Table 1 revealed the presence of alkaloids, flavonoids, saponins, and steroids in appreciable quantities in all the parts of the plant. Phenols and phytate were slightly present in the roots.

Table 1. the qualitative phytochemical analysis of the leaf, seed and root of *Vigna subterranea*.

Parameter	Leaf	Seed	Root
Flavonoids	+++	+	++
Alkaloids	+++	+	++
Tannins	+++	+	+
Saponins	++	+	+++
Phenols	+	+	\pm
Steroids	++	++	++
Phytate	++	\pm	\pm

Key \pm =slightly present, + = present, ++ = moderately present, +++= abundantly present

The presence steroids are very important because of their relationship with sex hormones [29, 30]. Saponins were most abundant in the seed and they are known to prevent excessive absorption of cholesterol thereby reducing the risk of cardiovascular diseases, they are said to also inhibit cancerous cells without killing the normal cell in the process [31, 32]. The leaf, seed and root of *Vigna subterranea* contained appreciable presence of flavonoids. Flavonoids has long been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative, anti-carcinogenic, and antimicrobial activities as well as to affect some aspect of mammalian metabolism [33,34]. Flavonoids in intestinal tract lower the risk of heart diseases [35].

These antimicrobial phytochemicals (especially, alkaloids, tannins and flavonoids) act by binding with the cell walls and inactivate the enzymes [36]. The presence of the phytochemical compounds in the leaf, seed and root of *Vigna subterranea* could make the plant a potential source of useful drug.

Quantitative Phytochemical Analysis

Quantitative Phytochemical Analysis of the leaf, seed and root of *V. subterranea* in percentages are shown in Table 2.

Table 2 shows the results (mean \pm standard deviation) of pooled sample determinations. Data on the same row with similar superscript alphabets are not statistically significant at $p < 0.05$. Data on the same row with different alphabets are statistically significant at $p < 0.05$.

Table 2: The quantitative phytochemical analysis of the leaf, seed and root of *V. subterranea* (%)

Sample	Alkaloids (%)	Saponins (%)	Flavonoids (%)	Steroids (%)
Leaf	0.340 ^c ±0.023	0.293 ^b ±0.012	0.373 ^c ±0.012	0.327 ^c ±0.013
Seed	0.167 ^a ±0.012	0.127 ^a ±0.012	0.233 ^a ±0.012	0.153 ^a ±0.012
Root	0.233 ^b ±0.012	0.433 ^c ±0.025	0.293 ^b ±0.042	0.227 ^b ±0.042
P-value	0.01	0.01	0.01	0.01

The alkaloid contents of the leaf, seed and root of *V. subterranea* were (0.34, 0.167, 0.233) % respectively at $P < 0.05$. Alkaloids are precursors of amino acids and are known to protect the plant from herbivorous animals as well as being pharmacologically active. Alkaloids contents of the seed ranging from 0.42 to 0.52% has been reported. [37]. Alkaloids which was abundantly present in root, are known to possess antimicrobial, antihypertensive, antifungal, anti-inflammatory, antifibrogenic and anti-diarrheal effect [38]. Several authors have also reported the analgesic properties of alkaloids. [15, 38] Flavonoids contents of the leaf, seed and root of *V. subterranea* were (0.373, 0.233 and 0.293) % respectively. Flavonoids, are potent antioxidants and free radical scavengers which prevent oxidative cell damage and possess strong anticancer activities. The leaf, seed and root of *V. subterranea* contained appreciable presence of flavonoids. Flavonoids has long been recognized to possess anti- allergic, antiviral, anti-proliferative, anti-carcinogenic, and antimicrobial activities as well as affect some aspect of mammalian metabolism [33, 33,39]. The saponins were found to follow the trend leaf > root > seed at 0.433, 0.293 and 0.127 respectively. It has been reported to have anti-hyper cholesterol because saponins prevent the excessive intestinal absorption of cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension [40]. The value

0.127, obtained for the seed is lower than 2.30 for *V. subterranea* seed in the literature [8, 41]. Saponins also have cardiac depressant properties and appear to kill or inhibit cancer cells without killing the normal cells in the process [42].

Proximate Composition

Proximate Composition of the leaf, seed and root of *Vigna subterranea* (%) are shown in Table 3. Values show the mean of triplicate analysis of \pm standard deviation; figures with different superscript down the column were significantly different ($p < 0.05$ theoretical).

The crude protein content (%) of the leaf, seed and root were 11.79 ± 0.1 , 18.96 ± 0.20 and 5.43 ± 0.18 respectively. The protein content of the seed showed moderately high protein content of 18.96% which is similar to 18.65% reported in the literature, [8] and 19.60% reported by Fadahunsi and Sanni [44] for the seed respectively. However, this value was lower than the (78.75%) reported by Yagoub and Abdalla [45] for the seed. The protein content of the leaf (11.79%) and root (5.43%) were higher than (3.3%) recorded by the USDA Nutrient Database for Standard Reference for leaves and roots of most legumes as no other studies were found on these parts of the plant under study to serve as reference [46].

Table 3: Proximate composition of the seed, leaf and root of *V. subterranea* (%)

Sample	Protein	Fat	Fiber	Ash	MC	CHO
Leaf	11.79 ^b ±0.10	2.57 ^c ±0.08	13.22 ^b ±0.22	3.09 ^c ±0.12	6.13 ^a ±0.05	60.53 ^c ±4.13
Seed	18.96 ^c ±0.20	1.69 ^b ±0.02	6.91 ^a ±0.03	2.89 ^b ±0.04	9.27 ^c ±0.05	60.26 ^b ±0.19
Root	5.43 ^a ±0.18	1.21 ^a ±0.03	28.84 ^c ±0.26	2.25 ^a ±0.01	6.26 ^b ±0.14	55.92 ^a ±0.02
p-value calculated	0.01	0.01	0.01	0.01	0.01	0.01

The high content of proteins in the various parts of *V. subterranean* can serve as media for microorganisms, feed for animal and humans with moderate protein needs. The plant is considered as a good source of protein because it provides more than 12% of calorific value from protein [19].

The ash content (an indicator for mineral elements) of the seed was found to be $2.89 \pm 0.04\%$ comparable to 3.48% reported by Andzouana, [8], 3.25% [9] and 3.26% [47]. That of the leaf and root were $3.09 \pm 0.12\%$ and $2.25 \pm 0.01\%$ respectively, these fall within the acceptable range recommended for animal feeds and human consumption and can as well serve as microbial media without mineral supplement. The carbohydrate content (%) of the leaf, seed and root were $60.53 \pm 0.13\%$, $60.26 \pm 0.19\%$ and $55.92 \pm 0.02\%$ respectively. The seed was found to be similar in content to 56.15% reported by a study conducted in 2013 [9]. Carbohydrate constitutes a major class of naturally occurring organic compounds which are essential for the maintenance of life in plant and animals and provide raw materials

for many industries [48]. All the parts of the plants are good sources of carbohydrates when consumed because they meet the Recommended Dietary Allowance (RDA) values of 45 -65% [49].

The moisture content (%) of the leaf, seed and root of *Vigna subterranea* were $6.13 \pm 0.05\%$, $9.12 \pm 0.05\%$ and $6.26 \pm 0.14\%$ respectively. Moisture content is an index of water activity of many foods. The observed values imply that the various parts of plant may have a long shelf life since microorganisms that cause spoilage do not

thrive in foods having low moisture content. The moisture content of the seed (9.27%) is similar to the value of moisture content ranging from 5 - 11% reported in literature for different *Vigna subterranea* seeds [50].

The fat content of the leaf, seed and root of *Vigna subterranea* were $2.57 \pm 0.08\%$, $1.69 \pm 0.02\%$ and $1.21 \pm 0.03\%$ respectively. The fat content of the seed was lower than previously reported 7.8 % and 6 -8 % [8 and 48]. Dietary fat functions in the increase of palatability of food by absorbing and retaining flavours. A diet having 1-2 % of its calorific energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as cancer and aging [51].

The fibre content of the leaf, seed and root of *Vigna subterranea* were $13.22 \pm 0.22\%$, $6.91 \pm 0.03\%$ and $28.84 \pm 0.26\%$ respectively. This is an indication that the various parts of the plant are good sources of crude fibre when consumed. Adequate intake of dietary fibre can lower the serum cholesterol, heart diseases, hypertension, constipation, diabetes and breast cancer [52].

Mineral Composition

Mineral Composition of the leaf, seed and root of *Vigna subterranea* (mg/100g) are shown in **Table 4**.

The result revealed that sodium (19.93), phosphorus (30.93), calcium (9.35) and iron (3.27) mg/100g were the most abundant in the leaf sample. Calcium (82.83), sodium (27.38), phosphorus (32.63) and iron (6.09) were the most abundant in the seed mg/100g while magnesium (58.40), potassium (70.67), were the most abundant in the root respectively.

Table 4: The mineral compositions of the leaf, seed and root of *V. subterranea* (mg/100g)

Sample	Ca	Mg	K	Na	P	Fe	Zn
Leaf	9.35 ^a ±2.31	1.26 ^a ±2.77	2.85 ^b ±1.73	19.93 ^b ±2.14	30.93 ^b ±0.99	3.27 ^b ±0.04	0.79 ^a ±0.03
Seed	82.83 ^c ±2.3	1.34 ^b ±1.39	1.12 ^a ±0.00	27.38 ^c ±1.13	32.63 ^c ±0.40	6.09 ^c ±0.02	1.07 ^a ±0.09
Root	13.36 ^b ±2.3	58.40 ^c ±1.39	70.67 ^c ±2.31	10.01 ^a ±0.30	18.93 ^a ±0.23	1.37 ^a ±0.02	0.48 ^a ±0.00
p-value calculated	0.01	0.01	0.01	0.01	0.01	0.01	0.414

Value show mean of triplicate analysis of \pm standard deviation; figures with different superscript down the column were significantly different ($p < 0.05$ theoretical). As observed in the study. Zinc was detected in a low concentration for leaf 0.79, seed 1.07 and root 0.48 mg/100g respectively. The ratio of sodium to potassium in the leaf (6.99) and the seed (24.45) is greater than 1 respectively but less than 1 in the root (0.14); therefore, consumption of the leaf and seed may cause high blood pressure because Na: K is more than the recommended value (Na: K \leq 1) by FND [47] while the root can be employed medically [40]. The samples are very good source of Ca and P which are important for growth and maintenance of bones, teeth and muscles. [53]. The Ca/P ratio in the seed and the root are 2.54 and 0.71 respectively. In animals, a Ca/P ratio above 2.0 help to increase the absorption of Ca in the small intestine. Food is considered 'good' if the ratio Ca/P is greater than 1 and 'poor' if less than 0.5 [54] This result indicates that the seeds leaves and roots are good sources of both calcium and phosphorous. Zinc and Iron content of all the studied parts were within the acceptable standard and the parts can be regarded as good sources of these elements. Zinc and iron are essential trace element required only in minute amount by the human body for important biochemical functions. Zinc stimulates the synthesis of metallothionein. Zinc is involved in normal functioning of immune system and is associated with protein metabolism [55]. Iron is required for haemoglobin, formation, normal functioning of central nervous system and in the oxidation of carbohydrates, protein and fats [56]. They are nutritionally and medically important based on their contribution to human physiology and requirement in health management to avoid metal deficiency [57].

Vitamin Content Vitamin Content of the leaf, seed and root of *Vigna subterranea* (mg/100g) are shown in Table 5. Value show mean of triplicate analysis of \pm standard deviation; figures with different superscript down the column were significantly different ($p < 0.05$ theoretical). Table 5 shows the vitamin content of the leaf, seed, and root of *Vigna subterranea* in mg/100g. Vitamin C for the three parts of the plant was predominantly present for the leaf (6.453), seed (2.053) and root (2.640) mg/100g respectively followed by vitamin B₃ leaf (1.560), seed (2.123) and root (0.820) mg/100g. The vitamin C content of the leaf was high and therefore could be a dietary source of vitamin C for both animals and humans. Vitamin B₂ was the least among the vitamins determined for the leaf (0.113), seed (0.153) and root (0.067) mg/100g. Vitamins are powerful anti-oxidants which help to prevent molecular damage caused by oxidation in that protection offered may help prevent diseases such as cancer, cardiovascular diseases and muscular degeneration [58].

Amino acid Composition Amino Acid Composition of the leaf, seed and root of *V. subterranea* (g/100 g) are shown in Fig. 1 below.

In figure 1, There seems to be an agreeable trend in the total amino acid constituents of the various parts under study with each part containing similar amino acid in relatively similar quantities and glutamic acid having the highest occurrence in the leaves, seeds and roots respectively. This is closely followed by aspartic acid and leucin.

The Composition of the essential and non-essential amino acids (g/100g) in the leaf, seed and root of *V. subterranea* are shown in Fig. 2 and 3 respectively.

Table 5: Vitamin B and C content of the leaf, seed and root of *V. subterranea* (mg/100g).

Sample	Vitamin B1	Vitamin B2	Vitamin B3	Vitamin C
Leaf	0.313 ^b \pm 0.006	0.113 ^b \pm 0.012	1.560 ^b \pm 0.080	6.453 ^c \pm 1.016
Seed	0.413 ^c \pm 0.031	0.153 ^c \pm 0.012	2.123 ^c \pm 0.012	2.053 ^a \pm 0.508
Root	0.267 ^a \pm 0.012	0.067 ^a \pm 0.012	0.820 ^a \pm 0.020	2.640 ^b \pm 0.000
p-value calculated	0.01	0.01	0.01	0.01

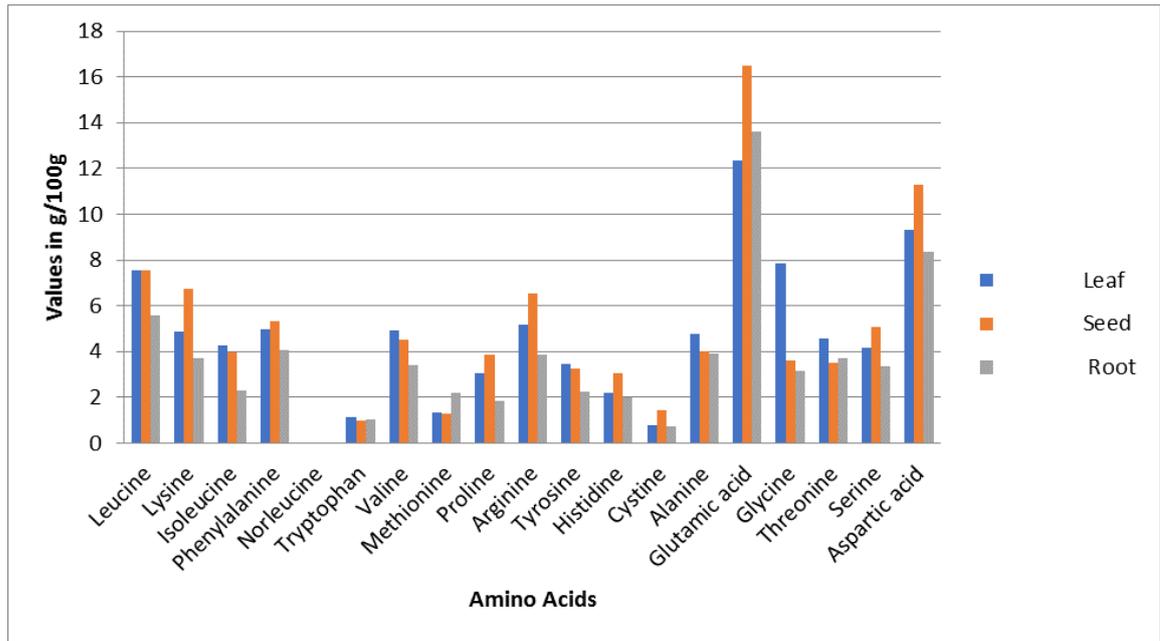


Fig. 1: The total amino acid composition of the leaves, seeds and roots of *V. subterranea*

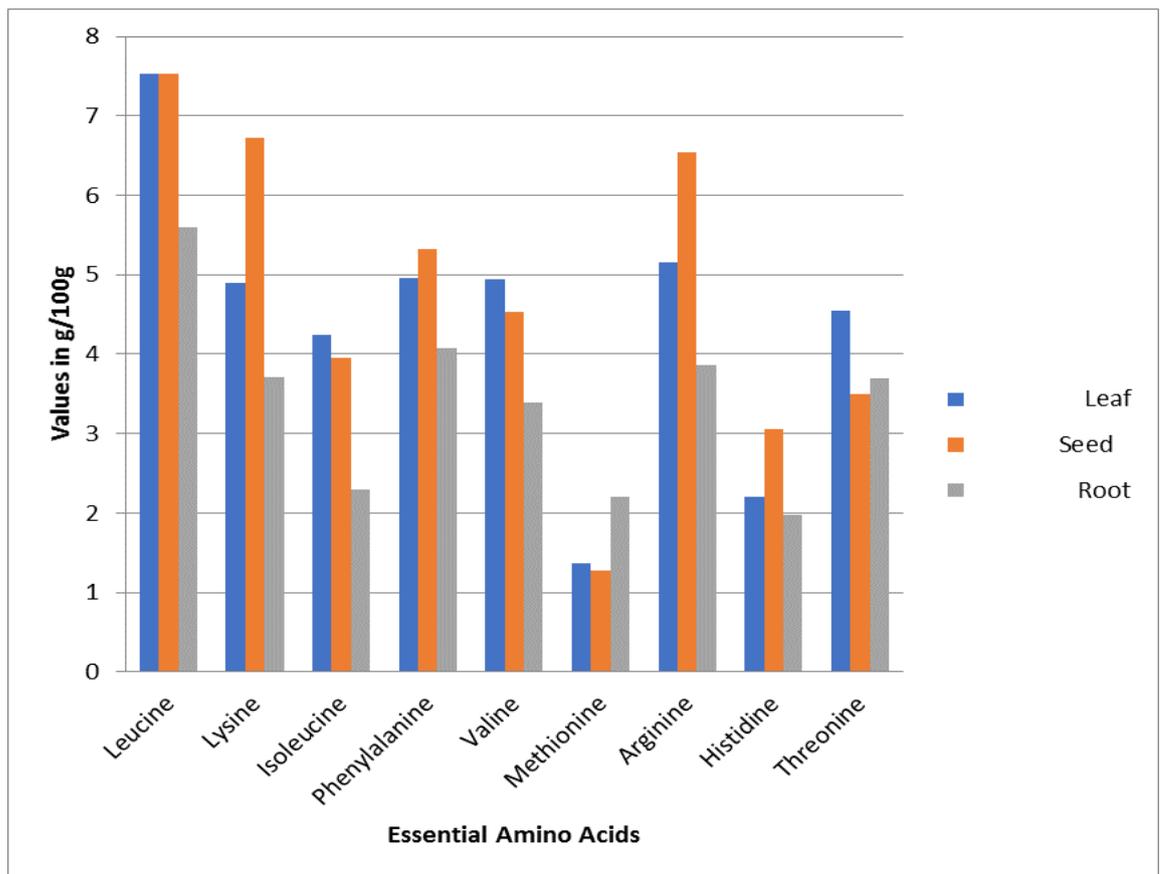


Fig. 2: The essential amino acid composition of the leaves, seeds and roots of *V. subterranea*

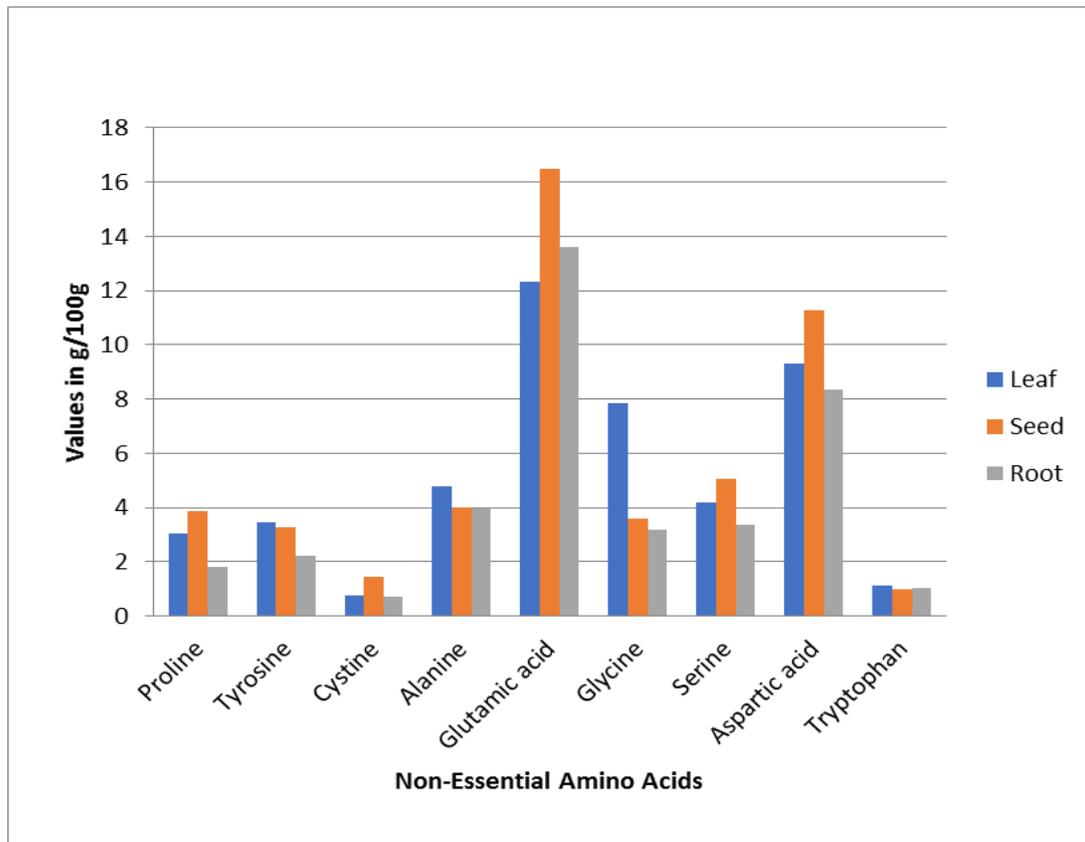


Fig. 3: The non -essential composition of the leaves, seeds and roots of *V. subterranea*

Fig. 1,2 and **3** depict the total amino acid, essential amino acids and non-essential amino acids composition of the leaf, seed and root of *V. subterranea*. Glutamic acid (12.34, 16.50 and 13.62) g/100 g was the predominant amino acid found in all the parts of the plant and this amino acid is essential for immune system, digestion and brain health. Next to this is the aspartic acid (9.30, 11.29 and 8.34) g/100 g, leucine (7.53, 7.53 and 5.60 g/100 g), lysine (4.90, 6.73 and 3.71) g/100 g, Arginine (5.16, 6.54 and 3.87) g/100 g for the leaf, seed and root respectively. The values of amino acids showed that cysteine (0.78, 1.45 and 0.72) and tryptophan (1.15, 0.97 and 1.05) for the leaf, seed and root respectively were in the lowest levels. The total essential amino acids and total non-essential amino acids for the leaf, seed and root were (39.85, 42.44 and 30.82) g/100 g and (46.84, 50.05 and 38.25) g/100 g respectively and essential to non-essential amino acids (E/N) ratio was 0.85, 0.85 and 0.81 for the three samples. The total essential amino acid and non-essential amino acid was lower when compared to the value of total essential (345.30) and total non-essential

(329.10) obtained for *P. Mildbraedi* [42]. The values of leucine (7.53, 7.53, and 5.60) g/100g for the leaf, seed and root is similar to the leucine composition for the raw (6.90), soaked (6.98) and cooked (7.60) *V. subterranea* [9]. As most edible legumes, lysine of the leaf (4.90), seed (6.73) and root (3.71) of *V. subterranea* was higher than sulphur amino acids (cystine, 0.78, 1.5 and 0.72) and (methionine 1.36, 1.28 and 2.21) [60]. When comparing the essential amino acids in the leaf, seed and root with the recommended (FAO/WHO) provisional pattern, all the parts were found to be rich in amino acids contents. Each of the parts can be used as potential source of protein for human beings. The seeds were superior with respect to aspartic acid, proline, lysine, leucine, phenylalanine, histidine and arginine [9, 30].

Antimicrobial Activities

The result of the antimicrobial activities of n-hexane, ethanol, and aqueous extracts of leaf, seed and root of *Vigna subterranea* using Agar-well diffusion method (IZD in mm) is shown in **Table 6** below.

Table 6: The anti-microbial activities of n-hexane, ethanol and aqueous extract of the leaf, seed and root of *V. subterranea* (mm)

Microbial Isolate	HR	ER	AR	HL	EL	AL	HS	ES	AS
<i>P. aeruginosa</i>	24.0±1.41	15.5±0.50	0	14.5±0.50	12.5±0.50	0	0	0	0
<i>S. aureus</i>	0	13.0±0.00	0	0	8.0±1.41	0	0	0	0
<i>Klebsiella sp.</i>	0	0	13.0±0.00	0	0	12.0±1.41	0	0	13.0±0.00
<i>E. coli</i>	0	0	0	0	0	0	0	0	0
<i>C. albicans</i>	14.5±0.50	0	0	0	0	0	0	0	0
<i>A. niger</i>	0	0	0	0	7.0±0.00	0	0	0	0

Keys: IZD = Inhibition Zone Diameter, HR = n-Hexane Root extract, ER = Ethanol Root extract, AR = Aqueous Root extract, HL = n-Hexane Leaf extract, EL = Ethanol Leaf extract, AL = Aqueous Leaf extract, HS = n-Hexane Seed extract, ES = Ethanol Seed extract, AS = Aqueous Seed extract

In the table 6 above, n-hexane and ethanol extracts of the root gave Inhibition Zone Diameter (IZD) of 24.0 ± 1.41 mm and 15.5 ± 0.50 mm respectively, against *Pseudomonas aeruginosa*. Also, n-hexane and ethanol extracts of the leaf gave IZD of 14.50 ± 0.50 mm and 12.50 ± 0.50 mm respectively, against *P. aeruginosa*. While n-hexane extracts of the root and leaves gave no promising results (0 mm) against *Staphylococcus aureus*, ethanol extracts of the root and leaf gave IZD of 13.0 ± 0.00 mm and 8.0 ± 1.41 mm respectively, against *S. aureus*. Furthermore, n-hexane root extract gave IZD of 14.5 ± 0.50 mm against *Candida albicans* and ethanol leaf extract gave IZD of 7.0 ± 0.00 mm against *Aspergillus niger*. Aqueous root, leaf and seed extracts gave IZD of 13.0 ± 0.00 mm, 12.0 ± 1.41 mm and 13.0 ± 0.00 mm respectively against *Klebsiella sp.*

Among the three solvents used for the extraction, n-hexane exhibited the highest antimicrobial activity: 24.0 ± 1.41 mm against *P. aeruginosa* in root extract; 14.5 ± 0.50 mm against *P. aeruginosa* in leaf extract and 14.5 ± 0.50 mm against *C. albicans* in root extract, whereas water exhibited the least activity, 13.0 ± 0.00 mm against *Klebsiella sp.* in both root and seed extracts. Plant extracts obtained using organic solvents give more potent and consistent antimicrobial activity result than aqueous extract [61, 62].

Comparing the activities of the three parts of the plant studied, the root exhibited the highest

antimicrobial activity: 24.0 ± 1.41 mm against *P. aeruginosa* in n-hexane extract; 15.5 ± 0.50 mm against *P. aeruginosa* in ethanol extract; 14.5 ± 0.50 mm against *C. albicans* in n-hexane extract; and 13.0 ± 0.00 mm against *S. aureus* in ethanol extract, whereas the seed exhibited the lowest activity (13.0 ± 0.00 mm) against *Klebsiella sp.* in aqueous extract. Though, bioactive compounds are normally accumulated as secondary metabolites in all plant cells, their concentration vary according to the plant parts, season, climate and growth phase [32]. Higher concentrations of secondary metabolites (alkaloids, tannins, flavonoids, saponins, etc.) that are responsible for the antimicrobial activity of plants occur in barks, heartwood, roots, branch bases and wound tissues [63, 24].

However, based on the inhibition zone diameter interpretation chart (www.clsi.org) [64] which considers only inhibition zone of 17 mm or above as susceptible for most antimicrobial agents, only n-hexane root extract which gave IZD of 24.0 ± 1.41 mm against *P. aeruginosa*, was considered susceptible; hence was subjected to Minimum Inhibitory Concentration and Minimum Bactericidal Concentration determinations.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

The MIC and MBC of n-hexane root extract against *P. aeruginosa* are shown in **Table 7**.

Table 7: MIC and MBC of n-hexane root extract against *P. aeruginosa*.

	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7	PC	NC	Blk
Concentration of extract (mg/L)	400	200	100	50	25	12.5	6.25	0	400	0
Volume of inoculum (mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0	0
MIC Results	C	C	C	T	T	T	T	T	C	C
MBC Results	NG	NG	G	-	-	-	-	G	NG	NG

Keys: PC = Positive control, NC = Negative control, Blk = Blank, T = Turbid (visible bacteria growth), C = Clear (no visible bacteria growth), G = Bacteria growth, NG = No bacteria growth.

From **Table 7**, it was observed that tube three (3) gave no visible bacteria growth in the broth test, but gave bacteria growth on the agar plate; while tube two (2) gave no bacteria growth both in the broth test and on the agar plate, therefore the MIC and MBC of n-hexane root extract of *V. subterranea* against *P. aeruginosa* used in this study are 100 mg/L and 200 mg/L respectively. Antimicrobial activities with the MIC values equal to or less than 1000mg/L for crude extracts exhibits good activity.

Conclusion

The three parts of the plant (leaf, seed and root) used in this study were found to contain comparable phytochemicals with the leaves having the highest percentage concentration of alkaloids, saponins flavonoids and tannins. The proximate analysis showed that the seed possess the highest percentage content of proteins, the leaf had the highest percentage of ash and carbohydrates while the root had the highest percentage crude fibre. Calcium and phosphorous were found to be most abundant in the seed, Potassium and magnesium were the most in the root. However, the ratio of Na: k in the root was found to be better than that of the seed and leaf for human consumption. Vitamin B3 was most abundant in the root while Vitamin C content of leaf was the highest. The antimicrobial studies using four different solvents on the various parts showed that the n-hexane root extract had the high activity against the bacteria *Pseudomonas aeruginosa* with MIC and MBC of 100mg/l and 200mg/l respectively which is considered a very strong activity and should be a candidate for potential drug discovery. The leaves and roots of *V. subterranea* have similar biochemical properties as seen in this study as the seeds termed 'super food' and hence, should be utilized as food supplement in animal feeds.

Acknowledgement.

The authors wish to acknowledge the staff of the Medical Laboratory Unit of Iyienu Mission Hospital for supplying microorganisms used in this study

Conflict of Interest.

The authors declare that there is no conflict of interests during the time of research and the publication of this study.

References

1. S. F Adebayo, Effect of soaking time on the proximate, mineral compositions and Anti-nutritional factors of lima bean. *Food science and Quality Management*. 27 (2014)224-6085.
2. O.B Uzochina, Nutrient and Anti-nutrients potentials of brown pigeon-pea (*Cajanus cajan Var bicolor*) seed flour. *Nig. Food J.* 27 (2009)10-16.
3. K. Vyaykumari, K. Siddhuraj, and K. Janardhanan, Effects of domestic processing the levels of certain antinutrients in *Prosopis chilensis* (Molina) Stunz seeds. *Food Chem.* 59 (3) (2009) 367-371.
4. E.A. Udensi, N.U Arisa and E. Ikpa Effects of soaking and boiling and autoclaving on the nutritional quality of *Mucuna Flagellipes* ("ukpo"). *African J.Biochem.Res.*4(2) (2010) 47-50.
5. Y. Y. Murevanhema and V.A. Jideani. Bambara groundnut (*Vigna subterranea* (L)verdc) milk as a Probiotic beverage a review. *Crit. Rev. Food Sci. nutria* 53 (2013) 954-967.
6. S.L. Okonkwo and M.F. Opara The analysis of bambara nut (*Voandzeia subterranea* (L) Thouars for sustainability in *African. Research Journal of Applied science* 5 (2010) 394-396.
7. M.A Mune, Minka, S.R. Mbome, L and F.X Etoa Nutritional potential of bambara bean protein concentrate. *Pakistan Journal of Nutritional* 10 (2011) 112-119

8. M. Andzouana, J.B. Mombouh. and Attibayeba Chemical and Phyto-chemical compositions of *Voandzeia subterranea* seeds. *Pakistan Journal of biological sciences*,17 (2014) 1083-1088
9. A.M. Mazahib, M. O. Nuha, I.S. Salawa and E.E. Babiker. Some Nutritional Attributes of bambara groundnut as influenced by Domestic processing. *International Food Research Journal* 20 (2013) 1165-1171.
10. J. Mubaiwa, V. Fogliano, C. Chidewe and A.R Linneman. Bambara groundnut (*Vigna subterranea* Verdc) flour: A functional ingredient to favour the use of an unexploited sustainable protein source. *PLOS ONE* 13 (2018) e 025776, 1-19
11. M. Brink, G.M. Ramolemana, and K.P. Sibuya. *Vigna subterranean (L) Verdc*, In Brink, M. and Belay, G. (Editors) Plant Resources of tropical African I cereal and pulses. PROTA Foundation (2006).
12. N.G. Majola, A.S. Gerano and H Shimelis Bambara groundnut (*V. subterranea* [l] Verdc), Production, Utilization and genetic improvement in Sub-Saharan Africa. *Agronomy* 11 (2021) 1345
13. B.O. Obadoni and P.O. Ochuko. Phytochemical Studies and comparative Efficacy of the crude extracts of some homoeostatic plants in Edo and Delta States of Nigeria. *Global J. Pure and Applied science* 8 (2001) 203-208
14. B.A. Bohm and M.R. Kocipai-Abyazni. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium reticulatum* and *V. calycinum* (Ericaceae). *Pac. Jour. of Sci.*, 48. (1994) 458-463.
15. J.B. Harborne. Phytochemistry, Acad. Press London, 21. (1993) 2785.
16. S.I. Umeh and A. S. Ogbuagu. A hand book of Laboratory Analysis. Fab Anieh Nig. Ltd. Awka, Anambra State. Nigeria. (2012) 44-46.
17. A. O. A. C. Association of Official Analytical Chemists. Official Methods of Analysis. 15th Edition, Association of Official Analytical Chemists, Washington D.C. USA. (2004) 176-190.
18. A. O. A. C. Association of Official Analytical Chemists. Official methods of analysis, 18th Ed., Washington D C, USA (2006) 200-210.
19. I.D. Pearson. Chemical Analysis of food, 7th ed., London: Churchill Livingstone. (1976) 52-78.
20. S.K. C Chang. Protein Analysis. In: Food Analysis, Nielson, S. S. (ed.) Kluwer Academic Plenum Publisher, New York (2003). 156.
21. G. D. Sadler and P. A. Murphy. PH and Titratable Acidity. In: Food Analysis. S. S. Neilson, ed. Kluwer Academic. New York. Chapter 13. (2003) 46-55.
22. C. S. James Experimental method on Analytical Chemistry of foods. Chapman and Hall, New York. (1995) 75-84.
23. L.V. Benitez. Amino Acid and fatty acid profiles in aquaculture nutrition studies p. 23-25 in S.S. De Silva (ed). Fish Nutrition Research in Asia. Proceedings of the third Asian Nutrition Network Meeting. Asian Fish. Society Special Publication, Asian Fisheries Society, Manila Philippines.11 (1989) 166
24. FAO/ WHO Protein quality evaluation Report of Joint FAO/WHO expert consultation). FAO, Food and Nutrition paper. FAO/WHO Rome Italy. (1991).
25. A.A. Ajiboyeand and G.K. Oyejobi, In vitro Antimicrobial Activities of *Vigna subterranea*. *Journal of Antimicrobial Agents* .3 (1) (2017)132-136
26. T. Selvamohan, and V. Ramadas. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Advances in Applied Science Research*. Pelagia Research Library India 3(5) (2012) 3374-3381.
27. S. Magaldi, C. Mata-Essayag, C. Hartungde, O. Carolina and O. Yudith Well diffusion for antifungal susceptibility testing. *J. Infect. Dis.*, 8(2004) 39-4.
28. G. Holla, R. Yeluri and A. K. Munshi Evaluation of minimum inhibitory and minimum bactericidal concentration of nano-silver base inorganic antimicrobial agent (Novaron^(R)) against *Streptococcus mutans*. *Contemp Clin Dent*; 3 (2012) 288-293.
29. E. Bell VitaminD₃ promotes immune function on the skin. <http://www.signalinggateway.org/update/updates>. (2008). Assessed 2017.
30. D.E. Okwu, Evaluation of chemical composition spices and flavouring agents. *Global Journal of Pure and Applied Science*. 7(2001) 455-459.
31. W. H. Lewis and M. P. Elvin- Lewis Medicinal plants as sources of new therapeutics. *Ann. Mo. bot. Gard.*, 82(1995) 16-24.
32. G. E. Trease and W.C. Evans, Pharmacognosy. 11th Edition, Tindal Ltd, London. (1985). pp:60-75.
33. F. I Akaneme, Identification and preliminary phyto-chemical analysis of herbs that can arrest threatened miscarriage in Orba and Nsukka towns

- of Enugu state, *African Journal of biotechnology*, 7 (1) (2008) 006-011
34. T. P. Cushine, and A. J. Lamb, Anti-microbial Activity of flavonoids: *Int. J. Antimicro. Agents*. 26(5) (2005) 343-356.
 35. D.E. Okwu, Phytochemicals and vitamin content of indigenous species of South Eastern Nigeria. *Nigeria.J. Sustain.Agric. Environ.* 6(1) (2004) 30-37.
 36. P. Tiwari, B. Kumar M. Kaur, O. Kaur, and H. Kau, *Phytochemical Screening and extraction. A review, Int. Pharm. Sci.* 1(1) (2011) 98-106.
 37. K.E Ayeni, and S.A. Yahaya, Phytochemical screening of three medicinal plants neem leaf (*Azadirachta indica*), hibiscus leaf (*Hibiscus rosasinensis*) and spear grass leaf (*Imperata cylindrical*). *Cont. Jour. of Pharm. Sci.* 4 (2010) 47- 50.
 38. , S. Ghosal B.N. Klishna –Prasad, and V. Laskimi Nutritional Composition of *Sonchus spp (S. asperl., S. oleraceus. L., S. terries L.)*. *Journal of Food Science. Agric.* 76 (1996) 628-632
 39. , L.M. Antherden, Text books of pharmaceutical chem. 8thEdn Oxford University Press London, (1969) 813-814.
 40. J.W Fahey, *Moringa oleifera* A Review of the medical evidence for its nutritional therapeutical and prophylactic properties, *Trees Life Journal* 15 (1) (2005) 1-15.
 41. O.K Ndukwe, and A. Ikpeama, Comparative Evaluation of the phytochemical and proximate constituents of Oha (*Pterocarpus soyansil*) and Ntururopa (*Pterocarpus santalinoides*) leaves. *Int. Journ. of Academic Research in progressive Edu. and Dev.* 2 (2013) 22-31
 42. R. O. Akinyeye, A. Oluwadunsi, and A. Omoyeni, Proximate, mineral, anti-nutrients, Phyto-chemical screening and amino acid compositions of the leaves of *Pterocarpus mildbraedi* Harm. *J. Environ. Agric Food chem.*, 9 (2010) 1322-1333
 43. B. Bhagya, K. R. Sridhar, S. Seena, and B. Bhat, Nutritional qualities of ripened beans of Mangrove wild legume *Canavalia cathatica* Thouars. *J. Agric Techn.* 3 (2007) 255-274
 44. I.F. Fadahunsi, and A.I. Sani, Chemical and biochemical bambara nut (*Voandzeia subterranea (L) Thouars*) during fermentation to Tempeh. *Elect. J. environ. Agric. Food chem.*, 9 (2010) 275-283.
 45. A.A. Yagoub, and A.A. Abdalla *Effect of domestic processing methods on chemical, in vitro digestibility of protein and starch and functional properties of bambara groundnut (Voandzeia subterranea) seed* *Research journal of Agriculture and Biological science* 3 (2007) 24-34
 46. R. Hall, Kale, *Brassica oleraceae* (Acephala Group). US database for Standard reference, Release for standard reference, Release 12 March, 1998 Nutrition guide. <http://www.nurition.about.com>.
 47. J.O. Ogunyi, M. Wirth, and D. I. Osuigwe *Nutrient composition of some tropical legumes capable of substituting fish meal in fish diets. J. Agric.Rural Dev.Trop. Subtropics*, 104 (2003) 143-149.
 48. P.O Ebun-Oluwa,. and A. S. Alade Nutritional Potential of Berlandier Nettle spurge (*Jatropha cathatica*) seed. *Pakistan Journal of Nutrition.* 6(2007) 345-348.
 49. F.N.D Food and Nutrition Board Institute of medicines, National Academy of Sciences. Dietary reference intake for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acid (micro nutrients) (2002). www.nap.edu
 50. P.C. Ojimelukwe, Cooking characteristics of four cultivars of bambara groundnuts seeds and starch isolate. *J. Food Biochem.* 23(1999) 109-117.
 51. B.S Anita, E.J. Akpan, P.A. Okon, and I.U. Umolen, Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves, *Pak. J. Nutri.*, 5 (2006) 166-168
 52. H. Ishida, H. Suzuno, N. Sugiyama, S. Innami, and T. Todokora. National evaluation of chemical component of leaves stalks and stem of sweet potatoes. *Ipomea batata poir.* Food Chemistry. 68 (2000) 359-367.
 53. J.C. Okaka, N.T.A. Enoch, and N.C.A. Okaka, Food and Human Nutrition. O.J.C. Academic Publishers, Enugu, Nigeria (2006) 135-153.
 54. E.I. Adeyeye, and P.A. Aye, Proximate composition and Phytochemical constituents of selected plants. *Pakistan Journal of nutrition*, 4 (2005) 187-196.
 55. N.D. Ibrahim, E.M. Abdurahman and G. Ibrahim, Elemental Analysis of the leaves of *Veronia amygdaleina* and its biological evaluation in rats. *Nigerian Journal of Natural Products Medicine.* 5 (2001) 13- 16.
 56. F. Adeleye and M.K.O. Otokiki Proximate composition and some Nutritionally Valuable Minerals of two varieties of *Capsicum annu.* *Discovery innovations.* 11(1999) 75-81.

57. G. M. Wardlaw and A. M. Smith Contemporary Nutrition. 6thEdn., McGraw- Hill, Higher Education, England. (2006) 353-367.
58. S. Islam, M. Yoshimoto, S. Yahara, S. Okuno, K. Ishiguro, and O. Yamakawa Identification and characterization of the polyphenolic composition in sweet potato [(*Ipomoea batata L.*) genotype *J. Agric. Food chem.*, 50 (2002) 3718-3722.
59. B. Bhagya, K. R. Sridhar, S. Seena and B. Bhat Nutritional qualities of ripened beans of Mangrove wild legume *Canavalia cathartica Thouars. J. Agric Techn. 3* (2007) 255-274
60. B. Nasir, H. Fatima, M. Ahmed and I. U. Haq, Recent trends and methods in antimicrobial drug discovery from plant sources. *Austin J. Microbiol.* 1 (2015) 1002
61. S. F. Van Vuuren, *Antimicrobial activity of South African Medicinal Plants. J. Ethnopharmacology.* 11(2008) 462-472.
62. N. S. Ncube, A. Afolayan, and A.I. Okoh. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afri. J. Biotechnol.* 7 (2005) 1797-1806
63. R. N. Okigbo, C. L. Anuagasi, and J. E. Amadi Advances in selected medicinal and aromatic plants indigenous to *African. J. Med. Plants Res.* 3 (2009) 86-95.
64. Global Laboratory Standard for a Healthier world <http://www.clsi.org> Assessed Oct 2020

HOW TO CITE THIS ARTICLE?

Adaora Stellamaris Ogbuagu, Chinwe Chioma Maduka, Isaac Obi Okerulu, Chukwuebuka Thankgod Onyema, Chidi Onyeizugbe and Anthonia Uzoamaka Emezie, Comparative Phytochemical, Nutritional and Antimicrobial Screening of the Seed, Leaf and Root of *Vigna Subterranea*. *Prog. Chem. Biochem. Res.* 5(2) (2022) 182-195

DOI: 10.22034/pcbr.2022.314226.1201

URL: http://www.pcbiochemres.com/article_149529.html

