Progress in Chemical and Biochemical Research

Journal homepage: www.pcbiochemres.com

Original Research Article

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

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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 (6.453mg/100g) while vitamin B3 was found to be highest in the seeds(2.123mg/100g) The total essential amino acids (g/100g) in seed, leaf and root were 42.44, 39.85, and 30.82 respectively, while total non-essential amino acids (g/100g) 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 ± 1.41 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

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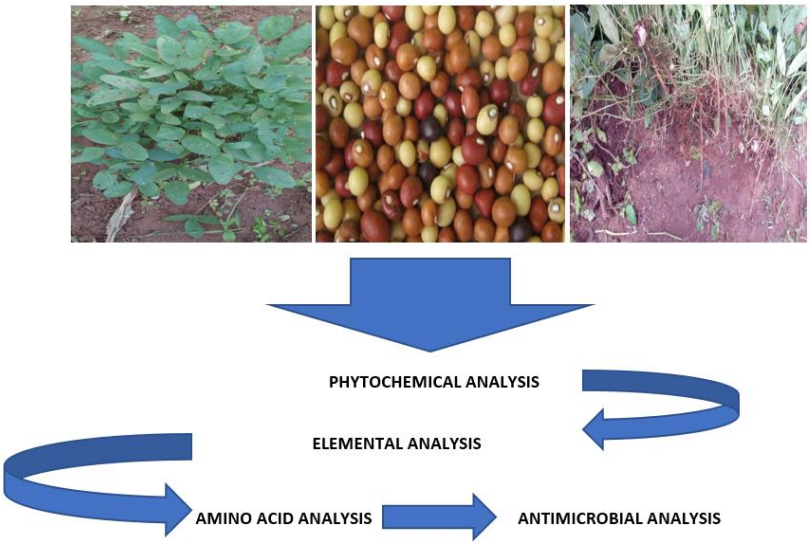
GRAPHICAL ABSTRACT



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 ^{o}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 ¼ of the original volume by evaporation over a steam bath. Alkaloid in the extract was precipitated by addition of NH_4OH 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^{o}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].

mg of flavonoid= Abs x 50/1000 (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].

mg of Saponin (mg/100) = Reading from the curve x Dilution factor x 100/1000 (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 coo 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 NH_4OH. 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 fiber content was assessed using Wende method [22]. Riboflavin, niacin, thiamine and ascorbic acid in each of the samples were determined spectroph-otometrically [17].

Determination of Amino acid

The Amino acids determination was conducted by defatting one gram of each sample using chloroform/ methanol in the ratio 2:1. The defatted sample (100 mg) was hydrolyzed at 105 ± 5 ^{0}C for 22 hours, hydrolyzed sample (about 60 microlitre) was analyzed for amino acids by loading into the Applied Biosystem PTH Amino Acid Analyzer 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^{o}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 ± 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	+	+	±
Steroids	++	++	++
Phytate	++	±	±

Key±=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 ± 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	$0.340^{c}\pm0.023$	$0.293^{b}\pm0.012$	$0.373^{c}\pm0.012$	$0.327^{c}\pm0.013$
Seed	$0.167^{a}\pm0.012$	$0.127^{a}\pm0.012$	$0.233^{a}\pm0.012$	$0.153^{a}\pm0.012$
Root	$0.233^{b}\pm0.012$	$0.433^{c}\pm0.025$	$0.293^{b}\pm0.042$	$0.227^{b}\pm0.042$
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 ± 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 ± 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 ± 0.12% and 2.25 ± 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 ± 0.13%, 60.26 ± 0.19% and 55.92 ± 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 ± 0.05%, 9.12 ± 0.05% and 6.26 ± 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 ± 0.08%, 1.69 ± 0.02% and 1.21 ± 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 fiber content of the leaf, seed and root of *Vigna subterranea* were 13.22 ± 0.22%, 6.91 ± 0.03% and 28.84 ± 0.26% respectively. This is an indication that the various parts of the plant are good sources of crude fiber when consumed. Adequate intake of dietary fiber 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 ± 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 ≤ 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 than1 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 ± 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 B_3 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 B_2 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	$0.313^{b}\pm0.006$	$0.113^{b}\pm0.012$	$1.560^{b}\pm0.080$	$6.453^{c}\pm1.016$
Seed	$0.413^{c}\pm0.031$	$0.153^{c}\pm0.012$	$2.123^{c}\pm0.012$	$2.053^{a}\pm0.508$
Root	$0.267^{a}\pm0.012$	$0.067^{a}\pm0.012$	$0.820^{a}\pm0.020$	$2.640^{b}\pm0.000$
p-value calculated	0.01	0.01	0.01	0.01

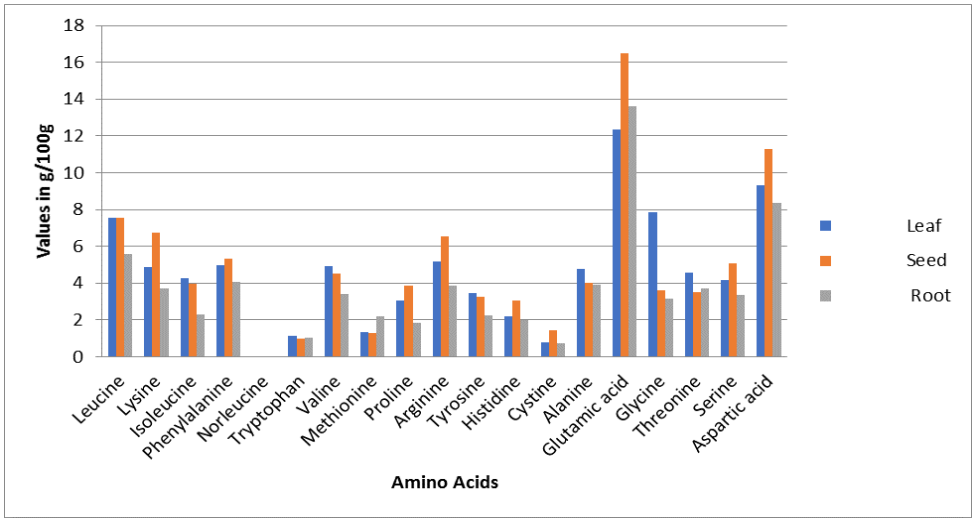


Fig. 1: The total amino acid composition of the leaves, seeds and roots of *V. subterranea*

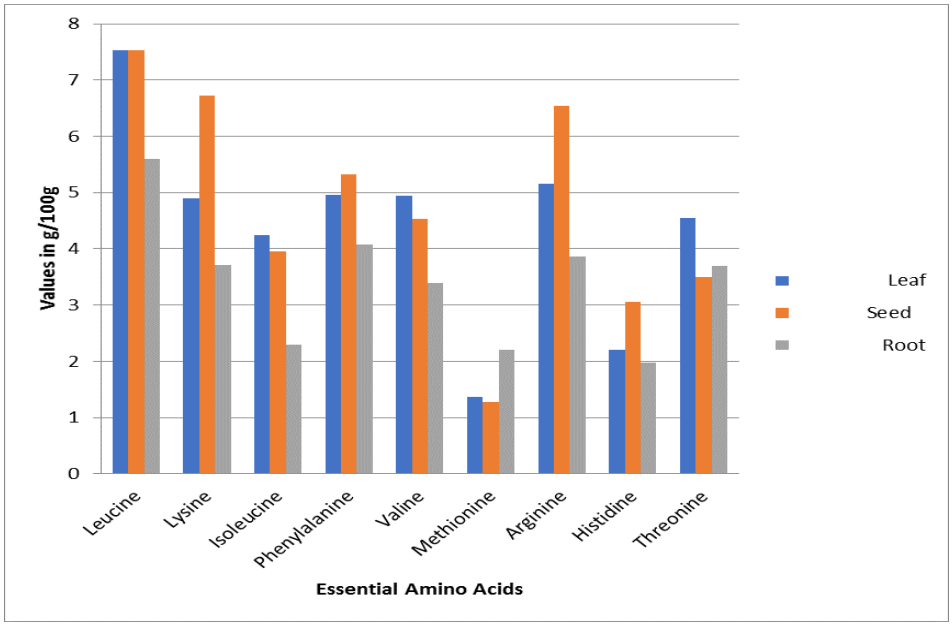


Fig. 2: The essential amino acid composition of the leaves, seeds and roots o *V. subterrane*

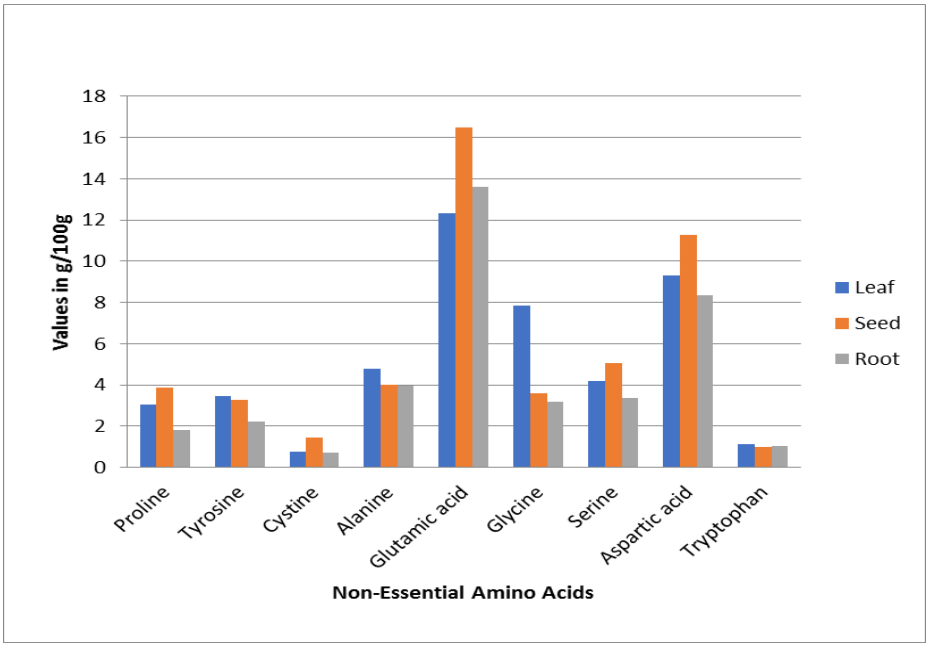


Fig. 3: The non -essential composition of the leaves, seeds and roots of *V. subterranea*

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
P. aeruginosa	24.0±1.41	15.5±0.50	0	14.5±0.50	12.5±0.50	0	0	0	0
S. aureus	0	13.0±0.00	0	0	8.0±1.41	0	0	0	0
Klebsiella sp.	0	0	13.0±0.00	0	0	12.0±1.41	0	0	13.0±0.00
E. coli	0	0	0	0	0	0	0	0	0
C. albicans	14.5±0.50	0	0	0	0	0	0	0	0
A. niger	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 ± 1.41mm and 15.5 ± 0.50mm 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 ± 1.41mm 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 ± 1.41mm 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 ± 1.41mm against *P. aeruginosa* in root extract; 14.5 ± 0.50 mm against *P. aeruginosa* in leaf extract and 14.5 ± 0.50mm 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 ± 1.41mm 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**.

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.

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.

Conclusion

The three parts of the plant (leaf, seed and root) used in this study were found to contain comparable phytocompounds 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 leave had the highest percentage of ash and carbohydrates while the root had the highest percentage crude fiber. 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 hight 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.

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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