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RESEARCH ARTICLE

Phytochemical analysis and GC-MS based bioactive compounds determination of 60 days Nigerian *Vigna Radiata* aqueous root extract

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Abstract: In the present study, the sturdy root of Nigerian *Vigna radiata* (L.) commonly called mung bean was investigated for the phytochemical content. This was necessitated as a result of limited information observed on the phytochemical content of fully matured Nigerian *Vigna radiata* root. 60 days old *Vigna radiata* (L.) plant, a newly introduced crop in Nigeria, was harvested from the farm of National Biotechnology and Research Development Agency, Abagana Centre, Nigeria. The roots were neatly separated from the plant, rinsed well with distilled water, air dried and grounded into flour. The 60 days old Nigerian *Vigna radiata* root flour sample (NVrR) subjected to preliminary phytochemical assay revealed the presence of 12 bioactive compounds with a remarkable high percentage concentration of 26. 780% recorded for flavonoids content. Tannins also recorded appreciable value of 8.927% while values $< 5\%$ were noted for the remaining compounds. Further confirmation of the actual bioactive compounds present in 60 days old NVrR through GC-MS studies, generated 30 observable peaks with 28 bioactive compounds identified through spectrum matching with MassHunter\Library\NIST14.L spectral database. The major component, eluted at RT 23.565 (peak area 33.38%) revealed a bioactive compound which has been reported as an active ingredient in the production of detergents and biodiesel. This discovery represents a groundbreaking innovation in the utilization of NVrR for the production of briquettes, offering a cost-effective alternative energy source. Isolation of the identified compounds may prove the NVrR an important raw material for industrial productions.

Keywords: mung bean, *Vigna radiata*, phytochemicals, GC-MS

1 Introduction

The roots of plants, commonly called 'mkpologwu' in Igbo tribe's language in Nigeria with the last part of the word '-ogwu' meaning 'drug' signifies the ancient use of roots of plants in the treatment of many health defects in Nigeria. Most of these plants with already established efficiency as herbal drugs are generally called medicinal plants. *Vigna radiata*, commonly known as mung bean, is a medicinal food crop whose seeds and sprouts have been extensively studied for their therapeutic potentials. Research has demonstrated trending efficacy of *Vigna radiata* seeds and sprouts in the treatment of various diseases, such as diabetes [1], hypertention [2], Cancer [3], inflammation [3] and many other reported therapeutic activities. However, despite the wealth of knowledge on the seeds and sprouts of this plant, there is a notable dearth of information regarding the medicinal properties and potential applications of the roots of *Vigna radiata.*

Vigna radiata plant (Figure 1), a leguminous crop from the fabaceae family with a short growth cycle of 50–60 days [4], originated from India and is now being cultivated all over the world [5] but lesser known in Nigeria [4]. The sturdy roots are commonly regarded as waste materials, here in Nigeria, due to their tender and fragile nature which has been perceived as unsuitable for practical applications. Consequently, these roots are frequently discarded, despite their potential utility. Therefore, to explore the bioactive compounds of *Vigna radiata* root which may have possible therapeutic benefits and uses with the focus of identifying innovative and valuable applications for this currently underutilized resource, a preliminary quantitative phytochemical analysis on the crude root flour sample and a GC-MS based bioactive compounds

analysis of the aqueous extract of 60 days old Nigerian *Vigna radiata* (L.) root were investigated in the present study.

(a) (b)

Figure 1 (a) and (b) : (a) Vigna radiata plantation, (b) Vigna radiata roots, from National Biotechnology Research and Development Agency demonstration farm, Abagana, Anambra State, Nigeria.

2 Materials and methods

2.1 Materials and reagents

All the reagents and solvents were of analytical grades and were bought from Sigma Aldrich U S A. These included hexane, methanol, ethanol, conc. NH_4OH, 3,5-DNS (Dinitro Salicylic acid), sodium carbonate solution, tannic acid, acetic acid, concentrated ammonia solution, HCl, conc. ethyl acetate, 0.3% ammonium thiocynate solution, $AgNO_3$, HNO_3, KCN, ammonium thiocynate solution, iron (111) chloride solution, $CaCl_2$ solution, H_2S0_4 solution**,** Amylalcohol**,** chloroform**,** acetic anhydride, anhydrous sodium sulphate.

2.2 Sample collection and preparation

Vigna radiata (L.). plants were selectively harvested on the 30^{th} August, 2023 which was the 60^{th}post planting date, from the farm of National Biotechnology and Research Centre, Abagana, Anambra State, Nigeria The root was neatly separated from the plant, rinsed in distilled water, air dried for 3weeks and milled into fine powder by using high power blender and grinder from QRSA product with brand number QBL-8008 pro2. The grinded *Vigna radiata* (L.) root flour was put in an air tight container and kept for further analysis.

2.2.1 Preliminary phytochemical analysis

Crude sample of the 60 days old Nigerian *Vigna radiata* (L.) root flour (the test sample) was subjected to preliminary Phytochemical studies for the quantities of Saponins, cardiac glycosides, tannins, alkaloids, flavonoids, phytates, cyanogenic glycosides, oxalates, anthocyanins, phenols and hemagglutinins present in the sample using standard test protocols.

2.2.2 Determination of saponins

Exactly 5g of the test sample was put into 20% acetic acid in ethanol and allowed to stand in a water-bath at 50^0C for 24 hours. This was filtered and the extract was concentrated using a water-bath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was complete. The solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content of the test sample was weighed and calculated in percentage [6] as shown in equation 1

$$\%\,\text{saponin content} = \frac{(\text{weight of filter paper} + \text{residue}) - (\text{weight of filter paper})}{\text{Weight of sample analysed}} \times 100 \quad (1)$$

2.2.3 Determination of cardiac glycosides

Wang and Filled method [7] was used. 1 ml of methanolic extract of the test sample was added 1 ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1 ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper of the sample was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50^0C till it was dried and the weight of the filter paper with the sample residue was noted.

The cardiac glycoside present in the test sample was calculated in percentage as shown in equation 2.

$$\%\,\text{cardiac glycoside} = \frac{(\text{weight of filter paper} + \text{residue}) - (\text{weight of filter paper})}{\text{Weight of sample analyzed}} \times 100 \quad (2)$$

2.2.4 Determination of tannins

Tannins content of the test sample was determined by the folin-Dennis spectrophotometric method [8]. 5.0 g of the test sample was dispersed in 100 ml of distilled water. The mixture was shaken for 30 mins at room temperature and filtered using Whatmann filter paper (No 42). The residue from the sample was washed further with the distilled water until 100 ml filtrate was obtained. 2 ml of the extract was mixed with equal volume of folin-Dennis reagent in a 50 ml volumetric flask. 2 ml of saturated sodium carbonate solution was added to the mixture before dilution to the 50 ml mark and allowed to incubate for 90 minutes at room temperature.

Meanwhile, a standard Tannin solution was prepared (with tannic acid) and diluted to the same 50 ml mark for the test sample. The diluted standard was treated as described for the sample. After incubation, the absorbance of the standard and sample were measured at 760 nm in a spectrophotometer. The tannin content of the test sample was calculated using the formula as shown in equation 3.

$$\%\,\text{Tannins} = \frac{100}{\text{W}} \times \frac{\text{au}}{\text{as}} \times \frac{\text{C}}{1000} \times \frac{\text{Vf}}{\text{Va}} \times \text{D} \quad (3)$$

Where,
W = Weight of sample analyzed;
au = Absorbance of the test sample;
as = Absorbance of the standard tannin solution;
C = Concentration of standard tannin solution in mg/ml;
Vf = Total filtrate (extract) volume;
Va = Volume of filtrate analyzed.

2.2.5 Determination of alkaloids

The alkaline precipitation gravimetric method [9] was used. 10 g of the test sample was dispersed in 100 ml of 10% acetic acid in ethanol solution at 1:10 weight by volume ratio. The mixture was shaken vigorously and allowed to stand for 4 hours at room temperature with shaking every 30 minutes. At the end of this period, the mixture was filtered through Whatman filter paper (no. 42). The filtered extract was concentrated by evapouration to a quarter of its original volume. The extract was treated with drop-wise addition of the concentrated ammonia solution to precipitate the alkaloids. Ammonia was continuously added until it was in excess.

The precipitated alkaloid was filtered using Whatman filter paper (No. 2). After washing with 1% NH_4OH solution, the precipitated alkaloid was dried at 60^oC and weighed after cooling in a dessicator. The alkaloid content present in the test sample was calculated as shown in equation 4.

$$\%\,\text{Alkaloids} = \frac{(\text{W}_2 - \text{W}_1)}{\text{Weight of sample}} \times 100 \quad (4)$$

Where,
W_1 = weight of sample;
W_2 = weight of filter paper and alkaloid precipitate.

2.2.6 Determination of flavonoids

The method described by Harborne [9] was used to determine the flavonoid content of the test sample. 5 g of the test sample was boiled in 100 ml of 2 ml HCl solution for 40 min. It was allowed to cool at room temperature before being filtered through Whatman filter paper (No. 42) to obtain the extract.

Flavonoid in the extract was then precipitated by dropwise addition of concentrated ethyl acetate until in excess. The flavonoid precipitate was recovered in weighed filter paper following filtration. After drying in the oven and cooling in a dessicator, the weight of flavonoid was obtained by difference and expressed as a percentage of the sample analysed. It was calculated as shown in equation 5.

$$\%\,\text{Flavonoid} = \frac{100 \times (\text{W}_2 - \text{W}_1)}{\text{W}} \quad (5)$$

Where,
W = weight of sample used;
W_1 = weight of empty filter paper;
W_2 = weight of filter paper and flavonoid precipitate.

2.2.7 Determination of phytates

Phytates content in the test sample was determined using the method of Young and Greaves [10]. 0.2 g of the test sample was weighed into 250 ml conical flasks. The sample was soaked in 100 ml of 2% concentrated HCl for 3 hours and then filtered. 50 ml of the filtrate was laced in 250 ml beaker and 100 ml distilled water added. 10 ml of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (111) chloride solution which contained 0.00195 g iron per 1 ml. The phytic acid content of the test sample was calculated in percentage as shown in equation 6.

$$\% \text{ Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100}{\text{Wt of sample}} \quad (6)$$

2.2.8 Determination of cynogenic glycosides

Acid Titration Method [11], was used in the determination of the quantity of cyanogenic glycoside present in the test sample. 10 g of the test sample was put into 800 ml kjeldahl flask and 100 ml H_2O was added to it and was macerated at room temperature for 2 hours. After that, 100 ml of H_2O was added and steam distilled, collection of the distillate was done in 20 ml 0.02 N $AgNO_3$ acidified with 1 ml HNO_3. Before distillation, the tip of the condenser was adjusted appropriately so that it dips below surface of liquid in receiver. When 150 ml has passed over the setup, the distillate was filtered through gooch wash receiver and gooch with little H_2O. Then titration with excess $AgNO_3$ in the filtrate and washings with 0.02 N KCN was done, using Fe alum indicator. The quantity of cyanogenic glycoside present was calculated using equation 7.

$$1 \text{ ml } 0.02 \text{ N } AgNO_3 = 0.54 \text{ mg HCN} \quad (7)$$

2.2.9 Determination of oxalates by titration method

This determination involves three major steps; digestion, oxalate precipitation and permanganate titration

2.2.9.1 Digestion

2 g of the test sample was suspended in 190 ml of distilled water in a 250 ml volumetric flask and 10 ml of 6 M HCl was introduced in it before digesting at 100^0C for 1 hour, was cooled, and then made up to 250 ml mark before filtration.

2.2.9.2 Oxalate precipitation

125 ml of the sample filtrate was measured into a beaker and four drops of methyl red indicator added. This was followed by the addition of NH_4OH solution (dropwise) until the test solution changes from salmon pink colour to a faint yellow colour (pH4-4.5). Each portion was then heated to 90^0C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90^0C and 10 ml of 5% $CaCl_2$ solution was added while being stirred constantly. After heating, the system was cooled and left overnight at 25^0C. The resulting solution was then centrifuge at 2500 rpm for 5minutes. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2S0_4 solution.

2.2.9.3 Permanganate titration

The total filtration resulting from digestion of 2 g of the test sample flour was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMNO_4$ solution to a faint pink colour which persists for 30 s (Harborne, 1993). The calcium oxalate content of the test sample was calculated the formular as written in equation 8.

$$\text{Oxalate content } (\text{mg}/100\text{g}) = \frac{\text{T} \times (\text{Vme})\ (\text{Df}) \times 10^5}{(\text{ME}) \times \text{Mf}} (\text{mg}/100\text{g}) \quad (8)$$

Where,
T = titre of $KMnO_4$(ml);
Vme = volume-mass equivalent (i.e. 1 ml of 0.05 m $KMnO_4$ solution is equivalent to 0.00225 g of anhydrous oxalic acid);

Df = dilution factor Vt/A;
Vt = total volume of each sample titrate (300 ml);
A = aliquot used (125 ml);
ME = molar equivalent of $KMnO_4$in oxalate ($KMnO_4$redox reaction);
Mf = mass of sample used.

2.2.10 Determination of anthocyanins

According to Harborne (1973) [9], 5.0 g of the test sample was boiled in 100 ml of 2 M HCl for 30 minutes. The hydrolysate formed was filtered individually using whatmann filter paper. The filtrate was transferred into the separation funnel and equal volume of ethyl acetate added, mixed and allowed to separate into two layers. The ethyl acetate layer was recovered from each system while the aqeous layer was discarded. The extract was dried over a steam bath and was then treated with 10 ml of conc. Amylalcohol to extract the anthocyanin. After filtration, the alcohol extract was dried. The weight of anthocyanin in the test sample was determined and expressed as percentage of original sample using equation 9.

$$(\%)\ \text{Anthocyanin} = \frac{\text{weight of Anthocyanin}}{\text{Wt of original sample}} \times 100 \tag{9}$$

2.2.11 Determination of steroids

1.0 g of the test sample was weighed and mixed in 100 ml of distilled water in a conical flask. The mixture was filtered and the filtrate eluted with 0.1 N ammonium hydroxide solution. 2 ml of the eluent was put in a test tube and mixed with 2 ml of chloroform. 3 ml of ice cold acetic anhydride was added to the mixture in the flask. 2 drops of (200 mg/dl) standard sterol solution was prepared and treated as described for test as blank. The absorbance of standadrd and sample were measured, zeroing the spectrophotometer with blank at 420 nm. The steroid content of the sample was calculated as stated in equation 10.

$$\text{Quantity of steroids (mg/100 ml)} = \frac{\text{Absorbance of test} \times \text{Conc of std.}}{\text{Absorbance of std.}} \tag{10}$$

2.2.12 Determination of phenols

The quantity of phenols in the test sample was determined using the spectrophotometer method. The 10 g of the sample was boiled with 50 ml of $(CH_3CH_2)_20$ for 15 min. 5 ml of the boiled sample was then pipette into 50 ml flask, and 10 ml of distilled water was added. After the addition of distilled water, 2 ml of NH_4OH solution and 5 ml of concentrated $CH_3(CH_2)_3CH_2OH$ was added to the mixture. It was made up to the mark and left for 30 min to react for colour development and was measured at 505 nm wavelength using spectrophotometer.

2.2.13 Determination of hemagglutinins

2 g of the test sample weighed into a beaker, was added 20 ml 0f 0.9% NaCl and the suspension shaken vigorously for 1 min. The supernatant was left to stand for 1 hour and was then centrifuged at 2000 rpm for 10 mins and the suspension filtered. The supernatant was collected and used as crude agglutination extract. Absorbance of the extract was read at 420 nm.

2.3 Preparation of Plant extracts

100 g of the test sample (60 days Nigerian *V. radiata* (L.) root flour) was placed in sohxlet apparatus and was added 500 mls of distilled water and this extraction process was allowed to operate for 12 hours at 64 ^oC. The extract was filtered through Whatmann filter paper number 41 (110 mm). Concentration of the resulting solution of the extract was done in a vaccum to dry the aqueous root extract and stored in a refrigerator for further use.

2.3.1 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

1g of the aqueous extract of the test sample (60 days old *V. radiata* (L.) root flour) was weighed and transferred into test tube and 25 mls of ethanol was added. The test tube was allowed to react in a hotplate at 60^0C for 90 mins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water and 3ml of hexane, which was all transferred to the funnel. The extract combined and washed three times with 10 ml of 10% v/v ethanol aqueous solution. The solution obtained was dried with anhydrous sodium sulphate and the solvent was evaporated. The sample was solubilized in 1000 ul of pyridine of which 200 ul was transferred to a vial for GC-MS analysis.

The GC–MS analysis of bioactive compounds from the extract was done using Agilent Technologies GC systems with GC-220 model (Varian, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length $\times$ 250 μm in diameter $\times$ 0.25 μm in thickness of film). Spectroscopic detection by GC–MS involved an electron ionization system which utilized high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with flow rate of 1 ml/min. The initial temperature was set at 50–150°C with increasing rate of 3°C/min and holding time of about 10 min. Finally, the temperature was increased to 300°C at 10°C/min. One microliter of the prepared 1% of the extracts diluted with respective solvents was injected in a splitless mode. Relative quantity of the bioactive compounds present in the aqueous extracts of Nigerian *Vigna radiata* (L.) root was expressed as percentage based on peak area (concentration of the compound) produced in the chromatogram.

2.3.2 Identification of chemical constituents

Bioactive compounds contained in the aqueous extracts of *V. radiata* (L.) root flour was identified based on GC retention time and percentage area of the spectrum on HP-5MS column and matching of the spectrum with the spectra of the known components stored in the NIST library (D:\MassHunter\Library\NIST14.L).

3 Results

3.1 The preliminary phytochemical analysis of 60 days old Nigerian *Vigna radiata* root flour

The preliminary quantitative phytochemical analysis of the crude 60 days old Nigerian *Vigna radiata* (L.) root flour sample revealed the presence of 12 bioactive compounds. Notably, flavonoids was found to be a predominant class of bioactive compounds, accounting for an exceptional 26.780% of the total 5g of the root flour sample used in the analysis. This remarkable high flavonoids content is a distinctive characteristic that can distinguish the *Vigna radiata* root as a valuable source of these compounds. The revealed bioactive compounds with their corresponding quantitative contents in 60 days old Nigerian *Vigna radiata* root are as shown in Table 1.

Table 1 Preliminary quantitative phytochemical analysis of 60 days old Nigerian *Vigna radiata* L. roots

Phytochemical constituents	Nigerian V.radiata roots flour sample
Saponin (%)	3.156
Cardiac glycosides (%)	2.371
Tannins (%)	8.927
Alkaloids (%)	1.881
Falvonoids (%)	26.780
Phytates (%)	0.335
Cyanogenic glycosides (mg)	1.782
Oxalates (mg/kg)	0.489
Anthocyanins (%)	4.690
Steroids (mg/kg)	3.904
Phenols (mg/kg)	4.055
Hemagglutinin (mg/kg)	0.179

This value observed in the flavonoids content of the 60 days old Nigerian *Vigna radiata* root is significantly higher than that reported in the phytochemical screening of 60 days old Nigerian *Vigna radiata* leaf which recorded a value of 7.624% [4]. Eriyanto et al., (2019) [12] also reported a value of 2.35 mg/g for the flavonoids content of mung bean seed. Therefore the Nigerian *Vigna radiata* root has the highest value for flavonoids content than that reported for the leaf and the seed.

3.2 GC-MS analysis of the aqueous extract of Nigerian *Vigna radiata* L. root flour

A total of 30 observable peaks with total peak area of 100% were revealed in the spectrum generated from the GC-MS analysis of the 60 days old Nigerian *Vigna radiata* aqueous root extract sample as shown in Figure 2.

The result obtained from this spectrum, when matched with MassHunter\Library\NIST14.L

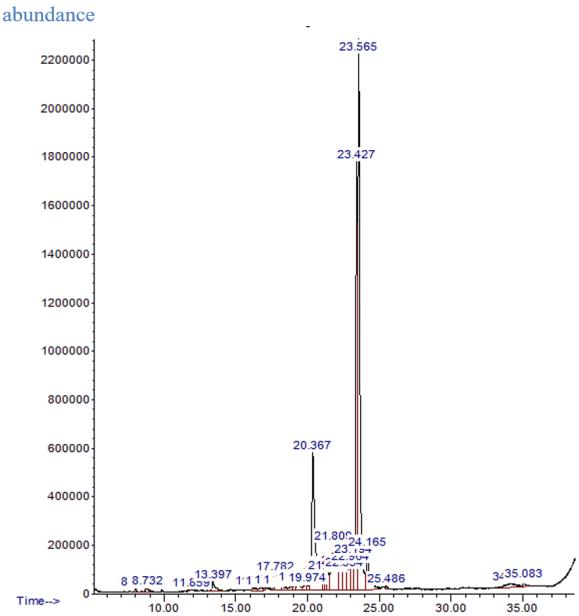

Figure 2 Total Ion Chromatogram (TIC) of aqueous extract of 60 days Nigerian *Vigna radiata* root

spectral database, revealed the presence of 28 bioactive compounds. Notably, two compounds, Heptadecyl heptafluorobutyrate and 1-Octadecene with total peak area of 0.48% and 7.93% respectively, exhibited repeat elution, with each being detected twice. This observation accounted for the presence of duplicate peaks which may be indicative of compound isomerism. The major component, identified as 11-Octadecenoic acid, methyl ester was eluted at RT 23.565 (peak area 33.38%). Appreciable quantities of 9,12-Octadecadienoic acid methyl ester and Hexadecanoic acid methyl ester were also identified at RT 23.427 (peak area 21.45%) and RT 20.367 (peak area 14.61%) respectively. The remaining identified compounds have peak area $< 3\%$ with the exception of Methyl stearate eluted at RT 24.165 (peak area 3.7%). All the bioactive compounds with their various retention time (RT), peak area (% composition) and their reported therapeutic activities / general uses are detailed in Table 2.

Table 2 Details of the compounds identified from GC-MS analysis of aqueous extract of *Vigna radiata* root

PEAK	RT	COMPOUND NAME	% Composition	Biological activities uses
1	8.000	Cyclopentane, 1,1'-[3-(2-cyclopentylethyl)-1,5-pentanediyl]bis-	0.18	No report
2	8.732	Cyclooctane, methyl-	0.43	Used in organic synthesis
3	11.859	Acetic acid n-octadecyl ester	0.08	Flavoring agents, plasticizer and solvent
4	13.397	5-Tetradecene, (Z)-	1.02	Precursor for synthesis
5	16.273	Heptadecyl heptafluorobutyrate	0.33	Surfactant and synthetic intermediate
6	16.715	7-Hexadecenal, (Z)-	0.33	Biochemical signaling, food additive, cosmetic, flavoring agent.
7	17.016	Disparlure	0.16	Pest control
8	17.641	Heptadecyl heptafluorobutyrate	0.15	Surfactant and synthetic intermediate
9	17.782	1-Octadecene	1.52	Not reported
10	18.276	Trichloroacetic acid, tetradecyl ester	0.45	Skin treatment, cosmetic and dermatology.
11	18.858	9-Eicosene, (E)-	0.42	Antimicrobial, antibacterial.
12	19.071	Cyclohexane, 1-(cyclohexylmethyl)- 4-ethyl-, trans-	0.31	Solvent
13	19.324	Oxirane, [(dodecyloxy)methyl]-	0.83	Not reported
14	19.835	Pentadecafluorooctanoic acid, octadecyl ester	0.44	Surfactant, coating
15	19.974	Heptadecanoic acid, heptadecyl ester	0.31	Peptide purification
16	20.367	Hexadecanoic acid, methyl ester	14.61	Solvent, flavoring agen, cosmetics.
17	21.108	Heptafluorobutyric acid, pentadecyl ester	0.68	Not reported
18	21.253	cis-Vaccenic acid	0.93	Antioxidant, Health benefit, nutritional supplement and Cosmetics.
19	21.386	2- Bromopropionic acid, pentadecyl ester	1.28	Chemical synthesis
20	21.809	1-Octadecene	6.41	Not reported
21	22.292	Cyclohexane, 1-(1,5-dimethylhexyl) -4-(4-methylpentyl)-	2.20	Solvent
22	22.554	Acetic acid, chloro-, hexadecyl ester	1.76	Flavoring agent, plasticizer, herbicide
23	22.964	1-Cyclohexylnonene	2.31	Organic synthesis
24	23.194	(Z)-Tetradec-11-en-1-yl 2,2,2-trifluoroacetate	2.31	Insecticides
25	23.427	9,12-Octadecadienoic acid, methylester	21.45	Lubricant, cosmetics and grease and biofuel
26	23.565	11-Octadecenoic acid, methyl ester	33.38	Antidiarrheal, biofuel
27	24.165	Methyl stearate	3.70	Antimicrobial, antioxidant, anti-inflammation, food additive, grease –and lubtricant.
28	25.486	Heptafluorobutyric acid, n-tetradecyl ester	0.22	Not reported
29	34.202	9,17-Octadecadienal, (Z)-	1.03	Anti-inflammatory, cardiovascular health, and immune responses, –food additive and cosmetics.
30	35.083	Squalene	0.33	Antioxidant, anti-inflammatory effect, skin and hair protection.

From Table 2, 11-Octadecenoic acid, methyl ester, 9,12-Octadecadienoic acid, methylester and Hexadecanoic acid, methyl ester recorded remarkable high values that can distinguish the *Vigna radiata* root as a very important bioresource.

4 Discussion

The preliminary phytochemical analysis revealed a remarkable high content of flavonoids, a class of bioactive compounds renowned for their antioxidant, anti-inflammatory, and potential health promoting properties [3]. The observed flavonoids content was found to be significantly elevated, indicating a substantial presence of these beneficial compounds. This finding suggests that 60 days old Nigerian *Vigna radiata* root possesses considerable potential for various applications, including neutraceutical, pharmaceutical and cosmeceutical usage. The two majorly found bioactive compounds, 11-Octadecenoic acid, methyl ester and 9,12-Octadecadienoic acid, methyl ester which are fatty acid methyl esters (FAME), identified through GC-MS analysis of aqueous extract of 60 days old Nigerian Vigna radiata root have several reported therapeutic activities and general uses of which Anneken et al.,(2006) [13] reported the use of FAME in the production of detergents and biodiesel. This discovery represents a groundbreaking innovation in the utilization of Vigna radiata root flour for the production of briquettes, offering a cost-effective alternative energy source for cooking. This novel application will have significant implications for provision of affordable energy solutions to low-income households, who are unable to afford cooking gas due to increase in the cost of living in Nigeria. The use of Vigna radiata dry root briquettes as a substitute for cooking gas has the potential to alleviate energy poverty, promote energy security, and contribute to sustainable national development. Further research and development are warranted to optimize the production process and explore large-scale implementation.

5 Conclusion

The discovery of the bioactive compounds in the 60 days old Nigerian *Vigna radiata* root (NVrR) through these studies on both the crude flour sample and the aqueous extract, has unveiled novel ways for the utilization of N*Vr*R. Notably, the NVrR which recorded outstanding values of two of the identified bioactive compounds with reported industrial usage for the production of biodiesel, can be employed as sustainable source of biofuel in industrial energy production which will help in reducing the cost of living of the citizens especially in Nigeria where inflation has caused a tremendous increase in the standard of living. Furthermore, the identification of flavonoids, with reported therapeutic activities, in the crude sample of 60 days old NVrR suggests potential applications as an anti-inflammatory agent, warranting further exploration and development. These research findings have marked NVrR a very important raw material for pharmaceutical, cosmeceutical and industrial productions thereby portraying the utility potentials of NVrR which was formerly considered a waste farm product.

Conflicts of interest

The authors declare that they have no conflict of interest.

References

[1] Yao Y, Chen F, Wang M, et al. Antidiabetic Activity of Mung Bean Extracts in Diabetic KK-Ay Mice. Journal of Agricultural and Food Chemistry. 2008, 56(19): 8869-8873. https://doi.org/10.1021/jf8009238

[2] Sonklin C, Alashi MA, Laohakunjit N, et al. Identification of antihypertensive peptides from mung bean protein hydrolysate and their effects in spontaneously hypertensive rats. Journal of Functional Foods. 2020, 64: 103635. https://doi.org/10.1016/j.jff.2019.103635

[3] Kabré WJ d'Arc, Dah-Nouvlessounon D, Hama F, et al. Anti-Inflammatory and Anti-Colon Cancer Activities of Mung Bean Grown in Burkina Faso. Di Giacomo C, ed. Evidence-Based Complementary and Alternative Medicine. 2022, 2022: 1-11. https://doi.org/10.1155/2022/7873572

[4] Mmuta EC, Echekoba CA, Igwe OS, et al. Phytochemical screening and GC-MS analysis of bioactive compounds present in methanolic extracts of 60 days old Nigerian Vigna Radiata leaves. Journal of

Pharmaceutical and Biopharmaceutical Research. 2024, 6(1): 459-467.
https://doi.org/10.25082/jpbr.2024.01.002

[5] Oplinger ES, Hardman LL, Kaminski AR, et al. Mungbean. Alternative Field Crops Manual. 1990.
https://www.hort.purdue.edu

[6] Obadoni BO, Ochuko PO. Phytochemical Studies And Comparative Efficacy Of The Crude Extracts Of Some Haemostatic Plants In Edo And Delta States Of Nigeria. Global Journal of Pure and Applied Sciences. 2002, 8(2).
https://doi.org/10.4314/gjpas.v8i2.16033

[7] Wang L, Liu LF, Wang JY, et al. A strategy to identify and quantify closely related adulterant herbal materials by mass spectrometry-based partial least squares regression. Analytica Chimica Acta. 2017, 977: 28-35.
https://doi.org/10.1016/j.aca.2017.04.023

[8] Pearson D. Chemical Analysis of Foods. Churchil livingstone, London, UK 7th ed. 1976, 137-140.

[9] Harborne JB. Methods of Plant Analysis. Phytochemical Methods. Published online 1973: 1-32.
https://doi.org/10.1007/978-94-009-5921-7_1

[10] Young SM, Greaves JE. Influence of variety and treatment on phytin content of wheat. Journal of Food Science. 1940, 5(1): 103-108.
https://doi.org/10.1111/j.1365-2621.1940.tb17171.x

[11] AOAC International; Official methods of analysis of AOAC international (17th ed.). Gaithersburg, 2002.

[12] Yusnawan E, Sutrisno S, Kristiono A. Total Phenolic Content and Antioxidant Activity of Mung bean Seed Cultivars from Optimized Extraction Treatment. Buletin Palawija. 2019, 17(1): 1.
https://doi.org/10.21082/bulpa.v17n1.2019.p1-9

[13] Anneken DJ, Both S, Christoph R, et al. Fatty Acids. Ullmann's Encyclopedia of Industrial Chemistry. Published online December 15, 2006.
https://doi.org/10.1002/14356007.a10_245.pub2