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RESEARCH ARTICLE

Phytochemical screening and GC-MS analysis of bioactive compounds present in methanolic extracts of 60 days old Nigerian *Vigna Radiata* leaves

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Abstract: Leaves of *Vigna radiata* (L.) are regarded as by-products due to the relatively low emphasis attributed to them when compared to the seeds and sprouts. They are usually left on the farm as waste products or for animals to graze on them, especially here in Nigeria; therefore, the need to investigate its various phytochemical content emerged, which will result in its optimum utilization. In the present study, 60 days old *Vigna radiata* (L.) leaves were harvested from *V. radiata* plants cultivated at the National Biotechnology and Research Development Centre Abagana, Anambra State, Nigeria. They were processed and milled into flour. Part of the milled flour was subjected to preliminary quantitative phytochemical screening, which revealed the presence of steroids as the major phytochemical content out of 12 bioactive compounds assessed with a value of 19.298%. The gas chromatography – mass spectrometry (GC-MS) used to determine the actual bioactive compounds present in the methanolic extracts of Nigerian *V. radiata* leaves (MEN*Vr*L) revealed the presence of 53 bioactive compounds with 58 peaks, covering a total peak area of 100% and these compounds were identified through spectrum matching with National Institute Standard and Technology (NIST) database. 2,4-Di-tert-butylphenol was identified as the major compound present in MEN*Vr*L with a peak area of 7.66%. Further isolation of these bioactive compounds may prove the leaves a rich source of pharmaceutical, biological, and cosmetologically important raw materials, for the formulation of new effective drugs and other related products.

Keywords: *Vigna radiata*, phytochemical screening, GC-MS

1 Introduction

Plants synthesize secondary metabolites called phytochemicals with the primary function of chemical defence against insects, environmental stress, and microorganisms [1]. These phytochemicals have been utilised in different countries as traditional medicine since ancient times [2]. The therapeutic activity, a medicinal property of a plant, can be predicted by the identification of its phytochemical constituents [3] which can differ in terms of quantities despite being the same species but grown in different environmental conditions [4]. These variations are governed by several factors like latitude, longitude, rainfall, temperature, quality of soil, and many other factors like habitats, cultural practices, etc. [5, 6]. Plant parts such as the leaves, flowers, stems, barks, roots, and seeds that are prone to insects, pests, microbial attacks, and the harsh environment have more amounts of phytochemicals than other parts of the plants [7]. Hence the need to screen the phytochemical content of the 60 days old Nigerian *Vigna radiata* (L.), leaves.

Vigna radiata (L.) commonly known as mung bean, is a leguminous crop from the Fabaceae family that has a short growth cycle of about 50 - 90 days. It originated from India and is now being cultivated all over the world [8]. Much emphasis has been laid on the different uses of the seeds and the sprouts of *Vigna radiata* (L.), which have been reported to be functional foods that contain balanced nutrients, including a high content of digestible protein, dietary fibre, minerals, vitamins, and significant amounts of bioactive compounds [9]. The leaves are used as foliage for feeding livestock, and the young leaves are eaten as vegetables by humans [10, 11]. In Nigeria, the leaves are mainly used as green manure, which is left to decay in the farm as a by-product after harvesting the seeds. To explore more cosmetological, biological, and pharmacological

properties of the leaves, a preliminary phytochemical screening on the crude flour sample and GC-MS -based compound analysis of the methanolic extract of 60 days old Nigerian *Vigna radiata* (L.) leaves were investigated in the present study.

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, and 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 the National Biotechnology and Research Centre, Abagana, Anambra State, Nigeria, after the removal of the dry pods. The leaves were separated from the stem, rinsed in distilled water, air dried for 3 weeks and milled into fine powder by using a high power blender and grinder from QRSA with brand number QBL-8008 Pro2. The grinded *Vigna radiata* (L.) leaves flour was put in an air tight container and kept for further analysis.

2.2.1 Preliminary phytochemical analysis

Crude sample of *V. radiata* (L.) leaf 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 heamaglutin present in the sample using standard test protocols.

2.2.2 Determination of saponins

Exactly 5 g of the test sample was put into 20% acetic acid in ethanol and allowed to stand in a waterbath at 50^oC for 24 hours. This was filtered, and the extract was concentrated using a waterbath 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 [12] 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 [13] was used. 1 ml of methanolic extract of the test sample was added 1 ml of a 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^oC 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 [14]. 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 of filtrate was obtained.

Exactly 2 ml of the extract was mixed with an 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.

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

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 [15] was used. 10 g of the test sample was dispersed in 100 ml of 10% acetic acid in an ethanol solution at a 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 evaporation 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.

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

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 [15] was used to determine the flavonoid content of the test sample. 5 g of the test sample was boiled in 100 ml of 2 m 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.

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

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 [16]. A 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 a 250 ml beaker, and 100 ml of distilled water was added. 10 ml of 0.3% ammonium thiocynate solution was added as an indicator and titrated with a standard iron (III) chloride solution, which

contained 0.00195 g of 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 [17] 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 an 800 ml Kjeldahl flask, and 100 ml of H_2O was added to it. It 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 of 0.02 N $AgNO_3$ acidified with 1 ml of HNO_3. Before distillation, the tip of the condenser was adjusted appropriately so that it dipped below the surface of liquid in the receiver. When 150 ml has passed over the setup, the distillate was filtered through the 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 were done using a 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.

2.2.10 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.

2.2.11 Oxalate precipitation

About 125 ml of the sample filtrate was measured into a beaker, and four drops of methyl red indicator were added. This was followed by the addition of NH_4OH solution (dropwise) until the test solution changed from a salmon pink colour to a faint yellow colour (pH 4 – 4.5). Each portion was then heated to 90^0C, cooled, and filtered to remove precipitate containing ferrous ions. 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 centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

2.2.12 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 were heated until near boiling and then titrated against a 0.05 M standardised $KMNO_4$ solution to a faint pink colour, which persists for 30 s [15]. The calcium oxalate content of the test sample was calculated using the formula as written in equation 8.

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

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.

2.2.13 Determination of anthocyanin

According to Harborne (1973) [15], 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 an equal volume of ethyl

acetate was added, mixed, and allowed to separate into two layers. The ethyl acetate layer was recovered from each system, while the aqueous layer was discarded. The extract was dried over a steam bath and was then treated with 10 ml of conc. Amyl alcohol to extract the anthocyanin. After filtration, the alcohol extract was dried. The weight of anthocyanin in the test sample was determined and expressed as a percentage of the original samples using Equation 9.

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

2.2.14 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 solutions. 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 were prepared and treated as described for the test as blank. The absorbance of the standard and sample were measured, zeroing the spectrophotometer with a blank at 420 nm. The steroid content of the sample was calculated as stated in equation 10.

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

2.2.15 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 $(CH_3CH_2)_2O$ for 15 min. 5 ml of the boiled sample was then pipetted into a 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 $CH_3(CH_2)_3CH_2OH$ were 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.

2.2.16 Determination of hemagglutinin

2 g of the test sample weighed into a beaker, was added 20 mls 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 (Nigerian *V. radiata* (L.) leaves flour) was placed in a sohxlet apparatus and 500 ml of methanol were added, 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 vacuum to dry the methanolic extract and stored in a refrigerator for further use.

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

1 g of the methanolic extract of the test sample (*V. radiata* (L.) leaf flour) was weighed and transferred into the test tube, and 25 ml of ethanol was added. The test tube was allowed to react in a hotplate at 60^0C for 90 min. After the reaction time, the reaction product contained in the test tube was transferred to a separation funnel. The tube was washed successfully with 20 ml of ethanol, 10 ml of cold water, 10 ml of hot water, and 3 ml 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 ionisation system that utilised high energy electrons (70 eV). Pure helium gas (99.995%) was used as the carrier gas with a flow rate of 1 ml/min. The initial temperature was set at 50–150 $^\circ$C with an increasing rate of 3°C/min and a 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

methanolic extracts of Nigerian *Vigna radiata* (L.) leaves 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 metanolic extracts of *V. radiata* (L.) leaf flour were identified based on GC retention time and percentage area of each spectra on HP-5MS column and matching of the spectra with the spectrum of the known components stored in the NIST library (Replib and Mainlab data of GC–MS systems).

3 Results

The result of the preliminary quantitative phytochemical analysis of the crude 60 days old Nigerian *Vigna radiata* (L.) leaf flour sample revealed the presence of 12 bioactive compounds, with steroids showing the highest value of 19.298%, followed by tannins with a value of 9.378%. Flavonoids and cardiac glycosides follow the decrease in value trend, with values of 7.624% and 4.348% respectively. Among all the 12 bioactive compounds revealed, phytates have the lowest value of 0.416%. All the values of the 12 bioactive compounds are as shown in Table 1.

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

Phytochemical constituents	Nigerian *V.radiata* Leave flour sample
Saponins (%)	3.062
Cardiac glycosides (%)	4.348
Tannins (%)	9.378
Alkaloids (%)	1.346
Flavonoids (%)	7.624
Phytates (%)	0.416
Cyanogenic glycosides (mg)	1.080
Oxalates (%)	0.501
Anthocyanins (%)	1.239
Steroids (%)	19.298
Phenols (mg/kg)	2.574
Hemagglutinin (mg/kg)	0.479

Mariswamy *et al.* (2011) [18] reported that more degree of steroid diversity has been observed in vegetative parts when compared with the reproductive parts of plants in general, which is in support of the results obtained in this quantitative preliminary phytochemical screening.

3.1 GC-MS analysis of the methanolic extract of the leaves flour of Nigerian *Vigna radiata* L.

The GC-MS spectrum of the methanolic extracts of Nigerian *Vigna radiata* (L.) leaf (MEN*Vr*L) generated 58 peaks as shown in Fig. 1. Moreover, the mass spectra of elucidated compounds from the MEN*Vr*L were matched with the NIST/NBS spectral database. The list of identified compounds covering 100% peak areas (PA) is as shown in Table 2 with their various retention times (RT), molecular formulas, molecular weights, reported biological activities, and the spectrum generated shown in Figure 1.

The result of the GC-MS analysis revealed the presence of 53 compounds from 100% peak area (PA) out of 58 peaks generated. Benzene, 1-methyl-3-(1-methylethyl)- (RT 7.203 mins; PA 3.34% and RT 7.290 mins; PA 1.56%), Heptadecane, 2,6,10,14-tetramethyl- (RT 9.274 mins; PA 3.03% and RT 9.694 mins; PA 1.67%), undecane, 5-methyl- (RT 10.027 mins; PA 0.32% and RT 10.102 mins; PA 1.11%), Hexadecane (RT 8.646 mins; PA 0.26% and RT 22.860 mins; PA 0.45%) and 1-Docosene (RT 31.812 mins; PA 1.65% and RT 32.968 mins; PA 0.77%) were each elucidated two times with different RT.

From the spectrum, 14-octadecenoic acid, methylester was elucidated with the highest peak at RT 31.195 mins and 6.81% area, but in the NIST/NBS spectra database, matching of the spectrum revealed 2,4-Di-tert-butylphenol as the major compound present in NME*Vr*L with RT 20.976 mins and PA of 7.66%. Other compounds elucidated, following the trend of decreasing concentrations, are Benzene, 1-methyl-3-(1-methylethyl)- with summed PA of 4.90, Heptadecane, 2,6,10,14-tetramethyl with a summed PA of 4.70; Decane,2- methyl- with RT 9.341; PA 4.67; and Decane, 2,3,5,8-tetramethyl- with a RT 8.857; PA 4.18. The remaining –

Table 2 Details of the compounds identified from GC-MS analysis of a methanolic extract of *Vigna radiate* leaf

PEAK	RT (mins)	Compound	Molecular Weight	Peak area (%)	Biological activity and general use
1	6.301	.beta.-Pinene	136.2340	0.22	Used in fragrances, essential oils, and the production of other aroma compounds.
2	6.360	Benzene, 1,2,4-trimethyl-	120.1916	0.45	Sterilizing agent, dye production, perfumes, gasoline additive and resins.
3	6.494	Octane, 4-ethyl-	142.2817	0.92	Sterilizing agent, dye production, perfumes, gasoline additive and resins.
4	8.846	Benzene, 1,4-dichloro-	147.002	1.43	Disinfectant: Used for its antimicrobial properties. Pesticide: Effective against certain pests and as deodorant
5	6.957	(+)-2-Carene	136.2340	1.21	Essential oil
6	7.203	Benzene, 1-methyl-3-(1-methylethyl)	134.2182	3.24	Sterilizing agent, found in essential oil
7	7.290	Benzene, 1-methyl-3-(1-methylethyl)	134.2182	1.26	Sterilizing agent, found in essential oil
8	7.947	.gamma.-Terpinene	136.2340	0.40	Antioxidant, Anti-inflammatory.
9	8.165	(+)-4-Carene	136.2340	6.17	Essential oil
10	8.247	Decane, 3,4-dimethyl-	170.3348	1.12	Sterilizing agent, found in essential oil
11	8.381	Decane, 3,4-dimethyl-	170.3348	2.86	Sterilizing agent, found in essential oil
12	8.536	Nonane	128.2551	2.18	Sterilizing agent, essential oil, perfumes, gasoline additive and resins.
13	8.595	Decane	142.2817	0.61	Major component of diesel fuel and paint production.
14	8.646	Hexadecane	226.4412	0.86	Radiolabeling exosomes, hydrogels, and positron emission tomography.
15	8.700	Tetradecane, 5-methyl-	212.4146	1.22	Reference compound for determining cetane numbers in diesel fuels.
16	8.916	Dodecane, 3-methyl-	184.3614	2.43	Antiviral, antioxidant, insecticidal antihyperglycemic,Cleaning agent, lubricants and fuels.
17	8.959	Decane, 2,3,5,8-tetramethyl-	198.3880	4.18	Cytotoxic effect, anti-inflammatory, antimicrobial, antioxidant and neuroprotective activity.
18	9.044	2,6-Dimethyldecane	170.3348	1.58	Cytotoxic effects, antioxidant and potential antimicrobial properties. It can be used as fuels solvents, cleaning agents and lubricant
19	9.120	Hexane, 2,3,4-trimethyl-	128.2551	1.89	Cytotoxic effects, antioxidant and potential antimicrobial properties. It can be used as fuels solvents, cleaning agents and lubricant
20	9.179	Undecane, 4-methyl-	170.3348	2.59	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
21	9.274	Heptadecane, 2,6,10,14-tetramethyl	296.5741	3.03	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
22	9.341	Decane, 2-methyl-	156.3083	4.04	Anti-inflammatory, Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent,
23	9.486	Carbonic acid, prop-1-en-2-yl tetradecyl ester	298.4608	1.05	Surfactant amd emulsifier, antimicrobial and antifungal, anti-inflammatory and antioxidant, skin and hair care, food and beverage use
24	9.545	Hexane, 3,3-dimethyl-	114.2285	1.07	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
25	9.637	Carbonic acid, nonyl vinyl ester	214.3013	1.21	Surfactant amd emulsifier, antimicrobial and antifungal, anti-inflammatory and antioxidant, skin and hair care, food and beverage use, potential use in pharmaceuticals and biotechnology
26	9.694	Heptadecane, 2,6,10,14-tetramethyl	296.5741	1.53	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
27	9.735	Octane, 2,3,7-trimethyl-	156.3083	1.79	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
28	9.808	Dodecane, 2,6,11-trimethyl-	212.4146	1.99	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
29	10.027	Undecane, 5-methyl-	170.3348	0.88	Cytotoxicity effects, antioxidant, antimicrobial effect. Lubricants, fuels solvent and cleaning agent.
30	10.102	Undecane, 5-methyl-	170.3348	1.11	Cytotoxicity effects, antioxidant, antimicrobial effect. Lubricants, fuels solvent and cleaning agent.
31	10.161	2,6-Dimethyldecane	170.3348	1.09	Antimicrobial activity, cosmetics and skincare, fuel.
32	12.026	1-Dodecene	168.3190	0.44	Antioxidant, antimicrobial, surfactant and detergent.
33	12.263	Dodecane	170.3348	1.48	Cytotoxicity, antimicrobial activity, cleaning and lubricant,
34	15.111	Tridecane	184.3614	1.57	Antimicrobial activity, cleaning agent and lubricant.
35	15.278	Thymol	150.2176	0.46	Antioxidant, antimicrobial, anticancer activity, flavoring and preservative, cosmetic and personal care.
36	17.625	7-Tetradecene, (Z)-	196.3721	1.43	Antimicrobial activity, cytotoxicity, fuel moisturizers.
37	17.829	Tetradecane	198.3880	1.32	Antimicrobial, antioxidant, food additives and flavoring agen
38	18.346	Aromandendrene	204.3511	0.99	Anticancer, antimicrobial, anti oxidant and anti inflammation. Essential oil cosmetics and personal care
39	20.103	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylet henyl)-, [2R-(2.alpha.,4a.alpha.,8 a.beta.)]-	204.3511	0.40	Antimicrobial, antioxidant, cytotoxic, anti-inflammatory, anti-protozoal, and anti-platelet aggregation effects
40	20.413	Pentadecane	212.4146	0.78	Anti-inflammatory, antiproliferative, non cytototoxic, antimicrobial,
41	20.976	2,4-Di-tert-butylphenol	206.3239	7.66	volatile or essential oils, antimicrobial activity, phosphite antioxidant.
42	22.690	1-Nonadecene	266.5050	2.73	Phosphite antioxidant, antimicrobial
43	22.860	Hexadecane	226.4412	0.45	Radiolabeling exosomes, hydrogels, and positron emission tomography.
44	27.258	1-Octadecene	252.4784	2.78	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
45	29.559	Hexadecanoic acid, methyl ester	270.4507	1.80	Cytotoxicity effects, antioxidant, insecticide activity, skin penetration enhancer, flavoring and fragrance agent
46	30.018	Dibutyl phthalate	278.3435	1.61	Anti-inflammatory, antiproliferative, non cytototoxic, antimicrobial,
47	30.256	Cycloeicosane	280.5316	2.67	Anti-inflammatory, antiproliferative, non cytototoxic, antimicrobial,
48	30.432	Indazol-4-one, 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro-	306.4	0.40	anti-inflammatory, antimicrobial, anti-HIV, anticancer, hypoglycemic, antiprotozoal, and antihypertensive effects2.
49	31.151	8,11-Octadecadienoic acid, methyl ester	294.4721	0.85	Antibacteria
50	31.195	14-Octadecenoic acid, methyl ester	296.5	6.81	Anticancer, antimicrobial, anti oxidant and anti inflammation. Essential oil cosmetics and personal care
51	31.380	Methyl stearate	298.5038	1.66	Antimicrobial, antioxidant, anti-inflammation, food additive, grease and lubtricant.
52	31.663	Ethyl Oleate	310.5145	0.39	Antimicrobial, antioxidant, anti-inflammation, food additive, grease and lubtricant.
53	31.812	1-Docosene	308.5848	1.65	Antioxidant, antimicrobial, surfactant and detergent.
54	31.951	11-Methyloctadec-12-enoic acid, methyl ester	312.5312.5	0.32	Antimicrobial, antioxidant, anti-inflammation, food additive, grease and lubtricant.
55	32.968	1-Docosene	308.5848	0.77	Antioxidant, antimicrobial, surfactant and detergent.
56	34.006	Diisooctyl phthalate	390.5561	1.88	Plasticizer, adhesives, sealant, estrogenic activity.
57	34.213	1-Hexacosene	364.6911	0.41	Antimicrobial, antioxidant and anti-inflammatory activity. Can also be used as a lubricant and grease, cosmetics and personal care.
58	36.312	Squalene	410.730	0.53	Antioxidant, anti-inflammatory effect, skin and hair protection.

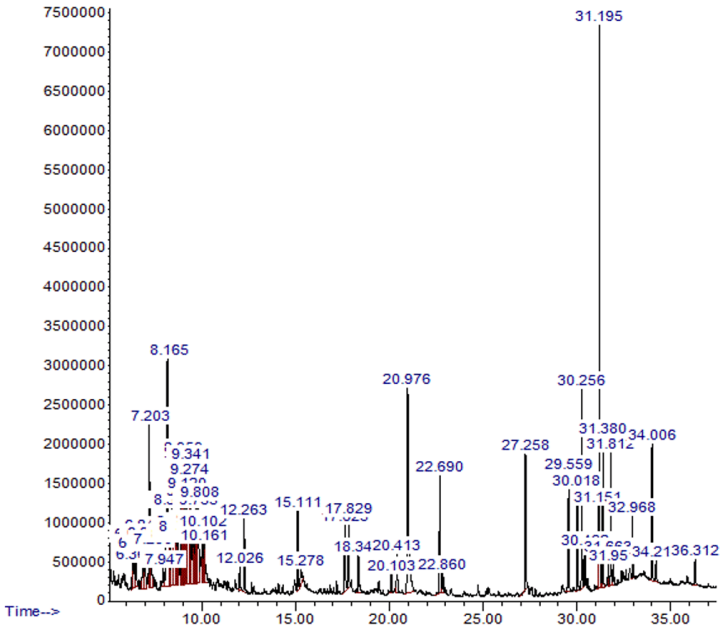

Figure 1 Total Ion Chromatogram (TIC) of methanolic extract of 60 days *Vigna radiata* leaf

compounds elucidated with PA less than 3% were also noted with .beta.-pinene as the least compound elucidated at RT 6.301 and PA 0.25%.

4 Discussion

The preliminary quantitative analysis of the crude sample of 60 days old Nigerian *Vigna radiata* leaves (N*Vr*L) revealed a high content of steroids. It has been reported that steroids have an important role in growth, development, sexual differentiation, and reproduction [19], which serves as a clue to their usage as a potential drug for alleviating reproduction defects, especially in Nigeria, where fertility among the citizens is declining as a result of poor nutrition and increased stress due to economic hardship [20]. Tannins, which were revealed to have a significant amount in the preliminary study, were reported to have antioxidant, anti-inflammatory, anti-cancer, and neuroprotective properties but should be taken with moderation because of the side effects like digestive problems reported to be associated with excess consumption of tannins [21]. With this interesting development of this existence of vital chemical contents, the MEN*Vr* leaves, subjected to GC-MS analysis to further investigate the actual bioactive compounds with reported therapeutic properties that were identified for further studies, revealed the presence of 53 bioactive compounds. This has justified the N*Vr* leaves as an important raw material for the formulation of drugs and other related chemical products, thereby paving a new way for its maximum utilization. The various retention times (mins), peak areas (%), molecular weights, reported biological activities, and uses of the identified bioactive compounds are well detailed in Table 2.

5 Conclusion

The leaves of *Vigna radiata* (L.), commonly being used as green manure in Nigeria, through these investigations on both the crude sample and the methanolic extract of the Nigerian *Vigna radiata* (L.) leaves, have been proven to contain different bioactive compounds with significant reported therapeutic properties that can be used in drug formulations and cosmetological productions. Excessive consumption of the leaves is not encouraged because of the appreciable tannin content which has been reported to cause digestive disorders when consumed in excess. The MEN*Vr* leaves are therefore highly recommended for further research on isolation and application of these research findings in drug and other related product formulations.

Data availability

All data discussed in this study are available in the current tables and figures.

Conflict of interest

The authors declare no competing interests.

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