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Original Article

Analysis of Bioactive Constituents of n-Hexane and Methanol Fractions of *Vernonia calvoana* Extracts using Gas Chromatography-Mass Spectrometry

Iwara A. Iwara*, Godwin O. Igile, Friday E. Uboh, Mbeh U. Eteng, Patrick .E. Ebong

ABSTRACT

Aim: Bioactive constituents of n-hexane and methanol fractions of *Vernonia calvoana* (VC) leaves were evaluated using Gas chromatography-Mass spectrometry. **Method:** The leaves were harvested, cleaned and air dried for 7 days. They were ground to yield 5 kg weight powder which was macerated and extracted in 8000 mL of 80% ethanol for 48 hours, yielding 310.3g (6.2%) of crude extract after evaporation of the solvent. The paste (251.8g) was subjected to column chromatography over silica gel (mesh 60 – 120 µm) and eluted with 1.5L of n-hexane and methanol respectively. The fractions were analyzed for bioactive constituents using Gas chromatography-Mass spectrometry (GC-MS) (30 m x 0.25 mm ID x 0.25 µm film thickness). **Result:** The results of GC-MS analysis showed the presences of twelve compounds with phytol (46.67%), 8,11,14-eicosatrienoic acid (33.40%), octadecanoic acid (11.25%), pentadecane carboxylic acid (10.69%), 9, 12, 15-octadecatrien-1-ol (8.12%) and ethyl palmitate (7.68%) in the n-hexane fraction, while methanol fraction was observed to have 14 compounds with oleic acid (33.40%), hexadecanoic acid (12.49%), 2-butanone (14.32%), palmitadehyde (8.36%), 11-octadecenoic acid (5.56%), Z-4-nonadecen-1-ol-acetate (5.36%) and limonene Oxide (4.37%) as well as other compounds in trace concentrations. **Conclusion:** it may be concluded from the results that, extracts of *Vernonia calvoana* contain various bioactive components that may be exploited as a good source of new drug for pharmaceutical industries.

Key words: Bioactive constituents, n-hexane, Methanol, *Vernonia calvoana*.

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INTRODUCTION

Vernonia calvoana. (VC), known by local consumers as' Ekeke", (Yakurr), in English, it is called sweet bitter leaf or bitter leaf while the French call it Vernonie douce or vernonie and Bayangi or Ndole in Cameroonians. It is popularly eaten raw and fresh as a local delicacy with or without palm oil in pepper sauce because the vegetable imparts a sweet taste like sugar in the tongue after its consumption. It serves as a component of traditional salad among the indigenous consumers. The vegetable is less bitter than the sister plant (*Vernonia amygdalina*), and yet both plants are used for the same ethno-medicinal purposes both as food and for traditional treatment of diseases such as malaria, diabetes, and gastrointestinal problems, in some part of south-south of Nigeria.[1] Chemical evaluations of this plants by[1] have revealed high levels of antioxidant vitamins (A, C, E and B-complex), mineral elements (Fe, Se, Zn, Cu, Cr and Mn) and phytochemical compounds (polyphenols, flavonoids and tannins) in the leaves of VC. Presences of this active nutritional antioxidants like vitamins A, C and E; the microelements Cr, Se, Mn, Co, Cu and Fe; and phytochemicals, such as the flavonoids, phenolic compounds, saponins and tannins have been reported widely to exhibit therapeutic properties against disease like cancer, mutagenesis, cardiovascular disease, diabetes etc. Preliminary pharmacological studies carried out in experimental models have validated the plant to have hypoglycemic and hypolipidemic activity.[2] Therefore, this present study was designed to evaluate the bioactive constituents of n-hexane and methanol fractions of *Vernonia calvoana* (VC) leaves using Gas Chromatography-Mass Spectrometry.

MATERIALS AND METHODS

Sample collection and preparation

Fresh leaves of *Vernonia calvoana* were harvested from a farm in Ugep, in Yakurr L.G.A of Cross River State, Nigeria. The leaves were collected in the morning hours, cleaned and air dried for 7 days after which they were grounded into powdered form. A measured quantity of 5 kg of powered leaves was extracted via cool maceration in 8000 mL using 8 liters of 80% ethanol for 48 hours. The extract was further double filtered with Chess cloth, then with

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filtered paper (Whatman 4 filtered paper) and the residue obtained was further extracted with 4000mL of 80% ethanol. The filtrate (extract) was then concentrated under reduced pressure at 45°C in a rotary evaporator to 10% volume and then to complete dryness using water bath yielding 310.3 g (6.2%) of crude extract. The crude extract (paste) obtained was subjected to fractionation.

Fractionation of plant extract using column chromatography

The crude extract (251.8.g) was chromatographically eluted with n-hexane and methanol in a column packed with silica gel of mesh 60-120 (Oxford laboratory reagent, Mumbai-400 002, India, Batch No: 1386). The fractions were collected and evaporated in rotary evaporator at 50°C to 10% of its original volume, and was further evaporated to paste form in a water bath at 50°C. The fractions were stored in a freezer at -4°C for further experiments.

Gas Chromatography–Mass Spectrum (GC-MS) analysis / identification of fractionated extract

Identification of fractionated samples was carried out using GC-MS -QP2010 plus (SHIMADZU-JAPAN), comprising of AOC-20i autosampler and gas chromatograph interfaced with a mass spectrometer. The assay conditions were as follows: fused silica capillary column (Rastek RT x 5Ms; 30m x 0.25mm ID x 0.25um film thickness) composing of 5% diphenyl 95% dimethylpolysiloxane; column oven temperature at 80°C, injection temperature maintained at 250°C; injection mode split; pressure of 108kpa; total flow of 6.2ml/min at 1ml/min; column flow of 1.58 ml/min; split ratio of 1.0 and solvent cut time of 2.50 min. Mass spectra were taken at a start time of 3.0 min and end time of 27.0min; while the ACQ mode- Scan was carried out at event time (0.50 sec), with scan speed 1250m/s.

Identification of component

The confirmation and interpretation of the mass spectrum GC-MS were done using the database of National Institute of Standards and Technology (NSIT) consisting of more than 62000 patterns. The spectrum of the unknown samples was compared with that of the known components that are stored in the library, and their molecular weights, names and the structural formulas were then elucidated.

RESULTS

The result of Gas Chromatographic-Mass Spectroscopy analyses of n-hexane fraction of the leave of *Vernonia calvoana* are presented in Table 1, with individual structures of the compounds, their names, percentage peak area, molecular weight, retention time, and the molecular formula in Figure 1a-g. From the result, twelve (12) compounds were identified to be present and based on percentage abundance, phytol (46.67%), 8,11,14-eicosatrienoic acid (33.40%), octadecanoic acid (11.25%), 1-pentadecanecarboxylic acid (10.69%), 9, 12, 15-octadecatrien-1-ol (8.12%) and ethyl palmitate (7.68%) were found to be present in significant percentages as well as other compounds in trace percentages. Also presented in Table 2 and Figure 2a-h are the chromatogram and identified compounds of methanol fraction of *Vernonia calvoana*, their names, percentage peak area, molecular weight, retention time, molecular formula, and structures of individual compounds. The results showed the presence of fourteen (14) compounds in the methanol fraction of *Vernonia calvoana* leaves. The names and percentage composition of some of these compounds were; Oleic acid (33.40%), 2-butanone (14.32%), hexadecanoic acid (12.49%), palmitadehyde (8.36%), Z-4-nonadecen-1-ol-acetate (5.36%), 11-octadecenoic acid (5.56%), limonene Oxide (4.37%) were found to be present in significant percentages as well as other compounds in traced percentages.

DISCUSSION

Green plants are known to represent a reservoir of effective chemotherapeutic agents with more systemic and easily biodegradable potentials. This study report shows the presence of many secondary metabolites in *V. calvoana* leaves that may be responsible for their pharmacological activities acclaimed by the local consumers. Fourteen compounds were found to be present in the methanol fraction of *V. calvoana* leaves. Based on percentage abundance, the methanol fraction of the leaves was observed to contain oleic acid, hexadecanoic acid, palmitaldehyde, 11-Octadecacenoic acid, Z-4-Nonadecen-1-ol-acetate and Limonene oxide; with oleic acid showing the highest percentage of abundance.

Oleic, hexadecanoic and octadecanoic acids have been reported to possess hypocholesterolemic, antioxidant and lubricating activity.[3] Oleic acid is a common monounsaturated fatty acid in human diet, and its consumption has been associated with decreased low-density lipoprotein (LDL) cholesterol, and possibly increased high-density lipoprotein (HDL) cholesterol.[4] Also palmitaldehyde, a derivative of palmitic acid. Authors,[5] have docu-

Table 1: Phytocomponents identified in the n-Hexane fraction of *Vernonia calvoana* GC-MS.

RT	Name of compound	Molecular formula	Molecular weight	Peak area %
9.3	2,furanmethanol	$C_5H_{10}O_2$	102	0.35
16.4	2-tridecen-1-ol	$C_{13}H_{26}O$	198	1.39
19.1	Hexadecanoic acid	$C_{18}H_{36}O_2$	284	7.68
19.5	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	10.69
21.0	Phytol	$C_{20}H_{40}O$	296	46.67
21.4	1.E-11-13 octadecatriene acetate	$C_{18}H_{32}$	248	3.55
21.6	9,12,15-octadecatrien-1-ol	$C_{18}H_{32}O$	264	8.12
21.7	Octadecanoic acid	$C_{20}H_{40}O_2$	312	11.25
23.7	Nonadecane	$C_{20}H_{42}$	282	3.34
25.3	Z,Z-10, 12-hexadecadien-1-ol acetate	$C_{18}H_{32}O_2$	280	3.17
25.4	8,11,14-eicosatrienoic acid	$C_{18}H_{34}O_2$	282	33.40
25.5	Docasanoic acid	$C_{10}H_{23}NO$	173	3.12

RT = Retention time100

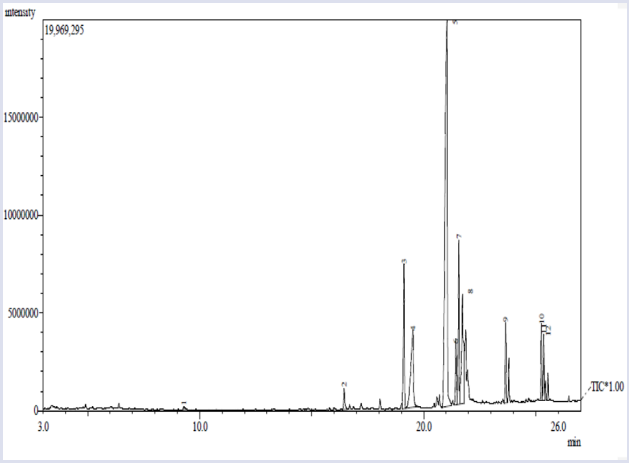

Figure 1a: Chromatogram of n-Hexane Fraction of *Vernonia calvoana*.

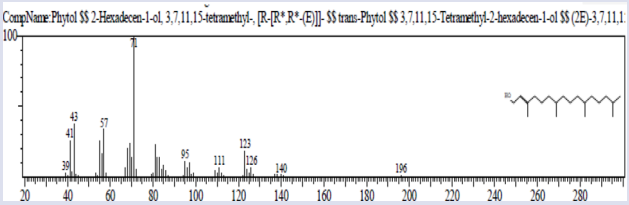

Figure 1b: Phytol

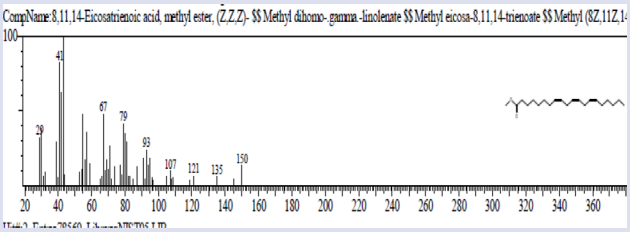

Figure 1c: 8, 11,14-Eicosatrienoic acid.

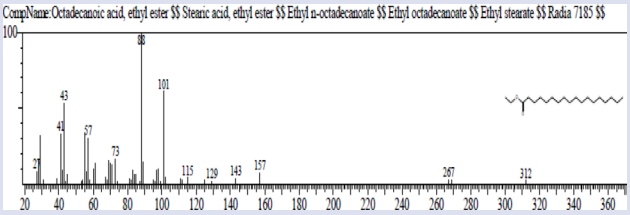

Figure 1d: Octadecanoic acid.

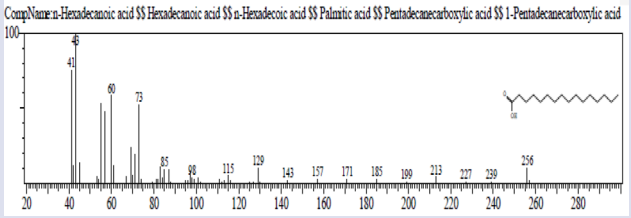

Figure 1e: n-Hexadecanoic acid.

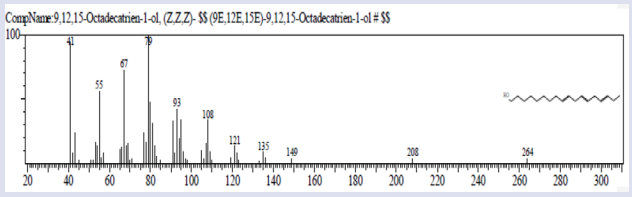

Figure 1f: 9, 12, 15- octadecatrien-1-ol.

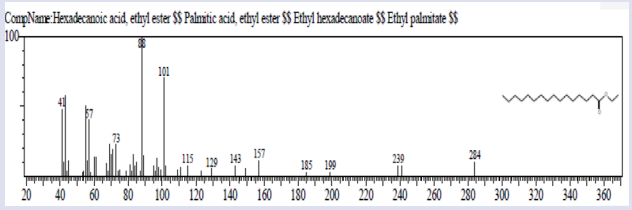

Figure 1g: Hexadecanoic acid.

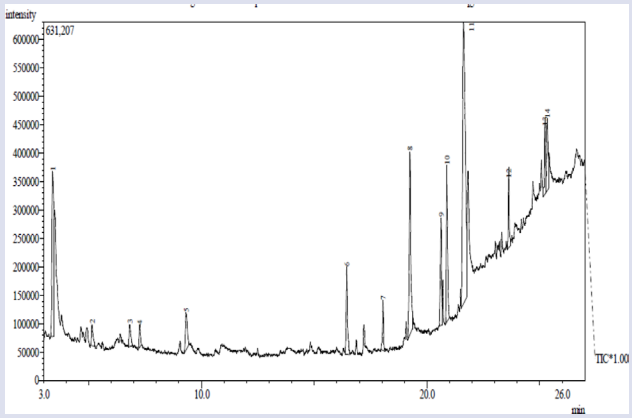

Figure 2a: Chromatogram of Methanol Fraction of *Vernonia calvoana*.

mented the stabilization of human erythrocyte membrane by palmitic acid. D-limonene a derivative of Limonene is used in the manufacture of food and some medicines. Particularly, D-limonene has been reported to be useful as a flavoring agent to mask the bitter taste of alkaloids, and as a fragrance in perfumery and adhesive lotions (http://www.cosmeticsinfo.org/ingredient/Limonene). In contrast, l-limonene is known to have a piney, turpentine-like odor. In natural and alternative medicine, d-limonene is used to relieve gastroesophageal reflux disease and heartburns.[6]

Also in this present study, the GC-MS analysis of n-hexane fraction of *V. calvoana* leaves showed the presence of twelve compounds. From the peak percentage of percentage abundance, phytol was observed to be highest, followed by 8, 11, 14-Eicosatrienoic acid, Octadecanoic acid, 1-pentadecanecarboxylic acid, ethyl palmitate and 9, 12, 15-Octadecatriene. The remaining compounds were found to be present at trace percentages. Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E[7] and vitamin K_1[8] which serve as antioxidant and clotting component of the blood, respectively. Phytanic acid, a chlorophyll metabolite from phytol has the potentials in regulating glucose metabolism. Particularly, it is a natural ligand of the peroxisome proliferator-activated receptor (PPAR) that is known to regulate hepatic glucose homeostasis.[9] It acts by activating PPARγ which can activate GLUT2 gene, and glucokinase mRNA; effect that facilitate hepatic glucose influx.[10-11] Eicosatrienoic acid is a polyunsaturated fatty acid found in various natural sources. Methyl

Table 2: Phytocomponents identified in the methanol fraction of *Vernonia calvoana* GC-MS.

RT	Name of compound	Molecular formula	Molecular weight	Peak area %
3.40	2, butanone	$C_4H_8O_2$	88	14.32
5.15	1,3,5-cycloheptatriene	C_9H_{12}	120	1.51
6.81	Propanoic acid	$C_7H_{12}O_{12}$	128	1.50
7.25	I-hydroxy-3-methjy-2-butanone	$C_7H_{12}O_2$	102	1.21
9.32	Butanoic acid	$C_5H_{10}O_2$	102	3.14
16.44	Z-4-Nonadecen-1-ol-acetate (new)	$C_7H_{12}O_2$	128	5.36
18.04	Pentanoic acid	$C_{12}H_{40}O_2$	328	2.42
19.24	n-Hexadecenoic acid	$C1_6H_{32}O_2$	256	12.49
20.62	11-Octadecenoic acid	$C1_9H_{36}O_2$	296	5.56
20.88	Palmitaldehyde	$C_{26}H_{54}O_2$	398	8.38
21.62	Oleic acid acid	$C_{12}H_{23}O_2$	282	33.40
23.63	Hydroxylamine	$C_{10}H_{23}NO$	173	3.12
25.23	9-Methyl-Z-Z-10-12 hexadecadlen-1-01	$C_{19}H_{34}O_2$	294	3.22
25.33	Limonene oxide	$C_{10}H_{16}O$	152	4.37

RT = Retention Time 100

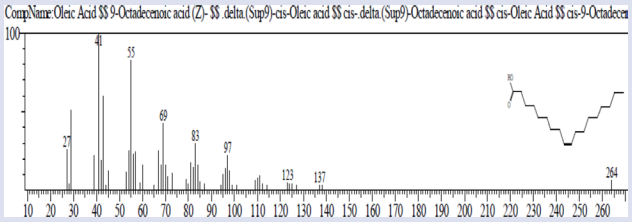

Figure 2b: Oleic acid.

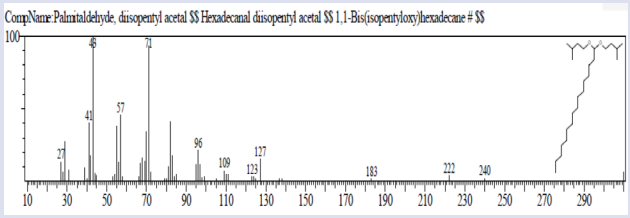

Figure 2e: Palmitaldehyde

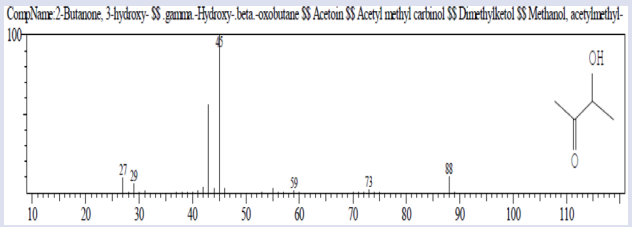

Figure 2c: 2-Butanone.

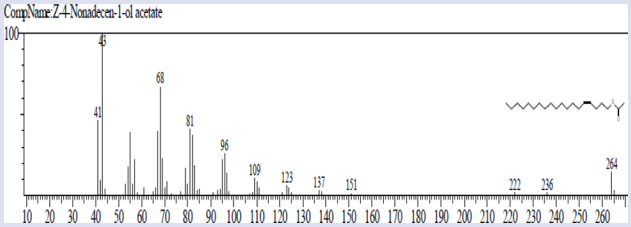

Figure 2f: Z-$_ Nonadecan-1-ol acetae.

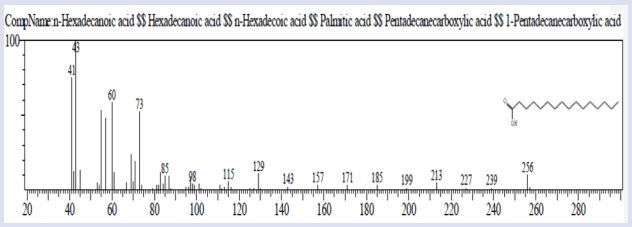

Figure 2d: n-Hexadecanoic acid.

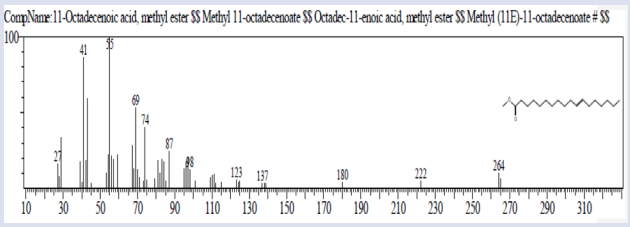

Figure 2g: 11-Octadecanoic acid.

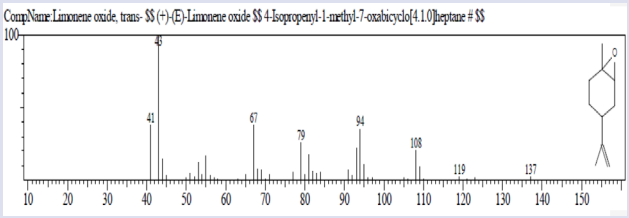

Figure 2h: Limonene oxide.

ester Eicosatrienoic acid was observed when applied topically to reduce inflammatory processes, by potentially removing arachidonic acid from phospholipid pools thus reducing the formation of inflammatory products such as prostaglandin E2 and leukotrienes.[12]

CONCLUSION

The GC-MS analysis of n-hexane fraction of *Vernonia calvoana* has revealed many components from this species of Vernonia with significant bioactivities. The genus (Vernonia) is well known for its traditional medicinal uses including cancer, hepatitis, inflammation of the uterus, uterine cancer, rheumatism, dysentery, stomachache, diarrhea, fever, etc.

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CONFLICT OF INTEREST

Authors have no conflict of interest, financial or otherwise

ABBREVIATIONS

GC-MS: Gas chromatography-Mass spectrometry; **VC:** *Vernonia calvoana*; **Glut 2:** Glucose transport system 2; **mRNA:** Messenger Ribonucleic acid; **L.G.A:** Local Government Area.

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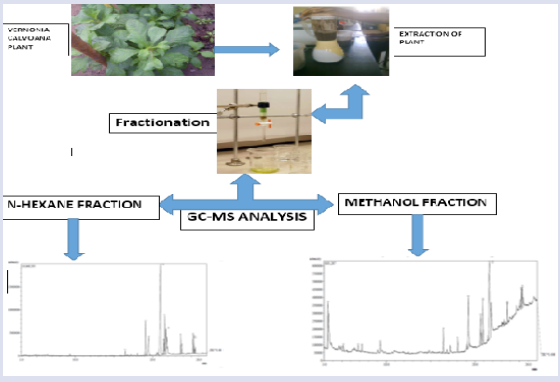
GRAPHICAL ABSTRACT



SUMMARY

- The bioactive constituents of n-hexane and methanol fractions of *Vernonia calvoana* was evaluated using GC-MS . Twelve compounds were identified in the n-hexane with phytol presentin appreciable percentage thus serve as the representative of this fraction. Fourteen compounds were identified in the methanol fraction and based on their percentage abundance, oleic acid was present in appreciable percentage and serving as the representative of this fraction. With the presence of this compounds in both fractions, extracts from this plant may be elected as a good source of new drugs for chemical and therapeutic studies.

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