WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 2, 35-49. Research Article ISSN 2277– 7105

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS OF PHYTOCOMPONENTS IN THE ROOT, STEM BARK AND LEAF OF *VERNONIA AMYGDALINA*

Longe Adeteju Olufunmilayo[1]*, Momoh Johnson Oshiobugie[1] and [2]Asoro Iroghama Iyobosa

[1]*Department of Science Laboratory Technology (Biochemistry Unit), School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria.

[2]Department of Biochemistry, College of Medicine, Idi-Araba, University of Lagos, Akoka Lagos, Nigeria.

Article Received on
25 Nov. 2016,

Revised on 15 Dec. 2016,
Accepted on 05 Jan. 2017

DOI: 10.20959/wjpr20172-7701

***Corresponding Author**
Longe Adeteju Olufunmilayo
Department of Science Laboratory Technology (Biochemistry Unit), School of Pure and Applied Sciences, Lagos State Polytechnic, Ikorodu, Lagos, Nigeria.

ABSTRACT

Vernonia amygdalina is a medicinal plant widely used in traditional medicine in Africa and Asia for the treatment of ailments such as diabetes, inflammation, microbial infections and it has hypoglycaemic properties. The chemical constituents of the methanolic extract of *Vernonia amygdalina* root, stem bark and leaf were investigated using Gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis of the *Vernonia amygdalina* root, stem bark and leaf extract revealed the existence of the GC-MS chromatogram of twenty six peaks present. Fifteen chemical compounds were identified in the root of *V amygdalina*, six were found in the stem bark. while five were identified in the leaf of the plant by GC-MS analysis. The result of the analysis showed that *Vernonia amygdalina* contains important pharmacologically important bioactive compounds. The presence of these bioactive compounds justifies the uses of the plant for various traditional medicines.

KEYWORDS: Bioactive compounds, GC-MS analysis, methanol extract and *Vernonia amygdalina*

INTRODUCTION

Plants are useful to man as source of food, medication and raw materials for Industries. Many parts of plants such as fruits, seeds, barks, roots, fruits and flowers have been used as medicaments to cure various diseases that afflict man and other animals (Phyllistin *et al.*, 2000).

Vernonia amygdalina grows throughout tropical Africa and has been domesticated in some parts of Nigeria. The leaves have been found to be relevance in traditional folk medicine as anthelmintics, antimalarial, antimicrobial, anticancer and as a laxative herb (Akah et al., 2009). The taxonomic classification of *Vernonia amygdalina* is as follows: Kingdom: plantae, Division: Angiosperms, Order : Asterales, Family: Asteraceae, Genius: Vernonia, Species*: V. amygdalina.* It has a variety of names in various languages. It is commonly called "Bitter leaf" in English language, "Shuwaka" in Hausa language, "Onugbu" in Igbo language, "Ewuro" in Yoruba language, "Oriwo" in Edo and "Chusa-doki" in Hausa (Egedigwe 2010).

The aim of this study is to identify some of the bioactive compounds of in *Vernonia amygdalina* using GC-MS technique with the possibility of discovering compound(s) of therapeutic value.

MATERIALS AND METHODS

Collection and identification of Plant material

The *Vernonia amygdalina* plant was obtained from Ikorodu in Lagos State.

The plant was authenticated from the department of Botany, University of Lagos, Nigeria. Authentication number for the *Vernonia amygdalina* was 6945.

Preparation of root, stem bark and leaf extract of *Vernonia amygdalina*

The root, stem bark and leaf of *Vernonia amygdalina* were obtained from Ikorodu, washed separately, air dried under shade in the Laboratory, pulverised to coarse power using industrial blender.2g each of the root, stem bark and leaf of the grounded *Vernonia amygdalina* plant material were placed in timble and later placed in a Soxhlet extractor with 30mls methanol and heated using heating mantle at 100^{o}C for 3 hours. The extracts were poured into separate beakers and concentrated with ultra sonic bath at 60^{o}C for one hour. The remaining extract was treated with anhydrous sodium sulphate to absorb the water in the

samples and later treated with silica gel which helps to remove impurities in the samples. The extracts were later used for GC-MS analysis.

GC-MS analysis of the root, stem bark and leaf of *Vernonia amygdalina*

GC-MS analysis of the plant was carried out on an Agilent technology 7890 GC system equipped with a mass spectrometric detector (MSD). Ms model is agilent technology 5975 ms, the column used is HP-5MS agilent technology, length of the column is 30 m, internal diameter 0.320 mm, thickness of 0.25 μm. Volume of sample injected is 1μL. Oven temperature program with initial temperature of 80^{o}C to hold for 2 minutes at 10^{o}C/min to final temperature of 240^{o}C to hold for 6 minutes with injector temperature of 250^{o}C. The mobile phase is helium gas while the stationary phase is column.

Detection of components

Analysis of mass spectrum GC-MS was conducted by the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unidentified component was compared with the spectrum of the identified components stored in the NIST library. The name, molecular weight, structure of the components in the test material was ascertained (Principle 1991, Strenhagen *et al.*, 1974 and Jennings and Shibamoto 1980).

RESULT

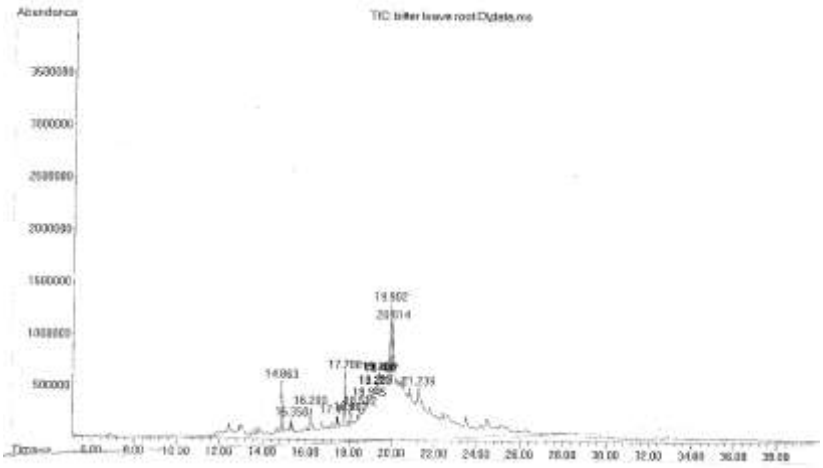

Figure 1. GC-MS Chromatogram of methanol root extract of *Vernonia amygdalina*

Table. 1 Phytocomponents identified in the methanol root extract of *Vernonia amygdalina*.

SN	Retention Time	Name of the compound	Molecular formulae	Molecular Weight (g/mol)	Peak Area (%)	Activity
1	14.866	2,4-Hexadiene, 2,3-dimethyl-	C_8H_{14}	110.1968	11.94	NF
2	15.352	Octadecane	$C_{18}H_{38}$	254.502	2.01	NF
3	16.199	1,2 benzenedicarboxylic acid,butyl,2-ethylhexyl ester	$C_{20}H_{30}O_4$	334.4498	3.97	NF
4	17.418	Dichloroacetic acid, heptadecyl ester	$C_{19}H_{36}Cl_2O_2$	367.395	0.99	Antiviral [Ara *et al.*, 2012]
5	17.790	1H-Naphtho [(2,1-b] pyran, 3-ethenyldo decahydro-3,4a,7,7, 10a-pentamethyl-, [3S-(3.alpha.,4a.alpha., 6a.beta.,10a.alpha., 10b.beta.)]-	$C_{20}H_{34}O$	290.4834	14.58	NF
6	18.070	Kaur-16-ene	$C_{20}H_{32}$	272.4681	2.40	NF
7	18.511	Cis-13-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48794 g/mol	1.25	NF
8	18.945	Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	1.39	antiinflammatory, hemolytic, pesticide, antioxidant and 5-alphareductase inhibitor.
9	19.209	Cis-vaccenic acid	$C_{18}H_{34}O_2$	282.46	4.32	NF
10	19.226	Cis-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	0.37	Antiinflammatory, nematicide, hypocholesterolemic,anticancer, antiarthritic, hepatoprotective, insectifuge, antiacne, 5-Alpha reductase inhibitor,antiandrogenic and anticoronary.
11	19.363	Oleic acid	$C_{18}H_{34}O_2$	282.461	3.65	Use to lower cholesterol, LDL-CHol and possibly increased HDL-CHOL.
12	19.409	9-Octadecenoic acid, (E)	$C_{18}H_{34}O_2$	282.4614	2.08	Antiviral [Helmy *et al.*, 2007]
13	19.489	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614	1.51	NF

14	19.901	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	$C_{11}H_{14}O_3$	194.2271	25.14	Anti-inflammatory, antidiarrhoeic, antidiabetic, antilipolytic and antispasmodic (Bilal ahmad *et al.*, 2015)
15	20.051	11,13-dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.4614	17.67	NF

NF mean not found

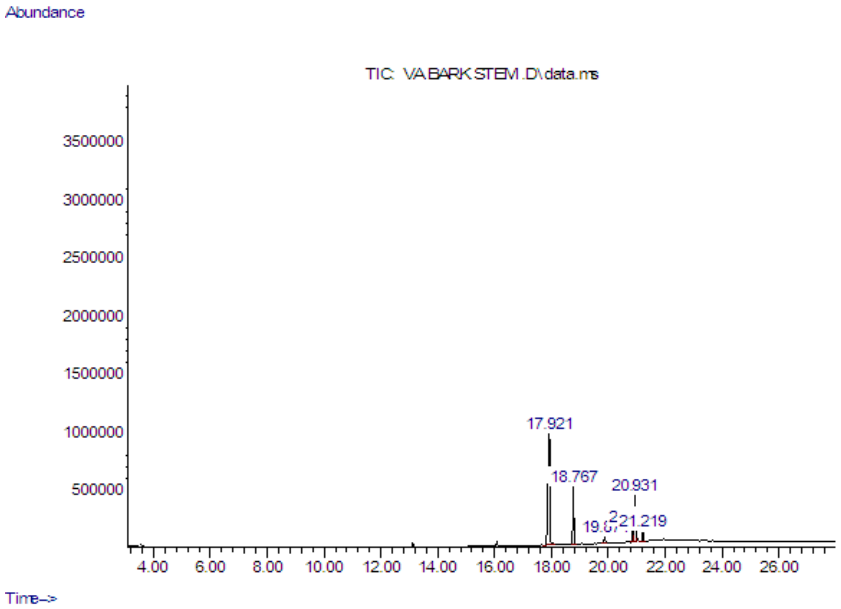

Figure2. GC-MS Chromatogram of methanol stem bark extract of *Vernonia amygdalina*

Table. 2 Phytocomponents identified in the methanol stem bark extract of *Vernonia amygdalina*.

SN	Retention Time	Name of the compound	Molecular formulae	Molecular Weight	Peak Area (%)	Activity
1	17.924	Caffeine	$C_8H_{10}N_4O_2$	194.19 g/mol	59.97	NF
2	18.765	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.4507	16.52	Anti-oxidant, antimicrobial, decrease blood cholesterol and anti-inflammatory [Akpuaka *et al.*, 2013, Hema *et al.*, 2011].
3	19.869	2-Cyclopenten-1-one, 2,3-dimethyl	$C_7H_{10}O$	110.1537	2.41	NF
4	20.865	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721	4.24	Antiinflammatory Nematicide, Antiacne, Antihistaminic, Insectifuge, antiCancer,

						Hypocholesterolemic, Hepatoprotective, and Antiarthritic (Ha *etal.*, 1990 and Johnson *et al.*, 2011)
5	20.928	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.4879	13.82	Antioxidant and anti cancer [Hema *et al.*, 2011 and Syeda *et al.*, 2011].
6	21.220	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	3.03	They are used as solvents or cosolvents, oil carrier in agricultural industry.

NF mean not found

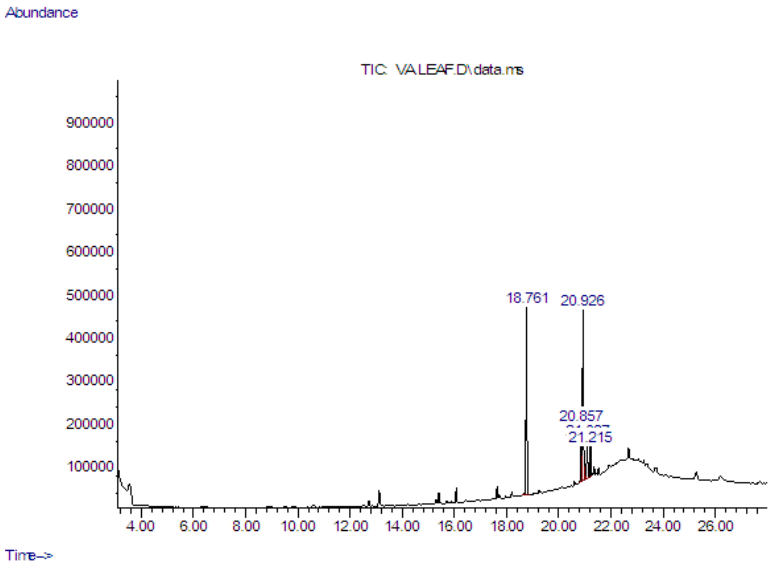

Figure3. GC-MS Chromatogram of methanol leaf extract of *Vernonia amygdalina*

Table.3. Phytocomponents identified in the methanol leaf extract of *Vernonia amygdalina*.

SN	Retention Time	Name of the compound	Molecular formulae	Molecular Weight	Peak Area (%)	Activity
1	18.759	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.4507	38.34	Antioxidant and antifungal (Akpuaka *et al.*, 2013)
2	20.859	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721	11.34	Antiinflammatory Nematicide, Antiacne, Antihistaminic, Insectifuge, antiCancer, Hypocholesterolemic, Hepatoprotective, and Antiarthritic (Ha *etal.*, 1990 and Johnson *et al.*, 2011)
3	20.928	10-Octadecenoic	$C_{19}H_{36}O_2$	296.4879	35.59	NF

		acid, methyl ester				
4	21.088	Hexadecylpentyl ether	$C_{21}H_{44}O$	312.5735	8.95	NF
5	21.214	Methyl stearate	$C_{19}H_{38}O_2$	298.5038	5.78	They are used as solvents or cosolvents and oil carrier in agricultural industry.

NF mean not found

Figure 4 to Figure 27 below show the structures and the mass spectrums of the different compounds found in the root, stem bark and leaf of *Vernonia amygdalina.*

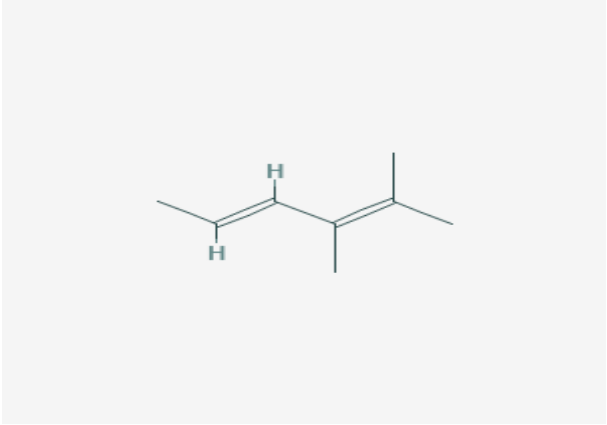

Figure 4. Structure of 2,4-Hexadiene, 2,3-dimethyl-

Figure 5. Structure of Octadecane

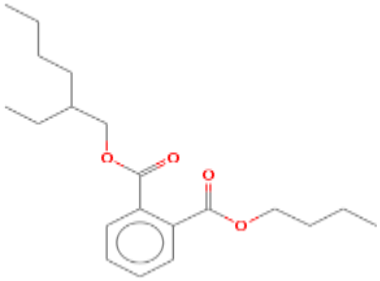

Figure 6. Structure of 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester

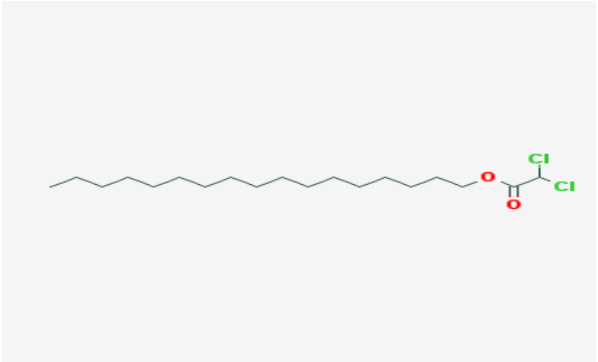

Figure 7. Structure of Dichloroacetic acid, heptadecyl ester

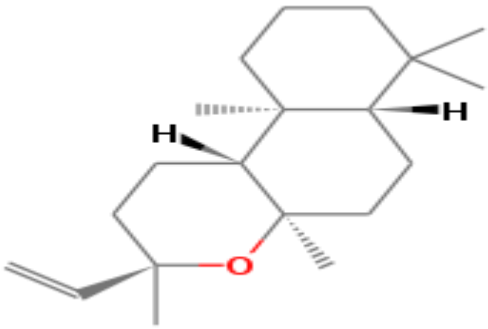

Figure 8. Structure of 1H-Naphtho[2,1-b]pyran, 3 ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3S-(3α,4aα,6aβ,10aα,10bβ)]-

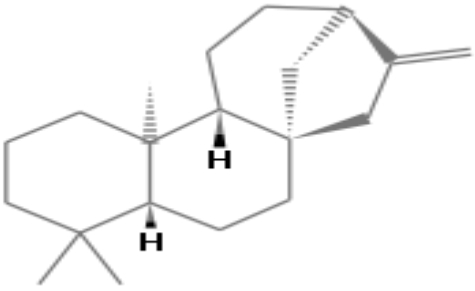

Figure 9. Structure of Kaur-16-ene

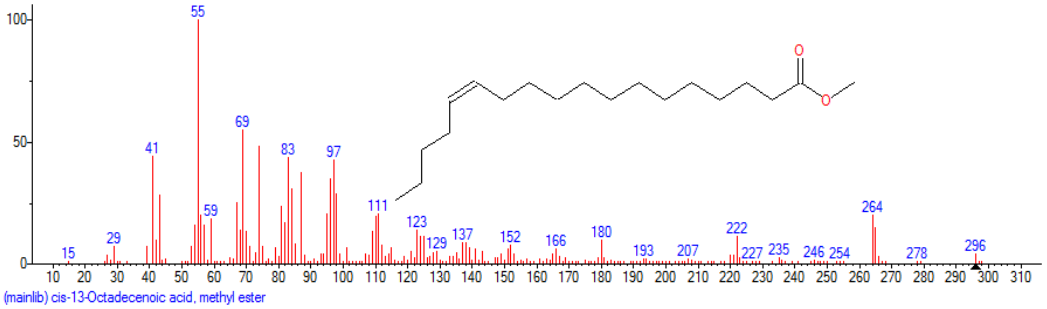

Figure 10. Mass spectrum of cis-13-Octadecenoic acid, methyl ester structure.

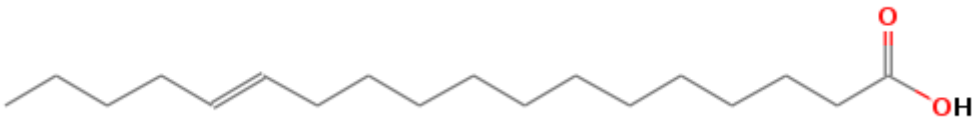

Figure 11. Structure of Trans-13-octadecenoic acid

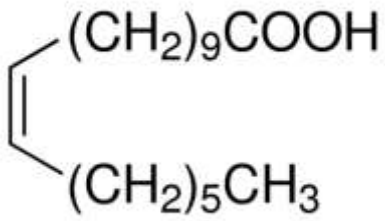

Figure 12. Structure of *cis*-Vaccenic acid

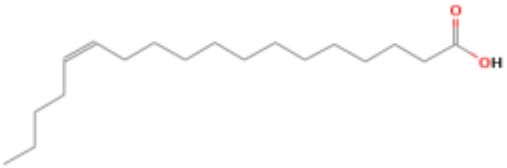

Figure 13. Structure of cis-13-Octadecenoic acid

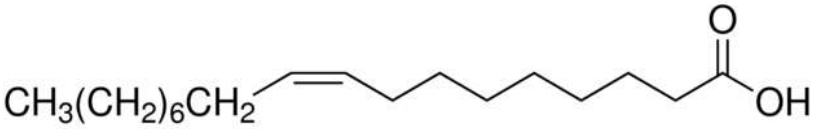

Figure 14. Structure of Oleic acid

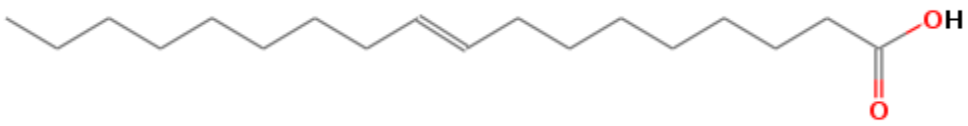

Figure 15. Structure of 9-Octadecenoic acid, (E)-

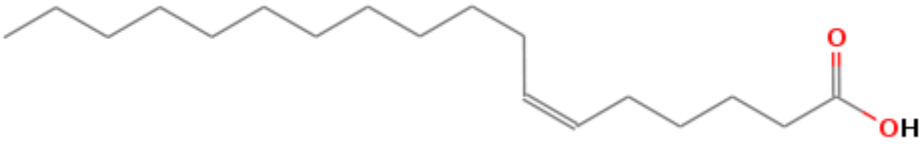

Figure 16. Structure of 6-octadecenoic acid

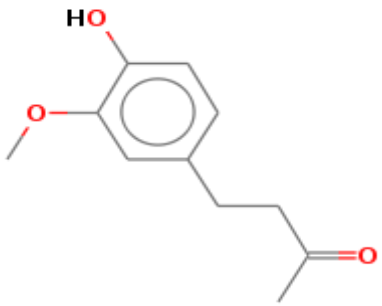

Figure 17. Structure of 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-

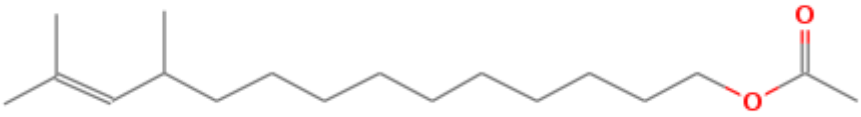

Figure 18. Structure of 11,13-Dimethyl-12-tetradecen-1-ol acetate

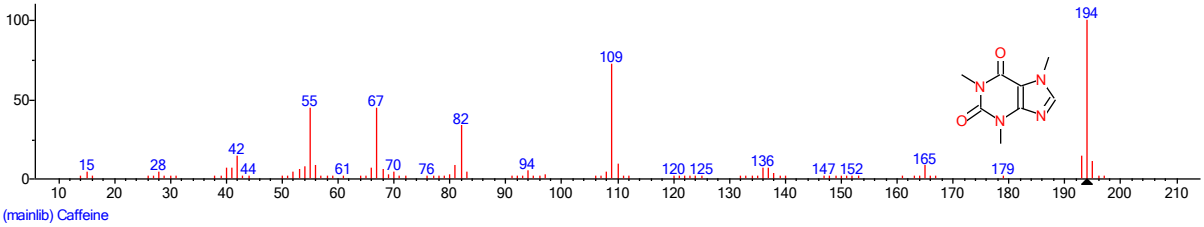

Figure 19. Mass spectrum of Caffeine

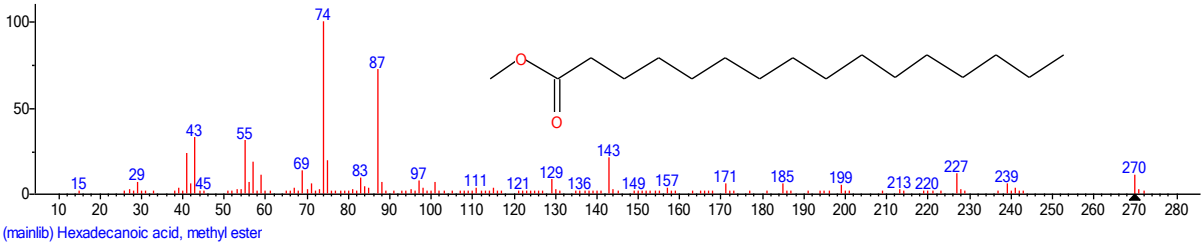

Figure 20. Mass spectrum of Hexadecanoic acid, methyl ester

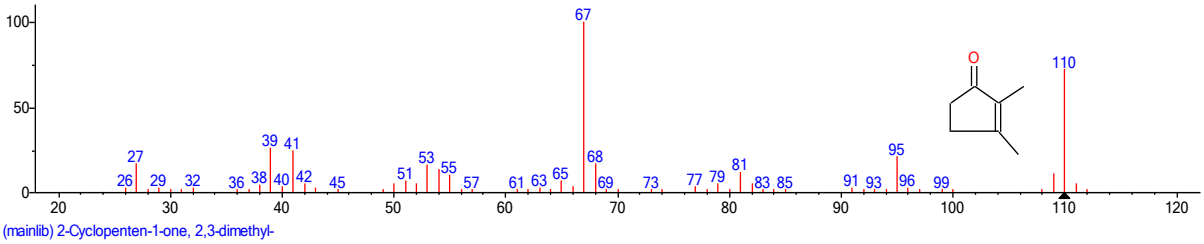

Figure 21. Mass spectrum of 2-Cyclopenten-1-one, 2,3-dimethyl-

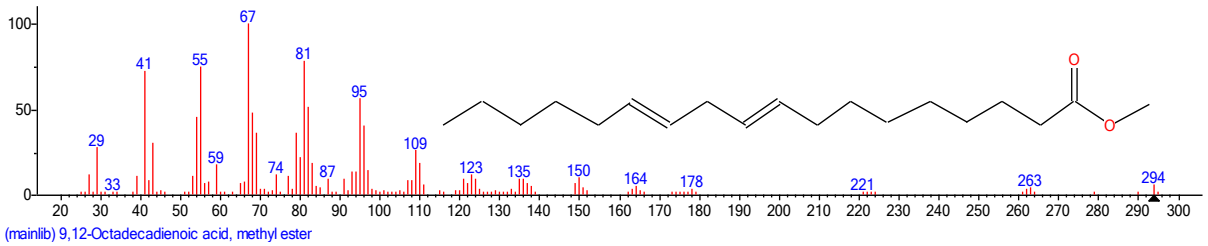

Figure 22. Mass spectrum of 9,12-Octadecadienoic acid, methyl ester

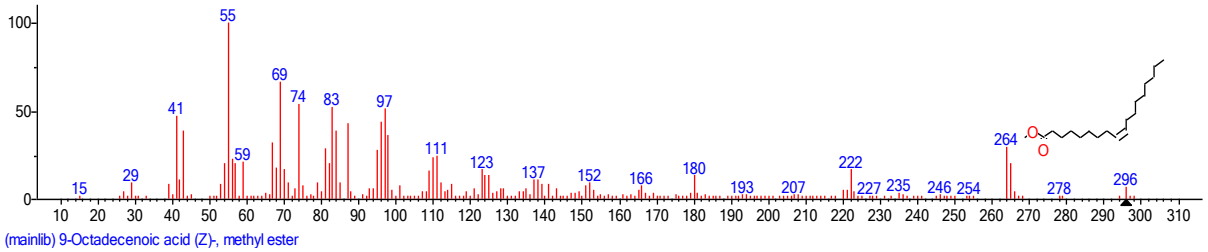

Figure 23. Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester

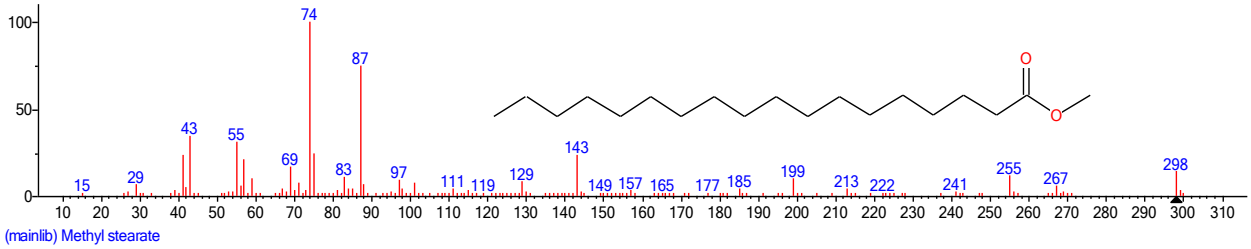

Figure 24. Mass spectrum of Methyl stearate

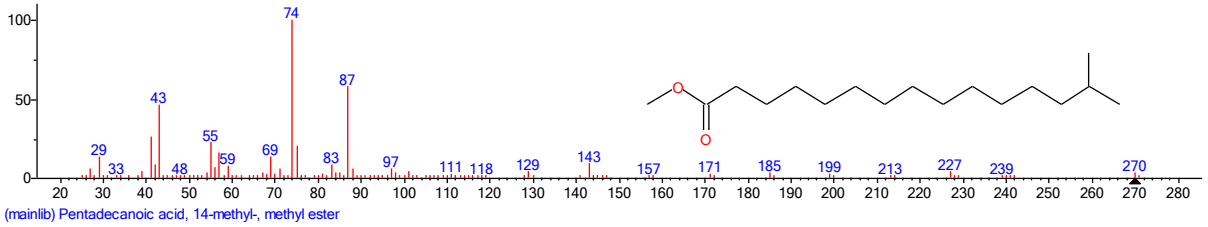

Figure 25. Mass spectrum of Pentadecanoic acid, 14-methyl-, methyl ester

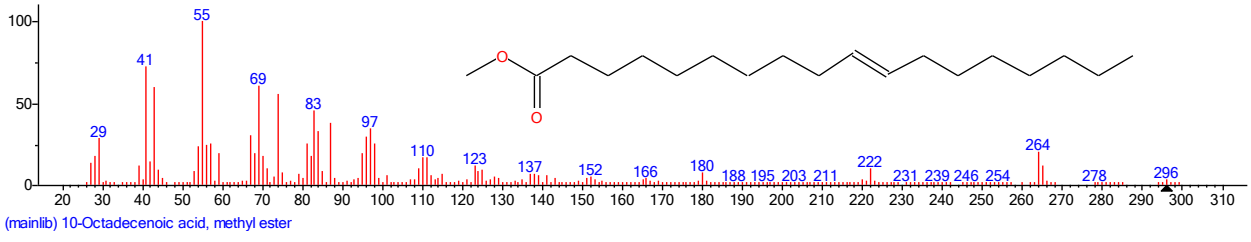

Figure 26. Mass spectrum of 10-Octadecenoic acid, methyl ester

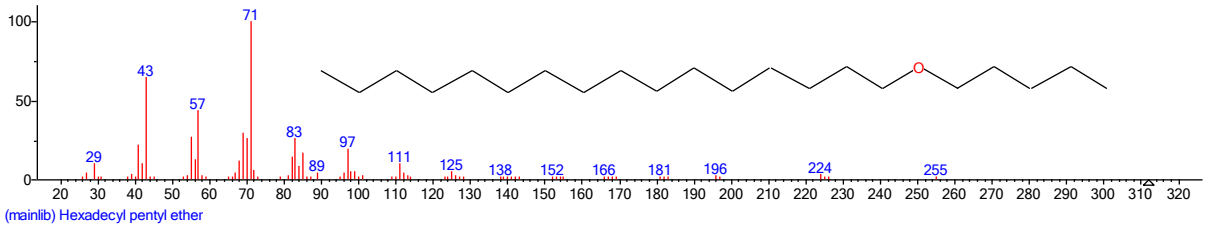

Figure 27. Mass spectrum of Hexadecylpentyl ether

DISCUSSION

Studies have shown that plants have been an important source of medicine. They are source of many potential drugs mainly on traditional remedies such as herbs used as popular folk medicines (Sathyaprabha *et al.*, 2010).

Gas chromatography coupled with mass spectrometry (GC-MS) is an established technique for reliable identification of bioactive compounds existing in medicinal plants including volatile matter, long chain and branched chain hydrocarbons, alcohols, acids, esters [Cong *et al.*, 2007, Kumar *et al.*, 2014, Johnson *et al.*, 2011.

The bio activity components were identified and characterized and interpretation on mass spectrum GC–MS conducted using the database of National Institute Standard and Technology (NIST) which is having more than 62,000 patterns. Besides that, the characteristic fragmentation patterns greatly helped in the identification of a particular class of compounds [Mass Spectrometry Data Centre; 1974].

The identified bioactive compounds of the methanol extract of the root, stem bark and leaf of *Vernonia amygdalina,* their retention time, peak area, molecular formulae, molecular weight, and their activities are shown in Table 1, 2 and 3 above.

In the present study, 26 compounds were identified of which some of the compounds are of medicinal important. Out of which 15 compounds are found in the root, they are: 2,4-Hexadiene, 2,3-dimethyl-, Octadecane, 1,2 benzenedicarboxylic acid,butyl,2-ethylhexyl ester, Dichloroacetic acid, heptadecyl ester, 1H-Naphtho [(2,1-b] pyran, 3-ethenyldo decahydro-3,4a,7,7,10a-pentamethyl-,[3S-(3.alpha.,4a.alpha., 6a.beta.,10a.alpha., 10b.beta.)], Kaur-16-ene, Cis-13-octadecenoic acid, methyl ester, Trans-13-octadecenoic acid, Cis-vaccenic acid, Cis-13-octadecenoic acid, Oleic acid, 9-Octadecenoic acid, (E), 6-Octadecenoic acid, 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- and 11,13-dimethyl-12-tetradecen-1-ol acetate. The stem bark contain six compounds: They include: Caffeine, Hexadecanoic acid, methyl

ester, 2-Cyclopenten-1-one, 2,3-dimethyl, 9,12-Octadecadienoic acid, methyl ester , 9-Octadecenoic acid (Z)-, methyl ester and Methyl stearate. Five compounds were also found in the leaf, they are: Pentadecanoic acid, 14-methyl-, methyl ester, 9, 12-Octadecadienoic acid, methyl ester, 10-Octadecenoic acid, methyl ester, Hexadecylpentyl ether and Methyl stearate. The compounds obtained from the GC-MS analysis were of biological important. Some of the medicinal uses of the compounds found in the root, stem bark and leaf of the plant have been shown in Table 1, 2 and 3 respectively. Studies have shown that compound like Oleic acid reduces blood pressure (Ruiz-Gutiérrez et al. 1996), prevent ulcerative colitis. (de Silva *et al.* 2014), protects cell membranes from free radicals. (Haug *et al.*2007). Cis-*13-Octadecenoic acid* is reported to have therapeutic importance in the treatment of dopaminergic cell loss and the motor sequelae of Parkinson disease.(Alfred *et al.,* 2005).

CONCLUSION

This study helps to predict the formula and structure of active molecules in the plant that can be used as drugs. The result also enhances the traditional uses of the plant.

ACKNOWLEDGEMENT

This research work was supported by Tertiary Education Trust Fund (TETFUND) grant from Nigeria. The authors are grateful to the Management of Lagos State Polytechnic for their support and assistance.

REFERENCES

1. Akah, P.A., Alemji J.A., Salawu,O.A., Okoye,T.C., Offiah, N.V. Effects of *Vernonia amygdalina* on Biochemical and Hematological Parameters in Diabetic Rats. Asian Journal of Medical Sciences., 2009; 1(3): 108-113.
2. Akpuaka, A., Ekwenchi, M.M., Dashak, D.A., Dildar, A., Biological Activities of Characterized Isolates of n-Hexane Extract of *Azadirachta indica* A. Juss (Neem) Leaves. New York Sci. J; 2013; 6(6):119-124.
3. Ara, I., Bukhari, N..A., Aref, N.M., Shinwari, M.M. A., Bakir, M.A.. Antiviral Activities of *streptomycetes* Against Tobacco Mosaic virus (TMV) in *Datura* Plant: Evaluation of Different Organic Compounds in Their Metabolites. African J. of Biotechnol., 2012; 11(8): 2130-2138.
4. Alfred, H., Lisa, W., Nancy, B., Suzanne, H., Josh, W.K., Giridher, A.R., Martin, G. Long-chain fatty acids increase cellular dopamine in an immortalized cellline (MN9D) derived from mouse mesencephalon. Neuroscience Letters, 2005; 376; 35-39.

5. Bilal A., Muneeb, U., Rehman, I. A., Ahmad A., Saiema R.,, Showkat A.B, Insha A., Ishraq H., Sheikh B., Manzoor, R.,. *Review Article* A Review on Pharmacological Properties of Zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone). Hindawi Publishing Corporation Scientific World Journal Volume; 2015, Article ID 816364, 6 pages.
6. Cong, Z., Meiling, Q., Qinglong, S., Shan, Z., Ruonong, F. J. Pharm. Biomed. Anal; 2007; 44: 464.
7. Egedigwe, C.A. "*Effects of Dietary Incorporation of Vernonia amygdalina and Vernonia colorata on Blood Lipid Profile and Relative Organ Weights in Albino Rats",* M.Sc. Dissertaton, Department of Biochemistry, Michael Opara University of Agriculture Umudike, Nigeria; 2010.
8. Ha, Y.L, Storkson, J., Pariza, M.W. Inhibition of benzo (a) pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res; 1990; 1097-1101.
9. Helmy, W.A., Abd-Alla, H.I., Amer, H., El-Safty, M.M. Chemical Composition and '*In-vitro*' Antiviral Activity of *Azadirachta indica* A. Juss (Neem) Leaves and Fruits against Newcastle Disease Virus and Infectious Bursal Disease Virus. Australian J. Basic Appl. Sci; 2007; 1(4): 801-812.
10. Hema, R, Kumaravel, S., Alagusundaram. *J. Am. Sci*. 2011; 7; 27.
11. Haug, A., Høstmark, A.T., Harstad, O.M. Bovine milk in human nutrition – a review. Lipids in Health and Disease 2007; 6: 25. Pubmed.
12. Jennings, W., Shibamoto, T. Quality of Flavour and Fragrance Volatiles by Glass Capillary Gas Chromatography. Academic Press, New York, NY 1980.
13. Johnson, M., Mariswamy. Y,, Gnaraj, W.F., Chromatographic finger print analysis of steroids in *Aerva lanasa* L. by HPTLC technique. Asian Pal. J. Trop. Biomedicine.; 2011; 1: 428-433.
14. Mass Spectrometry Data Centre. Eight peak index of mass spectra: the eight most abundant ions in 31,101 mass spectra, indexed by molecular weight, elemental composition and most abundant ions (4 volume set). 2nd ed. Aldermaston: Mass Spectrometry Data Centre; 1974.
15. Phyllistin, A.B., James, F.B. Tips for preventing food poisoning "Herbs" American No.1 Guide Natural health, 3rd edn. Publ. Averge.2000, No.9 pp. 383-386.
16. Principe, P. Monetising the pharmacological benefits of plants. US Environmental protection Agency, Washington, D.C. 2005; pp. 1991.

17. Ruiz-Gutiérrez, V., Muriana, F.J, Guerrero, A.Cert AM, Villar J. Plasma lipids, erythrocyte membrane lipids, and blood pressure of hypertensive women after ingestion of dietary oleic acid from two different sources. J Hypertens; 1996; 14: 1483–1490. Pubmed
18. Sathyaprabha, G., Kumaravel, S., Ruffina, D.,. Praveen Kumar, P. A. Comparative Study on Antioxidant, Proximate Analysis, Antimicrobial Activity and Phytochemical Analysis of Aloe vera and Cissus quadranghlaris by GC-MS. *J. Pharma Re;2010;*. 3: 2970-2973.
19. Sermakkani, M., Thangapandian, V. GC-MS analysis of Cassia italic a leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research; 2012; 5(2): 90-94.
20. Kumar, A., Kumari, P.S., Somasundaram, T. Gas Chromatography-Mass Spectrum (GC-MS) analysis of bioactive components of the methanol extract of *Halophyte, Sesuvium portulacastrum L.* IJAPBC; 2014; 3(3): 766-772.
21. Silva, P.S., Luben, R., Shrestha, S.S, Khaw, K.T., Hart, A.R. Dietary arachidonic and oleic acid intake in ulcerative colitis etiology: a prospective cohort study using 7-day food diaries. Eur J Gastroenterol Hepatol. 2014; 26: 11-8. Pubmed.
22. Stenhagen, E., Abrahamson, S., McLafferty, F. Registry of Spectral Data. J. Wiley and Sons, New York, NY, 1974.
23. Syeda, F.A., Habib-ur-Rahman, A.M., Khan, Choudahry M.I, Atta-Ur-Rahman. *Inter.J. Genetics Mol. Biol*. 2011; 3: 95.
24. "You Can Control Your Cholesterol: A Guide to Low-Cholesterol Living". Merck & Co. *Inc*. Retrieved; 2009-03-14.