Investigation of Bioactive Phytochemical Compounds from Aqueous Ethanol Extracts of Leaves of *Phyllanthus amarus* Schum and Thonn by Gas Chromatography-Mass Spectrometry (GC-MS)

Patience O. Adomi

Department of Medical Microbiology and Parasitology, College of Health Sciences,
Delta State University, Abraka, Nigeria.

E-mail: Padomi.adomi07@gmail.com

ABSTRACT

The GC-MS analysis of extracts obtained from aqueous ethanol solution, purified in chloroform, yielded ten compounds, namely decane; 1, 2, 3-Trimethybenzene; Isooctane (ethynyloxy) ;ethyltridecanoate; n-hexadecanoic acid; 3,7,11,15-tetramethy1-2-hexadecane-1-ol; 9,12,15-octadecatrienoic acid; octadecanoic acid; methyoxy acetic acid; 2-methylphenyl ester and Benzene, 4-ethyl, 1,2-dimethyoxy. The compound 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol, has not been reported in *P. amarus* leaf crude extract before now. Possible isolation of individual compounds and the toxicity analysis could lead to discovery of valuable drugs for combating diseases.

(Keywords: aqueous ethanol extract, GC-MS analysis, 3, 7, 11, 15-tetramethyl-2-hexadecen`-1-ol, *Phyllanthus amarus)*

INTRODUCTION

Phyllanthus amarus is a small plant with numerous leaves on lateral branches on the stem that gives the plant the appearance of having pinnate leaves (Akobundu and Aguakwa, 1998). The stem is round, woody, at the base, horizontally branched, smooth and greenish (Igwe and Okwunodulu, 2014).

The leaf is 3.0-11.0 by 1.5 – 6.0mm, elliptic oblong to obviate, obtuse or minutely apiculate at apex, obtuse or slightly inequilateral at base (Thyagerajan ,et al., 1988; Kiran, 2011). The inflorescence is auxiliary and composed of one male flower and one female flower in each axil (Akobundu and Aguakwa, 1998) the flowers are greenish and rather small, up to 1.5mm in diameter.

The fruit is a round capsule, brownish, 1.5-2m wide and occurs in leaf axis on the lower side of the lateral branches. Each capsule consists of six small seeds (Kiran, 2011).

The active constituents of the parts of the plant include lignans, glycosides, flavonoids, alkaloids, ellagitannins, and phenylpropanoids found in the leaf, stem, and root of the plant. Sterols, flavonoid and lipids are also present in the plant (Akobundu and Aguakwa, 1998). The aim of study was to investigate the chemical component of aqueous-ethanol extract of *P. amarus* leaf using the Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

MATERIALS AND METHODS

The leaves of *P. amarus* were collected in the month of February to May, 2012 from the wild in Abraka, Ethiope East Local Government Area of Delta State. The plant was transported in a polythene bag to the Botany department of Delta State University Abraka, for identification.

Plant Extraction

The leaves of *P. amarus* was washed in distilled water and air dried in the laboratory. The dried plant was pulverized to powder using mortar and a pestle. 500g powdered plant was soaked with distilled water and ethanol in a mass ratio of 1:2. The modified method of Stantovic *et al.* (1993) and Novkovic *et al.* (2014) was adopted. The well homogenized mixture was put in plastic bag and sealed and then left to ferment at 37^{o}C for 48 hours.

The fermented plant material (100g) and the extracting solvent 50 vol.% ethanol, was added to the extracting vessel equipped with a stirrer.

The plant material was macerated for one hour at room temperature. The liquid extract was separated from the plant material by vacuum filtration. The liquid extracts obtained were mixed in a separating funnel. The filtration cake of the exhausted plant material was washed 3 times with ethanol (100mL). Washing solutions were added to the total macerate in the separating funnel. The liquid-liquid extract was performed four times with 1 x 1:2 and 3 x 1:4 within 20 minutes in separating funnels.

The extract was passed through a chromatographic column packed with silica gel (60-120 mesh) to purify it using chloroform alone as eluting solvent. The resulting extract was evaporated to dryness in a rotary evaporator and then subjected to GCMS analysis.

Gas Chromatography-Mass Spectrometry GC-MS Analysis

GC-MS analysis was carried out on a GC-MS-QP 2010 plus Shimadzu system and Gas Chromatography interfaced to a Mass Spectrometer (GC-MS) instrument. The injector temperature was 250.00^{0}C, Column Elite-1 fused silica; Capillary column (30m x 0.25mm ID x 11df composed of 100% d-methyl polysiloxane) for GC-MS detection, an electron ionization system with ionization energy of 70ev was used . The carrier gas was helium gas used at a constant flow rate 1ml/min. ion source temperature was 230^{o}C. The oven temperature was programmed from 80^{o}C to 200^{o}C at 10^{o}C/min and then held isothermally for10min and finally raised to 280^{o}C at holding time of 5min. Mass spectra were taken at 70ev, scan event 0.5s and scan range 40-600Da. Total GC running time was 25 minutes.

The components of the extract was identified by matching with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature (Mamza, et al.,2012; Sermakkani and Thangapadian, 2012).

Identification of Component

Interpretation on mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology (NISIT) having more than 62, 000 patterns. The mass spectrum of the known components was compared with spectrum of the unknown components, the name, molecular weight, and structure of the component of the test materials were ascertained.

RESULTS

The compounds present in the extracts obtained using 50%vol aqueous ethanol solution and 95% chloroform and trichloroethylene by Liquid-Liquid extraction after maceration and percolation as revealed by GCMS were ten active principles with their retention time (RT), molecular formula, molecular weight (mw), and peak area in percentage, molecular mass of the compounds are shown in Table 1 and Figures 1 and 2.

The GCMS experiment identified the following compounds Decane, 1,2,3, Trimethylbenzene, Isooctane, Ethyl tridecanoate, n-hexadecanoic aci, 3,7,11,15-tetramethyl-2-hexadecane-1-ol, 9,12,15-octadecatrienoic acid, octadecanoic acid, octadecanoic acid, methyxy acetic acid, 2-methylphenyl ester, Benezene, and 4-ethylethyl-1,2-dimethoxy.

Table 1: Phytochemicals Identified in the Leaf Extract of *Phyllanthus amarus* by GC-MS.

Chromatogram peak	Compound name	Molecular formula	molecular weight	Retention time (min)	Peak area %	Nature of compound
1	Decane	$C_{10}H_{22}$	142	4.44	1.61	alkane
2.	1,2,3,Trimethylbenzene	$C_{19}H1_2$	120	4.65	1.75	unknown
3.	Isooctane, (ethanyloxy)	$C_{11}H_{24}$	156	5.735	0.83	unknown
4.	ethyl tridecanoate	$C_{16}H_{30}O_2$	242	18.48	1.87	unknown
5.	n-hexadecanoic	$C_{16}H_{32}O_2$	256	18.65	13.64	fatty acid
6.	3,7,11,15-tetramethyl-2-hexadecane-1-ol	$C_{20}H_{40}O$	296	20.53	5.41	Terpene alcohol
7.	9,12,15-octadecatrienoic acid	$C_{18}H_{30}O_2$	273	21.43	12.73	Fatty acid
8.	octadecanoic acid	$C_{18}H_{36}O_2$	284	21.5	8.94	Stearic acid
9.	methoxy acetic acid, 2-methylphenyl ester	$C_{10}H_{14}O_2$	166	25.88	33.08	Unknown
10.	Benzene, 4-ethylethyl-1,2-dymethoxy	$C_{10}H_{12}O_3$	180	23.52	20.14	Unknown

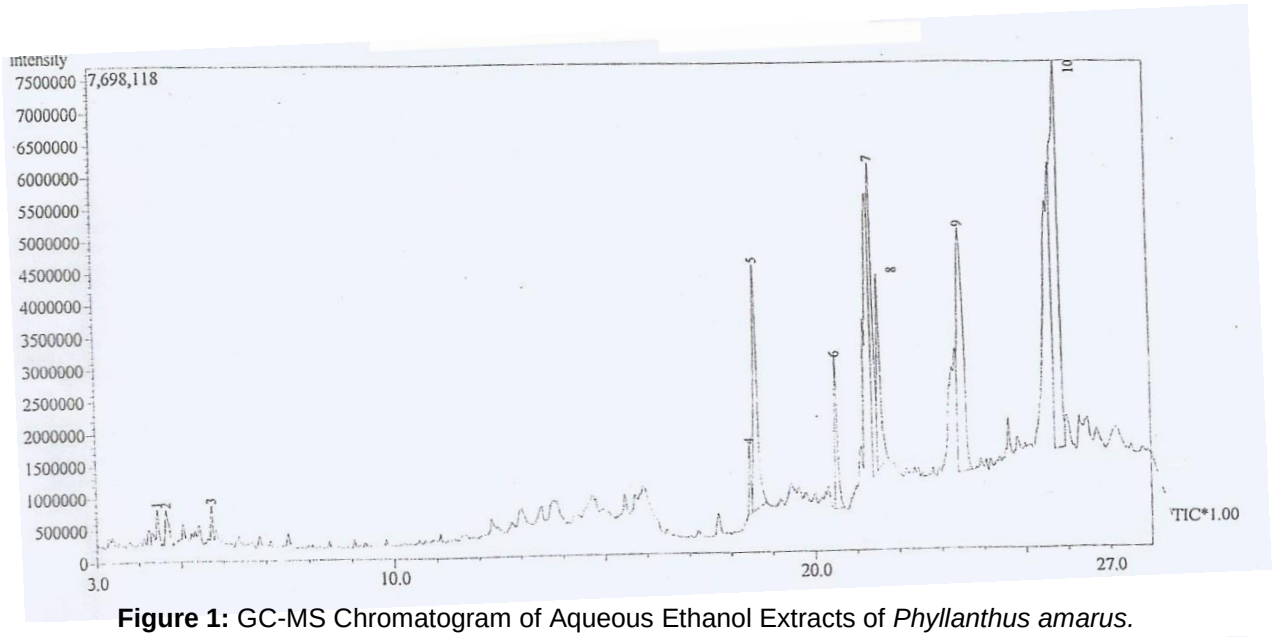


Figure 1: GC-MS Chromatogram of Aqueous Ethanol Extracts of *Phyllanthus amarus.*

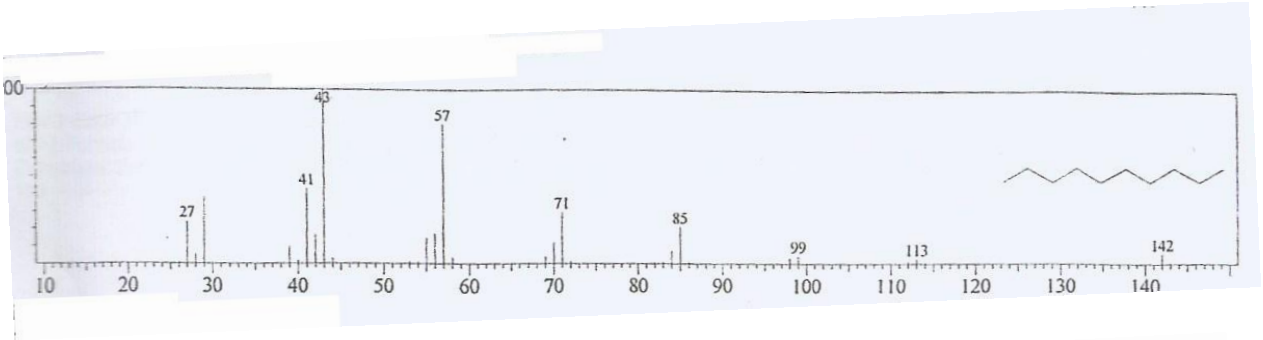


Figure 2a: Mass Spectra of Decane.

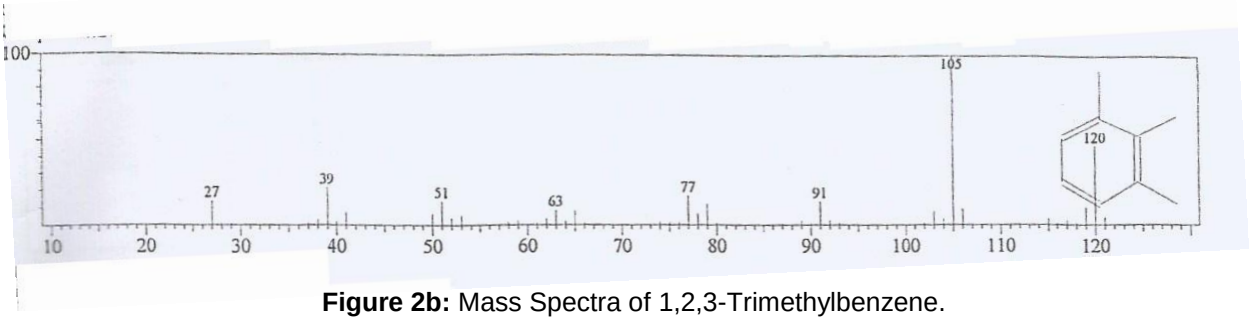


Figure 2b: Mass Spectra of 1,2,3-Trimethylbenzene.

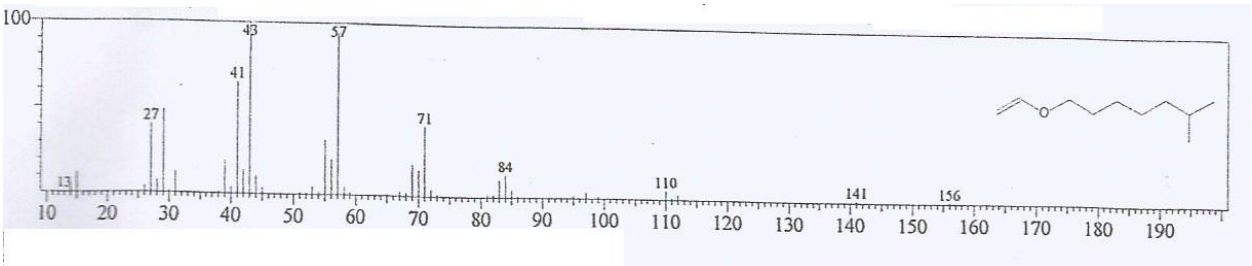


Figure 2c: Mass Spectra of Isooctane, (Ethenyloxy).

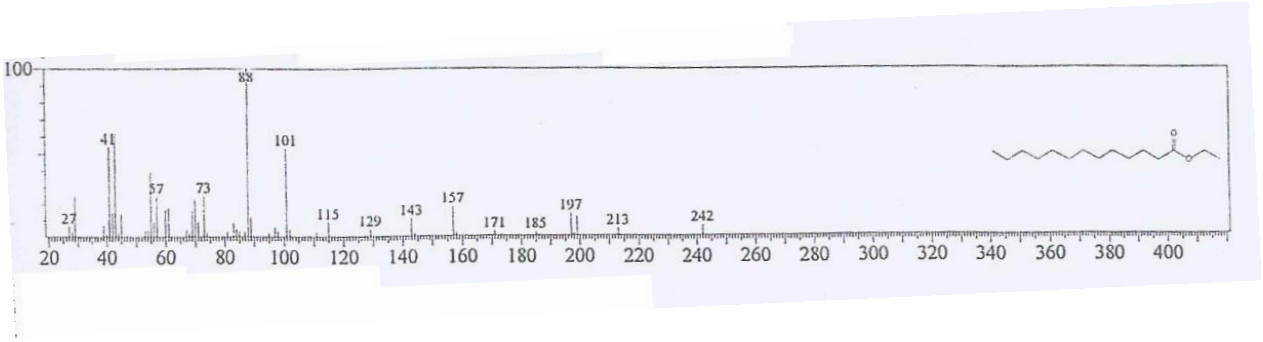


Figure 2d: Mass Spectra of Ethyltridecanoate.

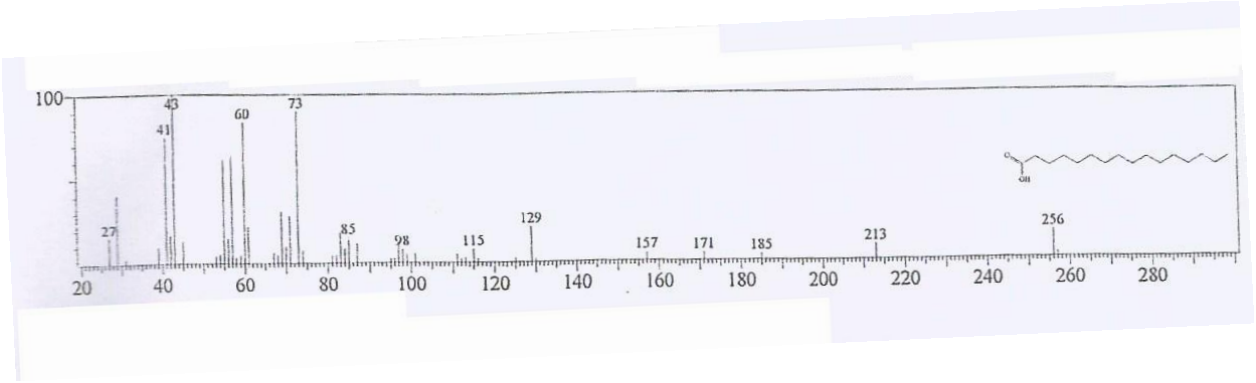


Figure 2e: Mass Spectra of n-Hexadecanoic Acid.

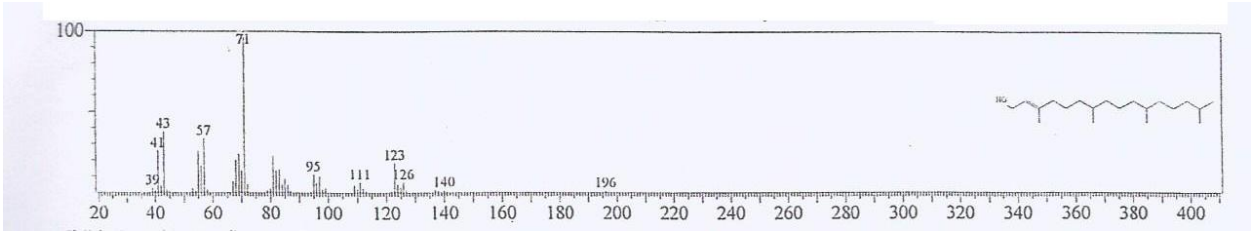


Figure 2f: Mass Spectra of 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol.

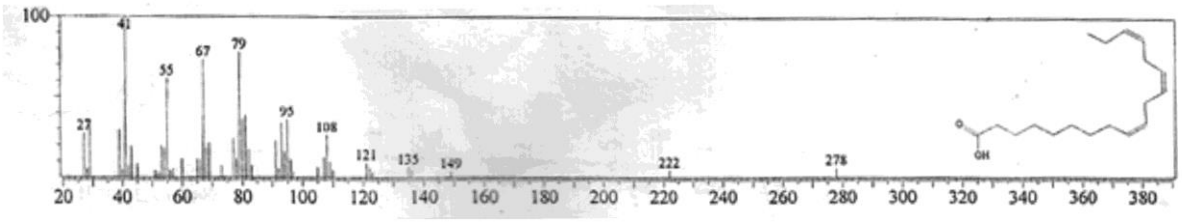


Figure 2g: Mass Spectra of 9,12,15-Octodecatrienoic Acid.

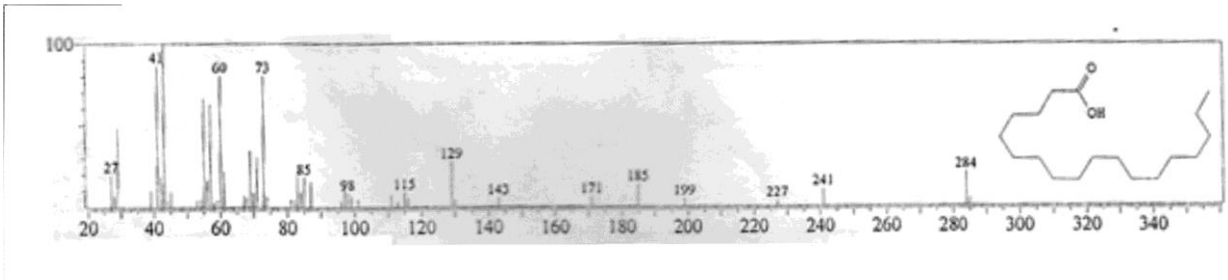


Figure 2h: Mass Spectra of Octadecanoic Acid.

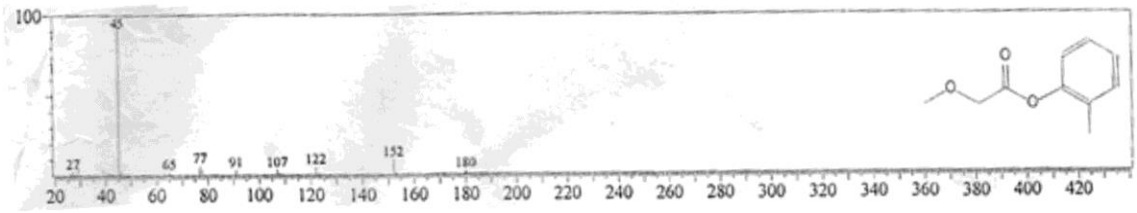


Figure 2i: Mass Spectra of Methylacetic Acid, 2-Methylphenylester.

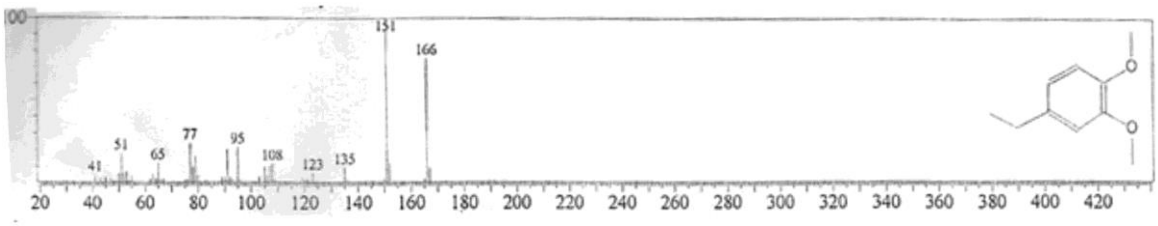


Figure 2j: Mass Spectra of Benzene, 4-Ethyl-1,2-Dimethoxy.

DISCUSSION

Medicinal plants contain components of medical value hence their use in traditional medicine. The phytochemical constituents which are responsible for their potency against disease have to be detected and possibly isolated for use as lead for synthesis of drugs. The need to discover and purify active components of plant to ameliorate and eradicate effects of agents of human disease and affliction in this era of immunodeficiency and emergence of disease difficult to tract cannot be overemphasized.

Gas-chromatography coupled with mass spectrometry (GC-MS) is one of the best technique to identify the constituents of volatile matter, long chain, branched chain hydrocarbon, esters (Sermakkani and Thangapadian, 2012). GC-MS also provide precise information in qualitative analysis from plants (Cong , 2007). The GC-MS analysis showed the presence of ten compounds.

N-Hexadecanoic acid is a fatty acid that has antioxidant, hypocholesterilemic, flavour and 5-alpha-reductase inhibitor properties.(see table and figure).

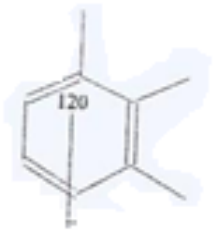
Anti-malaria activity has also been reported (Sermakkani and Thangapadian, 2012). The presence of n-hexadecanoic acid has been reported in *Phillanthusamarus* [2] and other crude extracts obtain form other plant species (Akpuaka, et al., 2013).

The mechanism of action of fatty acid have been observed to completely inhibit oxygen uptake or

stimulate uptake of amino acid into the cells in a dose dependent manner (Orhan, et al., ,2011; Shivakumar, et al., 2014). Additionally, fatty acid, intercalate in the phospholipid bilayer of microbes due to their lipophilicity thus increasing the permeability of the cell membrane, dissipation of the proton-motive force and leakages of organic ions leading to cell death (Lambert, 2001; Shivakumar, et al., 2014).

Decane also detected in the extract, is an alkane and possess anti-fungal and antibacterial activity (Gholamreza , 2012). 3, 7, 11, 15 – tetramethyl-2-hexadecen-1-ol is a terpene alcohol which possess antimicrobial activity. Terpene alcohols; include any of three isomeric alcohol which occur naturally in essential oils of certain plants and are used as solvent in perfumes, soaps and medicine (Unsaturated polylsoprenoids, n.d).

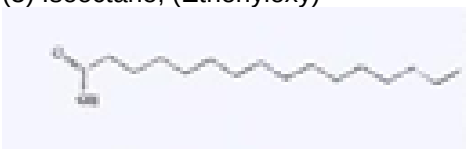
(1) Decane



(2) 1,2,3-Trimethylbenzene

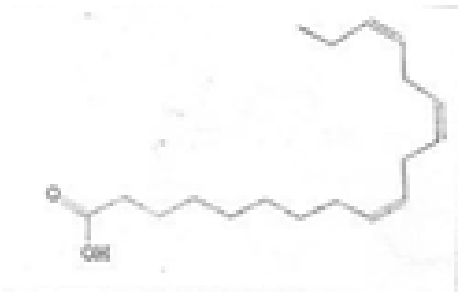
(3) Isooctane, (Ethenyloxy)

(4) Ethyltridecanoate

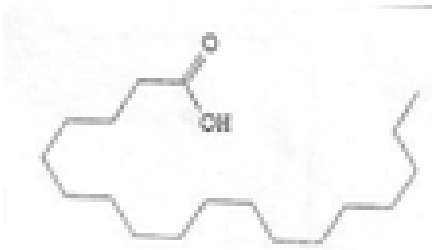


(5) n-Hexadecanoic Acid

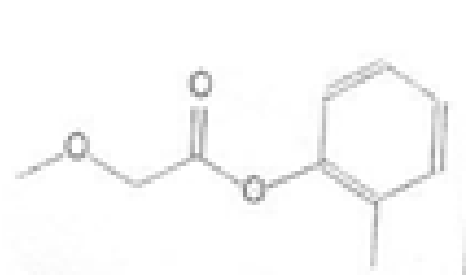
(6) 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol



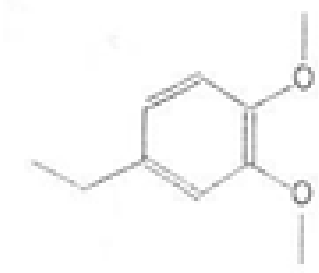
(7) 9,12,15-Octedecatrienoic Acid



(8) Octadecanoic Acid



(9) Methylacetic Acid, 2-Methylphenylester



(10) Benzene, 4-Ethyl-1,2-Dimethoxy

The presence of 3, 7, 11, 15 –tetramethyl-2-hexadecen-1-o1, have not been reported before in *Phyllathus amarus* Schum and Thonn. Leaf however, the presence have been reported in plant like *Mentha piperita*, *Cycas circinalis* L., and *Ionidium suffruticosum*. Ging and *Costus pictus* D. Don (Sathuran, 2012, Hossian ,2014; Kumar and Kumar, 2014).

Octadecanoic acid, stearic acid, 1, 2, 3 trimethybenzene, isooctane, (ethnyloxy) ethylphelonyl ester and Benzene, 4-ethyl-1, 2 –dimethoxy were also detected.

CONCLUSION

The GC-MS analysis of extracts obtained from aqueous ethanol solution purified in 95% chloroform yielded ten compounds including 3,7, 11, 15- tetramethyl-2-hexadecen-1-ol which have not been reported in *P. amarus* Schum and Thonn before now.

ACKNOWLEDGEMENT

The author acknowledges the technical assistance of Mr. Aghogho Eruemrejovwo of the Chemistry Department, Faculty of science Delta State University, Abraka.

REFERENCES

1. Akobundu, I.O. and C.W. Aguakwa. 1998. *A Handbook of West African Weeds 2nd Edition*. Int. Inst. of Tropical Agric.: Ibadan, Nigeria. 270-271.

2. Igwe, O.U. and F.U. Okwunodulu. 2014. "Investigation of Bioactive Phytochemical Compounds from the Chloroform Extract of the Leaves of *Phyllanthus amarus* by GC-MS. Technique". *International Joint of Chemistry and Phamaceutical Science*. 2(1):554-560.

3. Thyagerajan, S.P., S. Subramanians, T. Thrunaksunder, P.S. Venketeshwera, and B.S. Blumberg. 1988. "Effect of *Phyllathus amarus* in Chronic Carrier of Hepatitis B Virus". *Lancet*. 2:764.

4. Kiran, D., A. Rohilla, S. Rohilla, and M.U. Khan. 2011. "Pleiotropic Multifaceted Therapeutic Potentials of *Phyllanthus amarus*". *International Journal of Pharmaceutical and Biological Achievers*. 2(2):610-614.

5. Stankovic, M., S. Djordevic, S. Stankovic, and O. Stojanovic. 1983. "Enzymatic Transformation of Primary Glycosides from the *Digitalis lonate* Ehrh". *Hem. Ind*. 37:241-247.

6. Novkovic, V.M., L.P. Stanojevc, M.D. Cakic, V.B. Veljkovic, and M.Z. Stankovic. 2014. "Separation of Digoxin by Liquid-Liquid Extraction form Extraction of Foxglove Secondary Glycosides". *Hem. Ind.* 68(2):161-170.

7. Mamza, U.T, O.A. Sodips, and I.Z. Khan. 2012. "Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Bioactive Components of *Phyllanthus amarus* Leaves". *International Research Journal of Plant Science*. 3(10):208-215.

8. Sermakkani, M. and V. Thangapadian. 2012. "GC-MS Analysis of *Cassia kitatalica* Leaf Methanol Extract". *Asian Journal of Pharmaceutical and Clinical Research*. 5(2):91-94.

9. Cong, Z., Q. Meiling, S. Qinglong, Z. Shan, and F. Ruonong. 2007. "Analysis of the Volatile Compounds in *Ligusticum chuanxiong*Hort. using HS-SPME GC-MS". *Journal of Pharmaceutical and Biomedical Analysis*.44 (2):464-470.

10. Akpuaka, A., M.M. Ekwnchi, D.A. Deshale, and A. Dilder. 2013. "Biological Activities of Characterized Isolation of n-Hexane Extraction of *Azadirachta Indica* Ajuss (Neem) Leaves". *Nature and Science*. 11(5).

11. Orhan, I., B. Ozcelik, and B. Sener. 2011. "Evaluation of Antibacterial, Antifungal, Antiviral and Antioxidant Potentials of some Edible Oils and their Fatty Acids Profiles". *Turkish Journal of Biology*. 39(2):251-258.

12. Shivakumar, M.S., R. Srinivasan, and D. Natarajan. 2014. "*Phyllanthus wightianus Müll*. *Arg*. A Potential Source for Natural Antimicrobial Agents". *Biomed Research International Article*.

13. Lambert, N.J,W,. P.N. Skandemis, P.J. Coote, and G.J.C. Nychas. 2001. "A Study of the Minimum Inhibition Concentration and Mode of Action of Oregano Essential Oil Thymol and Carvacrol". *Journal of Applied Microbiology*. 91(3):453-462.

14. Gholamreza, A., J. Mohammed, and S. Ehsan. 2012. "Antimicrobial Activity and Chemical Composition of Essential Oil from the Seed of *Artemisia aucheri* Boss". *J. Nat pharm. Prod.* 7(1):11-15.

15. "Unsaturated Polylsoprenoids (prenols or polyprenols) (n.d) available at WWW.

cyberlipid.orgsimple.simpoo3html. isopen . Accessed 1st May 2015.

16. Hossian, M.A., A.L. Aarmed, A.S. Orimi, A.M. Weli, Q. Al-Riyami, Q, Al-Sabahi, and J.N. Al-Sabahi. 2014. “Studies on Free-Radical Sovereignty Activity and Identification of Certain Ingredients of Different Plant Crude Extracts of *Mentha pipersitic* Collected form sur Sultanate of Oman”. *Journal of Coastal Life Medicine*. 2(10): 2805-810.

17. Sathuran, M, A. Vignesh, R. Thongan P. Palani, R. Rengasamy, and K. Murugesan. 2012. “In vitro Antioxidant and Anticancer Potential of Bark of *Costrus pictus* D.Don”. *Asian Pacific Journal of Tropical Biomedicine*. 20(2):5741-5749.

18. Kumar, S.B. and J.V. Kumar. 2014. “GCMS Analysis Bioacetene Constituent for C*ycas circinalis* Land Ionidium Suffracticussion Ging”. *Int J. Pharm Sci Rev, Res.* 28(2):197-201.

SUGGESTED CITATION

Adomi, P.O. 2017. “Investigation of Bioactive Phytochemical Compounds from Aqueous Ethanol Extracts of Leaves of *Phyllanthus amarus* Schum and Thonn by Gas Chromatography-Mass Spectrometry (GC-MS)”. *Pacific Journal of Science and Technology*. 18(2):284-291.