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Chemical Constituents of Methanol Leaf Extract of *Aspilia africana* C.D. Adams by GC MS

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Abstract: *The therapeutic value of Aspilia africana (Asteraceace), commonly known as ''hemorrhage plant'' has been recognized as a component of traditional medication for the treatment of various human ailments. In Nigeria, a large number of medicinal plants have been used for many centuries for treating various diseases. Medicinal plants have been as remedies for human diseases because of its chemical contents of therapeutic value. Most traditional medicines are developed from nature. Thus plants remain a major source of medicinal compounds A. africana, which is widespread in Africa, is used in traditional medicine to stop bleeding from wounds, clean the surfaces of sores. The current study was undertaken to evaluate the phytochemical constituents of Methanol leaf extract of Aspilia Africana C.D. Adams by Gas Chromatography –Mass Spectrometry. The GC-MS analysis revealed the presence of 34 bioactive compounds such as flavonoids, alcohols, aldehydes, aromatic compounds, fatty acid methyl esters, terpenoids, phenolics, and steroids that can be postulated for antibacterial activity. The prevailing compounds are: Betulin (24.912%), Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienylester (8.087%), 2,5,5,8a-Tetramethyl-4-methylene-6,7,8, 8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide (7.074 %), 2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol (7.848%), Cholestan-3-ol, 2-methylene-, (3,5)- (4.446%), 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one (3.579 %), 3-Hydroxy-α-ionene (3.361 %), n-Hexadecanoic acid (3.253 %), 3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo [4.1.0] heptan-2-one (2.219 %),.*

Keywords: *GC-MS, photochemical constituent, methanol extract, anti-microbial, Aspilia africana*

1. Introduction

In developing countries, communities rely heavily on traditional herbal medicines in order to meet their primary health care needs. In many industrialized countries herbal medicines are gaining popularity as alternative and complimentary therapies. Some of the plants are used as food or medicine. These plants exhibit a wide range of biological and pharmacological activities such as anti-cancer, anti inflammatory, diuretic, oxytocic, laxative, antispasmodic, antihypertensive, anti-diabetic, and anti-microbial functions.[1]

Their role is twofold in the development of medicines and served as a natural blue print for the development of new drugs [2-3] Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as communicable diseases [4]. Medicinal plants represent a rich source of antimicrobial agents [2] [5].According to World Health Organization (WHO) more than 80 % of the world's population relies on traditional medicine for their primary healthcare needs. Medical plants contain large varieties of chemical substances, which possess important therapeutic properties that could be utilized in the treatment of human diseases. The potential of higher plants as source of new drugs is still largely unexplored. Among the estimated 250,000 –500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller [6].

Aspilia africana is one of such medicinal plants, which are fast gaining recognition. It is a semi woody herb occurring throughout the regions of the savannah and tropical Africa on wastelands [7]. It is known by various names among the Nigerian populace (*Orangila* in Igbo, *Tozalin* in Hausa, *Yunyun* in Yoruba and "Edemedong" in Efik). The plant has been reported in literature to possess antimicrobial [8], haemostatic [9], anti-inflammatory [10] and anti-fertility [11] activity. The wound healing and anti-ulcer activity of *Aspilia africana* n-hexane and methanolic extracts have also been reported [12-13][10].

In South-eastern Nigeria, all the parts of *Aspilia Africana* are widely used in traditional medicine, the leaves are claimed to be effective in the treatment of a number of health conditions. The infusion from the crushed leaves is applied on the wound, throughout many African communities, to stop bleeding and for cleaning the surfaces of sores. Historically, *Aspilia africana* was used in Mbaise and most Igbo speaking parts of South eastern Nigeria to prevent conception, suggesting potential contraceptive and anti-fertility properties[14]

Taxonomical classification of *Aspilia Africana* L

Kingdom	**:**	Plantae
Phylum	:	Spermatophyta
Subphylum	:	Angiospermae
Order	**:**	Asterales
Family	:	Asteraceae
Genus	:	Aspilia
Species	:	*Aspilia africana*

Fig1. *Aspilia africana leaves and flowers*

2. Materials and Methods

2.1. Collection and Authentication of Plant Material

Wild growing *Aspilia africana* plants fresh leaves were collected from Abia state university, Uturu Nigeria. farmland on 26th July, 2018 and identified by Dr E.O Emmanuel, a plant taxonomist. Voucher specimens [1769] were deposited at the herbarium section of Department of Plant Science and Biotechnology, Abia State University.

2.2. Preparation of Leaf Extracts

The Fresh harvested plant leaves were washed with tap water and rinsed with sterile distilled water. The fresh plant leaves sample was air dried on the laboratory bench for ten days at temperature below 30 ^{0}C to avoid decomposition of thermo labile compounds. The sample was milled using and electric blender to coarse powder and powdered sample was kept in a clean closed container pending extraction. 50 g of pulverized leaf material was mixed with 250 ml of solvent (95 % methanol) and kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper The extract was concentrated under reduced pressure using Digital Heidolph Rotary evaporator (4000 series) and the supernatant plant extract was decanted after complete removal of the solvent

2.3. GC –MS Analysis

2.3.1. Preparation of Extract

Two micro liters of the methanol leaf extract was employed in GC–MS analysis, for the identification of compounds.

2.3.2. GC – MS Analysis Conditions

The GC-MS analysis of the methanol leaf extrac of *Aspilia africana* was carried out using a HP 7890 GC instrument integrated with an Agilent 5975C MSD mass spectrometer (Aligent, Santa Clara, CA, USA). The capillary column was an Agilent HP-5MS (30.m x 0.25mm i.d. x 0.25 NM film thickness), helium (Purity > 99.999 %) was used as the carrier gas, and the flow rate was 1 ML/min. The injector temperature was 2500c, and the injection mode was splitless. The G.C oven temperature was held at 50 ^{0}C for 5min, which was increased to 210 0at a rate of 30C/min, maintained at 210 ^{0}Cfor 3 min, and finally increased to 230 ^{0}C at 15^{0}C/min. The mass spectrometer conditions were as follow: [12:13:14] ionization energy, 70 Ev; ion Source temperature, 230 ^{0}C; quadrupole temperature, 150 ^{0}C; quadrupole mass spectrometer scan range 30 – 500 atomic mass units (amu); solvent delay time 2.8 min.

2.4. Components Identification

The components of the chloroform extract of *Ageratum conyzoides* was identified by matching the peaks with computer Wiley Ms. libraries and confirmed by comparing mass spectra of the peaks and those from literature (Ahuchaogu *et al.*, 2018.)

3. RESULTS AND DISCUSSION

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas liquid chromatography and mass spectrometry to separate different substances within a test sample based on their retention time [16]. In recent times, GC-MS has become well recognized as a key technological platform for secondary metabolite profiling in both plant and non-plant materials [17]

The bioactive components present in the leaf methanol extract of the plant were identified by GC–MS analysis. The gas chromatogram shows the relative concentrations of various compounds eluted as a function of their retention time (Fig. 2). Identification of the compounds was done by comparing their mass spectra and retention indices with those given in the literature and with the authentic samples. The methanol extract of the leaves of Aspilia africana on GC-MS analysis showed thirty four. The 34 compounds were identified namely; 1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl)-(1.173%), Indolizine (1.093%), Falcarinol (0.955%), Caryophylla-4(12),8(13)-dien-5α-ol (0.861 %), 3-Hydroxy-α-ionene (2.194%), 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one (3.579 %), 1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one (0.816 %), 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,(R)-(1.574%),2,5-Octadecadiynoic acid, methyl ester (1.320%), Panaxydol (1.391 %), Cyclododecanone, 2-methylene-(1.614 %), 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-(2.101%), 3-Hydroxy-α-ionene (3.361%), Cholestan-3-ol, 2-methylene-,(3,5)-(4.446%), 3,4,4-Trimet hyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one(2.219%),2-Methyl-4-(2,6,6-trime thylcyclohex-1-enyl)but-2-en-1-ol (1.332%), [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester (0.79%), Cholestan-3-ol, 2-methylene-, (3,5)- (2.400 %),7-Hydroxyfarnesen (2.518%},2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-ylhydroperoxide (7.074 %),1-Oxaspiro[2.5]octa ne,5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-,(2.564%), 2,6,8-Trimethylbicyclo [4.2.0]oct-2-ene-1,8-diol (7.848 %), 17-Octadecynoic acid (1.367 %), 2,5-Octadecadiynoic acid, methylester (1.355%), Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester (8.087 %),9,12-Octadecad ienoyl chloride, (Z,Z)-(1.111), Cyclopropanebutanoic acid,2-[[2-[[2-[(2-pentylcyclopropyl)methyl] cyclopropyl] methyl]cyclopropyl]methyl]-, methyl ester(1.648%), n-Hexadecanoic acid (3.253%), 8, 11,14-Eicosatrienoic acid, (Z,Z,Z)- (0.816 %), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol(1.488%), 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-(0.879%), Tributyl acetylcitrate (0.888%),1H-Cycloprop [e]azule n-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1aα,4aα,7β, 7aβ,7bα)]-(0.973%), Betulin (24.912%).

Among the identified compounds n-, hexadecanoic acid is reported to have anti-inflammatory , Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic,5-Alpha reductase inhibitor , potent mosquito larvicide [18], Betulin:antibacterial, antioxidant, antitumor, cancer-preventive, immunostimulant, chemopreventive, lipoxygenase-inhibitor, pesticide [19], Indolizine; the biological activities include antimicrobial activity, antioxidant activity, anti-inflammatory activity , anticonvulsant activity, enzymes inhibition, activity and activity as calcium entry blocker [20]. Panaxynol, antitumor [21]. Falcarinol: antimicrobial properties [22-23] The presence of various bioactive compounds justifies the use of the whole flower for various ailments by traditional practitioners

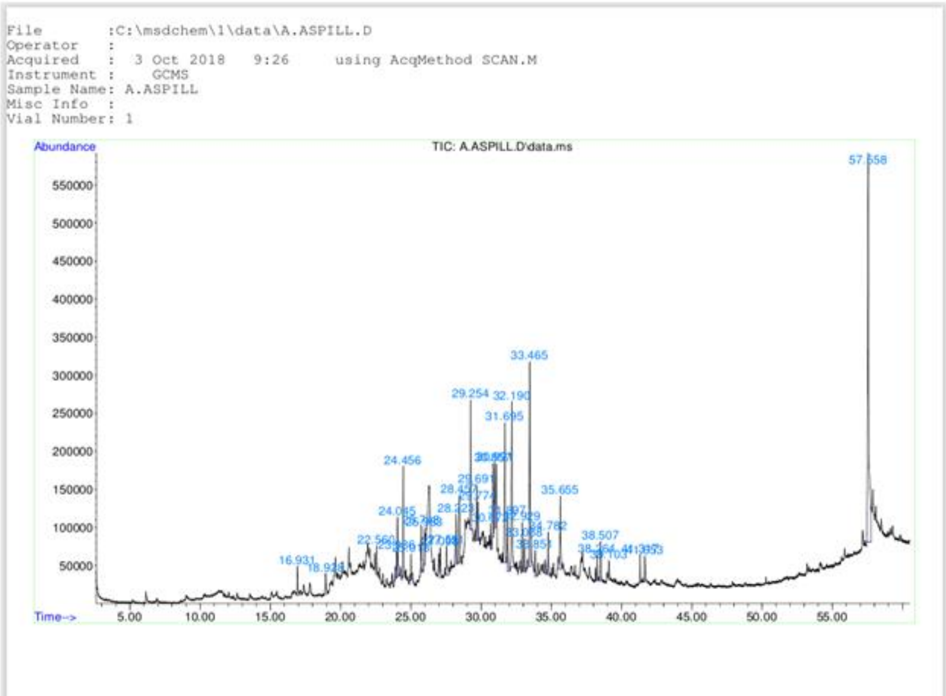
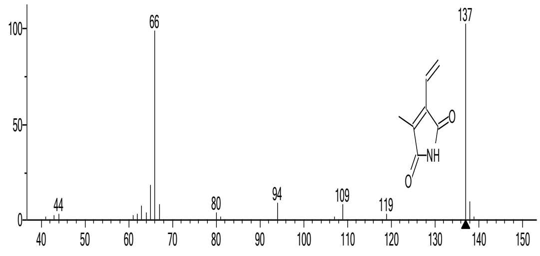


Fig2. *GC-MS Chromatogram of Methanol extract of the leaves of Aspilia Africana*

Table1. *Components detected in the plant of methanol extract of Aspilia africana MW: Molecular Weight, RT: Retention Time*

SN	RT	COMPONENT	FORMULA	MW	%
1	16.931	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl	$C_7H_7NO_2$	137	1.173
2	18.928	Indolizine	C_8H_7N	117	1.093
3	22.560	Falcarinol	$C_{17}H_{24}O$	244	0.955
4	23.986	Caryophylla-4(12),8(13)-dien-5α-ol	$C_{15}H_{24}O$	220	0.861
5	24.045	3-Hydroxy-α-ionene	$C_{13}H_{20}O_2$	208	2.194
6	24.456	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	$C_{13}H_{20}O$	192	3.579
7	25.018	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	0.816
8	25.748	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	$C_{11}H_{16}O_2$	180	1.574
9	25.983	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	1.320
10	27.098	Panaxydol	$C_{17}H_{24}O_2$	260	1.391
11	27.551	Cyclododecanone, 2-methylene-	$C_{13}H_{22}O$	194	1.614
12	28.223	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	2.101
13	28.457	3-Hydroxy-α-ionene	$C_{13}H_{20}O_2$	208	3.361
14	29.254	Cholestan-3-ol, 2-methylene-, (3,5)-	$C_{28}H_{48}O$	400	4.446
15	29.691	3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one	$C_{14}H_{20}O_2$	220	2.219
16	29.774	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	$C_{14}H_{24}O$	208	1.332
17	30.672	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$	322	0.79%
18	30.857	Cholestan-3-ol, 2-methylene-, (3,5)-	$C_{28}H_{48}O$	400	2.400
19	30.991	7-Hydroxyfarnesen	$C_{15}H_{24}O$	220	2.518
20	31.695	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	$C_{14}H_{22}O_3$	238	7.074

21	31.897	1-Oxaspiro[2.5]octane, 5,5-dimethyl-4-(3-methyl-1,3-butadienyl)-	$C_{14}H_{22}O$	206	2.564
22	32.190	2,6,8-Trimethylbicyclo[4.2.0]oct-2-ene-1,8-diol	$C_{11}H_{18}O_2$	182	7.848
23	32.929	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	1.367
24	33.088	2,5-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	1.355
25	33.465	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	$C_{17}H_{30}O_4$	298	8.087
26	33.851	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	1.111
27	34.782	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$	374	1.648
28	35.655	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	3.253
29	38.264	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.816
30	38.507	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	1.488
31	39.103	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	0.879
32	41.317	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	402	0.888
33	41.653	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-	$C_{15}H_{24}O$	220	0.973
34	57.558	Betulin	$C_{30}H_{50}O_2$	442	24.912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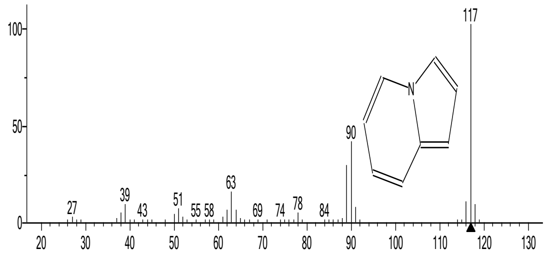
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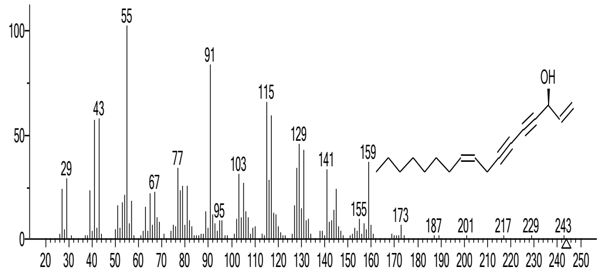
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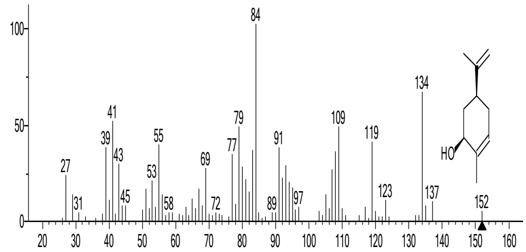
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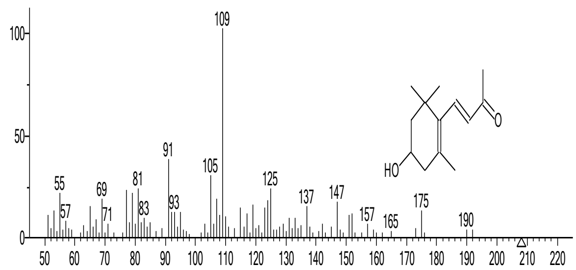
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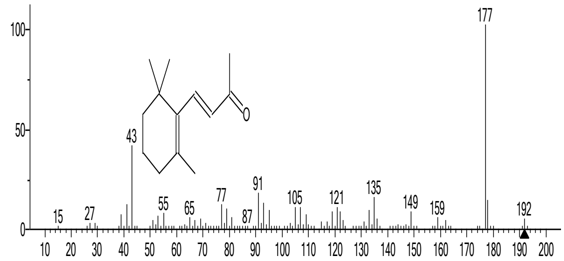
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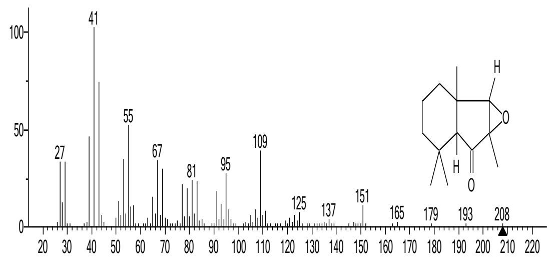
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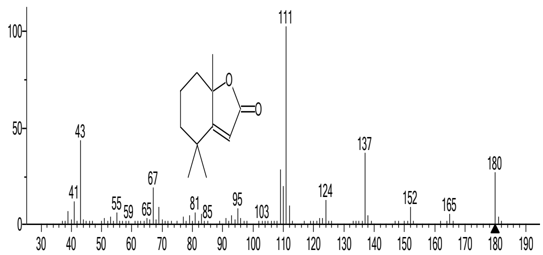
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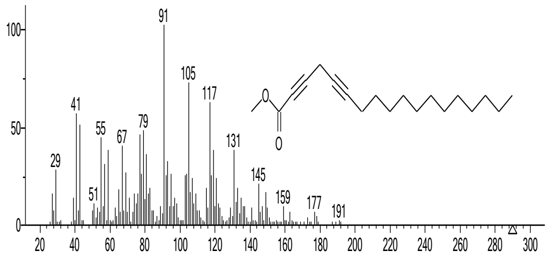

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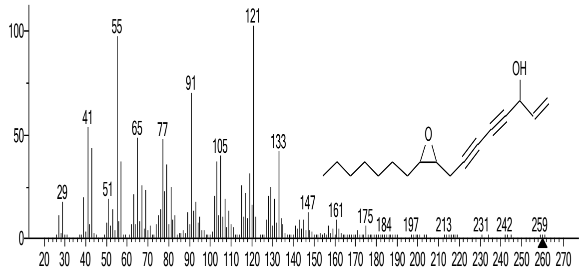

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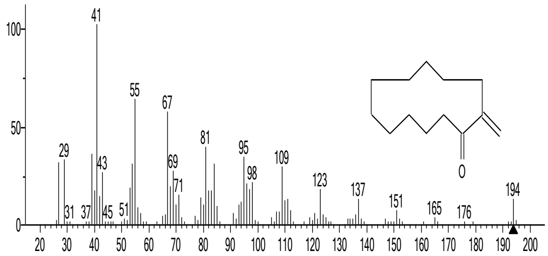

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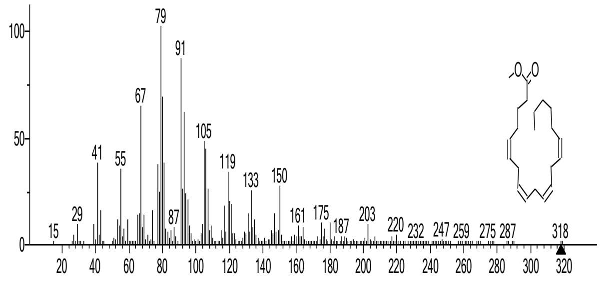

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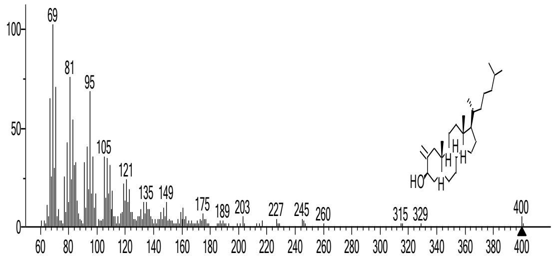

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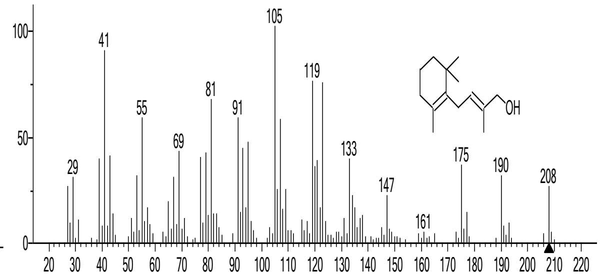

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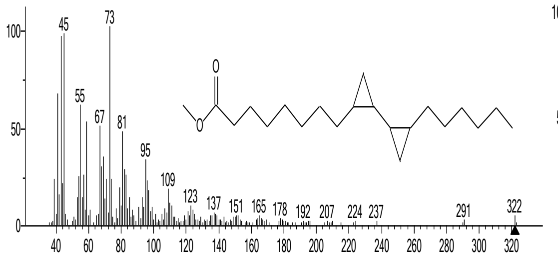

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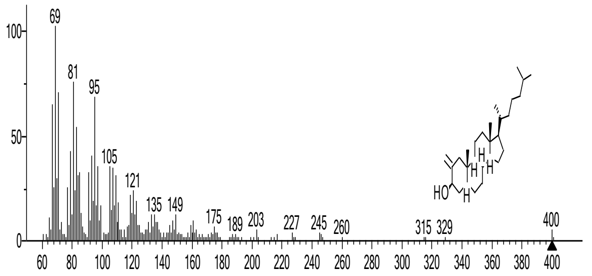

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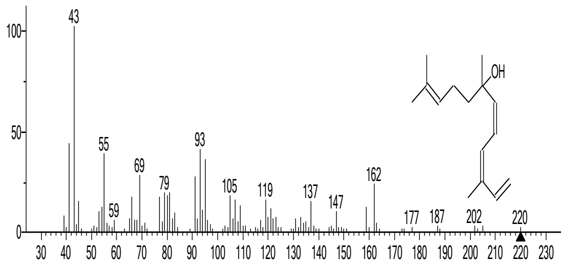

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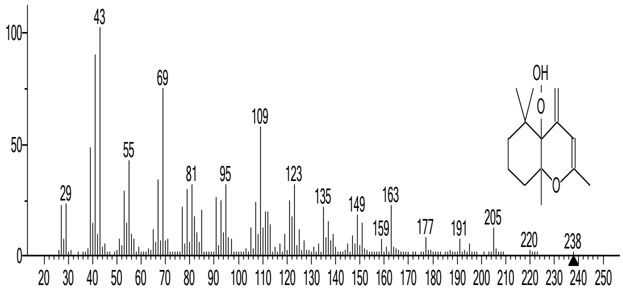

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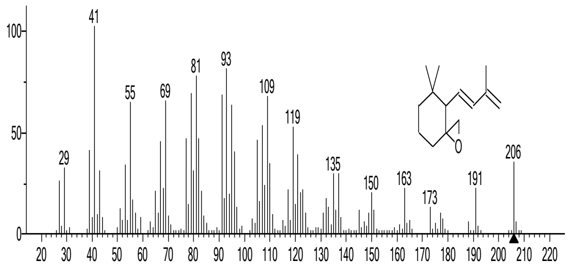

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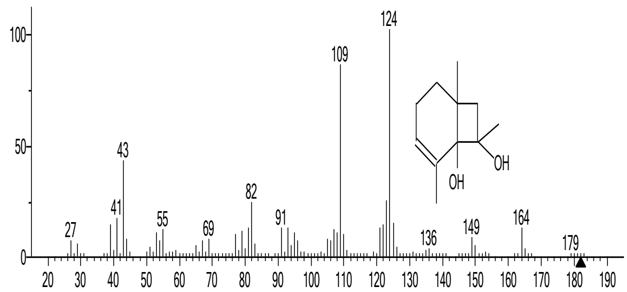

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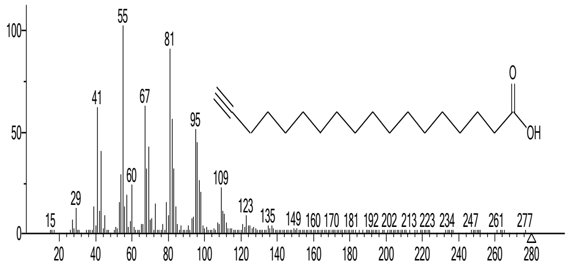
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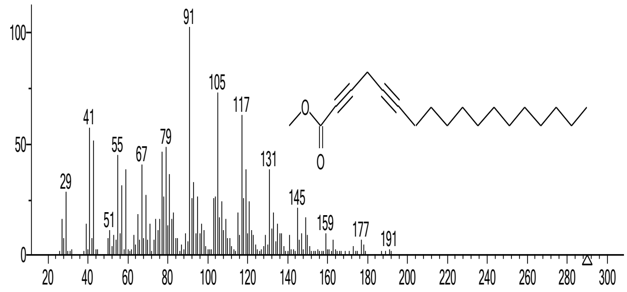
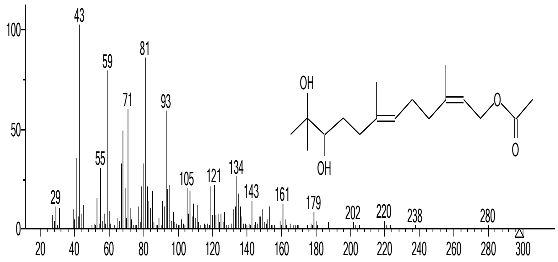
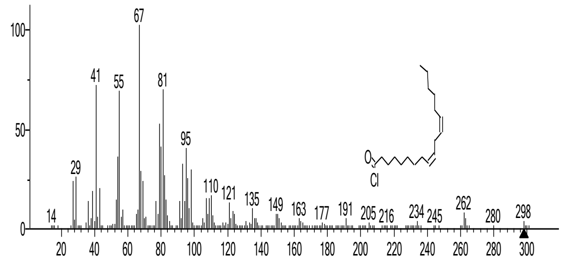
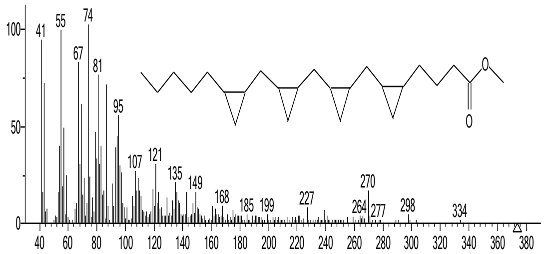
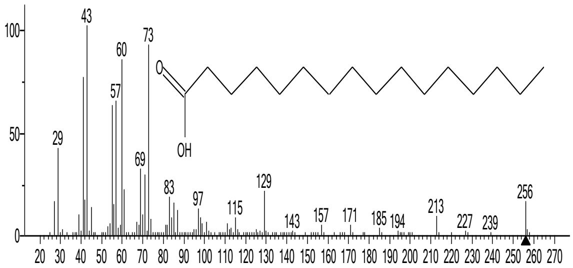
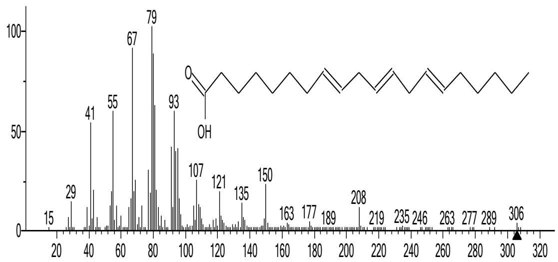

(mainlib) 2,5-Octadecadiynoic acid, methyl ester

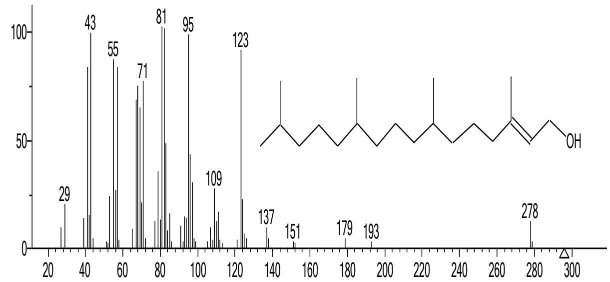

(mainlib) Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester

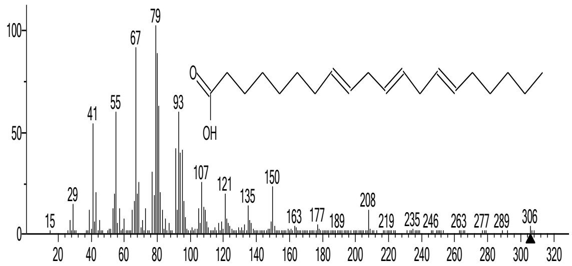

(mainlib) 9,12-Octadecadienoyl chloride, (Z,Z)-

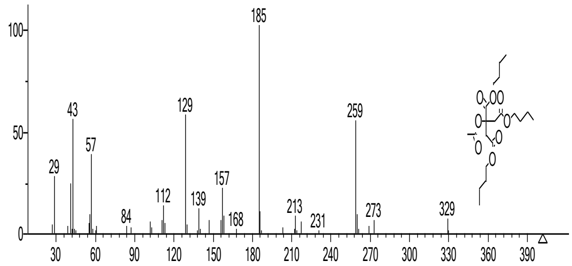

(mainlib) Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester

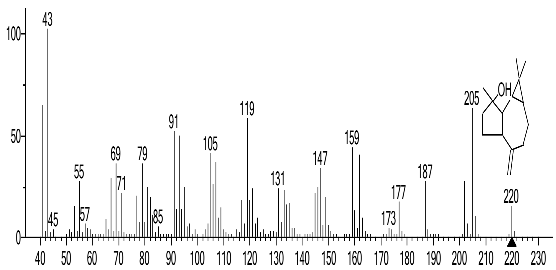

(mainlib) n-Hexadecanoic acid

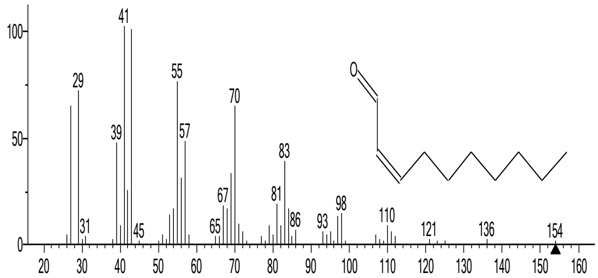

(mainlib) 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-


(mainlib) 3,7,11,15-Tetramethyl-2-hexadecen-1-ol


(mainlib) 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-


(mainlib) Tributyl acetylcitrate


(mainlib) 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aα,4aα,7β,7aβ,7bα)]-


(mainlib) 2-Decenal, (Z)-

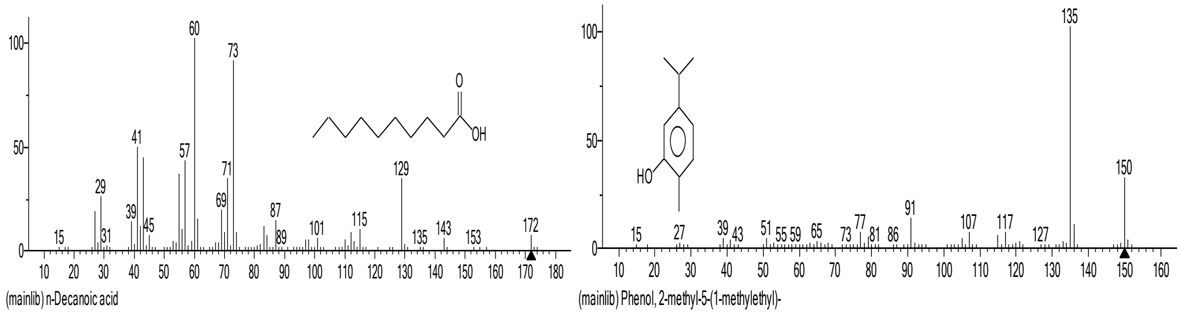


Fig3. *GC-MS Chromatogram of the extract*

4. CONCLUSION

In the present study thirty four chemical constituents have been identified from Methanolic extract of the plant leaves of *Aspilia africana* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds on the methanol leaf extract can be attributed to its widely use in folklore medicines, especially in arresting wound bleeding, inhibiting the growth of microbial wound contaminants and accelerating wound healing which suggest good potentials for use in wound care. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Aspilia africana* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance

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