Phytochemical Profiling and GC-MS Characterization of Vernonia amygdalina Leaf Extract

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Abstract

Vernonia amygdalina, a tropical plant in Africa is consumed as vegetable and possess high medicinal value. Traditionally it is used in the treatment of various ailments. The present investigation was to identify the various phytoconstituents present in this plant by qualitative phytochemical analysis and by using gas chromatography -mass spectrometry. The leaves of *v. amygdalina* were extracted with methanol at room temperature for 72 hours. The concentrated extract was subjected to GC-MS analysis to detect the phytoconstituents. Totally 79 compounds were identified. This preliminary study gives an idea to isolate the major bioactive compound present in the plant which might be responsible for its therapeutic efficiency.

Keywords: *Vernonia amygdalina*, GC-MS, Bioactive compound. Phytoconstituents, Bitter leaf

Introduction

Medicinal plants have been used as a source of drugs by millions of people around the world for several years. Yet, demand for many wild plant species is still increasing with human needs and as these quickly heal the symptoms of illnesses. One among such tropical plant is Vernonia amygdalina. V. amygdalina, is a potent medicinal plant found growing predominantly in tropical Africa. Due to its bitter taste and flavor, it is commonly known as bitter leaf [1]. The plant is widely used as an active anticancer, antibacterial, antimalarial [2-5] and antiparastic agent. [6, 7]. The leaves of V. amygdalina are consumed as green leafy vegetable in regular diet as they serve as excellent appetizer and extract from the leaves aid digestion. [8]. The leaves are also used for breast milk enhancement in nursing mothers [9], treatment of fever in poultry [10] and helminthosis in livestock [11], The roots and twigs of the plant are given for treating wounds [12,13]. It was also reported that V. amygdalina has hypoglycaemic activity as their results reported a close-dependent reduction in fasting blood sugar level in alloxan-induced diabetic rats after treatment with different concentrations of the aqueous leaf extracts [14]. Hence the main purpose of the present study was to identify the phytocompounds in the methanolic leaf extract of *V. amygdalina* by qualitative screening of phytochemicals and to identify each specific compound with their concentrations by Gas Chromatography – Mass Spectrum (GC-MS) analysis [15].

Materials And Methods

Collection Of Plant Material

Fresh leaves of bitter leaf (V. amygdalina) were collected from Afikpo Ebonyi State, Nigeria and was authenticated by Dr. Kalavathy, Botanist. The leaves were washed thoroughly with water to remove dust and dried under the shade at room temperature for seven days.

Plant Extract Preparation

The shade dried leaves of *V. amygdalina* were ground using kitchen blender to obtain the course powder and kept in an air tight container till further use. 500 g of the powdered plant material was defatted with petroleum ether (60-80°C) using a soxhlet extractor and then it was successively extracted with methanol for 72 h. The extract obtained was filtered and concentrated under reduced pressure in a rotary evaporator at 180 rpm. The crude extracts thus obtained were used for the phytochemical analysis and GC-MS analysis to find the bioactive components present in plant leaves.

Phytochemical Screening Of Leaves Extract

The leaf extract of *V. amygdalina* was screened for various phytochemical constituents using standard methods [9-11] as described in Table 1.

Gas Chromatography-Mass Spectrometry Analysis

The crude bioactive methanolic extract of V. amygdalina leaves was subjected to GC–MS analysis. The conditions used for the GC–MS analysis are presented in Table 2. The spectrum of the crude component was compared with the spectrum of the known components in the National Institute Standard and Technology (NIST) library. The molecular weight, name, chemical structure and molecular formula of the components of the test material were ascertained.

Results And Discussion

The yield of the plant extract was found to be 9.8%.

Phytochemical Screening

The crude extract of *V.amygdalina* obtained by soxhlet extraction was used for the phytochemical screening and GC-MS analysis to identify the phytoconstituents and bioactive components present in plant leaves of *V.amygdalina*. The results of phytochemical analysis (table 3) showed the presence of alkaloids, Cardiac Glycosides, Flavonoids, Saponin, Steroid, Carbohydrates, presence of oil and Fat, phenolic compounds, tannins and terpenoids. Phlobatannin were absent in the leaf extract.

Gas Chromatography – Mass Spectrometry

One of the most precise method to identify secondary metabolites present in any plant extract is Gas chromatography – mass spectrometry. The crude methanol extract of *V.amygdalina* was subjected to GC-MS to detect various compounds with the help of NIST library.. The bioactive compounds present in methanol extract obtained from *V.amygdalina* leaves are shown in Table 4. Totally 79 compounds were identified. The GC chromatograms of the extract presented in Figure 1 shows the retention time in the column and the detected peaks which correspond to the bioactive compounds present in the extract. Their identification and characterization were based on their elution order in a HP-5MS column. The elution time, molecular formula and the amount of these bioactive compounds are also given. The major constituents identified in the extract were dimethyl propanedioate, dimethyl pentanedioate, cis- 9,cis-12-octadecadienoic acid, pentanedioic acid and many other compounds were identified as low level.

Conclusion

From this preliminary study it is observed that the presence of various phytocompounds detected after GC-MS analysis justifies the use of *V.amygdalina* for treatment of various ailments by traditional practitioner. Earlier reports and the present study confirm the identified phytoconstituents to be highly bioactive. Therefore, the anticancer, antibacterial, antimalarial and antiparasitic activity of the plant might be due to the presence of above shown metabolites. Hence, *V.amygdalina* plant could be recommended as a plant of phytopharmaceutical importance and its highest therapeutic efficacy can be explored by pharmaceutical companies in order to develop safe drugs for various ailments. We also have put effort to exploit the biomedical applications of this plant to their full utilization by purifying and characterizing the principle active constituent responsible for its therapeutic efficiency.

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Table 1: Phytochemical tests for plant extracts

Tannins	The extract was treated with 10 % alcoholic $FeCl_3$	The blue-black or green color indicates the presence of tannins
Flavonoids	The extract was treated with magnesium ribbon and concentrated Hydrochloric acid	Orange red colour indicates the presence of flavonoids
Terpenoids	The extract was dissolved in 2 ml chloroform and 3 ml of concentrated sulphuric acid.	Formation of reddish brown color of the interface indicates the presence of terpenoids
Saponins	The extract was mixed with 2 ml of distilled water and was allowed to stand for 10 minutes	Appearance of stable froth indicates the presence of saponins.
Steroids	The extract was dissolved in 2 ml of chloroform in a dry test tube. To it 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid are added	Appearance of red, then blue and finally bluish green in colour indicates the presence of steroids.
Phlobatannins	To 2 ml of the extract few drops of dilute Hcl was added	Appearance of red precipitate indicates the presence of phlobatannins.
Carbohydrates	The extract was dissolved in 5 ml distilled water and filtered. 1 ml of filtrate solution is treated with Benedict's reagent and heated gently.	Formation of rediish precipitate indicates the presence of reducing sugars
Cardiac Glycosides	The extract was treated with 1 ml of $FeCl_3$ reagent and 99ml of glacial acetic acid. To this solution, a few drops of concentrated H_2SO_4 was added.	The presence of greenish blue color within few minutes indicates the presence of deoxy sugar of cardiac glycosides

Alkaloid	The extract was dissolved in 2N HCl acid and filtered. To the filtrate Wagner's reagent (iodine in potassium iodide) was added	Formation of brown/reddish precipitate indicates the presence of alkaloids
Test for proteins & amino acids	To the extract 0.25 % w/v of ninhydrin reagent was added and boiled for few minutes	Formation of blue violet color indicates the presence of amino acids or protein.
Fixed oil and fat	Small quantity of the extract was pressed between two filter papers	Oil stain on the paper indicates the presence of fixed oil
Tannins	The extract was kept on filter paper and sprayed with Ferric chloride –potassium ferricyanide reagent	Appearance of blue color indicates the presence of tannin.

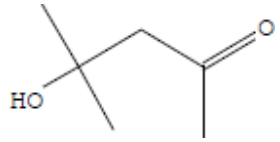
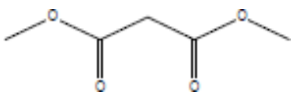
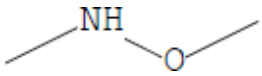
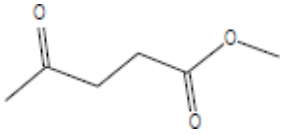
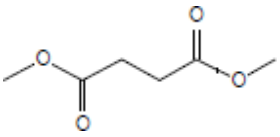
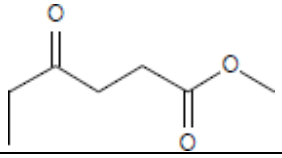
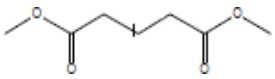
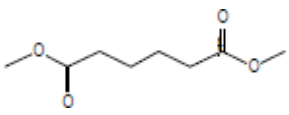
Table 2: Gc-Ms Conditions

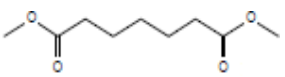
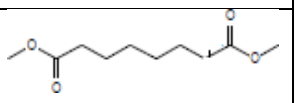
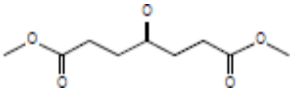
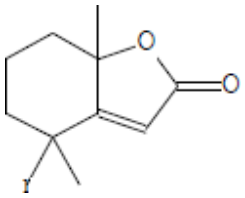
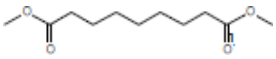
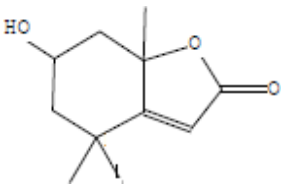
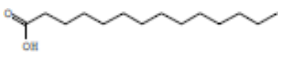
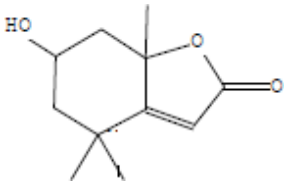
GC Programme	
Column	HP-5MS (5 % phenyl methyl siloxane)
Equipment	Agilent Technologies (GCMS-QP2010 Plus)
Carrier Gas	Helium gas 1 ml/min, splitless mode
Column Oven Temp.	60.0 °C
Injection Temp.	250.00 °C
Injection Mode	Split
Flow Control Mode	Linear Velocity
Pressure	56.7 kPa
Total Flow	8.9 mL/min
Column Flow	0.99 mL/min
Linear Velocity	36.3 cm/sec
Purge Flow	3.0 mL/min
Split Ratio	5
Sample Injection	1 µl
Detector	Mass detector
Oven Temperature programme	60 °C (1 min hold) up to 320 °C at the rate of 2 °C/min on hold
MS Programme	
Start Time	3.00min
End Time	48.00min
ACQ Mode	Scan
Event Time	0.50sec
Scan Speed	1666
Start m/z	50
End m/z	800.00`
Library used	NIST version—2011
Electron energy	70 eV
Mass Scan (m/z)	40–700 amu
Total MS run time	120 min

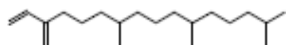
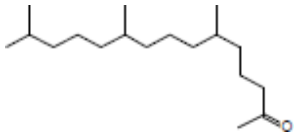
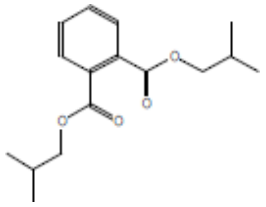
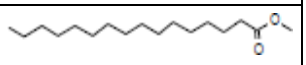
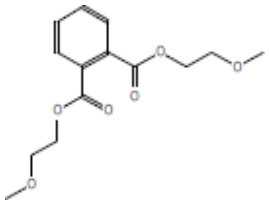
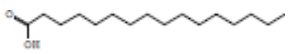
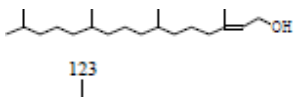
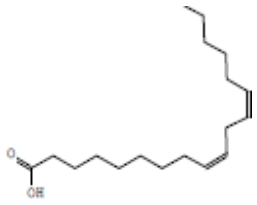
Table 3: results of phytochemical analysis of the v.amygdalina Extract

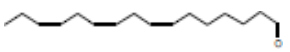
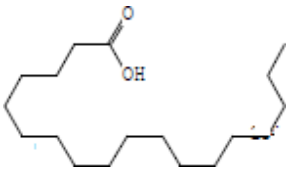
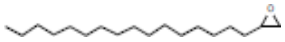
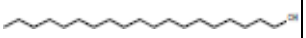
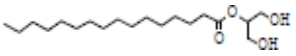
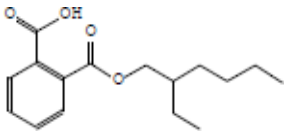
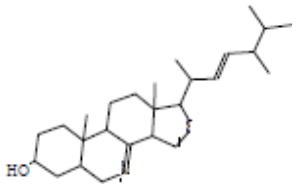
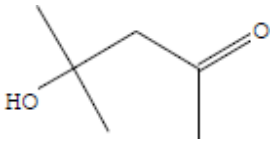
Phytochemical analysis	Name of the test	result
Tannins	Braymer's Test	++
Flavonoids	Shinoda's Test	++
Terpenoids	Salkowski Test	++
Saponins	Froth test	++
Steroids	Libermann Burchard test	++
Phlobatannins	Hcl test	--
Carbohydrates	Benedict's Test	++
Cardiac Glycosides	Keller – Killiani's test	++
Alkaloid	Wagner's test	++
Test for proteins & amino acids	Ninhydrin Test	--
Fixed oil and fat	Spot test	++
Tannins	Ferric chloride – potassium ferricyanide test	++

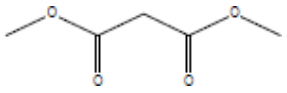
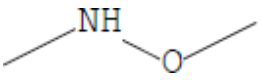
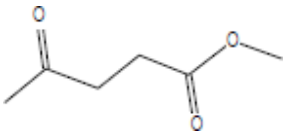
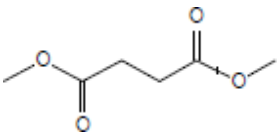
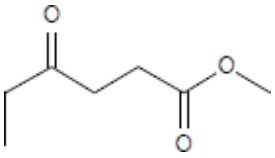
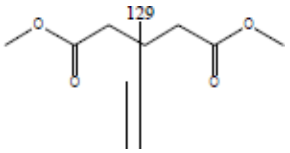
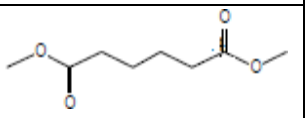
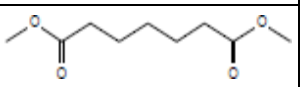
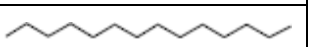
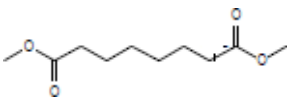
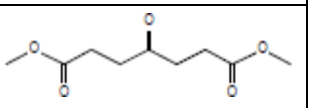
Table 4: Bioactive Compounds Identified In The Methanol Extract Of V.Amygdalina Leaves By Gc-Ms Analysis

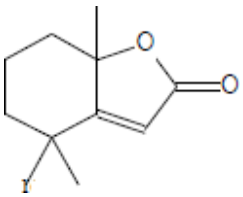
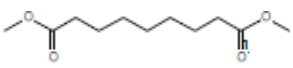
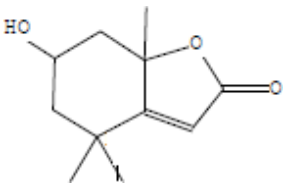
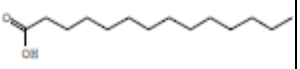
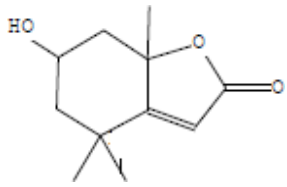
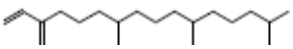
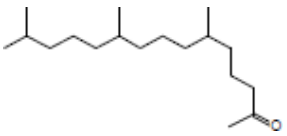
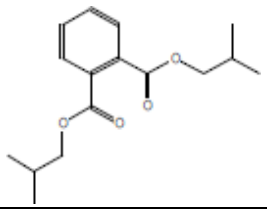
S.No	*Retention Time*	*Formula*	*Mol. Wt*	*Compound Name*	*Structure*
1.	3.358	C6H12O2	116	2-hydroxy-2-methyl-4-pentanone (diacetone)	
2.	4.367	C5H8O4	132	dimethyl propanedioate	
3.	4.958	C2H7NO	61	n,o-dimethyl-hydroxylamine	
4.	5.275	C6H10O3	130	4-oxopentanoic acid methyl ester	
5.	5.942	C6H10O4	146	dimethyl butanedioate	
6.	6.767	C7H12O3	144	4-oxohexanoic acid, methyl ester	
7.	7.650	C7H12O4	160	dimethyl pentanedioate	
8.	:9.692	C8H14O4	174	dimethyl adipate	

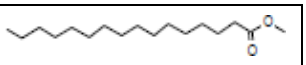
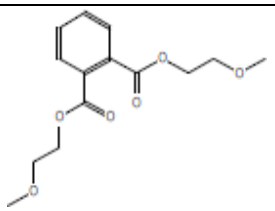
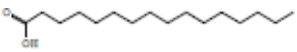
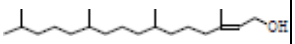
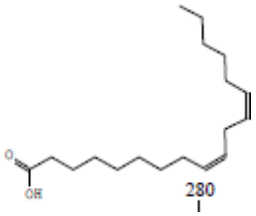
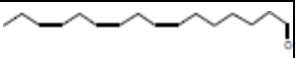
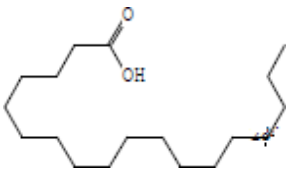
9.	11.850	C9H16O4	188	dimethyl heptanedioate	
10.	:13.217	C14H30	198	isotetradecane	
11.	14.133	C10H18O4	202	dimethyl suberate	
12.	14.675	C9H14O5	202	dimethyl 4-oxoheptanedioate	
13.	16.183	C11H16O2	180	(2,6,6-trimethyl-2-hydroxycyclohexylidene)acetic acid lactone	
14.	16.425(	C11H20O4	:216	dimethyl azelate	
15.	:17.733	C19H40	268	:nonadecane	
16.	20.658	C11H16O3	196	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6s-cis)-	
17.	21.150	C14H28O2	228	myristic acid	
18.	21.300	C11H16O3	196	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6s-cis)-	
19.	22.042	C18H38	254	octadecane	

20.	22.783	C20H38	278	2,6,10-trimethyl,14-ethylene-14-pentadecne	
21.	22.867	C18H36O	268	2-pentadecanone, 6,10,14-trimethyl-	
22.	23.200	C16H22O4	278	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	
23.	24.542	C17H34O2	270	methyl hexadecanoate	
24.	25.092	C14H18O6	282	1,2-benzenedicarboxylic acid, bis(2-methoxyethyl) ester	
25.	25.308	C16H32O2	256	palmitic acid	
26.	27.583	C14H30O	214	1-hydroxytetradecane	
27.	27.825	C8H14O	126	bicyclo[5.1.0]octan-3-ol, (1.alpha.,3.alpha.,7.alpha.)-	
28.	28.042	C20H40O	296	3,7,11,15-tetramethyl-2-hexadecen-1-ol	
29.	28.442	C18H32O2	280	cis-9,cis-12-octadecadienoic acid	

30.	28.550	C16H26O	234	cis,cis,cis-7,10,13-hexadecatrienal	
31.	28.975	C18H36O2	284	octadecanoic acid	
32.	30.633	C18H36O	268	1,2-epoxyoctadecane	
33.	31.192	C19H40O	284	1-nonadecanol	
34.	34.517	C20H42O	298	1-eicosanol	
35.	34.758	C19H38O4	330	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	
36.	35.100	C16H22O4	278	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	
37.	43.092	C33H68	464	tritriacontane	
38.	45.667	C28H46O	398	ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22e)-	
39.	46.533	C23H32O4	372	xyshalogenin	
40.	3.358	C6H12O2	116	2-hydroxy-2-methyl-4-pentanone (diacetone)	

41.	4.367	C5H8O4	132	dimethyl propanedioate	
42.	4.958	C2H7NO	61	n,o-dimethyl-hydroxylamine	
43.	5.275	C6H10O3	130	pentanoic acid, 4-oxo-, methyl ester	
44.	5.942	C6H10O4	146	dimethyl butanedioate	
45.	6.767	C7H12O3	144	4-oxohexanoic acid, methyl ester	
46.	7.650	C7H12O4	160	pentanedioic acid,	
47.	9.692	C8H14O4	174	hexanedioic acid, dimethyl ester	
48.	11.850	C9H16O4	188	dimethyl heptanedioate	
49.	13.217	C14H30	198	tetradecane	
50.	14.133	C10H18O4	202	dimethyl octanedioate	
51.	14.675	C9H14O5	202	dimethyl 4-oxoheptanedioate	

52.	16.183	C11H16O2	180	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	
53.	16.425	C11H20O4	216	nonanedioic acid, dimethyl ester	
54.	17.733	C19H40	268	nonadecane	
55.	20.658	C11H16O3	196	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6s-cis)-	
56.	21.150	C14H28O2	228	tetradecanoic acid	
57.	21.300	C11H16O3	196	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-6-hydroxy-4,4,7a-trimethyl-, (6s-cis)-	
58.	22.042	C18H38	254	octadecane	
59.	22.783	C20H38	278	2,6,10-trimethyl,14-ethylene-14-pentadecne	
60.	22.867	C18H36O	268	2-pentadecanone, 6,10,14-trimethyl	
61.	23.200	C16H22O4	278	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	

62.	24.542	C17H34O2	270	hexadecanoic acid, methyl ester	
63.	25.092	C14H18O6	282	1,2-benzenedicarboxylic acid, bis(2-methoxyethyl) ester	
64.	25.308	C16H32O2	256	n-hexadecanoic acid	
65.	27.583	C14H30O	214	1-tetradecanol	
66.	27.825	C8H14O	126	bicyclo[5.1.0]octan-3-ol, (1.alpha.,3.alpha.,7.alpha.)-	
67.	28.042	C20H40O	296	:2-hexadecen-1-ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]- (t-phytol) $$ 3,7,11,15-tetramethyl-2-hexadecen-1-ol	
68.	28.442	C18H32O2	280	`9,12-octadecadienoic acid (z,z)-	
69.	28.550	C16H26O	234	cis,cis,cis-7,10,13-hexadecatrienal	
70.	28.975	C18H36O2	284	octadecanoic acid	
71.	30.633	C18H36O	268	1,2-epoxyoctadecane	
72.	31.192	C19H40O	284	1-nonadecanol	

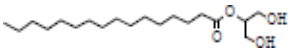
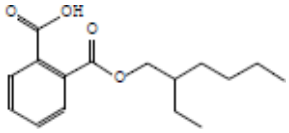
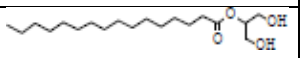
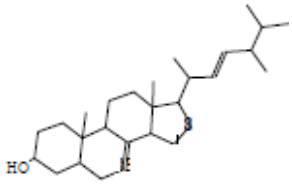
73.	34.517	C20H42O	298	1-eicosanol	
74.	34.758	C19H38O4	330	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	
75.	35.100	C16H22O4	278	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	
76.	37.875	C19H38O4	330	hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	
77.	43.092	C33H68	464	n-tritriacontane`	
78.	45.667	C28H46O	398	ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22e)-	
79.	46.533	C23H32O4	372	xyshalogenin	

Figure 1: Gc-Ms Chromatogram Of V.Amygdalina Leaf (Methanol) Extract