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ANTIBACTERIAL POTENTIAL AND GC-MS PROFILING OF METHANOLIC LEAF EXTRACT OF *VITEX NEGUNDO LINN*.

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ABSTRACT
The current study was designed to catalyze the primary metabolites and their authentification by using GC-MS analysis and antibacterial power of methanolic leaf extract of *Vitex negundo*. The antibacterial potential of the methanol leaf extract of *Vitex negundo* was tested against *Bacillus cereus*, *Proteus vulgaris* and *Proteus mirabilis* using by agar well diffusion method. The higher zone of inhibition (55.03mm) was observed against the bacterium *P.vulgaris* at 20µl and 25µl concentration of methanol leaf extract. GC-MS analysis confirms the occurrence of more than 16 different compounds in the methanol leaf extract of *Vitex negundo*.

KEYWORDS: *Vitex negundo*, Antibacterial activity, Disc diffusion assay, GC-MS.

INTRODUCTION
Systematic investigations of medicinal plants have started in many countries because of their contributions to health care. The primary benefits of using plant based medicines are relatively safer than synthetic alternatives, offering marked therapeutic benefits and more reasonable treatment. (Ahmad etal). Thus, over 50% of these modern drugs are of natural product origin and these natural products play an important role in the drug development in the pharmaceutical industry.(jeyachandran etal). In general, bacteria have the genetic ability to transfer and gain resistance to drugs used as therapeutic agents. The only way to prevent antibiotic resistance is by using new compounds which have not based on the existing synthetic anti-microbial agents.(shah etal). To promote the use of medicinal plants as potential sources of anti-microbial compounds, it is important to thoroughly find out their composition and activity and thus confirm their use. (Nair etal). Plants and their active constituents play an important role in the prevention of a variety of ailments. Vitex negundo Linn., Verbenaceae, known as Nirgundi in Hindi, grows gregariously in wastelands and is also planted as a hedge-plant. It is an erect, 2–5 m in height, slender tree with quadrangular branchlets distributed throughout India. The leaves have five leaflets in a palmately arrangement, which are lanceolate, 4–10 cm long, hairy beneath and pointed at both ends. The bluish purple flowers are numerous. The fruit is succulent, black when ripe, rounded and about 4 mm in diameter (Chopra, Nayar, & Chopra, 1956; Wealth of India, 1976). Although all parts V. negundo are used as medicine in the indigenous system of medicine, the leaves are the most potent for medicinal use. It is used for treatment of eye-disease, toothache, inflammation, leucoderma, enlargement of the spleen, skin-ulcers, in catarrhal fever, rheumatoid arthritis, gonorrhoea, and bronchitis. They are also used as tonics, vermifuge, lactagogue, emmenagogue, antibacterial, antipyretic and antihistaminic agents (Chaturvedi & Singh, 1965). Recently, attention has been focused on the medicinal properties of the extracts. The present study was aimed to evaluate the antibacterial potential of methanol leaf extract of ***Vitex negundo*** against bacterial pathogens and identify the secondary metabolites ***through GCMS study.*** The result also indicated that scientific studies carried out on medicinal plant having traditional claims of effectiveness might warrant fruitful results.

MATERIALS AND METHODS
Materials
The plant ***Vitex negundo*** Linn. was collected from Adhiparasakthi Agricultural College, Kalavai, Tamil Nadu. Methanol and required chemicals (HPLC grade) were procured from Merck, India. The plant was identified by the scientist of the Institute.

Methods
The leaves of Vitex negundo Linn was collected, washed, air dried in shadow and grinded by mixer grinder. After grinding, 5 gm of plant material was extracted in 50 ml of methanol solvent separately at

room temperature for 6 hours. The organic solvent was filtered by whatman filter paper till clear solution was obtained. Solvent was evaporated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure (vacuum) at 40-50˚C till complete dryness.

Extract yield percentage: The methanolic extract was transferred in preweighed beaker and was dried. Further the final weight of the beaker with extract was measured. Then the extract yield was calculated. Noted in Table1.
Extract yield % = ((Extract + Beaker weight) - (Empty beaker weight))/Sample weight *100.

Sample Preparation
6mg of methanolic extract of VN was suspended in 1 ml of methanol and from that 0.005, 0.010, 0.015, 0.020 ml and 0.025 ml was used for the test to give a concentration 30, 60, 90, 120 and 150 mcg each.

Microorganisms used and growth conditions
The following organisms were used in this study and they consist of both Gram positive and Gram negative bacteria: *Bacillus cereus*, *Proteus vulgaris* and *Proteus mirabilis*. The bacterial strains were maintained at 40^{o}C and their stock was kept in 10% glycerine saline at –20^{o}C. Antibacterial activity Sensitivity of different test bacterial strains to various extracts of ***Vitex negundo Linn*** was measured by agar well diffusion method.(Raman BV etal., 2005, raman BV etal 2009). Zone of inhibition was determined using Himedia zone of inhibition scale and results are expressed in millimeters (mm). For each combination of extract and the bacterial strain, the experiment was performed in triplicate. The bacteria with a clear zone of inhibition of more than 8 mm were considered to be sensitive.

GC-MS analysis
The chromatographic procedure was performed using GC-MS model MSQP2010 (Shimadzu, Kyoto, Japan) with auto sampler. 1000 ppm solution in methanol was prepared from methanolic extracts and 1 µL of methanolic extract was injected for analysis using DB - 5MS column (30 meter × 0.25 mm, film thickness 0.25µm). Helium gas was used at flow rate1ml/min. as a carrier gas. The analysis was carried out using oven programming of initial temperature 50˚C for 2 minutes followed by ramp rate of 20˚C/minute up to 130˚C followed by ramp of 12˚C/min. to a temperature of 180˚C, finally raised temperature to 280˚C at 3˚C per minute and hold for 15 minutes. The ion source temperature was set at 250˚C. The injection port temperature was set as a 250˚C and the total run time was 58.5 minute. The instrument was operated in electron impact (EI) mode with electron energy 70ev. Confirmation of analytes was done by SIM (selective ion mode) mode.(abirami etal 2012, prajapathi R etal., 2014).

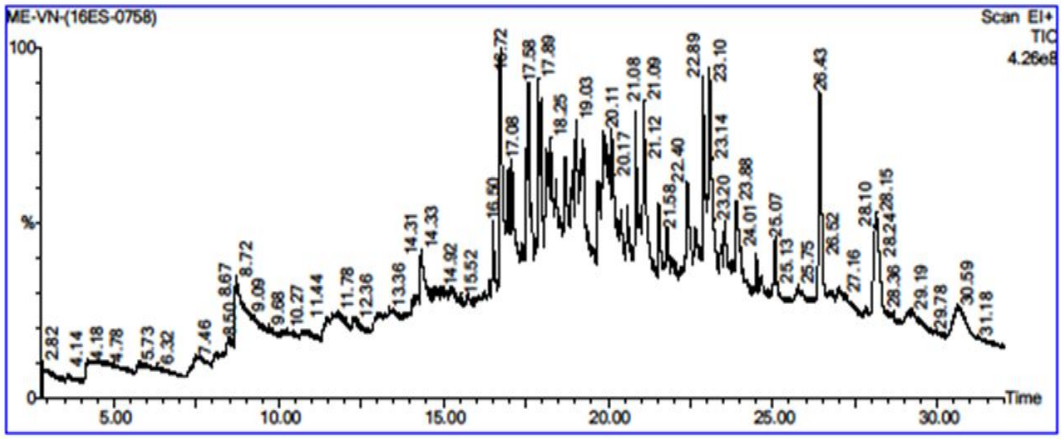
RESULT AND DISCUSSION

Antibacterial activity of methanolic leaf extract of *Vitex nigundo linn.*
The antibacterial activity of methanolic leaf extracts was evaluated against three opportunistic bacterial pathogens. The results of antibacterial activity of different concentrations of ***Vitex nigundo linn leaf extract*** were summarized in Table 2. The results revealed that methanolic leaf extract of ***Vitex nigundo linn*** showed the maximum activity against *P.vulgaris* at 20 and 25 µl concentration. The antibacterial activity was distinctly varied against the bacterial test pathogens. Kanamycin (30 µg) was used as a reference standard. The results of well diffusion method and minimum inhibitory concentration shown in figure 6, 7 and in Table 2. From the results, it is identified that the methanolic leaf extract of ***Vitex nigundo linn*** exhibited significant antibacterial activity when compared with the standard antibiotics tested.

Assessment of phytocomponents in methanolic leaf extract of *Vitex nigundo linn*
P.vulgaris showed the significant antibacterial activity. Consequently, it was subjected to GC–MS analysis. The mass spectrum of methanol extract showed more than 16 prominent peaks. The major phytoconstituents were phosphonic acid, ledol, benzene methanol 1 – t – butyl – 4 (adamantly-1) benzene, Z-5,17 – octadecadiene – 1-ol acetate, 6, 11 – Undecadiene, 1-acetoxy – 3,7-dimethyl, 4,(2,2,6-trimethyl – bicycle(4.1.0) hept – 1-yl)-butane – 2-one, heptacosone, dotriacontane, hydroxyl dehydro steric acid showed in figures 8.

Qualitative Report

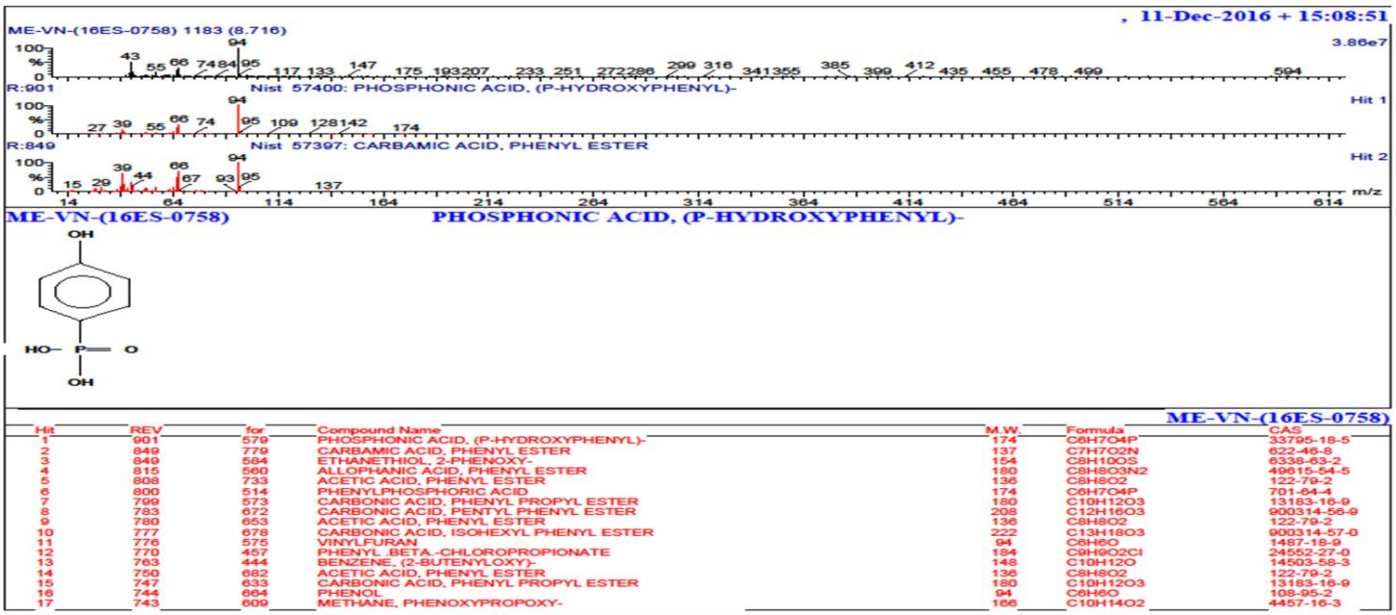
File: C:\TURBOMASS\2016.PRO\Data\ME-VN-(16ES-0758).raw
Acquired: 11-Dec-16 03:08:51 PM Printed: 20-Dec-16 09:58 AM
Description:
GC/MS Method: GC: METHOD-1.mth MS: METHOD-1.EXP Page 1 of 1
Sample ID: ME-VN-(16ES-0758) Vial Number: 4



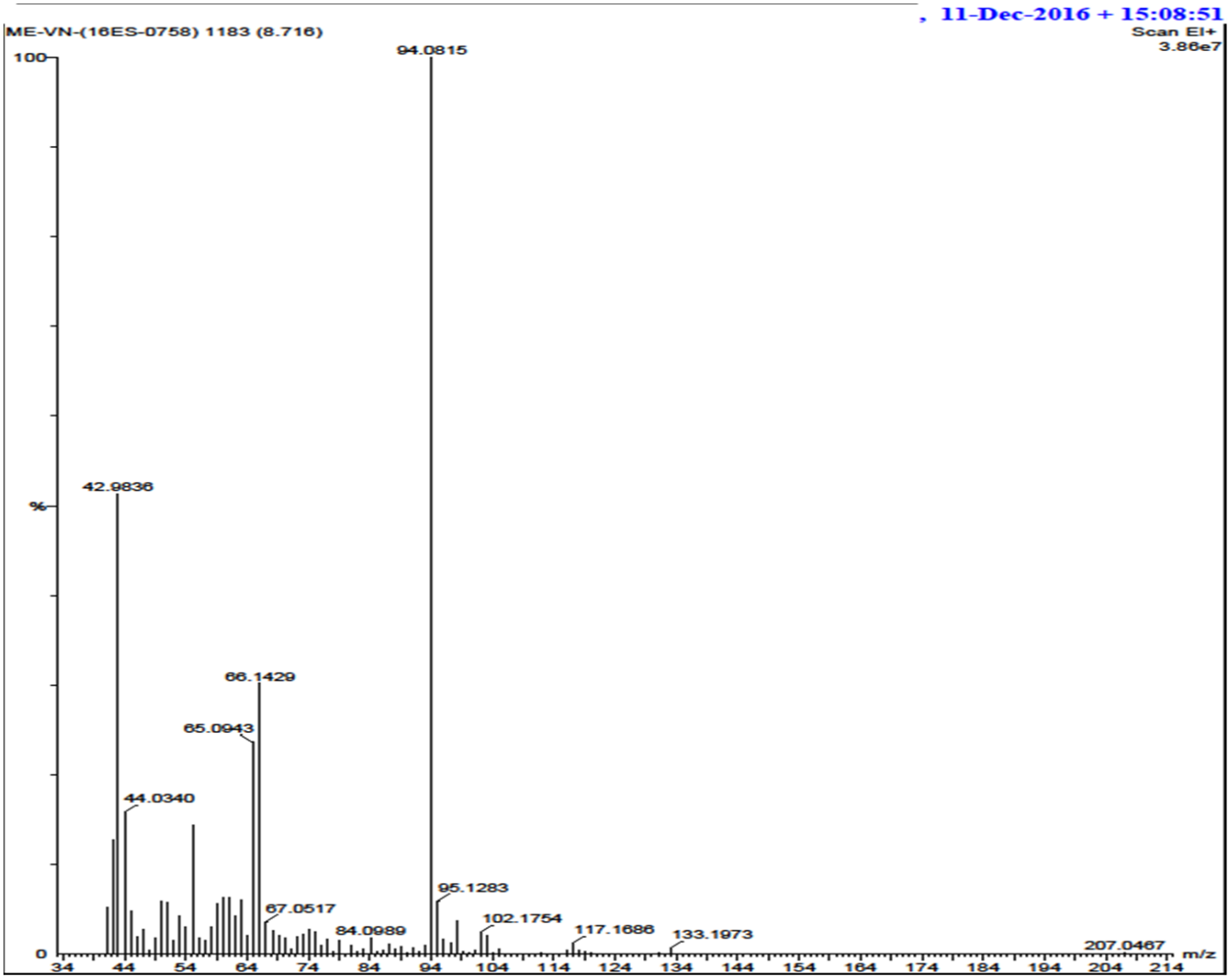
#	RT	Scan	Height	Area	Area %	Norm %
1	8.716	1183	84,031,136	38,545,300.0	13.607	100.00
2	14.333	2306	63,814,992	8,131,971.5	2.871	21.10
3	16.724	2784	291,537,376	26,909,288.0	9.499	69.81
4	16.974	2834	131,878,488	12,450,566.0	4.395	32.30
5	17.084	2856	136,212,400	12,510,705.0	4.417	32.46
6	19.250	3289	104,280,000	10,334,188.0	3.648	26.81
7	19.855	3410	108,226,008	21,277,508.0	7.511	55.20
8	20.105	3460	116,934,088	9,349,389.0	3.301	24.26
9	20.831	3605	172,088,352	15,010,450.0	5.299	38.94
10	21.086	3656	185,753,408	16,920,506.0	5.973	43.90
11	22.416	3922	104,642,848	10,684,571.0	3.772	27.72
12	22.886	4016	219,656,608	16,577,576.0	5.852	43.01
13	23.072	4053	233,424,944	27,418,176.0	9.679	71.13
14	23.882	4215	84,661,112	9,883,107.0	3.489	25.64
15	26.428	4724	250,164,976	23,759,578.0	8.388	61.64
16	28.154	5069	124,024,704	23,508,688.0	8.299	60.99

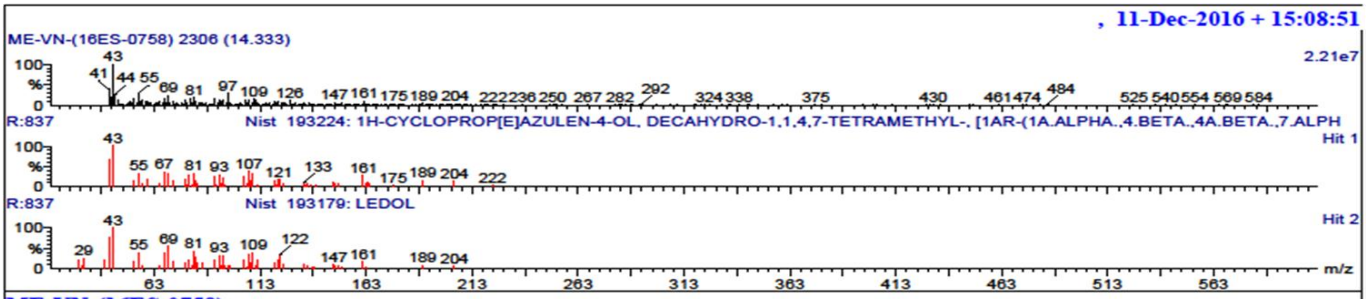
Inst() ACQUISITION PARAMETERS
Oven: Initial temp 60°C for 2 min, ramp 10°C/min to 300°C, hold 6 min, InjAauto=260°C, Volume=0 µL, Split=10:1, Carrier Gas=He, Solvent Delay=2.80 min, Transfer Temp=240°C, Source Temp=240°C, Scan: 40 to 600Da, Column 30.0m x 250µm

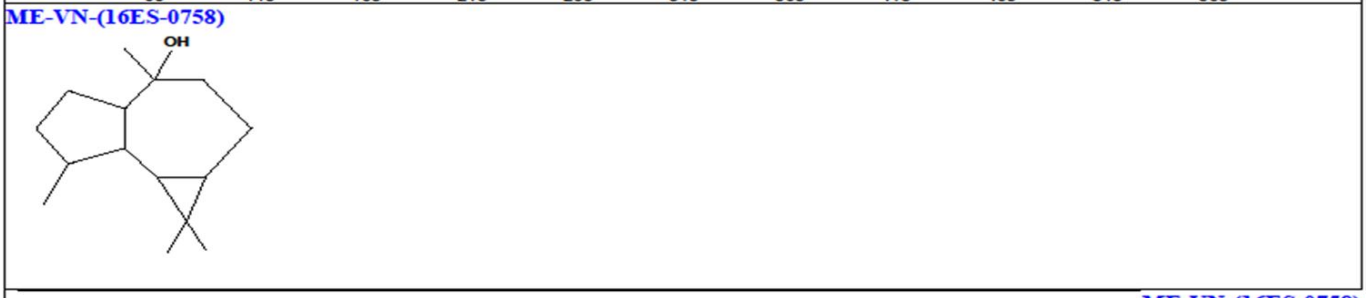
Figure 8: GC-MS profiling along with chromatogram of methanolic leaf extract of *Vitex negundo linn*



Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	901	579	PHOSPHONIC ACID, (P-HYDROXYPHENYL)-	174	C6H7O4P	33795-18-5
2	849	779	CARBAMIC ACID, PHENYL ESTER	137	C7H7O2N	622-46-8
3	849	584	ETHANETHIOL, 2-PHENOXY-	154	C8H10OS	6338-63-2
4	815	560	ALLOPHANIC ACID, PHENYL ESTER	180	C8H8O3N2	49615-54-5
5	808	733	ACETIC ACID, PHENYL ESTER	136	C8H8O2	122-79-2
6	800	514	PHENYLPHOSPHORIC ACID	174	C6H7O4P	701-64-4
7	799	573	CARBONIC ACID, PHENYL PROPYL ESTER	180	C10H12O3	13183-16-9
8	783	672	CARBONIC ACID, PENTYL PHENYL ESTER	208	C12H16O3	900314-56-9
9	780	653	ACETIC ACID, PHENYL ESTER	136	C8H8O2	122-79-2
10	777	678	CARBONIC ACID, ISOHEXYL PHENYL ESTER	222	C13H18O3	900314-57-0
11	776	575	VINYLFURAN	94	C6H6O	1487-18-9
12	770	457	PHENYL, BETA.-CHLOROPROPIONATE	184	C9H9O2Cl	24552-27-0
13	763	444	BENZENE, (2-BUTENYLOXY)-	148	C10H12O	14503-58-3
14	750	682	ACETIC ACID, PHENYL ESTER	136	C8H8O2	122-79-2
15	747	633	CARBONIC ACID, PHENYL PROPYL ESTER	180	C10H12O3	13183-16-9
16	744	664	PHENOL	94	C6H6O	108-95-2
17	743	609	METHANE, PHENOXYPROPOXY-	166	C10H14O2	4457-16-3

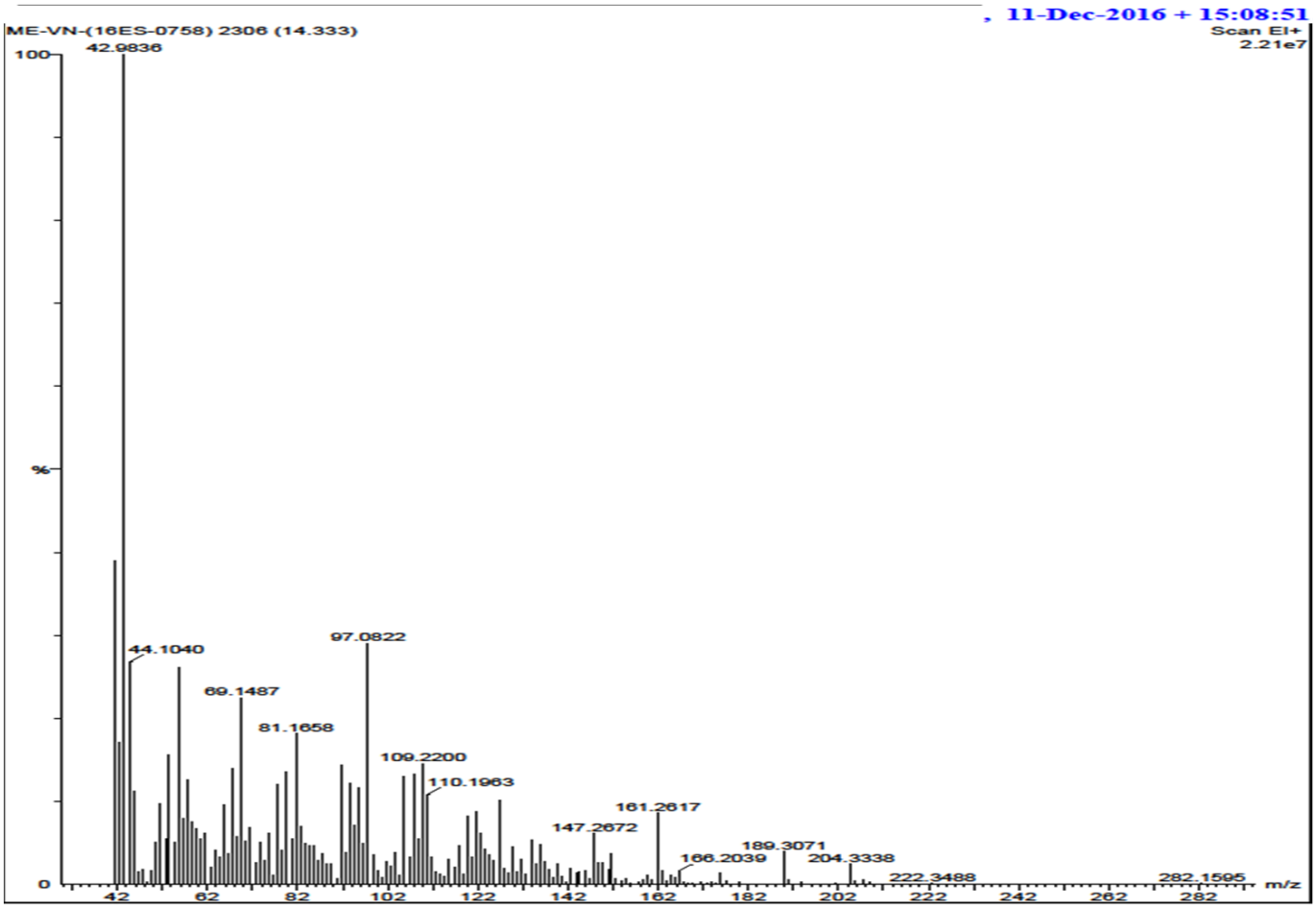


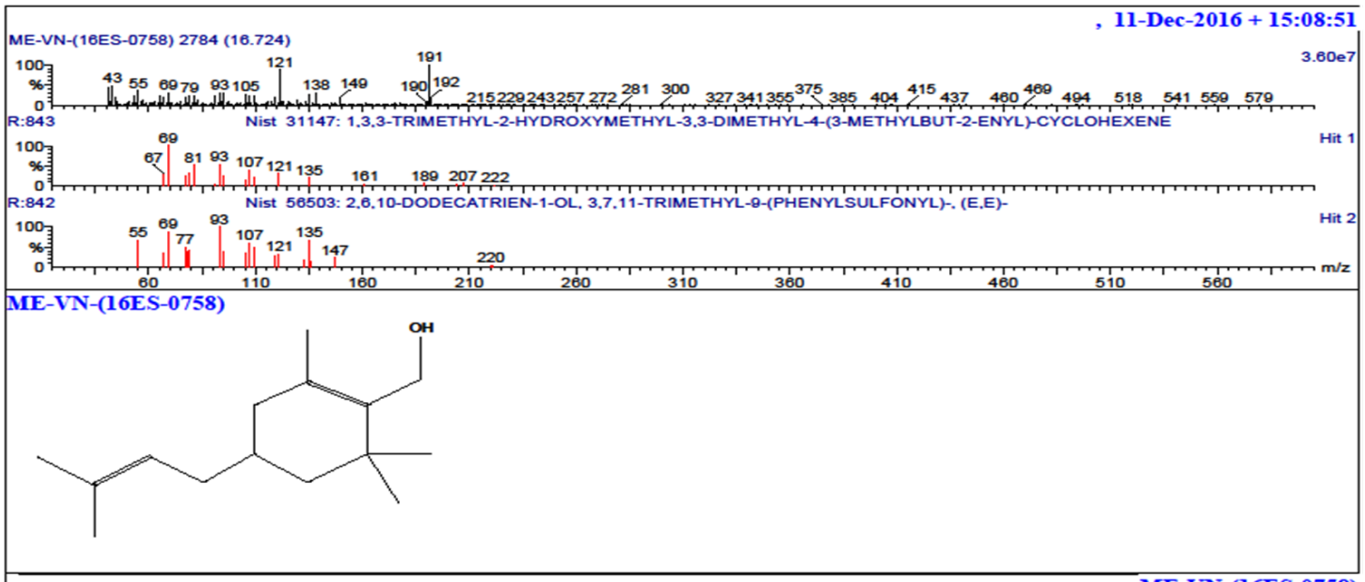




ME-VN-(16ES-0758)

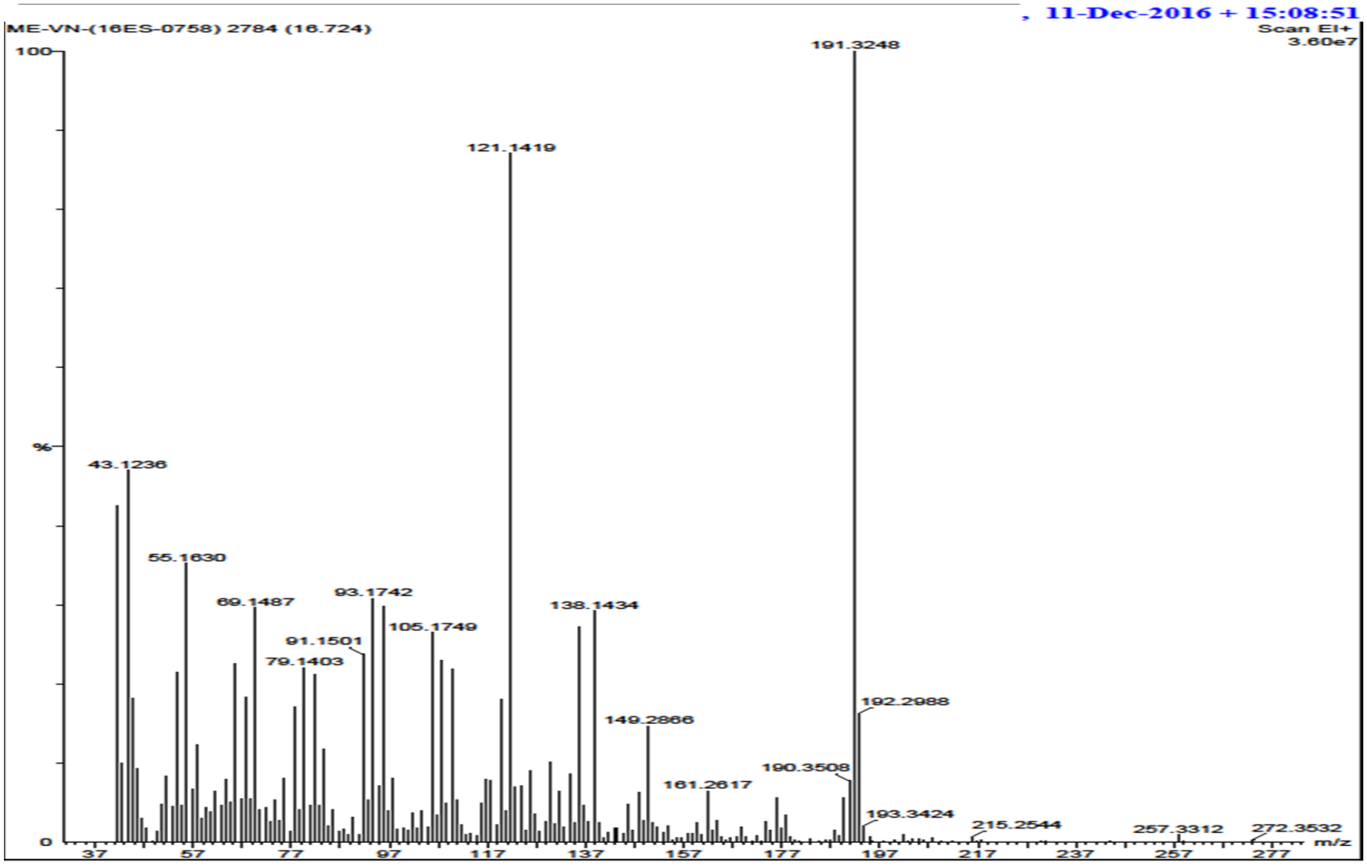
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	837	632	1H-CYCLOPROP[E]AZULEN-4-OL, DECAHYDRO-1,1,4,7-TETRAMETHYL-, [1AR-(1A.A	222	C15H26O	552-02-3
2	837	643	LEDOL	222	C15H26O	577-27-5
3	829	602	LEDOL	222	C15H26O	577-27-5
4	829	602	VERIDIFLOROL	222	C15H26O	900122-17-3
5	827	649	3-BUTEN-2-ONE, 4-(3-HYDROXY-6,6-DIMETHYL-2-METHYLENECYCLOHEXYL)-	208	C13H20O2	900142-31-3
6	820	619	1H-CYCLOPROP[E]AZULEN-4-OL, DECAHYDRO-1,1,4,7-TETRAMETHYL-, [1AR-(1A.A	222	C15H26O	552-02-3
7	819	600	1H-CYCLOPROP[E]AZULEN-4-OL, DECAHYDRO-1,1,4,7-TETRAMETHYL-, [1AR-(1A.A	222	C15H26O	552-02-3
8	804	584	GLOBULOL	222	C15H26O	51371-47-2
9	786	571	7-DEHYDROCHOLESTERYL ISOCAPROATE	482	C33H54O2	900251-07-6
10	780	534	CYCLOARTANOL	428	C30H52O	4657-58-3
11	771	519	9,19-CYCLOLANOST-24-EN-3-OL, ACETATE, (3.BETA.)-	468	C32H52O2	1259-10-5
12	769	566	LEDENE OXIDE-(I)	220	C15H24O	900151-93-3
13	765	602	5,8-DECADIEN-2-ONE, 5,9-DIMETHYL-, (E)-	180	C12H20O	130876-99-2
14	763	584	3,5,9-TRIMETHYL-DECA-2,4,8-TRIEN-1-OL	194	C13H22O	900188-00-3
15	757	566	1H-BENZOCYCLOHEPTEN-7-OL, 2,3,4,4A,5,6,7,8-OCTAHYDRO-1,1,4A,7-TETRAMET	222	C15H26O	6892-80-4
16	754	512	9,19-CYCLOERGOST-24(28)-EN-3-OL, 4,14-DIMETHYL-, ACETATE, (3.BETA.,4.ALPH	468	C32H52O2	10376-42-8
17	753	568	1,6-DIMETHYL-9-(1-METHYLETHYLIDENE)-5,12-DIOXATRICYCLO[9.1.0.0(4,6)]DODE	250	C15H22O3	900072-83-2

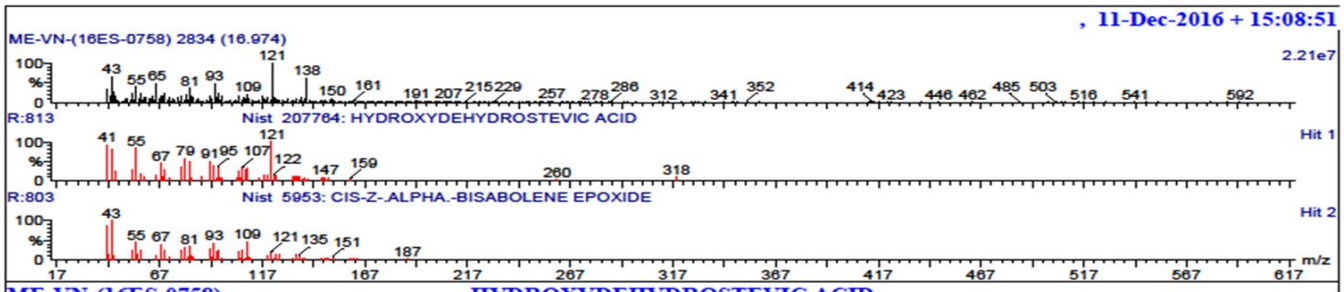


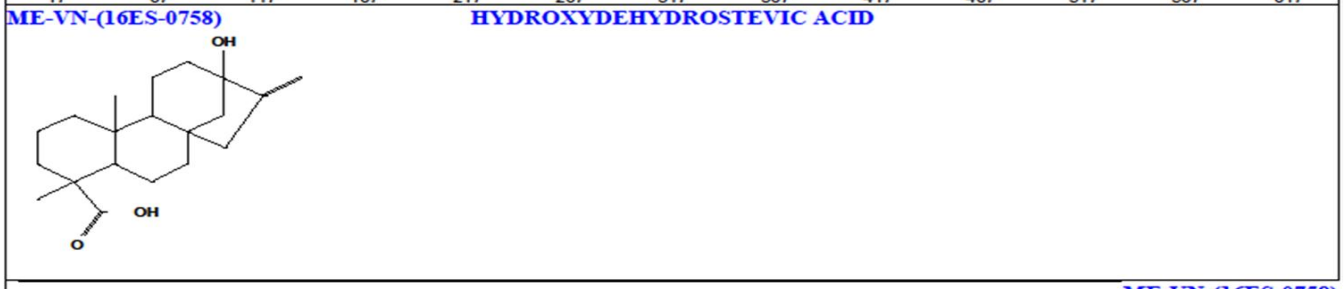


ME-VN-(16ES-0758)

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	843	402	1,3,3-TRIMETHYL-2-HYDROXYMETHYL-3,3-DIMETHYL-4-(3-METHYLBUT-2-ENYL)-C	222	C15H26O	900144-10-7
2	842	412	2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL-9-(PHENYLSULFONYL)-, (E,E)-	362	C21H30O3S	57683-67-7
3	818	641	(7A-ISOPROPENYL-4,5-DIMETHYLOCTAHYDROINDEN-4-YL)METHANOL	222	C15H26O	900187-02-9
4	790	533	9,19-CYCLOLANOST-24-EN-3-OL, ACETATE, (3.BETA.)-	468	C32H52O2	1259-10-5
5	788	488	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXEN	222	C15H26O	900144-10-5
6	761	639	7A-ISOPROPENYL-4,5-DIMETHYLOCTAHYDROINDENE-4-CARBOXYLIC ACID	236	C15H24O2	900192-14-7
7	754	461	DIHYDROTACHYSTEROL	398	C28H46O	67-96-9
8	754	596	5-(7A-ISOPROPENYL-4,5-DIMETHYL-OCTAHYDROINDEN-4-YL)-3-METHYL-PENT-2-	290	C20H34O	900193-54-0
9	748	334	(E,E,E)-(5-PHENYLSULFONYLGERANYL)GERANIOL	430	C26H38O3S	67428-43-7
10	746	582	5-(1-ISOPROPENYL-4,5-DIMETHYLBICYCLO[4.3.0]NONAN-5-YL)-3-METHYL-2-PENT	332	C22H36O2	900195-69-8
11	741	561	5-(7A-ISOPROPENYL-4,5-DIMETHYL-OCTAHYDROINDEN-4-YL)-3-METHYL-PENT-2-	288	C20H32O	900193-54-1
12	741	655	4-(2,2-DIMETHYL-6-METHYLENECYCLOHEXYLIDENE)-3-METHYLBUTAN-2-ONE	206	C14H22O	93175-74-7
13	741	613	TRICYCLO[4.3.0.0(7,9)]NONANE, 2,2,5,5,8,8-HEXAMETHYL-, (1.ALPHA.,6.BETA.,7.AL	206	C15H26	54832-82-5
14	736	553	TRICYCLO[3.1.0.0(2,4)]HEXANE, 3,3,6,6-TETRAETHYL-, TRANS--	192	C14H24	900150-14-4
15	726	420	1,4,5,6-TETRAHYDROCYCLOPENTAPYRAZOLE-3-CARBOXYLIC ACID, (1-ADAMANT	326	C19H26ON4	900265-46-6
16	719	571	2-(4A,8-DIMETHYL-6-OXO-1,2,3,4,4A,5,6,8A-OCTAHYDRO-NAPHTHALEN-2-YL)-PRO	234	C15H22O2	900190-21-8
17	714	438	3-O-ACETYL-6-METHOXY-CYCLOARTENOL	498	C33H54O3	900286-40-9

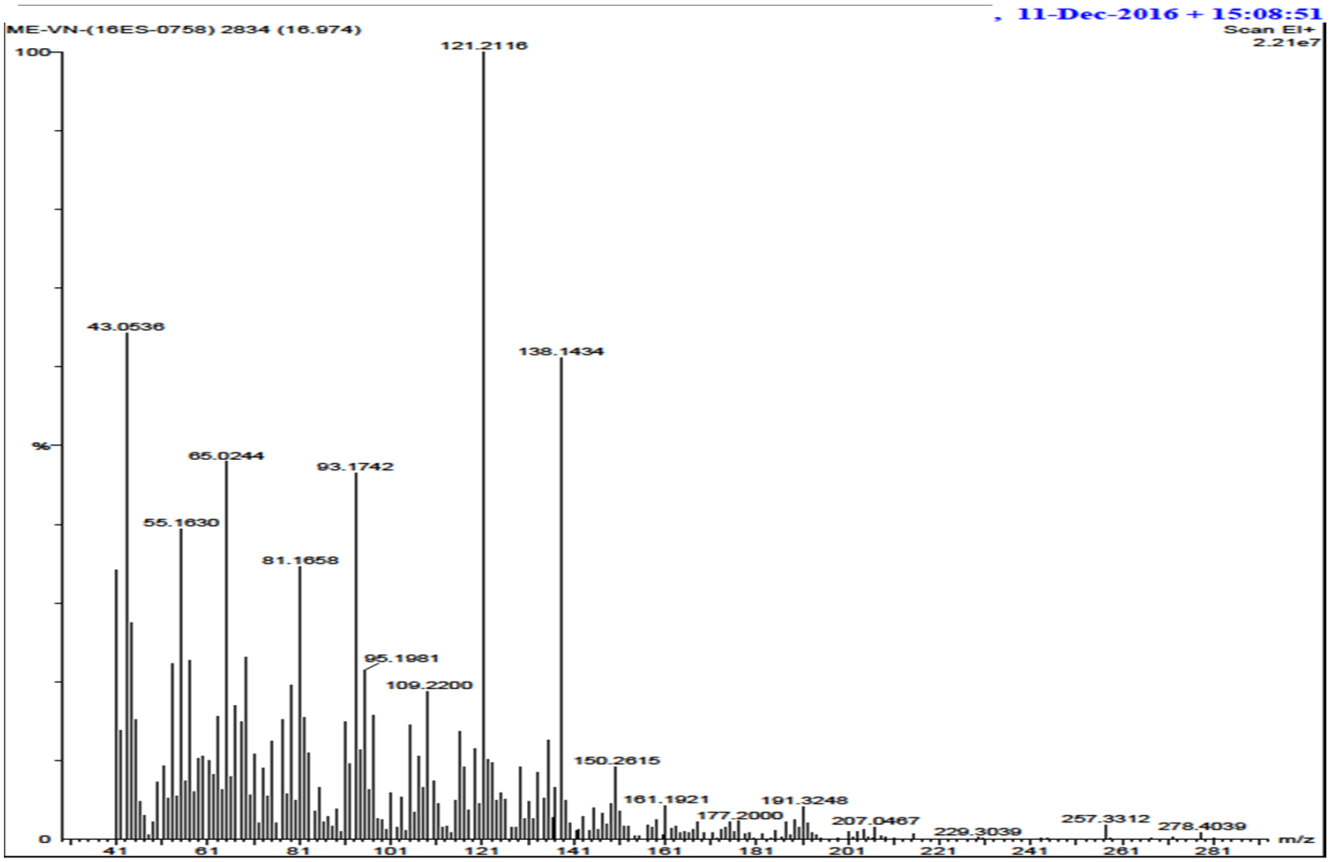


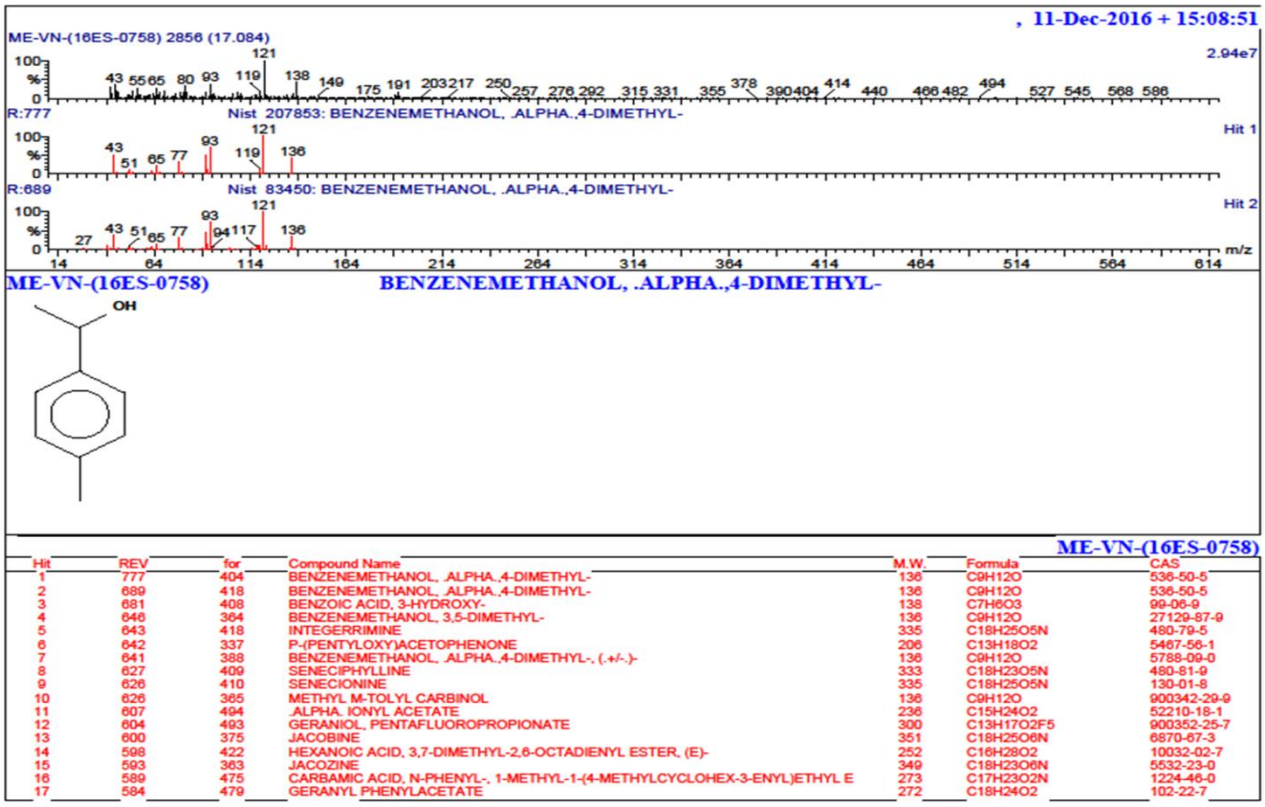




ME-VN-(16ES-0758)

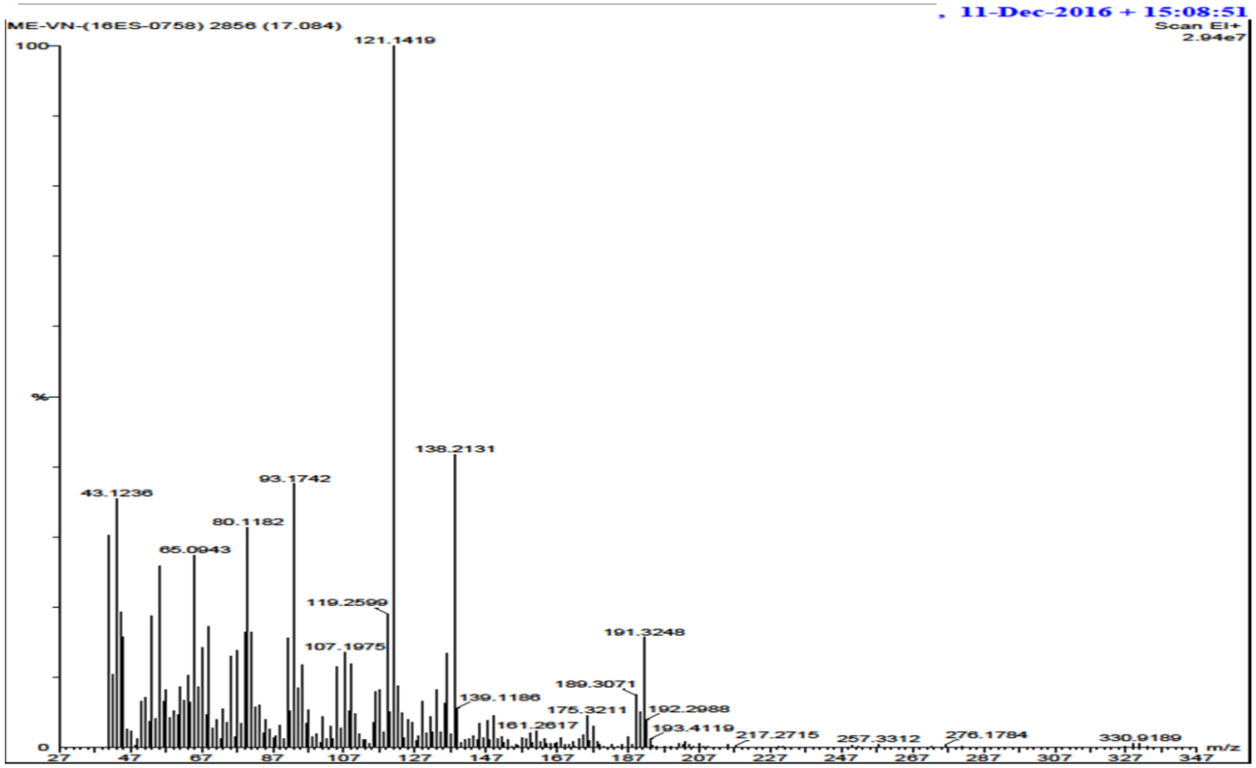
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	813	612	HYDROXYDEHYDROSTEVIC ACID	318	C20H30O3	471-80-7
2	803	612	CIS-Z-.ALPHA.-BISABOLENE EPOXIDE	220	C15H24O	900131-71-2
3	779	612	3,6-OCTADIEN-1-OL, 3,7-DIMETHYL-, (Z)-	154	C10H18O	5944-20-7
4	747	505	LEDENE OXIDE-(I)	220	C15H24O	900151-93-3
5	742	479	PREGNAN-20-ONE, 3-HYDROXY-, (3.BETA.)-	318	C21H34O2	4406-37-5
6	739	463	ISOSTEVIOL	318	C20H30O3	27975-19-5
7	720	425	1,2-CYCLOHEXANEDIMETHANOL, 3-(ACETYLOXY)-1,2-DIMETHYL-, DIACETATE	314	C16H26O6	55905-48-1
8	717	572	MENTHYL SALICYLATE	276	C17H24O3	89-46-3
9	715	552	3,6-OCTADIEN-1-OL, 3,7-DIMETHYL-, (Z)-	154	C10H18O	5944-20-7
10	715	438	9,19-CYCLOLANOST-24-EN-3-OL, ACETATE, (3.BETA.)-	468	C32H52O2	1259-10-5
11	715	450	ISOSTEVIOL ACETATE	360	C22H32O4	900256-11-1
12	714	429	METHYL 3-ACETOXY-12-KETO-18-NORCHOLANATE	432	C26H40O5	900251-87-8
13	711	463	EPIGLOBULOL	222	C15H26O	900150-05-1
14	711	440	DIHYDRO-ISOSTEVIOL METHYL ESTER	334	C21H34O3	900256-06-8
15	706	490	2,2,4-TRIMETHYL-3-(3,8,12,16-TETRAMETHYL-HEPTADECA-3,7,11,15-TETRAENYL)-	428	C30H52O	900194-01-4
16	705	459	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2
17	701	473	DIEPI-.ALPHA.-CEDRENE EPOXIDE	220	C15H24O	900151-96-9

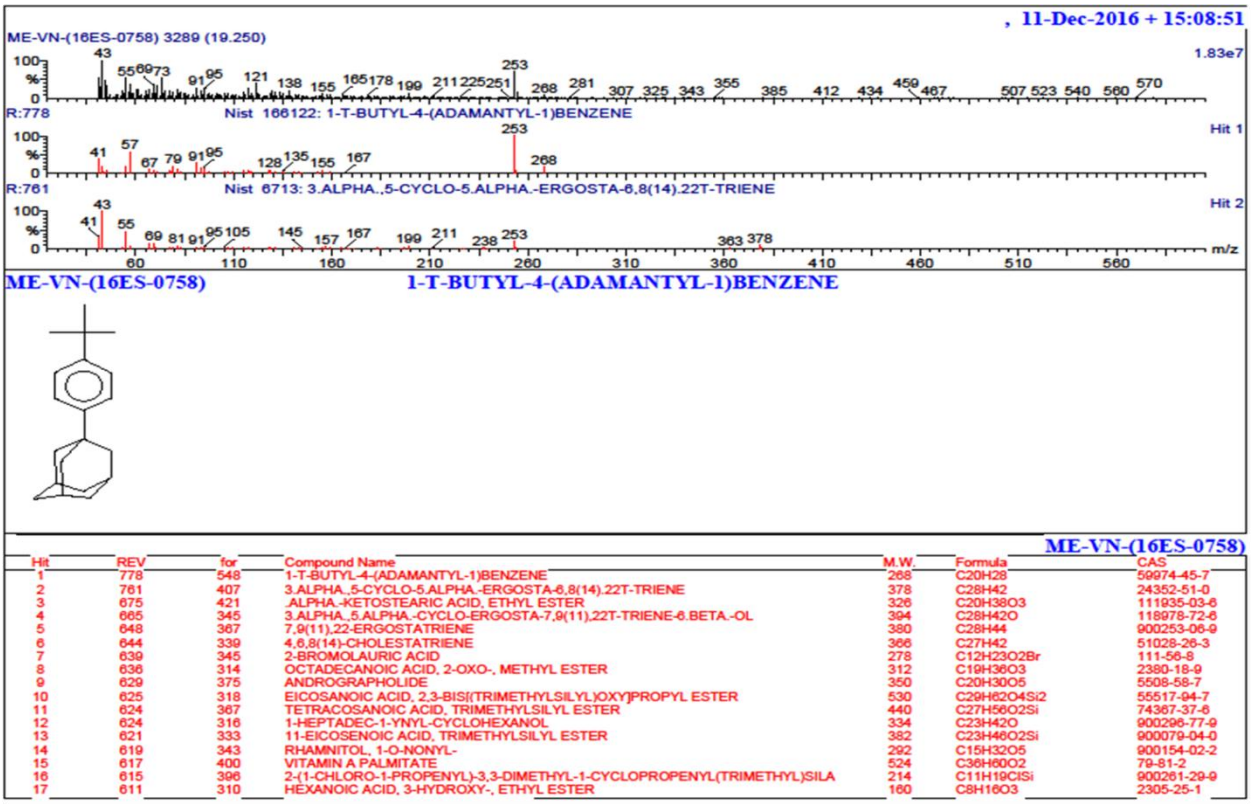




ME-VN-(16ES-0758)

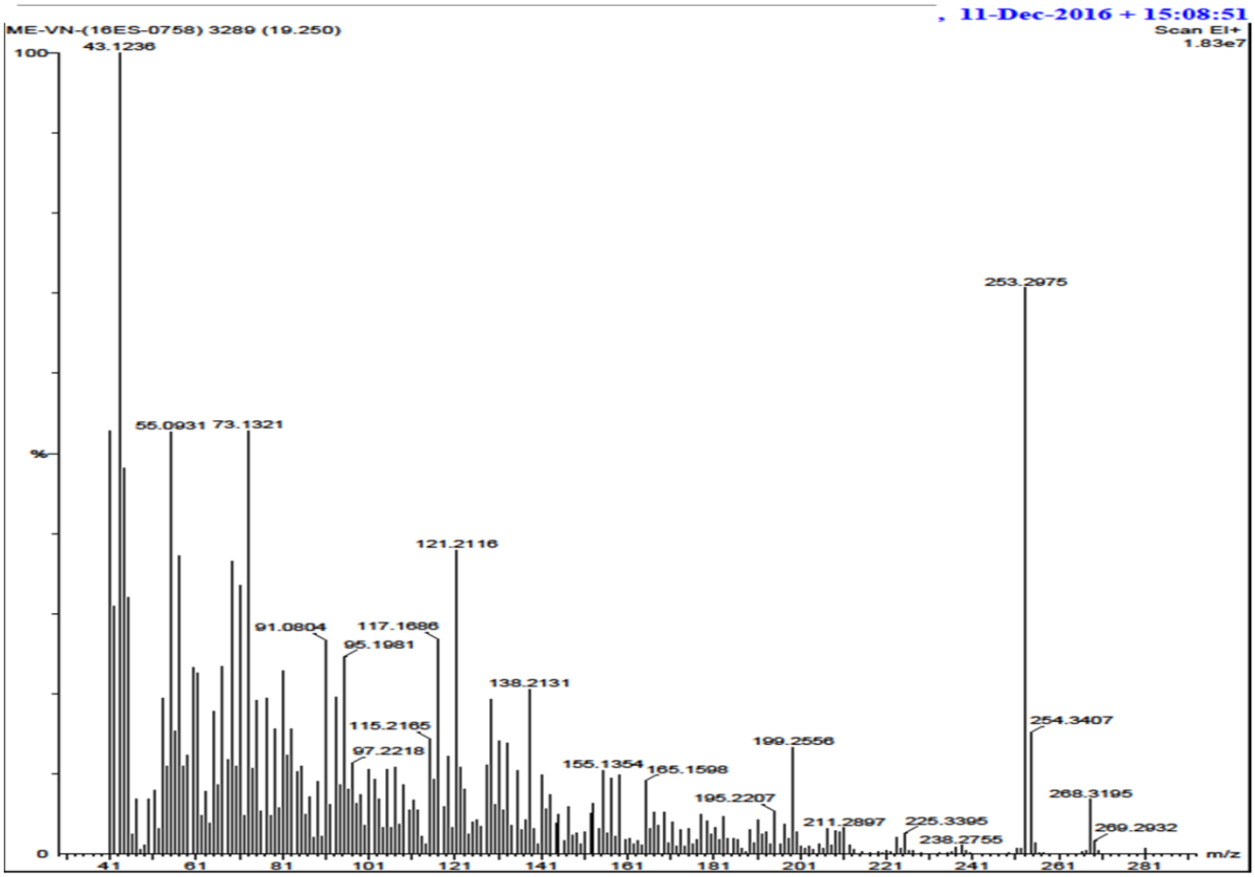
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	777	404	BENZENEMETHANOL, .ALPHA.,4-DIMETHYL-	136	C9H12O	536-50-5
2	689	418	BENZENEMETHANOL, .ALPHA.,4-DIMETHYL-	136	C9H12O	536-50-5
3	681	408	BENZOIC ACID, 3-HYDROXY-	138	C7H6O3	99-06-9
4	646	364	BENZENEMETHANOL, 3,5-DIMETHYL-	136	C9H12O	27129-87-9
5	643	418	INTEGERRIMINE	335	C18H25O5N	480-79-5
6	642	337	P-(PENTYLOXY)ACETOPHENONE	206	C13H18O2	5467-56-1
7	641	388	BENZENEMETHANOL, .ALPHA.,4-DIMETHYL-, (.+/-.)-	136	C9H12O	5788-09-0
8	627	409	SENECIPHYLLINE	333	C18H23O5N	480-81-9
9	626	410	SENECIONINE	335	C18H25O5N	130-01-8
10	626	365	METHYL M-TOLYL CARBINOL	136	C9H12O	900342-29-9
11	607	494	.ALPHA. IONYL ACETATE	236	C15H24O2	52210-18-1
12	604	493	GERANIOL, PENTAFLUOROPROPIONATE	300	C13H17O2F5	900352-25-7
13	600	375	JACOBINE	351	C18H25O6N	6870-67-3
14	598	422	HEXANOIC ACID, 3,7-DIMETHYL-2,6-OCTADIENYL ESTER, (E)-	252	C16H28O2	10032-02-7
15	593	363	JACOZINE	349	C18H23O6N	5532-23-0
16	589	475	CARBAMIC ACID, N-PHENYL-, 1-METHYL-1-(4-METHYLCYCLOHEX-3-ENYL)ETHYL E	273	C17H23O2N	1224-46-0
17	584	479	GERANYL PHENYLACETATE	272	C18H24O2	102-22-7

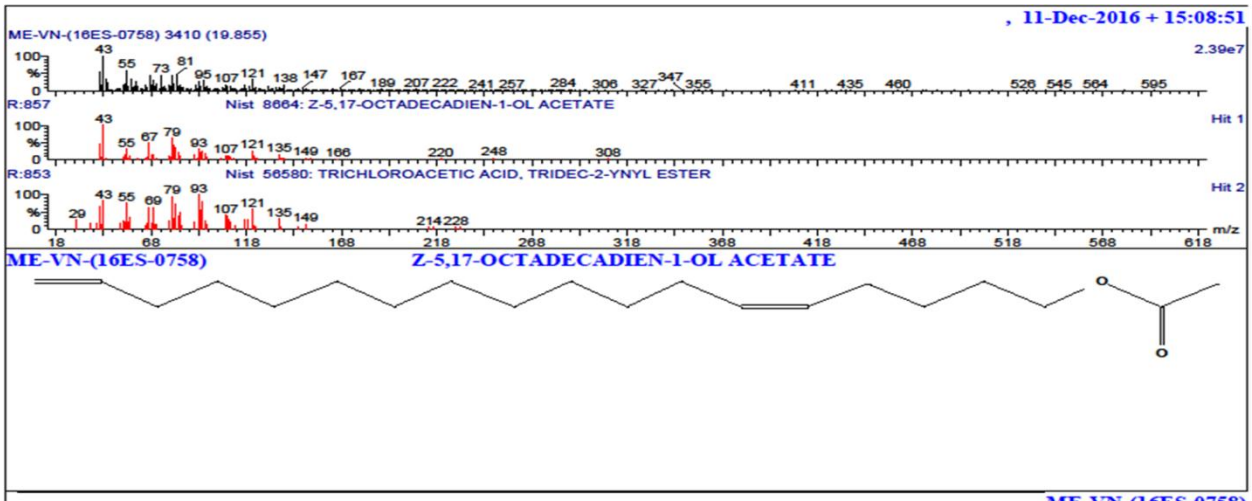




ME-VN-(16ES-0758)

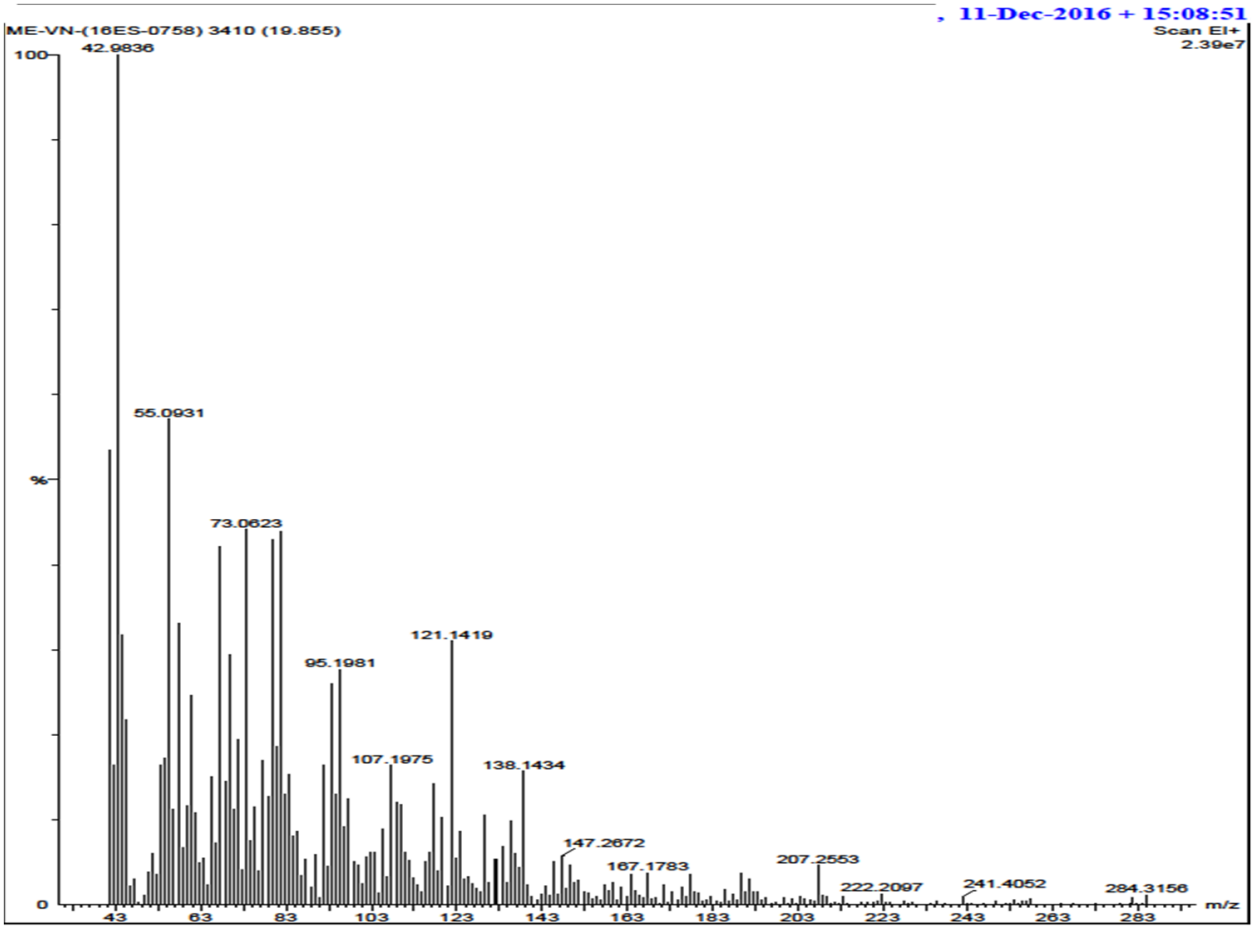
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	778	548	1-T-BUTYL-4-(ADAMANTYL-1)BENZENE	268	C20H28	59974-45-7
2	761	407	3.ALPHA.,5-CYCLO-5.ALPHA.-ERGOSTA-6,8(14).22T-TRIENE	378	C28H42	24352-51-0
3	675	421	.ALPHA.-KETOSTEARIC ACID, ETHYL ESTER	326	C20H38O3	111935-03-6
4	665	345	3.ALPHA.,5.ALPHA.-CYCLO-ERGOSTA-7,9(11),22T-TRIENE-6.BETA.-OL	394	C28H42O	118978-72-6
5	648	367	7,9(11),22-ERGOSTATRIENE	380	C28H44	900253-06-9
6	644	339	4,6,8(14)-CHOLESTATRIENE	366	C27H42	51028-26-3
7	639	345	2-BROMOLAURIC ACID	278	C12H23O2Br	111-56-8
8	636	314	OCTADECANOIC ACID, 2-OXO-, METHYL ESTER	312	C19H36O3	2380-18-9
9	629	375	ANDROGRAPHOLIDE	350	C20H30O5	5508-58-7
10	625	318	EICOSANOIC ACID, 2,3-BIS[(TRIMETHYLSILYL)OXY]PROPYL ESTER	530	C29H62O4Si2	55517-94-7
11	624	367	TETRACOSANOIC ACID, TRIMETHYLSILYL ESTER	440	C27H56O2Si	74367-37-6
12	624	316	1-HEPTADEC-1-YNYL-CYCLOHEXANOL	334	C23H42O	900296-77-9
13	621	333	11-EICOSENOIC ACID, TRIMETHYLSILYL ESTER	382	C23H46O2Si	900079-04-0
14	619	343	RHAMNITOL, 1-O-NONYL-	292	C15H32O5	900154-02-2
15	617	400	VITAMIN A PALMITATE	524	C36H60O2	79-81-2
16	615	396	2-(1-CHLORO-1-PROPENYL)-3,3-DIMETHYL-1-CYCLOPROPENYL(TRIMETHYL)SILA	214	C11H19ClSi	900261-29-9
17	611	310	HEXANOIC ACID, 3-HYDROXY-, ETHYL ESTER	160	C8H16O3	2305-25-1

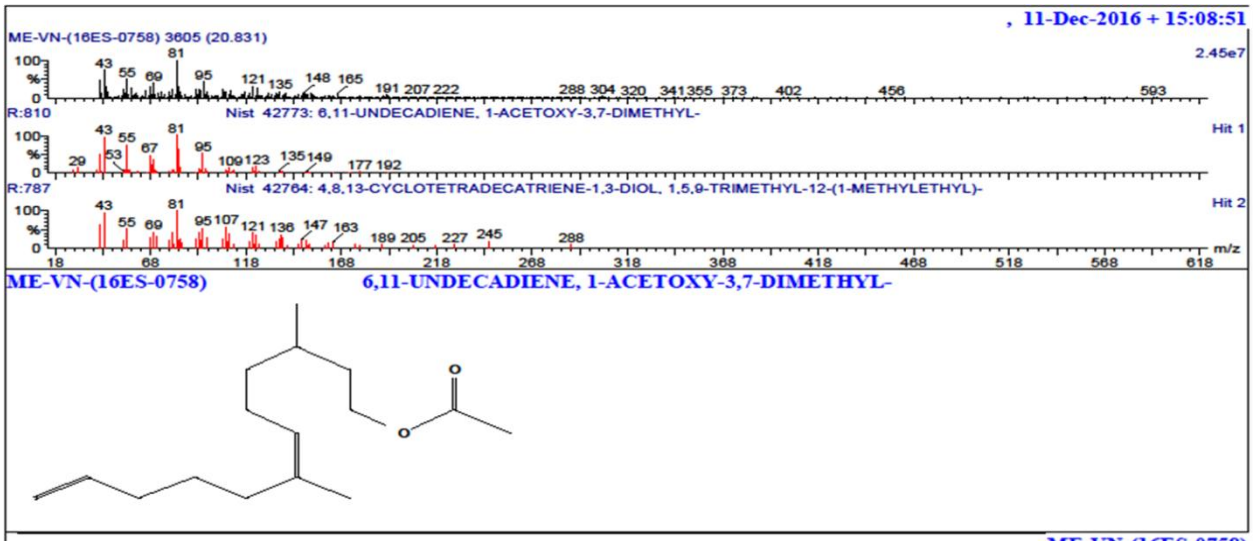




ME-VN-(16ES-0758)

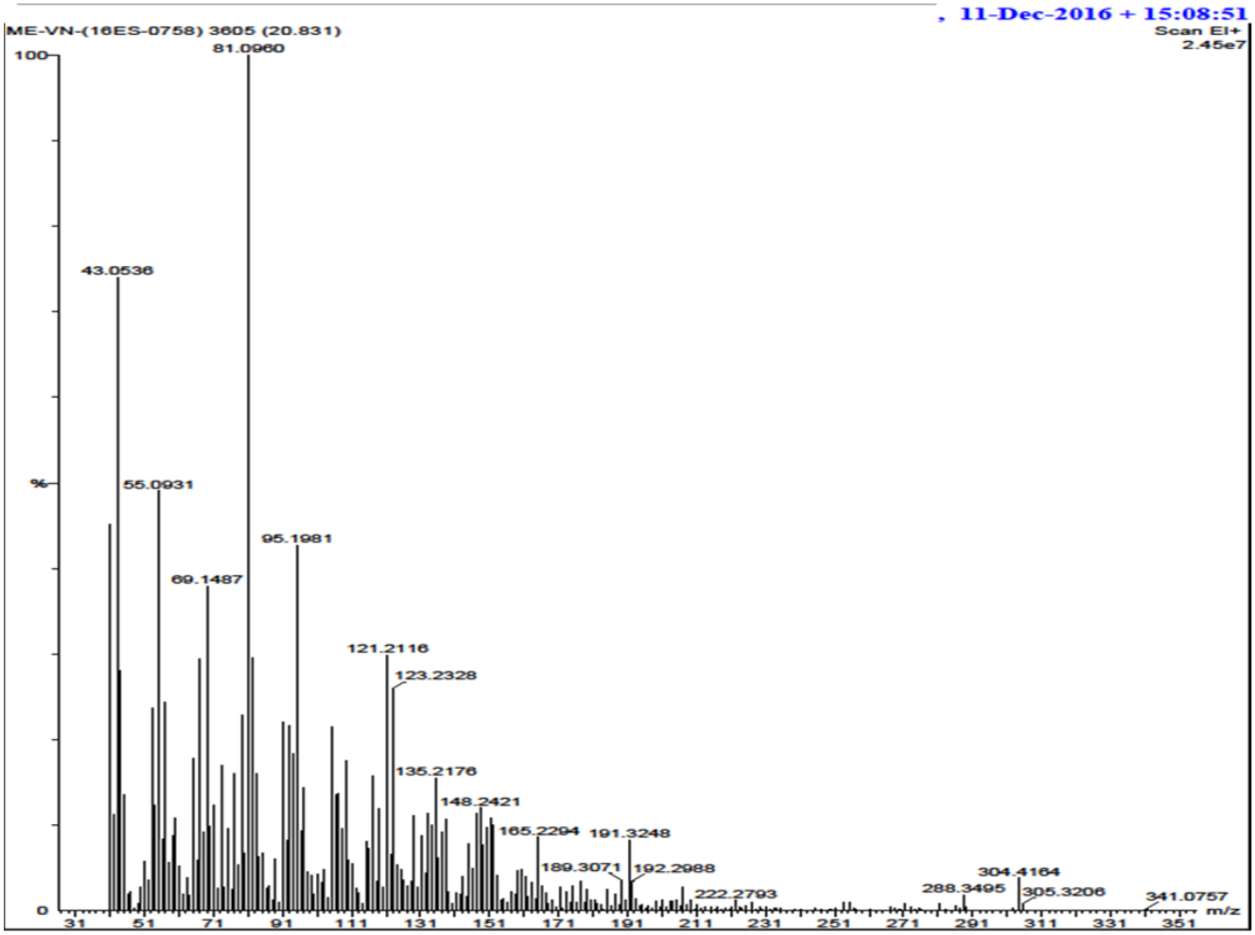
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	857	657	Z-5,17-OCTADECADIEN-1-OL ACETATE	308	C20H36O2	900131-06-9
2	853	657	TRICHLOROACETIC ACID, TRIDEC-2-YNYL ESTER	340	C15H23O2Cl3	900299-26-2
3	846	655	CHOLESTANE, 4,5-EPOXY-, (4.ALPHA.,5.ALPHA.)-	386	C27H46O	6079-19-2
4	842	585	11-DODECYN-1-OL ACETATE	224	C14H24O2	53596-78-4
5	842	580	2,2-DIBROMOCHOLESTANONE	542	C27H44OBr2	97370-79-1
6	842	636	Z,Z-4,6-NONADECADIEN-1-OL ACETATE	322	C21H38O2	900131-08-4
7	839	646	3-HEPTADECEN-5-YNE, (Z)-	234	C17H30	74744-55-1
8	837	566	2-BROMOCHOLESTANONE	464	C27H45OBr	22164-15-4
9	833	640	5-PENTADECEN-7-YNE, (Z)-	206	C15H26	74744-50-6
10	829	642	7-OXABICYCLO[4.1.0]HEPTANE, 1-METHYL-4-(2-METHYLOXIRANYL)-	168	C10H16O2	96-08-2
11	828	507	9,12,15-OCTADECATRIENOIC ACID, ETHYL ESTER, (Z,Z,Z)-	306	C20H34O2	1191-41-9
12	824	584	3-TETRADECEN-5-YNE, (E)-	192	C14H24	74744-44-8
13	820	674	7-OXABICYCLO[4.1.0]HEPTANE, 1-METHYL-4-(2-METHYLOXIRANYL)-	168	C10H16O2	96-08-2
14	820	610	1-NAPHTHALENEPROPANOL, .ALPHA.-ETHYLDECAHYDRO-5-(HYDROXYMETHYL)-.	308	C20H36O2	72401-52-6
15	818	569	KAURAN-18-AL, 17-(ACETYLOXY)-, (4.BETA.)-	346	C22H34O3	55902-84-6
16	817	454	METHYL 11,14,17-EICOSATRIENOATE	320	C21H36O2	900336-41-3
17	817	437	METHYL 2-HYDROXY-OCTADECA-9,12,15-TRIENOATE	308	C19H32O3	900336-39-1

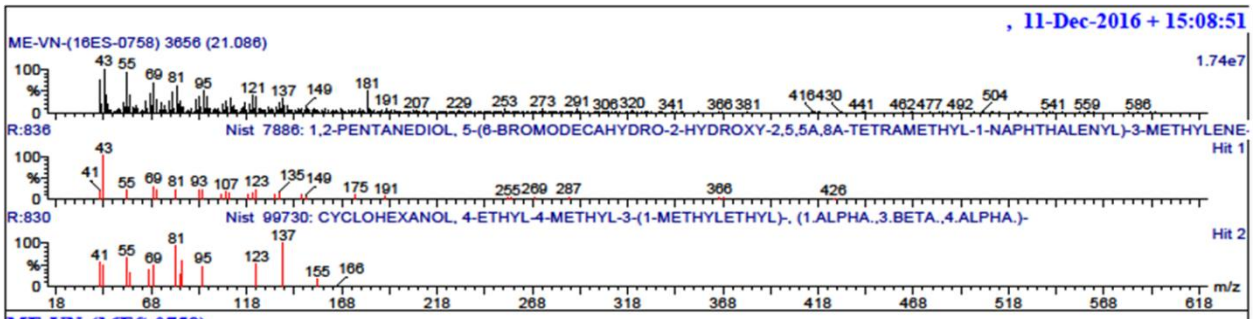


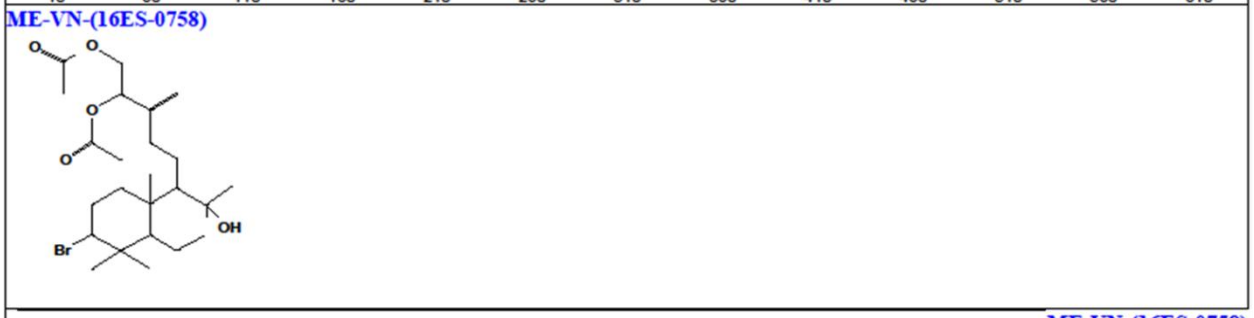


ME-VN-(16ES-0758)

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	810	615	6,11-UNDECADIENE, 1-ACETOXY-3,7-DIMETHYL-	252	C16H28O2	900150-66-0
2	787	649	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2
3	774	410	URS-12-ENE-1,2,11-TRIOL, 3-(ACETYLOXY)-, (1.BETA.,2.ALPHA.,3.BETA.,11.ALPHA.)	516	C32H52O5	108657-34-7
4	771	501	1,2-PENTANEDIOL, 5-(6-BROMODECAHYDRO-2-HYDROXY-2,5,5A,8A-TETRAMETHY	486	C24H39O5Br	115334-14-0
5	762	624	EPIGLOBULOL	222	C15H26O	900150-05-1
6	756	530	2,6-OCTADIENE, 2,6-DIMETHYL-	138	C10H18	2792-39-4
7	747	595	2,2,4-TRIMETHYL-3-(3,8,12,16-TETRAMETHYL-HEPTADECA-3,7,11,15-TETRAENYL)-	428	C30H52O	900194-01-4
8	746	503	6-OCTEN-1-OL, 3,7-DIMETHYL-, ACETATE	198	C12H22O2	150-84-5
9	743	540	CYCLOHEXANOL, 5-METHYL-2-(1-METHYLETHYL)-, ACETATE	198	C12H22O2	16409-45-3
10	740	499	6-OCTEN-1-OL, 3,7-DIMETHYL-, ACETATE	198	C12H22O2	150-84-5
11	738	537	6-OCTEN-1-OL, 3,7-DIMETHYL-, ACETATE	198	C12H22O2	150-84-5
12	734	513	3,7-DIMETHYL-6-NONEN-1-OL ACETATE	212	C13H24O2	900131-34-9
13	733	533	3,5-OCTADIENE, 2,7-DIMETHYL-, (Z,Z)-	138	C10H18	28980-73-6
14	733	547	BICYCLO[3.1.1]HEPTANE-2-METHANOL, 6,6-DIMETHYL-, [1S-(1.ALPHA.,2.ALPHA.,5.	154	C10H18O	53369-17-8
15	732	533	CYCLOHEXANE, 2-CHLORO-4-METHYL-1-(1-METHYLETHYL)-, [1S-(1.ALPHA.,2.BET	174	C10H19Cl	16052-42-9
16	732	516	O-TRIFLUOROACETYL-MENTHOL	252	C12H19O2F3	28587-50-0
17	731	493	MENTHYL ACETATE	198	C12H22O2	89-48-5

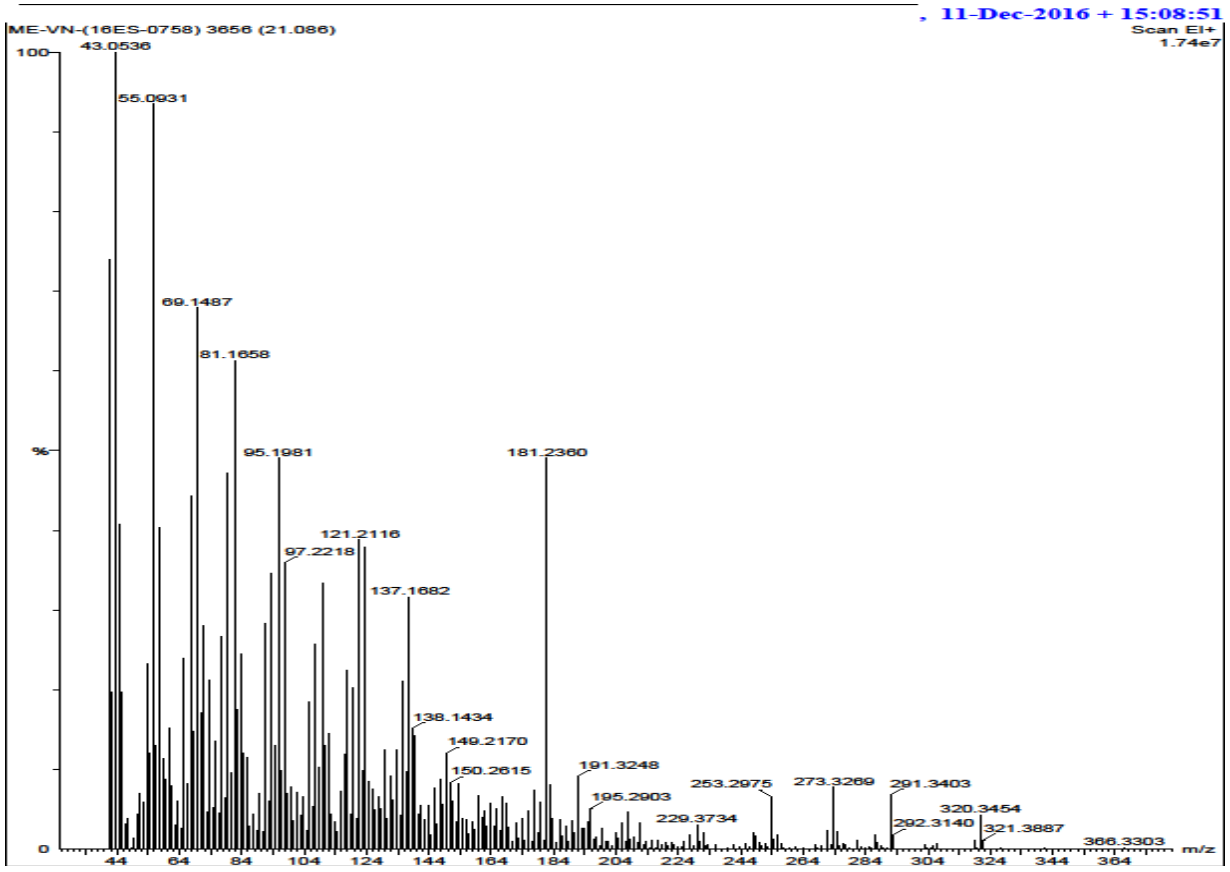


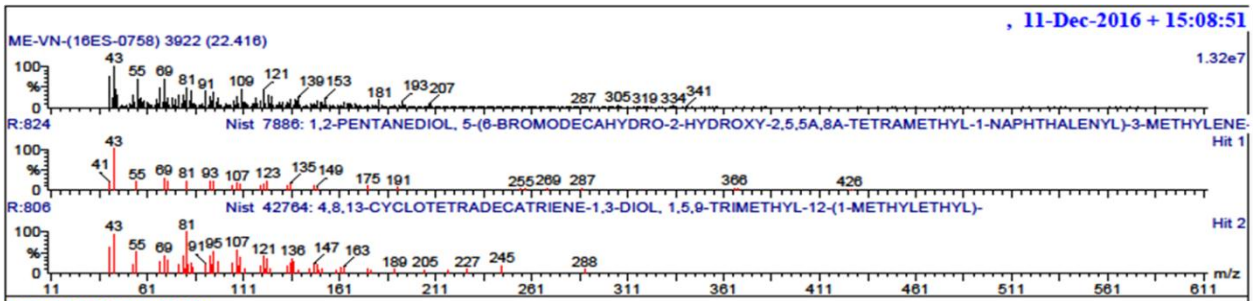


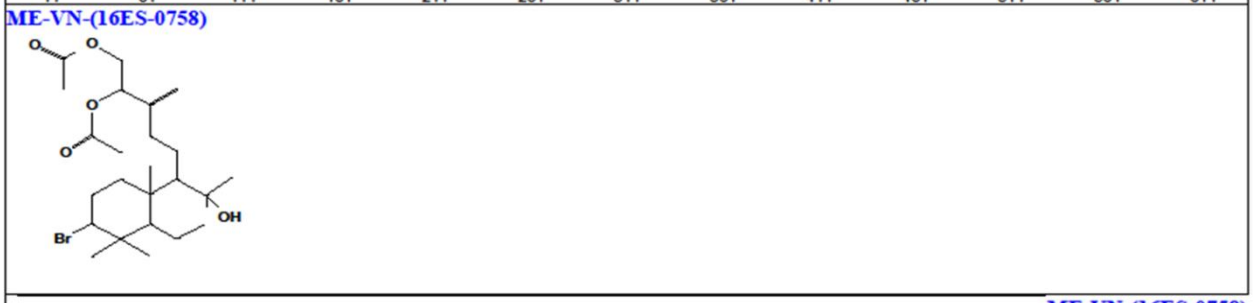


ME-VN-(16ES-0758)

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	836	517	1,2-PENTANEDIOL, 5-(6-BROMODECAHYDRO-2-HYDROXY-2,5,5A,8A-TETRAMETHY	486	C24H39O5Br	115334-14-0
2	830	434	CYCLOHEXANOL, 4-ETHYL-4-METHYL-3-(1-METHYLETHYL)-, (1.ALPHA.,3.BETA.,4.A	184	C12H24O	55869-53-9
3	827	433	CYCLOHEXANOL, 4-ETHYL-4-METHYL-3-(1-METHYLETHYL)-, (1.ALPHA.,3.ALPHA.,4.	184	C12H24O	55869-52-8
4	819	445	1-FORMYL-2,2,6-TRIMETHYL-3-(3-METHYL-BUT-2-ENYL)-6-CYCLOHEXENE	220	C15H24O	108287-18-9
5	802	721	ETHANONE, 1-[2-(5-HYDROXY-1,1-DIMETHYLHEXYL)-3-METHYL-2-CYCLOPROPEN-	224	C14H24O2	90165-05-2
6	799	629	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2
7	788	514	D:A-FRIEDOOLEANAN-28-AL, 3-OXO-	440	C30H48O2	14440-40-5
8	787	368	URS-12-ENE-1,2,11-TRIOL, 3-(ACETYLOXY)-, (1.BETA.,2.ALPHA.,3.BETA.,11.ALPHA.)	516	C32H52O5	108657-34-7
9	786	622	2,2,4-TRIMETHYL-3-(3,8,12,16-TETRAMETHYL-HEPTADECA-3,7,11,15-TETRAENYL)-	428	C30H52O	900194-01-4
10	782	685	1-CYCLOHEXENE-1-BUTANOL, 2,6,6-TRIMETHYL-	196	C13H24O	54344-91-1
11	778	606	ACETIC ACID, 1-[2-(2,2,6-TRIMETHYL-BICYCLO[4.1.0]HEPT-1-YL)-ETHYL]-VINYL EST	250	C16H26O2	77143-23-8
12	772	568	1,1':3',1"-TERCYCLOPENTANE, 2'-DODECYL-	374	C27H50	55282-68-3
13	761	563	2,5,5,8A-TETRAMETHYL-4-METHYLENE-6,7,8,8A-TETRAHYDRO-4H,5H-CHROMEN-4	238	C14H22O3	900192-73-0
14	753	485	ERGOST-25-ENE-3,5,6,12-TETROL, (3.BETA.,5.ALPHA.,6.BETA.,12.BETA.)-	448	C28H48O4	56052-97-2
15	747	581	2,4A,8,8-TETRAMETHYLDECAHYDROCYCLOPROPA[D]NAPHTHALENE	206	C15H26	74022-04-1
16	747	551	1,6,10,14,18,22-TETRACOSAHEXAEN-3-OL, 2,6,10,15,19,23-HEXAMETHYL-, (ALL-E)-	426	C30H50O	54159-46-5
17	745	574	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2

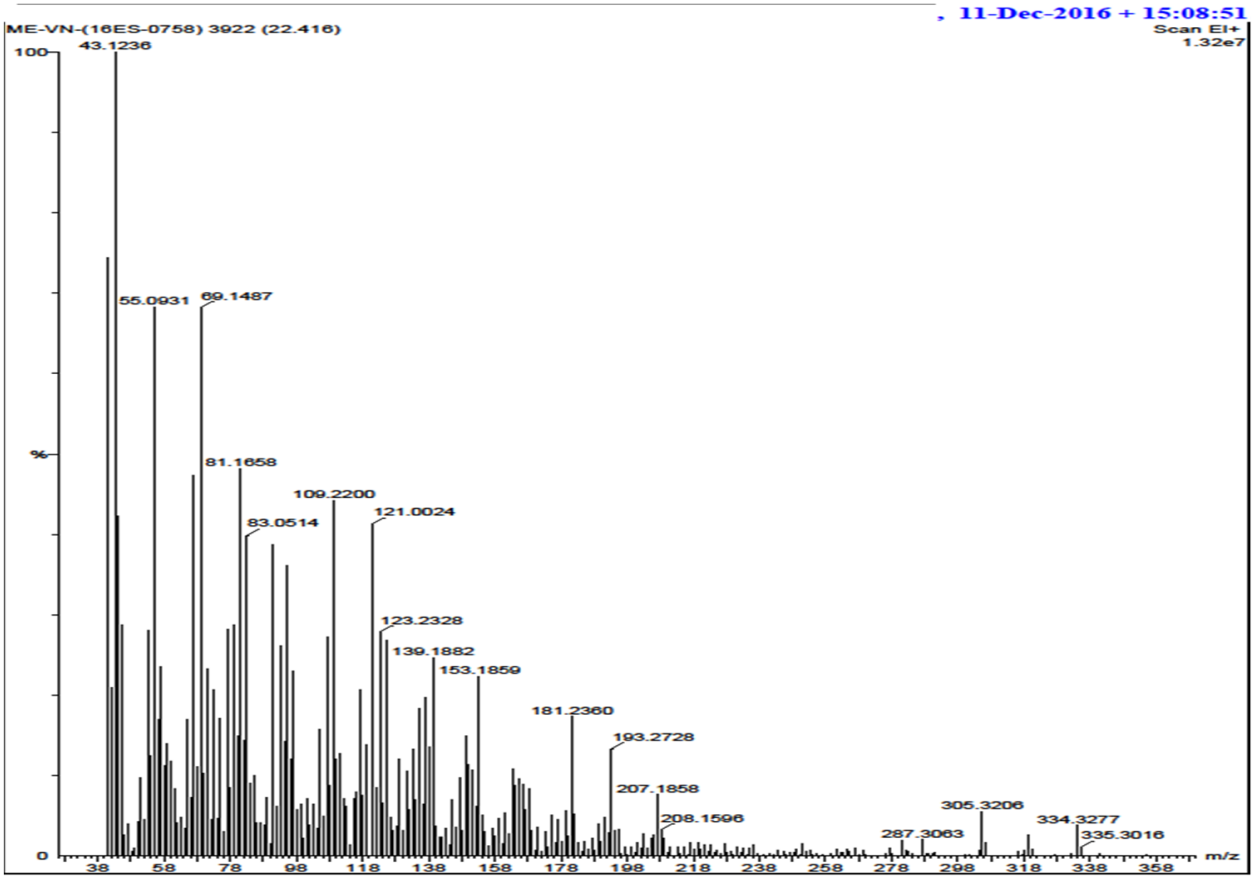


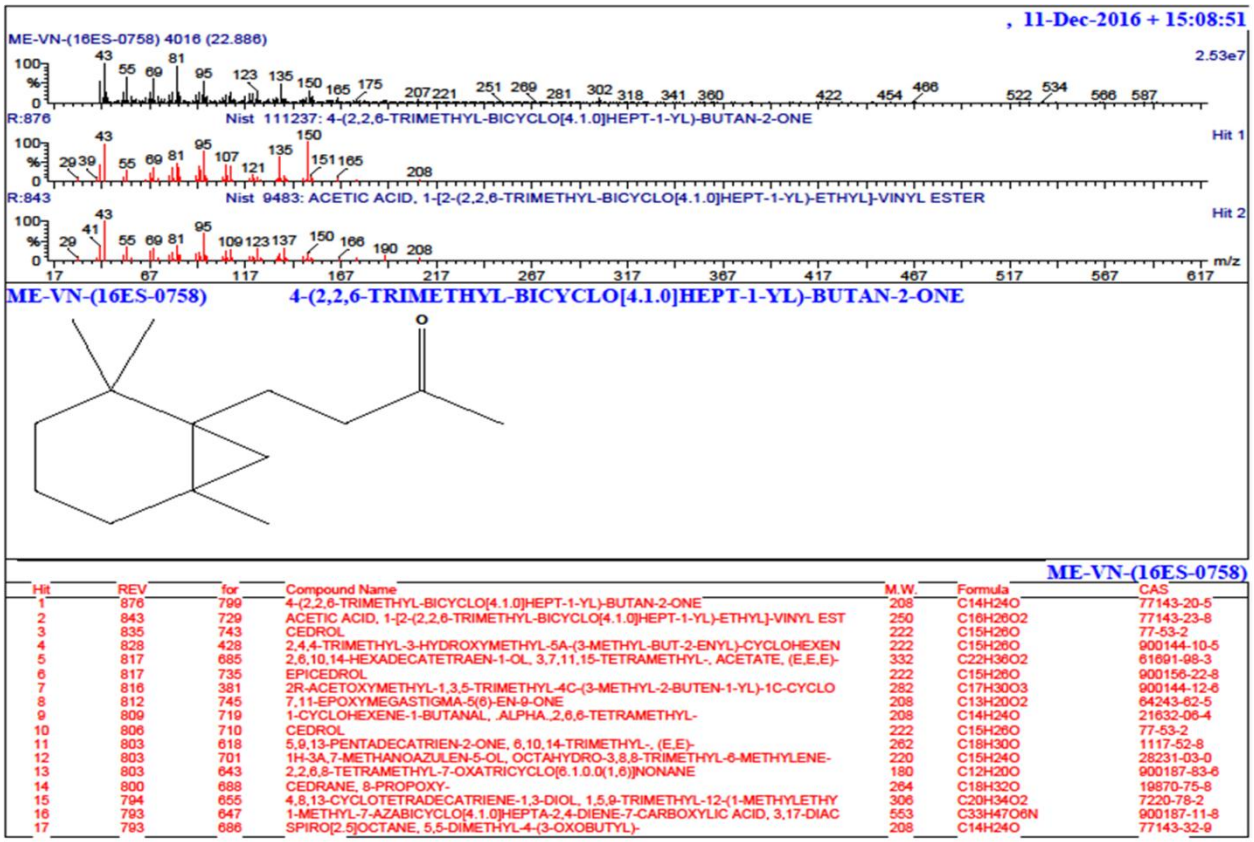




ME-VN-(16ES-0758)

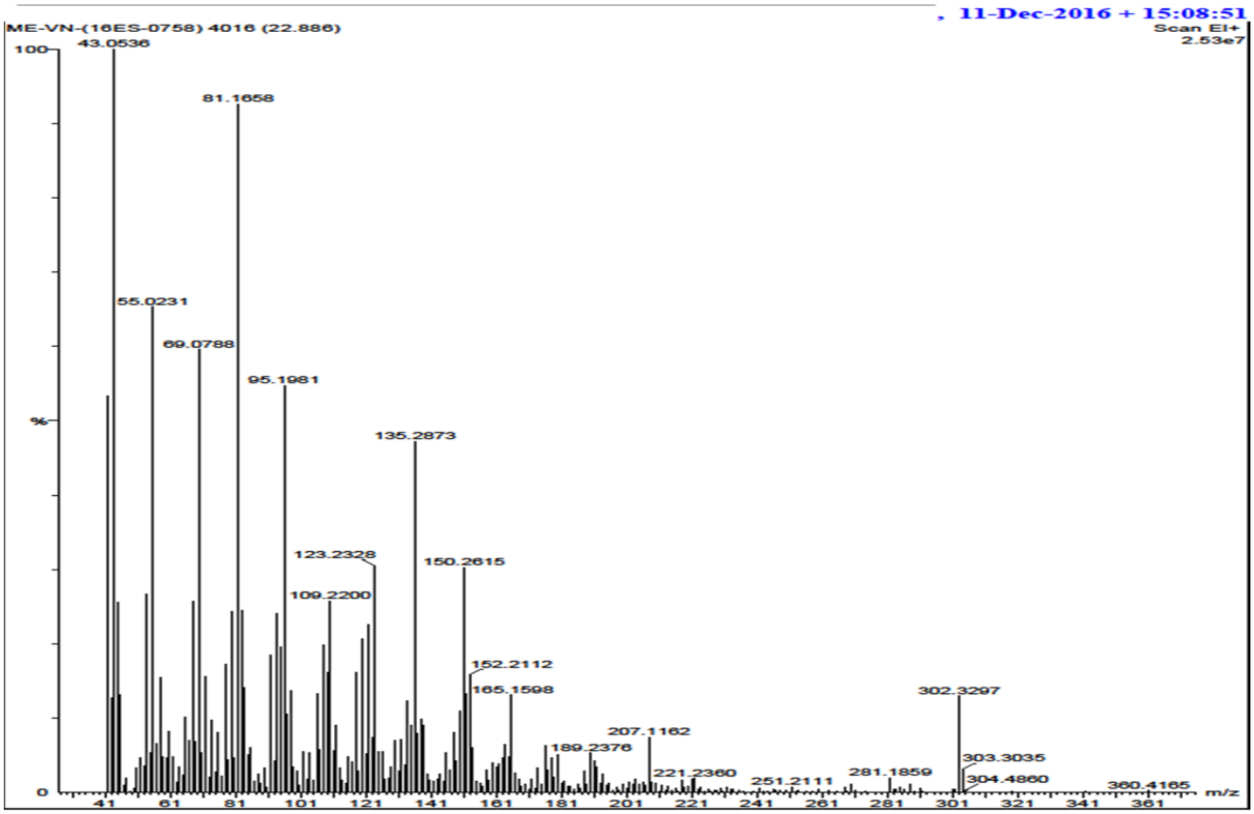
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	824	498	1,2-PENTANEDIOL, 5-(6-BROMODECAHYDRO-2-HYDROXY-2,5,5A,8A-TETRAMETHY	486	C24H39O5Br	115334-14-0
2	806	640	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2
3	798	634	TRICYCLO[20.8.0.0(7,16)]TRIACONTANE, 1(22),7(16)-DIEPOXY-	444	C30H52O2	900155-90-7
4	790	623	CIS-Z-.ALPHA.-BISABOLENE EPOXIDE	220	C15H24O	900131-71-2
5	783	575	9,19-CYCLOERGOST-24(28)-EN-3-OL, 4,14-DIMETHYL-, ACETATE, (3.BETA.,4.ALPH	468	C32H52O2	10376-42-8
6	781	549	ISOSTEVIOL	318	C20H30O3	27975-19-5
7	778	615	3,5,9-TRIMETHYL-DECA-2,4,8-TRIEN-1-OL	194	C13H22O	900188-00-3
8	778	535	9,19-CYCLOLANOST-24-EN-3-OL, ACETATE, (3.BETA.)-	468	C32H52O2	1259-10-5
9	775	591	2,5,5,8A-TETRAMETHYL-4-METHYLENE-6,7,8,8A-TETRAHYDRO-4H,5H-CHROMEN-4	238	C14H22O3	900192-73-0
10	771	547	PREGNAN-20-ONE, 3-HYDROXY-, (3.BETA.)-	318	C21H34O2	4406-37-5
11	771	577	THUNBERGOL	290	C20H34O	25269-17-4
12	769	581	(1R,2S,8R,8AR)-8-HYDROXY-1-(2-HYDROXYETHYL)-1,2,5,5-TETRAMETHYL-TRANS-	254	C16H30O2	900298-98-3
13	766	616	ETHYL ISO-ALLOCHOLATE	436	C26H44O5	900043-05-3
14	765	560	HYDROXYDEHYDROSTEVIC ACID	318	C20H30O3	471-80-7
15	765	577	2,2,4-TRIMETHYL-3-(3,8,12,16-TETRAMETHYL-HEPTADECA-3,7,11,15-TETRAENYL)-	428	C30H52O	900194-01-4
16	758	342	URS-12-ENE-1,2,11-TRIOL, 3-(ACETYLOXY)-, (1.BETA.,2.ALPHA.,3.BETA.,11.ALPHA.)	516	C32H52O5	108657-34-7
17	756	594	ACETIC ACID, 2-(2-ACETOXY-2,5,5,8A-TETRAMETHYLDECALIN-1-YL)-	310	C18H30O4	900296-70-3

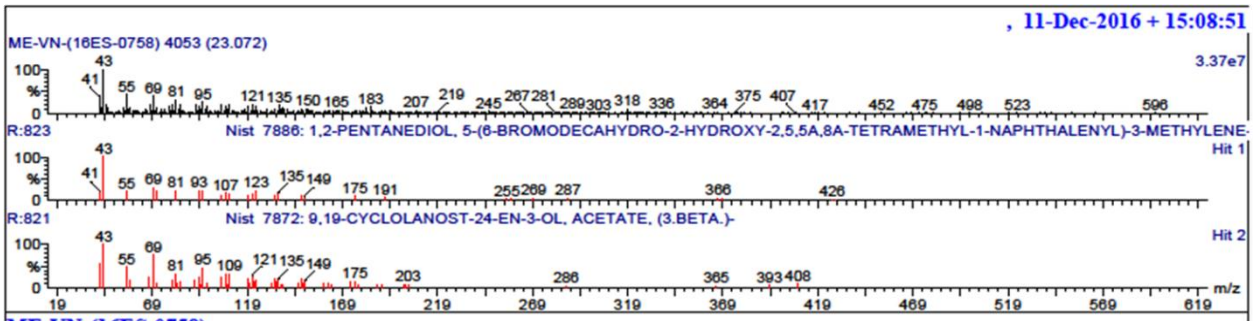


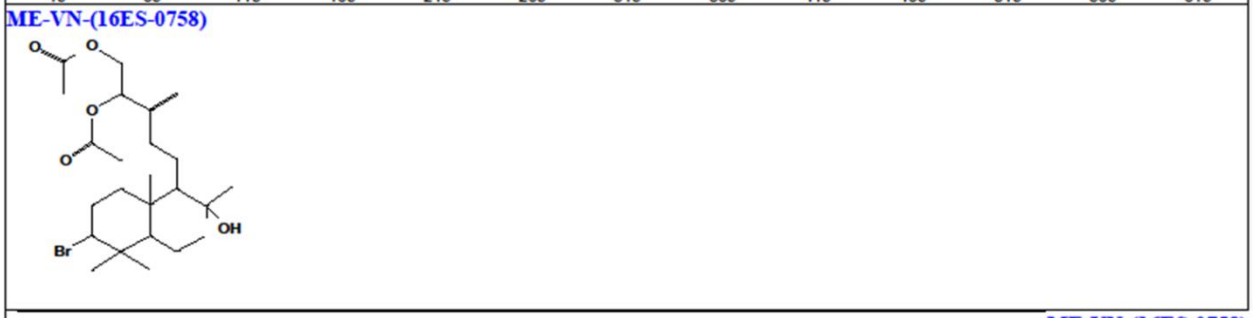


ME-VN-(16ES-0758)

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	876	799	4-(2,2,6-TRIMETHYL-BICYCLO[4.1.0]HEPT-1-YL)-BUTAN-2-ONE	208	C14H24O	77143-20-5
2	843	729	ACETIC ACID, 1-[2-(2,2,6-TRIMETHYL-BICYCLO[4.1.0]HEPT-1-YL)-ETHYL]-VINYL EST	250	C16H26O2	77143-23-8
3	835	743	CEDROL	222	C15H26O	77-53-2
4	828	428	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXEN	222	C15H26O	900144-10-5
5	817	685	2,6,10,14-HEXADECATETRAEN-1-OL, 3,7,11,15-TETRAMETHYL-, ACETATE, (E,E,E)-	332	C22H36O2	61691-98-3
6	817	735	EPICEDROL	222	C15H26O	900156-22-8
7	816	381	2R-ACETOXYMETHYL-1,3,5-TRIMETHYL-4C-(3-METHYL-2-BUTEN-1-YL)-1C-CYCLO	282	C17H30O3	900144-12-6
8	812	745	7,11-EPOXYMEGASTIGMA-5(6)-EN-9-ONE	208	C13H20O2	64243-62-5
9	809	719	1-CYCLOHEXENE-1-BUTANAL, .ALPHA.,2,6,6-TETRAMETHYL-	208	C14H24O	21632-06-4
10	806	710	CEDROL	222	C15H26O	77-53-2
11	803	618	5,9,13-PENTADECATRIEN-2-ONE, 6,10,14-TRIMETHYL-, (E,E)-	262	C18H30O	1117-52-8
12	803	701	1H-3A,7-METHANOAZULEN-5-OL, OCTAHYDRO-3,8,8-TRIMETHYL-6-METHYLENE-	220	C15H24O	28231-03-0
13	803	643	2,2,6,8-TETRAMETHYL-7-OXATRICYCLO[6.1.0.0(1,6)]NONANE	180	C12H20O	900187-83-6
14	800	688	CEDRANE, 8-PROPOXY-	264	C18H32O	19870-75-8
15	794	655	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2
16	793	647	1-METHYL-7-AZABICYCLO[4.1.0]HEPTA-2,4-DIENE-7-CARBOXYLIC ACID, 3,17-DIAC	553	C33H47O6N	900187-11-8
17	793	686	SPIRO[2.5]OCTANE, 5,5-DIMETHYL-4-(3-OXOBUTYL)-	208	C14H24O	77143-32-9

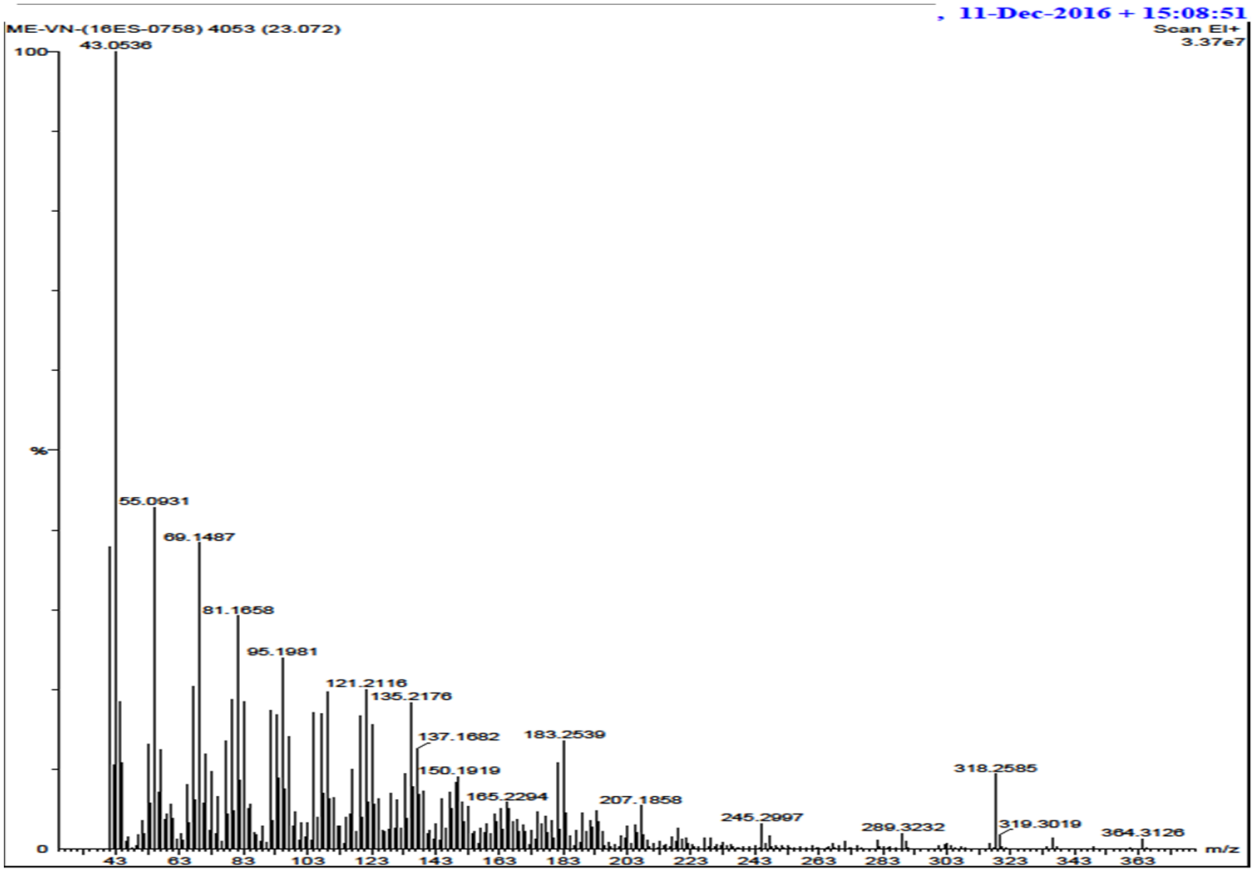


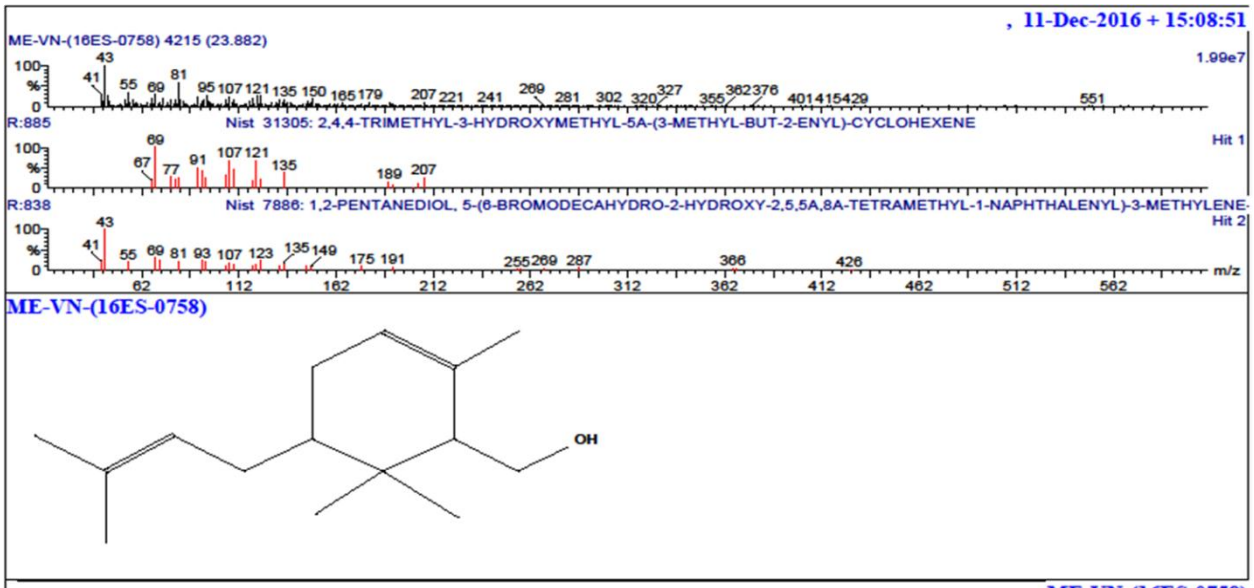




ME-VN-(16ES-0758)

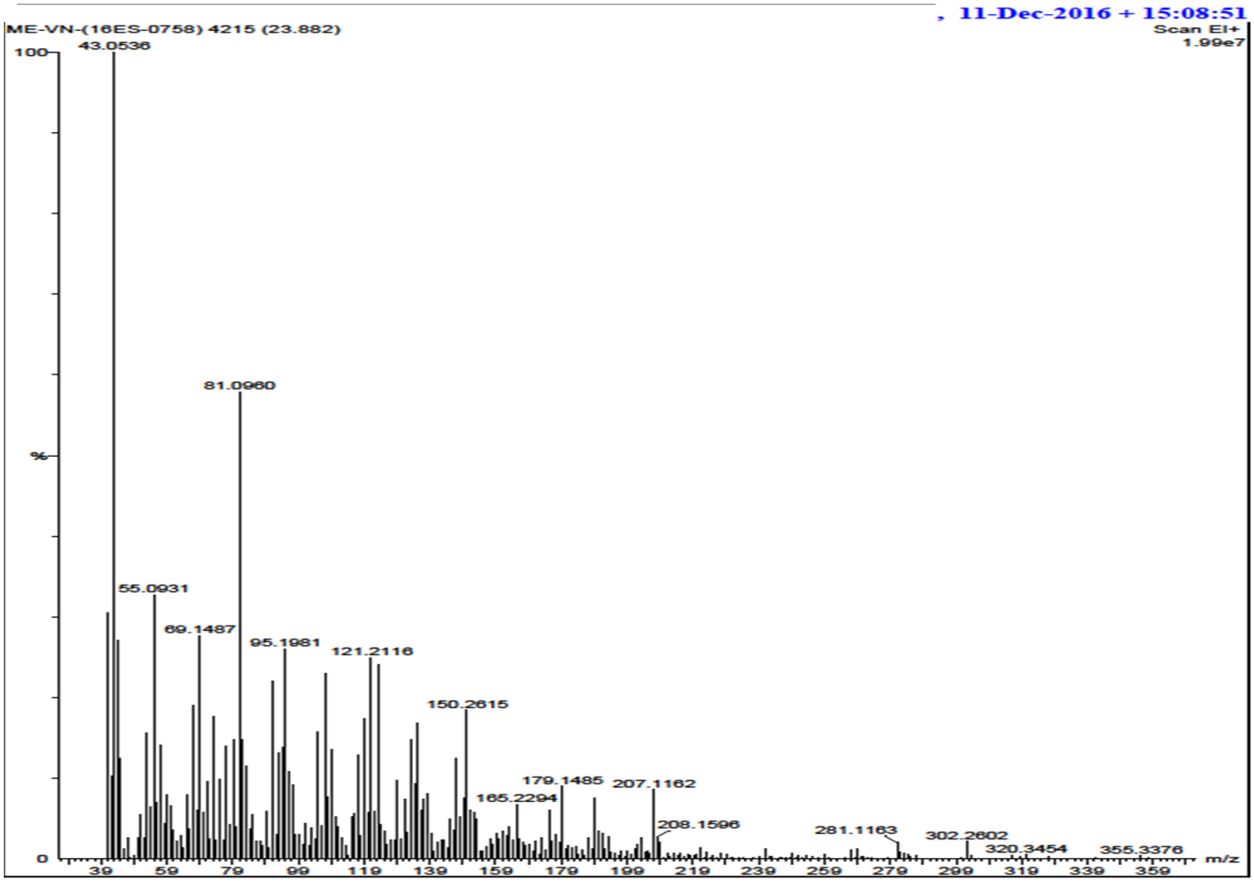
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	823	558	1,2-PENTANEDIOL, 5-(6-BROMODECAHYDRO-2-HYDROXY-2,5,5A,8A-TETRAMETHY	486	C24H39O5Br	115334-14-0
2	821	637	9,19-CYCLOLANOST-24-EN-3-OL, ACETATE, (3.BETA.)-	468	C32H52O2	1259-10-5
3	818	698	ACETIC ACID, 1-[2-(2,2,6-TRIMETHYL-BICYCLO[4.1.0]HEPT-1-YL)-ETHYL]-VINYL EST	250	C16H26O2	77143-23-8
4	814	371	2-ISOPROPYL-5-METHYLCYCLOHEXYL 3-(1-(4-CHLOROPHENYL)-3-OXOBUTYL)-C	524	C30H33O6Cl	900143-59-5
5	812	654	9,19-CYCLOERGOST-24(28)-EN-3-OL, 4,14-DIMETHYL-, ACETATE, (3.BETA.,4.ALPH	468	C32H52O2	10376-42-8
6	808	685	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2
7	800	613	METHYL 3-ACETOXY-12-KETO-18-NORCHOLANATE	432	C26H40O5	900251-87-8
8	793	428	URS-12-ENE-1,2,11-TRIOL, 3-(ACETYLOXY)-, (1.BETA.,2.ALPHA.,3.BETA.,11.ALPHA.)	516	C32H52O5	108657-34-7
9	791	649	THUNBERGOL	290	C20H34O	25269-17-4
10	786	627	2,6,10,14-HEXADECATETRAEN-1-OL, 3,7,11,15-TETRAMETHYL-, ACETATE, (E,E,E)-	332	C22H36O2	61691-98-3
11	785	618	1,2-PENTANEDIOL, 5-(6-BROMODECAHYDRO-2-HYDROXY-2,5,5A,8A-TETRAMETHY	402	C20H35O3Br	115346-29-7
12	782	587	9,19-CYCLOLANOSTAN-3-OL, ACETATE, (3.BETA.)-	470	C32H54O2	4575-74-0
13	781	593	CHOLESTA-22,24-DIEN-5-OL, 4,4-DIMETHYL-	412	C29H48O	900128-66-1
14	781	608	2,2-DIBROMOCHOLESTANONE	542	C27H44OBr2	97370-79-1
15	771	633	ANDROSTAN-3-ONE, 17-HYDROXY-2,4-DIMETHYL-, (2.ALPHA.,4.ALPHA.,5.ALPHA.,1	318	C21H34O2	54550-08-2
16	769	542	LANOSTA-8,24-DIEN-3-OL, ACETATE, (3.BETA.)-	468	C32H52O2	2671-68-3
17	762	581	CYCLOARTANOL	428	C30H52O	4657-58-3

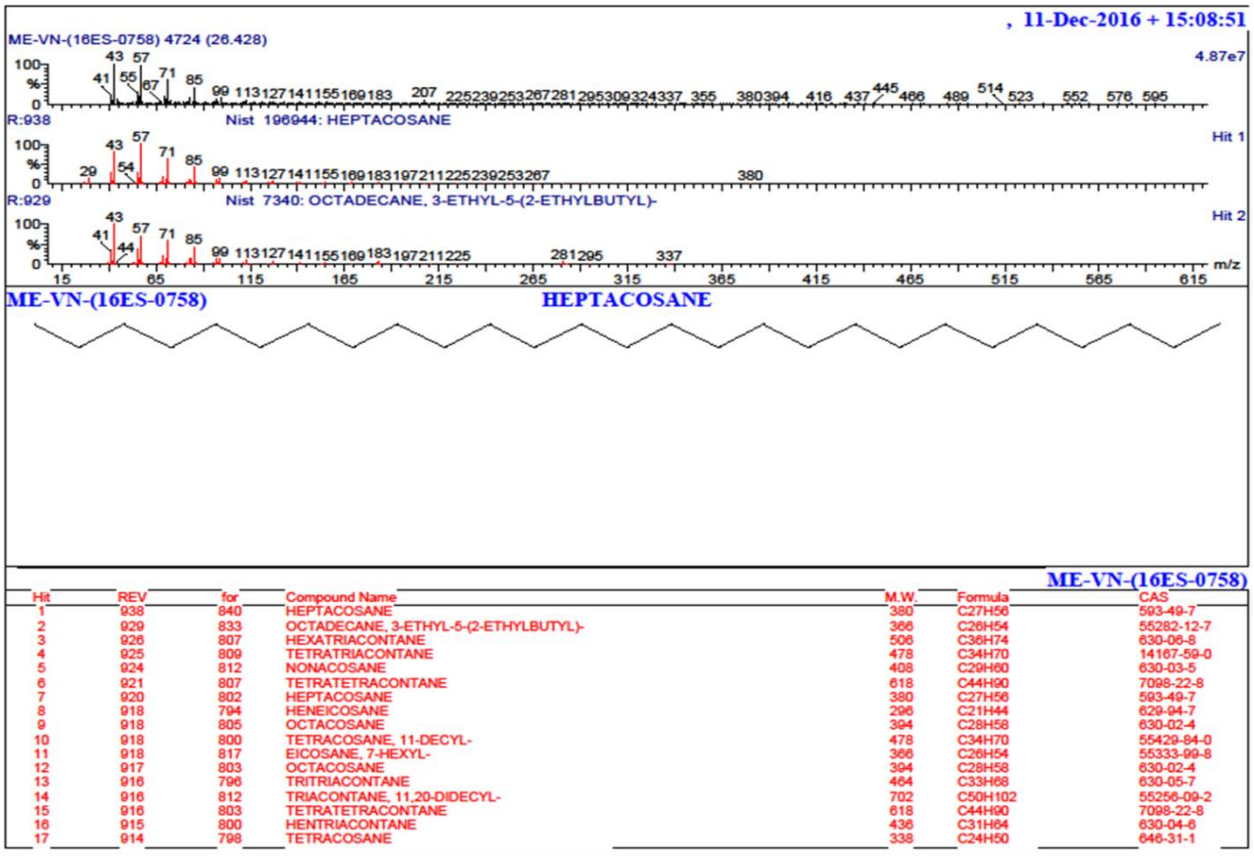




ME-VN-(16ES-0758)

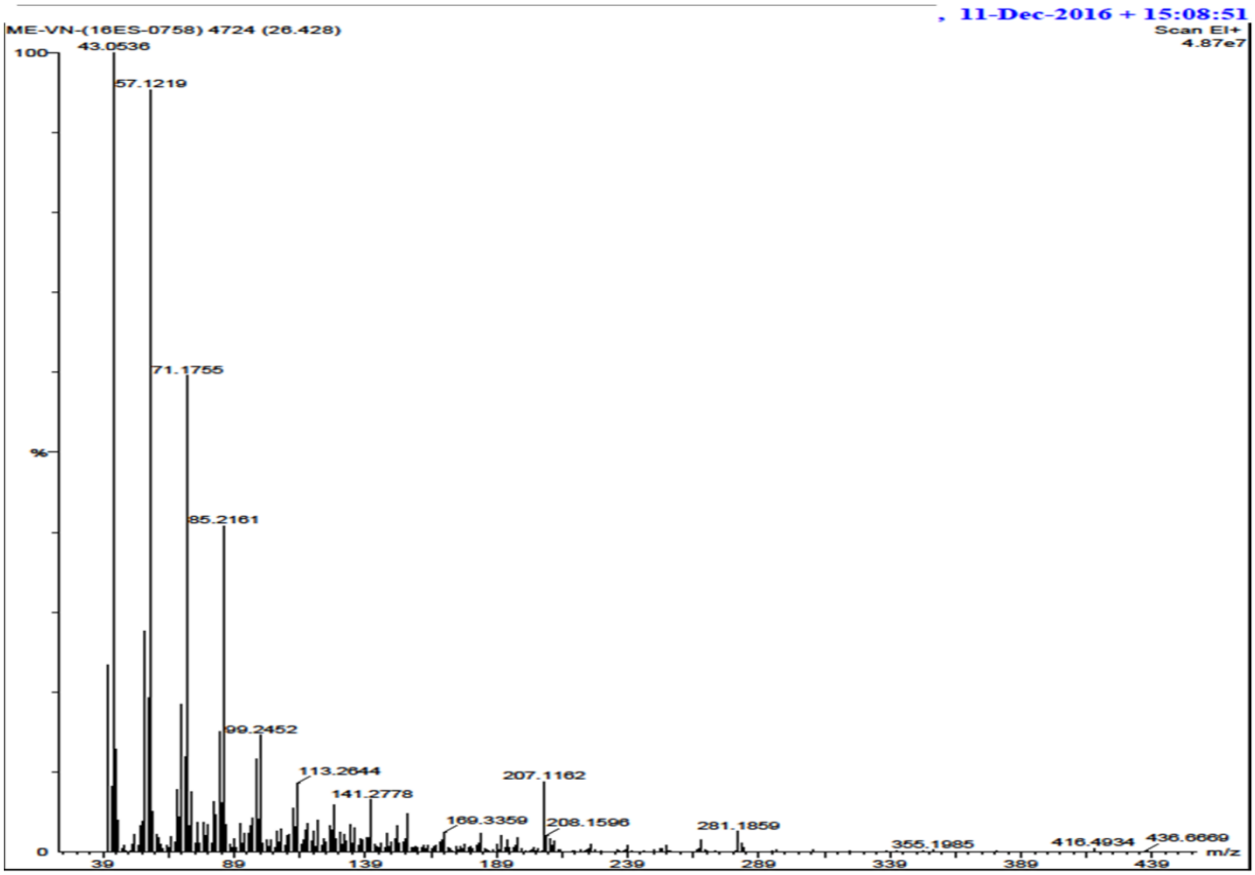
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	885	439	2,4,4-TRIMETHYL-3-HYDROXYMETHYL-5A-(3-METHYL-BUT-2-ENYL)-CYCLOHEXEN	222	C15H26O	900144-10-5
2	838	558	1,2-PENTANEDIOL, 5-(6-BROMODECAHYDRO-2-HYDROXY-2,5,5A,8A-TETRAMETHY	486	C24H39O5Br	115334-14-0
3	818	621	9,19-CYCLOLANOST-24-EN-3-OL, ACETATE, (3.BETA.)-	468	C32H52O2	1259-10-5
4	802	662	ACETIC ACID, 1-[2-(2,2,6-TRIMETHYL-BICYCLO[4.1.0]HEPT-1-YL)-ETHYL]-VINYL EST	250	C16H26O2	77143-23-8
5	795	657	4-(2,2,6-TRIMETHYL-BICYCLO[4.1.0]HEPT-1-YL)-BUTAN-2-ONE	208	C14H24O	77143-20-5
6	788	651	SPIRO[2.5]OCTANE, 5,5-DIMETHYL-4-(3-OXOBUTYL)-	208	C14H24O	77143-32-9
7	784	584	1,2-PENTANEDIOL, 5-(6-BROMODECAHYDRO-2-HYDROXY-2,5,5A,8A-TETRAMETHY	402	C20H35O3Br	115346-29-7
8	776	419	URS-12-ENE-1,2,11-TRIOL, 3-(ACETYLOXY)-, (1.BETA.,2.ALPHA.,3.BETA.,11.ALPHA.)	516	C32H52O5	108657-34-7
9	768	605	4,8,13-CYCLOTETRADECATRIENE-1,3-DIOL, 1,5,9-TRIMETHYL-12-(1-METHYLETHY	306	C20H34O2	7220-78-2
10	766	596	2,6,10,14-HEXADECATETRAEN-1-OL, 3,7,11,15-TETRAMETHYL-, ACETATE, (E,E,E)-	332	C22H36O2	61691-98-3
11	762	512	6.BETA.BICYCLO[4.3.0]NONANE, 5.BETA.-IODOMETHYL-1.BETA.-ISOPROPENYL-4.	332	C15H25I	900195-85-9
12	758	535	3-O-ACETYL-6-METHOXY-CYCLOARTENOL	498	C33H54O3	900286-40-9
13	756	551	THUNBERGOL	290	C20H34O	25269-17-4
14	754	606	1-CYCLOHEXENE-1-BUTANAL, .ALPHA.,2,6,6-TETRAMETHYL-	208	C14H24O	21632-06-4
15	750	564	2-HYDROXY-2,4,4-TRIMETHYL-3-(3-METHYLBUTA-1,3-DIENYL)CYCLOHEXANONE	222	C14H22O2	900191-17-4
16	747	610	7,11-EPOXYMEGASTIGMA-5(6)-EN-9-ONE	208	C13H20O2	64243-62-5
17	744	505	SPIRO[4.5]DEC-6-EN-1-OL, 2,6,10,10-TETRAMETHYL	208	C14H24O	900197-15-1

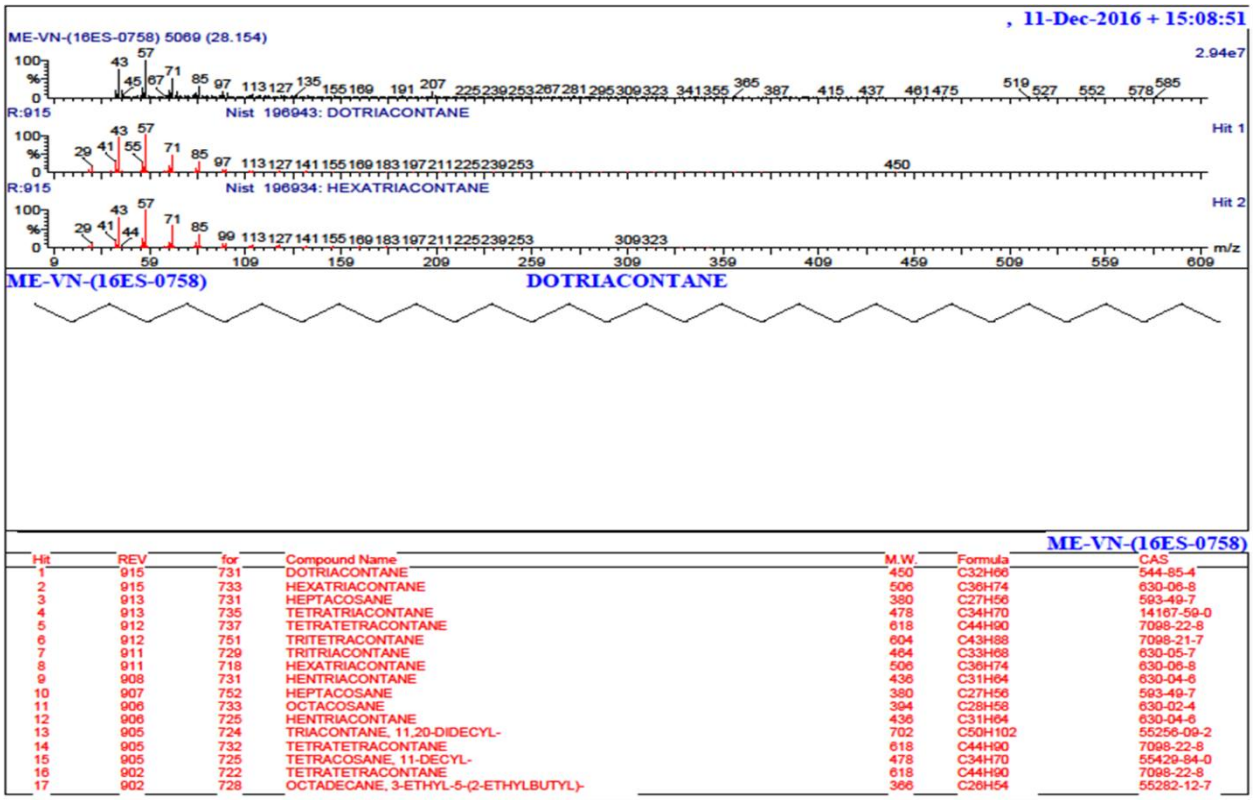




ME-VN-(16ES-0758)

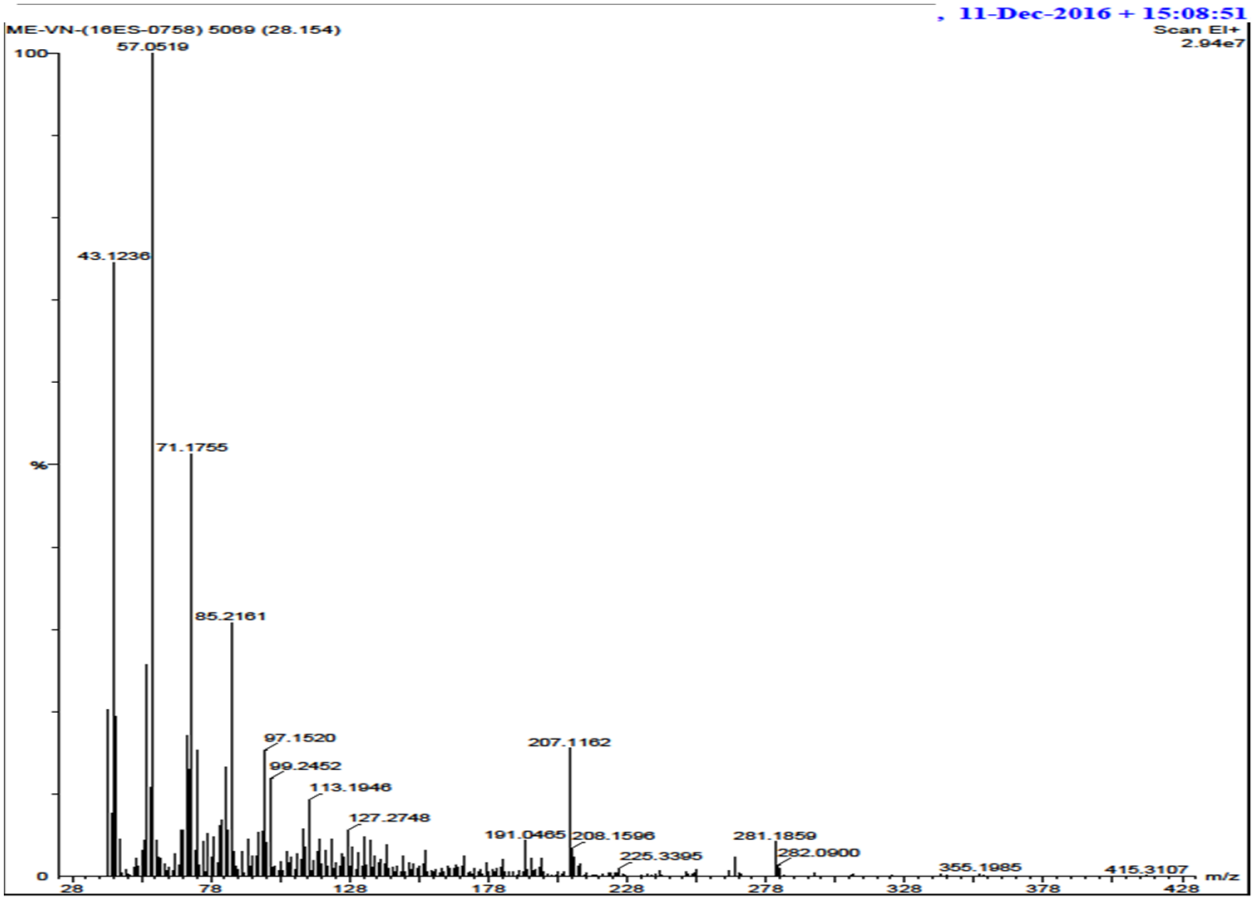
Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	938	840	HEPTACOSANE	380	C27H56	593-49-7
2	929	833	OCTADECANE, 3-ETHYL-5-(2-ETHYLBUTYL)-	366	C26H54	55282-12-7
3	926	807	HEXATRIACONTANE	506	C36H74	630-06-8
4	925	809	TETRATRIACONTANE	478	C34H70	14167-59-0
5	924	812	NONACOSANE	408	C29H60	630-03-5
6	921	807	TETRATETRACONTANE	618	C44H90	7098-22-8
7	920	802	HEPTACOSANE	380	C27H56	593-49-7
8	918	794	HENEICOSANE	296	C21H44	629-94-7
9	918	805	OCTACOSANE	394	C28H58	630-02-4
10	918	800	TETRACOSANE, 11-DECYL-	478	C34H70	55429-84-0
11	918	817	EICOSANE, 7-HEXYL-	366	C26H54	55333-99-8
12	917	803	OCTACOSANE	394	C28H58	630-02-4
13	916	796	TRITRIACONTANE	464	C33H68	630-05-7
14	916	812	TRIACONTANE, 11,20-DIDECYL-	702	C50H102	55256-09-2
15	916	803	TETRATETRACONTANE	618	C44H90	7098-22-8
16	915	800	HENTRIACONTANE	436	C31H64	630-04-6
17	914	798	TETRACOSANE	338	C24H50	646-31-1





ME-VN-(16ES-0758)

Hit	REV	for	Compound Name	M.W.	Formula	CAS
1	915	731	DOTRIACONTANE	450	C32H66	544-85-4
2	915	733	HEXATRIACONTANE	506	C36H74	630-06-8
3	913	731	HEPTACOSANE	380	C27H56	593-49-7
4	913	735	TETRATRIACONTANE	478	C34H70	14167-59-0
5	912	737	TETRATETRACONTANE	618	C44H90	7098-22-8
6	912	751	TRITETRACONTANE	604	C43H88	7098-21-7
7	911	729	TRITRIACONTANE	464	C33H68	630-05-7
8	911	718	HEXATRIACONTANE	506	C36H74	630-06-8
9	908	731	HENTRIACONTANE	436	C31H64	630-04-6
10	907	752	HEPTACOSANE	380	C27H56	593-49-7
11	906	733	OCTACOSANE	394	C28H58	630-02-4
12	906	725	HENTRIACONTANE	436	C31H64	630-04-6
13	905	724	TRIACONTANE, 11,20-DIDECYL-	702	C50H102	55256-09-2
14	905	732	TETRATETRACONTANE	618	C44H90	7098-22-8
15	905	725	TETRACOSANE, 11-DECYL-	478	C34H70	55429-84-0
16	902	722	TETRATETRACONTANE	618	C44H90	7098-22-8
17	902	728	OCTADECANE, 3-ETHYL-5-(2-ETHYLBUTYL)-	366	C26H54	55282-12-7



Result

Figure 1: *Vitex negundo*

Figure 2: Dried leaf powder of *Vitex negundo*

Figure 3: Soxhlet extraction using methanol

Figure 4: Rotary vacuum evaporator

Figure 5: Methanolic extract of VN (After drying)

Antibacterial activity for the methanolic extract of VN

i. Well diffusion method:

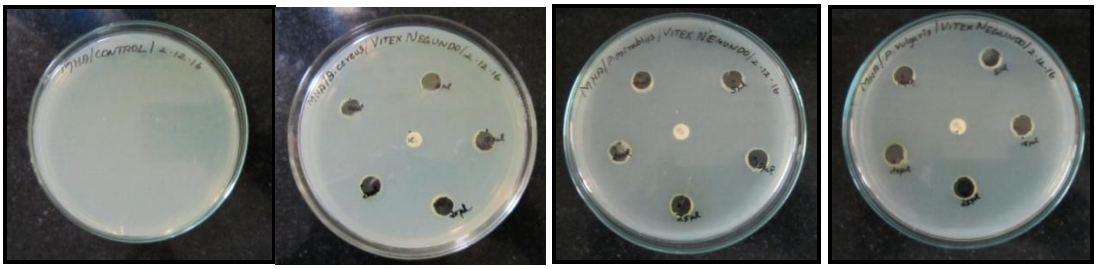


Figure 6: Antibacterial activity for VN (methanolic extract)
***B.cereus, P.mirabilis, P.vulgaris* (before inoculation)**

Figure 7: Antibacterial activity for VN (methanolic extract)
B.cereus, P.mirabilis, P.vulgaris (after inoculation)

Table 1: Extractive yield (%) of VN

Name of the extract	Empty beaker wt (g)	Sample + beaker wt (g)	Sample wt (g)	Extract yield % (25g)	Extract yield % (100g)
Methanol	30.1662	34.0001	25	3.8339	15.34

Table 2: Antibacterial activity – Zone of Inhibition (mm)

INHIBITION ZONE(mm)							
Bacteria	**Methanolic extract (VN)**					**R**	**MIC (µg/ml)**
	5 (µl)	**10 (µl)**	**15 (µl)**	**20 (µl)**	**25 (µl)**		
B.cereus	0	0	0	0	0	24	0
P.mirabilis	0	0	0	0	0	18	0
P.vulgaris	0	0	0	12	13	7	55.03

DISCUSSION

In recent years, the interest for the study of the organic compounds found in plants and their activity has increased. The GC–MS is an ideal technique for qualitative and quantitative analysis of active components in plant. The aim of the present study was to confirm the phytochemicals present in the plant extracts and to evaluate their antibacterial activity. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic key. Different phytochemicals have been found to have a broad range of activities, which may help in protection against chronic diseases.(Liu RH., 2003)

The discovery of novel antimicrobial metabolites from medicinal plants is an important alternative to overcome the increasing levels of drug resistance by human pathogens. Due to the world's urgent need for new antibiotics and chemotherapeutic agents, growing interest is taken into the research on the chemistry of medicinal plants. Antimicrobial activity of medicinal plants and their phytoconstituents have been deliberated in the late 19th century [L.L. Zaika., 1998]. In the present study, leaf extract of ***Vitex nigundo linn*** was evaluated for antibacterial activity against opportunistic bacterial pathogens *Bacillus cereus*, *Proteus vulgaris* and *Proteus mirabilis*. The results revealed that the methanolic leaf extract of ***Vitex nigundo linn*** showed considerable antibacterial activity against *P.vulgaris*.

GC–MS analysis plays a key role in the analysis of components of plant origin. Generally, the plant materials are highly complexes, which make GC–MS well suited for their analysis because of its high sensitivity and selectivity. It is considered to be the gold standard in scientific analysis (M. Sajewicz, ETAL., 2009, K. Balamurugan ETAL., 2012). Several phytochemical screening studies have been carried out in different parts of the world by using GC–MS [D. Dubey ETAL., 2014, G.M. Doshi ETAL., 2015). The methanolic leaf extract of ***Vitex nigundo linn*** used for bioactive compounds investigation using the GC–MS analysis.

In this study, we reported for the first time a high resolution GC–MS method for the evaluation of the chemical constituents of methanolic leaf extract of ***Vitex nigundo linn***. This accurate and sensitive analysis of methanolic leaf extract of ***Vitex nigundo linn*** revealed the various phytoconstituents present in the extracts. The major bioactive compounds in methanolic leaf extract of ***Vitex nigundo linn is phosphonic acid.*** It is the major compound present in this plant extract. Ledol, benzene methanol 1 – t – butyl – 4 (adamantly-1) benzene, Z-5,17 – octadecadiene – 1-ol acetate, 6, 11 – Undecadiene, 1-acetoxy – 3,7-dimethyl, 4,(2,2,6-trimethyl – bicycle(4.1.0) hept – 1-yl)-butane – 2-one, heptacosone, dotriacontane, hydroxyl dehydro steric acid another major compounds in the studied plant extracts.

The results of the present investigation also complement the ethnobotanical usage of the studied plants which possess several phytoconstituents with biological activity. Based on the present investigation, it is concluded that methanolic leaf extract of ***Vitex nigundo linn*** have potential source of bioactive compounds with great pharmaceutical value. The study can be extended for separation and *in vitro*, *in vivo* evaluation of bioactive compounds in novel drug discovery.

CONCLUSION

In the present study, more than sixteen chemical constituents have been confirmed from the methanolic leaf extract of ***Vitex nigundo linn*** using GC-MS analysis. The antibacterial study result indicated that the lowest MIC against *B.cereus and P.mirabilis* was observed in methanol extract, against kanamycin. The results also revealed that the methanolic leaf extract of ***Vitex nigundo linn*** showed considerable antibacterial activity against *P.vulgaris*. The presence of various phytochemical constituents justifies the use of plant for various ailments by traditional practitioners. Further studies are needed on these extracts in order to isolate, identify, characterize and elucidate the structure of these compounds.

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