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Research Article

Cyclamen persicum: Methanolic Extract Using Gas Chromatography-Mass Spectrometry (GC-MS) Technique

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ABSTRACT

Cyclamen was traditionally classified in the family Primulaceae, was reclassified in the subfamily Myrsinoideae within the family Primulaceae. The objective of this study was analysis of the secondary metabolite products. Bioactives are chemical compounds often referred to as secondary metabolites. Thirty eight bioactive compounds were identified in the methanolic extract of *Cyclamen persicum*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Cyclamen persicum* revealed the existence of the3-Oxo-androsta-1,4-dien-17β-spiro-2'-3'-oxo-oxetane, 3,5-Dithiahexanol 5,5-dioxide, 1-(2-Nitrophenyl)piperazine, Oxime-,methoxy-phenyl- , Cyclohexene, 1-methyl-4-(1-methylethenyl)-,(S)-, D-Limonene , Fumaric acid,3-methylbut-3-enyl undecyl ester, Geranyl vinyl ether, 3,6,9,12-Tetraoxatetradecan -1-ol,14-[4-(1-,1,3,3-tetramethylbu, Cis-5,8,11,14,17-Eicosapentaenoic acid, α-Terpineol, 3-Allyl-6-methoxyphenol, 3-Cyclohexene-1-methanol,α,α,4-trimethyl-,acetate, Orcinol, 4,5-di-epi-aristolochene, Trans-calamenene, 3-(N,N-Dimethyllaurylammonio)propanesulfonate, Deoxyqinghaosu, Atranorin , N-[4-(4-Chlorophenyl)isothiazol-5-yl)-1-methylpiperidin-2-imine, 10-Heptadecen-8-ynoic acid , methyl ester, (E)-, 2-Pentadecanone,6,10,14-trimethyl-, Caffeine, 4,4,8-Trimethyltricyclo[6.3.1.0(1.5)]dodecane-2,9-diol , Bufa-20,22-dienolide, 3,14-dihydroxy-,(3β,5β)-, 1-(3-methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol , 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 9-Octadecenamide,(Z)-, 9,10-Secocholesta -5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-, Tributyl acetylcitrate, Cyproheptadine, 3,9-Epoxypregn-16-en-20-one , 3-methoxy-7,11,18-triacetoxy- , 17-Pentatriacontene, Phthalic acid , bis(7-methyloctyl) ester, Phthalic acid, di(6-ethyl-3-octyl) ester, Ergosterol, γ-Sitosterol and Friedelan-3-one.

Keywords: Spectral analysis, Compounds, GC-MS, *Cyclamen persicum*.

INTRODUCTION

Cyclamen is Medieval Latin, from earlier Latin cyclamīnos, because of the round tuber[1,2]. In English, the species of the genus are commonly called by the genus name. Is a genus of 23 species of perennial flowering plants in the family Primulaceae. Cyclamen species are native to Europe and the Mediterranean Basin east to Iran[3], with one species in Somalia. They grow from tubers and are valued for their flowers with upswept petals and variably patterned leaves. Species: *Cyclamen africanum, Cyclamen abchasicum, Cyclamen alpinum, Cyclamen balearicum, Cyclamen cilicium, Cyclamen colchicum, Cyclamen confusum, Cyclamen coum, Cyclamen creticum, Cyclamen cyprium, Cyclamen elegans, Cyclamen graecum, Cyclamen hederifolium, Cyclamen intaminatum, Cyclamen libanoticum, Cyclamen mirabile, Cyclamen parviflorum, Cyclamen persicum, Cyclamen pseudibericum, Cyclamen purpurascens, Cyclamen repandum, Cyclamen rhodium, Cyclamen rohlfsianum, and Cyclamen somalense*. In many languages, cyclamen species are colloquially called by a name like the English sowbread, because they are said to be eaten by pigs: pain de pourceau in French, pan porcino in Italian, varkensbrood in Dutch, "pigs' manjū" in Japanese. In addition, the saponins stimulate the sensitive receptors present in the nasal mucosa, inducing a nociceptive response transmitted by the trigeminal nerve[4-6]. The nasal mucosa is entirely innervated by the trigeminal nerve, and therefore the cholinergic response generated in the nasal cavity is observed throughout the nasal mucosa, favouring opening of the ostium, increasing glandular secretions and increasing ciliar movement in the entire area[7]. The accumulated secretions in the sinuses are consequently drained through the nose, providing rapid symptomatic relief of nasal congestion.

MATERIALS AND METHODS

Gas chromatography – Mass Spectrum analysis

Interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA). The database consists of more than 62,000 patterns of known compounds. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library[8-12].

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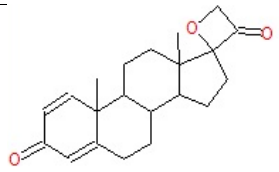
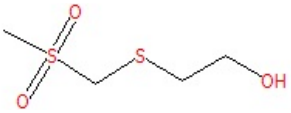
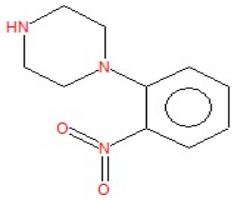
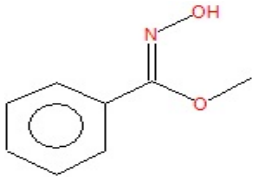
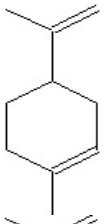
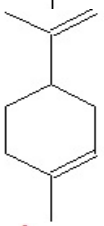
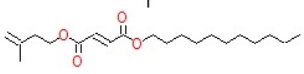
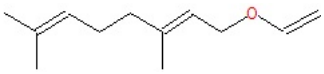
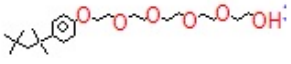
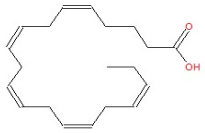
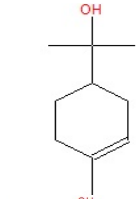
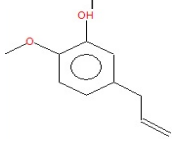
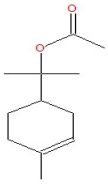
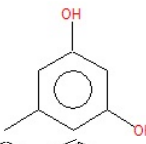
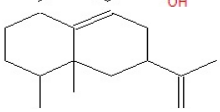
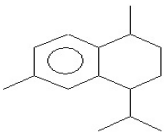
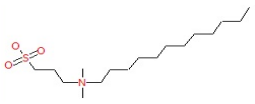
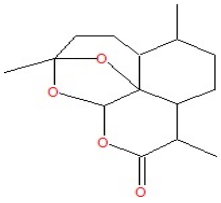
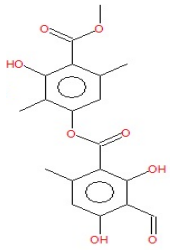
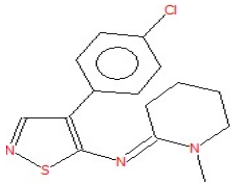
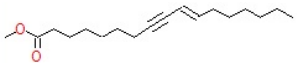
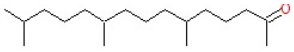
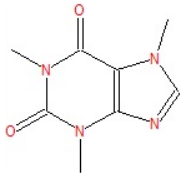
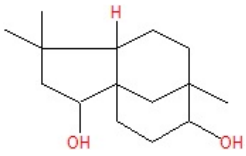
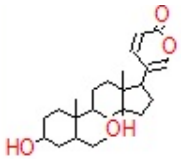
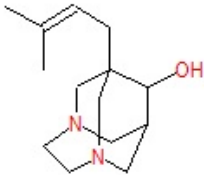
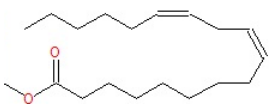
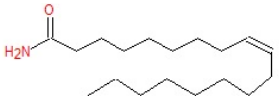
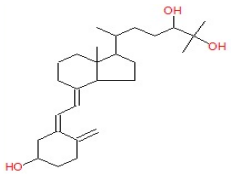
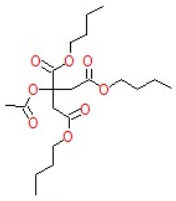
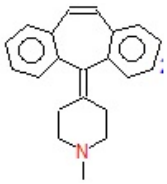
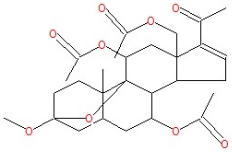
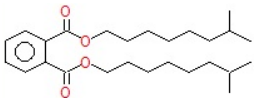
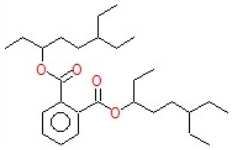
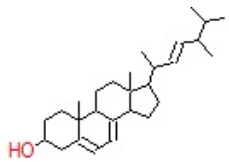
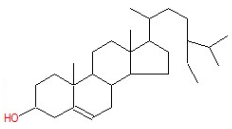
Cyclamen persicum GC–MS analysis were carried out in a GC system (Agilent 7890A series, USA). The flow rate of the carrier gas, helium (He) was set to beat 1 mL

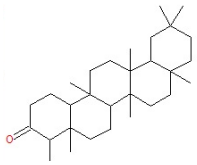
Table 1: Major phytochemical compounds identified in methanolic extract of Cyclamen persicum.

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragmentions	Pharmacological actions
1.	3-Oxo-androsta-1,4-dien-17β-spiro-2'-3'-oxo-oxetane	3.224	326	326.188194		55,77,91,105,122,147,159,173,199,227,281,326	New chemical compound
2.	3,5-Dithiahexanol 5,5-dioxide	3.287	170	170.007136		61,81,91,111,140,170	*anti*-tumor
3.	1-(2-Nitrophenyl) piperazine	3.779	207	207.100777		56,77,119,135,165,207	*anti-tumor*
4.	Oxime-,methoxy-phenyl-	3.917	151	151.063329		73,105,133,151	*anti*-inflammatory
5.	Cyclohexene , 1-methyl-4-(1-methylethenyl)-,(S)-	4.163	136	136.1252		53,68,79,93,136	New chemical compound
6.	D-Limonene	4.489	136	136.1252		53,68,79,93,136	*Anti*-stress *effects*
7.	Fumaric acid ,3-methylbut-3-enyl undecyl ester	5.072	338	338.24571		55,68,83,157,211,253	New chemical compound
8.	Geranyl vinyl ether	5.141	180	180.151415		53,69,81,93,136	Antioxidant, Antibacterial Activity
9.	3,6,9,12-Tetraoxatetradecan -1-	5.284	426	426.29814		57,69,89,113,135,161,175,207,223,249,267,	New chemical compound

	ol,14-[4-(1-,1,3,3-tetramethylbu					281,311,355,379,426	
10.	Cis-5,8,11,14,17-Eicosapentaenoic acid	5.587	302	302.22458		55,67,79,91,133,166,206,292	*anti*-inflammatory, antithrombotic and immunomodulatory *actions*
11.	α-Terpineol	5.919	154	154.135765		59,81,93,121,136	*Antimicrobial effect*
12.	3-Allyl-6-methoxyphenol	6.537	164	164.08373		55,65,77,103,149,164	*antimicrobial activity*
13.	3-Cyclohexene-1-methanol,α,α,4-trimethyl-,acetate	7.350	196	196.14633		68,81,93,121,136,181	*anti*-inflammatory *activity*
14.	Orcinol	7.819	124	124.0524297		55,67,77,95,107,124	*anti*-inflammatory agents
15.	4,5-di-epi-aristolochene	8.963	204	204.1878		79,105,161,189	New chemical compound
16.	Trans-calamenene	9.227	202	202.172151		159,202	*Antimicrobial* and *anti*-inflammatory
17.	3-(N,N-Dimethyllaurylammonio)propanesulfonate	9.358	335	335.249414		58,69,84,97,122,152,180,213	*anti*-bacterial activity
18.	Deoxyqinghaosu	9.902	266	266.15181		55,81,124,165,195,222,266	*anti*-malarial

19.	Atranorin	9.965	374	374.100168		53,77,136,150,164,179,196,374	*anti*-inflammatory agents
20.	N-[4-(4-Chlorophenyl)isothiazol-5-yl)-1-methylpiperidin-2-imine	11.904	305	305.075346		55,70,98,149,178,246,305	Unknown
21.	10-Heptadecen-8-ynoic acid, methyl ester , (E)-	11.967	278	278.22458		57,79,91,150,278	*anti*-inflammatory *activities*
22.	2-Pentadecanone ,6,10,14-trimethyl-	12.477	268	268.276615		58,71,85,165,210,250	*anti*-inflammatory, antioxidant
23.	Caffeine	12.854	194	194.080376		55,82,94,109,165,194	*anti*-inflammatory
24.	4,4,8-Trimethyltricyclo[6.3.1.0(1.5)]dodecane-2,9-diol	13.083	238	238.19328		55,67,107,164,182,220,238	*anti*-inflammatory *activities*
25.	Bufa-20,22-dienolide , 3,14-dihydroxy-,(3β,5β)-	13.501	386	386.24571		55,79,93,147,207,250,281,325,350,368	Unknown
26.	1-(3-methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol	14.582	236	236.188864		58,72,111,162,219,236	Unknown
27.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	14.886	294	294.25588		55,67,81,95,150,164,220,263,294	antibacterial *effects*
28.	9-Octadecenamide,(Z)-	15.738	281	281.271864		59,72,83,114,184,220,264,281	*anti*-bacterial activity

29.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-	16.162	416	416.329044		55,118,136,158,207,253,383,416	Unknown
30.	Tributyl acetylcitrate	16.373	402	402.225368		57,112,129,157,185,213,259,329	*antimicrobial activity*
31.	Cyproheptadine	17.689	287	287.1674		70,96,215,229,287	antihistamine
32.	3,9-Epoxypregn-16-en-20-one , 3-methoxy-7,11,18-triacetoxy-	18.027	518	518.251583		55,124,163,209,325,357,490	Unknown
33.	17-Pentatriacontene	18.187	490	490.547752		57,69,97,292,407,490	antimicrobial, *anti*-inflammatory, and anticancer properties
34.	Phthalic acid , bis(7-methyloctyl) ester	20.796	418	418.30831		57,71,85,127,149,167,231,275,293,347,418	*anti*-inflammatory activity
35.	Phthalic acid , di(6-ethyl-3-octyl) ester	21.369	446	446.33961		57,84,104,149,167,307	antimicrobial and *anti*-inflamatory activities
36.	Ergosterol	23.194	396	396.339216		55,69,119,143,211,253,271,293,337,363,396	*anti*-fungal
37.	γ-Sitosterol	24.081	414	414.386166		55,81,145,213,255,273,303,329,396,414	*anti*-inflammatory *activity*

38.	Friedelan-3-one	25.683	426	426.386166		55,69,109,163, 205,246,273,302,341,426	*anti-*microbial *properties*

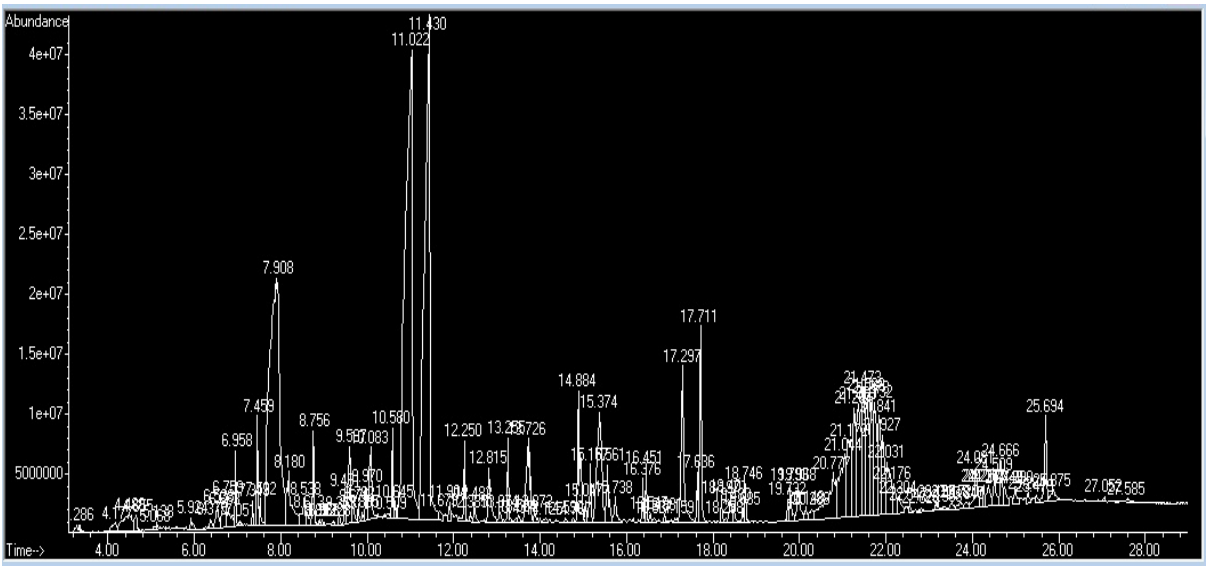


Figure 1: GC-MS chromatogram of methanolic extract of *Cyclamen persicum.*

min−1, split ratio was 1:50. The injector temperature was adjusted at 250◦C, while the detector temperature was fixed to280 Cº. The column temperature was kept at 40◦C for 1 min followed by linear programming to raise the temperature from 40◦to 120◦C (at 4 Cº min−1with 2 min hold time), 120 Cº to 170 Cº (at 6 Cº min−1with 1 min hold time) and 170 Cº to 200 Cº (at10◦C min−1with 1 min hold time). The transfer line was heated at 280 Cº. Two microliter of FAME sample was injected for analysis. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z[13-17].

Statistical analysis

Results of the study were based on analysis of variance (ANOVA) using Statistica Software. A significance level of 0.05 was used for all statistical tests [18].

RESULTS AND DISCUSSION

Identification of biochemical compounds

Analysis of compounds was carried out in methanolic extract of *Cyclamen persicum*, shown in Table 1. The GC-MS chromatogram of the peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Cyclamen persicum* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined as follows. All peaks were determined to be 3-Oxo-androsta-1,4-dien-17β-spiro-2'-3'-oxo-oxetane , 3,5-Dithiahexanol 5,5-dioxide , 1-(2-Nitrophenyl)piperazine , Oxime-,methoxy-phenyl- , Cyclohexene , 1-methyl-4-(1-methylethenyl)-,(S)- , D-Limonene , Fumaric acid ,3-methylbut-3-enyl undecyl ester , Geranyl vinyl ether , 3,6,9,12-Tetraoxatetradecan -1-ol,14-[4-(1-,1,3,3-tetramethylbu, Cis-5,8,11,14,17-Eicosapentaenoic acid, α-Terpineol , 3-Allyl-6-methoxyphenol , 3-Cyclohexene-1-methanol,α,α,4-trimethyl-,acetate , Orcinol , 4,5-di-epi-aristolochene, Trans-calamenene, 3-(N,N-Dimethyllaurylammonio)propanesulfonate , Deoxyqinghaosu, Atranorin, N-[4-(4-Chlorophenyl)isothiazol-5-yl)-1-methylpiperidin-2-imine , 10-Heptadecen-8-ynoic acid , methyl ester , (E)- , 2-Pentadecanone ,6,10,14-trimethyl- , Caffeine , 4,4,8-Trimethyltricyclo[6.3.1.0(1.5)]dodecane-2,9-diol , Bufa-20,22-dienolide , 3,14-dihydroxy-,(3β,5β)- , 1-(3-methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol , 9,12-Octadecadienoic acid (Z,Z)-, methyl ester , 9-Octadecenamide,(Z)- , 9,10-Secocholesta -5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- , Tributyl acetylcitrate , Cyproheptadine , 3,9-Epoxypregn-16-en-20-one , 3-methoxy-7,11,18-triacetoxy- , 17-Pentatriacontene , Phthalic acid , bis(7-methyloctyl) ester , Phthalic acid , di(6-ethyl-3-octyl) ester , Ergosterol , γ-Sitosterol and Friedelan-3-one Figure 2-38. Cyclamens are plants native to an area of southern Europe, northern Africa and western Asia bordering the Mediterranean Sea. The Cyclamen genus comprises around 20 species, the most familiar being C purpurascens, widely cultivated as a houseplant for its showy, dark green leaves flecked with silver, and nodding white, pink or red flowers with their familiar, reflexed petals. In medieval times cyclamen retained its plethora of uses, but became used increasingly in the treatment of rheumatic and arthritic conditions. Recent research has focused on reported anti-inflammatory and antinociceptive effects of cyclamen extracts. The roots contain triterpene glycosides known as

saponins and researchers at the University of Padua in Italy have found that extracts of the tubers of Cyclamen repandum show promising activity when tested on rats

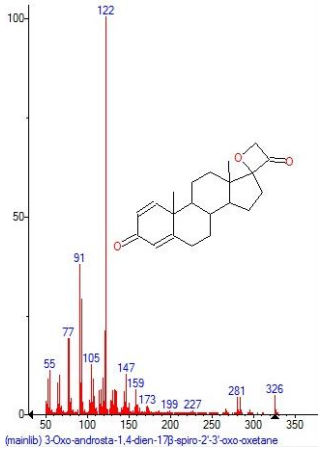


Figure 2: Mass spectrum of 3-Oxo-androsta-1,4-dien-17β-spiro-2'-3'-oxo-oxetane with Retention Time (RT)= 3.224

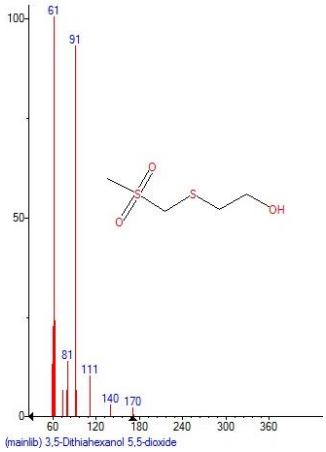


Figure 3: Mass spectrum of 3,5-Dithiahexanol 5,5-dioxide with Retention Time (RT)= 3.287

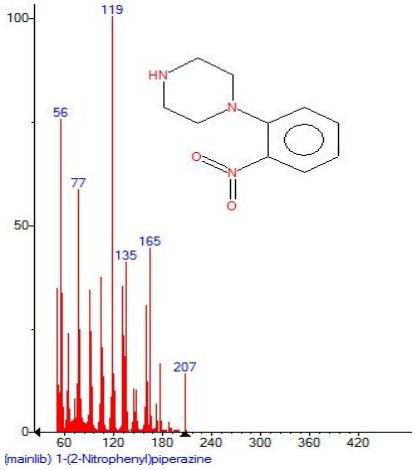


Figure 4: Mass spectrum of 1-(2-Nitrophenyl)piperazine with Retention Time (RT)= 3.779

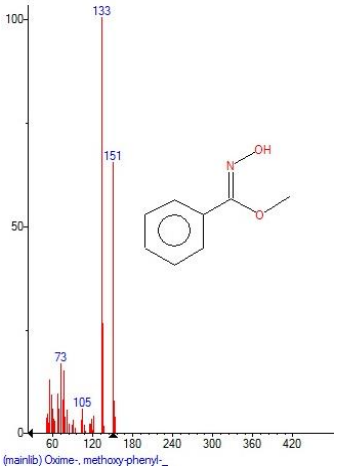


Figure 5: Mass spectrum of Oxime-,methoxy-phenyl- with Retention Time (RT)= 3.917

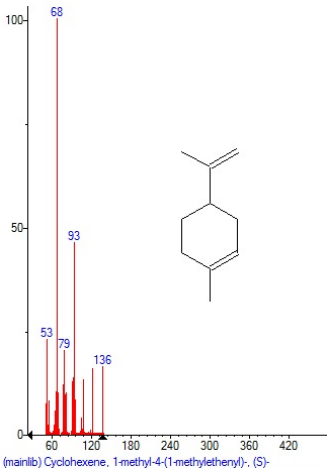


Figure 6: Mass spectrum of Cyclohexene , 1-methyl-4-(1-methylethenyl)-,(S)- with Retention Time (RT)= 4.163

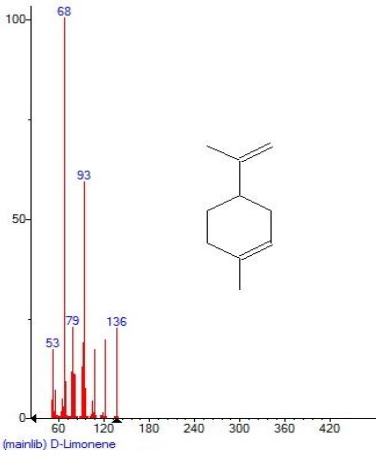


Figure 7: Mass spectrum of D-Limonene with Retention Time (RT)= 4.489

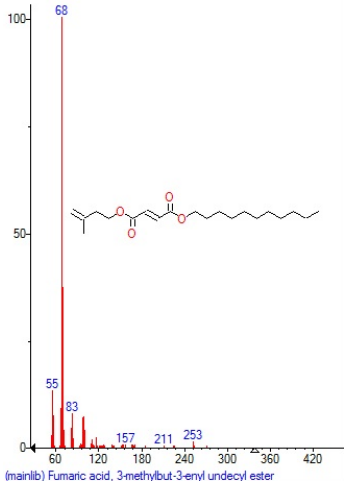


Figure 8: Mass spectrum of Fumaric acid ,3-methylbut-3-enyl undecyl ester with Retention Time (RT)= 5.072

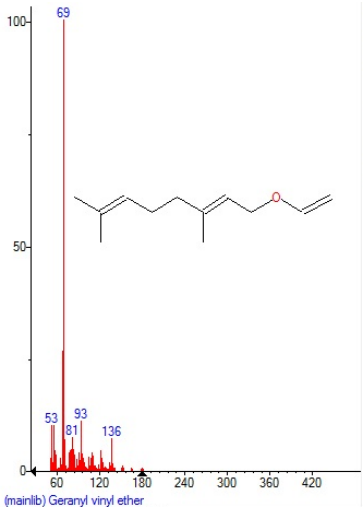


Figure 9: Mass spectrum of Geranyl vinyl ether with Retention Time (RT)= 5.141

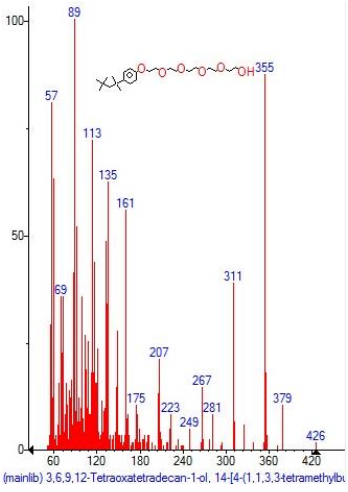


Figure 10: Mass spectrum of 3,6,9,12-Tetraoxatetradecan -1-ol,14-[4-(1-,1,3,3-tetramethylbu with Retention Time (RT)= 5.284

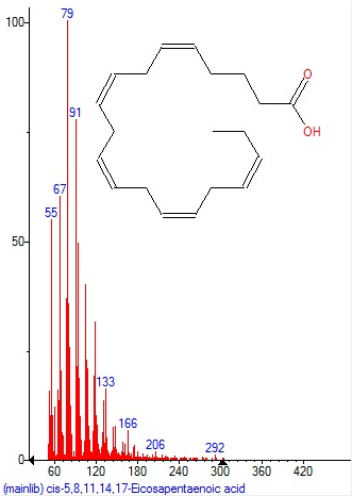


Figure 11: Mass spectrum of Cis-5,8,11,14,17-Eicosapentaenoic acid with Retention Time (RT)= 5.587

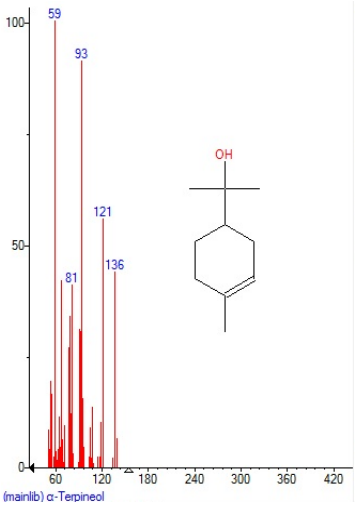


Figure 12: Mass spectrum of α-Terpineol with Retention Time (RT)= 5.919

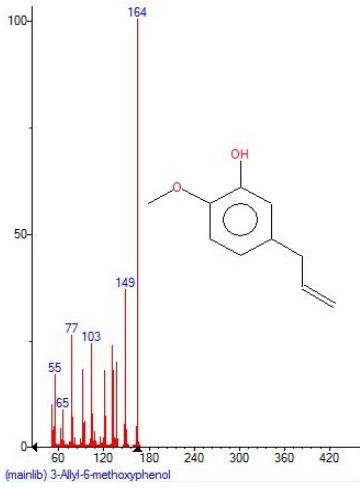


Figure 13: Mass spectrum of 3-Allyl-6-methoxyphenol with Retention Time (RT)= 6.537

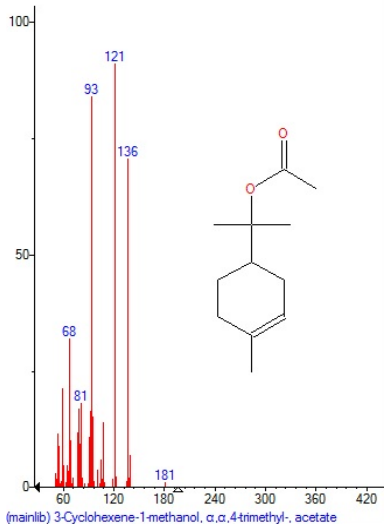


Figure 14: Mass spectrum of 3-Cyclohexene-1-methanol,α,α,4-trimethyl-,acetate with Retention Time (RT)= 7.350

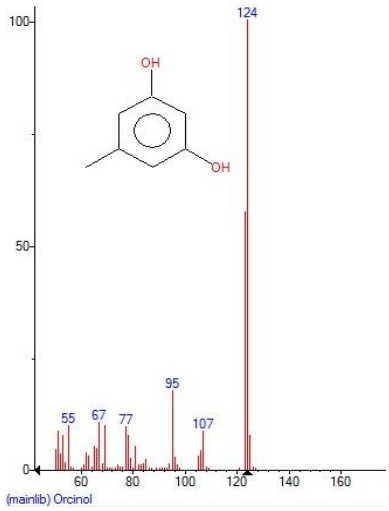


Figure 15: Mass spectrum of Orcinol with Retention Time (RT)= 7.819

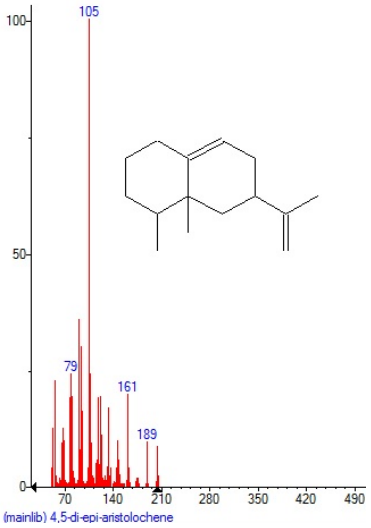


Figure 16: Mass spectrum of 4,5-di-epi-aristolochene with Retention Time (RT)= 8.963

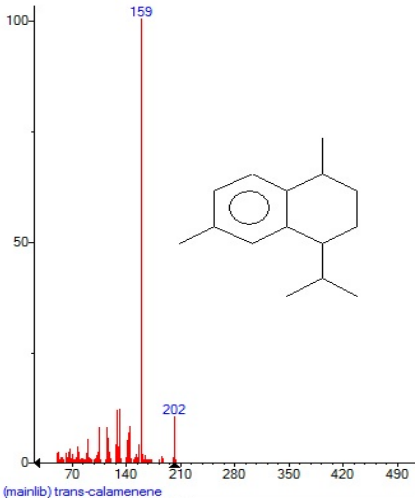


Figure 17: Mass spectrum of Trans-calamenene with Retention Time (RT)= 9.227

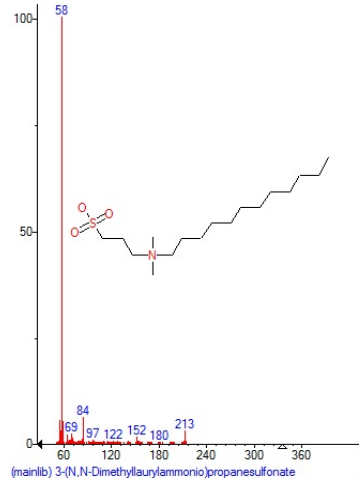


Figure 18: Mass spectrum of 3-(N,N-Dimethyllaurylammonio)propanesulfonate with Retention Time (RT)= 9.358

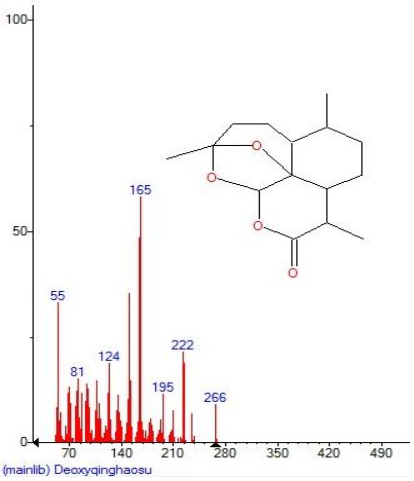


Figure 19: Mass spectrum of Deoxyqinghaosu with Retention Time (RT)= 9.902

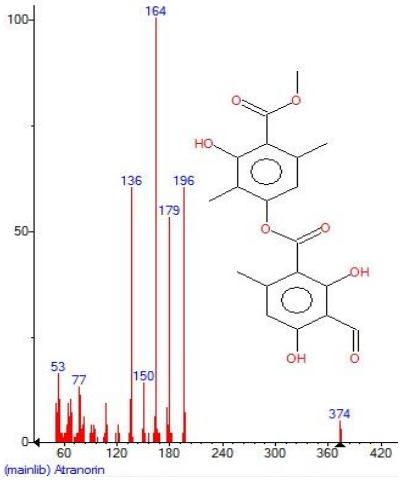


Figure 20: Mass spectrum of Atranorin with Retention Time (RT)= 9.965

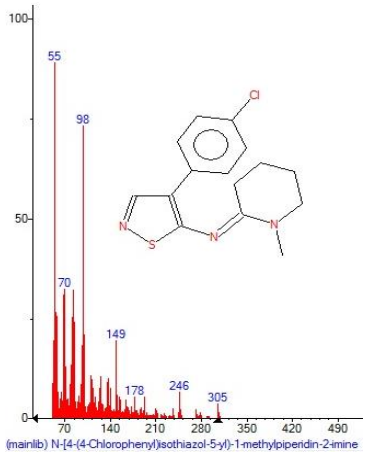


Figure 21: Mass spectrum of N-[4-(4-Chlorophenyl)isothiazol-5-yl)-1-methylpiperidin-2-imine with Retention Time (RT)= 11.904

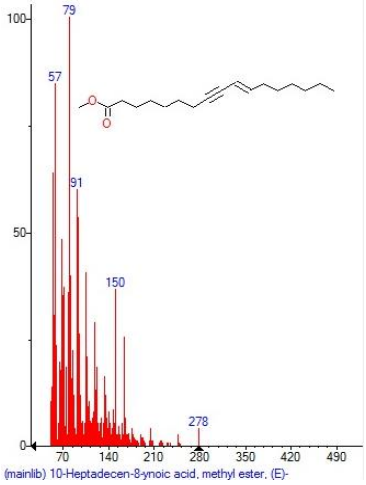


Figure 22: Mass spectrum of 10-Heptadecen-8-ynoic acid , methyl ester , (E)- with Retention Time (RT)= 11.967

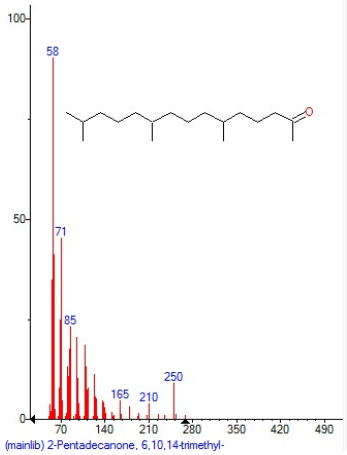


Figure 23: Mass spectrum of 2-Pentadecanone ,6,10,14-trimethyl- with Retention Time (RT)= 12.477

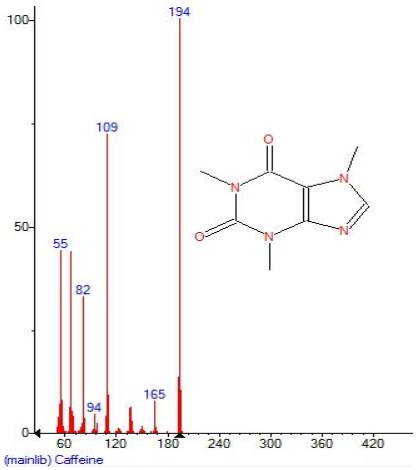


Figure 24: Mass spectrum of Caffeine with Retention Time (RT)= 12.854

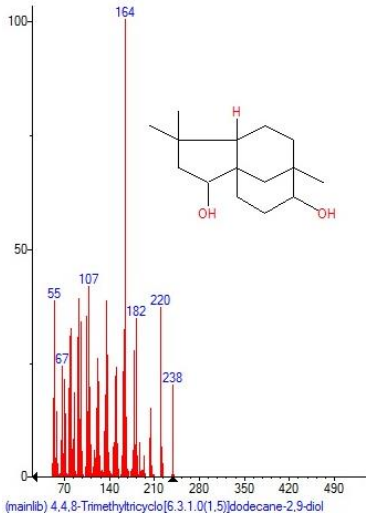


Figure 25: Mass spectrum of 4,4,8-Trimethyltricyclo[6.3.1.0(1.5)]dodecane-2,9-diol with Retention Time (RT)= 13.083

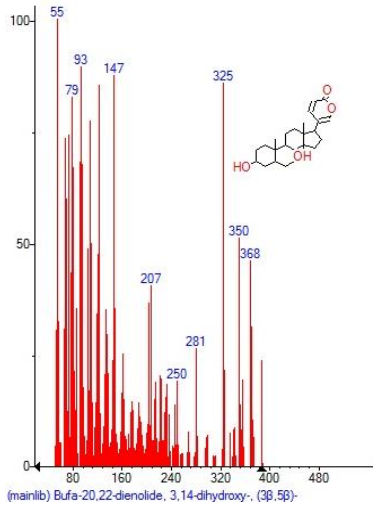


Figure 26: Mass spectrum of Bufa-20,22-dienolide , 3,14-dihydroxy-,(3β,5β)- with Retention Time (RT)= 13.501

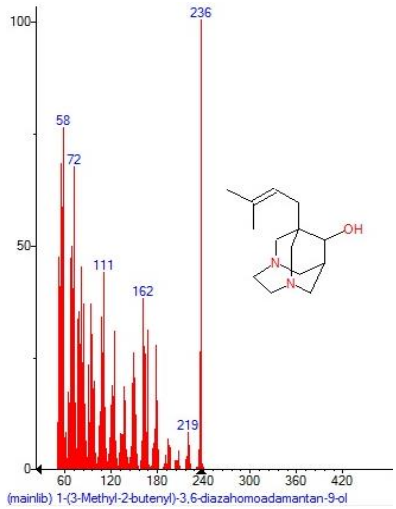


Figure 27: Mass spectrum of 1-(3-methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol with Retention Time (RT)= 14.582

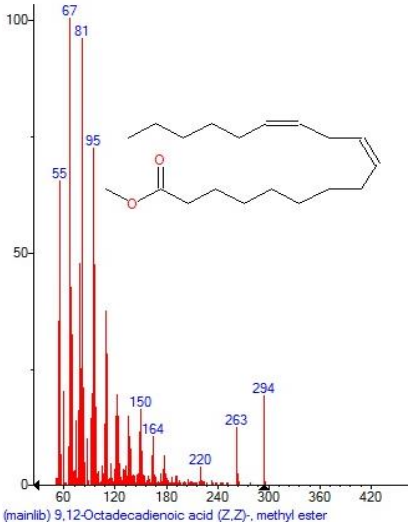


Figure 28: Mass spectrum of 9,12-Octadecadienoic acid (Z,Z)-, methyl ester with Retention Time (RT)= 14.886

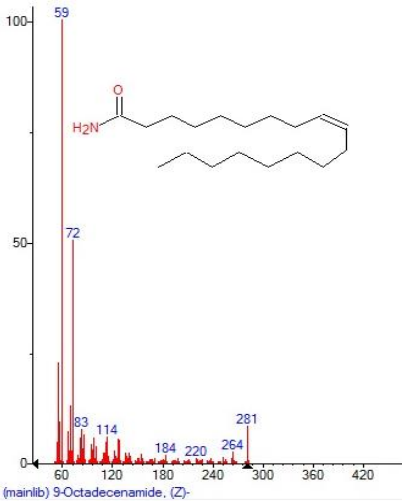


Figure 29: Mass spectrum of 9-Octadecenamide,(Z)- with Retention Time (RT)= 15.738

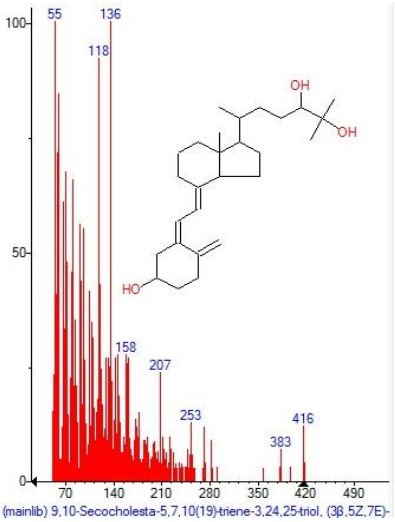


Figure 30: Mass spectrum of 9,10-Secocholesta -5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- with Retention Time (RT)= 16.162

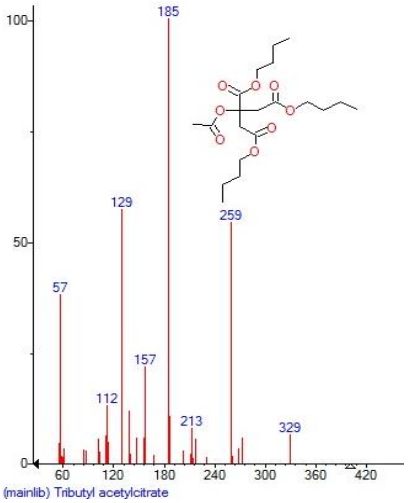


Figure 31: Mass spectrum of Tributyl acetylcitrate with Retention Time (RT)= 16.373

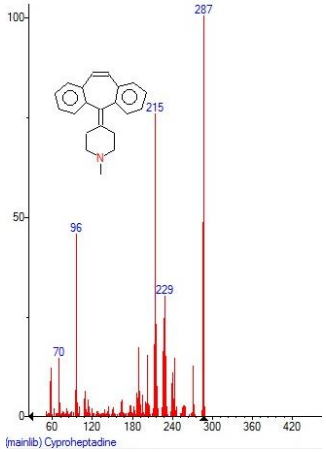


Figure 32: Mass spectrum of Cyproheptadine with Retention Time (RT)= 17.689

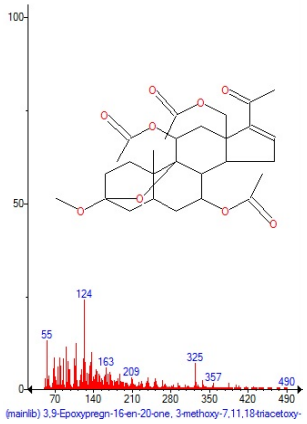


Figure 33: Mass spectrum of 3,9-Epoxypregn-16-en-20-one , 3-methoxy-7,11,18-triacetoxy- with Retention Time (RT)= 18.027

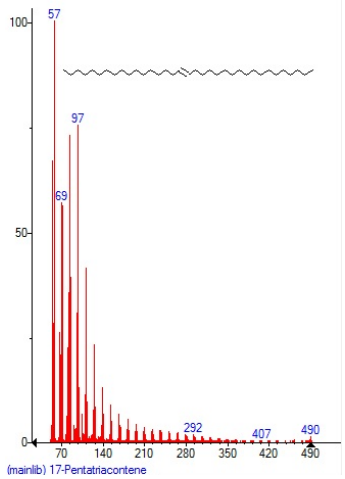


Figure 34: Mass spectrum of 17-Pentatriacontene with Retention Time (RT)= 18.187

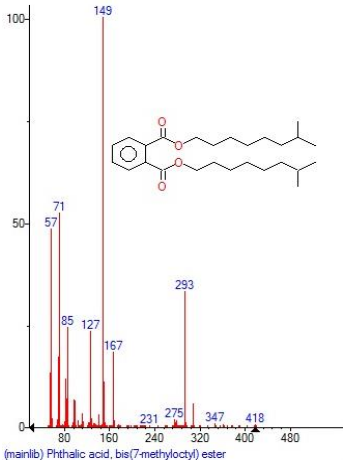


Figure 35: Mass spectrum of Phthalic acid , bis(7-methyloctyl) ester with Retention Time (RT)= 20.796

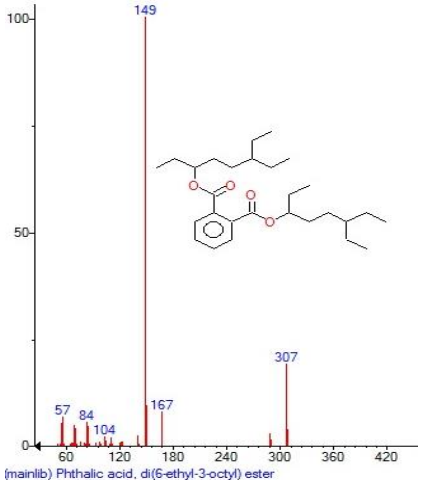


Figure36 : Mass spectrum of Phthalic acid , di(6-ethyl-3-octyl) ester with Retention Time (RT)= 21.369

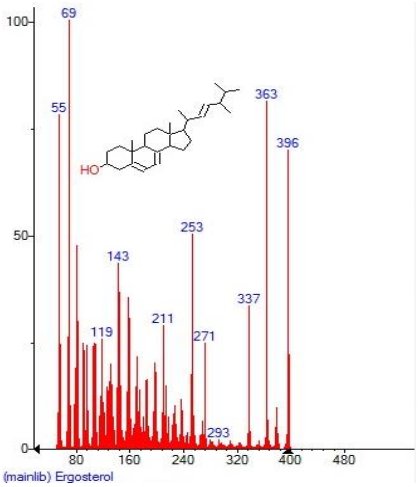


Figure 37: Mass spectrum of Ergosterol with Retention Time (RT)= 23.194

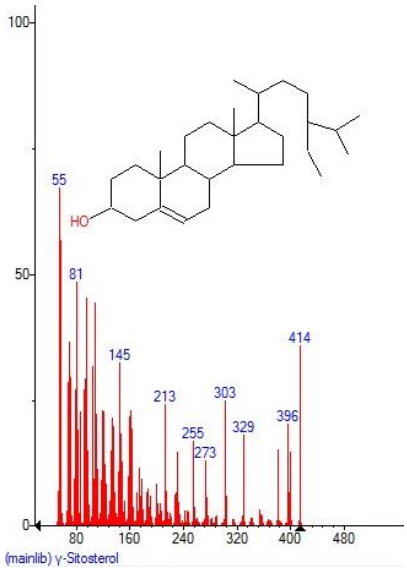


Figure 38: Mass spectrum of γ-Sitosterol with Retention Time (RT)= 24.081

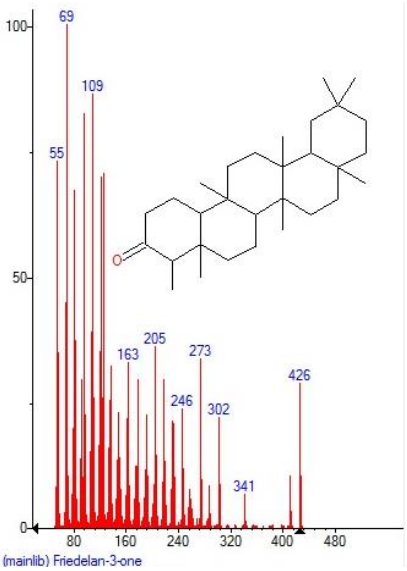


Figure 39: Mass spectrum of Friedelan-3-one with Retention Time (RT)= 25.683

and mice[19-23]. The researchers have isolated and identified the various glycosides and have carried out further in vitro studies measuring the anti-inflammatory properties of cyclamen extracts. They concentrated particularly upon the activity of a newly isolated saponin called repandoside. Results showed that repandoside is one of several saponins that did indeed mediate the inflammatory response by influencing the behaviour of human macrophages[24-29]. It is hoped that these compounds can be developed for future use in the treatment of inflammatory conditions.

ACKNOWLEDGEMENT

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