Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(4): 2668-2679
Received: 13-05-2019
Accepted: 15-06-2019

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Determination of bioactive components of *Pronus avium* L Leaf

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Abstract
Medicinal plants are a source of naturally active compounds used extensively in herbal medicine worldwide for treatment of many ailments**,** promote human health and longevity in many regions of the world since ancient times. Plant-derived bioactive compounds in the past few decades have become of great interest owing to their rich ethnomedicinal and nutraceutical potentials and versatile pharmaceutical applications The objective of the study was to determine the content of bioactive compounds using Gas Chromatography-Mass Spectrometry technique. GC-MS analysis confirmed the occurrence of a total 24 phytocompounds. These compounds belong to different chemical classes and most of them are reported to exhibit important biological activities. Some of the major compounds included, 2-Methyl-2-[(1-E,3Z-E,5E)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexenyl)-1,3,5-hexatrienyl]-1,3-dioxolane (29.09%), Cyclopentaneundecanoic acid, methyl ester (15.5%), 2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)]]-(2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)] (9.855%), The biological activity of these phytochemicals includes, Anticancer, Anti-inflammatory, Antiprotozoal, Anti-myelomic, Antileishmanial, Antioxidant and Antimigrain.

Keywords: Phytocompounds, extracts, methanol extract, *Pronus avium* and drugs

Introduction
Medicinal plants act as an indigenous source of new compounds possessing therapeutic value and can also be used in drug development [1]. Plants and their extracts have been be used world-wide for the treatment of disease and novel drug entities continue to be developed through research into their constituent [1-2]. It is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body. These natural compounds formed the base of modern drugs as we use today [3-5].
80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO [6]. Because of the growing recognition of natural products the demand for medicinal plants has been increasing all over the world. They have minimal toxicity, are cost effective and pharmacologically active, and provide an easy remedy for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effects [7]. The leaves and petioles had a higher concentration of dietary fiber, vitamin C, carotenoids and polyphenols as well as an antioxidant activity than the fruit [8]. *Pronus avium* belongs to the *Rosaceae* family as a subfamily, the *Prunoideae*. The genus *Prunus* includes about 430 species of deciduous or evergreen trees and shrubs naturally widespread throughout temperate regions While some species do not yield edible fruits and are used for decoration, others are grown commercially for fruit and "nut" production. Most of these species are originally from Asia or Southern Europe. Although several species of cherries exist, two popular cultivars are wild or sweet-cherry, and sour or tart-cherry. While sweet cherries belong to the species of *Prunus avium*, tart variety belongs to that of *Prunus cerasus*. Cherries are drupe fruits with a central “stony-hard” seed surrounded by fleshy edible pulp measuring about 2 cm in diameter. Externally they covered by bright "shiny" red or purple, thin peel [9]. Fruits of the sweet cherry (*Prunus avium* L.) are particularly rich in polyphenols (especially flavonoids, anthocyanins and hydroxycinnamic acids) as well as containing high levels of ascorbic acid and potassium [10-11].

Botanical Description
The leaves are alternate, simple ovoid-acute, 7–14 cm (2.8–5.5 in) long and 4–7 cm (1.6–2.8 in) broad, glabrous matt or sub-shiny green above, variably finely downy beneath, with a serrated margin and an acuminate tip, with a green or reddish petiole 2–3.5 cm (0.79–1.38 in)

long bearing two to five small red glands. The tip of each serrated edge of the leaves also bear small red glands [12].

Taxonomical classification

Kingdom	Plantae
Subkingdom	Viridiplantae
Superdivision	Embryophta
Division	Tracheophyta
Subdivision	Spermatophytina,
Class	Magnoliopsida
Superorder	Rosanae
Order	Rosales
Family	Rosaceae
Genus	*Prunus* L.

Fig 1: The leaves and fruits of *Pronus avium*

Materials and methods

Collection and Authentication of Plant Material

Prunus avium fresh leaves were collected from Eziama, Aba North Local Government area of Abia State, Nigeria on 7th July, 2018 and identified by Dr E.O Emmanuel, a plant taxonomist. Voucher specimen with herbarium number [1964] was deposited at the herbarium section of Department of Plant Science and Biotechnology, Abia State University.

Preparation of Leaf Extracts

The plant leaves were washed with running tap water for 5 min to remove the dust and debris and rinsed with sterile distilled water. The fresh plant leaves sample was air dried on the laboratory bench for ten days at temperature below 30 ^{0}C to avoid decomposition of thermo labile compounds. The sample was milled using and electric blender to coarse powder and powdered sample was kept in a clean closed container pending extraction. 50 g of pulverized leaf material was mixed with 250 ml of solvent (95% methanol) and kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper The extract was concentrated under reduced pressure using Digital Heidolph Rotary evaporator (4000 series) and the supernatant plant extract was decanted after complete removal of the solvent

GC –MS analysis

Preparation of extract

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

GC – MS Analysis Conditions

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

Components Identification

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

Results and Discussion

Nature has been a source of medicinal agents for thousands of years. Various medicinal plants have been used for years in daily life to treat disease all over the world [14].

Medicinal plants and their extracts with biological activity are the main resource for new chemical structures. They are useful in the development of compounds with a potential for use in pharmacology, agronomy and other fields of human health endeavour. The world of plants represents an untapped reservoir of drugs, The compounds present in the methanolic extract of *Prunus avium* were identified by GC-MS analysis presented in figure 1. The active principle Molecular Weight (MW), Concentration (%), Molecular Formula (MF), and Retention Time (RT) is presented in Table 1. Twenty four compounds were identified in the extract at RT 3.644, 4.558, 24.775, 28.885, 26.981, 27.098, 28.256, 28.885, 29.053, 29.800, 29.867, 30.412, 32.350, 32.484, 32.929, 33.642, 33.843, 33.843, 35.177, 38.146, 38.255, 38.255, 38.255.

The prevailing compounds were 2-Methyl-2-[(1-E,3Z-E,5E)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexenyl)-1,3,5-hexatrienyl]-1,3-dioxolane (29.09%), Cyclopentaneundecanoic acid, methyl ester (15.5%), 2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)]]-(2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)] (9.855%), Dispiro[2.0.2.1]heptane-1-carboxylic acid, 1-methyl-, methyl ester, (7.471%), Oxirane, tetradecyl, (5.872%), Neophytadiene, (4.840%), Parthenolide, (3.347%).

The gas chromatogram of the analysis of Methanol extract of the leaves of *Prunus avium* shown in Figure 2. The structures of some constituents are shown in figures 3. Neophytadiene is reported to be an antibacterial, antipyretic, analgesic, and anti-inflammatory, antimicrobial, antioxidant [15-17]. It is a fatty acid-related compound and plays an important role in competitive inhibition of lipoxygenase or cyclooxygenase in an inflammation reduction, resulting in decreased production of prostaglandins and leukotrienes [18]. Parthenolide is a major sesquiterpene lactone isolated from medicinal herbs *Tanacetum parthenium* (commonly known as feverfew) and from *Magnolia grandiflora* Feverfew has been traditionally used as a herbal medicine for the treatment of fever, arthritis, and migraine in Asia and Europe for centuries. Various biological properties of Parthenolide includes, Anticancer, Anti-inflammatory, Antiprotozoal, Anti-myelomic, Antileishmanial, Antioxidant and Antimigrain [19]. Cyclopentaneundecanoic acid, methyl ester is a fatty acid and has Antimicrobial activity [20].

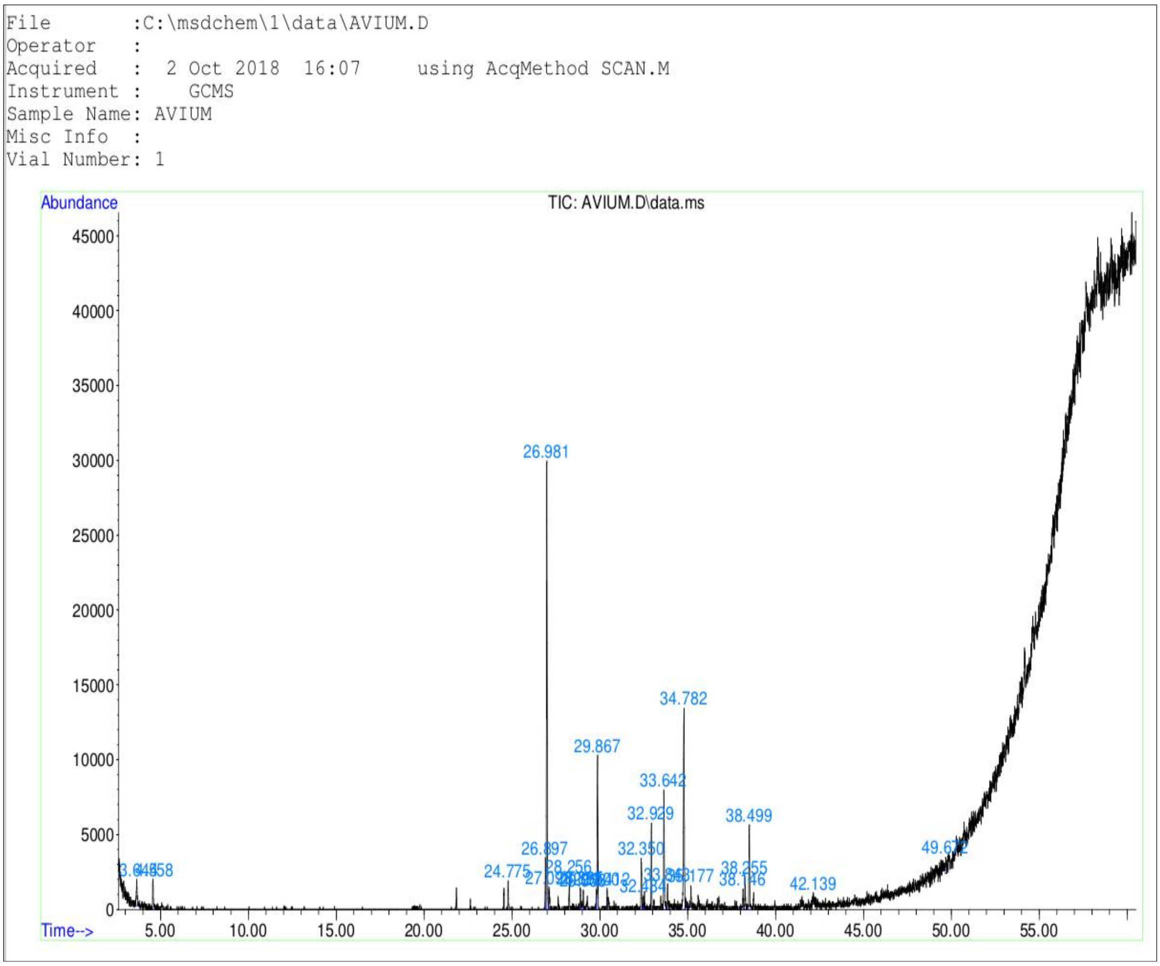
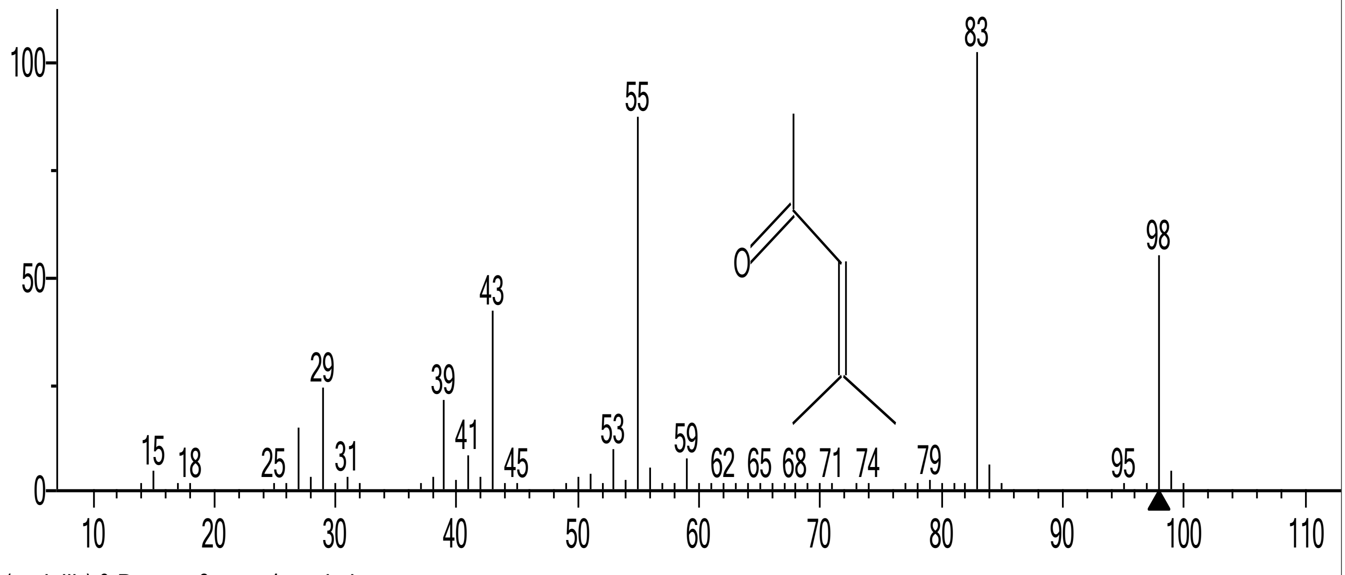


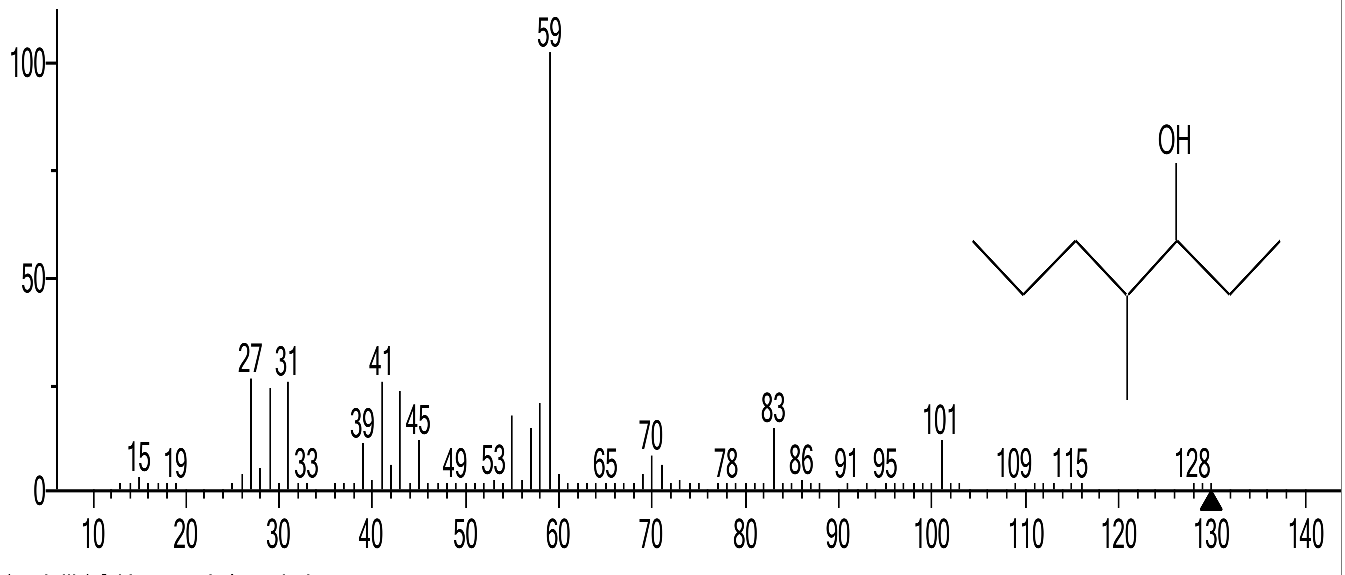
Fig 2: GC-MS Chromatogram of Methanol extract of the leaves of *Prunus avium*

Table 1: Components detected in the plant of methanol extract of *Prunus avium* MW: Molecular Weight, RT: Retention Time

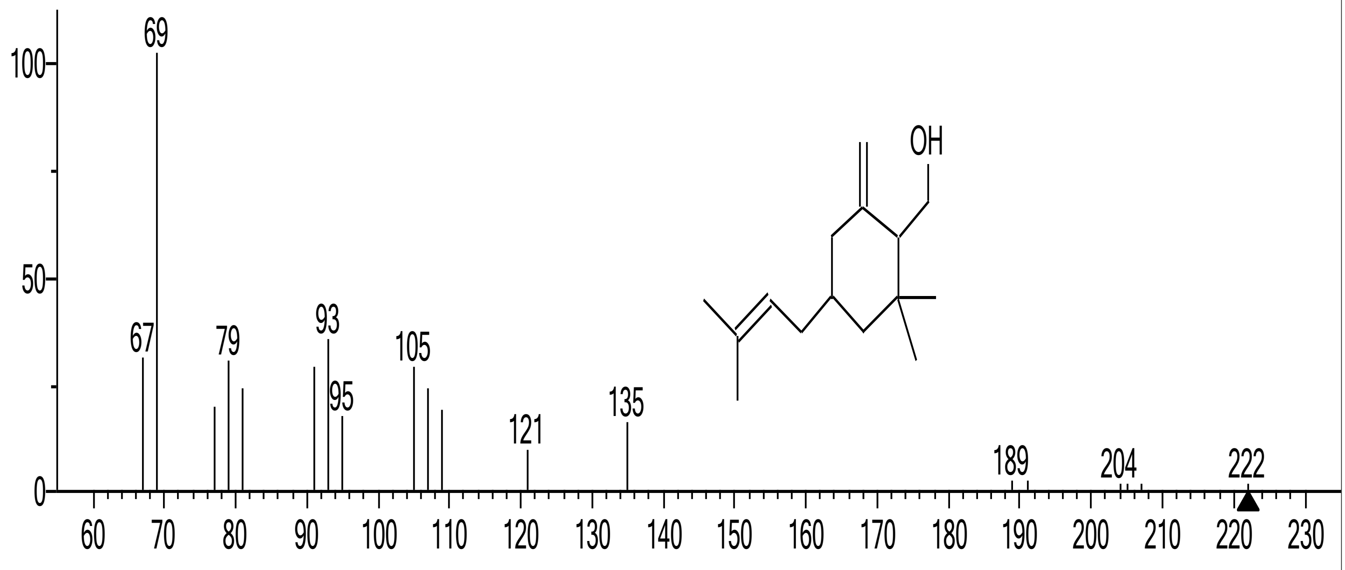
SN	RT	Component	Formula	MW	%
1	3.644	3-Penten-2-one, 4-methyl	C6H10O	98	1.919
2	4.558	3-Heptanol, 4-methyl	C8H18O	130	2.118
3	24.775	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	C15H26O	222	1.285
4	26.897	2-Methyl-2-[(1-E,3Z-E,5E)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexenyl)-1,3,5-hexatrienyl]-1,3-dioxolane	C20H30O2	302	2.642
5	26.981	2-Methyl-2-[(1-E,3Z-E,5E)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexenyl)-1,3,5-hexatrienyl]-1,3-dioxolane	C20H30O3	302	29.092
6	27.098	(3R,4aS,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene	C15H22	202	1.462
7	28.256	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	C15H26O	222	1.791
8	28.885	Benzhydrazide, N2-(2-allyloxybenzylideno)-	C17H16N2O2	280	1.300
9	29.053	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	C15H26O	222	1.074
10	29.800	2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)]]-	C15H26O2	238	0.929
11	29.867	2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)]]-	C15H26O3	239	9.855
12	30.412	3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-	C13H18O	190	1.299
13	32.350	Parthenolide	C15H20O3	248	3.347
14	32.484	Parthenolide	C15H20O4	249	0.968
15	32.929	Neophytadiene	C20H38	278	4.840
16	33.642	Dispiro[2.0.2.1]heptane-1-carboxylic acid, 1-methyl-, methyl ester	C10H14O2	166	7.471
17	33.843	Glutaric acid, di(myrtenyl) ester	C25H36O4	400	1.265
18	34.782	Cyclopentaneundecanoic acid, methyl ester	C16H30O2	254	15.452
19	35.177	Heptane, 4-azido	C7H15N3	141	1.034
20	38.146	Ethane, 1,2-dicyclopropyl	C8H14	110	1.216
21	38.255	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1α,2β,5α)]-	C10H18	138	1.850
22	38.499	Oxirane, tetradecyl	C16H32O	240	5.872
23	42.139	10-Pentadecen-5-yn-1-ol, (E)-	C15H26O	222	0.915
24	49.672	4-tert-Octylphenol, TMS derivative	C17H30OSi	278	1.004



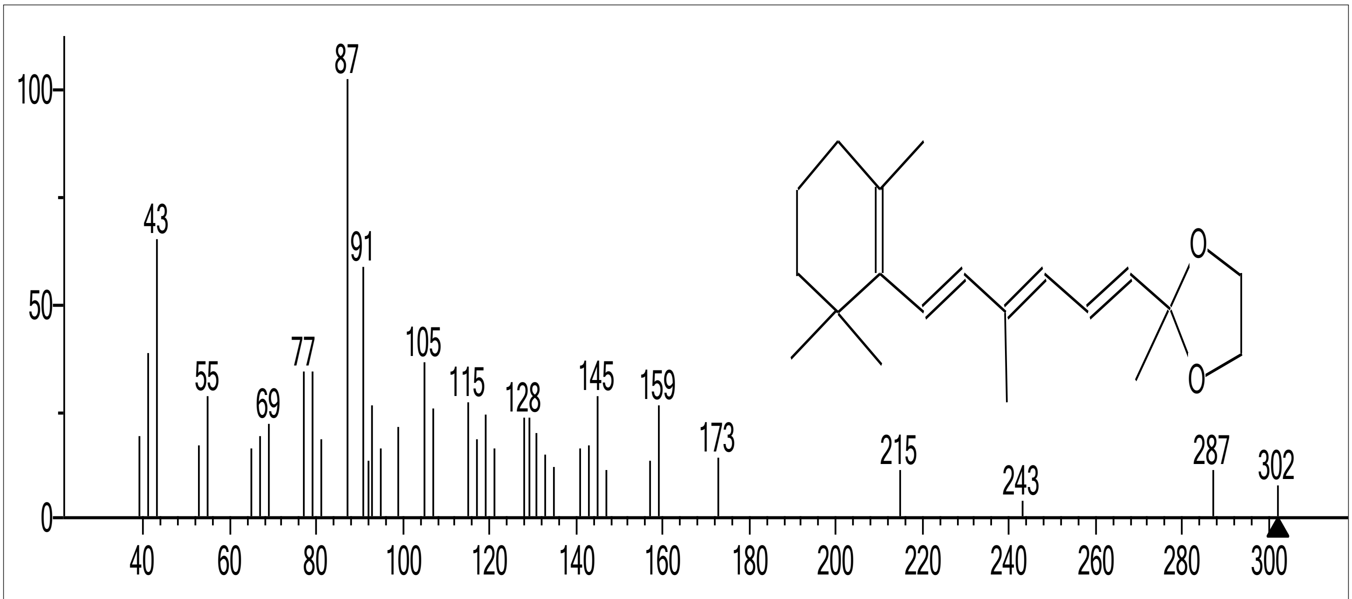
(mainlib) 3-Penten-2-one, 4-methyl-



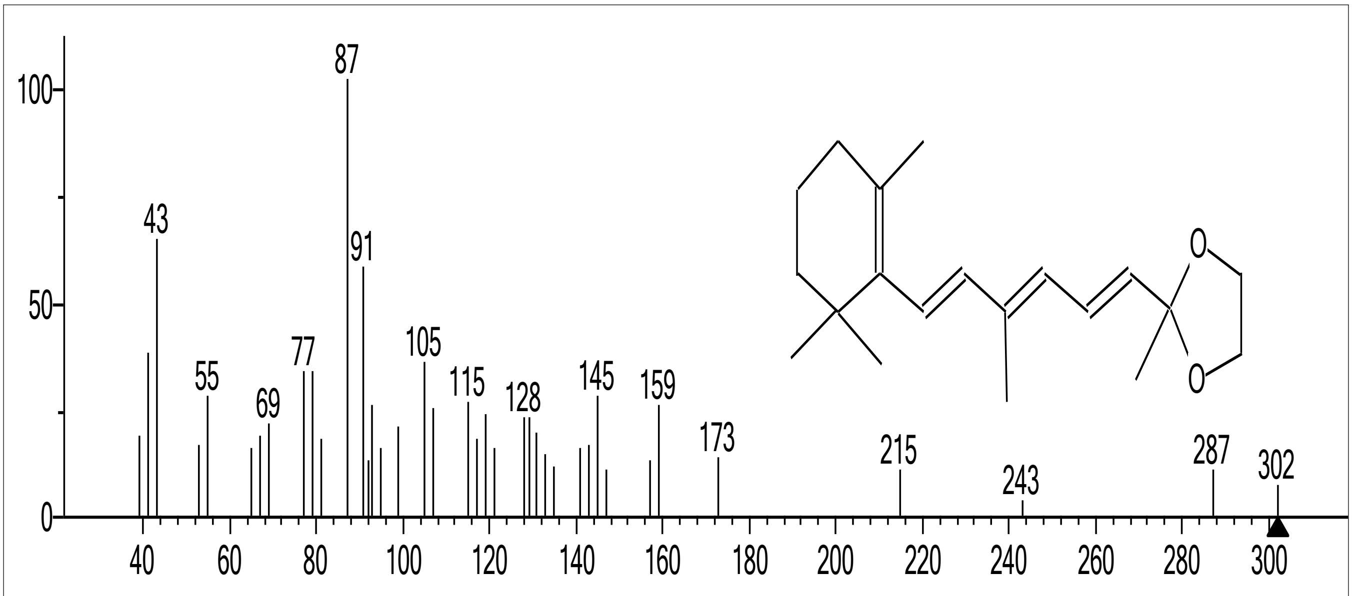
(mainlib) 3-Heptanol, 4-methyl-



(mainlib) 1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane



(mainlib) 2-Methyl-2-[(1-E,3Z-E,5E)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexenyl)-1,3,5-hexatrienyl]-1,3-dioxolane



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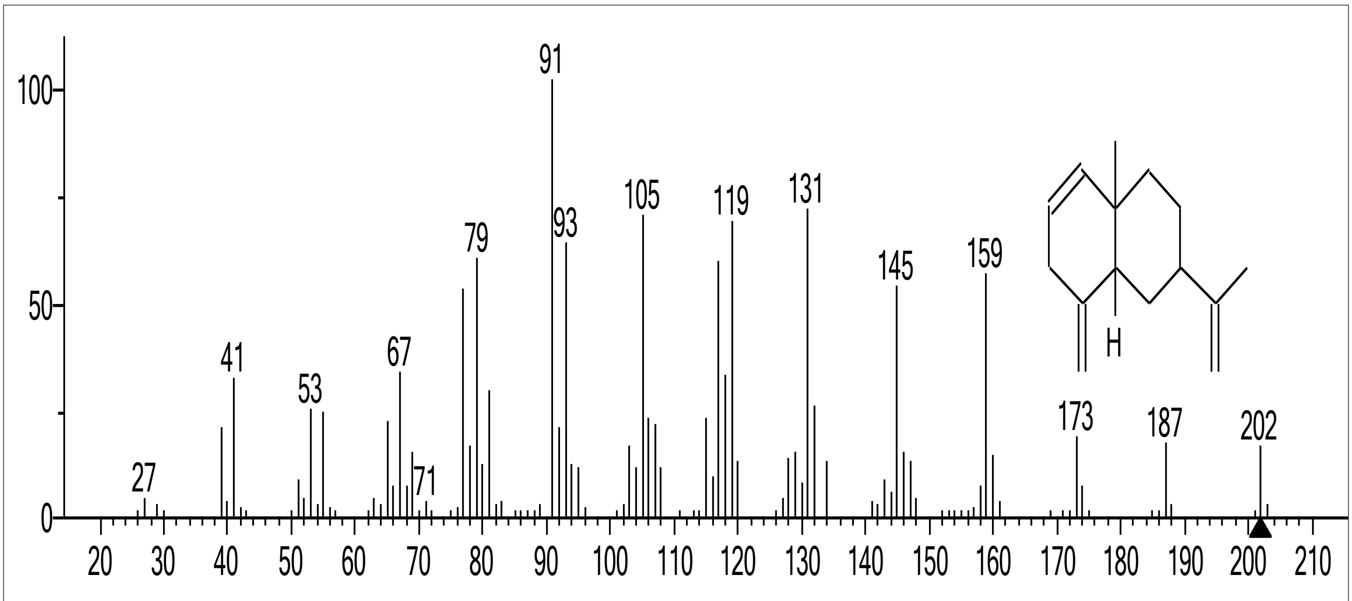
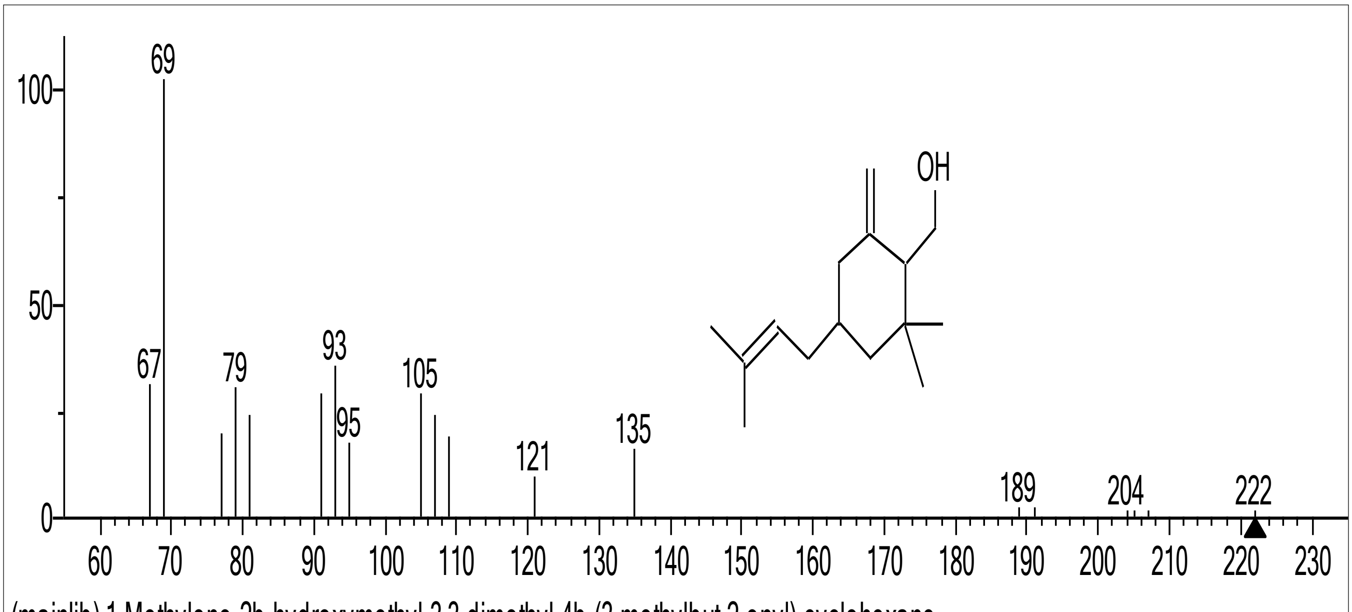


(mainlib) (3R,4aS,8aS)-8a-Methyl-5-methylene-3-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene



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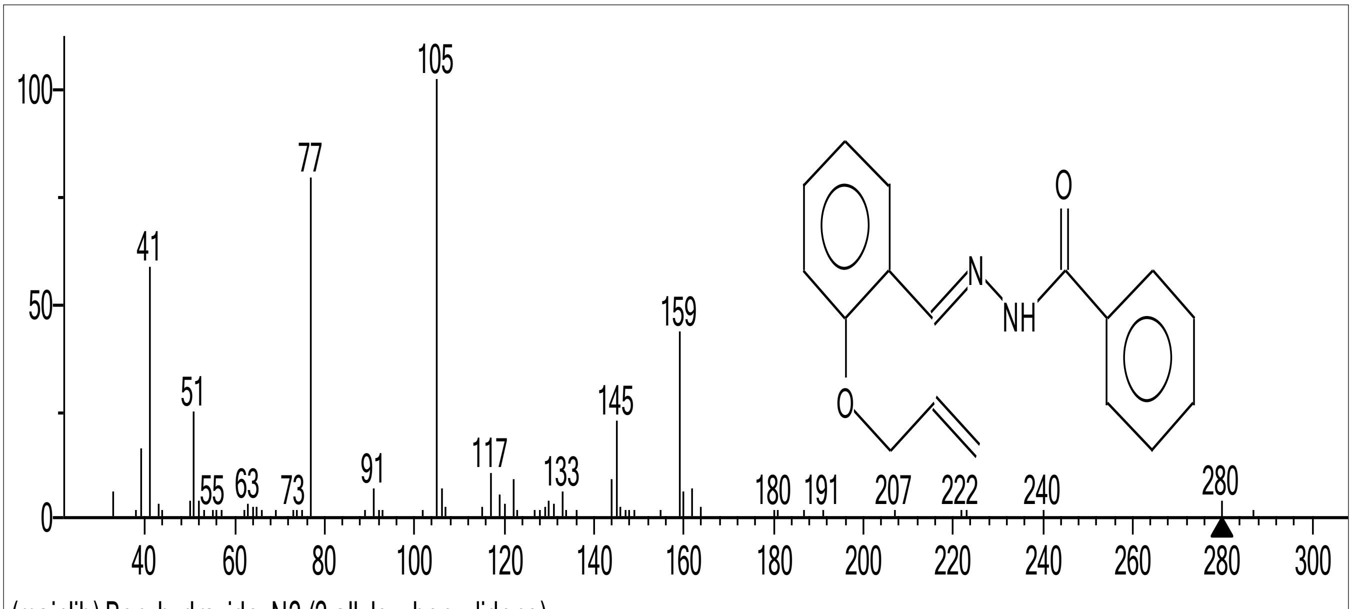
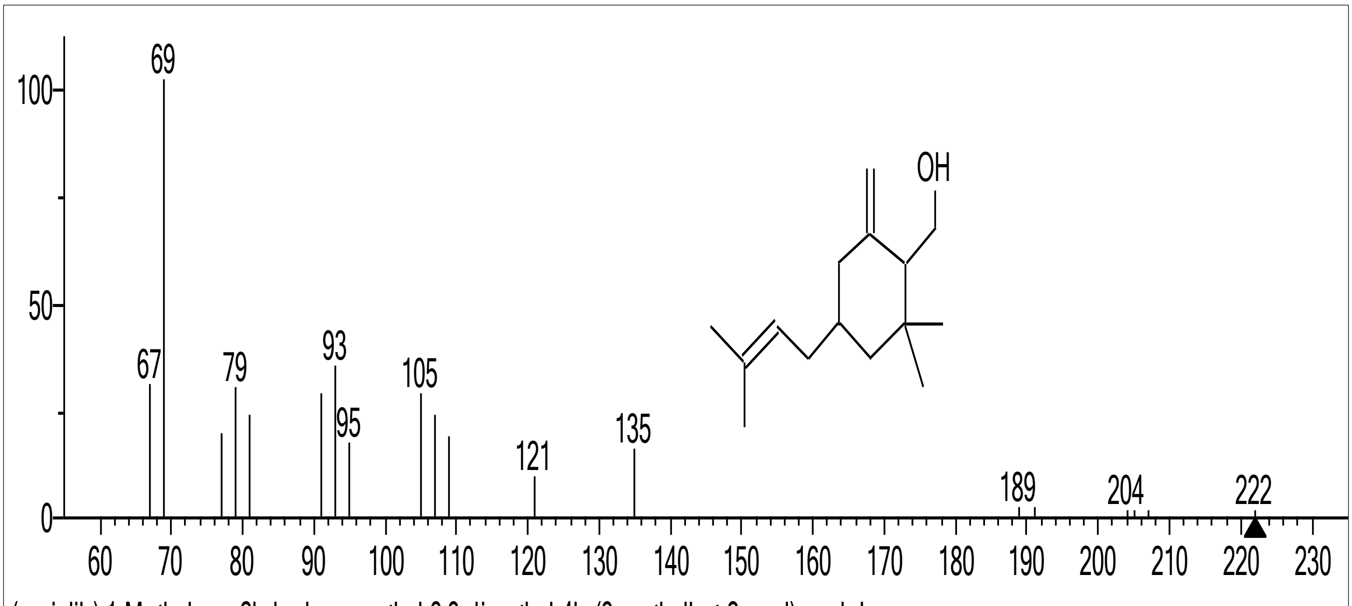


(mainlib) Benzhydrazide, N2-(2-allyloxybenzylideno)-



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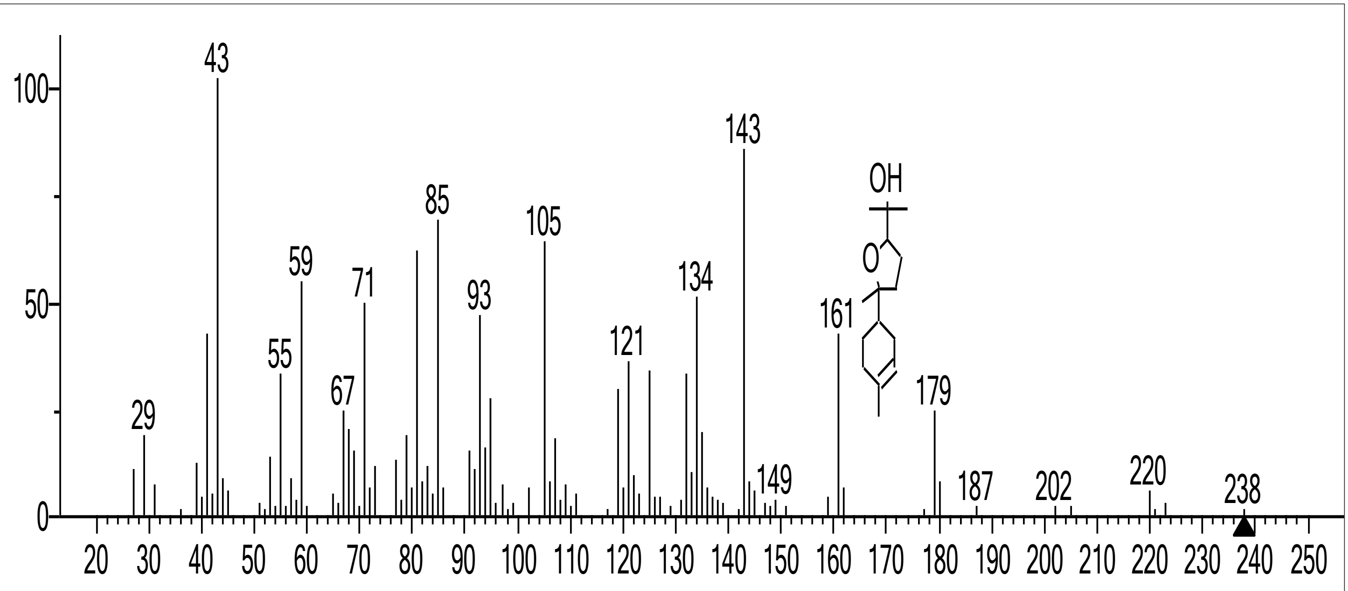
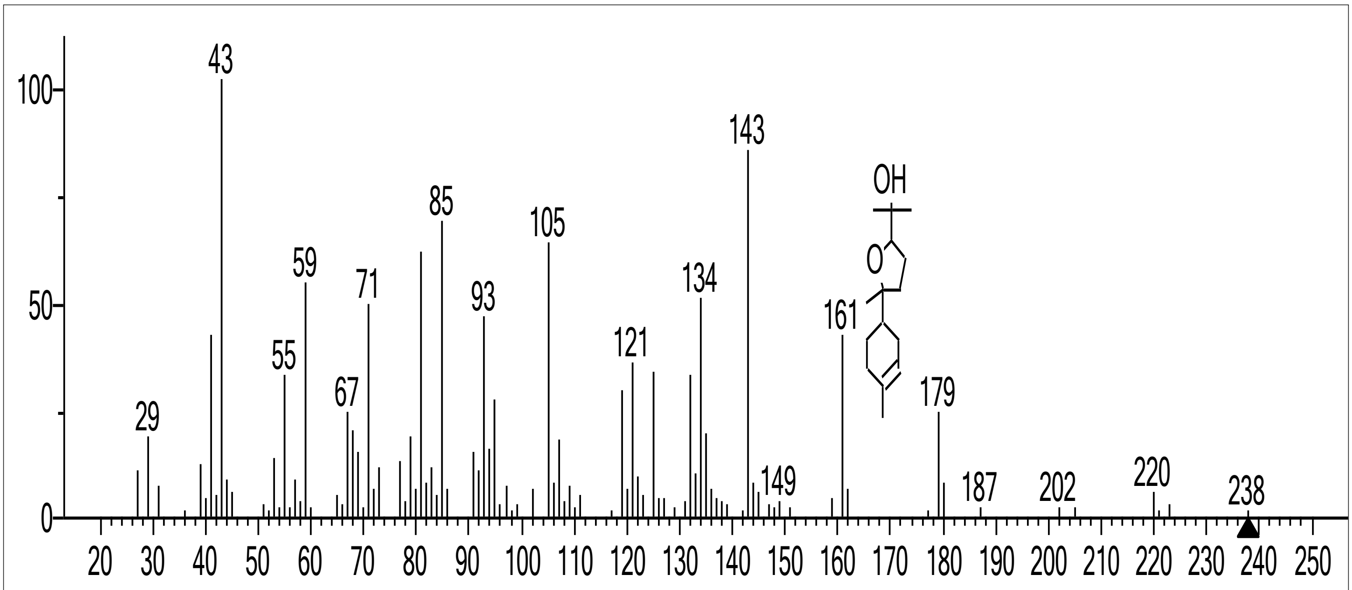


(mainlib) 2-Furanmethanol, tetrahydro-α,α,5-trimethyl-5-(4-methyl-3-cyclohexen-1-yl)-, [2S-[2α,5β(R*)]]-



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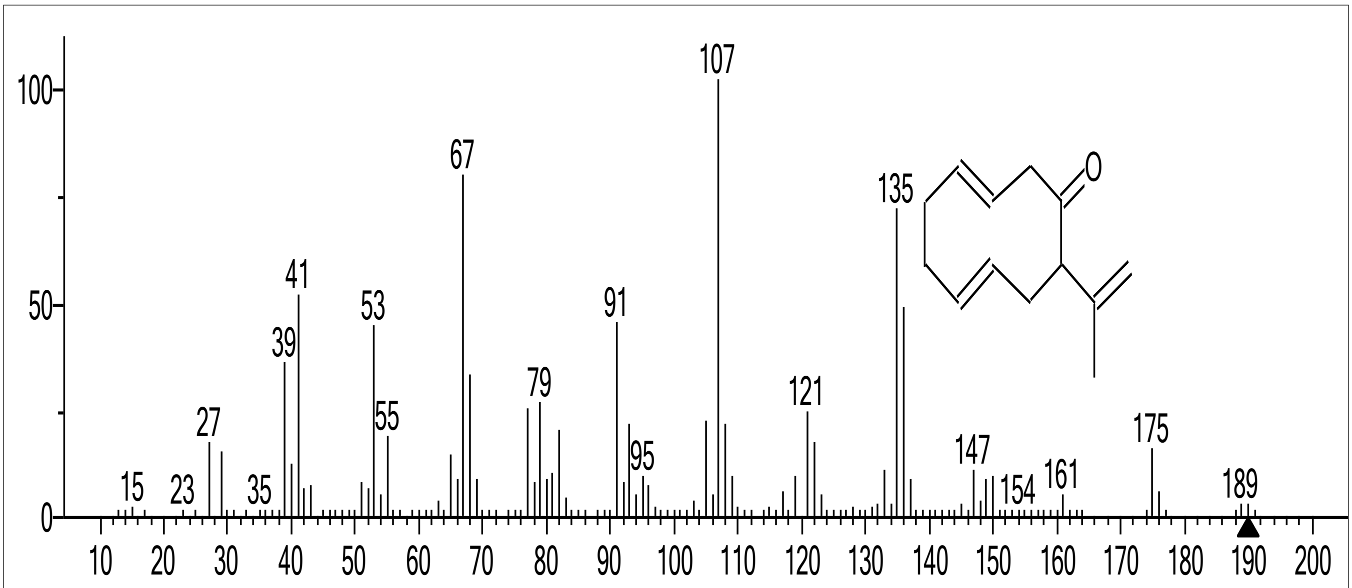
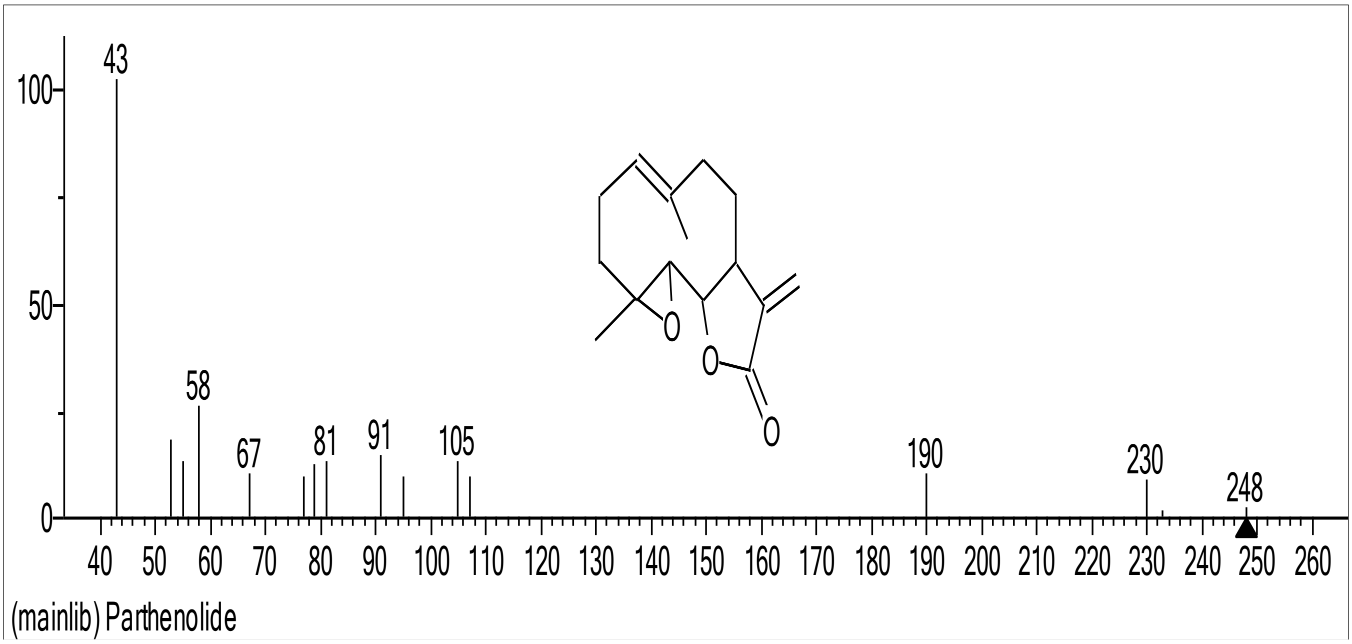


(mainlib) 3,7-Cyclodecadien-1-one, 10-(1-methylethenyl)-, (E,E)-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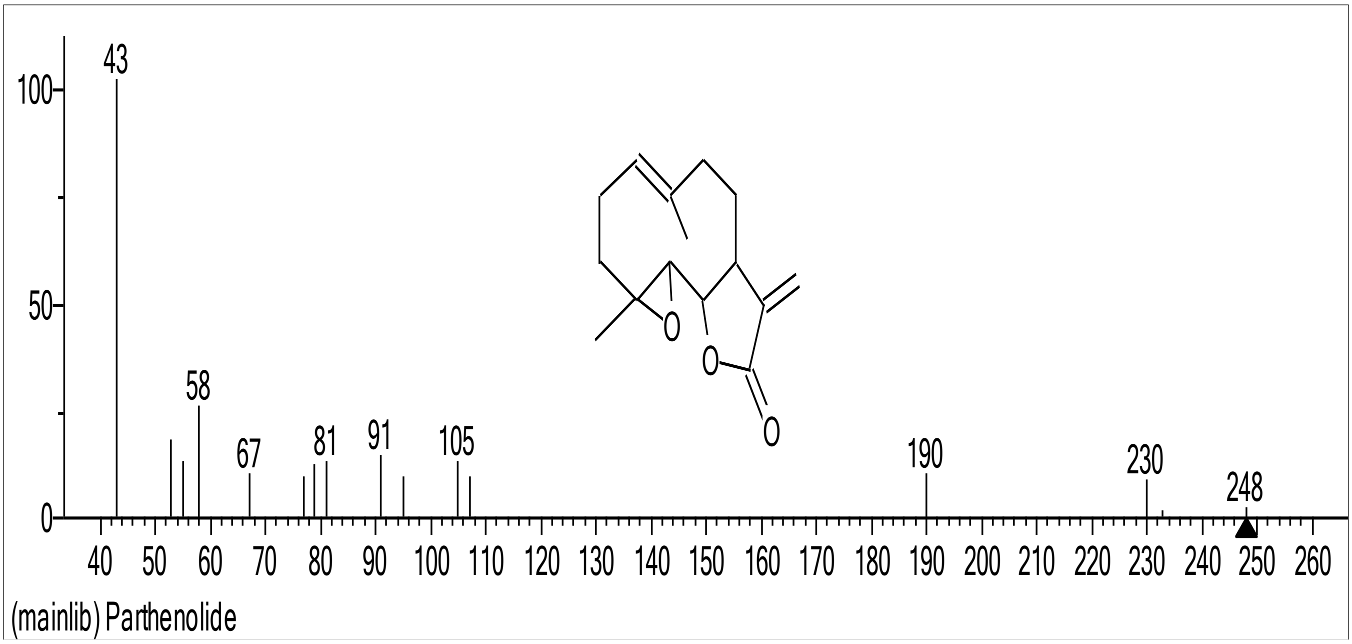
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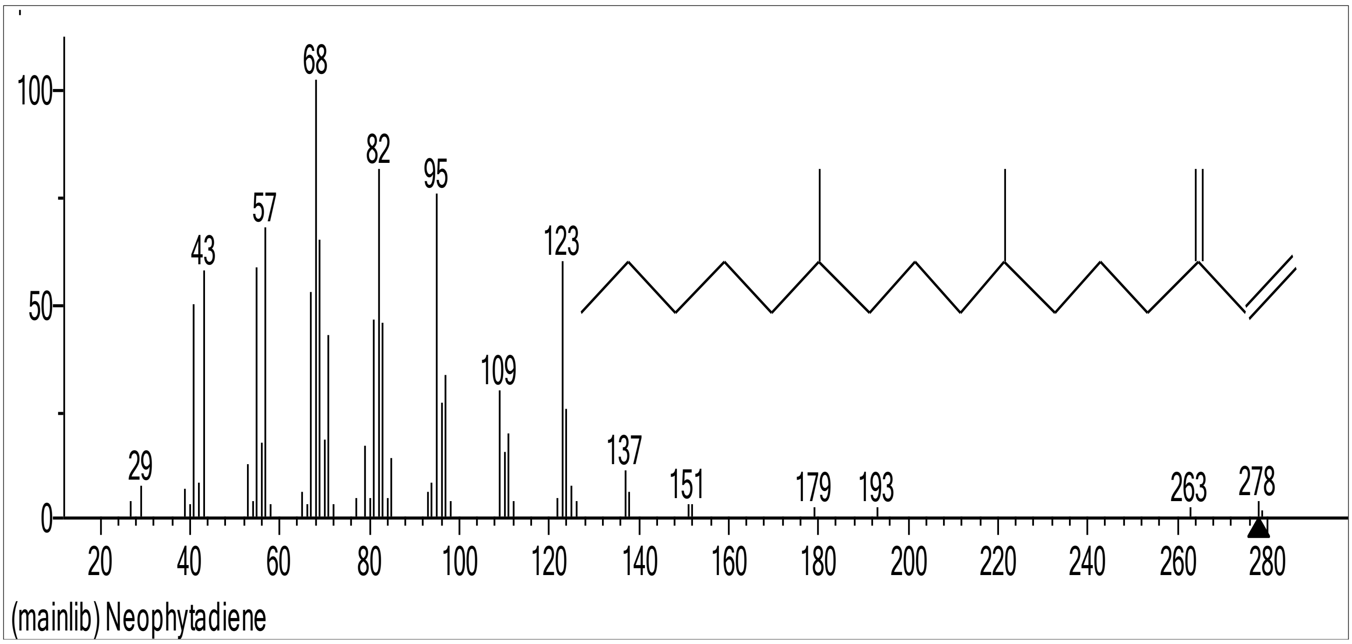
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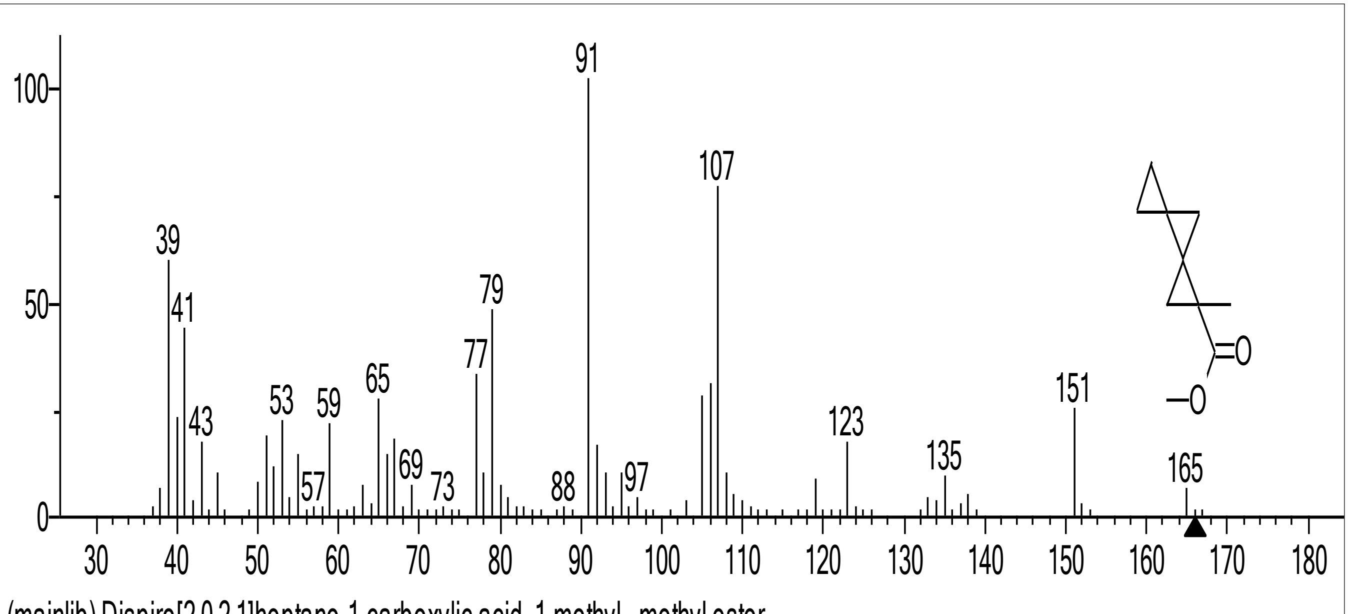
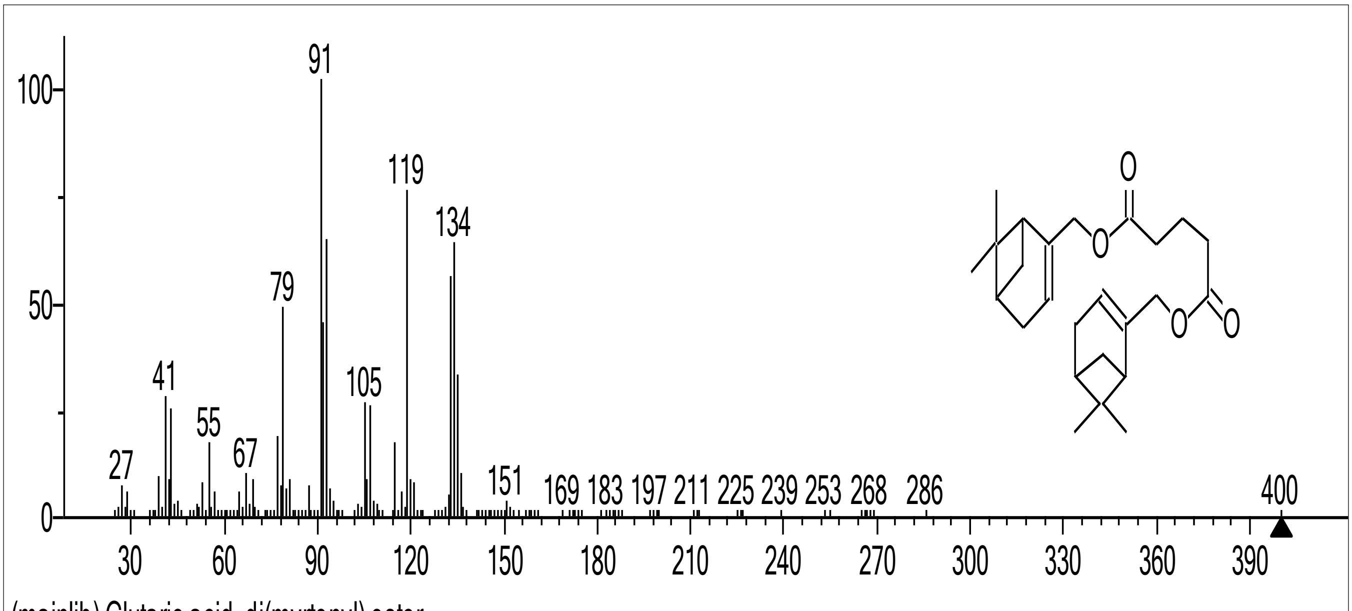
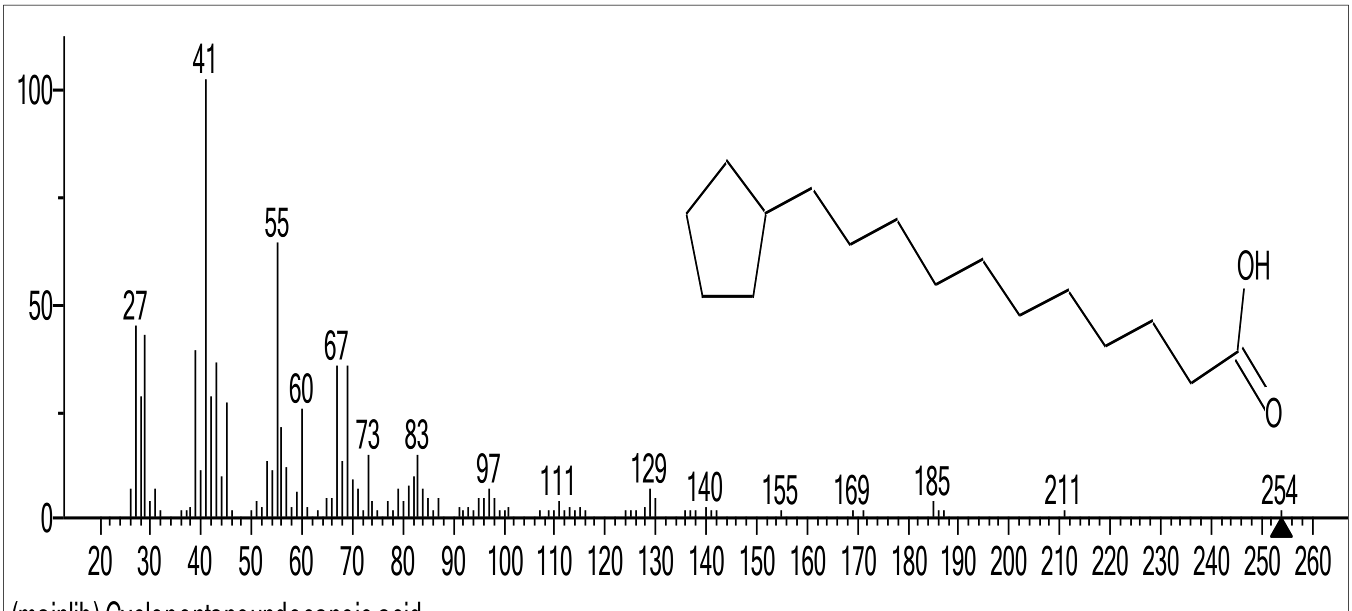
(mainlib) Neophytadiene



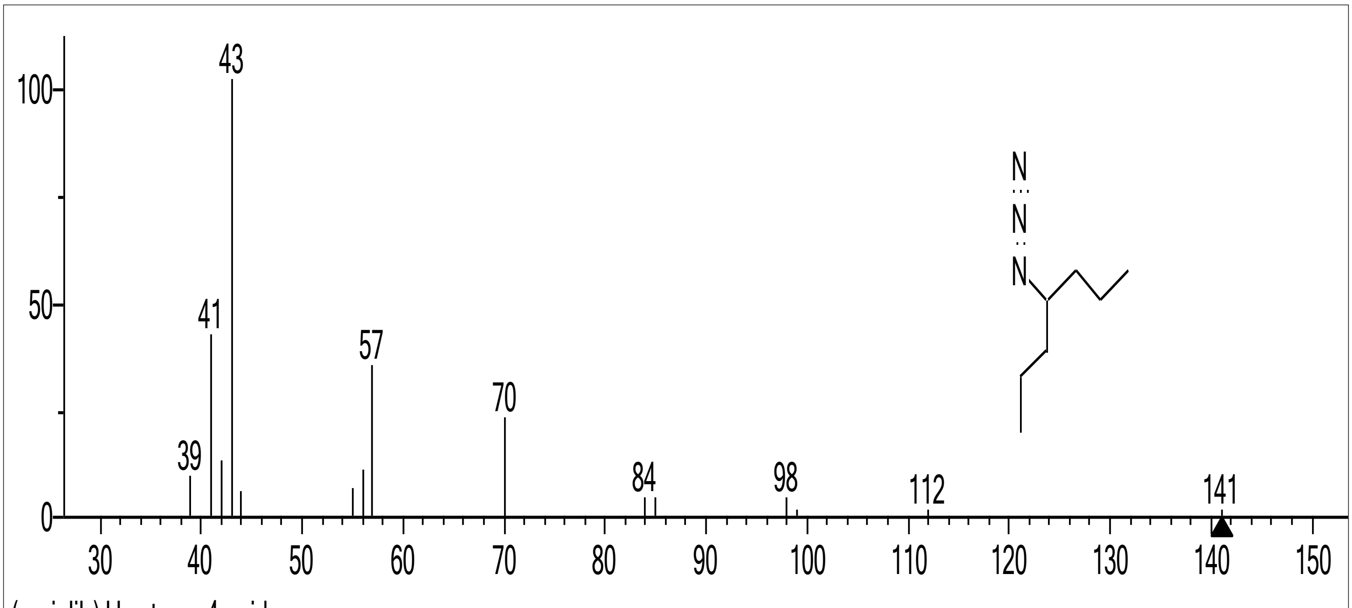
(mainlib) Dispiro[2.0.2.1]heptane-1-carboxylic acid, 1-methyl-, methyl ester



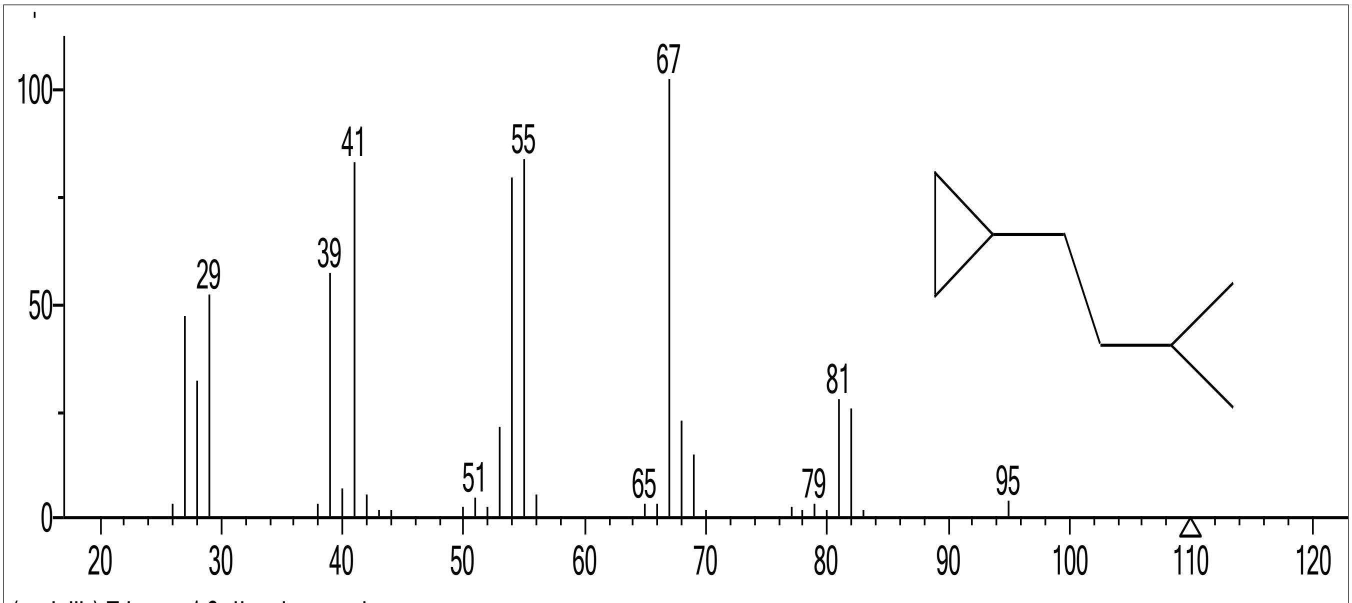
(mainlib) Glutaric acid, di(myrtenyl) ester



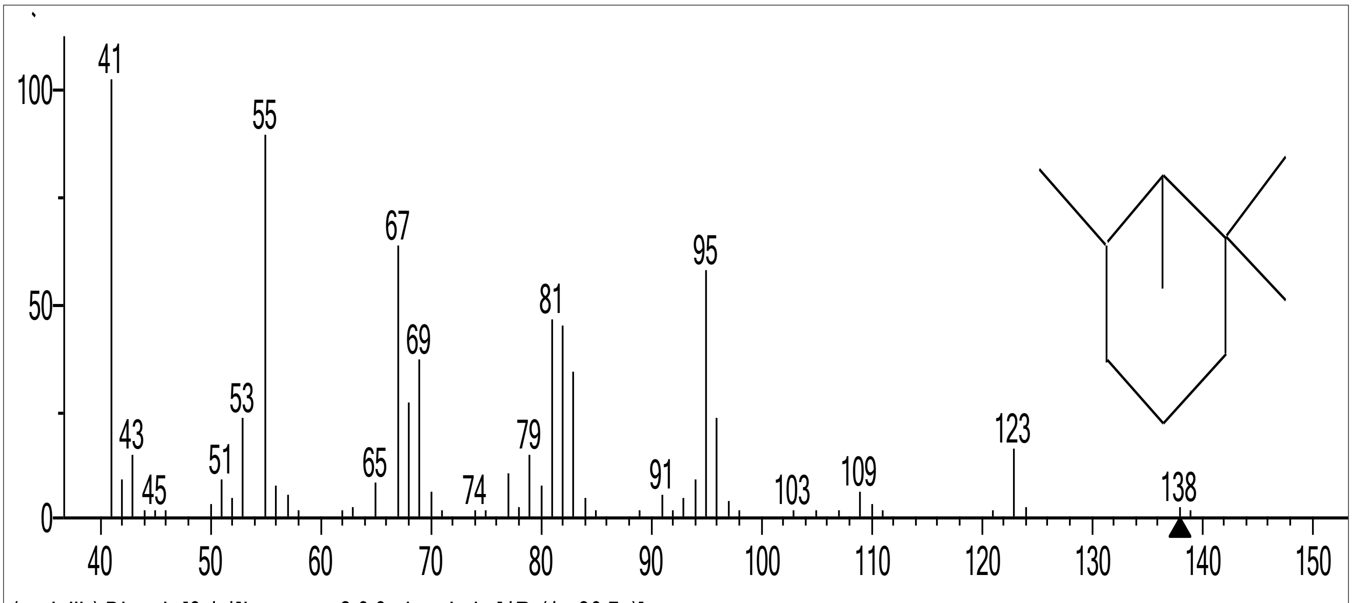
(mainlib) Cyclopentaneundecanoic acid



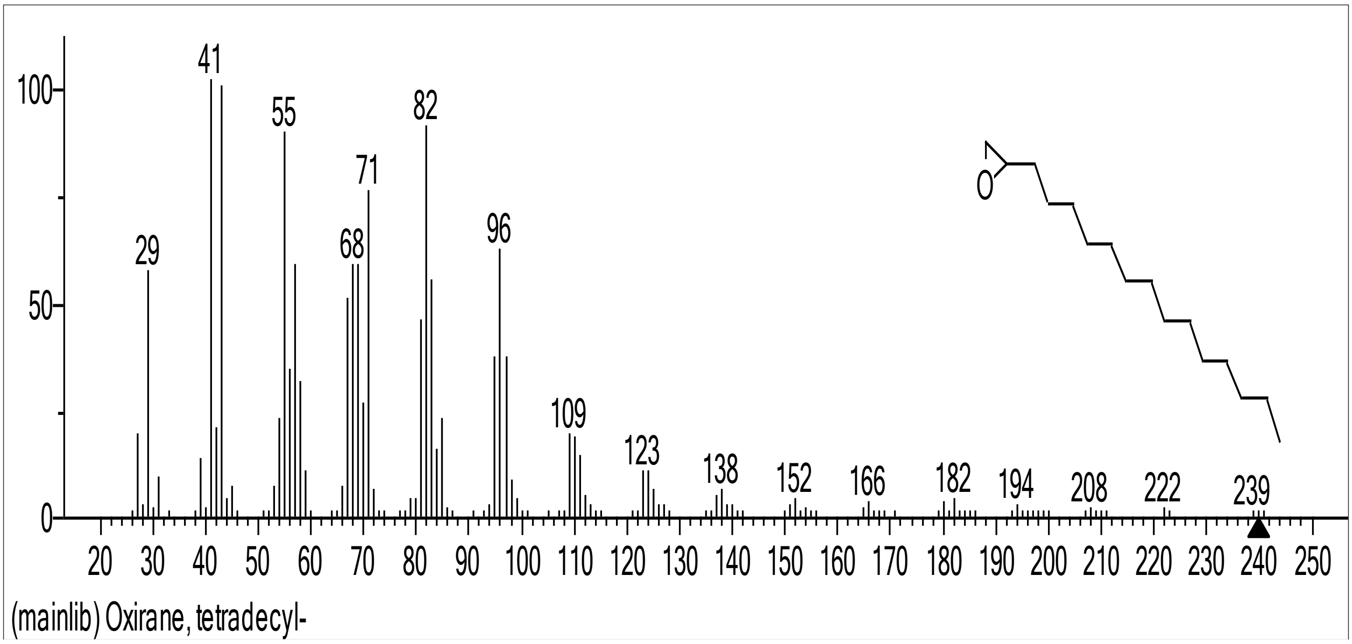
(mainlib) Heptane, 4-azido-

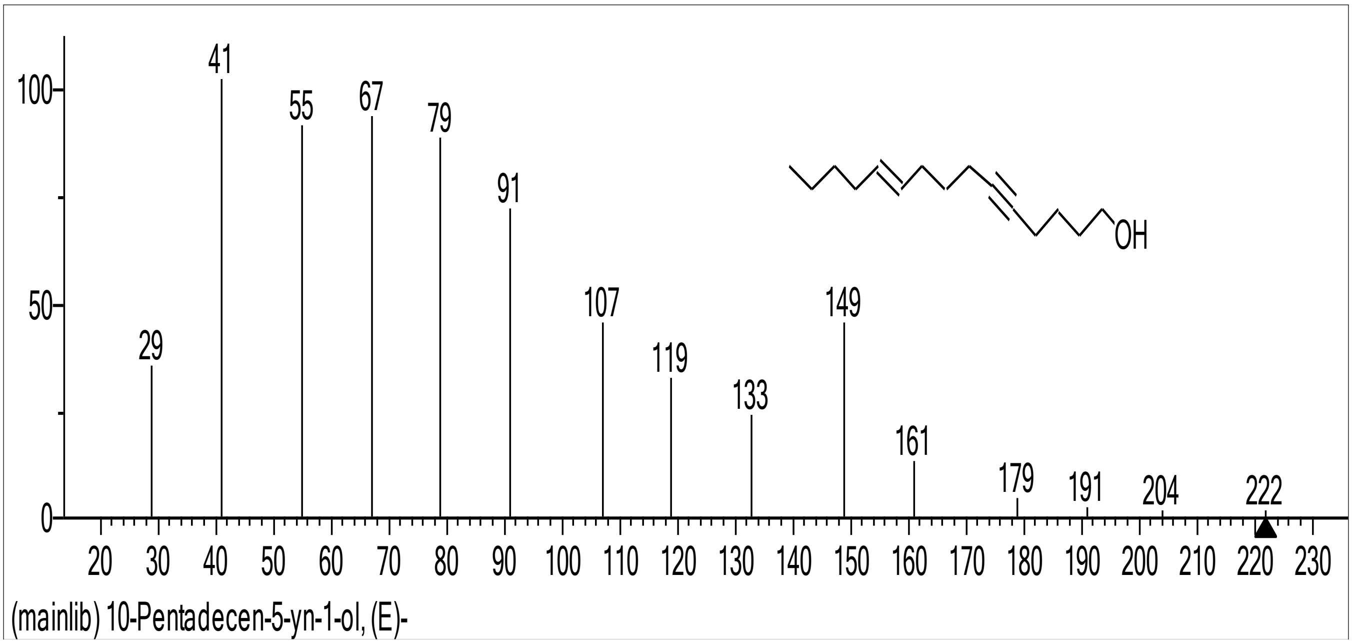


(mainlib) Ethane, 1,2-dicyclopropyl-



(mainlib) Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-, [1R-(1α,2β,5α)]-





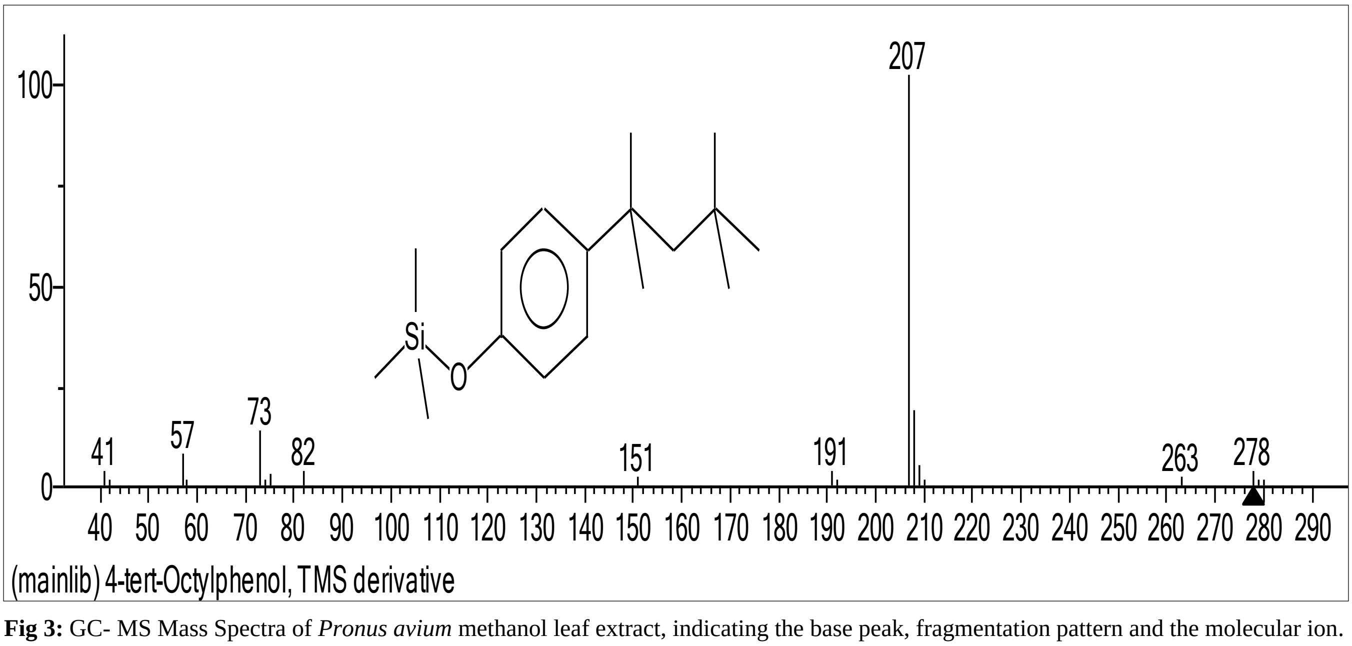


Fig 3: GC- MS Mass Spectra of *Pronus avium* methanol leaf extract, indicating the base peak, fragmentation pattern and the molecular ion.

Conclusion

In the present study twenty four chemical constituents have been identified from Methanolic extract of the plant leaves of *Prunus avium* by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds on the methanol leaf extract can be attributed to its widely use in forklore medicines. The phytochemical analysis of the medicinal plants are important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for the treatment of various diseases. The presence of these metabolites lends credence to the claims of the use of plants for the treatment of diseases by trado-medical doctors. However isolation of individual phytochemical constituents and subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Prunus avium* contains various bioactive compounds. So it is recommended as a plant of phytopharmaceutical importance.

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