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Research Article

Anti-bacterial, Antifungal Activity and Chemical Analysis of *Punica grantanum* (Pomegranate peel) Using GC-MS and FTIR Spectroscopy

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ABSTRACT

Medicinal plants are important source of antibacterial compounds. These plants contain secondary metabolites such as alkaloids, flavonoids, steroids, phenolics, terpenes and volatile oils The objective of this research was to determine the chemical composition of methanolic peel extract. The phytochemical compound screened by GC-MS method. Twentyseven bioactive phytochemical compounds were identified in the methanolic extract of *Punica granatum*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, MS Fragment- ions and Pharmacological actions. GC-MS analysis of *Punica granatum* revealed the existence of the 2H-Pyran,2,2´-[1,10-decanediylbis(oxy)]bis[tetrahydro-, 6-Oxa-bicyclo[3.1.0]hexan-3-one, 2,5-Furandione, 3-methyl-, 2-Furancarboxaldehyde,5-methyl, D-Glucose, 6-O-α-D-galactopyranosyl, D-Limonene, Lactose, DL-Arabinose, 5-Methyl -2- pyrazinylmethanol, 6-Acetyl-ß-d-mannose, α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw)-ß-D-fruc, 4-Hexenal,6-hydroxy-4-methyl-,dimethyl acetal, acetate, (Z), 4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl, 4-Chloro-3-n-hexyltetrahydropyran, 4-Methyl itaconate, 5-Hydroxymethylfurfural, 4,6-di-tert-butyl-m-cresol, 3-butyl-4-nitro-pent-4-enoic acid , methyl ester, 1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl, n-Hexadecanoic acid, Estra-1,3,5(10)-trien-17ß-ol, Cis-Vaccenic acid, 9-Octadecenamide, 8,14-Seco-,3,19-epoxyandrostane-8,14-dione,17-acetoxy -3ß-meth, Dasycarpidan-1-methanol,acetate(ester), α-Tocopheryl acetate and γ-Sitosterol. The FTIR analysis of *Punica granatum* peel proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro Compounds, Alkanes, H-bonded H-X group, Hydrogen bonded Alcohols and Phenols. *Punica granatum* was highly active against *Aspergillus fumigatus* (7.00±0.150). Bioactive compounds of *Punica granatum* was assayed for in vitro antibacterial activity against *Proteus mirabilis, Pseudomonas aerogenosa, Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* using the diffusion method in agar. The zone of inhibition were compared with different standard antibiotics. The diameters of inhibition zones ranged from 5.91±0.200 to 1.00±0.110 mm for all treatments.

Key words: Antifungal, Antibacterial activity, *Punica granatum,* Gas chromatography-mass spectrometry, Fourier-transform infrared spectroscopy, Phytochemicals.

INTRODUCTION

Pomegranate (*Punica granatum* L.), is one of oldest fruit trees known to human. The pomegranate (*Punica granatum* L.) is among historic native horticultural plants of Iraq which have been cultivated in different regions of the country. This plant is native from Iran to the Himalayas in northern India and also cultivated over in all over Mediterranean region[1-6]. It is widely used in traditional medicine to cure inflammation, diabetes, cardiac disease, AIDS, ischemia and cancer. Modern research has shown that the pomegranate contains polyphenols and anthocyanidins that are powerful free-radical scavengers and are more effective against disease than are those in green tea. Thus different extraction methods and different solvents will elute different bioactive compounds. GC-MS analysis revealed the presence of 36 compounds. n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)-, 3,4-Difluorobenzoic acid, 4-dodecyl ester, Stigmasterol and 5-Hydroxymethylfurfural. The pomegranate has also been shown to induce programmed cell death and to inhibit tumor invasion, proliferation and angiogenesis. It targets several proteins in the cell-signaling pathway. The unique biochemistry of the pomegranate tree is quiet intriguing. In addition to the high levels of antioxidant-rich tannis and flavonids in its juice and peel, the crushed and dry seeds of its fruit produce distinct oil, about 60% of which is a very rare 18-carbon fatty acid, also referred to as punicic acid. Two new beta-sitosterol esters have been isolated by Bagri et al., (2009)[7], from the flowers of *Punica granatum* Linn. several compounds in *Punica granatum* were previously reported by several authors, Di hydroxyl pyridine, N-Nitroso-2-methyl oxazolidine, 2,5-Furandicarboxaldehyde, Undecane, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy- 6-methyl[8-9], Hesperetin[10] and

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Table 1: Major phytochemical compounds identified in methanolic extract of *Punica grantanum*.

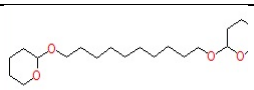
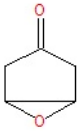
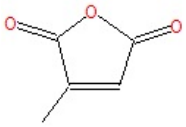
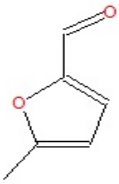
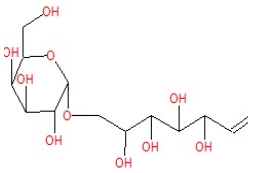
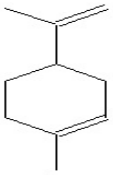
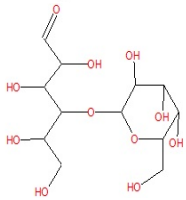
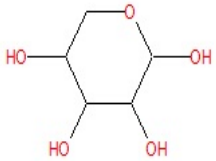
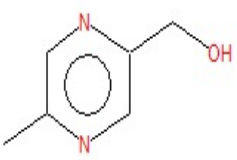
S. No	Phytochemical compound	RT (min)	Formula	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	Pharma-cological actions
1.	2H-Pyran,2,2´-[1,10-decanediyl bis(oxy)]bis[tetrahydro-	3.287	$C_{20}H_{38}O_4$	342	342.27701		55,85,101, 157,187,227,257	Anti-bacterial and anti-fungal effects
2.	6-Oxa-bicyclo[3.1.0]hexan-3-one	3.367	$C_5H_6O_2$	98	98.0367794		55,69,98	Anti-trypanosoma activity
3.	2,5-Furandione, 3-methyl-	3.476	$C_5H_4O_3$	112	112.016044		53,68,96,112	Anticancer effect
4.	2-Furancarboxaldehyde, 5-methyl-	3.670	$C_6H_6O_2$	110	110.0367794		53,81,95,110	Biological properties including significant antibacterial and anti-fungal effects
5.	D-Glucose,6-O-α-D-galactopyranosyl	3.779	$C_{12}H_{22}O_{11}$	342	342.11621		60,73,85,110,126,182,212,261	Anti-inflammatory
6.	D-Limonene	3.945	$C_{10}H_{16}$	136	136.1252		53,68,79,93,136	Anti-stress effects
7.	Lactose	4.489	$C_{12}H_{22}O_{11}$	342	342.11621		60,73,91,97,126,145,163,191	Anti-hypertensive and Anti-microbial
8.	DL-Arabinose	4.603	$C_5H_{10}O_5$	150	150.052823		60,85,149	Anti-tumor Effect
9.	5-Methyl -2-pyrazinylmethanol	4.878	$C_6H_8N_2O$	124	124.063663		55,66,79,95,124	New chemical compound

Table 1: Major phytochemical compounds identified in methanolic extract of *Punica grantanum*.

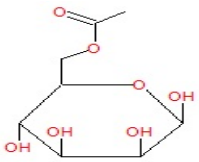
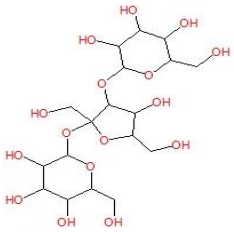
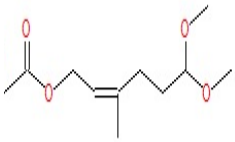
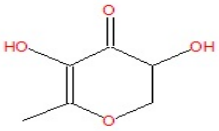
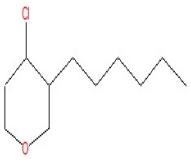
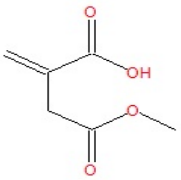
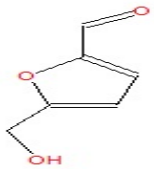
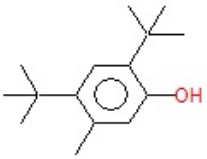
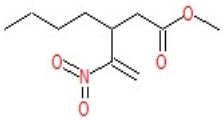
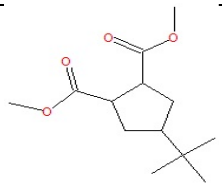
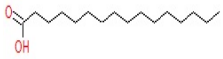
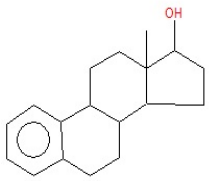
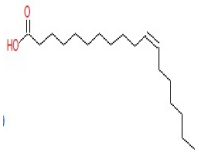
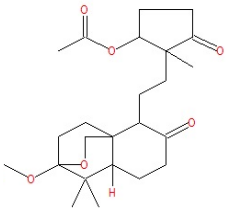
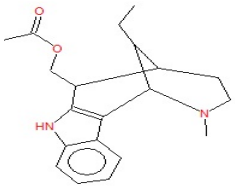
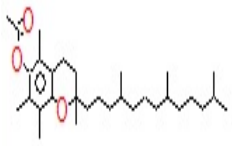
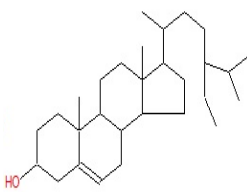
S. No	Phytochemical compound	RT (min)	Formula	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	Pharma-cological actions
10.	6-Acetyl-ß-d-mannose	5.009	$C_8H_{14}O_7$	222	222.073953		60,73,81,97,109,126,144,173,192	Anti-inflammatory and diuretic effects
11.	α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw)-ß-D-fruc	5.204	$C_{18}H_{32}O_{16}$	504	504.169035		60,73,85,97,113,126,145,163,179,199	Anti-diabetic activity
12.	4-Hexenal,6-hydroxy-4-methyl-,dimethyl acetal, acetate , (Z)-	5.370	$C_{11}H_{20}O_4$	216	216.136159		58,67,75,84,93,110,138,152,184,215	Unknown
13.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	5.753	$C_6H_8O_4$	144	144.042258		55,72,85,101,115,144	Anti-oxidant, anti-microbial, laxative, and anti-cancer activities
14.	4-Chloro-3-n-hexyltetrahydropyran	6.108	$C_{11}H_{21}ClO$	204	204.128093		55,69,83,97,125,150,168,203	Biological properties like anti-inflammatory action
15.	4-Methyl itaconate	6.383	$C_6H_8O_4$	144	144.042258		59,68,85,99,113,126,144	Anti-cancer *activity*
16.	5-Hydroxymethylfurfural	6.961	$C_6H_6O_3$	126	126.031694		53,69,81,97,126	Antioxidant
17.	4,6-di-tert-butyl-m-cresol	10.359	$C_{15}H_{24}O$	220	220.182715		57,67,91,149,163,189,205,220	Antioxidants, and anti-inflammatory agents
18.	3-butyl-4-nitro-pent-4-enoic acid , methyl ester	12.774	$C_{10}H_{17}NO_4$	215	215.115758		55,67,74,85,95,109,125,137,169,184,198	Anti-carcinogenic

Table 1: Major phytochemical compounds identified in methanolic extract of *Punica grantanum*.

S. No	Phytochemical compound	RT (min)	Formula	Mol. Wt.	Exact Mass	Chemical structure	MS Fragment-ions	Pharma-cological actions
19.	1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)- ,dimethyl	13.512	$C_{13}H_{22}O_4$	242	242.151809		57,67,81,107,126,135,154,186,211,242	Antioxidant
20.	n-Hexadecanoic acid	15.212	$C_{16}H_{32}O_2$	256	256.24023		60,73,83,97,115,129,157,185,213,227,256	Anti-inflammatory, antispasmodic, anticancer and antiviral
21.	Estra-1,3,5(10)-trien-17ß-ol	15.349	$C_{18}H_{24}O$	256	256.182714		57,73,85,97,185,213,256	Antitumor, anti-inflammatory, antioxidant and antibacterial activities
22.	Cis-Vaccenic acid	16.882	$C_{18}H_{34}O_2$	282	282.25588		55,69,83,97,111,123,165,193,222,264,282	Anti-inflammatory effects
23.	9-Octadecenamide	17.243	$C_{18}H_{35}NO$	281	281.271864		55,72,83,122,136,150,220,281	Anti-inflammatory effects
24.	8,14-Seco-,3,19-epoxyandrostane-8,14-dione,17-acetoxy -3ß-meth	21.134	$C_{24}H_{36}O_6$	420	420.251188		55,69,83,96,111,149,177,209,265,304,360,420	Anti-cancer
25.	Dasycarpidan-1-methanol,acetate(ester)	21.546	$C_{20}H_{26}N_2O_2$	326	326.199429		69,97,124,180,222,256,326	Anti-inflammatory
26.	α-Tocopheryl acetate	26.398	$C_{14}H_{52}O_3$	472	472.391645		57,69,121,165,207,247,288,330,372,430,472	Anti-inflammatory
27.	γ-Sitosterol	29.992	$C_{29}H_{50}O$	414	414.386166		55,69,81,145,161,213,255,303,329,381,396,414	Anti-tumor and chemopreventive activity

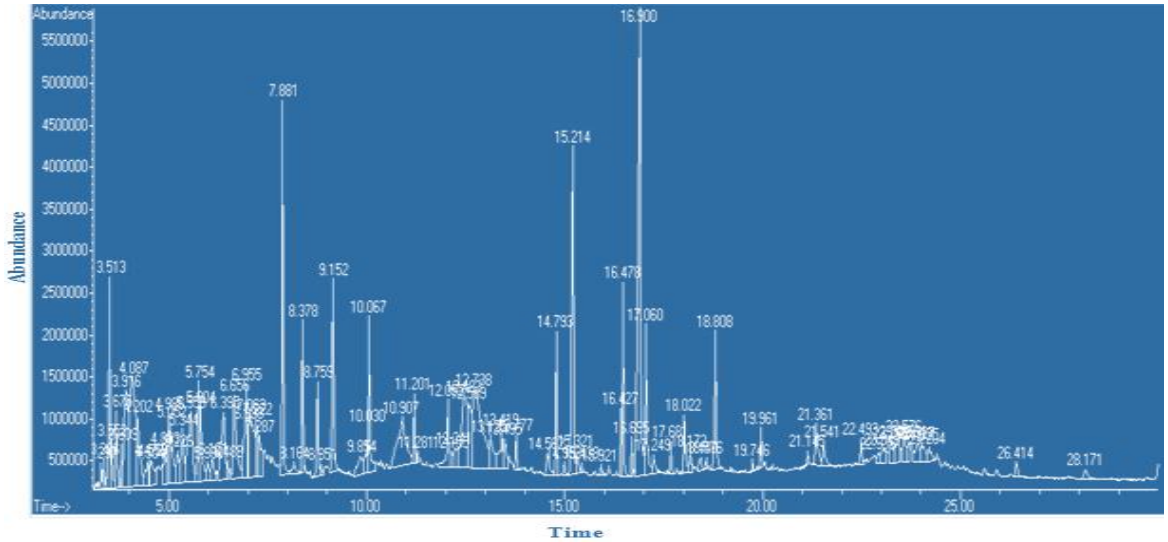


Figure 1: GC-MS chromatogram of methanolic extract of *Punica granatuml*.

Table 2: FT-IR peak values of methanolic extract of *Punica granatuml*.

No.	Peak (Wave number cm-[1])	Intensity	Bond	Functional group assignment	Group frequency
1.	671.23	66.703	C-H	Alkenes	675-995
2.	688.59	67.042	C-H	Alkenes	675-995
3.	707.88	68.216	C-H	Alkenes	675-995
4.	754.17	69.954	C-H	Alkenes	675-995
5.	802.39	73.793	C-H	Alkenes	675-995
6.	875.68	72.802	C-H	Alkenes	675-995
7.	921.97	72.057	C-H	Alkenes	675-995
8.	1016.49	45.588	C-F stretch	Aliphatic fluoro compounds	1000-10150
9.	1145.72	70.738	C-F stretch	Aliphatic fluoro compounds	1000-10150
10.	1226.73	71.554	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
11.	1317.38	71.787	NO2	Nitro Compounds	1300-1370
12.	1338.60	70.830	NO2	Nitro Compounds	1300-1370
13.	1608.63	75.464	-	Unknown	-
14.	2860.43	85.041	C-H	Alkanes	2850-2970
15.	2929.87	82.882	C-H	Alkanes	2850-2970
16.	3082.25	83.140	H-O	H-bonded H-X group	2500-3500
17.	3176.76	80.314	H-O	H-bonded H-X group	2500-3500
18.	3219.19	79.272	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
19.	3246.20	78.835	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
20.	3265.49	78.558	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600
21.	3334.92	78.477	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

MATERIALS and METHODS

Collection and preparation of plant material

The peels were dried at room temperature for five days and when properly dried then powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

Preparation of sample

About twelve grams of the plant sample powdered were soaked in 120 ml methanol individually. It was left for 72 hours so that alkaloids, flavonoids and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed[12,13].

Gas chromatography – Mass Spectrum analysis

Table 3: Zone of inhibition (mm) of test bacterial strains to *Punica granatuml* bioactive compounds and standard antibiotics.

Bacteria	Antibiotics / Plant (*Punica granatuml)*			
	Punica granatuml	Streptomycin	Rifambin	Cefotoxime
Pseudomonas eurogenosa	5.91±0.200	2.72±0.350	2.66±0.200	3.920±0.200
Escherichia coli	4.00±0.250	4.90±0.200	2.71±0.310	3.93±0.400
Klebsiella pneumonia	4.31±0.200	3.97±0.101	2.43±0.300	1.08±0.250
Staphylococcus aureus	2.54±0.600	2.11±0.390	1.00±0.110	2.96±0.400
Proteus mirabilis	2.00±0.210	3.99±0.200	1.78±0.270	2.60±0.310

Table 4: Zone of inhibition (mm) of *Aspergillus Spp.* test to *Punica granatuml* bioactive compounds and standard antibiotics.

Plant / Antibiotics	*Aspergillus Spp.*			
	Aspergillus niger	*Aspergillus terreus*	*Aspergillus flavus*	*Aspergillus fumigatus*
Punica granatuml	2.96±0.210	5.91±0.520	6.89±0.210	7.00±0.150
Amphotericin B	1.91±0.180	3.98±0.220	3.95±0.5	5.00±0.210
Fluconazol	3.99±0.211	3.86±0.25	2.90±0.451	5.10±0.310
Control	0.00	0.00	0.00	0.00

Squalene[11]. Evaluation of antibacterial and antifungal activity were the objectives of this research.The GC-MS analysis of the plant extract was made in a (QP 2010 Plus SHIMADZU) instrument under computer control at 70 eV. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the Retention time (RT). While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The M/Z (Mass / Charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using Gas Chromatography and Mass Spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV.The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. Compounds were identified by comparing their spectra to those of the Wiley and NIST/EPA/NIH mass spectral libraries[14-16].

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of the plant specimen was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 nm and 4000 nm[17,18].

Determination of antibacterial activity of crude bioactive compounds of Punica granatum.

The test pathogens (*Proteus mirabilis, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus)* were swabbed in Muller Hinton agar plates. 60µl of plant extract was loaded on the bored wells. The wells were bored in 0.5cm in diameter. The plates were incubated at 37C° for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured[19,20].

Determination of antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µl of the samples solutions (*Punica granatum*) was delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity

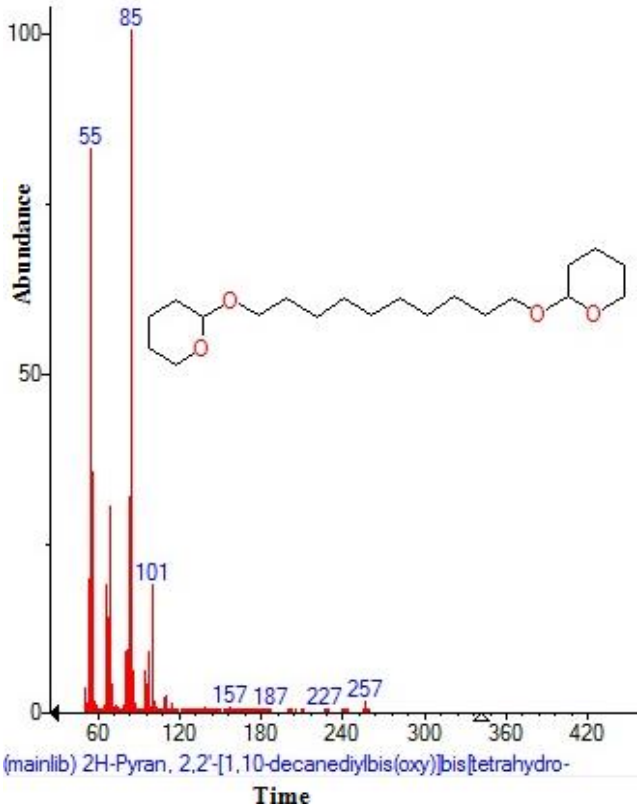


Figure 2: Mass spectrum of 2H-Pyran,2,2´-[1,10-decanediylbis(oxy)]bis[tetrahydro with Retention Time (RT)= 3.287

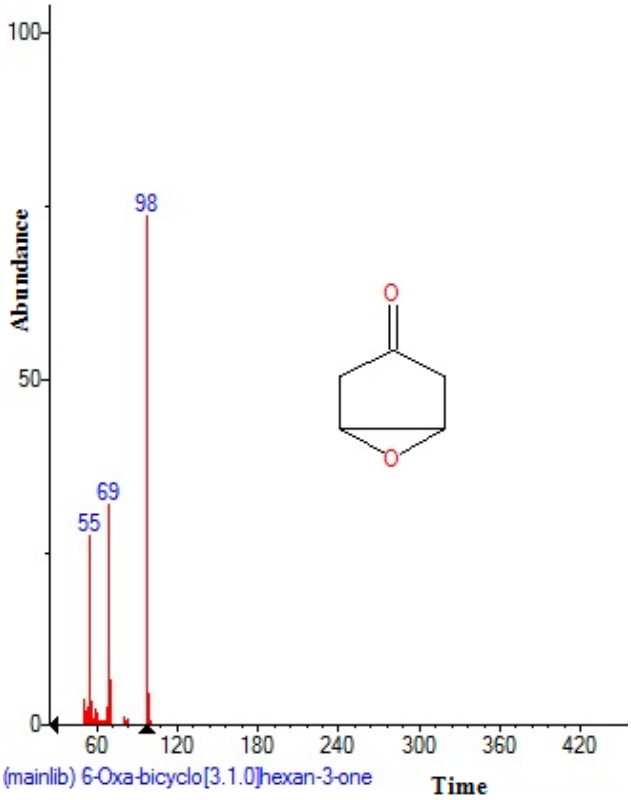


Figure 3: Mass spectrum of 6-Oxa-bicyclo[3.1.0]hexan-3-one with Retention Time (RT)= 3.367

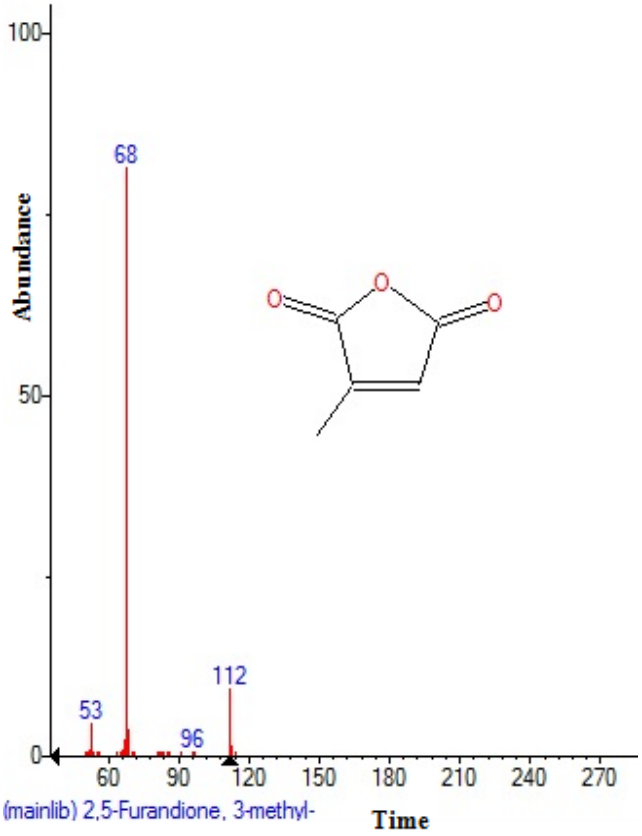


Figure 4: Mass spectrum of 2,5-Furandione, 3-methyl with Retention Time (RT)= 3.476

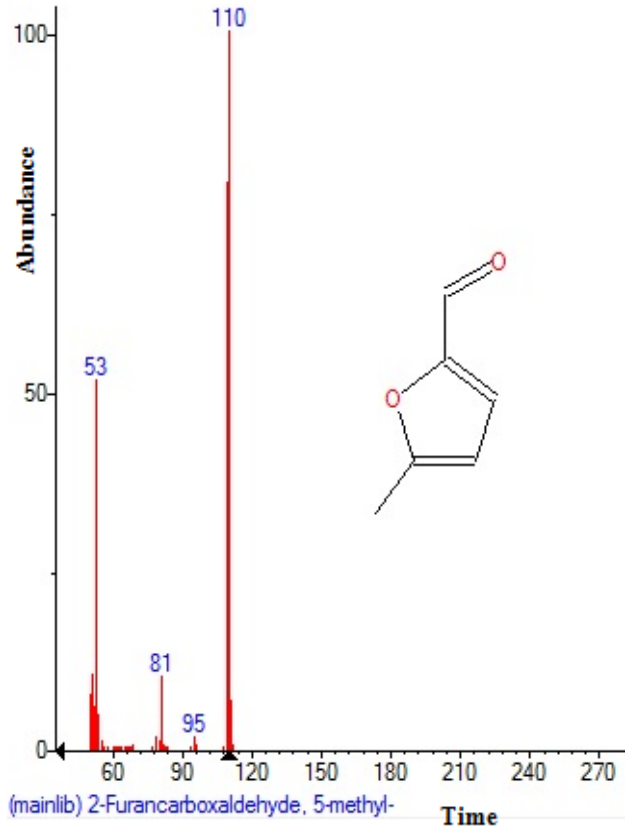


Figure 5: Mass spectrum of 2-Furancarboxaldehyde,5-methyl with Retention Time (RT)= 3.670

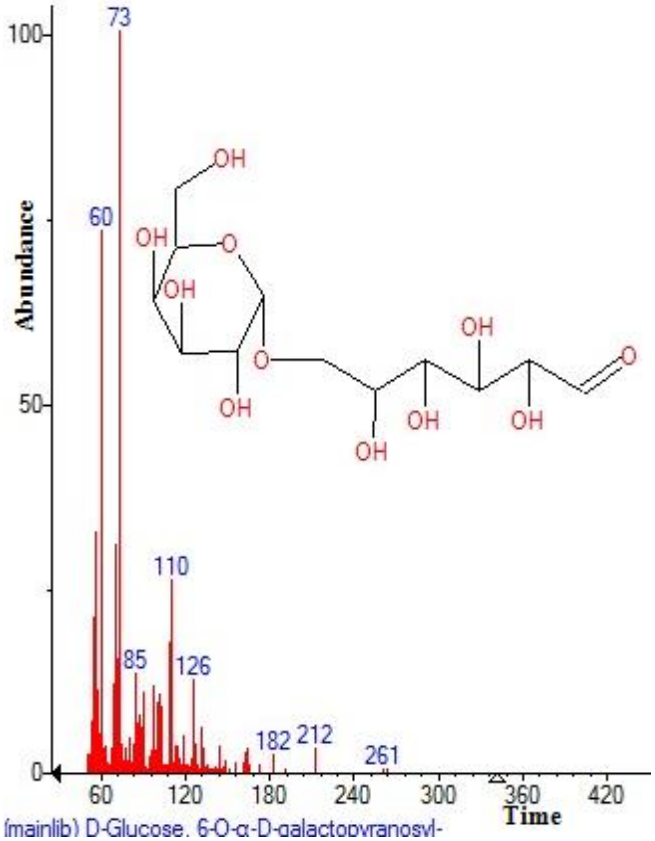


Figure 6: Mass spectrum of D-Glucose ,6-O-α-D-galactopyranosyl with Retention Time (RT)= 3.779

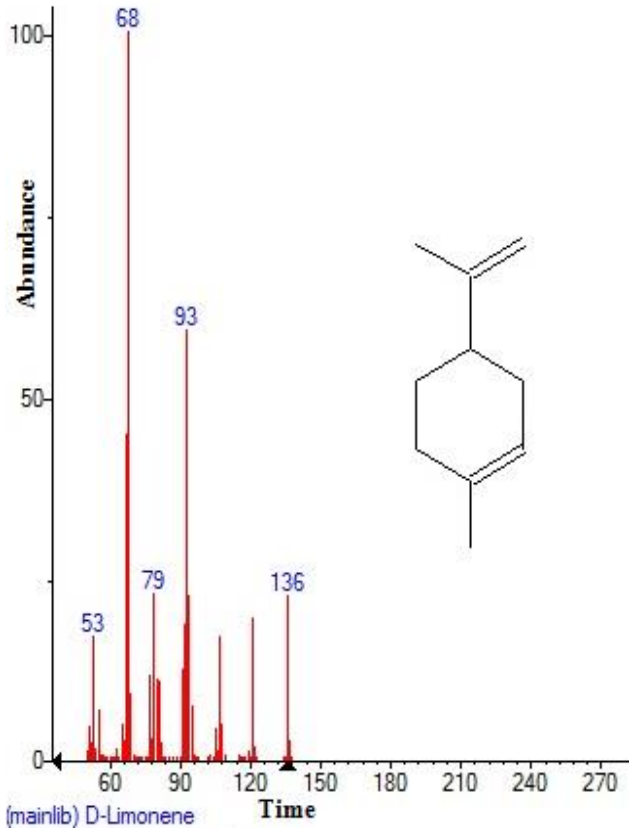


Figure 7: Mass spectrum of D-Limonene with Retention Time (RT)= 3.945

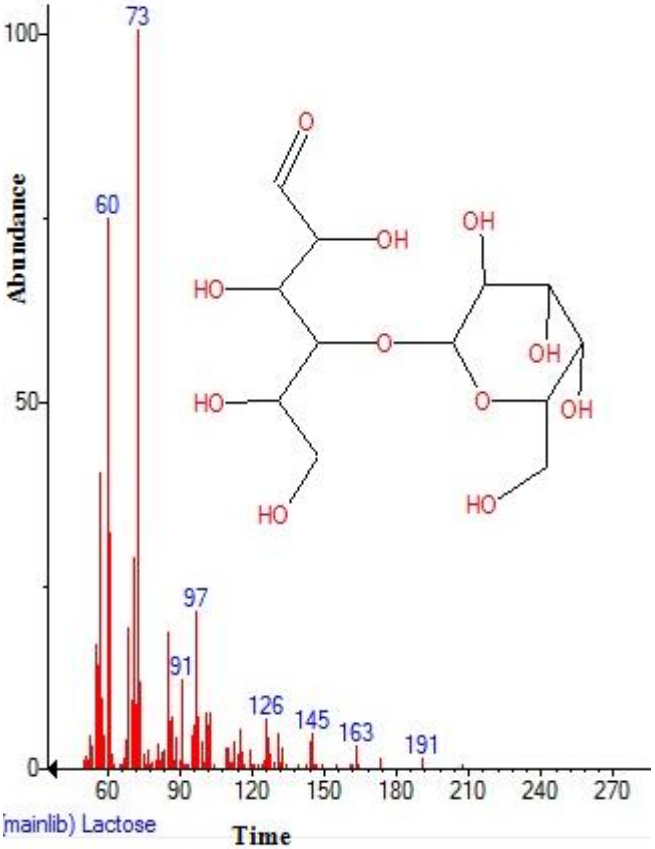


Figure 8: Mass spectrum of Lactose with Retention Time (RT)= 4.489

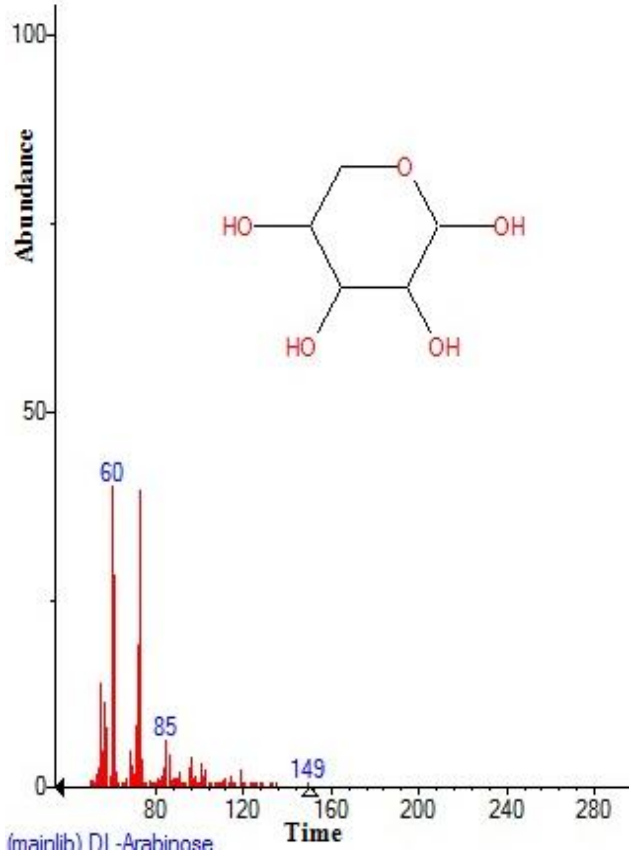


Figure 9: Mass spectrum of DL-Arabinose with Retention Time (RT)= 4.603

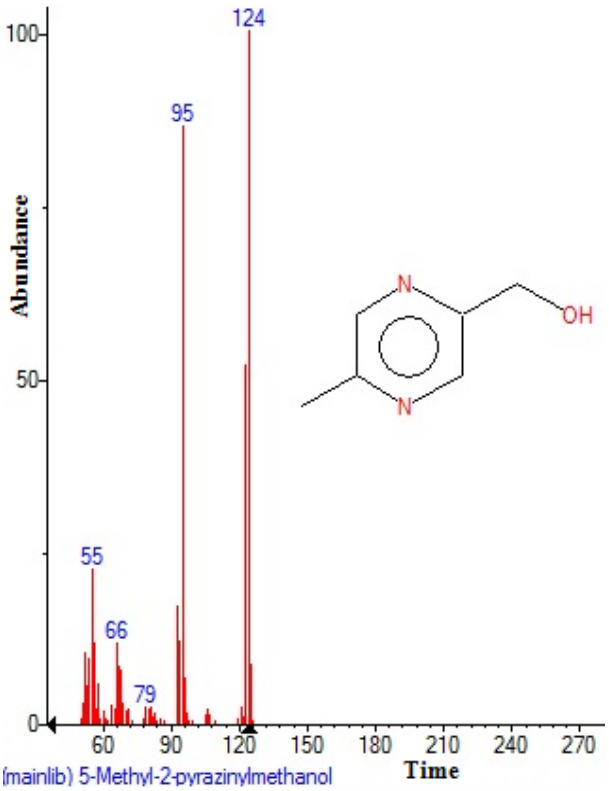


Figure 10: Mass spectrum of 5-Methyl -2-pyrazinylmethanol with Retention Time (RT)= 4.878

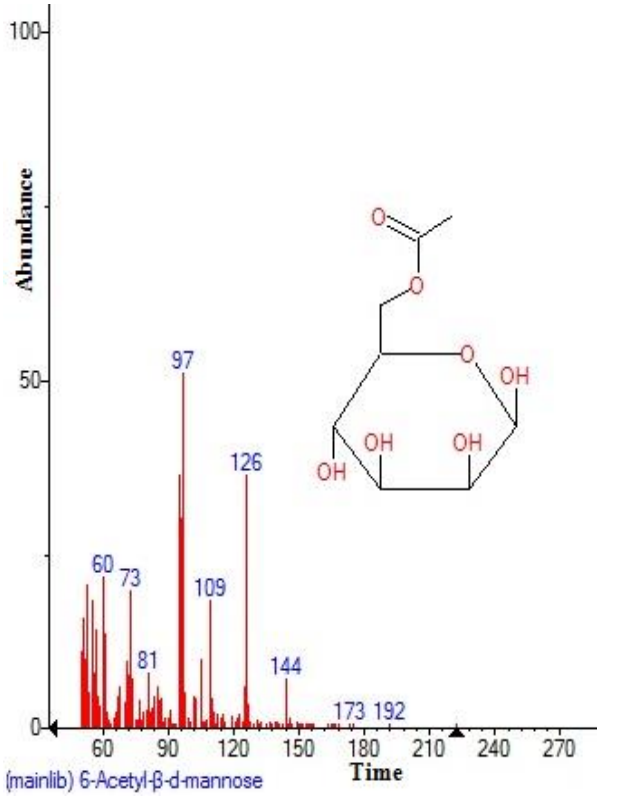


Figure 11: Mass spectrum of 6-Acetyl-ß-d-mannose with Retention Time (RT)= 5.009

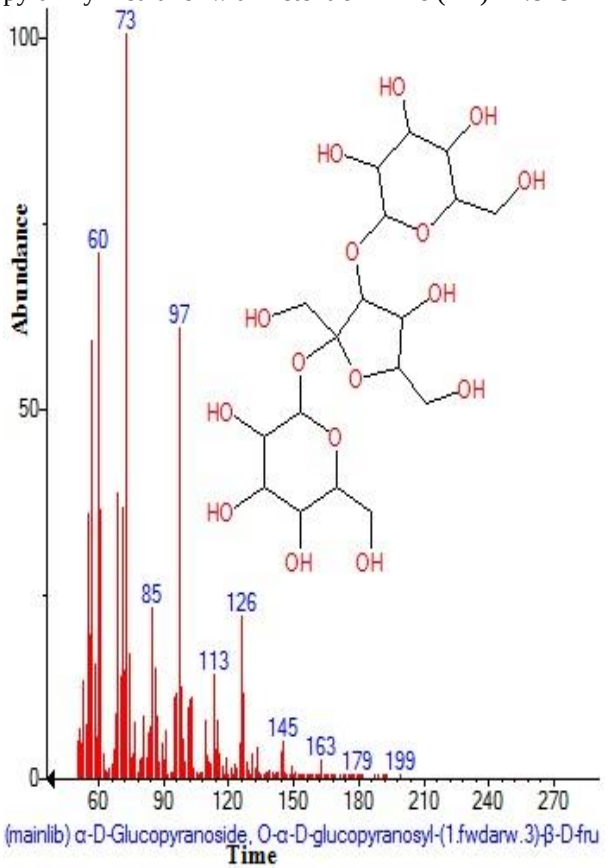


Figure 12: Mass spectrum of α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw)-ß-D-fruc with Retention Time (RT)= 5.204

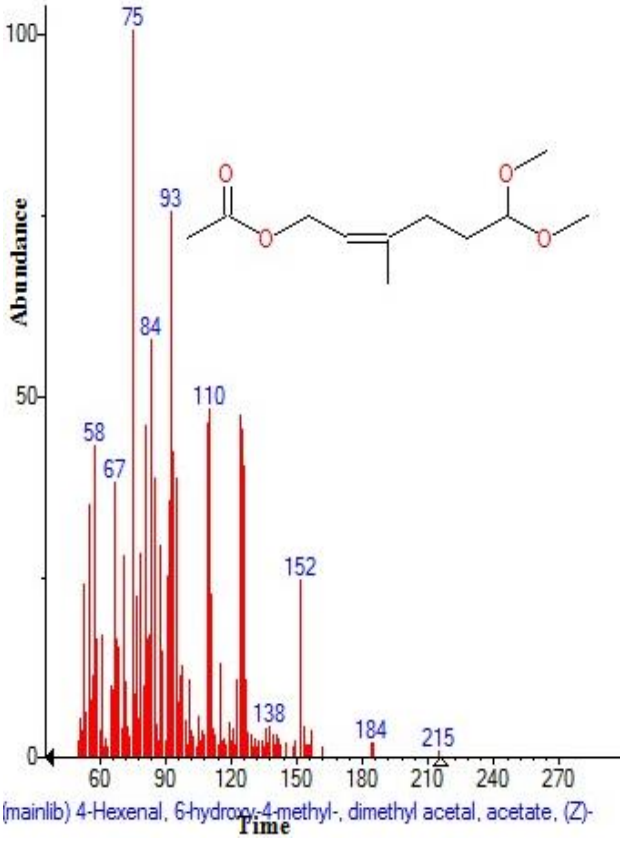


Figure 13: Mass spectrum of 4-Hexenal,6-hydroxy-4-methyl-, dimethyl acetal, acetate, (Z) with Retention Time (RT)= 5.370

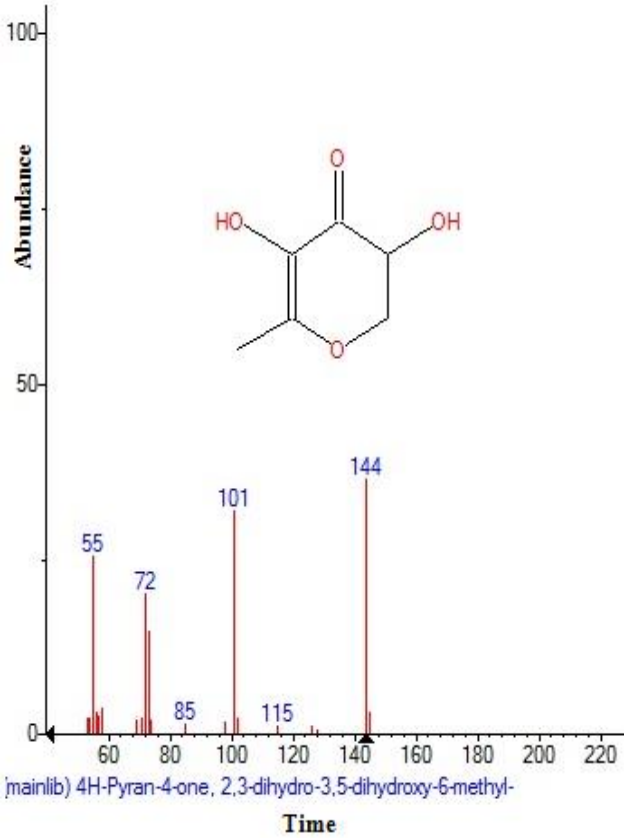


Figure 14: Mass spectrum of 4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl with Retention Time (RT)= 5.753

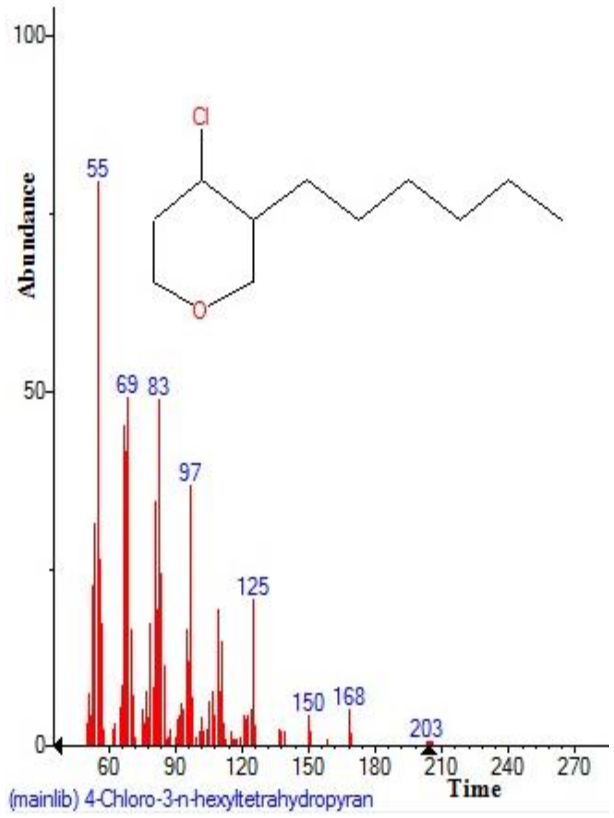


Figure 15: Mass spectrum of 4-Chloro-3-n-hexyltetrahydropyran with Retention Time (RT)= 6.108

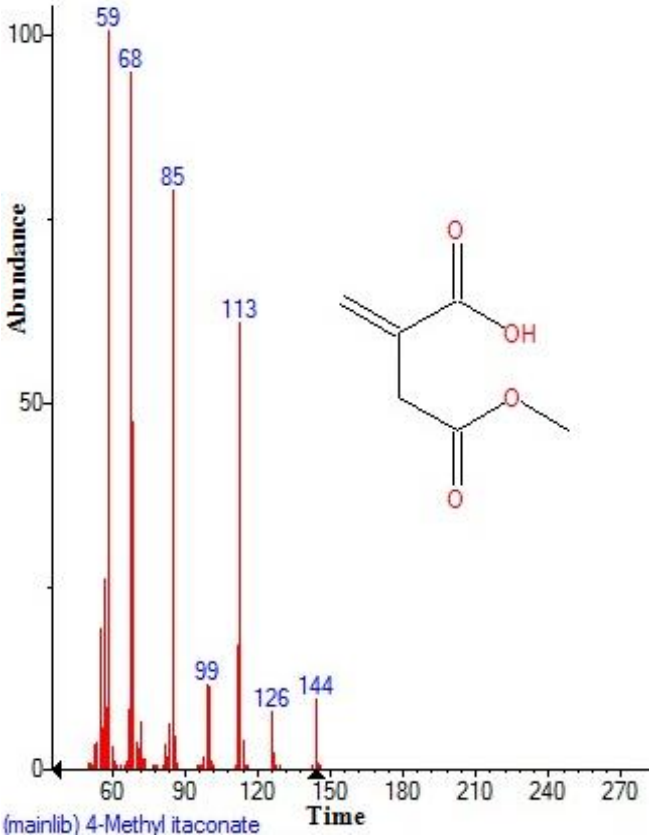


Figure 16: Mass spectrum of 4-Methyl itaconate with Retention Time (RT)= 6.383

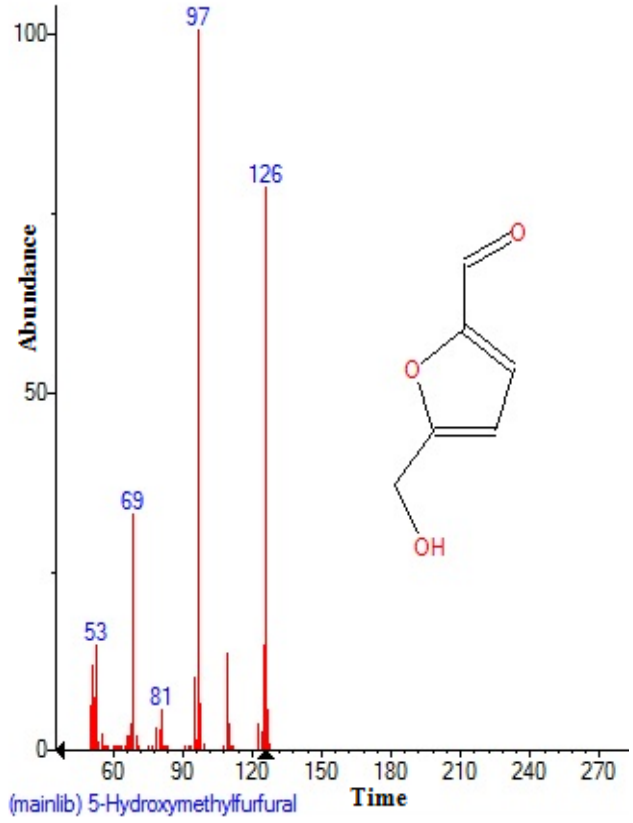


Figure 17: Mass spectrum of 5-Hydroxymethylfurfural with Retention Time (RT)= 6.961

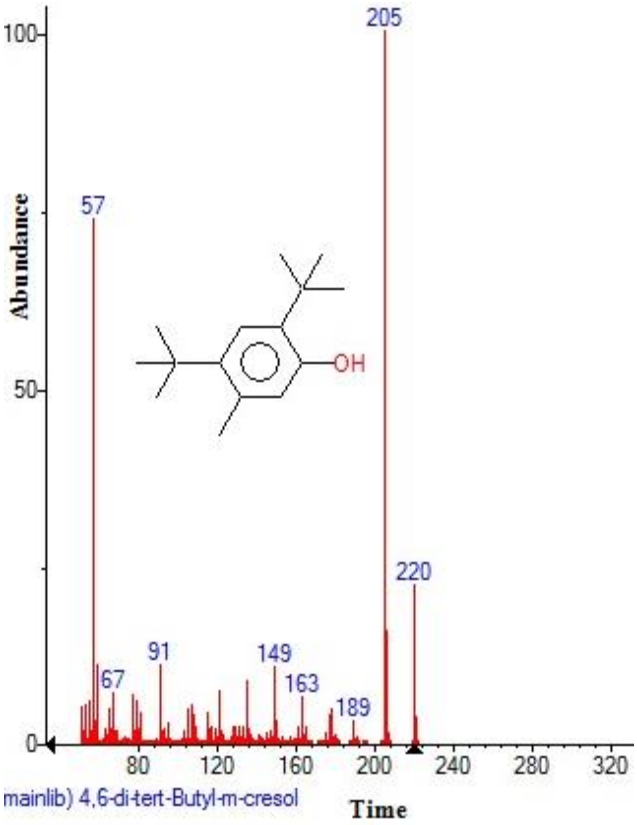


Figure 18: Mass spectrum of 4,6-di-tert-butyl-m-cresol with Retention Time (RT)= 10.359

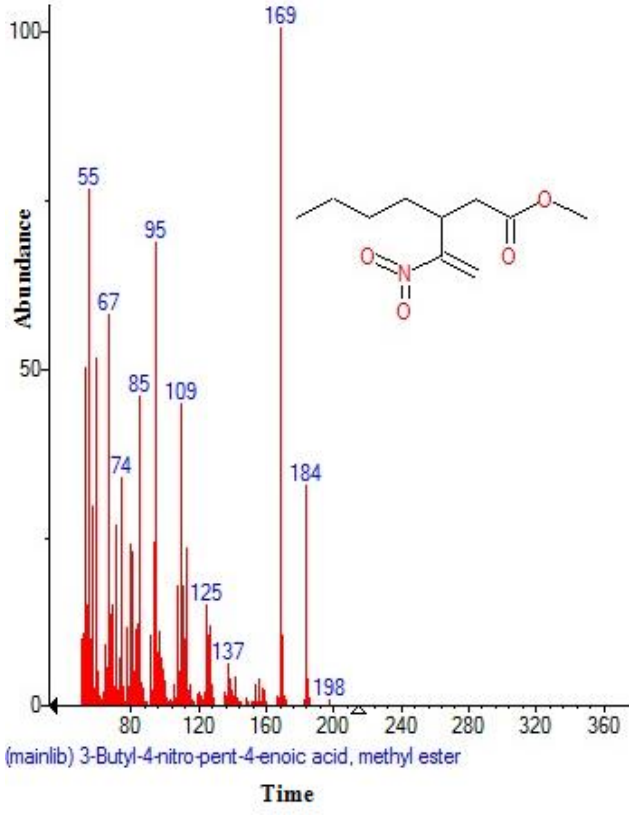


Figure 19: Mass spectrum of 3-butyl-4-nitro-pent-4-enoic acid, methyl ester with Retention Time (RT)= 12.774

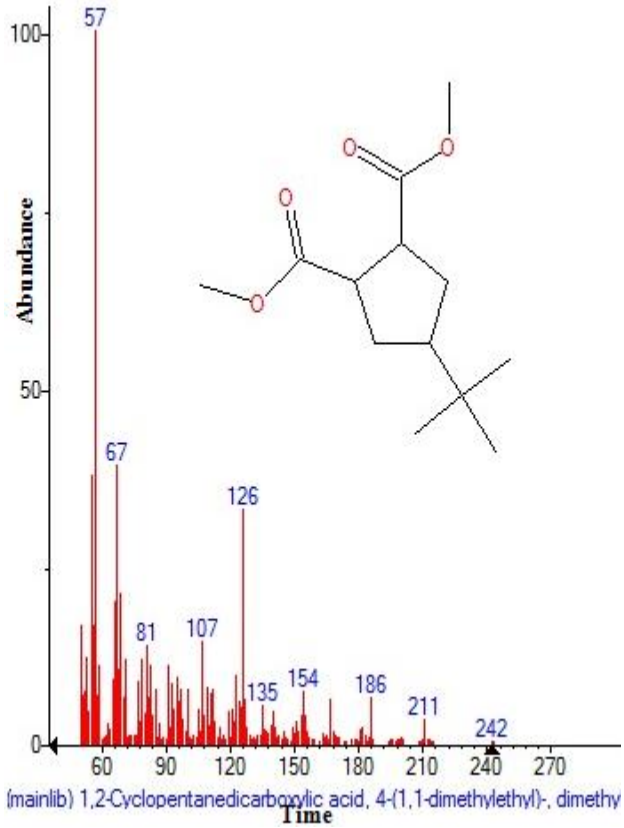


Figure 20: Mass spectrum of 1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-, dimethyl with Retention Time (RT)= 13.512

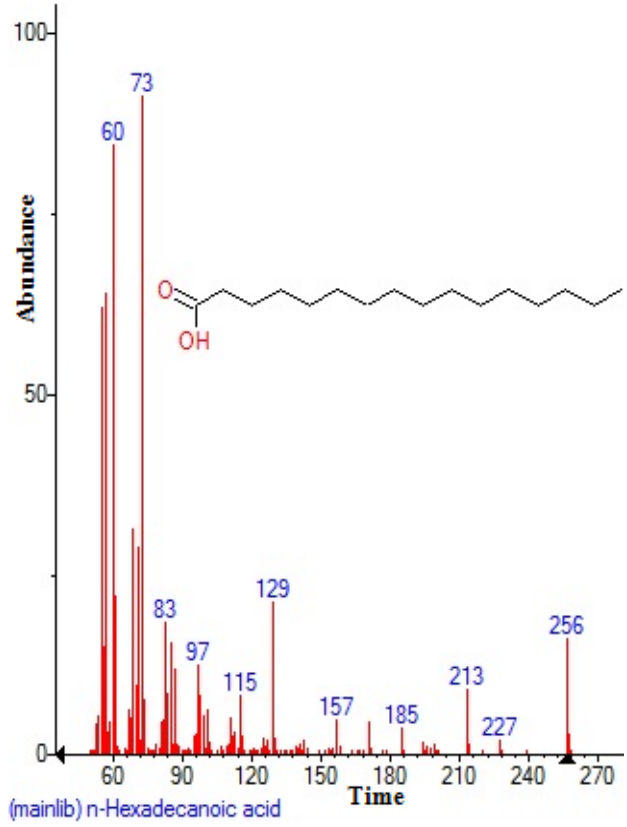


Figure 21: Mass spectrum of n-Hexadecanoic acid with Retention Time (RT)= 15.212

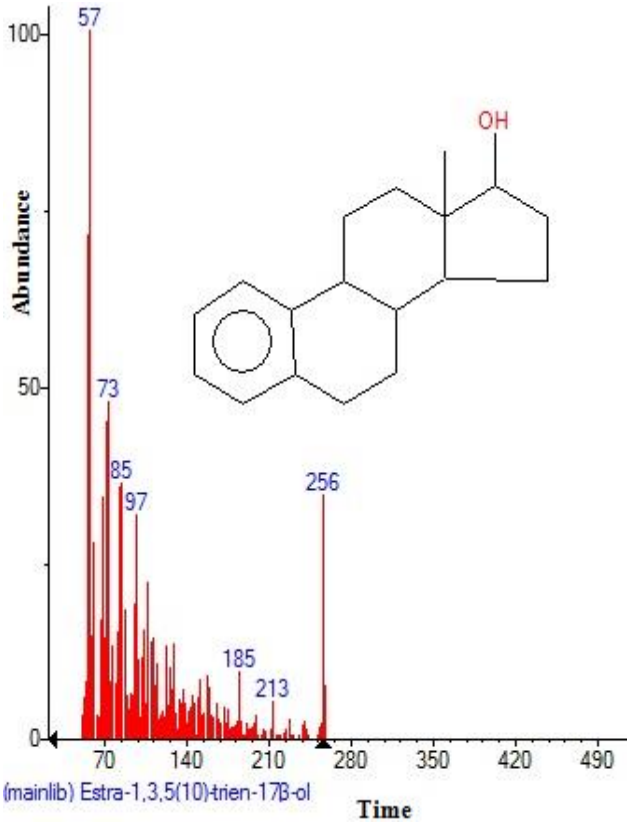


Figure 22: Mass spectrum of Estra-1,3,5(10)-trien-17ß-ol with Retention Time (RT)= 15.349

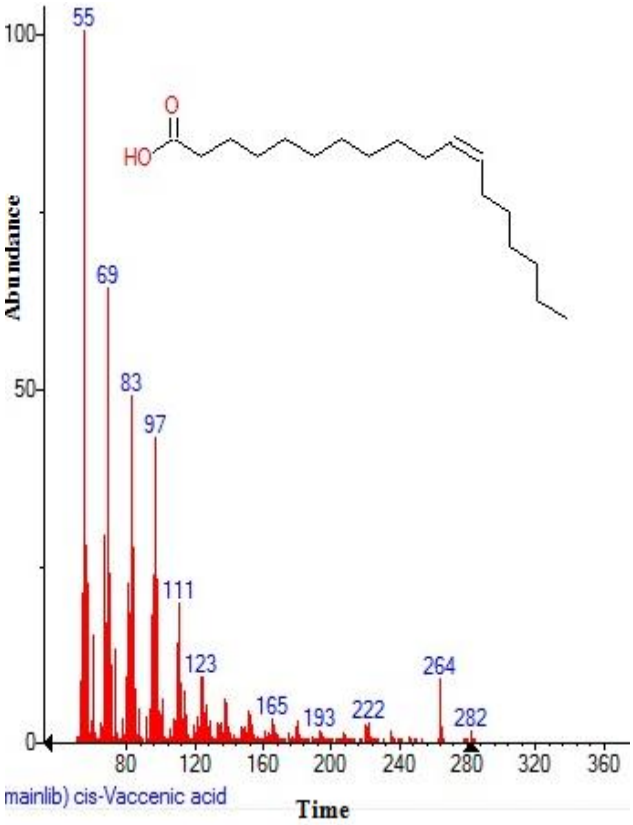


Figure 23: Mass spectrum of Cis-Vaccenic acid with Retention Time (RT)= 16.882

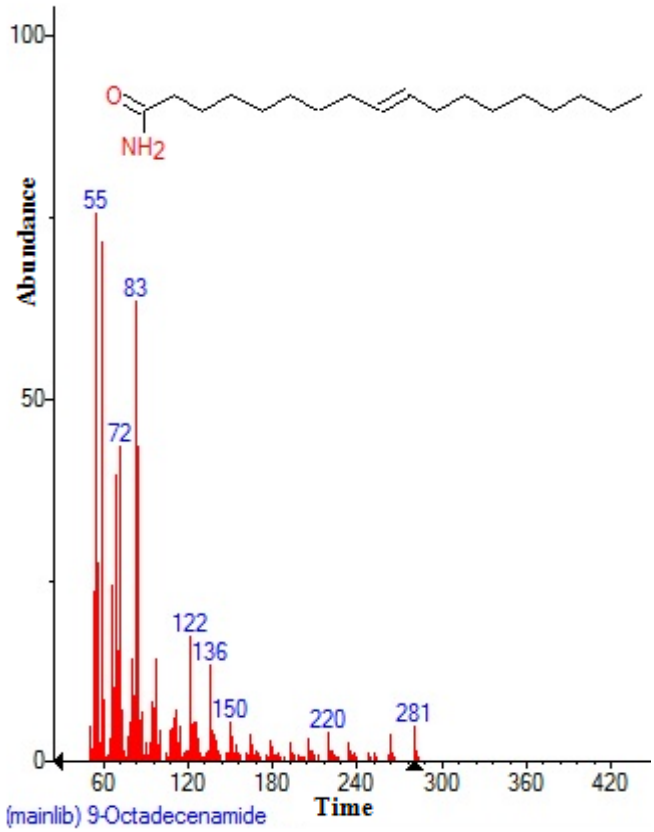


Figure 24: Mass spectrum of 9-Octadecenamide with Retention Time (RT)= 17.243

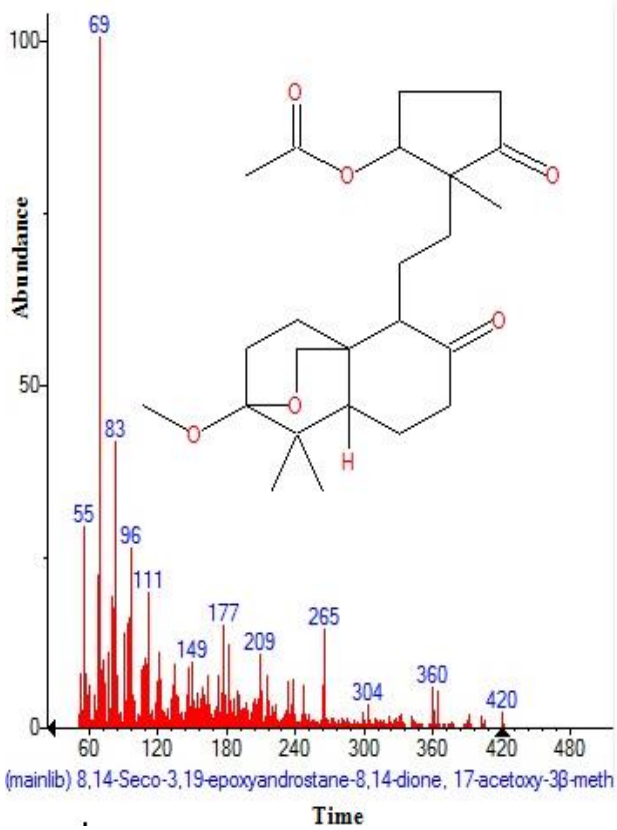


Figure 25: Mass spectrum of 8,14-Seco-,3,19-epoxyandrostane-8,14-dione,17-acetoxy -3ß-meth with Retention Time (RT)= 21.134

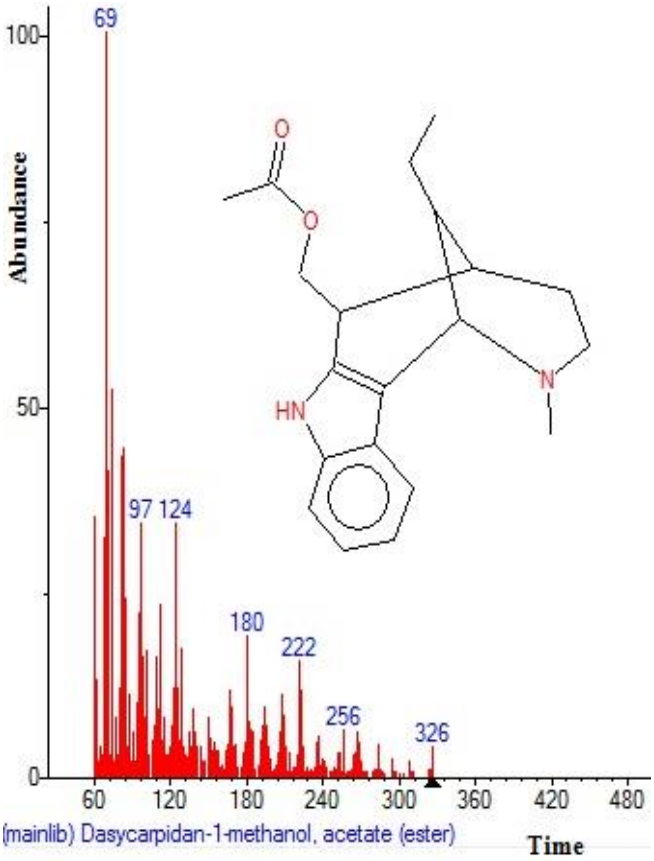


Figure 26: Mass spectrum of Dasycarpidan-1-methanol, acetate(ester) with Retention Time (RT)= 21.546

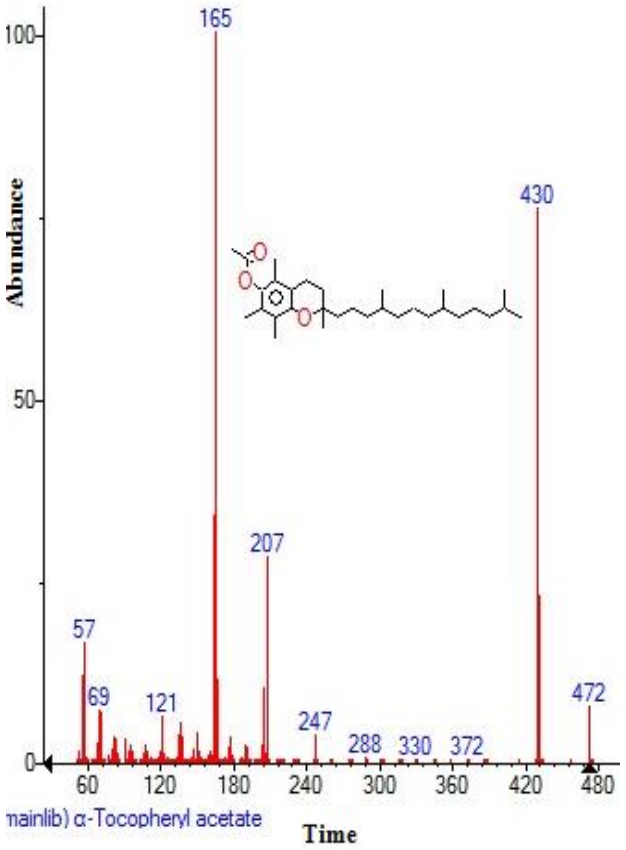


Figure 27: Mass spectrum of α-Tocopheryl acetate with Retention Time (RT)= 26.398

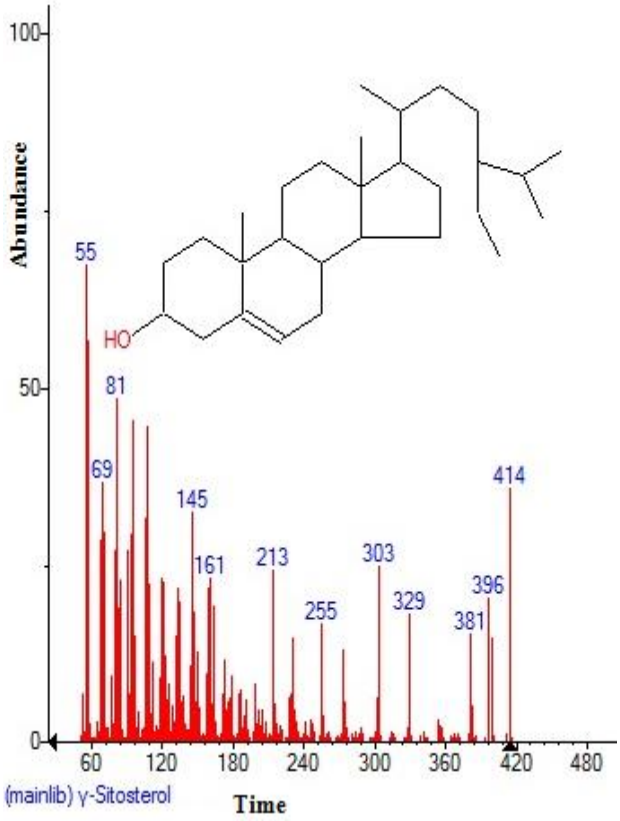


Figure 28: Mass spectrum of γ-Sitosterol with Retention Time (RT)= 29.992

was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software) Version 9.1

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RESULTS and DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic peel extract of *Punica granatum*, shown in Table 1. The GC-MS chromatogram of the 27 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Punica granatum* showed the presence of twentyseven major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be 2H-Pyran,2,2´-[1,10-decanediylbis(oxy)]bis[tetrahydro Figure 2. The second peak indicated to be 6-Oxa-bicyclo[3.1.0]hexan-3-one Figure 3. The next peaks considered to be 2,5-Furandione, 3-methyl-, 2-Furancarboxaldehyde,5-methyl, D-Glucose ,6-O-α-D-galactopyranosyl, D-Limonene, Lactose, DL-Arabinose, 5-Methyl -2- pyrazinylmethanol, 6-Acetyl-ß-d-mannose, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw)-ß-D-fruc, 4-Hexenal,6-hydroxy-4-methyl-,dimethyl acetal, acetate, (Z), 4H-Pyran-4-one, 2,3-dihydro- 3,5-dihydroxy-6-methyl, 4-Chloro-3-n-

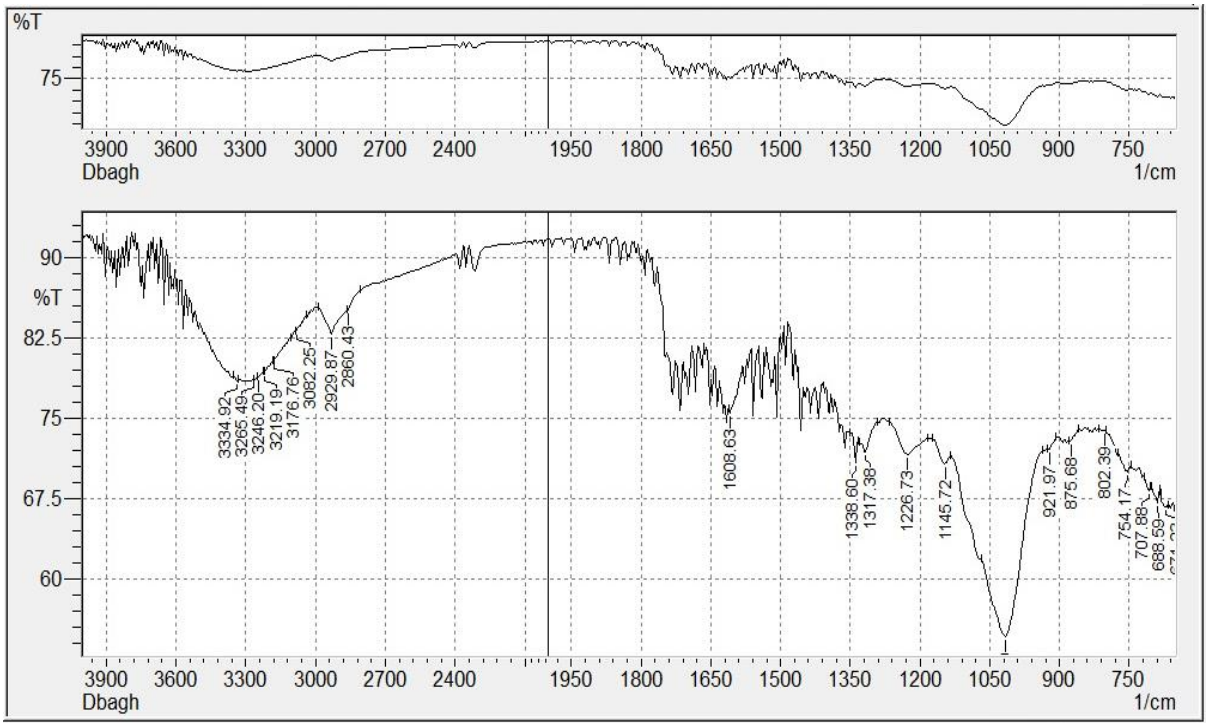

Figure 29: FT-IR profile of *Punica granatum*

hexyltetrahydropyran, 4-Methyl itaconate, 5-Hydroxymethylfurfural, 4,6-di-tert-butyl-m-cresol, 3-butyl-4-nitro-pent-4-enoic acid, methyl ester, 1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl, n-Hexadecanoic acid, Estra-1,3,5(10)-trien-17ß-ol, Cis-Vaccenic acid, 9-Octadecenamide, 8,14-Seco-,3,19- epoxyandrostane-8,14-dione,17-acetoxy -3ß-meth, Dasycarpidan-1-methanol,acetate(ester), α-Tocopheryl acetate and γ-Sitosterol (Figure 4-28). FTIR analysis of dry methanolic extract of *Punica granatum* peel proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro Compounds, Alkanes, H-bonded H-X group, Hydrogen bonded Alcohols and Phenols which shows major peaks at 671.23, 688.59, 707.88, 754.17, 802.39, 875.68, 921.97, 1016.49, 1145.72, 1226.73, 1317.38, 1338.60, 2860.43, 2929.87, 3082.25, 3176.76, 3219.19, 3246.20, 3265.49 and 3334.92 (Table 2; Figure 29). Yoshikazu *et al*. (2001) [21] investigated the inhibitory effect of several plant extracts on the production of verotoxin by enterohemorrhagic *Escherichia coli* O157: H7 (EHEC). Overall the effectiveness using of pomegranate seed oil is in health and possibly in preventing inflammation, brain disorders, diabetes, oxidative stress, hypoxia, hyperlipidemia (possibly decreased low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) cholesterol), cardiac disease, AIDS, ischemia and cancer (especially Skin, Colon, Breast, Prostate and lung) monounsaturated fat consumption has been associated with cholesterol[22-24].

Antibacterial and antifungal activity

Klebsiella pneumoniae, Pseudomonas aeroginosa, E.coli, Staphylococcus aeureus. and *Proteus mirabilis* were five clinical pathogens selected for antibacterial activity. Maximum zone formation against *Klebsiella pneumoniae,* Table 3. Methanolic extraction of plant showed notable antifungal activities against *Aspergillus niger*, *Asp. terreus*, *Asp. flavus*, and *Asp. fumigatus*, Table 4. *Punica granatuml* was very highly active against *Aspergillus fumigatus* (7.00±0.150). *Aspergillus* was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug amphotericin B and fluconazole to some extent.

CONCLUSION

Punica granatum is native plant of Iraq. It contain chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.

ACKNOWLGMENTS

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