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Research Article

Phytochemical Screening of Methanolic Leaves Extract of *Malva sylvestris*

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ABSTRACT

The objectives of this study were analysis of the secondary metabolite products and evaluation antibacterial activity. Bioactives are chemical compounds often referred to as secondary metabolites. Thirty six bioactive compounds were identified in the methanolic extract of *Malva sylvestris*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Malva sylvestris* revealed the existence of the1-Propanamine , 2-methyl-N-(2-methylpropyldene)- , Pyrrolidine,1-(1-oxo-2,5-octadecadienyl)- , Dimethyl sulfoxide , Cyclohexylamine ,N-ethyl- , N-(2-Methylbutylidene)isobutylamine , 1-Methyl-2-pyrrolideethanol , 2-(2-Hydroxyethyl)piperidine , 1-Butanamine , 2-methyl-N-(2-methylbutylidene)- , 4-(Pyrrolidin-2-ylcarbonyl)morpholine , Dithiocarbamate , S-methyl-,N-(2-methyl-3-oxobutyl)- , l-Gala-l-ido-octonic lactone , 1-(5'-methylfurfuryl)pyrrolidine , 2-Methoxy-4-vinylphenol , Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester) , 1-Naphthaienol , 1,2,3,4-tetrahydro-2,5,8-trimethyl- , Pterin-6-carboxylic acid , N-(2-Acetamido)iminodiacetic acid , N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic , Cyclopropanedodecanoic acid , 2-octyl-,methyl ester , Cholestan-3-ol,2-methylene-,(3β,5α)- , 3-(N,N-Dimethyllaurylammonio)propanesulfonate , Pyrazole[4,5-b]imidazole , 1-formyl-3-ethyl-6-β-d-ribofuranosyl- , Octahydrobenzo[b]pyran , 4a-acetoxy-5,5,8a-trimethyl- , Tetraacetyl-d-xylonic nitrile , 4,6-Heptadien-3-one,1,7-diphenyl- , Pentanoic acid ,2,2,4-trimethyl-3-carboxyisopropyl , isobutyl ester , D-Fructose , diethyl mercaptal, pentaacetate , Phytol , Hexadecanamide , Tributyl acetylcitrate , Cholestan-3-one,cyclic 1,2-ethanediyl aetal , (5β)- , Dasycarpidan-1-methanol, acetate (ester)- , 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol , (+)-y-Tocopherol, O-methyl- , Campesterol and Stigmasterol.

Keyword: *Malva sylvestris,* Gas chromatography, Phytochemical, Malvaceae.

INTRODUCTION

Malva sylvestris L. (Malvaceae family), annual or biennial herbaceous medicinal plant usually known as common mallow, is native to Asia, North Africa and Europe[1]. The high mucilage content of *Malva sylvestris* makes it an excellent demulcent that can be used for many applications. In the digestive tract the fruit mucilage can be used to heal and soothe inflammations such as gastritis, peptic ulcers, enteritis, and colitis[2]. *Malva sylvestris* L. has been used in folk medicine of Brazil and other countries for the treatment of colitis and stomatitis, in cases of chronic bronchitis, against furuncle and abscess, contusions and haemorrhoids as well as other dolorous and inflammatory processes[3-7].

Young leaves considered one of the culinary herbs in Palestine and in other Mediterranean countries, they are eaten raw in salads or consumed in soups and as boiled vegetables[8-13]. A pharmaceutical suspension, is thermodynamically unstable, thus, making it necessary to include in the dosage form, a stabilizer or suspending agent which reduces the rate of settling and permits easy redispersion of any settled particulate matter both by protective colloidal action and by increasing the consistency of the suspending medium. It is also widely recognized to have anti-inflammatory properties, some other pharmacological and clinical effects are frequently mentioned such as diuretic, laxative, antiseptic, antispasmodic, lenitive, choleretic, bronchodilator, expectorant, antitussive and antiacne activities[14-18].

Malva sylvestris is an herbaceous plant used in phitotherapy and widely distributed in Terpenoids, phenolic acids and anthocyanins were identified in water leaf extract of the plant. It is used for many purposes as medicine, nutrition, fibrous, green color dye, and cosmetic from centuries. Numbers of medical and pharmacologic researches about nettle are increased day by day. Nettle leaves contain anthocyanin glycosides, quercetin, rutin flavonoids, chlorophylll a, chlorophylll b, β-carotene, and lutein[19, 20].

MATERIALS AND METHODS

Malva sylvestris were were collected from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials. About eighteen grams of methanolic extract of *Malva sylvestris* powdered were soaked in one hundred mL methanol for ten hours in a

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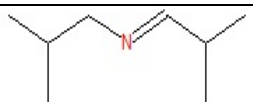
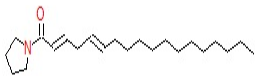
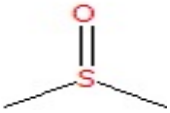
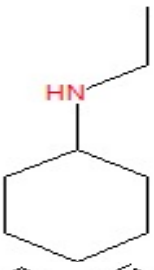
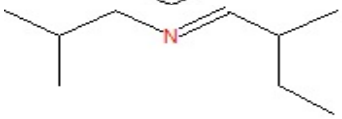
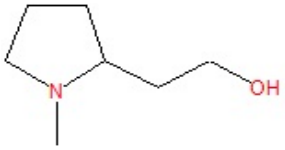
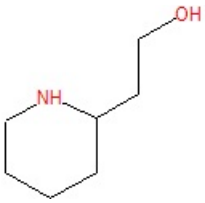
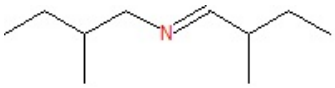
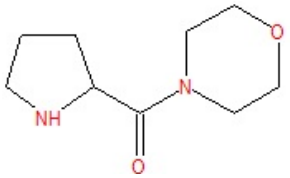
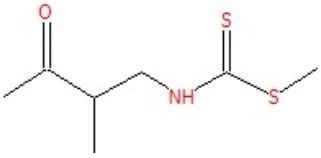
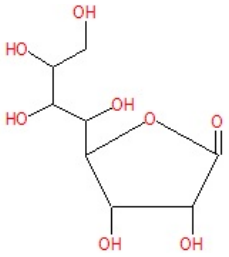
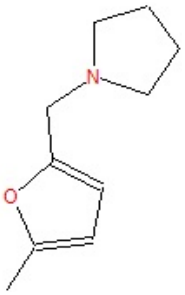
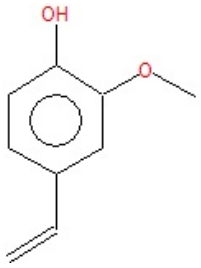
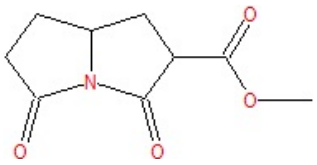
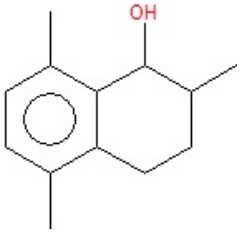
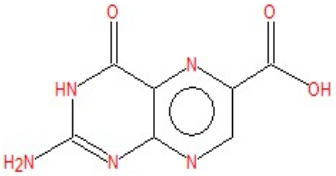
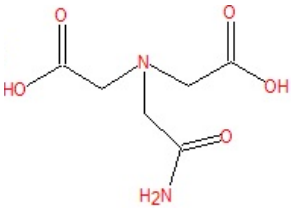
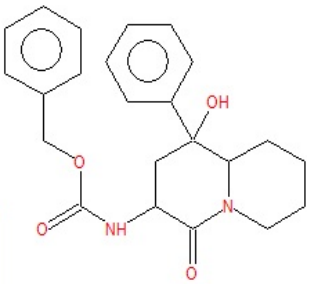
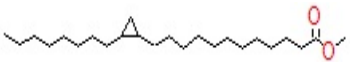
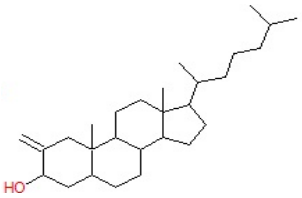
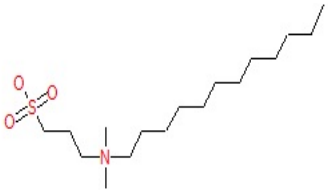
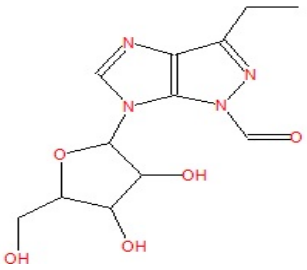
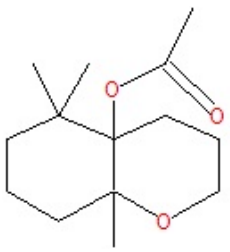
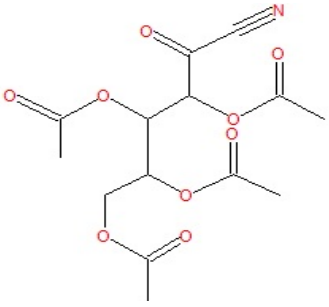
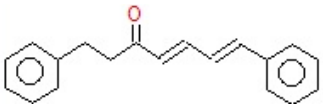
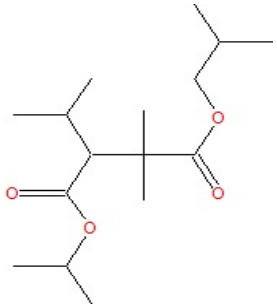
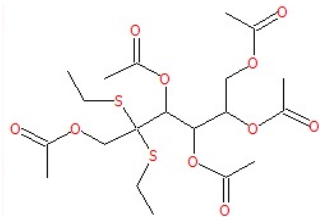
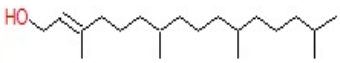
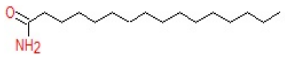
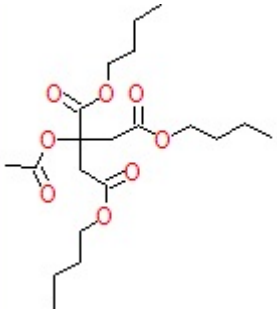
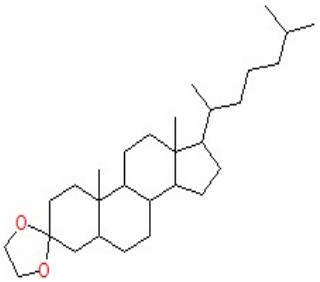
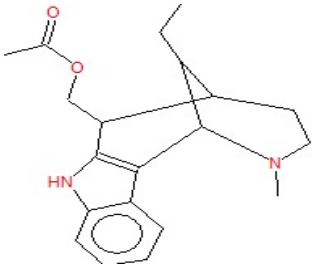
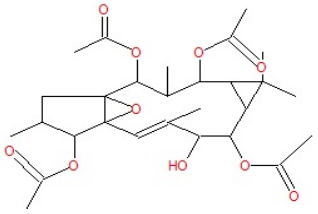
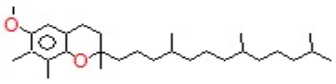
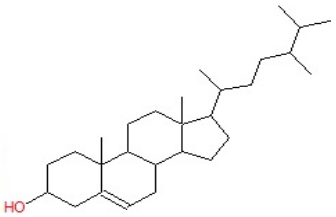
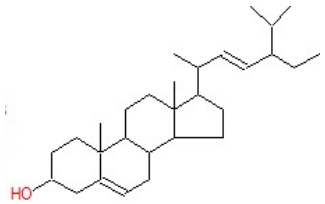
Table 1: Major phytochemical compounds identified in methanolic extract of *Malva sylvestris*.

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1.	1-Propanamine , 2-methyl-N-(2-methylpropyldene)-	3.218	127	127.1360993		57,84,112	Unknown
2.	Pyrrolidine,1-(1-oxo-2,5-octadecadienyl)-	3.396	333	333.303165		55,81,98,113,150,220,264,333	Unknown
3.	Dimethyl sulfoxide	3.476	78	78.013936		63,78	anti-inflammatory, and an antioxidant
4.	Cyclohexylamine ,N-ethyl-	3.590	127	127.1360993		55,71,84,98,127	anti-inflammatory and antioxidant
5.	N-(2-Methylbutylidene) isobutylamine	3.877	141	141.15175		57,69,98,113,126	anti-stereochemistry
6.	1-Methyl-2-pyrrolideethanol	4.306	129	129.115364		55,84,98,129	Unknown
7.	2-(2-Hydroxyethyl)piperidine	4.449	129	129.115364		56,84,98,128	Antimicrobial, Anti-malarial, Antibacterial
8.	1-Butanamine , 2-methyl-N-(2-methylbutylidene)-	4.563	155	155.167399		56,70,84,98,113,127,140,154	antimicrobial activity
9.	4-(Pyrrolidin-2-ylcarbonyl)morpholine	4.649	184	184.121178		56,70,86,114,142	antimicrobial activity
10.	Dithiocarbamate , S-methyl-,N-(2-methyl-3-oxobutyl)-	5.215	191	191.043856		57,85,143,191	anti-bacterial activity

11.	l-Gala-l-ido-octonic lactone	6.057	238	238.068868		61,73,84,112,127,142,159,189,220	anti-bacterial activity
12	1-(5'-methylfurfuryl)pyrrolidine	6.371	165	165.115364		95,122,165	Anti-oxidant
13	2-Methoxy-4-vinylphenol	7.041	150	150.06808		51,77,89,107,135	Antioxidant, anti microbial and anti-inflammatory
14	Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester)	7.361	197	197.068808		55,69,84,98,142,197	anti-tumor activity, anti-diabetic activity
15	1-Naphthaienol , 1,2,3,4-tetrahydro-2,5,8-trimethyl-	7.853	190	190.135765		51,77,11,142,157,172,190	Unknown
16	Pterin-6-carboxylic acid	7.916	207	207.039239		57,69,105,149,163,207	Anti-cancer, anti-viral
17	N-(2-Acetamido)imino diacetic acid	8.025	190	190.058971		71,101,127,146,172,190	Unknown

18	N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic	8.248	394	394.189257		55,84,105,138,166,196,238,282,394	anti-tumor activity, anti-diabetic activity
19	Cyclopropanedodecanoic acid , 2-octyl-,methyl ester	8.563	366	366.349781		5,69,118,66,334,366	antioxidants, anti-microbial
20	Cholestan-3-ol,2-methylene-,(3β,5α)-	8.626	400	400.370516		69,81,95,149,227,315,400	anti-inflammatory
21	3-(N,N-Dimethyllaurylammonio)propanesulfonate	8.906	335	335.249414		58,69,84,97,122,152,179,213	activity anti-oxidant and anti-inflammatory activities
22	Pyrazole[4,5-b]imidazole , 1-formyl-3-ethyl-6-β-d-ribofuranosyl-	9.101	296	296.11207		55,149,281	antimicrobial, anticancer
23	Octahydrobenzo[b]pyran , 4a-acetoxy-5,5,8a-trimethyl-	9.427	240	240.1725445		55,69,111,180,197,240	anti-inflammatory
24	Tetraacetyl-d-xylonic nitrile	9.604	343	343.090332		60,73,112,133,238,281	antioxidant and anti-inflammatory
25	4,6-Heptadien-3-one,1,7-diphenyl-	9.856	262	262.135765		51,77,91,128,157,262	anti-oxidative of compounds

26	Pentanoic acid ,2,2,4-trimethyl-3-carboxyisopropyl , isobutyl ester	9.948	286	286.214409		55,71,97,159,243	Unknown
27	D-Fructose, diethyl mercaptal, pentaacetate	11.693	496	496.14369		60,97,129,154,273,316,375,436	anti- bacterial activity
28	Phytol	15.074	296	296.307917		57,71,81,95,111,123,137,196,221,249,278	Antinociceptive and Antioxidant Activities
29	Hexadecanamide	15.961	255	255.256215		59,72,86,128,170,212,255	analgesic and anti-inflammatory
30	Tributyl acetylcitrate	16.413	402	402.225368		57,112,129,157,185,213,231,259,273,329	anti-bacterial
31	Cholestan-3-one,cyclic 1,2-ethanediyl aetal , (5β)-	18.925	430	430.38108		55,69,99,125,149,194,232,282,340,384,430	analgesic, anti-ulcer, anticancer
32	Dasycarpidan-1-methanol, acetate (ester)-	19.990	326	326.199429		69,97,180,222,256,326	inflammatory, anti-bacterial, anti-fungal, anti-diabetic, anti-cancer

33	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	22.296	536	536.262146		55,69,122, 207,236,29 7,357,417, 477,536	anti-bacterial activity
34	(+)-y-Tocopherol, O-methyl-	22.828	430	430.38108		57,69,137, 165,205,27 4,316,358, 430	anti-oxidant activity
35	Campesterol	23.531	400	400.370516		55,81,145, 213,255,28 9,315,382, 400	anti-inflammatory effects
36	Stigmasterol	23.737	412	412.370516		55,83,133, 213,255,30 0,351,369, 412	anti- microbial

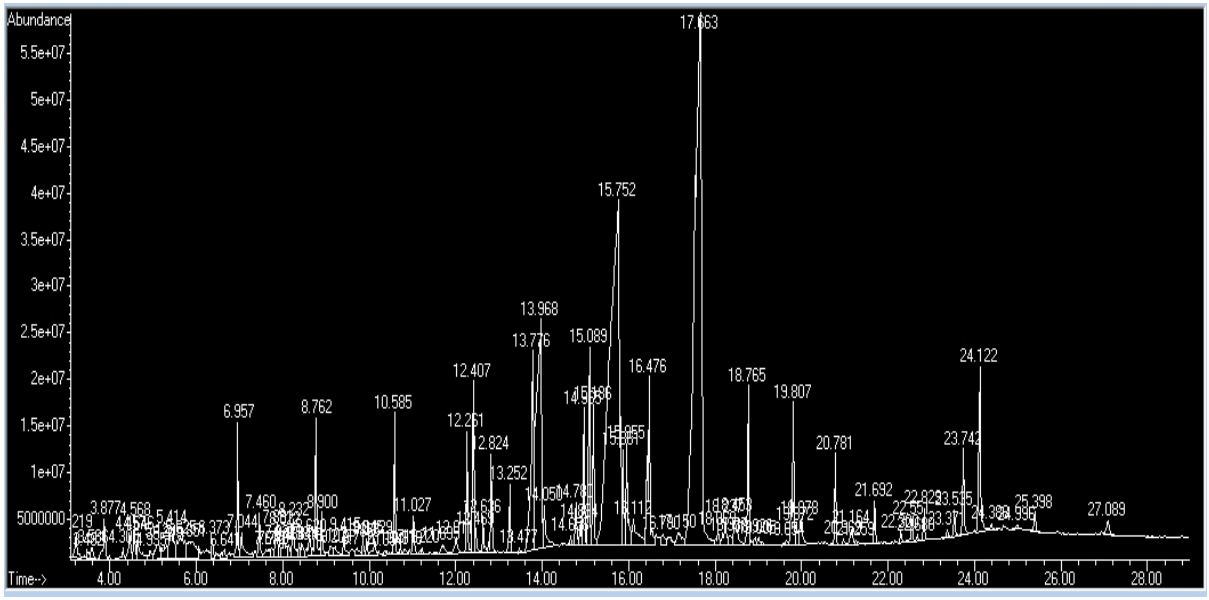


Figure 1: GC-MS chromatogram of methanolic extract of *Malva sylvestris*.

rotatory shaker. The filtrates were used for further phytochemical analysis[21-30]. It was again filtered through sodium sulphate in order to remove the traces of moisture.

Gas chromatography – mass spectrum analysis

GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. One µL of the methanol extract of *Malva sylvestris* was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. The time from when the injection was made (Initial time) to when elution occurred 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. 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

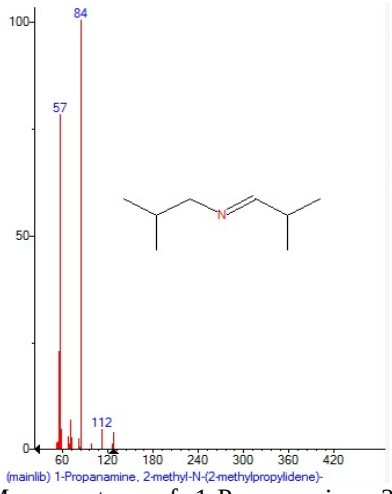


Figure 2: Mass spectrum of 1-Propanamine , 2-methyl-N-(2-methylpropyldene)- with Retention Time (RT)= 3.218.

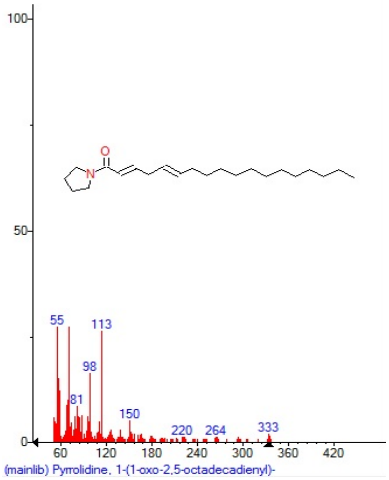


Figure 3: Mass spectrum of Pyrrolidine,1-(1-oxo-2,5-octadecadienyl)- with Retention Time (RT)= 3.396.

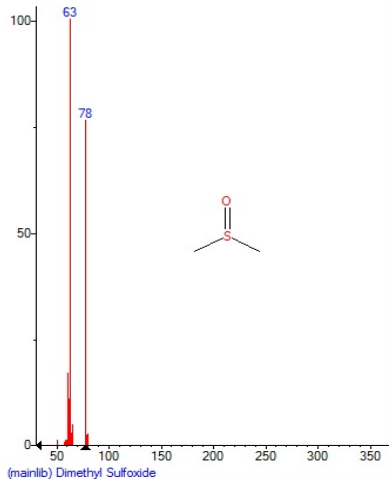


Figure 4: Mass spectrum of Dimethyl sulfoxide with Retention Time (RT)= 3.476 .

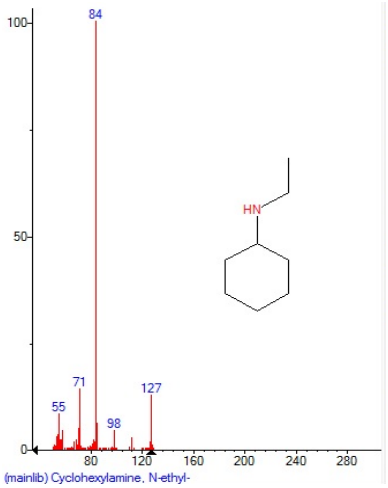


Figure 5: Mass spectrum of Cyclohexylamine ,N-ethyl- with Retention Time (RT)= 3.590.

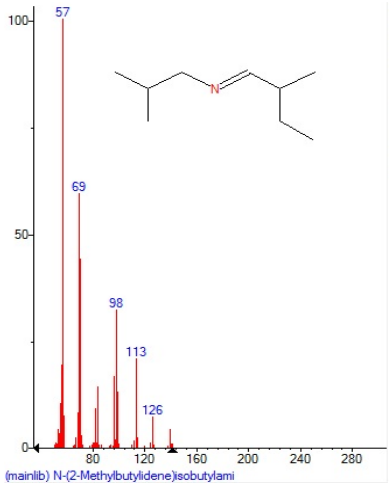


Figure 6: Mass spectrum of N-(2-Methylbutylidene)isobutylamine with Retention Time (RT)= 3.877.

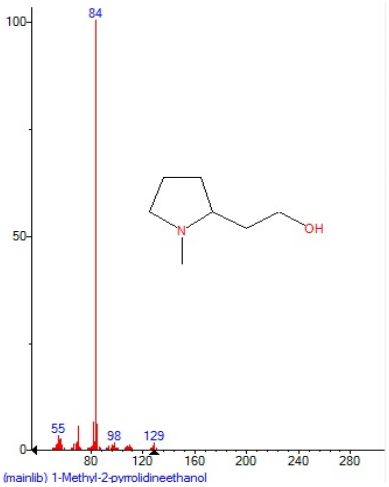


Figure 7: Mass spectrum of 1-Methyl-2-pyrrolideethanol with Retention Time (RT)= 4.306.

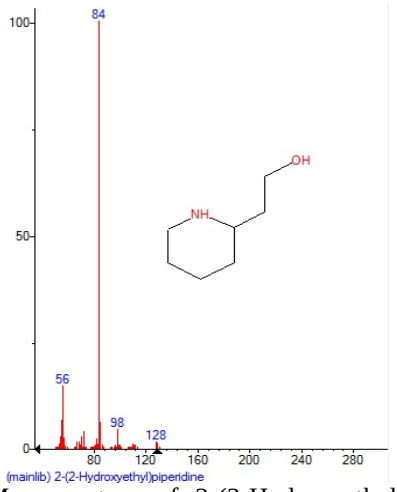


Figure 8: Mass spectrum of 2-(2-Hydroxyethyl)piperidine with Retention Time (RT)= 4.449.

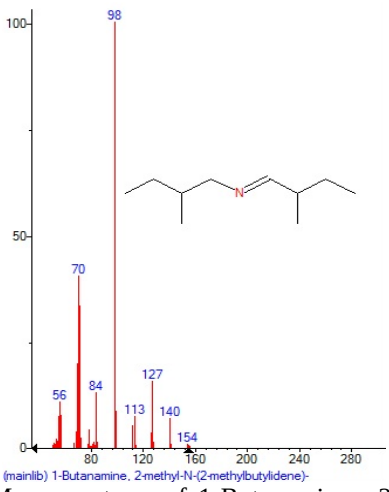


Figure 9: Mass spectrum of 1-Butanamine , 2-methyl-N-(2-methylbutylidene)-with Retention Time (RT)= 4.563.

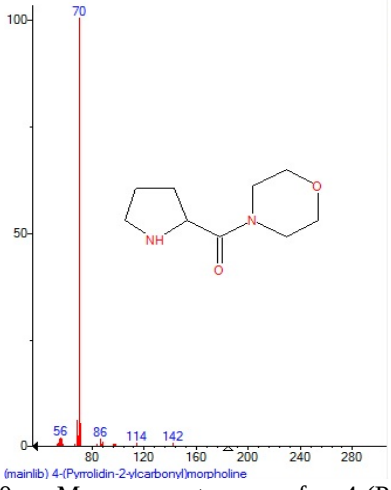


Figure 10: Mass spectrum of 4-(Pyrrolidin-2-ylcarbonyl)morpholine with Retention Time (RT)= 4.649.

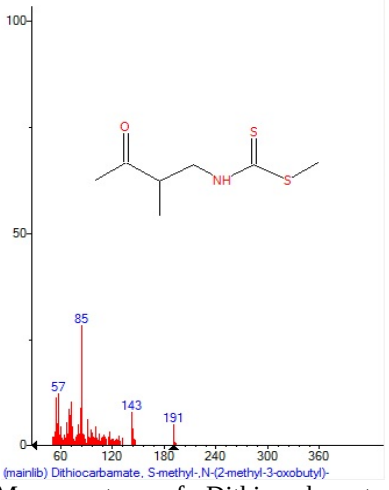


Figure 11: Mass spectrum of Dithiocarbamate , S-methyl-,N-(2-methyl-3-oxobutyl)- with Retention Time (RT)= 5.215.

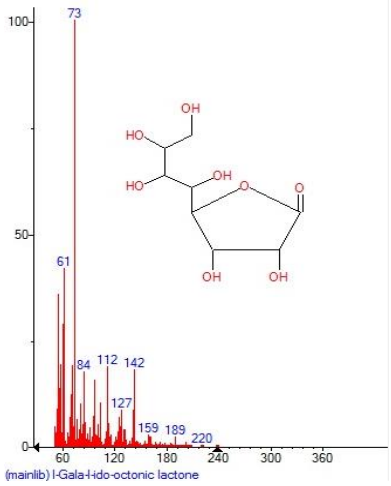


Figure 12: Mass spectrum of l-Gala-l-ido-octonic lactone with Retention Time (RT)= 6.057.

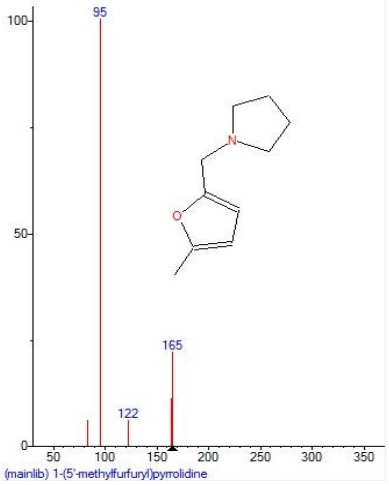


Figure 13: Mass spectrum of 1-(5'-methylfurfuryl)pyrrolidine with Retention Time (RT)= 6.371.

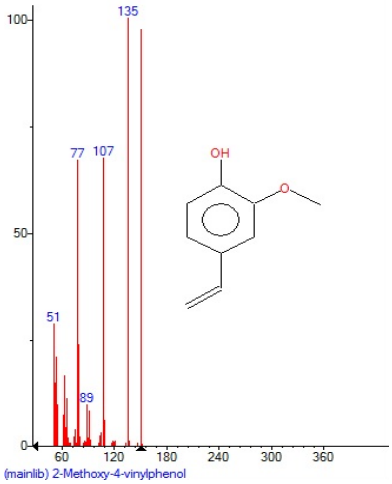


Figure 14: Mass spectrum of 2-Methoxy-4-vinylphenol with Retention Time (RT)= 7.041.

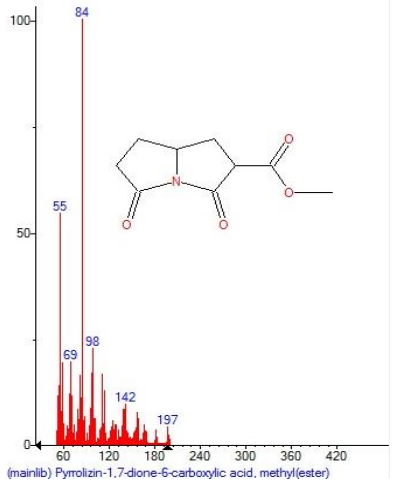


Figure 15: Mass spectrum of Pyrrolizin-1,7-dione-6-carboxylic acid methyl(ester) with Retention Time (RT)= 7.361.

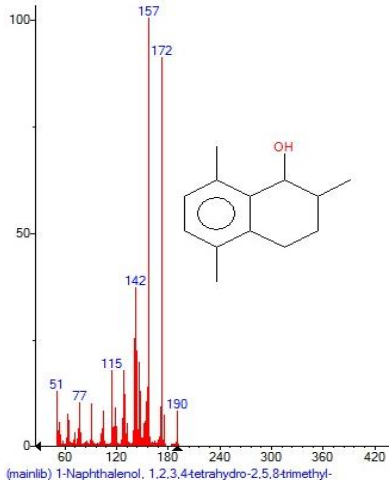


Figure 16: Mass spectrum of 1-Naphthaienol , 1,2,3,4-tetrahydro-2,5,8-trimethyl- with Retention Time (RT)= 7.853.

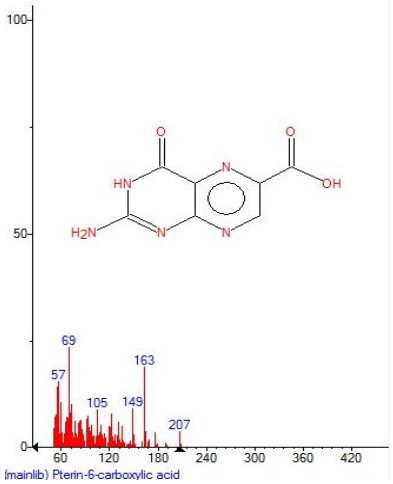


Figure 17: Mass spectrum of Pterin-6-carboxylic acid with Retention Time (RT)= 7.916.

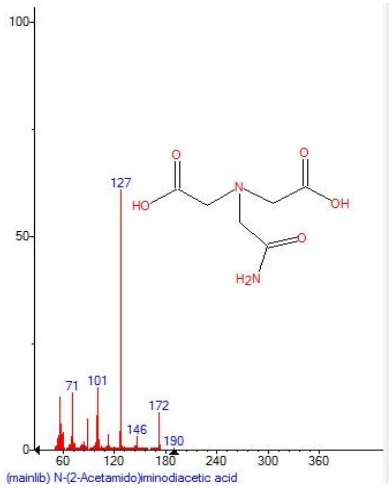


Figure 18: Mass spectrum of N-(2-Acetamido)iminodiacetic acid with Retention Time (RT)= 8.025.

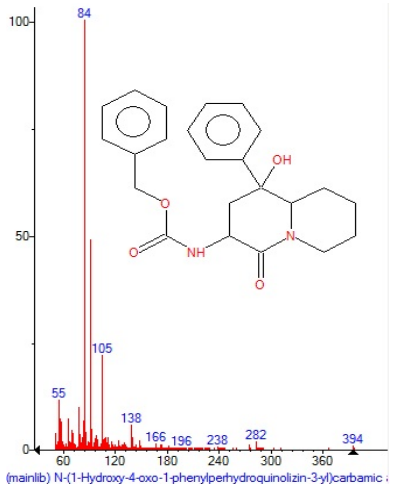


Figure 19: Mass spectrum of N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic with Retention Time (RT)= 8.248.

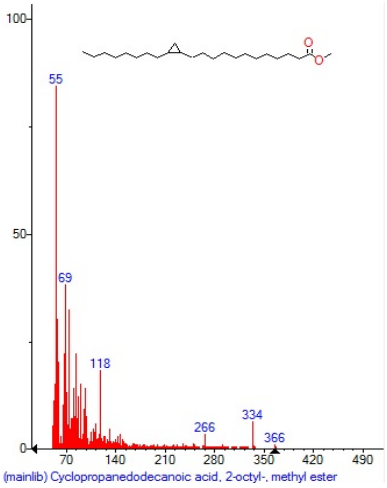


Figure 20: Mass spectrum of Cyclopropanedodecanoic acid , 2-octyl-,methyl ester with Retention Time (RT)= 8.563.

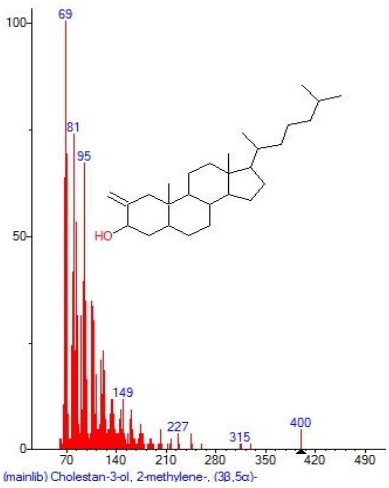


Figure 21: Mass spectrum of Cholestan-3-ol,2-methylene-,(3β,5α)-with Retention Time (RT)= 8.626.

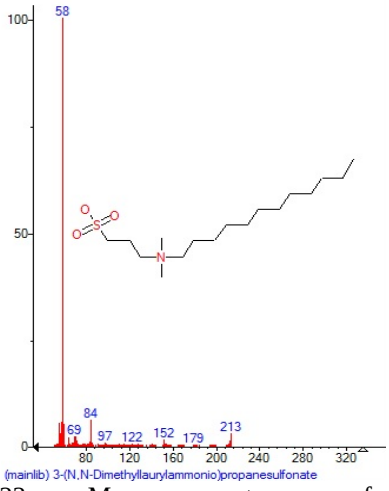


Figure 22: Mass spectrum of 3-(N,N-Dimethyllaurylammonio)propanesulfonate with Retention Time (RT)= 8.906.

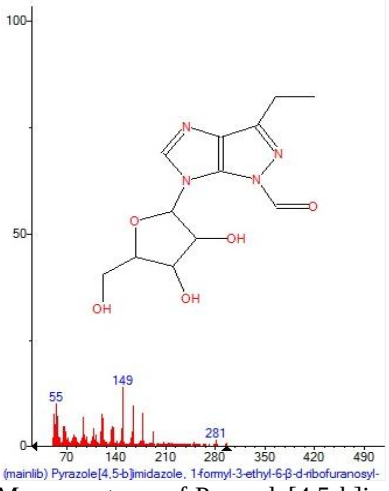


Figure 23: Mass spectrum of Pyrazole[4,5-b]imidazole , 1-formyl-3-ethyl-6-β-d-ribofuranosyl- with Retention Time (RT)= 9.101.

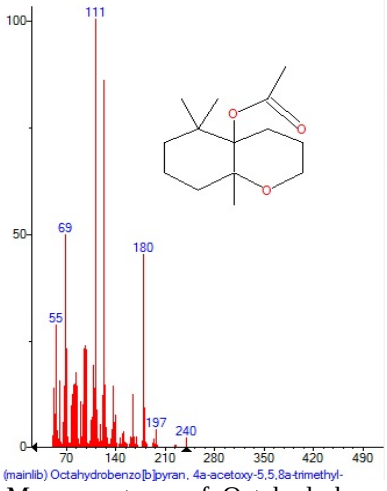


Figure 24: Mass spectrum of Octahydrobenzo[b]pyran , 4a-acetoxy-5,5,8a-trimethyl- with Retention Time (RT)= 9.427.

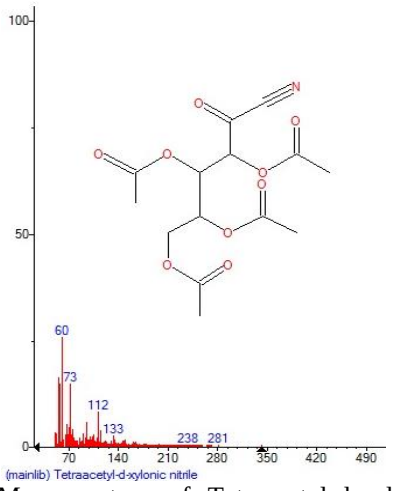


Figure 25: Mass spectrum of Tetraacetyl-d-xylonic nitrile with Retention Time (RT)= 9.604.

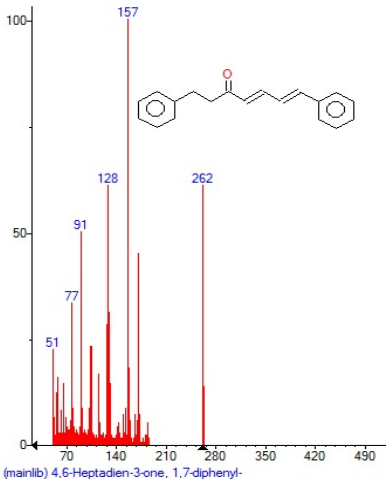


Figure 26: Mass spectrum of 4,6-Heptadien-3-one,1,7-diphenyl- with Retention Time (RT)= 9.856.

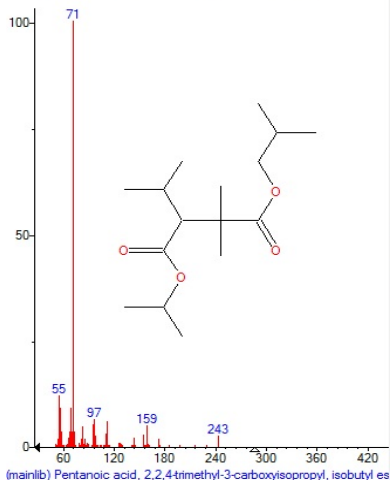


Figure 27: Mass spectrum of Pentanoic acid ,2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester with Retention Time (RT)= 9.948.

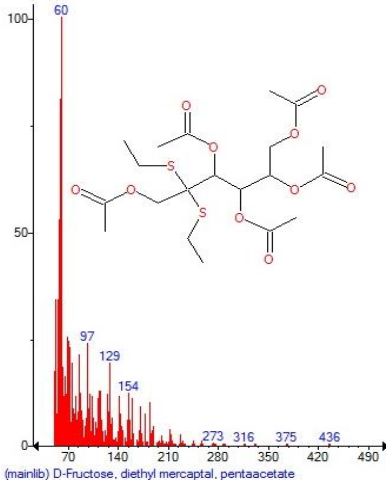


Figure 28: Mass spectrum of D-Fructose, diethyl mercaptal, pentaacetate with Retention Time (RT)= 11.693.

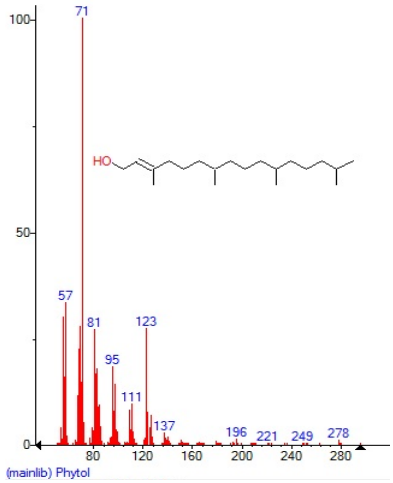


Figure 29: Mass spectrum of Phytol with Retention Time (RT)= 15.074.

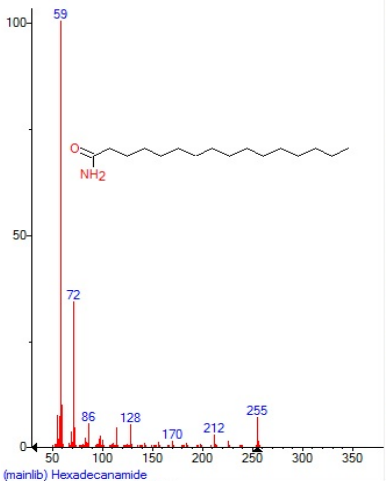


Figure 30: Mass spectrum of Hexadecanamide with Retention Time (RT)= 15.961.

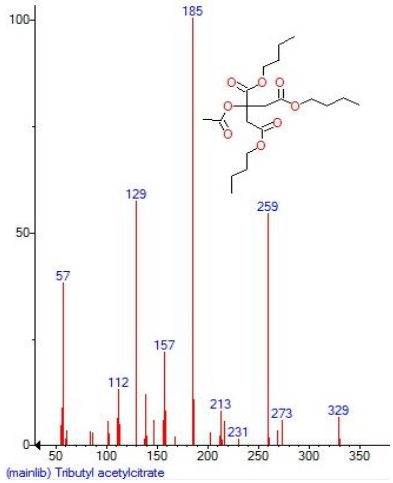


Figure 31: Mass spectrum of Tributyl acetylcitrate with Retention Time (RT)= 16.413.

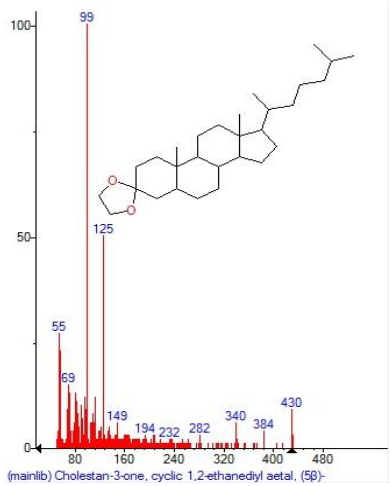


Figure 32: Mass spectrum of Cholestan-3-one,cyclic 1,2-ethanediyl aetal , (5β)- with Retention Time (RT)= 18.925.

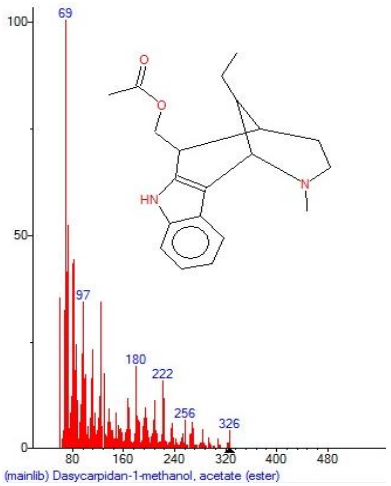


Figure 33: Mass spectrum of Dasycarpidan-1-methanol, acetate (ester)- with Retention Time (RT)= 19.990.

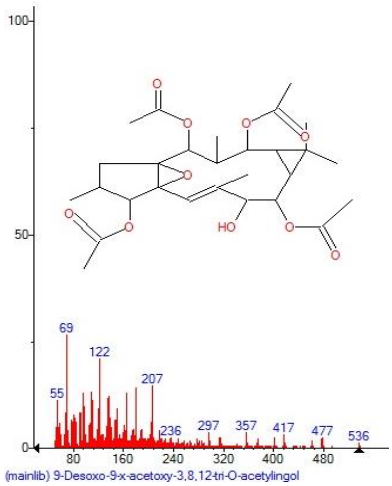


Figure 34: Mass spectrum of 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingolwith Retention Time (RT)= 22.296.

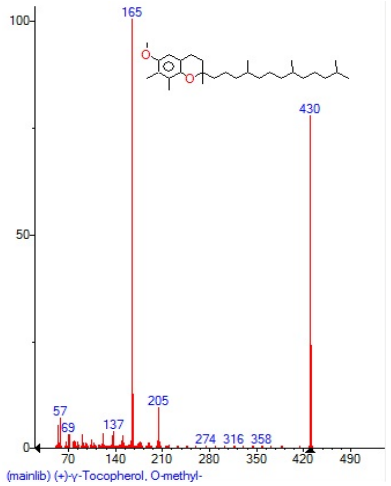


Figure 35: Mass spectrum of (+)-γ-Tocopherol, O-methyl- with Retention Time (RT)= 22.828.

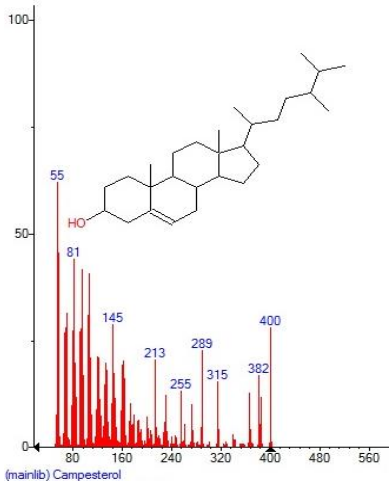


Figure 36: Mass spectrum of Campesterol with Retention Time (RT)= 23.531.

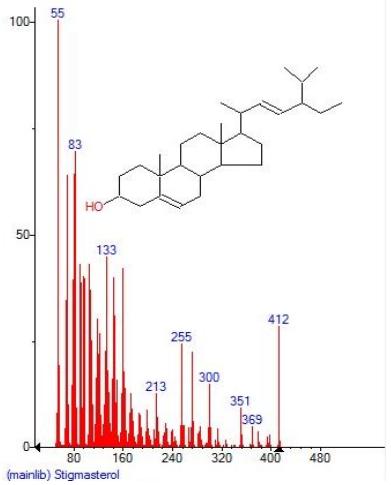


Figure 37: Mass spectrum of Stigmasterol with Retention Time (RT)= 23.737.

certain mass. Helium gas was used as a carrier as well as an eluent[31-51]. 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 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[52-61].

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Malva sylvestris*, shown in Table 1. The GC-MS chromatogram of the 31 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Malva sylvestris* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be Propanamine , 2-methyl-N-(2-methylpropyldene)- , Pyrrolidine,1-(1-oxo-2,5-octadecadienyl)- , Dimethyl sulfoxide , Cyclohexylamine ,N-ethyl- , N-(2-Methylbutylidene)isobutylamine , 1-Methyl-2-pyrrolideethanol , 2-(2-Hydroxyethyl)piperidine , 1-Butanamine , 2-methyl-N-(2-methylbutylidene)- , 4-(Pyrrolidin-2-ylcarbonyl)morpholine , Dithiocarbamate , S-methyl-,N-(2-methyl-3-oxobutyl)- , l-Gala-l-ido-octonic lactone , 1-(5'-methylfurfuryl)pyrrolidine , 2-Methoxy-4-vinylphenol , Pyrrolizin-1,7-dione-6-carboxylic acid , methyl(ester) , 1-Naphthaienol , 1,2,3,4-tetrahydro-2,5,8-trimethyl- , Pterin-6-carboxylic acid , N-(2-Acetamido)iminodiacetic acid , N-(1-Hydroxy-4-oxo-1-phenylperhydroquinolizin-3-yl)carbamic , Cyclopropanedodecanoic acid , 2-octyl-,methyl ester , Cholestan-3-ol,2-methylene-,(3β,5α)- , 3-(N,N-Dimethyllaurylammonio)propanesulfonate , Pyrazole[4,5-b]imidazole, 1-formyl-3-ethyl-6-β-d-ribofuranosyl-, Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-, Tetraacetyl-d-xylonic nitrile, 4,6-Heptadien-3-one,1,7-diphenyl-, Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl , isobutyl ester, D-Fructose, diethyl mercaptal, pentaacetate, Phytol, Hexadecanamide, Tributyl acetylcitrate, Cholestan-3-one,cyclic 1,2-ethanediyl aetal, (5β)- , Dasycarpidan-1-methanol, acetate (ester)- , 9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol , (+)-y-Tocopherol, O-methyl-, Campesterol and Stigmasterol (Figure 2-37). From ancient times, plants have been used for many purposes, including food, medicine, flavoring agents, cosmetics and other uses[62-68]. Antioxidant and antiprotozoic activities; quercetin is the major flavonoid in the human diet and has been reported to exhibit antioxidative. *M. sylvestris* extracts are reported for their radical scavenging effect. *Malva sylvestris* extracts are reported for their radical scavenging effect as well as *E. camaldulensis* and *C. sativa*; the later demonstrated also antineoplastic activity in B16 cells[69-71].

CONCLUSION

The results clearly shows that the plants which have the highest organic and aqueous extraction yields have the highest antioxidant activities. Wild natural *Malva sylvestris* plants leaves had slightly higher antioxidant activity than their cultivated species. Based on the above presented results the rareness of natural antioxidant use which is usually due to the cost and unavailability of the wild plant could be overcome by using cultivated species. The cultivated *Malva sylvestris* plants leaves, could be used as a possible new source of natural antioxidants in the food, nutraceuticals, pharmaceuticals and cosmetic industry.

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