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Research Article

Determination of Bioactive Compounds of Methanolic Extract of *Vitis vinifera* Using GC-MS

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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 three bioactive compounds were identified in the methanolic extract of *Vitis vinifera*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Vitis vinifera* revealed the existence of the Butanol , 2-nitro , α-D-Glucopyranoside , methyl 3,6-anhydro , Propanedioic acid , amino -, diethyl ester, DL-Arabinose , Hexadecenoic acid, Furfural, 1H-Pyrazole-1-carbothioamide , 3,5-dimethyl- , 2-Furanmethanol , 2(1H) Pyrazinone, o-Acetyl-L-serine , 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol, 6-Oxa-bicyclo[3.1.0]hexan-3-one , Acetic acid , 2,2'-[oxybis(2,1-ethanediyloxy)]bis- , Desulphosinigrin, D-Glucose , 6-o-α-D-galactopyranosyl-, Cyclohexene , 1-methyl-4-(1-methylethenyl)-,(S)-, -D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- , 2,5-Dimethyl-4-hydroxy-3(2H)-furanone , Cis-2-Ethyl-2-hexen-1-ol, Maltose , 7-Oxa-2-oxa-7-thiatricyclo[4.4.0.0(3,80]decan-4-ol , 1-Gala-l-ido-octonic lactone , 5-Hydroxymethylfurfural , Cyclohexene-1-methanol,α,α,4-trimethyl-,propanoate , Hydroxymethylfurfural , Octanamide,N-(2-mercaptoethyl)-, 1,2,4-Trioxolane-2-octanoic acid , 5-octyl-,methyl ester, 9-Octadecenoic acid , (2-phenyl-1,3-dioxolan-4-yl)methyl ester 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-, 13-Heptadecyn-1-ol Hexadecanoic acid , 1-(hydroxymethyl)-1,2-ethanediyl ester, 9-Octadecenamide ,(Z).

Keyword*:* bioactive compounds, GC-MS, *Vitis vinifera*, Vitaceae, Pharmacological actions.

INTRODUCTION

Vitis vinifera is a member of the Vitaceae family, native to southern Europe and Western Asia cultivated worldwide. Grape (*Vitis vinifera)* are considered rich sources of poly-phenolic compounds, mainly monomeric catechin and epicatechin, gallic acid, and polymeric and oligomeric procyanidins skins and seeds[1-4]. The seeds and the leaves of the grape vine are used in herbal medicines, whilst fruit is consumed as a dietary supplement. Recent studies have revealed that grape seed extract (GSE) has antioxidant and free radical scavenging, antidiabetic, cardioprotective, hepatoprotective, anti-carcinogenic, anti-microbial, and anti-viral activities. *Vitis vinifera* is used in conditions like hemorrhages, anemia, leprosy, skin diseases, syphilis, asthma, jaundice, bronchitis, anti-inflammatory, anticarcinogenic, platelet aggregation inhibiting, and metal chelating properties[6-9]. The grape seed extract (GSE) has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardioprotective, hepatoprotective, and neuroprotective effects. The grape seed extract (GSE) has been reported to possess a broad spectrum of pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardioprotective, hepatoprotective, and neuroprotective effects[10-13]. The seeds of the grape are used in herbal medicine and as a dietary supplement. Their composition and properties have been extensively investigated, with several reports of the presence of large amounts of phenolic compounds having antioxidant activities[14,15]. The grape seed polyphenols are flavan-3-ol derivatives and only 4% of grape polyphenols exist in grape pulp. In grape skin there is another type of polyphenol, called anthocyanins, which usually have a purple color and amount to ~30% of total polyphenols in grapes. Resveratrol is mainly contained in the skins of grapes.

MATERIALS AND METHODS

The leaves of *Vitis vinifera* were dried at room temperature for ten days and when properly dried then powdered using clean pestle and mortar. About fifteen grams of the plant sample powdered were soaked in 100 mL methanol individually. Then all the extracts were preserved in separate containers at 5 Cº for further experimentations[16-19].

Gas chromatography – Mass Spectrum analysis

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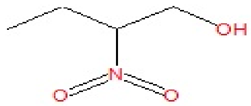
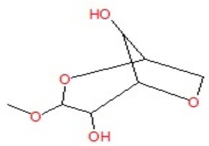
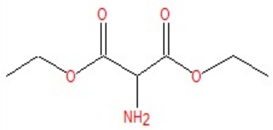
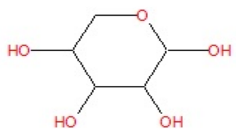
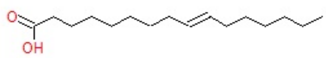
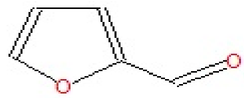
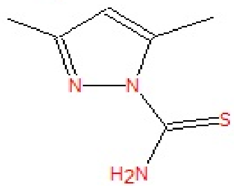
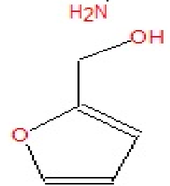
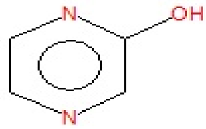
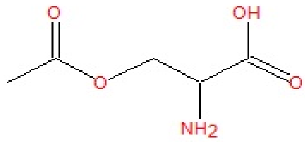
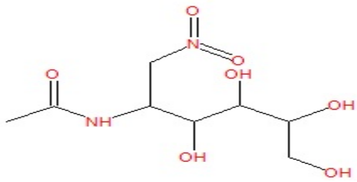
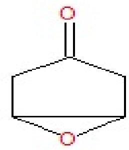
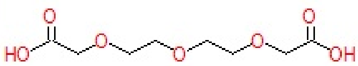
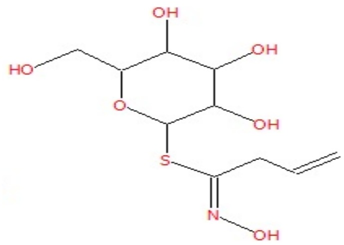
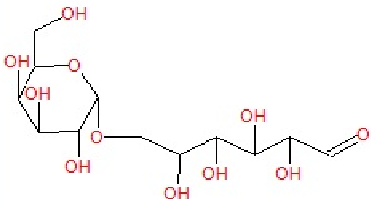
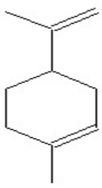
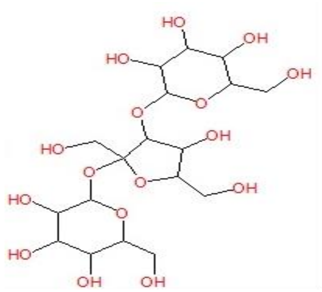
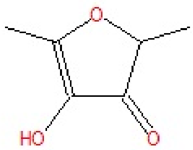
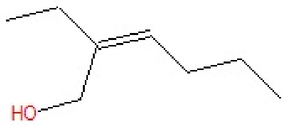
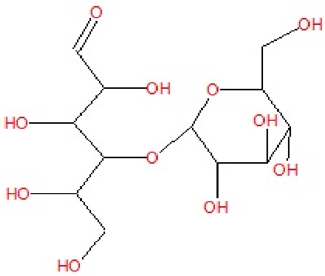
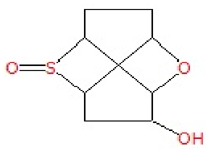
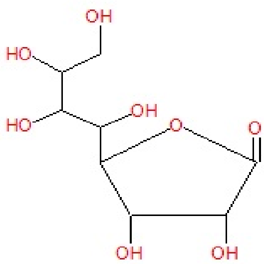
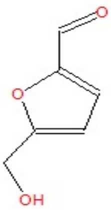
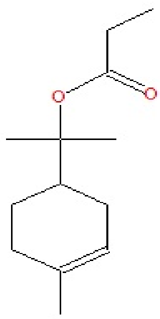
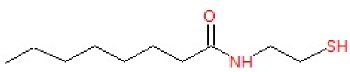
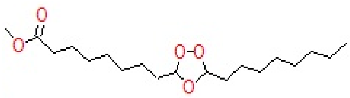
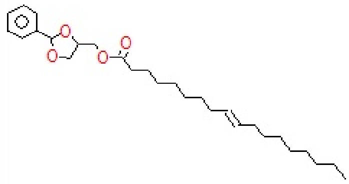
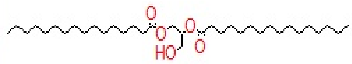
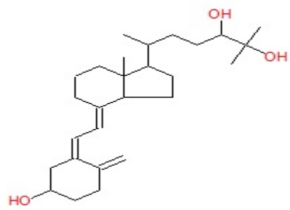
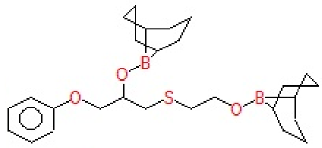
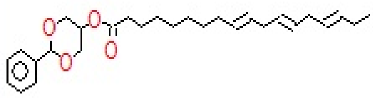
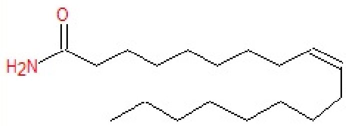
Vitis vinifera GC–MS analysis were carried out in a GC carrier gas, helium (He) was set to beat 1 mL min−1, split ratio was 1:50. The injector temperature was adjusted at 250◦C, while the detector temperature was fixed to280◦C[17-25]. The column temperature was kept at 40◦C for 1 min fol-lowed by linear 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 system (Agilent 7890 Aseries, USA). The flow rate of the analysis[26-42]. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z. Identification of compounds 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[43-54].

Table 1: Major phytochemical compounds identified in methanolic extract of *Vitis vinifera*.

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment- ions	Pharmacological actions
1.	1-Butanol , 2-nitro-	3.144	119	119.0582433		55,72,89,119	antibacterial, antifungal, anti-inflammatory
2.	α-D-Glucopyranoside, methyl 3,6-anhydro-	3.224	176	176.068474		57,73,102,145	Unknown
3.	Propanedioic acid , amino -, diethyl ester	3.253	175	175.084458		57,74,102,130,175	anti-inflammatory and analgesic activity
4.	DL-Arabinose	3.276	150	150.052823		60,85,135	Antibacterial and anti-Candida activities
5.	9-Hexadecenoic acid	3.333	254	254.22458		55,69,83,97,194,236,254	anti-inflammatory
6.	Furfural	3.384	96	96.021129		51,6796	Anti-inflammatory activity
7.	1H-Pyrazole-1-carbothioamide , 3,5-dimethyl-	3.436	155	155.051719		59,81,96,128,155	Anti-microbial activity
8.	2-Furanmethanol	3.516	98	98.0367794		53,69,81,98	Anti-inflammatory activity

9.	2(1H)-Pyrazinone	3.676	96	96.032363		53,68,79,96	Unknown
10.	o-Acetyl-L-serine	3.779	147	147.053158		60,74,87,102,129	anti-inducers
11.	1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	3.831	252	252.095751		60,86,98,114,161,219,252	Anti-insect *activity*
12.	6-Oxa-bicyclo[3.1.0]hexan-3-one	3.939	98	98.0367794		55,69,81,98	Antioxidant activity
13.	Acetic acid , 2,2'-[oxybis(2,1-ethanediyloxy)]bis-	3.750	222	222.073953		58,75,89,103,133	Uknown
14.	Desulphosinigrin	3.808	279	279.077658		60,73,85,103,127,145,163,213,262	anti-asthmatic
15.	D-Glucose , 6-o-α-D-galactopyranosyl-	4.174	342	342.11621		60,73,85,110,126,182,212,261	Anti-bacterial activity
16.	Cyclohexene , 1-methyl-4-(1-methylethenyl)-,(S)-	4.466	136	136.1252		53,68,79,93,136	Anti-bacterial activity
17.	α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β-	4.700	504	504.169035		60,73,85,97,113,126,145,173,192	anti-diabetic activity

18.	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	4.941	128	128.047344		57,72,85,94,128	antimicrobial effect
19.	Cis-2-Ethyl-2-hexen-1-ol	5.072	128	128.120115		55,67,85,95,110,128	Unknown
20.	Maltose	5.204	342	342.11621		60,73,85,97,126,163	anti-inflammatory effect
21.	7-Oxa-2-oxa-7-thiatricyclo[4.4.0.0(3,80]decan-4-ol	5.890	188	188.050715		55,67,83,95,121,139,171,188	Unknown
22.	1-Gala-l-ido-octonic lactone	6.051	238	238.068868		61,73,84,112,127,142,159,189,220	Anti-diabetic *activity*
23.	5-Hydroxymethylfurfural	6.657	126	126.031694		53,69,81,97,109,126	Anti-Inflammatory Agents
24.	3-Cyclohexene-1-methanol,α,α,4-trimethyl-,propanoate	7.344	210	210.16198		57,67,81,93,107,121,136,152,195	Unknown
25.	Octanamide,N-(2-mercaptoethyl)-	7.475	203	203.134385		57,72,85,127,144,170,203	Unknown
26.	1,2,4-Trioxolane-2-octanoic acid , 5-octyl-,methyl	9.799	344	344.256275		56,69,143,185,241,311	Unknown

	ester						
27.	9-Octadecenoic acid , (2-phenyl-1,3-dioxolan-4-yl)methyl ester	11.143	444	444.32396		55,73,105,122,179,221,264,283,338	antimicrobial, anti-inflammatory
28.	Hexadecanoic acid , 1-(hydroxymethyl)-1,2-ethanediyl ester	13.810	568	568.506676		57,98,129,213,239,256,313,331,423,507	Anti -microbial activity
29.	13-Heptadecyn-1-ol	15.051	252	252.245316		55,67,82,96,219	anti-inflammatory, antifungal
30.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-	15.709	416	416.329044		55,118,136,158,176,207,253,383,416	Unknown
31.	1,8-Dioxa-5-thiaoctane,8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-	15.818	468	468.304077		67,8195,133,151,207,223,331,348,427,468	Unknown
32.	9,12,15-Octadecatrienoic acid , 2-phenyl-1,3-dioxan-5-yl ester	16.081	440	440.29266		55,67,79,105,129,165,185,219,265,334,440	Antimicrobial and anti-inflammatory
33.	9-Octadecenamide ,(Z)-	17.294	281	281.271864		59,72,83,114,184,220,264,281	Unknown

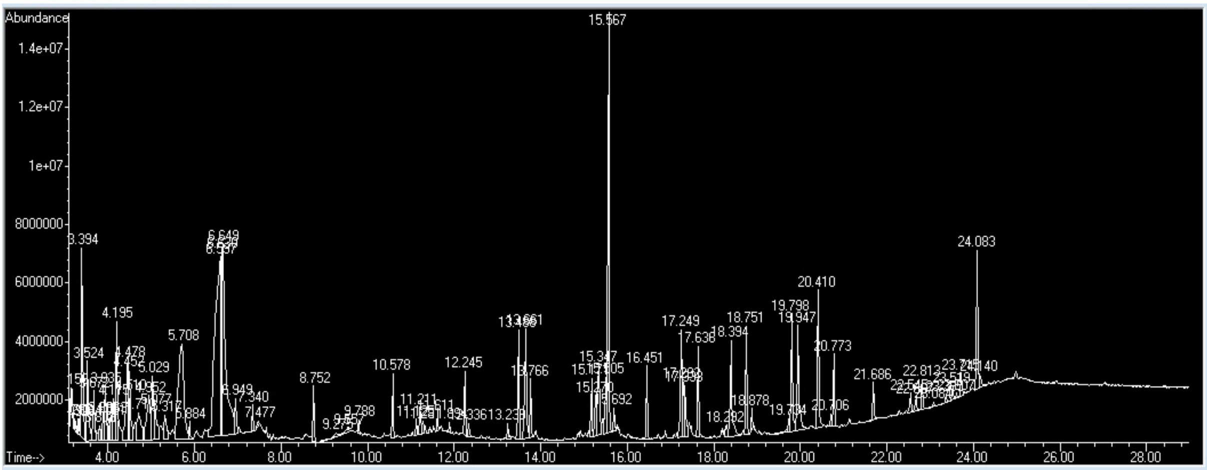


Figure 1: GC-MS chromatogram of methanolic extract of *Vitis vinifera*.

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic leaves extract of *Vitis vinifera,* shown in Table 1. The GC-MS chromatogram of the 33 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Vitis vinifera* showed the

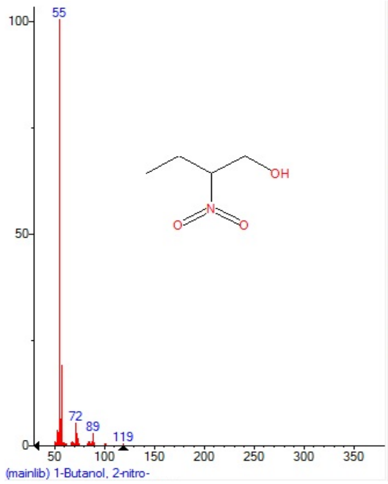


Figure 2: Mass spectrum of 1-Butanol , 2-nitro- with Retention Time (RT)= 3.144

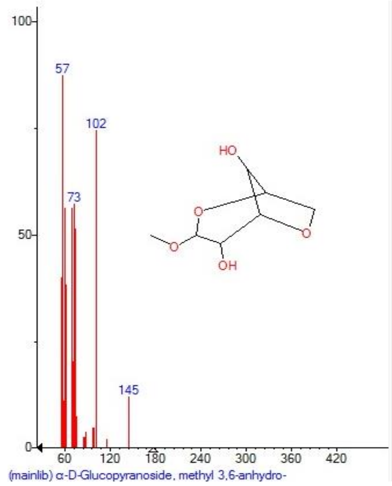


Figure 3: Mass spectrum of α-D-Glucopyranoside , methyl 3,6-anhydro- with Retention Time (RT)= 3.224

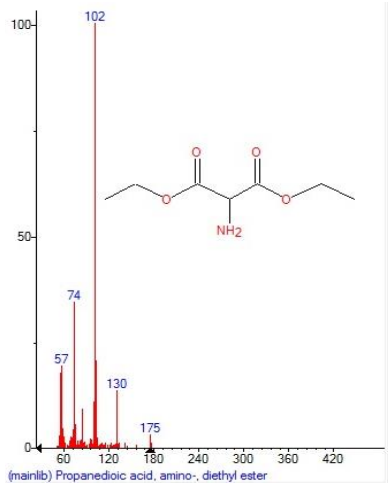


Figure 4 : Mass spectrum of Propanedioic acid , amino -, diethyl ester with Retention Time (RT)= 3.253

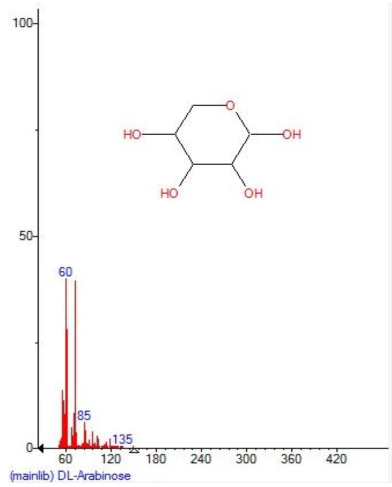


Figure 5 : Mass spectrum of DL-Arabinose with Retention Time (RT)= 3.276

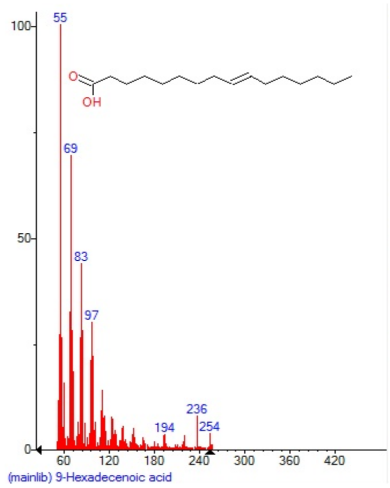


Figure 6: Mass spectrum of 9-Hexadecenoic acid with Retention Time (RT)= 3.33

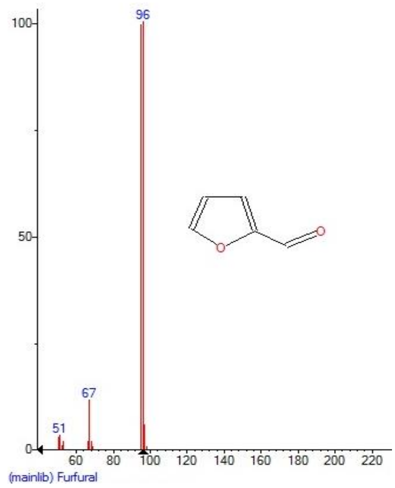


Figure 7: Mass spectrum of Furfural with Retention Time (RT)= 3.384

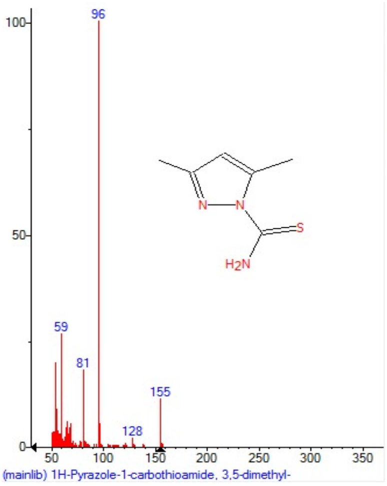


Figure 8: Mass spectrum of 1H-Pyrazole-1-carbothioamide , 3,5-dimethyl- with Retention Time (RT)= 3.436

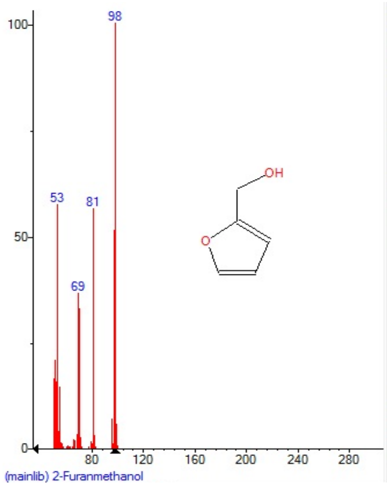


Figure 9: Mass spectrum of 2-Furanmethanol with Retention Time (RT)= 3.516

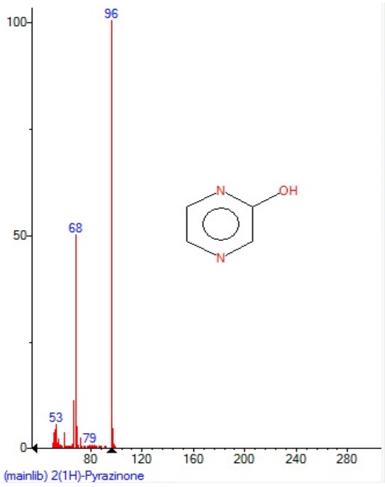


Figure 10: Mass spectrum of 2(1H)-Pyrazinone with Retention Time (RT)= 3.676

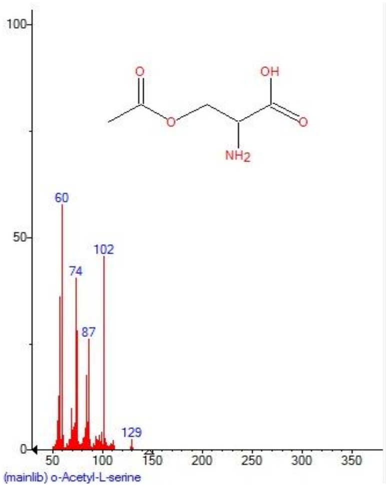


Figure 11: Mass spectrum of o-Acetyl-L-serine with Retention Time (RT)= 3.779

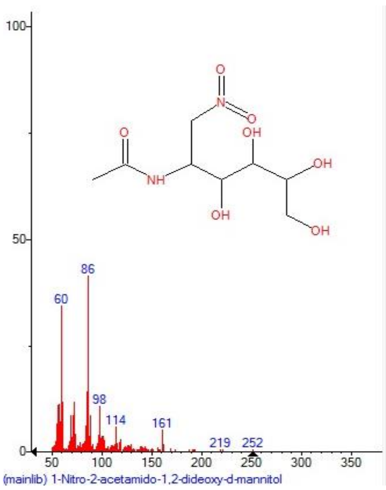


Figure 12: Mass spectrum of 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol with Retention Time (RT)= 3.831

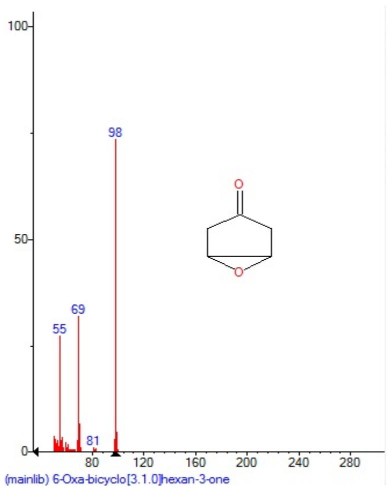


Figure 13: Mass spectrum of 6-Oxa-bicyclo[3.1.0]hexan-3-one with Retention Time (RT)= 3.939

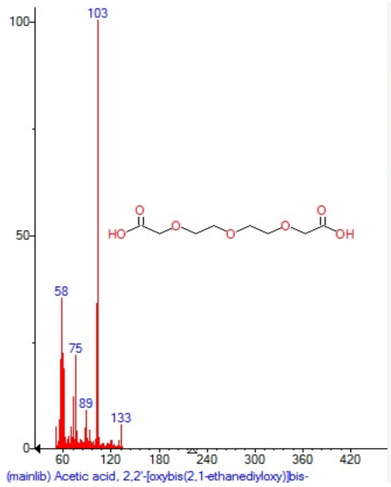


Figure 14: Mass spectrum of Acetic acid , 2,2'-[oxybis(2,1-ethanediyloxy)]bis- with Retention Time (RT)= 3.750

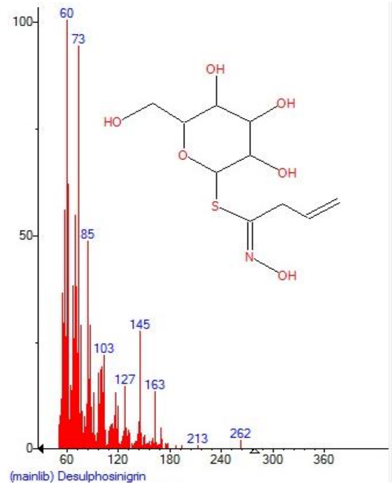


Figure 15: Mass spectrum of Desulphosinigrin with Retention Time (RT)= 3.808

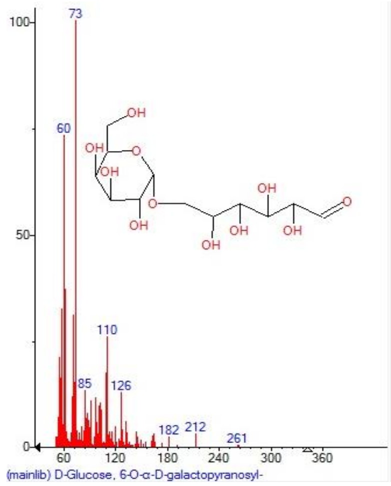


Figure 16: Mass spectrum of D-Glucose , 6-o-α-D-galactopyranosyl- with Retention Time (RT)= 4.174

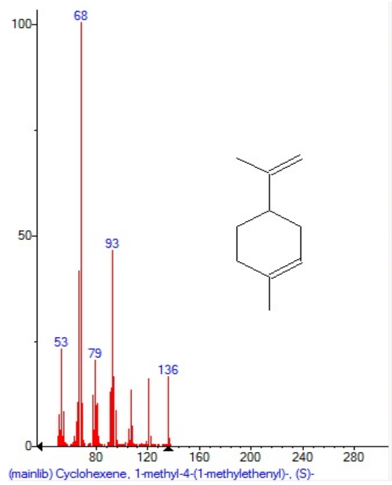


Figure 17: Mass spectrum of Cyclohexene , 1-methyl-4-(1-methylethenyl)-,(S)- with Retention Time (RT)= 4.466

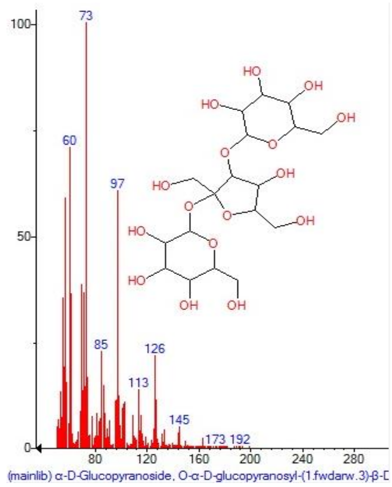


Figure 18: Mass spectrum of α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- with Retention Time (RT)= 4.700

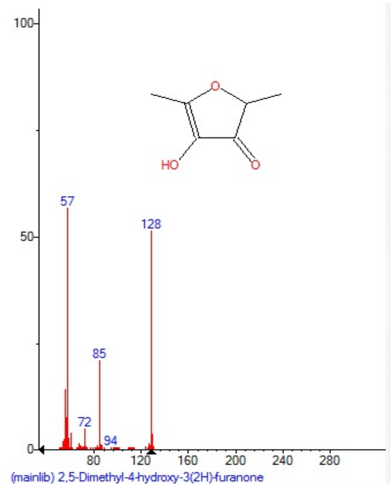


Figure 19: Mass spectrum of 2,5-Dimethyl-4-hydroxy-3(2H)-furanone with Retention Time (RT)= 4.941

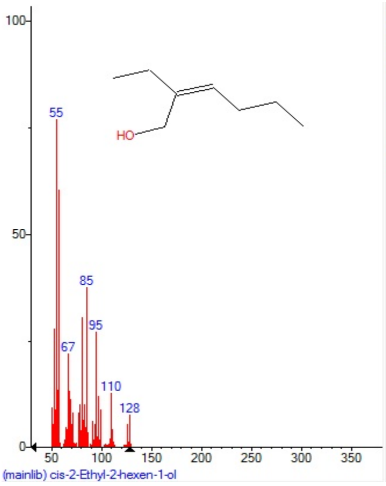


Figure 20: Mass spectrum of Cis-2-Ethyl-2-hexen-1-ol with Retention Time (RT)= 5.072

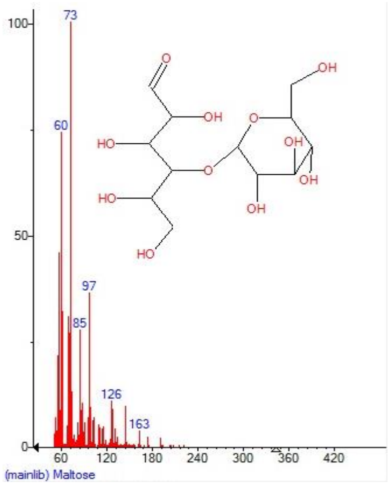


Figure 21 : Mass spectrum of Maltose with Retention Time (RT)= 5.204

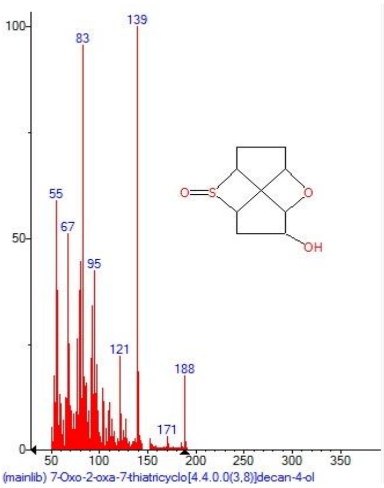


Figure 22: Mass spectrum of 7-Oxa-2-oxa-7-thiatricyclo[4.4.0.0(3,80]decan-4-ol with Retention Time (RT)= 5.890

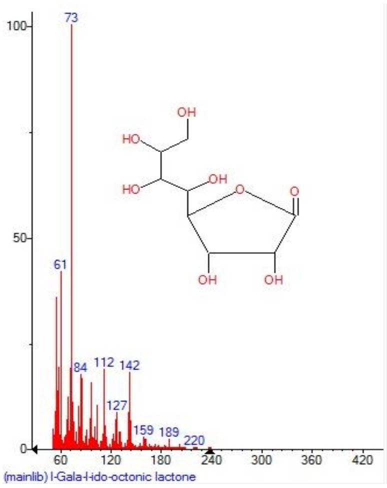


Figure 23: Mass spectrum of 1-Gala-l-ido-octonic lactone with Retention Time (RT)= 6.051

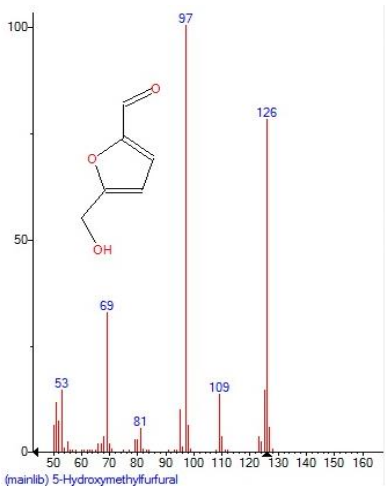


Figure 24: Mass spectrum of 5-Hydroxymethylfurfural with Retention Time (RT)= 6.657

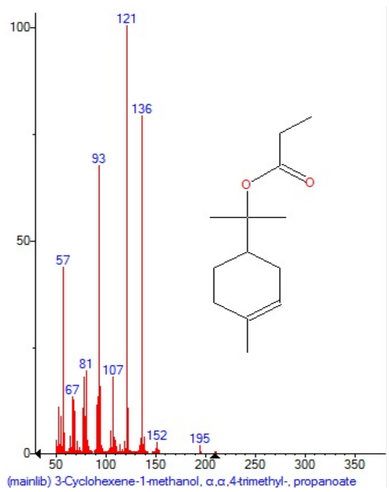


Figure 25: Mass spectrum of 3-Cyclohexene-1-methanol,α,α,4-trimethyl-,propanoate with Retention Time (RT)= 7.344

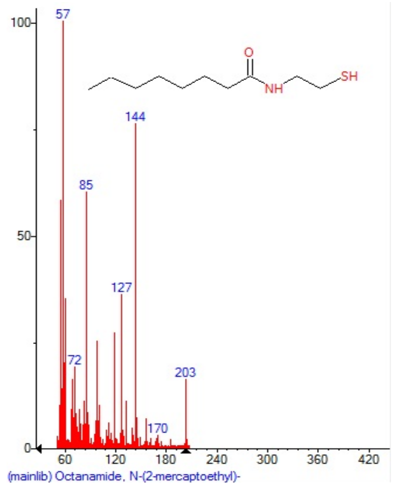


Figure 26: Mass spectrum of Octanamide,N-(2-mercaptoethyl)- with Retention Time (RT)= 7.475

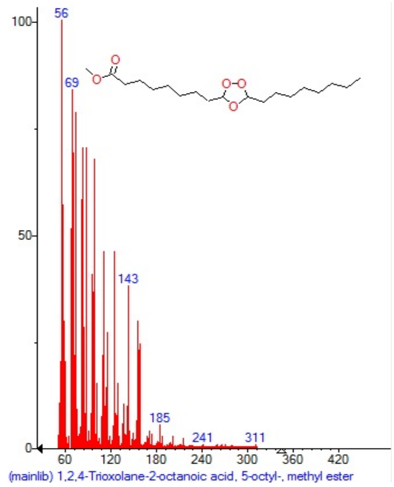


Figure 27: Mass spectrum of 1,2,4-Trioxolane-2-octanoic acid , 5-octyl-,methyl ester with Retention Time (RT)= 9.799

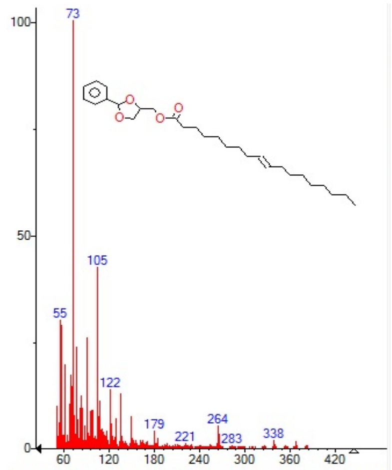


Figure 28: Mass spectrum of 9-Octadecenoic acid , (2-phenyl-1,3-dioxolan-4-yl)methyl ester with Retention Time (RT)= 11.143

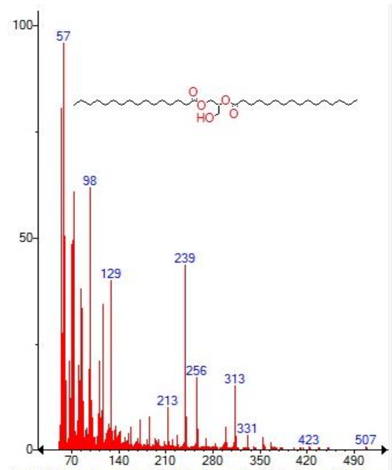


Figure 29: Mass spectrum of Hexadecanoic acid , 1-(hydroxymethyl)-1,2-ethanediyl ester with Retention Time (RT)= 13.810

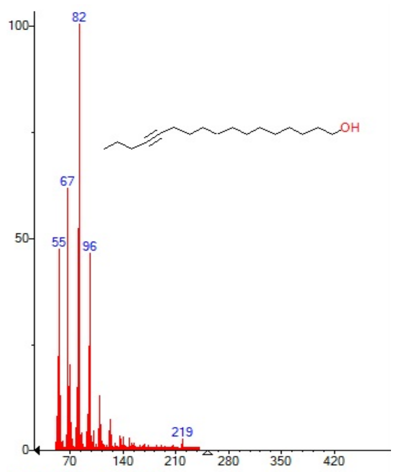


Figure 30: Mass spectrum of 13-Heptadecyn-1-ol with Retention Time (RT)= 15.051

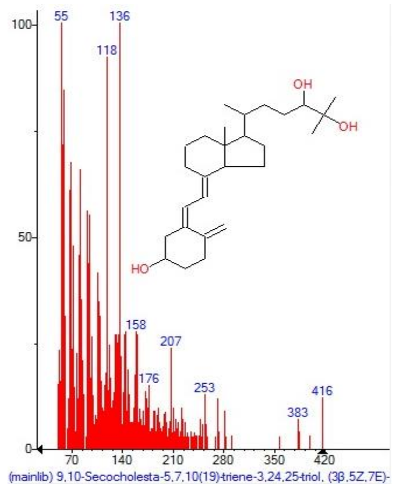


Figure 31: Mass spectrum of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- with Retention Time (RT)= 15.709

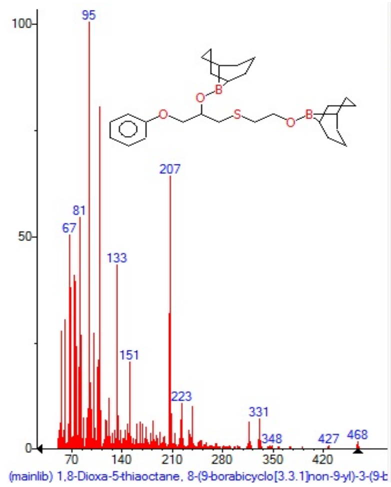


Figure 32: Mass spectrum of 1,8-Dioxa-5-thiaoctane,8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9- with Retention Time (RT)= 15.818

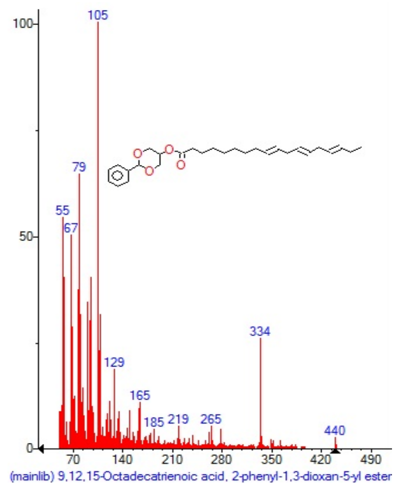


Figure 33: Mass spectrum of 9,12,15-Octadecatrienoic acid , 2-phenyl-1,3-dioxan-5-yl ester with Retention Time (RT)= 16.081

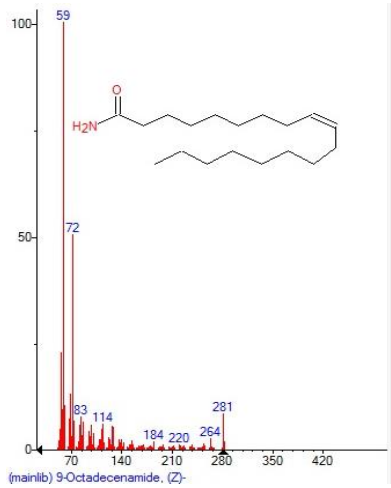


Figure 34: Mass spectrum of 9-Octadecenamide ,(Z)- with Retention Time (RT)= 17.294

presence of thirty three major peaks and the components corresponding to the peaks were determined as follows: Butanol, 2-nitro, α-D-Glucopyranoside, methyl 3,6-anhydro, Propanedioic acid, amino -, diethyl ester, DL-Arabinose, Hexadecenoic acid, Furfural, 1H-Pyrazole-1-carbothioamide, 3,5-dimethyl-, 2-Furanmethanol, 2(1H) Pyrazinone, o-Acetyl-L-serine , 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol, 6-Oxa-bicyclo[3.1.0]hexan-3-one, Acetic acid, 2,2'-[oxybis(2,1-ethanediyloxy)]bis-, Desulphosinigrin, D-Glucose, 6-o-α-D-galactopyranosyl-, Cyclohexene, 1-methyl-4-(1-methylethenyl)-,(S)-, -D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, Cis-2-Ethyl-2-hexen-1-ol, Maltose, 7-Oxa-2-oxa-7-thiatricyclo[4.4.0.0(3,80]decan-4-ol , 1-Gala-l-ido-octonic lactone, 5-Hydroxymethylfurfural, Cyclohexene-1-methanol,α,α,4-trimethyl-,propanoate, Hydroxymethylfurfural, Octanamide,N-(2-mercaptoethyl)-, 1,2,4-Trioxolane-2-octanoic acid, 5-octyl-,methyl ester, 9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-, 13-Heptadecyn-1-ol Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester, 9-Octadecenamide ,(Z). *Vitis vinifera* is used in conditions like hemorrhages, anemia, leprosy, skin diseases, syphilis, asthma, jaundice, bronchitis, anti-inflammatory, anti-carcinogenic, platelet aggregation inhibiting, and metal chelating properties[55]. *V. vinifera* seed contains lipid, protein, carbohydrates and 5-8% polyphenols. Several studies have indicated that extracts obtained from grape seed inhibit enzyme systems that are responsible for the production of free radicals, and that they have anti-mutagenic and anti-carcinogenic[56]. It has a protective effect on oxidant-induced production and deposition of extracellular matrix components. Hence the objective of the present study is to identify the phytochemical constituents of ethanolic extract of *Punica granatum* peel and *Vitis vinifera* seeds with the aid of GCMS technique[57].

CONCLUSION

Vitis vinifera is native plant of Iraq. In the present study determined that fortysix phytoconstituents were identified from methanol leaves extract of *Vitis vinifera* by gas chromatogram and mass spectrometry (GC-MS) analysis. *Vitis vinifera* leaves can be used as a promising multipurpose medicinal source whereas further clinical trial is required to prove its efficacy.

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