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-Glucoopyranoside, methyl 3,6-anhydro, Propanedioic acid, aminos, diethyl ester, DL-Arabinose, Hexadecenoic acid, Furfural, 1H-Pyrazole-1-carbothioamide, 3,5-dimethyl-, 2-Furannemthanol, 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-ethanediyl)]bis-, Desulphosinigrin, D-Gluco, 6-o-α-D-galactopyranosyl-, Cyclohexene, 1-methyl-4-(1-methylethenyl)-(S), -D-Glucoopyranoside, O-α-D-glucopyranosyl-(1.fwdarw;3)-β-, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 5,7,10(19)octanoic acid , 5-Octadecyn-1-ol, Maltose, 2-4,7-Oxa-2-oxa-7-thiatricyclo[4,4.0.0(3,8)]decan-4-ol, 1-Gala-1-ido-octonic lactone, 5-Hydroxymethylfurfural, Cyclohexene-1-methanol,a,a,4-trimethyl-propanoate, Hydroxymethylfurfural, Octanamide,N-(2-mercaptoethyl), 1,2,4-Trioxolane-2-octanoic acid, 5-octyl-methyl ester, 9-Octadeenoic acid, (2-phenyl-1,3-dioxolan-4-y)methyl ester 9,10-Secholesta-5,7,10(19)-triene-3,24,25-triol,(3f),SZ,SE-, 1-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. 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. 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. 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.

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 °C for further experimentations. Gas chromatography – Mass Spectrum analysis.
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 to 280°C. The column temperature was kept at 40°C for 1 min followed by linear programming to raise the temperature from 40°C to 120°C (at 4°C min−1 with 2 min hold time), 120°C to 170°C (at 6°C min−1 with 1 min hold time) and 170°C to 200°C (at 10°C min−1 with 1 min hold time). The transfer line was heated at 280°C. Two microliter of FAME sample was injected for analysis. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z. 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.

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

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Phytochemical compound</th>
<th>RT (min)</th>
<th>Molecular Weight</th>
<th>Exact Mass</th>
<th>Chemical structure</th>
<th>MS Fragment-ions</th>
<th>Pharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1-Butanol, 2-nitro-</td>
<td>3.144</td>
<td>119</td>
<td>119.05</td>
<td>82433</td>
<td>55,72,89,119</td>
<td>antibacterial, antifungal, anti-inflammatory</td>
</tr>
<tr>
<td>2.</td>
<td>α-D-Glucopyranoside, methyl 3,6-anhydro-</td>
<td>3.224</td>
<td>176</td>
<td>176.06</td>
<td>8474</td>
<td>57,73,10,2,145</td>
<td>Unknown</td>
</tr>
<tr>
<td>3.</td>
<td>Propanedioic acid, amino-, diethyl ester</td>
<td>3.253</td>
<td>175</td>
<td>175.08</td>
<td>4458</td>
<td>57,74,10,2,130,17,5</td>
<td>anti-inflammatory and analgesic activity</td>
</tr>
<tr>
<td>4.</td>
<td>DL-Arabinoa</td>
<td>3.276</td>
<td>150</td>
<td>150.05</td>
<td>2823</td>
<td>60,85,13,5</td>
<td>Antibacterial and anti-Candida activities</td>
</tr>
<tr>
<td>5.</td>
<td>9-Hexadecenoic acid</td>
<td>3.333</td>
<td>254</td>
<td>254.22</td>
<td>458</td>
<td>55,69,83,97,194,236,254,51,6796</td>
<td>anti-inflammatory</td>
</tr>
<tr>
<td>6.</td>
<td>Furfural</td>
<td>3.384</td>
<td>96</td>
<td>96.021</td>
<td>129</td>
<td>59,81,96,128,155,98</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>7.</td>
<td>1H-Pyrazole-1-carbothioamide, 3,5-dimethyl-</td>
<td>3.436</td>
<td>155</td>
<td>155.05</td>
<td>1719</td>
<td>53,69,81,98</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>8.</td>
<td>2-Furanmethanol</td>
<td>3.516</td>
<td>98</td>
<td>98.036</td>
<td>7794</td>
<td>53,69,81,98</td>
<td>Anti-inflammatory</td>
</tr>
</tbody>
</table>

System (Agilent 7890 Aseries, USA). The flow rate of the analysis was 6–42. 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.
<table>
<thead>
<tr>
<th>No.</th>
<th>Compound</th>
<th>MW</th>
<th>Exact Mass</th>
<th>Retention Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>2(1H)-Pyrazinone</td>
<td>3.676</td>
<td>96</td>
<td>96.032/363</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>o-Acetyl-L-serine</td>
<td>3.779</td>
<td>147</td>
<td>147.05/3158</td>
<td>anti-inducers</td>
</tr>
<tr>
<td>11</td>
<td>1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol</td>
<td>3.831</td>
<td>252</td>
<td>252.09/5751</td>
<td>Anti-insect activity</td>
</tr>
<tr>
<td>12</td>
<td>6-Oxa-bicyclo[3.1.0]hexan-3-one</td>
<td>3.939</td>
<td>98</td>
<td>98.036/7794</td>
<td>Antioxidant activity</td>
</tr>
<tr>
<td>13</td>
<td>Acetic acid, 2,2'-[oxybis(2,1-ethanediol)]bis-</td>
<td>3.750</td>
<td>222</td>
<td>222.07/3953</td>
<td>Uknown</td>
</tr>
<tr>
<td>14</td>
<td>Desulphosinigrin</td>
<td>3.808</td>
<td>279</td>
<td>279.07/7658</td>
<td>anti-asthmatic</td>
</tr>
<tr>
<td>15</td>
<td>D-Glucose, 6-o-α-D-galactopyranosyl</td>
<td>4.174</td>
<td>342</td>
<td>342.11/621</td>
<td>Anti-bacterial activity</td>
</tr>
<tr>
<td>16</td>
<td>Cyclohexene, 1-methyl-4-(1-methylethenyl)-(S)-</td>
<td>4.466</td>
<td>136</td>
<td>136.12/52</td>
<td>Anti-bacterial activity</td>
</tr>
<tr>
<td>17</td>
<td>α-D-Glucopyranoside, O-α-D-glucopyranosyl</td>
<td>4.700</td>
<td>504</td>
<td>504.16/9035</td>
<td>anti-diabetic activity</td>
</tr>
</tbody>
</table>
18. 2,5-Dimethyl-4-hydroxy-3(2H)-furanone 4.941 128 128.04 7344 57.72,85 antimicrobial effect

19. Cis-2-Ethyl-2-hexen-1-ol 5.072 128 128.12 0115 55.67,85 Unknown

20. Maltose 5.204 342 342.11 621 60.73,85 anti-inflammatory effect

21. 7-Oxa-2-oxa-7-thiatricyclo[4.4.0].[3,80]decan-4-ol 5.890 188 188.05 0715 55.67,83 Unknown

22. 1-Gala-l-ido-octonic lactone 6.051 238 238.06 8868 61.73,84 Anti-diabetic activity

23. 5-Hydroxymethyl urfural 6.657 126 126.03 1694 53.69,81 Anti-Inflammatory Agents

24. 3-Cyclohexene-1-methanol,α,α,4-trimethylpropanoate 7.344 210 210.16 198 57.67,81 Unknown

25. Octanamide,N-(2-mercaptoethyl)-1,2,4-trioxolane-2-octanoic acid, 5-octyl-methyl 7.475 203 203.13 4385 57.72,85 Unknown

26. 9.799 344 344.25 6275 56.69,14 Unknown
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-ethanediyoxy)]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-methyleneyl)-(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(3,8)0]decan-4-ol with Retention Time (RT) = 5.890

Figure 23: Mass spectrum of 1-Gala-1-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-Trioxolan-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-dioxalan-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
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-carboxioamide, 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-ethanediol)]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(3,8)decan-4-ol, 1-Gala-1-idooctonic 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. 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. 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 Panico granatum peel and Vitis vinifera seeds with the aid of GCMS technique.

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.

**ACKNOWLEDGMENT**
The authors thank the department of biology in college of nursing for providing all necessary facilities to conduct this study. Authors also thank Assist. Prof. Dr. Amean Al-yaasiri for assisting in extract preparation, and biochemical analysis.

**REFERENCES**


