Full Length Research Paper

Identification of five newly described bioactive chemical compounds in methanolic extract of Mentha viridis by using gas chromatography - mass spectrometry (GC-MS)

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The bioactive compounds were screened by gas chromatography-mass spectrometry (GC-MS) method. Twenty one bioactive phytochemical compounds were identified in the methanolic extract of Mentha viridis using GC-MS method. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula. GC-MS analysis of M. viridis revealed the existence of the 3,6-Octadecadiynoic acid, methyl ester, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, benzofuran, R-Limonene, 2-methoxy-4-vinylphenol, 2-hydroxy-5-methylbenaldehyde, tetra-acetyl-d-xylonic nitrile, Ficusin, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl, n-Hexadecanoic acid, 7-Methyl-Z-tetradecen-1-ol acetate, Ethyl 9,12,15-octadecatrienoate, Methyl 19-methyl-eicosanoate, Ethyl iso-allocholate, and Tocopherol. Five new bioactive chemical compounds 3-(N,N-Dimethylarylammonio), 1b,4a-eboxy-2H-cyclopenta [3,4]cyclopropal[8,9] cyclundec, 5H-Cyclopropa[3,4]benz [1,2-e]azulene-5-one, 2,2,4-Trimethyl-3-(3,8), 12,16-tetramethyl-hepta deca, and 4H-Cyclopropa[5,6]benz [1,2:7,8] azulene[5,6-b] oxiren-4-one are described and may in future be suitable sources for phytotherapy purposes. Mentha viridis contain chemical constitutions which may be useful for various herbal formulation exhibiting cardiac tonic, analgesic, antiasthamatic, anti-inflammatory and antipyretic properties.

Key words: Bioactive compounds, chromatography-mass spectrometry (GC-MS) analysis, Mentha viridis.

INTRODUCTION

Mentha viridis, (Lamiaceae family) commonly known as garden or green mint, is originally a native of the Mediterranean region (Grieve, 2013), and widely distributed in Euroasia, Australia and South Africa have...
been grown in damp or wet places (Gulluce et al., 2007; Mkaddem et al., 2009; Ozturk et al., 2009). Lamiaceae family consists of 200 genera and more than 4000 species (Ramesh et al., 2007). The phytochemical investigations on the Mentha species revealed that they possessed flavonoids and their glycosides, phenolic compounds, triterpenoids, steroids, and lignans (Monte et al., 1998; Areias et al., 2001; Ali et al., 2002; Zheng et al., 2007). Many species within this family are medicinal plants that apply in food, row and cooked forms and in human disease therapy (Santos et al., 2012).

*Mentha spp.* has been used for liver complaints due to its anti-inflammatory and treatment of bronchitis, nausea, flatulence, anorexia, and ulcerative colitis (Zhang et al., 2006). Essential oil formation in the plants is highly dependent on climatic conditions, especially day length, irradiance, temperature, and water supply (Franz and Novak, 2010). The leaves, flowers and stems of the Mentha species have been used as carminative, antispasmodic, antiemetic, stimulant, analgesic, and emmenagogue in traditional medicine all around the world. Their leaves have also been consumed as herbal tea and spice (Isçan et al., 2002).

*Mentha* species usually contain the monoterpene menthol in their constitutions, food products, menthol, and cosmetic (Simões et al., 2007; Oliveira et al., 2014). The chemical composition of the essential oils is influenced by factors such as leaf development and the emergence of new organs, which may lead to lower concentrations of these metabolites caused by translocation, as well as by effects such as seasonality, rain levels, and the stress to which the plant is exposed. These effects can directly influence the quantity and quality of the constituents in the essential oil (Gobbo et al., 2007; Mkaddem et al., 2009). The objective of this research was to determine the phytochemical composition of methanolic extract of *Mentha viridis*.

### Materials and Methods

**Collection and preparation of plant material**

*Mentha viridis* seeds were purchased from local market in Hilla city, middle of Iraq. After thorough cleaning and removal of foreign materials, the seeds were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use (Ameera et al., 2015; Huda et al., 2015a).

**Preparation of sample**

About seventeen grams of methanolic extract of *Mentha viridis* powdered were soaked in twenty five ml methanol for ten h in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis. It was again filtered through sodium sulphate in order to remove the traces of moisture (Huda et al., 2015b).

### Results and Discussion

Gas chromatography - mass spectrum analysis

The GC-MS analysis of the plant extract was made in a QP 2010 Plus SHIMADZU instrument under computer control at 70 eV (Mohammed and Imad, 2013). About 1 μl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. 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 1 ml per min. The electron gun of mass detector liberated electrons having energy of about 70 eV. The column employed here for the separation of components was Elite 1 (100% dimethyl poly siloxane) (Imad et al., 2014; Muhanned et al., 2015). 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.

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Figure 1. GC-MS chromatogram of methanolic extract of *Mentha viridis* leaves.

Figure 2. Mass spectrum of 3,6-Octadecadiynoic acid, methyl ester.
Figure 3. Mass spectrum of 2,5-Dimethyl-4-hydroxy-3(2H)-furanone.

Figure 4. Mass spectrum of 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl.
Figure 5. Mass spectrum of Benzofuran.

Figure 6. Mass spectrum of R-Limonene.
Figure 7. Mass spectrum of 2-Methoxy-4-vinylphenol.

Figure 8. Mass spectrum of 2-Hydroxy-5-methylbenaldehyde.
**Figure 9.** Mass spectrum of 3-(N,N-Dimethylallylammonio).

**Figure 10.** Mass spectrum of Tetraacetyl-d-xylonic nitrile.
Figure 11. Mass spectrum of Ficusin.

Figure 12. Mass spectrum of Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl.
Figure 13. Mass spectrum of n-Hexadecanoic acid.

Figure 14. Mass spectrum of 7-Methyl-Z-tetradecen-1-ol acetate.
Figure 15. Mass spectrum of Ethyl 9,12,15-octadecatrienoate.

Figure 16. Mass spectrum of Methyl 19-methyl-eicosanoate.
Figure 17. Mass spectrum of 1b,4a-epoxy-2H-cyclopenta[3,4]cyclopropal[8,9]cycloundec.

Figure 18. Mass spectrum of 5H-Cyclopropa[3,4]benz[1,2-e]azulene-5-one.
Figure 19. Mass spectrum of 2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca.

Figure 20. Mass spectrum of 4H-Cyclopropa[5,6]benz[1,2;7,8]azuleno[5,6-b]oxiren-4-one.
Figure 21. Mass spectrum of Ethyl iso-allocholate.

Figure 22. Mass spectrum of Tocopherol.
### Table 1. Major phytochemical compounds identified in methanolic extract of *Mentha viridis* leaves.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Phytochemical compound</th>
<th>RT(min)</th>
<th>Formula</th>
<th>Molecular weight</th>
<th>Exact mass</th>
<th>Chemical structure</th>
<th>Pharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3,6-Octadecadiynoic acid, methyl ester.</td>
<td>4.088</td>
<td>C_{19}H_{30}O_{2}</td>
<td>290</td>
<td>290.22458</td>
<td><img src="image1" alt="Image" /></td>
<td>Anti-asthma, pesticides, Neurons protective, anti-inflammatory and hepato-protective property</td>
</tr>
<tr>
<td>2.</td>
<td>2,5-Dimethyl-4-hydroxy-3(2H)-furanone.</td>
<td>4.775</td>
<td>C_{6}H_{8}O_{3}</td>
<td>128</td>
<td>128.047344</td>
<td><img src="image2" alt="Image" /></td>
<td>Anti-cataract effects and anti-oxidative activities</td>
</tr>
<tr>
<td>3.</td>
<td>4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl.</td>
<td>5.850</td>
<td>C_{6}H_{8}O_{4}</td>
<td>144</td>
<td>144.042258</td>
<td><img src="image3" alt="Image" /></td>
<td>Anti-diabetic and anti-oxidant activity</td>
</tr>
<tr>
<td>4.</td>
<td>Benzofuran.</td>
<td>6.686</td>
<td>C_{8}H_{8}O</td>
<td>120</td>
<td>120.0575147</td>
<td><img src="image4" alt="Image" /></td>
<td>Anti-inflammatory and analgesic effects</td>
</tr>
<tr>
<td>5.</td>
<td>R-Limonene.</td>
<td>7.006</td>
<td>C_{10}H_{16}O_{3}</td>
<td>184</td>
<td>184.109944</td>
<td><img src="image5" alt="Image" /></td>
<td>Anti-anxiety and anti-Inflammatory</td>
</tr>
</tbody>
</table>
### Table 1. Cont’d.

<table>
<thead>
<tr>
<th>No.</th>
<th>Compound Description</th>
<th>Molecular Formula</th>
<th>MW</th>
<th>Molecular Weight</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.</td>
<td>2-Methoxy-4-vinylphenol.</td>
<td>C9H10O2</td>
<td>150</td>
<td>150.06808</td>
<td>Anti-inflammatory effect</td>
</tr>
<tr>
<td>7.</td>
<td>2-Hydroxy-5-methylbenaldehyde.</td>
<td>C6H6O2</td>
<td>136</td>
<td>136.052424</td>
<td>Anti-Inflammatory and antioxidant</td>
</tr>
<tr>
<td>8.</td>
<td>3-(N,N-Dimethyllayrylammono).</td>
<td>C17H37NO3S</td>
<td>335</td>
<td>335.249414</td>
<td>New chemical compound (not found in PubChem Compound)</td>
</tr>
<tr>
<td>9.</td>
<td>Tetraacetyl-d-xylonic nitrile.</td>
<td>C14H17NO9</td>
<td>343</td>
<td>343.090332</td>
<td>Anti-tumor and anti-oxidant</td>
</tr>
<tr>
<td>10.</td>
<td>Ficusin.</td>
<td>C11H6O3</td>
<td>186</td>
<td>186.031694</td>
<td>Anti-oxidant and antimicrobial activity</td>
</tr>
<tr>
<td>No.</td>
<td>Name Description</td>
<td>Molecular Formula</td>
<td>Molecular Weight</td>
<td>Antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.</td>
<td>Anti cancer, anti-inflammatory, hepatoprotective</td>
</tr>
<tr>
<td>-----</td>
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<td>------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>11</td>
<td>Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl.</td>
<td>C₈H₇F₃O₂</td>
<td>206.055464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>n-Hexadecanoic acid.</td>
<td>C₁₆H₃₂O₂</td>
<td>256.24023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>7-Methyl-Z-tetradecen-1-ol acetate.</td>
<td>C₁₇H₃₂O₂</td>
<td>268.24023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Ethyl 9,12,15-octadecatrienoate.</td>
<td>C₂₀H₃₄O₂</td>
<td>306.25588</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Methyl 19-methyl-eicosanoate.</td>
<td>C₂₂H₄₄O₂</td>
<td>340.334131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>5H-Cyclopropa[3,4]benz [1,2-e]azulene-5-one.</td>
<td>C₂₈H₃₈O₁₁</td>
<td>548.22576</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Cont’d.

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical Compound</th>
<th>Molecular Formula</th>
<th>MW</th>
<th>Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.</td>
<td>2,2,4-Trimethyl-3-(3,8, 12,16-tetramethyl-heptadeca.</td>
<td>C_{30}H_{52}O</td>
<td>428</td>
<td><img src="image1.png" alt="Structure 1" /></td>
</tr>
<tr>
<td>19.</td>
<td>4H-Cyclopropa[5,6]benz [1,2,7,8]azuleno[5,6-b] oxiren-4-one.</td>
<td>C_{26}H_{34}O_{11}</td>
<td>522</td>
<td><img src="image2.png" alt="Structure 2" /></td>
</tr>
<tr>
<td>20.</td>
<td>Ethyl iso-allocholate.</td>
<td>C_{26}H_{44}O_{5}</td>
<td>436</td>
<td><img src="image3.png" alt="Structure 3" /></td>
</tr>
<tr>
<td>21.</td>
<td>Tocopherol</td>
<td>C_{29}H_{54}O_{2}</td>
<td>430</td>
<td><img src="image4.png" alt="Structure 4" /></td>
</tr>
</tbody>
</table>

(Mkaddem et al., 2009), while the study from Morocco showed that the oil contains high pulegone content (Talbaoui et al., 2012). The essential oil composition of *M. viridis* reported from Tunisie were carvone, 1,8- cineole, and limonene (Mkaddem et al., 2009), while that from Morocco contained high pulegone content (Talbaoui et al., 2012). The major compounds identified by Joshi and Sharma (2014) were cis-octimene (61.7%), limonene (10.5%), and trans-carveol (5%). The other minor constituents were α-selinene (1.7%), isodihydrocarveol acetate (1.5%), Z-jasmone (1.3%), 1,8-cineole (1.2%), and cis-carveol (1.0%). The essential oils of two species of mentha and showed strong antimicrobial activite (Hajlaoui et al., 2009). Studies of Bang (2007) examined biological activities of essential oil of mentha on pathogen of potato. Among the identified phytochemicals have the property of antimicrobial activities, antioxidant and inhibit several pathogenic parasites (Stainer et al., 1986; Singh et al., 1998; Prescott et al., 1999; Purohit and Vyas, 2004; Sasikumar et al., 2003; Santh, 2006; Sazada et al., 2009). However, further research is required to better understand the scientific and biotechnological basis values of applied phyto-
therapy.

Conclusion

*Mentha viridis* is native plant of Iraq. It contains chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthmatic.

**ACKNOWLEDGEMENT**

I thank Dr. Abdul-Kareem Al-Bermani, Lecturer, Department of Biology, for valuable suggestions and encouragement.

**Conflicts of interest**

Authors have none to declare.

**REFERENCES**


