Characterization of Non-Terpenoids in Marrubium crassidens Boiss. Essential Oil

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ARTICLE INFO

Article Type: Research Article

Article History:
Received: 20 April 2013
Revised: 15 May 2013
Accepted: 18 May 2013
ePublished: 20 August 2013

Keywords:
Marrubium crassidens
Lamiaceae
Oil-poor species
GC-MS
Acetophenone
Tolualdehyde

ABSTRACT

Purpose: Marrubium crassidens, a plant belonging to the family Lamiaceae, was studied for its volatile components present in the aerial parts of the plant during the flowering stage. Methods: The essential oil of the plant obtained through hydrodistillation of the dried plant material was assessed for its chemical composition by GC/MS and GC-FID analyses. Results: Twenty-five compounds were identified, which constituted 94.3% of the total oil composition. The major components were identified as, m-tolualdehyde (23.3%), acetophenone (15.8%), nonacosane (13.1%), docosane (7.2%), α-tolualdehyde (4.1%), β-caryophyllene (3.8%) and caryophyllene oxide (3.4%). Non-terpenoids with 75.7% were the most abundant components of the essential oil. Conclusion: Overall, M. crassidens essential oil revealed to include rather higher proportions of non-terpenoid compounds compared with other species of genus Marrubium.

Introduction

The family Lamiaceae with about 220 genera and almost 4000 species worldwide has always been the leader sources for culinary, vegetable and medicinal plants.1-2 In this regard, Marrubium a genus of about 40 species of flowering plants in this family has been holding a place of value in different cultures and herbal medicine with some known healing attributes.3-4 Cytotoxicity, immunomodulating, vasorelaxant, antispasmodic, hypolipidemic, hypoglycemic, and analgesic activities are some of the famous properties for species of genus Marrubium that have been reported by several studies.5-14 Although Marrubium species are mainly recognized for their non-volatile compounds, like diterpenes, polyphenols, steroids, phenylpropanoid glycosides and flavonoids, they happen to produce small amounts of essential oil and thus they are called as oil-poor species.15-17 Generally, essential oils from Marrubium sp. consist mostly of sesquiterpenes and studies concerning their antimicrobial and antioxidant activities are sparse.18-24 Nevertheless in our previous study on M. persicum, it was described substantially as a species distinct in chemical composition of the essential oil rich in non-terpenoid compounds from other species of Marrubium. According to the chemical diversity of essential oils within a genus indicating both quantitative and qualitative diversity in the composition of the essential oils, searching for the variety of naturally occurring volatile constituents in the essential oil of a plant appears to be of value. So as, based on flora Iranica, M. crassidens, endemic to the countries; Armenia, Azerbaijan, Turkey and Iran25 was selected to be further analyzed for its volatile constituents from the aerial parts of the plant that is grown in Azerbaijani province in Iran.

Materials and Methods

Plant material

Aerial parts of Marrubium crassidens were collected during the flowering stage from Chichaklou in East Azerbaijan province, in June 2011. A voucher specimen of the plant (Tbz-Fph-719) representing this collection has been deposited at the Herbarium of the Faculty of Pharmacy, Tabriz University of Medical science, Iran.

Essential oil extraction

Air-dried plant material of the aerial parts of M. crassidens was subjected to hydrodistillation using a Clevenger-type apparatus (Clevenger, 1928). Since

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the oil content was low in quantity, the distillation time was prolonged (3h) and xylene was used as an absorbing medium. The obtained essential oil was stored in sealed glass vial at 4-5 °C prior to analysis.

Gas Chromatography-Mass Spectrometry (GC-MS)
The essential oil was analyzed by GC-MS using a Shimadzu GC-MS-QP 5050A gas chromatograph fitted with a DB1 (methyl phenyl sylonane, 60 m x 0.25 mm i.d., 0.25 μm film thickness) capillary column. The GC was set at the following conditions with helium as the carrier gas; flow rate of 1.3 mL/min; linear velocity: 29.6 cm/s; Split ratio, 1:29; column temperature, 2 min in 60 °C, 50-260 °C at 3 °C/min; injector temperature, 240 °C, and 1 μL of volume injection of the essential oil. The MS operating parameters were as follows: ionization potential, 70 eV; ion source temperature; 270 °C; quadrupole 100 °C, Solvent delay 2 min, scan speed 2000 amu/s and scan range 30-600 amu, EV voltage 3000 volts.

Identification of the compounds
The identification of compounds was based on direct comparison of the retention indices and mass spectral data with those for the standards and by computer matching with the Wiley 229, Nist 107, Nist 21 Library, as well as by comparing the fragmentation patterns of the mass spectra with those reported in the literature.26 For quantification purpose, relative area percentages were obtained by FID without the use of correction factors, where the FID detector condition was set on a duplicate of the same column applying the same operational conditions.

Results and Discussions
The GC–MS analysis of the small amount of essential oil obtained through hydrodistillation led to the identification of 25 different components, representing 94.3% of the total oil constituents. All the identified compounds were arranged in order of their elution from the DB1-MS column and the retention indices whereas their percentage compositions were summarized in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>RI a</th>
<th>Compounds</th>
<th>Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>906</td>
<td>n-Nonane</td>
<td>2.1</td>
</tr>
<tr>
<td>2</td>
<td>933</td>
<td>Benzaldehyde</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>936</td>
<td>α-Pinene</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>966</td>
<td>1-Octen-3-ol</td>
<td>2.8</td>
</tr>
<tr>
<td>5</td>
<td>1004</td>
<td>Decane</td>
<td>0.3</td>
</tr>
<tr>
<td>6</td>
<td>1025</td>
<td>Limonene</td>
<td>0.6</td>
</tr>
<tr>
<td>7</td>
<td>1037</td>
<td>Acetophenone</td>
<td>15.8</td>
</tr>
<tr>
<td>8</td>
<td>1041</td>
<td>m-Tolualdehyde</td>
<td>23.3</td>
</tr>
<tr>
<td>9</td>
<td>1053</td>
<td>o-Tolualdehyde</td>
<td>4.1</td>
</tr>
<tr>
<td>10</td>
<td>1381</td>
<td>α-Cubebene</td>
<td>0.7</td>
</tr>
<tr>
<td>11</td>
<td>1424</td>
<td>β-Caryophyllene</td>
<td>3.8</td>
</tr>
<tr>
<td>12</td>
<td>1450</td>
<td>β-Farnesene</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td>1457</td>
<td>α-Humulene</td>
<td>0.4</td>
</tr>
<tr>
<td>14</td>
<td>1482</td>
<td>Germacrene D</td>
<td>2.6</td>
</tr>
<tr>
<td>15</td>
<td>1488</td>
<td>β-Selinene</td>
<td>0.6</td>
</tr>
<tr>
<td>16</td>
<td>1497</td>
<td>Bicyclogermacrene</td>
<td>1.9</td>
</tr>
<tr>
<td>17</td>
<td>1520</td>
<td>δ-Cadinene</td>
<td>0.4</td>
</tr>
<tr>
<td>18</td>
<td>1571</td>
<td>Spathulenol</td>
<td>0.9</td>
</tr>
<tr>
<td>19</td>
<td>1578</td>
<td>Caryophyllene oxide</td>
<td>3.4</td>
</tr>
<tr>
<td>20</td>
<td>1834</td>
<td>Hexahydrofarnesyl acetone</td>
<td>1.9</td>
</tr>
<tr>
<td>21</td>
<td>1945</td>
<td>Eicosanoic acid</td>
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</tr>
<tr>
<td>22</td>
<td>1989</td>
<td>Docosane</td>
<td>7.2</td>
</tr>
<tr>
<td>23</td>
<td>2592</td>
<td>Hexacosane b</td>
<td>2.0</td>
</tr>
<tr>
<td>24</td>
<td>2793</td>
<td>Octacosane b</td>
<td>1.9</td>
</tr>
<tr>
<td>25</td>
<td>2892</td>
<td>Nonacosane b</td>
<td>13.1</td>
</tr>
<tr>
<td>Total</td>
<td>94.3</td>
<td>Non-terpenoids</td>
<td>75.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Monoterpene hydrocarbons</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sesquiterpene hydrocarbons</td>
<td>11.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxygenated sesquiterpenes</td>
<td>4.3</td>
</tr>
</tbody>
</table>

a RI is the Retention Index relative to C8–C24 n-alkanes on the DB-1 column. b This compound was compared with an authentic sample.

It was established that the essential oil of M. crassidens was a complex mixture of mostly non-terpenoids (75.7%), sesquiterpene hydrocarbons (11.9%), oxygenated sesquiterpenes (4.3%) and monoterpenes...
hydrocarbons (2.4%). In spite of the absence of oxygenated monoterpenes, something to be expected in study of Marrubium species essential oil, our sample was characterized by the presence of alkanes, alkanolic acid derivatives and ketones in great amounts. As regards, m-tolualdehyde with 23.3%, and acetonophene with 15.8% were the two basic constituents present among the non-isoprenoid compounds of the essential oil. Moreover, β-caryophyllene (3.8%), germacrene D (2.6%), and bicyclogermacone (1.9%) were the leader sesquiterpene hydrocarbons that were present in the M. crassidens essential oil. The only identified two monoterpenes in the essential oil were α-pinene, and limonene with the relative percentages of 1.8 and 0.6%, respectively. Besides, caryophyllene oxide with 3.4% and spathulenol with 0.9% were the only components of the oxygenated sesquiterpen compound grouping.

Having reviewed previously reports of the essential oils from genus Marrubium, it was established that Marrubium species possess rather low amounts of aliphatic and non-terpenoid fractions in essential oils, in contrary to our findings.22-24,27 Owing to the fact that the production of terpenoid and non-isoprenoid compounds diverges early in the pathway of anabolic plant secondary compound synthesis,28,29 necessitates fundamental studies in advance especially in practical applications of the essential oils in fragrance and flavor industries, as well as in the pharmaceutical and chemical industries. In order to distinguish between the differences observed in the essential oil constituents of M. crassidens with other species of genus Marrubium additional studies could be of advantageous in determining the intrinsic (genetic, growth stage, etc.) and extrinsic (climatic, seasonal, environmental distillation processes, etc.) conditions affecting the relative biosynthesis pathways of the essential oils.

Conclusion
On the whole, M. crassidens in agreement with previous reports for other Marrubium species seems to be poor in essential oil content while it shares some similarities and also differences in constituents of the essential oil. Meanwhile, presence of relatively higher amounts of non-terpenoids; acetonophene with different isomers of tolualdehyde in the M. crassidens essential oil has been reported for the first time in this study for this plant.

Acknowledgments
The authors would like to thank Drug Applied Research Center of Tabriz University of Medical Sciences for recording the mass spectra. Financial support of this work by the Research Vice-Chancellor of Tabriz University of Medical Sciences is faithfully acknowledged. This article was written based on a data set of PhD. thesis, registered in Tabriz University of Medical Sciences (5/4/6651- NO. 71).

Conflict of Interest
The authors report no conflict of interest in this study.

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