Qualitative Screening of Secondary Metabolites from Sambucus Ebulus Roots by Multiple Analytical Techniques

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The interest in the separation of secondary metabolites in plants is huge, given the unlimited resource provided by the native species that are not yet evaluated. The aim of this study was to achieve a phychochemical study to explain the healing properties of Sambucus ebulus using modern analytical techniques (GC-MS, HPLC) in order to determine certain compounds with biological activity of the Sambucus ebulus species. Among the secondary metabolites identified in Sambucus ebulus exercising a wide range of biological activities on living organisms can be listed: quinic acid, 6-methyl-2-methoxy pyrazine, 2-methoxy-6-(2-propyl)-phenol. By HPLC was succeeded the separation of anthocyanins and two terpenoids: α - and β amyrin.

Keywords: Sambucus Ebulus, roots, GC-MS, HPLC, secondary metabolites

Secondary phytochemical compounds (secondary metabolites) are defined as chemicals without nutritional significance, found in relatively small amounts compared to primary metabolites, in plants, fruits, vegetables, grains and other plant products whose pharmaceutical properties have been correlated with reduced risk of disease.

It was proved that the presence of bioactive compounds in plant extracts is correlated with many in vitro biological activities: antibacterial, antifungal, or anticancer [1]. Due to imbalances in nutrition, environmental pollution and other disturbance among other things leading to a poor immune system, the current tendency is to consume products with intermediary role between medicine and food, and even supplementation of food with natural products rich in active principles.

In recent years, was increasingly invested in the development of analytical techniques and methods that allow the isolation of secondary metabolites present in various species used in folk medicine.

The aim of this study was to achieve a phychochemical study to explain the healing properties of Sambucus ebulus using modern analytical techniques (GC-MS, HPLC) in order to determine certain compounds with biological activity of the Sambucus ebulus species.

The interest in this species whose curative virtues have not been fully investigated was raised by various articles in the literature that investigated two other homologues of this species: Sambucus nigra [2] and Sambucus racemosa. Sambucus ebulus, similar to Sambucus nigra species is widespread in both Central and South America, in Asia, North Africa and USA. In Romania, the habitat of this species is extremely wide, being found in the lowlands and up in the mountains, especially on vacant lots and roadsides.

Although in our country the curative potential of this plant is relatively unknown, appearing a few mentions about the medicinal properties of this species only in 1973 [3], other countries such as Turkey and Iran paying special attention to this species. Thus, there are references in the literature about the use of leaves, flowers and fruits as expectorant, diuretic and purgative [4], leaves being used separately in various inflammatory diseases such as rheumatoid arthritis, fever, pulmonary edema, burning, or various open wounds [5]. Roots (Ébuli radix) are also mentioned in the literature as having bacteriostatic and diuretic action [6].

A study by Zakay-Rones et al. in 2004 [7] showed that people who received the extract from the fruit of S. ebulus species during convalescence, having contacted the influenza virus were recovered faster than those who received placebo, thus confirming the known antiviral properties of this plant.

Experimental part

Roots material

The raw material was collected from Craiova, Dolj, Romania during July-August 2010. After harvesting, roots were cleaned of soil and dust particles that may have influenced the analytical results. Roots were dried at temperatures between 22 – 24°C, in a dark room and were pulverized.

Reagents

All chemicals used for the study were of analytical grade. Double distilled water was used for all the analyses.

GC–MS analysis

Soxhlet extraction

10 g of fine powdered product was extracted with ethanol for 8 h in a Soxhlet extractor on a water bath. After cooling, the system was washed with alcohol, and the combined extracts were concentrated by distillation in a rotavapor. The yield (w/w) of the compounds extracted was found to be 9.49 %.

Equipment

Hewlett Packard 6890 gas chromatograph with 5973 mass spectrometer detector; Column: BD1 30 m x 0.25 mm x 1μm; carrier gas: Helium, 0.8 mL/min, constant flow; injection: 1μL, split ration: 188 : 1.290 oC; source temperature MS = 230°C; quadrupol MS = 150°C, interface MS = 300°C.

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HPLC analysis

The samples of *Sambucus ebulus* were analyzed by HPLC, using an Ultimate 3000 Dionex HPLC system, with UVD-3000 detector, C18 column (Acclaim 120 DIONEX, 250 mm length and 4 mm diameter), LPG-3400A pump.

Plant extraction

1 g of sample was weighted and mixed with 16 mL CH₃OH. Then, the mixture was maintained on water bath at 50 Celsius degrees for 5 h. The obtain solution was filtrated and analyzed by HPLC.

Chromatographic conditions

Separation of anthocyanins

Mobile phase: acetonitrile: acidified water (0.05% trifloracetic acid) = 25:75. Column temperature 20°C, flow rate 0.5 mL/min, wavelength 230 nm.

Separation of α and β – amyrin

Mobile phase: acetonitrile: acidified water (0.05% trifloracetic acid) = 70:30. Column temperature 40°C, flow rate 0.5 mL/min, wavelength 230 nm.

Results and discussions

After gas-chromatographic analysis 15 compounds were identified in the alcoholic extract with a probability higher than 70%, representing 82% of the identified compounds (table 1).

Figure 1 illustrates the gas chromatogram obtained for alcoholic extract. The gas chromatogram is abundant in peaks, not very well separated, so that in the absence of coupling with mass spectrometry would not allow the identification of chemical compounds in the extract.

Bio-medical considerations

It was found that this plant is rich in secondary metabolites, similar with other herbs used in medicine [8-9]. Our previous studies on *Sambucus ebulus* ethereal extract showed a wide number of secondary metabolites with a wide range of biological activities on living organisms: palmitic acid (hexadecanoate), stearic acid (octadecanoic acid t_R=28.52), (Z,9,12,15-octadecadieinoic) acid and linolenic acid (docosanoic acid) [10]. A special feature of this species is the presence of a highly increased number of higher alkanes: from pentadecane (C₁₅H₃₂) to eicosane (C₂₀H₴₂).

In the root alcoholic extract, other important compounds were identified: quinic acid, 6-methyl-2-methoxy pyrazine, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-2-methoxy-6 - (2-propyl)-phenol, etc. They were tested for their antimicrobial, anti-inflammatory, antioxidant, hipocolesterolice, anticancer, hepatoprotective [11-12]. Of these organic compounds, perhaps most important is quinic acid, a chiral compound with an intense inflammatory and immunostimulatory activity, commonly used in the pharmaceutical industry for the synthesis of new drugs such as Tamiflu.

<table>
<thead>
<tr>
<th>No.</th>
<th>Retention time</th>
<th>Compound</th>
<th>M₀</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3.26</td>
<td>acetic acid</td>
<td>C₂H₄O₂</td>
<td>60.05</td>
</tr>
<tr>
<td>2.</td>
<td>7.27</td>
<td>1,1-dithioxyethane</td>
<td>C₆H₁₁O₂</td>
<td>118.17</td>
</tr>
<tr>
<td>3.</td>
<td>9.50</td>
<td>furfural</td>
<td>C₆H₈O₄</td>
<td>96.08</td>
</tr>
<tr>
<td>4.</td>
<td>9.94</td>
<td>3-methylbutanoic acid</td>
<td>C₇H₁₄O₂</td>
<td>102.13</td>
</tr>
<tr>
<td>5.</td>
<td>10.57</td>
<td>1,3-dihydroxy-2-propanone</td>
<td>C₆H₁₂O₃</td>
<td>90.07</td>
</tr>
<tr>
<td>6.</td>
<td>16.03</td>
<td>2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one</td>
<td>C₈H₁₃O₅</td>
<td>144.12</td>
</tr>
<tr>
<td>7.</td>
<td>16.16</td>
<td>benzoic acid</td>
<td>C₆H₅CO₂H</td>
<td>122.12</td>
</tr>
<tr>
<td>8.</td>
<td>16.73</td>
<td>1,2-benzenediol</td>
<td>C₁₀H₁₂O₂</td>
<td>110.11</td>
</tr>
<tr>
<td>9.</td>
<td>16.93</td>
<td>5-hydroxyethyl-2-furanocarbaldehyde</td>
<td>C₈H₁₀O₃</td>
<td>126.11</td>
</tr>
<tr>
<td>10.</td>
<td>17.71</td>
<td>hydroquinone</td>
<td>C₆H₄O₂</td>
<td>110.11</td>
</tr>
<tr>
<td>11.</td>
<td>18.77</td>
<td>2-methoxy-4-rinilphenol</td>
<td>C₁₀H₁₀O₂</td>
<td>150.17</td>
</tr>
<tr>
<td>12.</td>
<td>19.29</td>
<td>D-galactose</td>
<td>C₆H₁₀O₅</td>
<td>180.15</td>
</tr>
<tr>
<td>13.</td>
<td>20.60</td>
<td>D (+)-alose</td>
<td>C₆H₁₀O₅</td>
<td>180.15</td>
</tr>
<tr>
<td>14.</td>
<td>20.62</td>
<td>1,6-anhydro-β-D-glucopyranose (levogluconan)</td>
<td>C₁₀H₁₈O₈</td>
<td>162.14</td>
</tr>
<tr>
<td>15.</td>
<td>22.04</td>
<td>D (+)-quinic acid</td>
<td>C₇H₁₀O₅</td>
<td>192.16</td>
</tr>
</tbody>
</table>

Fig.1.a. Gas chromatogram of the alcoholic extract of the roots of *Sambucus ebulus*: t_R: 0-13 min
Fig.1.b. Gas chromatogram of the alcoholic extract of the roots of *Sambucus ebulus*: b - t\textsubscript{R}: 13-18 min

Fig.1.c. Gas chromatogram of the alcoholic extract of the roots of *Sambucus ebulus*: c - t\textsubscript{R}: 18-30 min

**HPLC analysis**

**Separation of anthocyanins**

For the three reference compounds were obtained the following retention times: delphinidin - 6.28 min, pentunidin - 6.89 min and cyanidin 3,5-diglicoside - 7.35 min. Chromatographic characteristics obtained from HPLC analysis are shown in table 2 and the chromatograms obtained for the separation of anthocyanins and terpenoids are shown in figures 3 and 4.

The petunidin found in the root extract is readily soluble, is a potent inhibitor of free radicals having a very high antioxidant activity and thus explaining the anti-inflammatory and immunostimulant properties of *Sambucus ebulus*.

**Separation of terpenoids**

Retention times for the two reference compounds are: for α - amyrin - 3.48 min and for β - amyrin - 4.35 min. The presence of β – amyrin, known in the literature for its antinociceptive effects, in the roots extract is consistent with the therapeutic properties of the investigated species [13].
Conclusions

In this study was accomplished a qualitative study in order to identify secondary metabolites from *Sambucus ebulus* roots using two analytical techniques: GC-MS and HPLC. The found compounds explain the therapeutic properties of this herb suggesting that it can be successfully used for therapeutic purposes.

References

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