EVALUATION OF PHENOLIC ACID DERIVATIVES AND ESSENTIAL OIL CONTENT IN SOME MELISSA OFFICINALIS L. VARIETIES

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Abstract
Considering the importance of the active principles content in the vegetal product for determining its good quality, we investigated phenolic acid derivatives and essential oil levels, in different Melissa officinalis L. varieties cultivated in Cluj, Romania. The content of the essential oil, obtained by hydrodistillation, was between 0.03 – 0.10mL/100g fresh vegetal product. The phenolic acid derivatives concentrations were determined spectrophotometrically between 7.66 – 11.65 mg% (expressed in caffeic acid). We identified caftaric and caffeic acid in all samples by HPLC analysis and p-coumaric and ferulic acid only in the hydrolyzed samples.

Keywords: Melissa officinalis varieties, phenolic acid derivatives, essential oil

Introduction
The leaves of Melissa officinalis L. (Lamiaceae), also known as lemon balm, are used in traditional medicine for the symptomatic treatment of gastrointestinal disturbances, as adjuvant therapy for the pain associated to functional dyspepsia, for the symptomatic treatment of neurotonic disorders (minor sleeplessness). Recent studies have demonstrated the antioxidant and neuroprotective effects of different extracts from M. officinalis. That is why
this plant could be considered an effective agent in the prevention of various neurological diseases associated with oxidative stress [10, 12]. Balm essential oil is an antibacterial and antifungal agent, with spasmylytic and sedative properties. An important antiviral activity (herpes, vacciniaux) has been shown for the aqueous extract, probably due to the phenolic acids or their derivatives and to their interaction with viral proteins [1, 8, 14].

The aim of this paper was to study the content of essential oil and phenolic acid derivatives, depending on the variety of *Melissa officinalis*, in order to evaluate the quality of natural products. We have also identified by HPLC analysis the most important polyphenolic compounds from some varieties of *M. officinalis*.

**Materials and methods**

The material used for analysis was represented by the aerial parts harvested in May 2007, from 14 varieties of *M. officinalis*, which were cultivated in the experimental fields of University of Agricultural Sciences and Veterinary Medicine (UASVM) Cluj-Napoca, Romania [5]:
- **MCj, MCluj** – selections from local populations from Cluj county;
- **M349** - local variety of Melissa;
- **MTimisoara** - population from Timișoara county;
- **MLemona** - german variety Lemona;
- **MCitronella** - german variety Citronella;
- **6, 7, 8, 9, 10. MGermania0, MGermania1, MGermania2, MGermania3, MGermania4** - different german varieties;
- **MFranța** - french variety;
- **MCehia** - czech variety;
- **MPolonia** - polish variety.

The essential oils were obtained by hydrodistillation of fresh aerial parts of *M. officinalis* varieties [11].

The quantitative determinations of the phenolic acid derivatives were performed using a spectrophotometric method with the Arnow reagent and the results were expressed in mg caffeic acid [11]. Spectrophotometric analysis was performed using an UV-VIS JASCO V-530 spectrophotometer.

The identification and quantification of phenolic acid derivatives were performed by HPLC analysis [2, 3, 4, 6, 7, 9, 13]. HPLC analyses were carried out using an Agilent 1100 HPLC Series system (Agilent USA), binary pump. The separation was performed using a Zorbax SB-C18 reverse-phase column (100×3.0 mm i.d., 3.5 µm particles). The working temperature was 48°C and the detection of the compounds was performed at 330 nm (first 17 min from chromatogram) and 370 nm (from 17 min to 38
min) using a G1311A diode array detector system. The chromatographic data were processed using ChemStation software from Agilent, USA.

The mobile phase was a binary gradient methanol: KH₂PO₄ 40 mM, pH=2.3 with H₃PO₄ 85%. The elution started with a linear gradient beginning with 5% methanol and ending at 42% methanol, for 35 min; isocratic elution followed, for the next 3 min, with 42% methanol. The flow rate was 1 mL/min. and the injection volume was 5 µL. All solvents were filtered through 0.5 µm filters (Sartorius) and degassed through ultrasonication. Quantitative determinations were performed using an external standard method. The detection was carried out with an UV detector, at 330nm, 370nm.

**Standards:** caftaric acid from Dalton (USA), gentisic acid, ferulic acid, sinapic acid, from Roth (Germany), caffeic acid, chlorogenic acid, p-coumaric acid, from Sigma (Germany).

**Samples preparation:** dried natural products were extracted with methanol (1:10) after degreasing with chloroform in the Soxhlet apparatus.

In order to obtain more accurate data on the studied compounds, each sample was analyzed before and after acid hydrolysis. 2mL extractive solution was treated with 2 mL 2M hydrochloric acid and 0.2 mL ascorbic acid solution 100 mg/mL, and the mixtures were heated at 80°C on a water bath for 30 min, ultrasonicated for 15 min, and heated for another 30 min at 80°C. During the heating, 1mL methanol was added to the extraction mixture every 10 min, in order to ensure the permanent presence of methanol. The mixtures were centrifuged at 4000 rpm and the solutions were diluted with distilled water in a 10 mL volumetric flask and filtered through a 0.45 µm filter before injection.

**Results and discussion**

The essential oil, important for the antimicrobial properties, was determined in concentrations between 0.03-0.10mL/100g fresh natural product (table I). The optimal content indicated in the literature is 0.05 mL/100g, so that only the local variety McJ and the czech MČehia variety had an inferior quality (0.03-0.04 mL/100g).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Essential oil concentration (mL/100g natural product)</th>
<th>Sample</th>
<th>Essential oil concentration (mL/100g natural product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MČJ</td>
<td>0.04</td>
<td>MČGermany1</td>
<td>0.08</td>
</tr>
<tr>
<td>MČCluj</td>
<td>0.08</td>
<td>MČGermany2</td>
<td>0.05</td>
</tr>
<tr>
<td>MČXπ</td>
<td>0.06</td>
<td>MČGermany3</td>
<td>0.07</td>
</tr>
<tr>
<td>MČTimișoara</td>
<td>0.07</td>
<td>MČGermany4</td>
<td>0.06</td>
</tr>
<tr>
<td>MČLemona</td>
<td>0.09</td>
<td>MČPolonia</td>
<td>0.07</td>
</tr>
<tr>
<td>MČCitronella</td>
<td>0.10</td>
<td>MČFranţa</td>
<td>0.08</td>
</tr>
<tr>
<td>MČGermany0</td>
<td>0.07</td>
<td>MČCehia</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table I

Essential oil concentrations in fresh aerial parts of *Melissa officinalis* varieties
The content of phenolic derivatives was between 7.66 and 11.65 % (table II). The polish variety had the highest content of these compounds, the german varieties’ content was 8-11.3% and the lowest level was found in a local variety, MCJ (7.66 mg%). The levels of caffeic acid derivatives were higher than the limits from the literature (~4-5%) [1], so all the Melissa officinalis varieties are suitable for cultivation.

### Table II

The concentrations of phenolic acid derivatives (expressed in mg % caffeic acid)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of phenolic acid derivatives (mg%)</th>
<th>Sample</th>
<th>Concentration of phenolic acid derivatives (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCJ</td>
<td>7.6655</td>
<td>MGermania1</td>
<td>8.0488</td>
</tr>
<tr>
<td>MCluj</td>
<td>11.448</td>
<td>MGermania2</td>
<td>11.341</td>
</tr>
<tr>
<td>M349</td>
<td>10.487</td>
<td>MGermania3</td>
<td>9.423</td>
</tr>
<tr>
<td>MTimisoara</td>
<td>10.521</td>
<td>MGermania4</td>
<td>11.212</td>
</tr>
<tr>
<td>MCluj</td>
<td>9.355</td>
<td>MPolonia</td>
<td>11.652</td>
</tr>
<tr>
<td>MCluj</td>
<td>9.4223</td>
<td>MFranta</td>
<td>10.433</td>
</tr>
<tr>
<td>MGermani0</td>
<td>10.995</td>
<td>MChia</td>
<td>10.721</td>
</tr>
</tbody>
</table>

In order to analyse the phenolic acid derivatives from aerial parts of different *M. officinalis* varieties by HPLC, we used 7 standards: caftaric acid (Rt = 3.27), gentisic acid (Rt = 3.76), caffeic acid (Rt = 6.10), chlorogenic acid (Rt = 6.80), p-coumaric acid (Rt = 9.49), ferulic acid (Rt = 12.80), sinapic acid (Rt = 15.01). After the analysis, 4 phenolic compounds were identified in the studied extracts. The results of the compounds identification are presented in table III and the chromatographic profiles are shown in figure 1.

Two free cinnamic acid derivatives: p-coumaric acid (35-73mg/100g) and ferulic acid (36-56mg/100g) were identified and quantified in the hydrolyzed extracts of *M. officinalis*. Caffeic acid was identified and quantified in all extracts, before and after hydrolysis. The high quantities of caffeic acid in the hydrolyzed extracts (1280-1956 mg/100g) indicate its presence as esters with other acids or alcohols (as caftaric acid, for example). The caffeic acid is the major compound of the hydrolyzed extracts.

### Table III

Phenolic acid derivatives identified by HPLC (mg/100g natural product)

<table>
<thead>
<tr>
<th>Sample</th>
<th>caftaric acid NH / H</th>
<th>caffeic acid NH / H</th>
<th>p-coumaric acid NH / H</th>
<th>ferulic acid NH / H</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFranta</td>
<td>340.12 / 189.81</td>
<td>74.12 / 1954.38</td>
<td>- / 73.23</td>
<td>- / 55.66</td>
</tr>
<tr>
<td>MGermania1</td>
<td>312.43 / 221.45</td>
<td>63.21 / 1956.56</td>
<td>- / 52.14</td>
<td>- / 37.96</td>
</tr>
<tr>
<td>MGermania2</td>
<td>261.01 / 177.94</td>
<td>56.67 / 1921.66</td>
<td>- / 58.16</td>
<td>- / 40.99</td>
</tr>
<tr>
<td>MGermania3</td>
<td>241.23 / 154.21</td>
<td>56.67 / 1677.36</td>
<td>- / 49.73</td>
<td>- / 36.95</td>
</tr>
<tr>
<td>MPolina</td>
<td>281.58 / 150.26</td>
<td>46.79 / 1886.76</td>
<td>- / 61.17</td>
<td>- / 38.72</td>
</tr>
<tr>
<td>MTimisoara</td>
<td>245.19 / 181.91</td>
<td>44.02 / 1280.37</td>
<td>- / 35.89</td>
<td>- / 45.80</td>
</tr>
<tr>
<td>MTimisoara</td>
<td>324.29 / 197.72</td>
<td>47.94 / 1459.23</td>
<td>- / 50.33</td>
<td>- / 56.92</td>
</tr>
</tbody>
</table>

NH - before hydrolysis; H - after hydrolysis
The study of all HPLC chromatograms showed several other peaks which were not identified. Among these compounds, three major peaks were detected in all non-hydrolyzed extracts. The retention time of these compounds was between 15 and 27 min and they were not found in the hydrolyzed extracts. They may correspond to some flavonoidic glycosides.

**Conclusions**

In order to evaluate the quality of some vegetable products, we investigated the content of essential oil and phenolic acid derivatives in some *Melissa officinalis* varieties cultivated in Cluj. The levels of essential oil and caffeic acid derivatives were comparable with the literature data for almost all samples. Caffeic and caftaric acids were identified by HPLC in all varieties and *p*-coumaric and ferulic acids were determined only after hydrolysis. There were small variations between the amounts of these compounds, in the analyzed varieties.

**References**


*Manuscript received: November 10th 2009*