VARIABILITY OF THE ESSENTIAL OIL CONTENT IN LAVENDER (LAVANDULA ANGUSTIFOLIA P. MILL.)

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Abstract: The aim of this work was the determination of variability of the essential oil content in lavender obtained from different sources. The results indicate that significant differences were found both in the essential oil content and in its composition. The essential oil content varied in the range of 2.10–2.83 ml.100 g⁻¹. Thus, it is important to distinguish individual lavender types according their usage. The Czech Pharmacopoeia (2009) states that the lavender should contain 13 ml.kg⁻¹ of the essential oil.

Key Words: Lavender, essential oil, limonene, cineol, linalool, camphor, borneol, α-terpineol, linalyl acetate

INTRODUCTION
The lavender (Lavandula angustifolia L.) has refreshing effects; inhaling the scent of lavender soothes, relieves of depression and induces calmness. The generic name of lavender is derived from the Latin word “lavoy”, “to wash”. Thanks to its insecticide, repellent, and especially aromatic properties it is an ideal plant for use in the perfumery and cosmetics industry (Neugebauerová 2006, Small 2006). Lavender is a perennial hemixylum belonging to the Lamiaceae family (Brabenec 1981, Neugebauerová 2006). Czech Pharmacopoeia (2009) defines the lavender flower as a flower of the specie Lavandula angustifolia P. MILL. (Lavandula officinalis CHAIX.) (Lamiaceae), that has significant aromatic smell and must contain at least 13 ml.kg⁻¹ of the essential oil as a waterless drug.

Lavender essential oil is defined by the Czech Pharmacopoeia (2009) as a colourless or pale yellow clear liquid with characteristic odour obtained from the flowering tops of the specie Lavandula angustifolia P. MILL. (Lavandula officinalis CHAIX.) (Lamiaceae) by the means of steam distillation.

Lavender contains 0.5–1.5% of the essential oil, 12% of tannins, coumarins, flavonoids and in the leaves about 0.7% of ursolic acid. The essential oil is composed of linalyl acetate (8–18% in Lavandula angustifolia and 30–60% in Lavandula dentata), which is the main source of its smell, and also of bornyl acetate, α-terpineol, linalool, 1,8-cineol, camphor, geraniol and other compounds (Velgosová, Velgos 1988, Small 2006).

The drug acts mostly as a nervivum, sedativum, chologagum a external derivans. It is often used as a cure for migraine, for its beneficial effect on neurasthenia, hysteria, heart problems or insomnia. It has spasmolytic effects during the spasms, relieves of pain and colic, stimulates the digestion and increases the production of bile (Kresánek, Krejčí 1988).

It was demonstrated in clinical and preclinical studies that lavender oil and its constituents, particularly linalool, reduces anxiety (Tsang, Ho 2010, Perry et al. 2012).

The essential oil obtained from Lavandula angustifolia is of the highest quality, but often it is forged with the essential oil of L. latifolia or similar species that are much cheaper to produce. Most of the lavender oil found in shops is made of levandine (L. angustifolia × L. latifolia). This essential oil has a quality comparable to Lavandula angustifolia (Neugebauerová 2006, Small 2006).

As for the industrial products containing lavender tops, there is for example herbal tea blend Valofyt Neo - nervinum, Calmonal liquidum - a solution containing lavender extract used as external
antirheumatic, the essential oil is a component of ointment Rheumosin unguentum - derivans and an aromatic ointment Unguentum aromaticum SPOFA (Kresánek, Krejča 1988).

Estimated annual global production of the essential oil obtained from Lavandula angustifolia, lavandine and L. latifolia is 462 tons, 768 tons and 64 tons, respectively (Lawrence 1992).

MATERIAL AND METHODS

Lavender flower

Samples of materials designed for commercial use were obtained from the Leros, Ltd. Company, Prague-Zbraslav. Also the samples obtained from three different growers were evaluated.

Methods for the determination of contained compounds:

Determination of the content of lavender essential oil

Determination of the essential oil content was carried out according to a modified methodology given in the Czech Pharmacopoeia (2009). Czech Pharmacopoeia (2009) states that the essential oil content in herbal drugs must be determined by steam distillation. Essential oil is separated from the drug at high temperature and subsequently condensed in the condenser, gathered and collected in a special part of the condenser, the calibrated tube, above the surface of the aqueous phase. The essential oil is caught into xylene and the aqueous phase returns automatically to the distillation apparatus. The result is given as the essential oil content in ml.kg⁻¹ of dry matter.

The essential oil composition

Gas chromatograph Trace Ultra (Thermo Scientific) with the detector Trace DSQ (Thermo Scientific) was used for the lavender oil analysis. The separation was carried out at the capillary column SLB-5MS (60 m × 0.25 mm × 0.25 µm). Following temperature program was used for the measurement: \( T_1 = 50°C, t_1 = 0.1 \text{ min}, 3°C/\text{min} \) to \( T_2 = 150°C, t_2 = 10 \text{ min}, 10°C/\text{min} \) to \( T_3 = 200°C, t_3 = 5 \text{ min} \). Injector temperature was 250°C with split: 1 min. Ion source temperature was 200°C. 1 µl of the essential oil solution in hexane was injected to the column. Flow rate of the carrier gas, he, was 1.5 ml.min⁻¹. Ionisation energy 70 eV and scan m/z: 20–450 were used. Calibration curves and analyses performed by the method of external standard were processed and evaluated by the means of the Xcalibur software.

RESULTS AND DISCUSSION

The essential oil content and composition

6 samples of lavender flowers (Lavandulae flos) of different origin were analysed. Total essential oil content was determined, as well as selected essential oil components (limonene, cineol, linalool, camphor, borneol, α-terpineol and linalyl acetate).

Table 1 Variance analysis for the total essential oil content and selected essential oil components (limonen, cineol, linalool) in investigated variants of lavender

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>d.f.</th>
<th>Essential oil content [ml.100 g⁻¹]</th>
<th>Limonene [%]</th>
<th>Cineol [%]</th>
<th>Linalool [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MS</td>
<td></td>
</tr>
<tr>
<td>variant</td>
<td>5</td>
<td>0.36***</td>
<td>0.048***</td>
<td>131.399***</td>
<td>274.55***</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>0.00</td>
<td>0.001</td>
<td>0.431</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Note: * - \( p \leq 0.05 \); ** - \( p \leq 0.01 \); *** - \( p \leq 0.001 \)

Table 1, the variance analysis, shows very highly statistically significant effect of the variety both on the essential oil content variations and on individual essential oil components (limonen, cineol, linalool).
Table 2 Average values of total essential oil content and selected essential oil components (limonene, cineol, linalool) in investigated variants of lavender

<table>
<thead>
<tr>
<th>Variant</th>
<th>Essential oil content [ml.100 g⁻¹]</th>
<th>Limonene [%]</th>
<th>Cineol [%]</th>
<th>Linalool [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>14/1</td>
<td>2.83 d</td>
<td>0.50 b</td>
<td>20.77 bc</td>
<td>30.72 b</td>
</tr>
<tr>
<td>14/2</td>
<td>2.60 c</td>
<td>0.61 c</td>
<td>20.26 b</td>
<td>31.00 b</td>
</tr>
<tr>
<td>14/3</td>
<td>3.15 e</td>
<td>0.33 a</td>
<td>21.34 bc</td>
<td>29.80 b</td>
</tr>
<tr>
<td>14/4</td>
<td>2.10 a</td>
<td>0.77 d</td>
<td>21.00 bc</td>
<td>24.41 a</td>
</tr>
<tr>
<td>14/5</td>
<td>2.33 b</td>
<td>0.43 b</td>
<td>22.28 c</td>
<td>25.44 a</td>
</tr>
<tr>
<td>14/6</td>
<td>2.08 a</td>
<td>0.48 b</td>
<td>1.34 a</td>
<td>56.15 c</td>
</tr>
</tbody>
</table>

Note: Average values marked with different letters in columns vary on a statistically significant level at P=0.05

Statistically highest content of lavender essential oil was found in the samples of lavender variant 14/3 (3.15 ml.100 g⁻¹). On the other hand, the lowest content of lavender essential oil was found in the samples of the variant 14/6 (2.08 ml.100 g⁻¹). However, these samples were not statistically significantly different from the samples of lavender variant 14/4 (2.10 ml.100 g⁻¹), (see Table 2 and Figure 1).

The highest content of the essential oil component limonene was found in the samples of lavender variant 14/4 (0.77%). The samples of lavender variants 14/5 (0.43%), 14/6 (0.48%), 14/1 (0.50%) were not statistically significantly different from each other. The lowest content of limonene was found in the samples of lavender variant 14/6 (0.33%).

Samples of the lavender variant 14/6 are significant due to lowest, extremely low content of cineol (1.34%), in comparison with other investigated samples. These samples had higher content of linalool instead (56.15%). The ratio of camphor in the essential oil was statistically significantly lowest in these samples (0.76%) in comparison with other variants (see Table 4). The lavender variant 14/6 was statistically significantly different also as for the content of linalyl acetate (34.81%) (see Table 4). These samples were analysed repeatedly to prove that the results are correct.

The content of cineol was highest in the samples of lavender variety 14/5 (22.28%). The samples of the lavender variants 14/2 (20.26%), 14/1 (20.77%), 14/4 (21.00%), 14/3 (21.34%) were not statistically significantly different as for the cineol content.

The content of the essential oil component linalool varied in the samples of investigated lavender variants in the range from 24.41% to 31.00–56.15%. Similar content of linalool was found by Prusinowska, Smigielski (2015), 25.7–44.9%.

Figure 1 Total essential oil content in investigated variants of lavender
Table 3 Variance analysis for the total essential oil content and selected essential oil components (camphor, borneol, α-terpineol, linalyl acetate) in investigated variants of lavender.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>d.f.</th>
<th>Camphor [%]</th>
<th>Borneol [%]</th>
<th>α-terpineol [%]</th>
<th>Linalyl acetate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>variant</td>
<td>5</td>
<td>229.16***</td>
<td>4.4***</td>
<td>0.78***</td>
<td>154.41***</td>
</tr>
<tr>
<td>Error</td>
<td>6</td>
<td>1.02</td>
<td>0.13</td>
<td>0.01</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Note: * - p ≤ 0.05; ** - p ≤ 0.01; *** - p ≤ 0.001

All investigated essential oil components (camphor, borneol, α-terpineol, linalyl acetate) were very highly statistically significantly affected by the variant.

Table 4 Average values of total essential oil content and selected essential oil components (camphor, borneol, α-terpineol, linalyl acetate) in investigated variants of lavender.

<table>
<thead>
<tr>
<th>Variant</th>
<th>Camphor [%]</th>
<th>Borneol [%]</th>
<th>α-terpineol [%]</th>
<th>Linalyl acetate [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>14/1</td>
<td>26.25 b</td>
<td>6.99 b</td>
<td>3.12 d</td>
<td>11.64 A</td>
</tr>
<tr>
<td>14/2</td>
<td>25.98 b</td>
<td>7.02 b</td>
<td>2.84 bc</td>
<td>12.30 Ab</td>
</tr>
<tr>
<td>14/3</td>
<td>25.80 b</td>
<td>6.76 b</td>
<td>2.69 b</td>
<td>13.29 B</td>
</tr>
<tr>
<td>14/4</td>
<td>28.14 b</td>
<td>7.12 b</td>
<td>1.65 a</td>
<td>14.87 C</td>
</tr>
<tr>
<td>14/5</td>
<td>28.12 b</td>
<td>7.05 b</td>
<td>1.81 a</td>
<td>16.91 D</td>
</tr>
<tr>
<td>14/6</td>
<td>0.76 a</td>
<td>3.48 a</td>
<td>2.97 cd</td>
<td>34.81 E</td>
</tr>
</tbody>
</table>

Note: Average values marked with different letters in columns vary on a statistically significant level at P=0.05

Investigated samples of lavender variants 14/1–5 were not statistically significantly different as for the content of the essential oil component camphor. The camphor content varied in the range of 25.80–28.14% in these varieties (see table 4). With the exception of the sample 14/6 it was more than what was found by Carrasco et al. (2015). These authors observed the content of camphor in the range of 16–24% in the samples cultivated in Spain. Similar situation as for the camphor was found for another essential oil component, borneol. Only the samples of lavender variant 14/6 with the lowest content of 3.48% were statistically significantly different. All other investigated variants were not statistically significantly different from each other.

In opposite to the work of Prusinowska, Smigielski 2015 (4.0–6.6% of borneol) most of the samples showed higher content of the essential oil component borneol, which varied in the range of 3.48–7.12%. The highest content of this essential oil component was found in the samples of the variant 14/4. However, these samples were not statistically significantly different from other samples of lavender variants 14/1–14/5.

The highest content of α-terpineol was found in the samples of lavender variant 14/1 (3.12%), These samples were not statistically significantly different from the samples of lavender variant 14/6 (2.97%). The lowest content of the essential oil component α-terpineol was found in the samples of the lavender variant 14/4 (1.65%), but these samples were not statistically significantly different from from the variant 14/5 (1.81%). Prusinowska, Smigielski (2015) found higher percentage of α-terpineol, 4.1–8.5%.

The statistically lowest content of the essential oil component linalyl acetate was found in the samples of lavender variant 14/1 (11.64%); these samples were not statistically significantly different from the variant 14/2 (12.30%).

CONCLUSION

The total essential oil content was investigated in lavender flowers. The values varied in the range of 2.10–2.83 ml.100 g⁻¹ in the investigated variants. These values are in good accordance with the standards for pharmaceutical use given by the Czech Pharmacopoeia. The differences were found in the essential oil composition, mostly in the samples of the variant 14/6; it was a sample obtained from the area with lower total rainfall. The hypothesis that different essential oil composition could be caused by the locality with lower total rainfall will have to be verified in further years of observation.
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REFERENCES