THE VARIABILITY OF CONTAINED COMPOUNDS IN SELECTED MILK THISTLE [SILYBUM MARIANUM L. (GAERTN.)] VARIETIES CULTIVATED IN 2010–2015

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Abstract: The aim of this work was to demonstrate the variability of silymarin complex isolated from milk thistle [Silybum marianum (L.) Gaertn.] achenes. The silymarin complex was determined in 63 different samples of two home-grown varieties (Silyb and Mirel) originating from various environmental conditions and different years of harvest. The samples were analysed by the means of a reference method using HPLC (high performance liquid chromatography). The obtained results indicate that the year of harvest had statistically significant influence to both the amount and the composition of silymarin complex in the samples of the Mirel variety. The situation was similar for the samples of the Silyb variety, except of the isosilybin B component.

Key Words: silymarin, quality, flavonolignans, milk thistle

INTRODUCTION
The silymarin complex is a group of flavonolignan compounds contained in the pericarp and seed of milk thistle [Silybum marianum (L.) Gaertn.] achenes. It contains ca. 70–80% of silymarin flavonolignans and ca. 20–30% of chemically non-specified compounds, mostly polymeric and oxidised polyphenolic compounds (Gažák et al. 2007, Andrzejewska et al. 2011). According to the Pharmacopea Bohemica (2009) the official drug is a ripe fruit without pappus that have a minimal silymarin content of 1.5%, expressed as silybinin (C25H22O10; M, 482.4). The achenes usually contain 1–3% of silymarin in the dry weight, but the silymarin content can be also more than 8% (Karkanis et al. 2011). The base components of the silymarin complex are silybin A, silybin B, isosilybin A, isosilybin B, silychristin, silydianin and the flavonoid taxifolin (AbouZid 2012). According to Šeršeň et al. (2006) the silymarin complex has following distribution of individual components: 36.3% silybin, 15.9% silychristin, 5.9% silydianin, 5.1% isosilybin and 1.9% taxifolin. According to Nasrabadi et al. (2014) whole plant can be used for medicinal purposes (for phytopharmacy), but it is the achenes that have the highest content of active compounds.

Milk thistle has been known as a medicinal plant since the ancient times; 2000 years ago it was used as a treatment for liver dysfunctions. Nowadays it is a part of preparations for the treatment of diseases and disorders of liver, gallbladder and spleen, including in particular viral hepatitis (B, C), cirrhosis, hepatitis, gall colic. It also serves as protection against damage caused by chemical toxins and environmental pollutants like snake bites, insect bites, swallowed poisonous mushrooms and especially against liver damage caused by alcohol (Gažák et al. 2007, Kroll et al. 2007, Cardile et al. 2013). The basic effect of silybin on the human body is its ability to destroy free radicals and stabilize the cell walls, i.e. the cytoprotective effect, but it has also chemoprotective effects which inhibit carcinogenic effects of many chemicals. Silymarin complex has anti-allergic and anti-inflammatory effects, lowers the concentration of cholesterol in blood, it has anticancer effect, especially in the case of prostate cancer, supports and protects the liver during the treatment of HIV, helps against stress-induced gastric ulcers etc. (Flora et al. 1998, Šeršeň et al. 2006, Gažák et al. 2007, Stancheva et al. 2008, Cacho et al. 2013).
MATERIAL AND METHODS

Plant material sources

In the framework of this research, 63 samples of milk thistle achenes of two varieties – Silyb and Mirel – cultivated in the Czech Republic were investigated. The samples were grown in different provenances and years of harvest and were provided by growers and research organizations. Samples of the Mirel variety come from the harvest years 2011 and 2013–2015 and total 27 samples were evaluated. Samples of the Silyb variety come from the harvest years 2010–2014 and the evaluation of 36 samples was performed and included to the paper.

The silymarin complex and its basic components (silychristin, silydianin, silybin A, silybin B, isosilybin A, isosilybin B) were determined by the reference method.

The reference method for the determination of contained compound

For the sample processing, 20 mg of ground seeds was weighted and homogenised by the means of mortar and pestle with the addition of 0.5 ml isooctane and 0.5 ml methanol. 1.5 ml methanol and 1 ml isooctane were added to the sample after the homogenisation and 2 ml aliquot was centrifuged for 5 minutes at 14 000 rpm. 0.75 ml of lower phase was taken from each sample and analysed by the means of HPLC.

The analysis was performed by the means of the liquid chromatograph Dionex Ultimate 3000. The silymarin complex was separated using the Hypersil GOLD column 150 × 4.6 mm at 30 °C. 5 μl of the sample was injected to the column. The analysis was performed at the mobile phase flow rate 1 ml/min and the UV detection at 288 nm. The analytes were eluated at isocratic conditions with 65% of mobile phase A (0.1% formic acid) and 35% of mobile phase B (100% methanol). Time of one analysis was 45 minutes.

RESULTS AND DISCUSSION

The silymarin complex in milk thistle variety Mirel

27 samples of achenes of milk thistle variety Mirel of different provenances from the years of harvest 2011 and 2013–2015 were investigated.

Table 1 Variance analysis for the contained compounds of milk thistle variety Mirel in monitored harvest years 2011 and 2013–2015

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>d.f.</th>
<th>Silychristin</th>
<th>Silydianin</th>
<th>Silybin A</th>
<th>Silybin B</th>
<th>Isosilybin A</th>
<th>Isosilybin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>3</td>
<td>8.182***</td>
<td>0.7367***</td>
<td>5.368***</td>
<td>15.23***</td>
<td>0.5286***</td>
<td>0.089***</td>
</tr>
<tr>
<td>Error</td>
<td>80</td>
<td>0.696</td>
<td>0.1458</td>
<td>0.499</td>
<td>1.26</td>
<td>0.0684</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Note: * - p ≤ 0.05; ** - p ≤ 0.01; *** - p ≤ 0.001; [g/kg]

The analysis of variance in Table 1 shows a statistically very highly significant effect of the harvest year on the contents of all monitored components of the silymarin complex in the Mirel variety samples.

Table 2 The variability of contained compounds of milk thistle variety Mirel (g/kg) in monitored harvest years 2011 and 2013–2015

<table>
<thead>
<tr>
<th>Year</th>
<th>Silychristin</th>
<th>Silydianin</th>
<th>Silybin A</th>
<th>Silybin B</th>
<th>Isosilybin A</th>
<th>Isosilybin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>9.05 a</td>
<td>3.23 ab</td>
<td>8.20 a</td>
<td>12.19 a</td>
<td>2.79 a</td>
<td>0.65 a</td>
</tr>
<tr>
<td>2013</td>
<td>10.45 b</td>
<td>3.39 b</td>
<td>9.45 b</td>
<td>14.21 b</td>
<td>3.20 b</td>
<td>0.71 b</td>
</tr>
<tr>
<td>2014</td>
<td>10.25 b</td>
<td>2.98 a</td>
<td>9.52 b</td>
<td>14.10 b</td>
<td>3.06 b</td>
<td>0.70 ab</td>
</tr>
<tr>
<td>2015</td>
<td>10.78 b</td>
<td>3.08 a</td>
<td>9.39 b</td>
<td>14.47 b</td>
<td>3.15 b</td>
<td>0.81 c</td>
</tr>
</tbody>
</table>

Note: Average values marked with different letters in columns vary on a statistically significant level at p=0.05; [g/kg]

The samples of milk thistle variety Mirel were monitored in harvest years 2011 and 2013–2015. The lowest content of the flavonolignan silychristin that is a part of silymarin complex was found out in the harvest year 2011 (9.05 g/kg). The average content of silychristin in the year 2011 was statistically
significantly different from the samples investigated in harvest years 2013–2015 (10.45 g/kg, 10.25 g/kg and 10.78 g/kg, resp.). The lowest content of the silydianin component was found in the samples from the harvest year 2014 (2.98 g/kg), but the values were not statistically significantly different from the samples from the harvest year 2015 (3.39 g/kg). In the harvest year 2011 the lowest content of not only the silychristin component, but also of silybin A (8.20 g/kg), silybin B (12.19 g/kg), isosilybin A (2.79 g/kg) and isosilybin B (0.65 g/kg) was found out in the comparison with other monitored years of harvest. The highest content of silybin A was found in the harvest year 2014 (9.52 g/kg), but the values from these samples were not statistically significantly different from the samples taken in the harvest year 2013 (9.45 g/kg) and 2015 (9.39 g/kg). The content of silybin B, another component of the silymarin complex, was found higher in the years 2013–2015, in the range of 14.10–14.47 g/kg in comparison with above-given harvest year 2011. The highest content of isosilybin A was found in the harvest year 2013 (3.20 g/kg).

The silymarin complex in milk thistle variety Silyb

The samples of the Silyb variety were monitored in the harvest years 2010–2014. 36 samples of milk thistle achenes of the variety Silyb were used for the analyse.

Table 3 Variance analysis for the contained compounds of milk thistle variety Silyb in monitored harvest years 2010–2014

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>Silychristin</th>
<th>Silydianin</th>
<th>Silybin A</th>
<th>Silybin B</th>
<th>Isosilybin A</th>
<th>Isosilybin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>4</td>
<td>8.339***</td>
<td>1.888***</td>
<td>14.064***</td>
<td>14.250***</td>
<td>1.3354***</td>
</tr>
<tr>
<td>Error</td>
<td>113</td>
<td>0.487</td>
<td>0.0673</td>
<td>0.449</td>
<td>0.892</td>
<td>0.0586</td>
</tr>
</tbody>
</table>

Note: * - p ≤ 0.05; ** - p ≤ 0.01; *** - p ≤ 0.001; [g/kg]

As shown in the Table 3, the components of the silymarin complex in monitored samples of the Silyb variety were very highly significantly influenced by the year of harvest, except of the isosilybin B that was only influenced.

Table 4 The variability of contained compounds of milk thistle variety Silyb in monitored harvest years 2010–2014

<table>
<thead>
<tr>
<th>Year</th>
<th>Silychristin</th>
<th>Silydianin</th>
<th>Silybin A</th>
<th>Silybin B</th>
<th>Isosilybin A</th>
<th>Isosilybin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>9.51 a</td>
<td>2.59 a</td>
<td>8.32 a</td>
<td>12.87 a</td>
<td>2.80 a</td>
<td>0.55 a</td>
</tr>
<tr>
<td>2011</td>
<td>10.34 b</td>
<td>2.91 b</td>
<td>9.13 b</td>
<td>13.82 b</td>
<td>3.08 b</td>
<td>0.82 b</td>
</tr>
<tr>
<td>2012</td>
<td>10.21 abc</td>
<td>2.74 abcd</td>
<td>8.89 abc</td>
<td>14.00 abc</td>
<td>2.97 ab</td>
<td>0.85 ab</td>
</tr>
<tr>
<td>2013</td>
<td>10.88 c</td>
<td>3.06 d</td>
<td>10.02 d</td>
<td>14.67 c</td>
<td>3.34 c</td>
<td>0.66 ab</td>
</tr>
<tr>
<td>2014</td>
<td>10.10 b</td>
<td>2.42 c</td>
<td>9.84 cd</td>
<td>14.08 b</td>
<td>3.02 b</td>
<td>0.70 ab</td>
</tr>
</tbody>
</table>

Note: Average values marked with different letters in columns vary on a statistically significant level at p=0.05; [g/kg]

The content of silychristin found in the samples of the Silyb variety investigated in the years 2010–2014 varied in the range of 9.51–10.88 g/kg (see Table 4). The contents of silydianin, silybin A, silybin B and isosilybin A components were found to be statistically significantly highest in the year 2013 (3.06 g/kg, 10.02 g/kg, 14.67 g/kg and 3.34 g/kg, resp.). Thus, the obtained results indicate that the harvest year 2013 was the most favourable for investigated samples of the Silyb variety. On the other hand, the lowest values were found for the harvest year 2010.

The silymarin complex of milk thistle varieties Mirel and Silyb in comparable harvest years 2011, 2013 and 2014

Milk thistle samples of selected varieties Mirel and Silyb could be compared in harvest years 2011, 2013 and 2014. As shown in Table 5, the components silychristin, silydianin and silybin A+B were very highly significantly influenced both by the variety and by the year of harvest, but only silychristin and silybin B were very highly significantly influenced by the mutual interaction of both investigated factors.
Table 5 Variance analysis for the contained compounds of selected milk thistle varieties (Mirel and Silyb) in monitored harvest years 2011, 2013 and 2014

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>d.f.</th>
<th>Silychristin</th>
<th>Silydianin</th>
<th>Silybin A</th>
<th>Silybin B</th>
<th>Isosilybin A</th>
<th>Isosilybin B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>1</td>
<td>8.20***</td>
<td>5.136***</td>
<td>11.23***</td>
<td>14.02***</td>
<td>0.536**</td>
<td>0.047</td>
</tr>
<tr>
<td>Variety</td>
<td>2</td>
<td>10.99***</td>
<td>3.263***</td>
<td>13.63***</td>
<td>23.15***</td>
<td>1.503***</td>
<td>0.031</td>
</tr>
<tr>
<td>Variety * Year</td>
<td>2</td>
<td>4.45***</td>
<td>0.230</td>
<td>0.83</td>
<td>6.23***</td>
<td>0.224*</td>
<td>0.136</td>
</tr>
<tr>
<td>Error</td>
<td>146</td>
<td>0.51</td>
<td>0.109</td>
<td>0.43</td>
<td>0.94</td>
<td>0.057</td>
<td>0.078</td>
</tr>
</tbody>
</table>

Note: * - p ≤ 0.05; ** - p ≤ 0.01; *** - p ≤ 0.001; [g/kg]

Table 6 The variability of contained compounds of selected milk thistle varieties (Mirel and Silyb) in monitored harvest years 2011, 2013 and 2014

<table>
<thead>
<tr>
<th>Variety</th>
<th>Year</th>
<th>Silychristin</th>
<th>Silydianin</th>
<th>Silybin A</th>
<th>Silybin B</th>
<th>Isosilybin A</th>
<th>Isosilybin B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2011</td>
<td>10.34 b</td>
<td>2.91 b</td>
<td>9.13 b</td>
<td>13.82 b</td>
<td>3.08 bc</td>
<td>0.82 b</td>
</tr>
<tr>
<td></td>
<td>2013</td>
<td>10.88 c</td>
<td>3.06 bc</td>
<td>10.02 d</td>
<td>14.67 c</td>
<td>3.34 d</td>
<td>0.66 ab</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>10.10 b</td>
<td>2.42 a</td>
<td>9.84 cd</td>
<td>14.08 b</td>
<td>3.02 b</td>
<td>0.70 ab</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>10.44</td>
<td>2.80</td>
<td>9.66</td>
<td>14.19</td>
<td>3.15</td>
<td>0.72</td>
</tr>
</tbody>
</table>

|         | 2011 | 9.05 a       | 3.23 cd    | 8.20 a    | 12.19 a   | 2.79 a      | 0.65 a      |
|         | 2013 | 10.45 b      | 3.39 d     | 9.45 b    | 14.21 b   | 3.20 c      | 0.71 ab     |
|         | 2014 | 10.27 b      | 3.00 bc    | 9.54 bc   | 14.14 bc  | 3.06 b      | 0.70 ab     |
| Average |      | 9.93         | 3.21       | 9.06      | 13.51     | 3.02        | 0.69        |

Note: Average values marked with different letters in columns vary on a statistically significant level at p=0.05; [g/kg]

The comparison of Silyb and Mirel varieties in the Table 6 shows that the most represented component of the silymarin complex was silybin B with the content in the range of 12.19–14.67 g/kg. Both varieties gave the lowest content of silybin B in the harvest year 2011. The second most represented component was silychristin that was in average found more in the samples of the Silyb variety. The Mirel variety on the other hand showed higher content of the silydianin component.

The average content of individual components of the silymarin complex in the investigated samples is in good accordance with the results of Andrzejewska et al. (2011) that states the content of silychristin to be in the range of 0.58–0.88%, silydianin of 0.27–0.37%, silybinin A+B of 0.6–0.97% and isosilybin A+B of 0.21–0.28%. Thus, it is obvious that the monitored samples of Silyb and Mirel varieties cultivated and filed for legal protection in the Czech Republic are comparable with foreign varieties and in some harvest years can give even higher than average values of the silymarin complex.

Stancheva et al. (2008) states the content of silydianin+silychristin to vary in the range of 0.74–0.95% and silybin+isosilybinin in the range of 1.09–1.45%.

Results obtained in this work give the average content of silydianin+silychristin in the range of 1.23–1.39% and that of silybin+isosilybinin in the range of 2.38–2.87%. Thus, the content of silydianin+silychristin is comparable. The difference is evident in the content of silydianin+silychristin where higher values were found in the monitored samples of the Silyb and Mirel varieties; it could be caused by the oiliness of the achenes.

CONCLUSION

The silymarin complex was investigated in two different milk thistle varieties (Mirel and Silyb), which were grown in different provenances and in the harvest years 2010–2015. The average content of individual components of the silymarin complex in the studied samples was very highly statistically significantly affected by the cultivation year. Most represented ingredient of the silymarin complex was silybin B, its content in compared varieties ranged from 12.19 to 14.67 g/kg.
These results were compared with data published by several other authors. The comparison of the results shows that in some cases the silybin content was higher in monitored varieties. From that it can be concluded that the investigated varieties (Mirel, Silyb) are a high level one in terms of the quality of silymarin complex.

Analyses by HPLC reference method are quite time-consuming and costly. Therefore, it would be a benefit to verify and expand the possibilities of using the FT-NIR spectroscopy calibration model for rapid, non-destructive detection of silymarin complex as well as other important substances in milk thistle (oiliness, vitamin E, oleic acid and linoleic acid, etc.).

ACKNOWLEDGEMENTS
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REFERENCES