At present, the use of complex liquid herbal preparations has been increasing. Taking into consideration the needs of Lithuanian consumers, we chose to produce a new sedative, indigestion-relieving complex phytopreparation. For the preparation of the multi-component herbal extract, we selected herbal raw material (ginger rhizomes, cinnamon bark, rosemary leaves, and lavender flowers) with sedative and indigestion-relieving effects of its biologically active substances. During the selection of the raw material, attempts were made to achieve that the biologically active substances in the composition of herbal raw materials, i.e. essential oils (borneol, bisabolene, cineol, camphor, linalool, terpineol, eugenol, cinnamaldehyde, gingerol, shogaol, geraniol) and flavonoids (hyperforin, quercitin, isoquercitin, rutin), would be targeted at particular symptoms and would complement each other’s effect. Four herbal raw materials with positive effects of their active substances on digestive tract disorders were selected. According to literature, the main active substances of the component raw materials, i.e. ginger rhizomes, rosemary leaves, cinnamon bark, and lavender flowers are essential oils (1-8). All these raw materials can be used for the treatment and prophylaxis of digestion disorders.

The herbal raw material of St. John’s wort (Hyperici herba) with sedative and anti-depressive action of its biologically active substances was selected. It is one of the most popular herbs in Lithuania, which is attributed to the traditional group of medicinal plants. St. John’s wort accumulates up to 13 per cent of enzymes, 0.1 – 1.25 per cent of essential oils, up to 8 per cent of flavonoids (rutin, quercetin, quercitrin, hyperoside) as well as antrachinones (hypericin and its derivatives), hyperforin, carotinoids, and resins (2, 9, 10). Flavonoids, i.e. quercetin and quercitrin are distinguished by their sedative and antidepressive effects of monoamine oxydase A type. Xantones also act as monoamine oxidase A inhibitors: they inhibit anxiety. Biflavonoids possess great affinity to benzodiazepine receptors. They relieve anxiety and stimulate sleep (11-15). R. F. Weiss recommended St. John’s wort ‘for the treatment of somatogenic and psychogenic forms of depression’ (16). In the Republic of Lithuania, the following indications of St. John’s wort are approved: the complementary

* Correspondence: Kristina Ramanauskiené, tel +370 37 327255, +370 601 02567, e-mail povvis@gmail.com
remedy for the treatment of neurotic depressions, sleep disorders, and anxiety; the anti-inflammatory and wound-healing remedy (17). For the study of herbal extracts, the same methods of analysis as those for chemical drugs are applied, and their results are evaluated according to analogous criteria. High pressure liquid chromatography (HPLC) was selected as a reliable method of analysis. This method allows for a qualitative and quantitative analysis of components of herbal extracts (18-20). It seems relevant to analyze the chemical composition of the prepared extracts and to prove that St. John’s wort is a major source of flavonoids.

The aim of the study was to identify the amounts of rutin, hyperoside, quercetin and other flavonoids in a multi-component extract using the method of high pressure liquid chromatography, by estimating the correlation among the amounts of flavonoids in the extract.

**MATERIALS AND METHODS**

The object of the analysis is a multi-component herbal extract containing: herbs of St. John’s wort, ginger rhizomes, cinnamon bark, rosemary leaves, lavender flowers, and 70 % ethanol (Table 1).

For the qualitative and quantitative identification of flavonoids in the extracts of the crude drug, control extracts were prepared, with the ratio of crude drug and the vehicle matching the ratio of

<table>
<thead>
<tr>
<th>Active compounds, µg/mL</th>
<th>Extract of St. John’s wort</th>
<th>Extract of ginger rhizomes</th>
<th>Extract of cinnamon bark</th>
<th>Extract of lavender flowers</th>
<th>Extract of rosemary leaves</th>
<th>Extract of the mixture of herbal raw materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>700.04</td>
<td>1.11</td>
<td></td>
<td></td>
<td></td>
<td>568.1</td>
</tr>
<tr>
<td>Hyperoside</td>
<td>477.82</td>
<td>39.9</td>
<td>11.42</td>
<td>664.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rutin</td>
<td>531.49</td>
<td>11.68</td>
<td></td>
<td></td>
<td></td>
<td>658.22</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>169.58</td>
<td>1.96</td>
<td></td>
<td></td>
<td></td>
<td>171.86</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astragalain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keampferol</td>
<td></td>
<td>0.62</td>
<td>84.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercitrin</td>
<td></td>
<td>1.90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apigenin-7-glycosyde</td>
<td></td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitexin rhamnoside</td>
<td></td>
<td></td>
<td>19.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vicenin</td>
<td></td>
<td>20.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orientin</td>
<td></td>
<td>0.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The composition of herbal extracts.

<table>
<thead>
<tr>
<th>Composition of a multi-component extract</th>
<th>Ratio of crude drug and ethanol in a multi-component extract</th>
<th>Ratio of crude drug and ethanol in control extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. John’s wort herb (4 parts), Ginger rhizome (3 parts), Cinnamon bark (1 part), Rosemary leaves (1 part), Lavender flowers (1 part)</td>
<td>1. St. John’s wort herb 1 part, ethanol 12.5 parts 2. Ginger rhizome 1 part, ethanol 16.7</td>
<td>1. St. John’s wort herb extract, ratio 1:12.5 2. Ginger rhizome extract, ratio 1:16.7</td>
</tr>
<tr>
<td>1 part of crude drug mixture requires 5 parts of ethanol</td>
<td>3. Cinnamon bark 1 part, ethanol 50 parts 4. Rosemary leaves 1 part, ethanol 50 parts</td>
<td>3. Cinnamon bark extract, ratio 1:50 4. Rosemary leaves extract, ratio 1:50</td>
</tr>
<tr>
<td></td>
<td>5. Lavender flowers 1 part, ethanol 50 parts</td>
<td>5. Lavender flowers extract, ratio 1:50</td>
</tr>
</tbody>
</table>
The qualitative analysis of ethanol extracts of herbal raw... 329

the multi-component extract (Table 1). The method of the preparation was percolation (21-23). The solvent of the crude herbal materials is 70% ethanol. Identification of flavonoids was performed using high pressure liquid chromatography; the equipment used was a chromatograph Waters 2690 with the spectrophotometric detector Waters 2487; chromatographic column „X-Terra” RP18, 3.5 µm, column dimensions 3.0 × 150 mm; the volume of injected sample was 10 µl. Elution is gradient, i.e. the composition of the gradient changes with time. Eluent A: acetonitrile and trifluoroacetic acid (99.9:0.1, v/v). Eluent B: distilled water and trifluoroacetic acid (99.9:0.1, v/v). Gradient shift: from 0 min – 5% of eluent A and 95% of eluent B; from 45.0 min – 45% of eluent A and 55% of eluent B; from 60.0 min – 5% of eluent A and 95% of eluent B. The eluent flow rate was 0.4 mL/min. Preparation of the tested solution: the extract was filtered through a membrane filter with pore diameter of 5 µm and diluted with 70% vol. ethanol solution, ratio 1 : 1. Flavonoids were identified at 360 nm wavelength. The compounds were identified and their amounts in extract calculated according to the length of retention of the extracted components, peak areas, and ratios between individual peak areas and those of standard peaks.

Figure 1. The chromatogram of flavonoid identification by HPLC in the extract of the multi-component herbal mixture. 1 – chlorogenic acid; 2 – caffeic acid; 3 – vicenin; 4 – orientin; 5 – vitexin-4-rhamnoside; 6 – vitexin; 7 – isovitexin; 8 – rutin; 9 – hyperoside; 10 – apigenin-7-glucoside; 11 –astragalin; 12 – quercitrin; 13 – luteolin; 14 – quercetin; 15 – apigenin; 16 – kaempferol.

Figure 2. The chromatogram of flavonoid identification by HPLC in the control extraction of St. John’s wort. 1 – chlorogenic acid; 2 – caffeic acid; 3 – vicenin; 4 – orientin; 5 – vitexin-4-rhamnoside; 6 – vitexin; 7 – isovitexin; 8 – rutin; 9 – hyperoside; 10 – apigenin-7-glucoside; 11 –astragalin; 12 – quercitrin; 13 – luteolin; 14 – quercetin; 15 – apigenin; 16 – kaempferol.
RESULTS

In the prepared multi-component extract (MCE), the largest amounts of the following flavonoids were identified: hyperoside, quercetin, rutin; chlorogenic acid was also identified (Table 2). Figure 1 shows a chromatogram in which other active substances in addition to the above-mentioned flavonoids in the multi-component herbal extract were found. In the control extract of St. John’s wort (SJWCE), similar flavonoids were identified and their amounts measured (Table 2). Furthermore, not only flavonoids but also two phenolic acids — caffeic acid and chlorogenic acid — were identified in SJWCE (Fig. 2). The amount of chlorogenic acid was measured (Table 2). In the control extract of ginger (GCE), phenolic acids, i.e. caffeic acid and chlorogenic acid were identified and their amounts measured (Table 2). The results of the analysis have shown a small amount of flavonoids in ginger rhizome extract, which is congruent with the data in the literature (1-3, 5, 11). Flavonoids were not detected in this extract (Fig. 3).

In the control extract of cinnamon bark (CCE), very
small amounts of the following substances were identified: quercetin, apigenin-7-glycoside, quercitrin, and kaempferol (Table 2). In the chromatogram (Fig. 4), hyperoside, rutin, luteolin, chlorogenic and caffeic acids were identified; however, their amounts were not significant. The largest amount of biologically active substances in crude cinnamon belongs to essential oils (1-3, 11), and the amount of the identified flavonoids is not pharmacologically relevant (Table 2). In the control extract of lavender flowers (LCE), hyperoside, rutin, luteolin, chlorogenic, and caffeic acids were identified; however, their amounts were not significant. In the control extract of rosemary leaves (RCE), the following compounds and their amounts were identified: caffeic acid, isovitexin, luteolin (Fig. 6). A big part of the area under peaks belongs to some unidentified compounds released during 31 and 34-36 min., and the length of their release significantly differs from the release of the standards from the column (Fig. 6).

The study showed that the highest amount of flavonoids (hyperoside, rutin, quercetin) and chlorogenic acid was released from the control extract of St. John’s wort (Fig. 7). Figure 7 shows that the control extract of St. John’s wort yields the largest amount of quercetin and the smallest amount of chlorogenic acid. The identified amounts of flavonoids in the control extract of rosemary leaves (RCE), the following compounds and their amounts were identified: caffeic acid, isovitexin, luteolin (Fig. 6). A big part of the area under peaks belongs to some unidentified compounds released during 31 and 34-36 min., and the length of their release significantly differs from the release of the standards from the column (Fig. 6).
mon and rosemary are relatively small if compared to the control extract of St. John’s wort (Fig. 7). Therefore, it can be stated that the largest amount of flavonoids in the multi-component extract is extracted from crude herbs of St. John’s wort. This coincides with the data in the literature, in particular that the main flavonoids in the herbs of St. John’s wort are hyperoside, rutin and quercetin (2, 9, 10). The comparison of the findings in control extract and in the extract of the mixture of the herbal raw materials showed that the amount of flavonoids differed insignificantly, which supports our assumption that other components of the herbal mixture have no influence on the yield of flavonoids. The method of high pressure liquid chromatography has confirmed the compatibility of biologically active substances that were extracted from the mixtures of herbal raw materials.

CONCLUSION

Using the method of high pressure liquid chromatography it was found that the largest amount of flavonoids is extracted from crude herbs of St. John’s wort; therefore, the amount of flavonoids in a multi-component extract differs insignificantly from the flavonoid amount in the extract of St. John’s wort.

REFERENCES


Received: 5.01.2007