IDENTIFICATION AND DETERMINATION OF HYPERICINE IN THE EXTRACTS OF THE AMBER – HYPERICUM PERFORATUM L. SSP. ANGSTIFOLIUM

The extraction of plant species Hypericum perforatum L.ssp. angustifolium (amber) was carried out by the following methods: maceration, ultrasonic maceration and extraction with CO2 under high pressure. The highest yields of dry extract were achieved by the ultrasonic maceration method (23.3%) and the conventional maceration method (22.9%). Freytag's HPLC method was used to determine the hypericin content in methanol ultrasonic macerate (0.03%) and in methanol macerate (0.036%). In the extracts obtained by extraction by CO2 under high pressure no traces of hypericin were found. The results obtained indicate that the most convenient method for the extraction of hypericin is ultrasonic maceration with methanol as the extraction agent.

![Figure 1. The naphthodiantrones in the Hypericum perforatum L.](image)

Plant species of the Hypericum genus have been known for a long time as medicinal ones, and a special place among them belongs to Hypericum perforatum L.species (amber). The most recent investigations show that the area of application of amber is extremely wide. Besides traditional use as an antiseptic and wound healing agent, it is increasingly applied as an antidepressant [1] and virucidal drug, and even for the treatment of diseases such as AIDS [2]. Until now it has not been established which of amber’s ingredients has the most important curative power, but most frequently hypericin is cited as the bearer of curative properties.

Hypericin (hypericinum mycoporphyrin, cyclo Soros) is a derivative of naphthodiantrone, with the molecular formula C20H10O2 and the molecular weight 304.43. The violet crystals of the hypericin are red in alkaline aqueous solution at pH values lower than 11.5, while at pH values over 11.5 they are green with red fluorescence.

Besides hypericin in Hypericum perforatum L., there are a few photodynamically important substances from the group of naphthodiantrone characterized by red fluorescence. The most common are pseudo-hypericin, protohypericin and protopseudohypericin (Figure 1) [3].

The first paper in which hypericin is mentioned under the name of hypericinum was published by Buchner in 1830 [4]. The greatest contribution to the determination of the structural formulae of hypericin and its anthraquinonic derivatives was made by Backman [5-9]. The first methods for the identification and determination of hypericin in plant extracts, as well as the HPLC separation of hypericin from pseudohypericin was published by Freytag in 1964 [3]. The photometric determination of hypericin according to the Deutscher Arzneimittel Codex 1979, is carried out without separating hypericin from pseudohypericin and that is why significant errors are being made.

The aim of this paper was to investigate, by the HPLC method, the presence and the content of hypericin in different extracts of Hypericum perforatum L.ssp. angustifolium picked from the until now unknown locality of Sobina, on the mountain of Kristilovica (the surroundings of Vranje, South Serbia) in order to gain a better insight into its pharmacological value.

Freytag's HPLC method was used for chromatography of the extracts obtained by extraction: methanol maceration, ultrasonic methanol maceration, and with CO2 under high pressure.

EXPERIMENTAL CONDITIONS

To carry out the investigation, amber Hypericum perforatum L.ssp. angustifolium picked on June 19th, 1996 on the locality of Sobina, on the slopes of the mountain of Kristilovica (South Serbia, Yugoslavia) was used. The plant material was dried at room temperature to a moisture content below 11%, then ground by an electric grinder and screened through a 1 mm mesh sieve.
Extraction by the maceration method (extract No. 1) was carried out with a drug to solvent ratio of 1:10, for a period of 24 hours.

For the ultrasonic maceration method (extract No. 2) a "Sonic" ultrasonic apparatus of 150 W was used, also with a drug to solvent ratio of 1:10, for a period of 30 minutes.

The weight of the drug samples used for the maceration and ultrasonic maceration was 10 g each, and methanol p.a. was used as the extraction agent.

Extraction with CO₂ under high pressure was carried out on the apparatus HREP—High Pressure Extraction Plant, NOVA—Swiss, under the following conditions:

1. p = 100 bars, t = 40°C, t = 2 hours (extract No. 3)
2. p = 300 bars, t = 40°C, t = 2 hours (extract No. 4)

The weight of the tested samples of the drug was 50.0 g, the flow rate of CO₂ was 97.725 dm³/h (at 20°C) while the conditions in the separator were: p = 15 ± 1 bar and t = 22 ± 1°C.

The HPLC analysis of the samples was carried out under the following conditions [3]:
- the apparatus: Hewlett Packard 1100
- the column: Lichrosorb RP—18 (5 µm), (250 x 4 mm)
- the mobile phase: 317 g of methanol + 90 g of ethyl acetate + 57 g of 0.1 M NaH₂PO₄, pH = 2.1
- the flow rate: 0.8 cm³/min
- the detection wave length: 590 nm
- the injection volume: 20 µl
- the temperature: room
- the solvent: methanol

For the identification and determination of the hypericine, an internal standard of the Institute for Organic Chemistry in Göttingen (Laboratory of Prof. Dr. Hartmut Laatsch) was used.

RESULTS

The yields of the extracts of amber Hypericum perforatum L. ssp. angustifolium are shown in Table 1.

Table 1. Dry extract yields of amber Hypericum perforatum L. ssp. angustifolium

<table>
<thead>
<tr>
<th>Extract No</th>
<th>Yield (%)</th>
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<tbody>
<tr>
<td>1.</td>
<td>22.9</td>
</tr>
<tr>
<td>2.</td>
<td>23.3</td>
</tr>
<tr>
<td>3.</td>
<td>1.6</td>
</tr>
<tr>
<td>4.</td>
<td>2.8</td>
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</table>

It may be seen from the table that the highest yields of dry extracts were obtained by the methods of ultrasonic maceration and maceration, while considerably lower yields were obtained by extraction with CO₂ under high pressure. Comparing the yields for extracts Nos. 3 and 4, it may be concluded that when using the high pressure CO₂ extraction method higher yields were obtained when the pressure was increased. Figures 2 to 5 show HPLC chromatograms of the tested extracts.

The extracts obtained by maceration and ultrasonic maceration methods have hypercine contents of 0.038% and 0.039% respectively, while the extracts

Figure 2. The HPLC chromatogram of extract No 1

Figure 3. The HPLC chromatogram of extract No 2

Figure 4. The HPLC chromatogram of extract No 3
obtained by CO₂ under high pressure do not have a peak at the retention time of 8.2 minutes, which is characteristic for hypericins.

Ultrasonic maceration with methanol is recommended as the optimal method for the extraction of hypericins, since with much shorter extraction time a slightly higher yield is obtained as compared with conventional maceration.

CONCLUSIONS

1. For the extraction of amber Hypericum perforatum L. ssp. angustifolium three methods were used: extraction, ultrasonic extraction and extraction with CO₂ under high pressure. The highest yields of the dry extract were obtained using the ultrasonic maceration method (23.3%) and the maceration method (22.9%).

2. The HPLC method according to Freytag gave a content of hypericins of 0.039% in methanol ultrasonic macerate, and of 0.036% in methanol macerate. In the extracts obtained by CO₂ under high pressure no traces of hypericins were found.

3. With respect to the investigations carried out, the methanol ultrasonic maceration method can be recommended as the optimum method for the extraction of hypericins because of the considerably shorter testing time required and slightly higher yield than by the conventional maceration method.

REFERENCES


IZVOD

IDENTIFIKACIJA I ODREĐIVANJE HIPERICINA U EKSTRAKTIMA KANTARIONA HYPERICUM PERFORATUM L. SPP. ANGUSTIFOLIUM

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Ekstrakcija biljne vrste Hypericum perforatum L. ssp. angustifolium (kantarija) herba je vremena metoda: maceracije, ultrazujuće maceracije i ekstrakcije sa CO₂ pod visokim pritiskom. Najveći prihod svog ekstrakta su ostvareni primenom ultrazujuće maceracije (23,3%) i konvencitve maceracije (22,9%). HPLC metodom po Freytag–u je određen sadržaj hipericina u metanolnom ultrazujućem maceratu (0,039%) i metanolnom maceratu (0,036%). U ekstraktima obilježenim ekstrakcijom sa CO₂ pod visokim pritiskom nije utvrđeno prisustvo hipericina. Dobijeni rezultati ukazuju da je za ekstrakciju hipericina najpogodnija metoda ultrazujuće maceracije sa metanolom kao ekstragensom.

Ključne reči: hipericin • određivanje • ekstrakcija • kantarion •
Key words: hypericins • determination • amber • extraction •