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SCIENTIFIC PAPER

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

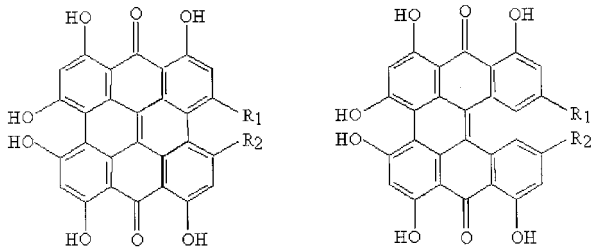
The extraction of plant species Hypericum perforatum L.ssp. angustifolium (amber) was carried out by the following methods: maceration, ultrasonic maceration and extraction with CO_2 under high pressure. The highest yields of dry extract were achieved by the ultrasonic maceration method (23.3%) and the conventional maceration method (22.9%). Freytag's HPLC method was used to determine the hypericine content in methanol ultrasonic macerate (0.039%) and in methanol macerate (0.036%). In the extracts obtained by extraction by CO_2 under high pressure no traces of hypericine were found. The results obtained indicate that the most convenient method for the extraction of hypericine is ultrasonic maceration with methanol as the extraction agent.

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 hypericine is cited as the bearer of curative properties.

Hypericine (hypericumrot mycoporphyrin, cyclosan) is a derivative of naphthodiantrone, with the molecular formula $C_{30}H_{16}O_8$ and the molecular weight 504.43. The violet crystals of the hypericine 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 hypericine 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-hypericine, protohypericine and protopseudohypericine (Figure 1) [3].

The first paper in which hypericine is mentioned under the name of hypercumrot was published by Buchner in 1830 [4]. The greatest contribution to the determination of the structural formulae of hypericine and its anthroquinonic derivatives was made by Brockman [5–9]. The first methods for the identification and determination of hypericine in plant extracts, as well as the HPLC separation of hypericine from pseudohypericine was published by Freytag in 1984 [3]. The photometric determination of hypericine according



	R_1	R_2
Hypericin	CH_3	CH_3
Pseudohypericin	CH_3	CH_2OH
Protohypericin	CH_3	CH_3
Protopseudohypericin	CH_3	CH_2OH

Figure 1. The naphthodiantrones in the Hypericum perforatum L.

to the Deutscher Arzneimittel Codex 1979. is carried out without separating hypericine from pseudohypericine 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 hypericine in different extracts of *Hypericum perforatum* L.*ssp. angustifolium* picked from the until now unknown locality of Sobina, on the mountain of Krstilovica (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 CO_2 under high pressure.

EXPERIMENTAL CONDITIONS

To carry out the investigation, amber *Hypericum perforatum* L.*ssp. angustifolium* picked on June 19th, 1998 on the locality of Sobina, on the slopes of the mountain of Krstilovica (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.

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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 CO_2 under high pressure was carried out on the apparatus HPEP–High Pressure Extraction Plant, NOVA–Swiss, under the following conditions.:

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

The weight of the tested samples of the drug was 50.0 g, the flow rate of CO_2 was 97.725 dm^3/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 NaH_2PO_4, pH = 2.1
- the flow rate: 0.8 cm^3/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

Extract No	Yield (%)
1.	22.9
2.	23.3
3.	1.6
4.	2.8

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 CO_2 under high pressure. Comparing the yields for extracts Nos. 3 and 4, it may be concluded that when using the high pressure CO_2 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 hypericine contents of 0.036% and 0.039% respectively, while the extracts

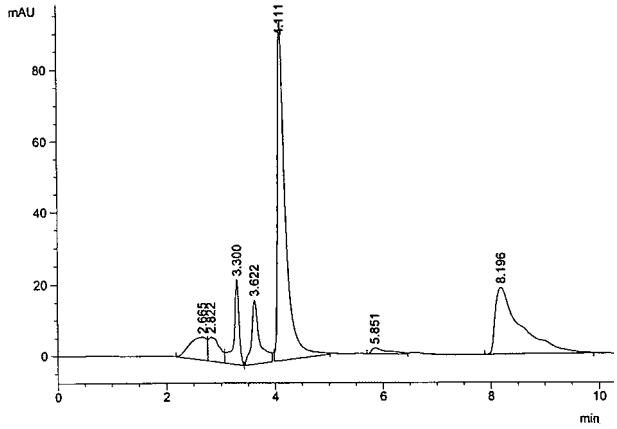

Figure 2. The HPLC chromatogram of extract No 1

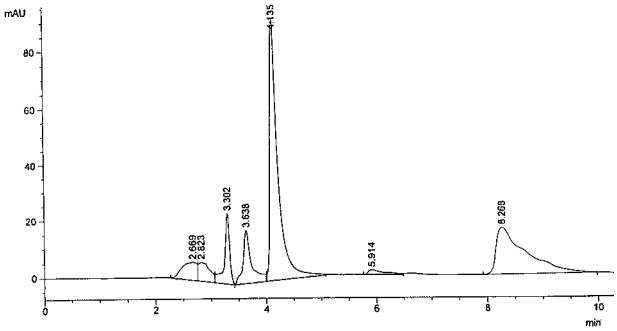

Figure 3. The HPLC chromatogram of extract No 2

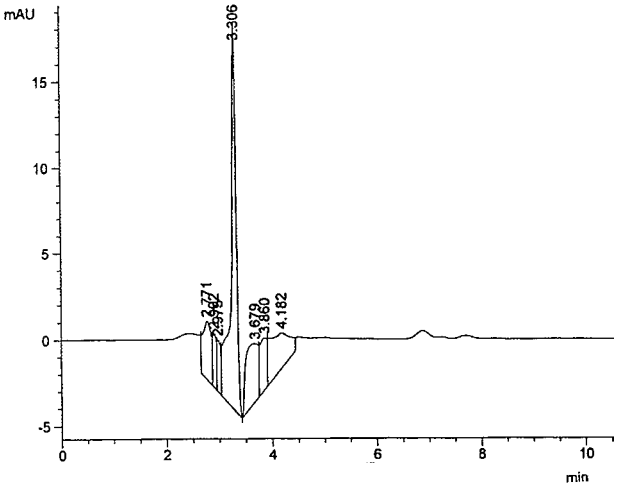

Figure 4. The HPLC chromatogram of extract No 3

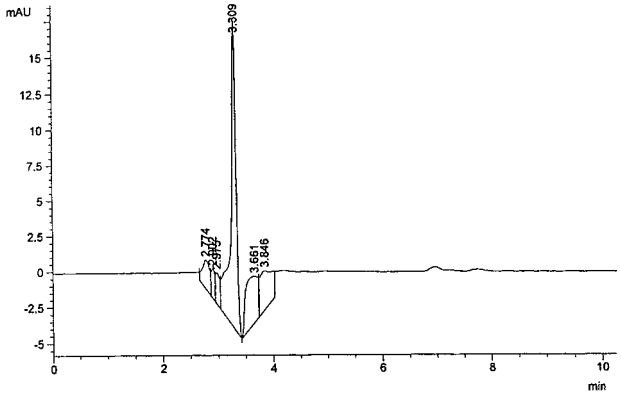

Figure 5. The HPLC chromatogram of extract No 4

obtained by CO_2 under high pressure do not have a peak at the retention time of 8.2 minutes, which is characteristic for hypericine.

Ultrasonic maceration with methanol is recommended as the optimal method for the extraction of hypericine, 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 CO_2 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 hypericine of 0.039 % in methanol ultrasonic macerate, and of 0.036 % in methanol macerate. In the extracts obtained by CO_2 under high pressure no traces of hypericine 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 hypericine because of the considerably shorter testing time required and slightly higher yield than by the conventional maceration method.

REFERENCES

[1] V. H. Muldner and M. Zoller, Antidepressieve Wirkung eines auf den Wirkstoffkomplex Hypericin standasierten Hypericum–extraktes, Aryneim.–Forsch. **34** (1984) 918

[2] D. Meruelo, G. Lavie and D. Lavie, Terapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: Aromatic polycyclic diopes hypericin and pseudohypericin, Proc. Ntl. Acad. Sci. **85** (1988) 5230–5234

[3] W. E. Freytag, Bestimmung von Hypericin und Nachweis von Pseudohypericin in hypericum perforatum L. durch HPLC, Deutsche Apothekerzeitung. **124** (46) (1984) 2383–2386

[4] A. Buchner, Buchners, s Report, Pharmacie. **34** (1830) 217

[5] H. Brockmann, M. N. Haschad et al., Über das Hyperizin, den photodynamisch wirksamen Farbstoff aus H. perforatum, Naturwissenschaften. **27** (1939) 50

[6] H. Brockmann and W. Sanne, Pseudohyperizin, ein neuer Hypeicum–Farbstoff, Naturwissenschaften. **40** (1953) 461

[7] H. Brockmann, Photodynamisch wirksame Pflancenfarbstoffe, Fortschrit der chem. org. Naturstoffe. **14** (1957) 141–177

[8] H. Brockmann and W. Sanne, Zur Kenntnis des Hypericins und Pseudohypericins, Chem. Berichte. **90** (1957) 2480–2491

[9] H. Brockmann and D. Spitcner, Die Konstitution des Pseudohypericins, Tetrahedron Letters. (1975) 37–40

IZVOD

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

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Ekstrakcija biljne vrste *Hypericum perforatum* L. *ssp. angustifolium* (kantarion) herba je vrena metodama: maceracije, ultrazvu*f*ne maceracije i ekstrakcije sa CO_2 pod visokim pritiskom. Najveći prinosi suvog ekstrakta su ostvareni primenom ultrazvučne maceracije (23,3 %) i konventivne maceracije (22,9 %). HPLC metodom po Freytag–u je određen sadržaj hipericina u metanolnom ultrazvučnom maceratu (0,039 %) i metanolnom maceratu (0,036 %). U ekstraktima dobijenim ekstrakcijom sa CO_2 pod visokim pritiskom nije utvrđeno prisustvo hipericina. Dobijeni rezultati ukazuju da je za ekstrakciju hipericina najpogodnija metoda ultrazvučne maceracije sa metanolom kao ekstragensom.

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