The analysis of the kinetics of extraction of resinoids and hypericines from the amber, *Hypericum perforatum* L.

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The kinetics of the extraction of the overall extracted materials (resinoids), total hypericine, hypericine and pseudohypericine from amber (*Hypericum perforatum* L.) was investigated by the procedure of maceration both with and without ultra-sound, using methanol as the extractant. It was found that the period of fast extraction with intensification of the extraction of resinoid by ultra-sound was significantly shorter (about 20 minutes) than was needed for the extraction without ultra-sound (about 5 h). Similar results were also obtained for the extraction of the other tested substances. It can be concluded that better drug exploitation can be achieved in a much shorter extraction time by intensification of the extraction using ultra-sound. By preparation of herbal material through pulverization, a significant grade of herbal tissue structure disintegration was achieved, so that turbulent mass transfer plays a dominant role in the extraction. The results show that the coefficient values of fast extraction (*b*) are approximately the same for all the investigated kinetics.

Keywords: *Hypericum perforatum* L., kinetics of extraction, resinoids, total hypericine, hypericine, pseudohypericine.

INTRODUCTION

Plant species of the *Hypericum* genus are used as traditional medical plants in various parts of the world. Among them, *Hypericum perforatum* L., occupres a special place and is the most intensively studied species.[1] The great interest in *H. perforatum* L. was provoked by investigations that proved that it could be used as a virucidal drug and, even more, for the treatment of such diseases as aids.[2] From a pharmacological point of view, the hypericins are at present the most interesting compounds of *H. perforatum* L. The information on the use of hypericine (Scheme 1) and pseudohypericine (Scheme 2) in the modern pharmaceutical industry and medicine is readily available.[2–7]
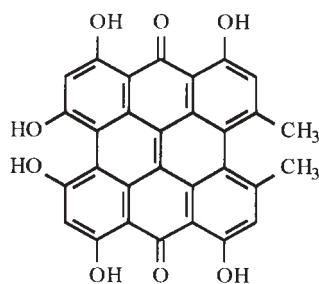
Scheme 1.
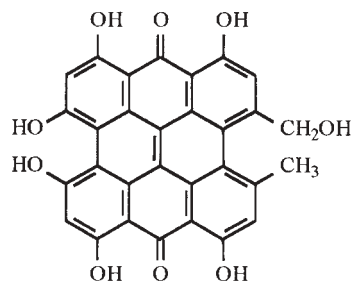
Scheme 2.

During extraction of the herbal material, prepared by pulverization, wetting must first occur, then dissolvability and the fast transfer of the substance mass from the destroyed cells (period of fast extraction), and then the transfer of mass from the cells of non-destroyed herbal tissue (period of mass transfer by molecular diffusion). The process of diffusion starts from the moment of drug wetting and permeation of the extracts within the cells. The quantity of extracted matter in the period of fast extraction designates basically the extraction operation rate, and is expressed through the coefficient of fast extraction, b.[8,9]

Although *H. perforatum* L. is an extremely interesting herb species for the modern pharmaceutical industry, not sufficient attention has been given to the question of the kinetics of the extraction of the bioactive components from *H. perforatum* L. The objective of this study was to analyze the kinetics of the extraction of resinoids, total hypericine, hypericine and pseudohypericine from *H. perforatum* L.

EXPERIMENTAL

Plant material

The investigation in this work was performed on amber *H. perforatum* L. *ssp. angustifolium* picked at the locality of Sobina (Vranje surroundings, South Serbia, Yugoslavia). The plant material was dried at room temperature. For drug pulverization an electric mill ($n = 1200$ min⁻¹, Ø strokes = 60 mm) was used for drug pulverization and the middle diameter of the particles (de) was determined by granulometric analysis (de) = 0.183×10^{-3} m).

The extraction obtained by process of maceration and intensification of the extraction by ultra-sound

The extraction of herbal material by the process of maceration was carried out with herbal material – dissolvent ratio (1:10, m/v). A Banderline electronic KG ultrasonic apparatus, 50/60 Hz, was employed for the intensification of the extraction. Methanol was used as a solvent in both cases.

Determination of the content of extract matter

Macererated plant material (10 g) was weighed into a 250 cm³ Erlenmeyer flask with a ground stopper and covered with 100 cm³ of the extraction agent. At the end of the maceration cycle, the extract was separated from the residual plant material by vacuum filtration. The solvent was evaporated under vacuum. The residue was dried in a vacuum oven at 50 ℃ until constant mass was attained.

Determination of the content of extracted matter in the starting drug, q_0

Macererated plant material (10 g) was weighed into a 250 cm³ Erlenmeyer flask with a ground stopper and covered with 100 cm³ of the extraction agent. The extraction was carried out by the ultrasonic maceration method for a period of 30 minutes. The already extracted drug was extracted two more time, by the same procedure, with fresh solvent. The value of q_0 was obtained by summation of the values of the dry residues.

The content of total hypericine in the extracts was determinated spectrophotometrically and by HPLC (as the sum of the content of hypericine and pseudohypericine).

Spectrophotometrically determination of total hypericine

Before the extraction of naphthodiantrone, the chlorophyll was removed with petrol ether. The herbal material (1 g) was extracted two times with petrol ether (2×10 min) in a ultra-sonic bath. The greenish petrol ether phase was rejected. The extraction of naphthodiantrone was carried out with 10 cm^3 of methanol and pyridine mixture (97 : 3) and left standing for 2 h. The absorbtion of sample of the final solution was measured at 590 nm, using a Perkin-Elmer Lambda 15 UV/VIS Spectrophotometer (UV WinLab Software). The calculation of the total hipericine was carried out using the formulation:[11]

$$c = \frac{E_{590} \; 1000 \; V}{E_{1cm}^{1\%} \; d \; 100}$$ (1)

Hypericine and pseudohypericine determination

The contents of hypericine and pseudohypericine in the extracts were determined by the HPLC method,[10] under the following conditions: apparatus: Knauer (two Knauer HPLC pumps 64; Shimadzu SPD-6A UV; spectrophotometric detector; Knauer HPLC software); column: Lichrospher 60 RP-select B (Merck), 5 μm; eluent: (A) methanol : ethyl acetate : 0.1 M sodium dihydrophosphate = 317 : 90 : 57 (m/m/m); (B) 0.1 M sodium dihydrophosphate; gradient: isocratic: (A) : (B) = 80 : 20; flow rate: 0.8 cm^3/min; sample volume: 20 μl; detection: 590 nm; retention time: $RT_{pseudohypericine} = 5.1$ min; $RT_{hypericine} = 13.3$ min.

The contents of hypericine and pseudohypericine were determined using calibration curves of the standard substances. There was a strong dependence between the peak surface area and concentration. Values of the correlation coefficient (*r*) for the calibration curves of hypericine and pseudohypericine were 0.999 and 1, respectively. Standards of the Institute for Organic Chemistry at Göttingen (The Laboratory of Prof. Dr. Hartmut Laatsch) were used.

Determination of the kinetic parameters of the extraction

The values of the parameters for fast extraction can be determined by investigation of the extraction kinetics. The quantity of extracted matter in the period of fast extraction basically defines the extraction rate and it is expressed through the coefficient of fast extraction, *b*. On the basis of experimental results and the equation:

$$\log \frac{q_i}{q_0} = \log (1 - b) - k\tau$$ (2)

which represents one of the solutions of the equation of non-stationary diffusion by Fika method of the least quadrant, the parameters *a* and *k*, as well as the coefficient of fast extraction, *b* ($b = 1–a$)[9] were calculated by analysis of dependence $\log q_i/q_0$ from τ.

RESULTS AND DISCUSSION

The results of the kinetic investigations of the extraction of extracted matter (resinoids) from amber by the application of the maceration procedure both with and without ultra-sound, are shown in Figs. 1 and 2. On the basis of the results of the kinetic investigations of the extraction system amber – methanol, it can be seen that the period of fast extraction was shorter when the extraction was intensified by ultra-sound (20 min) than during extraction without ultra-sound (5 h).

The dependencies of the yield (*Y*) of total hypericine, hypericine and pseudohypericine on the extraction time (τ) by the procedure of maceration both with and without the application of ultra-sound , are shown in Figs. 3 and 4. The content of total hypericine
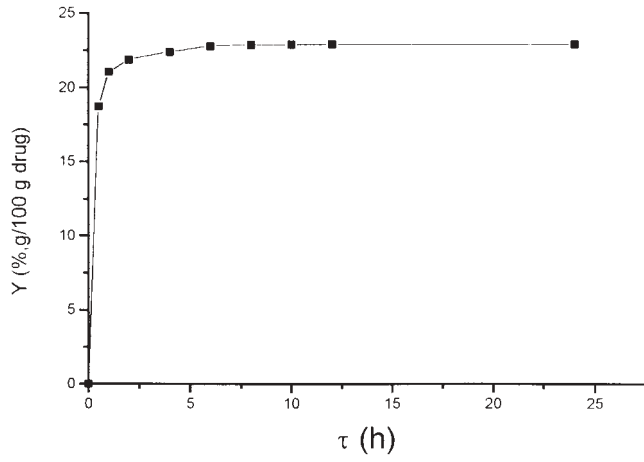
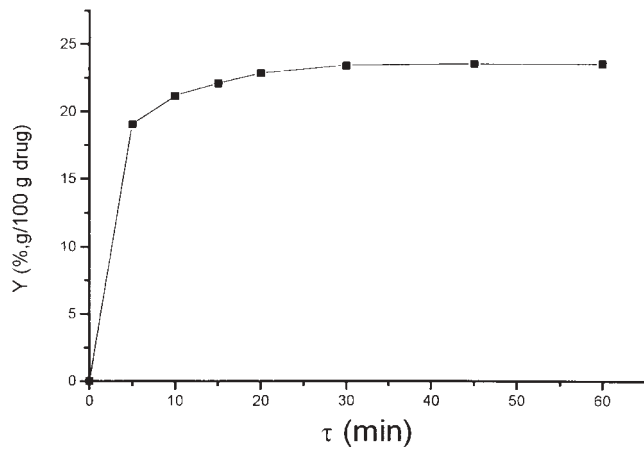Fig. 1. Dependence of *Y* on τ (extraction by the method of maceration).

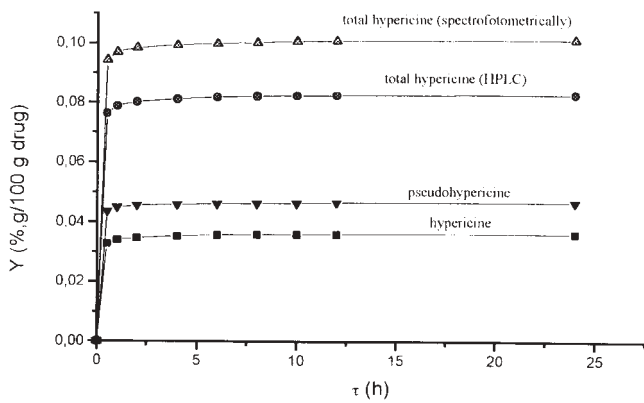Fig. 2. Depencence of *Y* on τ (extraction intensified by ultra-sound).

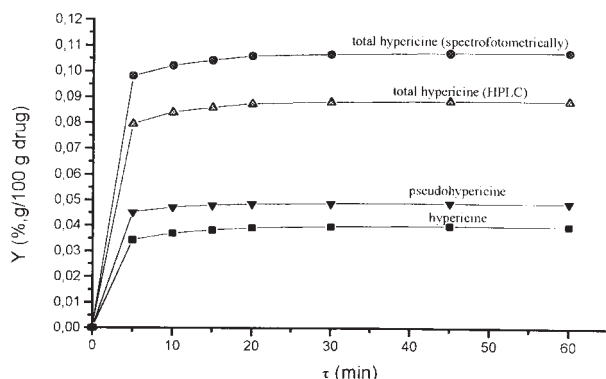Fig. 3. Dependence of *Y* on τ (extraction by the method of maceration).

Fig. 4. Dependence of Y on τ (extraction intensified by ultra-sound).

was determined by a spectrophotometric method.[11] The spectrophotometrically determined values are greater than those obtained by the addition of the content of hypericine and pseudohypericine, determined by HPLC (the increase in absorption originates from the presence of other accompanying substances which absorb at 590 nm).

TABLE I. The parameters k and b are defined by calculation (Eq. (2))

Extracted matters	Extraction procedure			
	Maceration		Intensification of extraction	
	b	k/h^{-1}	b	k/h^{-1}
Resinoid	0.789	8.85×10^{-2}	0.758	3.38×10^{-1}
Total hypericine (spectrophotometrically)	0.705	2.24×10^{-3}	0.729	1.01×10^{-1}
Total hypericine (HPLC)	0.753	3.54×10^{-3}	0.784	1.63×10^{-1}
Hypericine	0.733	3.92×10^{-3}	0.771	2.28×10^{-1}
Pseudohypericine	0.768	3.20×10^{-3}	0.794	1.10×10^{-1}

The contents of resinoid, total hypericine (spectrophotometrically), total hypericine (HPLC), hypericine and pseudohypericine in the starting drug, q_0, amount to 27.09, 0.1384, 0.1055, 0.0469 and 0.0586 (%, g/100 g drug), respectively. The calculated values of the coefficient of fast extraction (b) and the coefficient of slow extraction (k) during the extraction of resinoids, total hypericine, hypericine and pseudohypericine by the procedure of maceration both with and without ultra-sound are given in Table I. The obtained results show that the values of the coefficient of fast extraction in all the tested cases are approximately the same, because they refer to herbal material of a relatively high degree of pulverization. The obtained values of the coefficient of slow extraction are significantly larger than the intensification of the extraction by ultra-sound.

CONCLUSION

The obtained results show undoubtedly that the process of amber extraction was significantly activated by the application of ultra-sound. The curves of the change of the yield of extracted matters in dependence on the duration of extraction both with and without the application of ultra-sound have approximately the same shape. The values of the coefficient of fast extraction (*b*) in all the tested cases were approximately the same, because the starting to herbal material already of relatively high level of pulverization. It can be concluded that the dominant role in the extraction was the transference of mass.

SYMBOLS

b – coefficient of rapid extraction
c – total hypericine [mg]
d – thickness of cuvette [= 1 cm]
E_{590} – measured trial value at 590 nm, calculated on trial volume of 100 cm^3
$E^{1\%}_{1cm}$ – specific extinction coefficient [= 870]
k – slow extraction coefficient [h^{-1}]
q_0 – content of extractable matter in the starting drug [%]
q_i – content of extractable matter residue in the drug [%] after time τ
r – correlation coefficient
RT – retention time [min]
V – volume of the measured solution [cm^3]
τ – time [h]

ИЗВОД

АНАЛИЗА КИНЕТИКЕ ЕКСТРАКЦИЈЕ РЕЗИНОИДА И ХИПЕРИЦИНА ИЗ КАНТАРИОНА *HYPERICUM PERFORATUM* L.

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Испитиване су кинетике екстракције: укупних екстрактивних материја (резиноида), укупног хиперицина, хиперицина и псеудохиперицина из кантариона (*Hypericum perforatum* L.) поступком мацерације са и без ултразвука, коришћењем метанола као екстрагенса. Нађено је да се период брже екстракције при интензификацији екстракције резиноида ултразвуком завршава за знатно краће време (око 20 минута) него при екстракцији без ултразвука (око 5 сати). Слични резултати су добијени и при екстракцији осталих испитиваних супстанци. Произилази да се при интензификацији екстракције ултразвуком постиже боље исцрпљење дроге за знатко краће време екстракције. Припремом биљног материјала уситњавањем постигнут је знатан степен дезинтеграције структуре биљног ткива, тако да при екстракцији доминантну улогу има турбу-

лентни пренос масе. Резултати показују да су вредности коефицијента брзе екстракције (*b*) приближно исте за све испитиване екстракције.

REFERENCES

1. G. M. Kitanov, K. F. Blinova, *Chemistry of Natural Compounds* **23** (1987) 151
2. D. Meruelo, G. Lavie, D. Lavie, *Proc. Natl. Acad. Sci. USA* **85** (1988) 5230
3. G. Lavie, D. Mazur, D. Lavie, D. Maruelo, *Med. Res. Rev.* **15** (1995) 111
4. N. D. Weber, B. K. Murray, J. A. North, S. G. Wood, *Antiviral Chem. Chemother.* **5** (1994) 83
5. J. B. Hudson, I. Lopez-Bazzocchi, G. N. H. Towers, *Antiviral Res.* **15** (1991) 101
6. I. Takahashi, S. Nakanishi, E. Kobayashi, H. Nakano, K. Suzuki, T. Tamaoki, *Biochem. Biophys. Res. Comm.* **165** (1989) 1207
7. W. Zhang, R. E. Lawa, D. R. Hintona, Y. Z. Su, W. T. Couldwell, *Cancer Lett.* **96** (1995) 31
8. B. Pekić, D. Miljković, *Hemija i tehnologija kardiotoničnih glikozida*, Univerzitet u Novom Sadu, Tehnološki fakultet, Novi Sad, 1980, p. 158–172
9. V. D. Ponomarev, *Ekstragirovanie lekarstvennogo syr'ya,* Medicina, Moskva, 1976, p. 46–51
10. A. Šmelcerović, H. Laatsch, Ž. Lepojević, S. Đorđević, *Die Pharmazie* (in press)
11. H. Schutt, J. Hölzl, *Pharmazie* **49** (1994) 206.