

## IDENTIFICATION AND QUANTITATIVE DETERMINATION OF HYPERFORIN IN SOME *HYPERICUM* SPECIES

Daniela GÎTEA<sup>1</sup>, Laurian VLASE<sup>2</sup>, Mircea TĂMAȘ<sup>2</sup>, Ilioaara ONIGA<sup>2</sup>

<sup>1</sup>Facultatea de Medicină și Farmacie, Universitatea din Oradea,  
str. Armatei Romane, nr. 5, RO-410087 Oradea, România

<sup>2</sup>Facultatea de Farmacie, Universitatea de Medicină și Farmacie "Iuliu Hațieganu",  
str. I. Creangă, nr. 12, RO-400023, Cluj-Napoca, România  
e-mail: mtbotanica@yahoo.com

**Abstract:** Using HPLC/MS, hyperforin was identified and quantitatively determined in four species of *Hypericum* (St John's-wort, Johanniskraut) gathered from the wild flora of Bihor County: *H. perforatum* L., *H. maculatum* Crantz subsp. *typicum* Frohl., *H. tetrapterum* Fries and *H. hirsutum* L.

The identification was carried out by MS detection with standard hyperforin (Sigma-Aldrich), and quantitative determination by calibration curve in hyperforin, in negative ionisation. Hyperforin content was determined as 7.89% in *H. perforatum*, 0.077% in *H. maculatum*, 0.103% in *H. tetrapterum* and 0.67% in *H. hirsutum*.

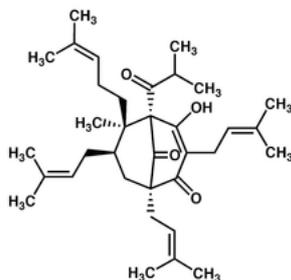
Considering that hyperforin is one of the most important active principles of *Hyperici herba*, and its very low content in the other species (from 100 to 10 times lower), the sole utilization of *H. perforatum* in phytotherapy may be justified.

**Keywords:** hyperforin, *Hypericum* spp., HPLC/MS, St John's-wort

### Introduction

The most famous of all *Hypericum* species is *H. perforatum* L., or Perforate St. John's-wort (Johanniskraut), which has been known as a medicinal plant from ancient times to the present day. The medicinal plant product obtained (*Hyperici herba*) represents the aerial parts of the plant, with stems, leaves and blossoms, and it has been used medicinally in the treatment of several gastric and hepatic conditions, and for healing wounds and burns [11,12]. Since the 1980s, it has been successfully used in the treatment of mild and medium depression [8]. Its active substances are considered to be a phyto-chemical complex represented by flavonoids, dianthrones (hypericins), hyperforin (a phloroglucinol derivative), essential oil, tannins, proanthocyanins and xanones [8,11,12]. Of those mentioned above, hyperforin is a relatively recent discovery (1971–1982), and has anti-microbial, anti-viral and anti-depressant properties [2,5,8,10].

*Hyperici herba* is extremely valued in phytotherapy, being an officinal product in every edition of the Romanian Pharmacopoeia, as well as in the European Pharmacopoeia [13,14]. In all of these, the species *H. perforatum* is the only one cited as a source of the raw material, without any indication as to why other species are not employed, although in reality other *Hypericum* species from the spontaneous flora are harvested and can be found in the product [1,9]. Consequently, we embarked on a botanical and chemical comparative study, of the officinal species, compared to other frequently used species. Not only could this help establish the identity of the officinal product, but also show the chemical differences and similarities between these species; moreover, it can provide an evaluation of their therapeutic potential. This paper presents the results regarding the identification and quantitative determination of hyperforin in four *Hypericum* species found in the spontaneous flora of Bihor County.



**Fig.1: Structure of hyperforin**

The first mention of hyperforin (Fig.1) was by Gurevici (1971), while Bîstrov *et al.* (1975) and Brontz *et al.* (1982) [2,3] indicated its structure. In 1986, Berghofer and Holzl determined the concentration of hyperforin in various parts of the plant, the highest being recorded in the buds, flowers and fruits (capsules), whereas in the stems and leaves the concentration is very low [1,6]. Holzl & Ostrowski (1987) mention the use of the HPLC technique for the identification and quantitative determination of hyperforin contained by *Hypericum* spp. [6]. The content of hyperforin increases from 2.5% in flower buds to 8.5% in the fruits. Other authors have reported concentrations of 3% and above [8,11]. A content of 5.46 mg/g was reported in *H. perforatum* plants from Turkey, and a content of 24.26 mg/g in plants found in Italy [7].

It is important to mention that the hyperforin contained by *Hypericum* species found in the Romanian flora has never been analyzed before, only studies regarding the content of hypericins and flavonoids being carried out so far [9].

### Material and Methods

The “herba” product originated from species of *Hypericum* gathered from the spontaneous flora of Bihor County was used for the analysis of hyperforin: *H. perforatum* L. (Cefa-Inand), *H. maculatum* Crantz subsp. *typicum* Frohl. (Stâna de Vale), *H. tetrapterum* Fries (Pietroasa-Chișcău) and *H. hirsutum* L. (Vadu Crișului) [4]. All the species were harvested during the flowering period, dried at room temperature and then ground to the degree of fineness of the VI (FR X) grinder. Alcoholic solutions of 1% were then prepared from these samples, established in a warm environment, temperature regulated in a water bath at 80°C for 30 minutes, in a flask with reflux refrigerator, using 96° alcohol.

The analysis of hyperforin was performed using HPLC/MS, employing as standard hyperforin (Sigma-Aldrich) in the following experimental conditions [6]:

#### Appliances:

HP 1100 Series binary pump;  
Autosampler HP 1100 Series;  
Termostat HP 1100 Series;  
Mass spectrometer Agilent Ion Trap 1100 VL.

#### HPLC work conditions:

Zorba SB-C 18 analytical column, 100 x 3 mm i.d., 3.5 μm, Agilent USA;  
On-line filter 0.2 μm (Agilent USA);  
Mobile phase: ammonium acetate 1 mM, acetonitrile (35:65 v/v);  
Flow capacity: 1 ml/min;  
Temperature: 45°C;  
Detection: negative electrospray MS/MS;  
Injection volume: 2 μl.

#### MS work conditions:

Source of ions: ESI (electrospray ionization);

Ionization mode: negative;  
Nebulizer: nitrogen, pressure 60 psi;  
Drying gas: nitrogen, 12 L/min, temperature 350° C;  
Analysis mode: monitoring transitions m/z (535.4; 313.1; 315.1; 383.1; 397.2; 466.2).

### Results and Discussions

The full scan of hyperforin is presented in figure 2. According to the molecular mass of hyperforin ( $M=536.4$ ), the ion expected in the case of positive ionization is the ion with m/z 537.4 and in case of negative ionization, the ion with m/z 535.4. This shows that hyperforin can be quantified through SM by both positive and negative ionization. After checking SM detection through the two types of ionization, the analysis of chromatograms (Fig. 3) reveals that the negative ionization of hyperforin ensures a better sensitivity of the method (S/N 64) compared to the positive one (S/M 18). The chromatogram of a hyperforin sample (1  $\mu\text{g/ml}$ ) following MS detection is presented in Fig. 4.

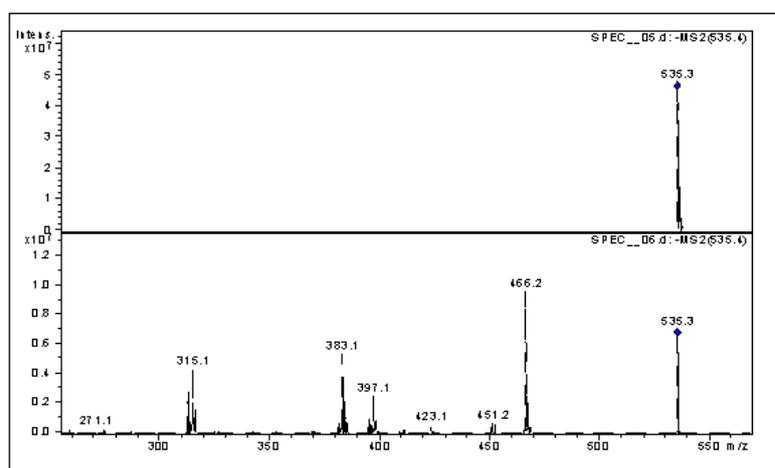


Fig. 2: Full scan (up) and fragmentary (down) spectra of hyperforin in the mobile phase, negative ionization

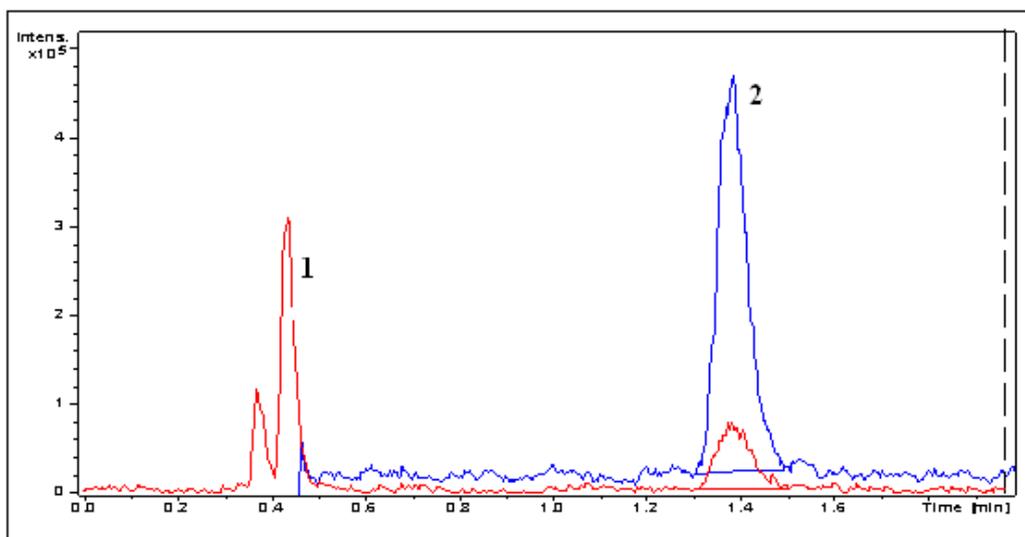
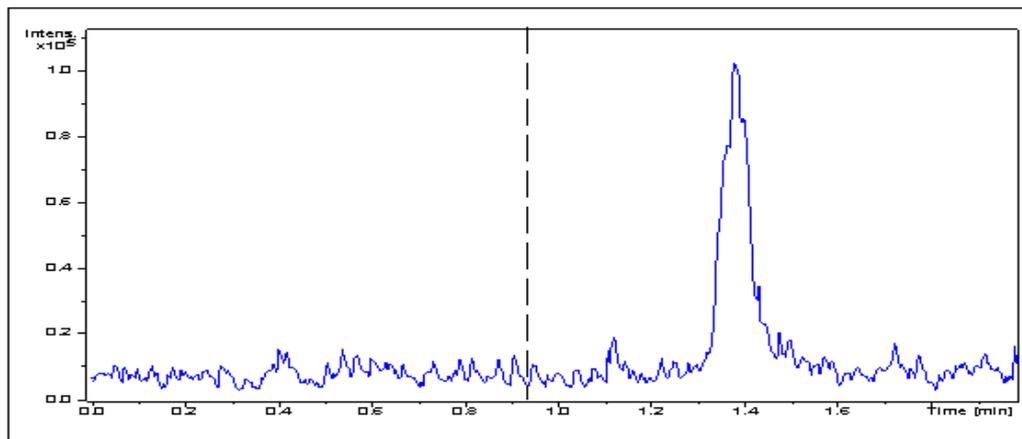


Fig. 3: Chromatogram of hyperforin through MS detection, standard solution (1-positive ionization; 2-negative ionization)

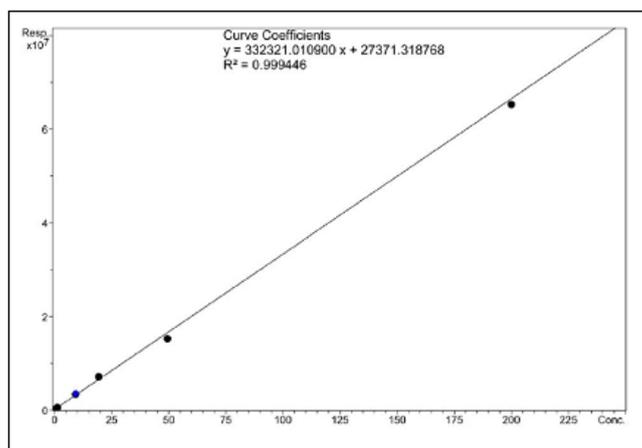


**Fig.4:** Chromatogram of hyperforin through MS detection, negative ionization, to the limit of quantification of the analytical method (1 µg/ml).

The calibration curve of hyperforin was established in the 1–200 µg/ml range of concentrations (Fig. 5)

**Table 1:** Concentrations of the hyperforin used for creating the calibration curve

Theoretical concentration µg/ml	Confirmed concentration µg/ml	Accuracy %
1.000	1.000	100.02
2.000	1.970	98.54
10.000	10.555	105.55
20.000	21.746	108.73
50.000	46.155	92.31
200.000	196.351	98.17



**Fig. 5:** Calibration curve of hyperforin

The initial extracts of 1% were diluted 1:10 before the analysis with methanol-water (80:20). The individual chromatograms of hyperforin from the four extracts, identified through SM detection, are presented in figure 6, while the concentrations of hyperforin in the diluted extracts, in the 1% ones and in the plant products are presented in Table 2.

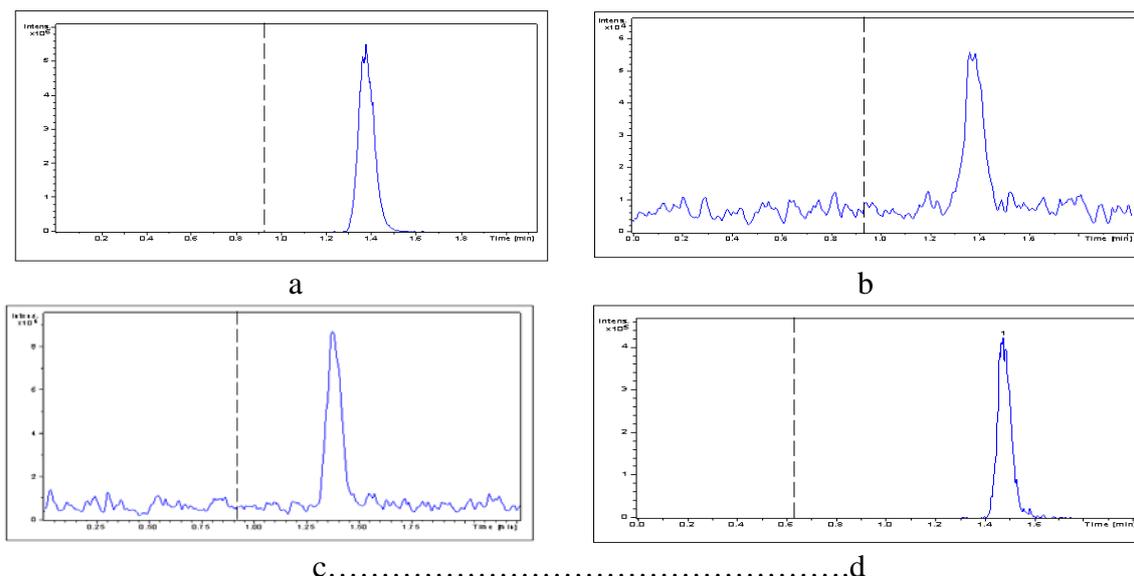


Fig. 6: Chromatograms of *H. perforatum* (a), *H. maculatum* (b), *H. tetrapterum* (c), *H. hirsutum* (d)

Table 2: Concentrations of hyperforin in the analyzed plant extracts

Sample	Species	Concentration of hyperforin (mg/100 ml of extract 1%)	Concentration of hyperforin in plant product %
1.	<i>H. perforatum</i>	78.911	7.891
2.	<i>H. maculatum</i>	0.771	0.077
3.	<i>H. tetrapterum</i>	1.038	0.103
4.	<i>H. hirsutum</i>	6.702	0.670

The analysis of the results clearly reveals the very high content (7.89%) of hyperforin in *H. perforatum*, while the other species have a much lower content, about 100 times lower (0.077%) in *H. maculatum*, about 70 times lower (0.103%) in *H. tetrapterum* and about 10 times lower (0.67%) in *H. hirsutum*. These results may explain why *H. perforatum* is the sole species used in phytotherapy, even though hyperforin was only discovered 30 years ago.

### Conclusions

The hyperforin content in four species of *Hypericum* harvested from the spontaneous flora of Bihor County was identified and determined for the first time in Romania with the aid of the HPLC/MS technique.

Of the four species, only *H. perforatum* has a high content of hyperforin (7.89%), whereas the other three species show a much lower content, 10–100 times lower than in the officinal species. This may account for the exclusive use of *H. perforatum* in phytotherapy.

### REFERENCES

- Berghofer, R., Holzl, J., 1986, Johanniskraut (*Hypericum perforatum*) - Prufung auf Verfalschung, *Dtsch.Apoth.Ztg.*, **126**: 2569-2573.
- Bistrov, N.S., Chernov, B.K., Dobrinin, V.N., Kolossov, M.N., 1975, The structure of hyperforin, *Tetrahedron Lett.*, **32**: 2791-94.
- Brontz, I., Greibrokk, P., Groth, P.A., Aasen, A.J., 1982, The relative stereochemistry of hyperforin - an antibiotic substance from *Hypericum perforatum*, *Tetrahedron Lett.*, **23**: 1299-1300.
- Ciocârlan, V., 2009, *Flora ilustrată a României*. Ed.Ceres, București: 502-504.
- Gurevich, A.I., Dobrinin, V.N., Kolossov, M.N., Popravko, S.A., Ryabova, I.D., Chernov, B.K., Derbentseva, N.A, Aizenman, B.E, Garagulya, A.D., 1971, Hyperforin, an antibiotic from *Hypericum perforatum* L, *Antibiotiki (Moskow)*, **16**: 510-513.

6. Holzl, J., Ostrowski, E., 1987, Johanniskraut - HPLC Analyse der wichtigen Inhaltstoffe und deren Variabilität in einer Population, *Dtsch. Apoth.Ztg.*, **127**: 1227-1230.
7. Maggi, F., Ferretti, G., Poceschi, N., Menghini, L., Riccutelli, M., 2004, Morphological, histochemical and phytochemical investigation of the genus *Hypericum* of the Central Italy, *Fitoterapia*, **75**: 702-711.
8. Miron, A., Stănescu, U., Hancianu, M., Aprotosoiaie, C., 2002, Bazele farmaceutice, farmacologice și clinice ale fitoterapiei. Ed."Gr.T.Popa" UMF Iași, vol.II: 25-30.
9. Oniga, I., Tămaș, M., Vlase, L., Toiu, A., Jula, R., 2008, Botanical and Phytochemical Studies of *Hypericum perforatum* L. and *H. maculatum* Crantz from Romania. In "Proc. 5th Conf. AMAPSEEC, Brno: 1-6.
10. Schempf, C.M., Pelcz, K., Wittmer, A., Schopf, Simon, J.C., 1999, Antibacterial activity of hyperforin from St.John's wort against multiresistent *Staphylococcus aureus* and Gram-positive bacteria, *Lancet*, **35**: 2129.
11. Stănescu, U., Miron, A., Hancianu, M., Aprotosoiaie, C., 2004, *Plantele medicinale de la A la Z*, vol.I., Ed."Gr.T.Popa" UMF Iași: 297-305.
12. Wichtl, M., Bisset, N.G., 1994, *Herbal Drugs and Phytopharmaceuticals*, MedPharm. Publ. Stuttgart: 273-275.
13. \*\*\*, 1993, *Farmacopeea Română*, Ed. X. Editura Medicală București: 483-484.
14. \*\*\*, 2008, *European Pharmacopoeia*, Council of Europe, Strasbourg: 2958-59.

### IDENTIFICAREA ȘI DETERMINAREA CANTITATIVĂ A HIPERFORINEI ÎN UNELE SPECII DE *HYPERICUM*

#### (Rezumat)

A fost identificată și determinată cantitativ, prin tehnica CLIP/SM, hiperforina, în 4 specii de *Hypericum* recoltate din flora spontană a județului Bihor: *H. perforatum* L., *H. maculatum* Crantz spp. *typicum* Frohl., *H. tetrapterum* Fries și *H. hirsutum* L.

Identificarea s-a realizat prin detecție SM față de hiperforina standard (Sigma-Aldrich), iar determinarea cantitativă, cu ajutorul unei curbe de calibrare în hiperforină, cu ionizare negativă. Am determinat un conținut de hiperforină de: 7,89% în *H. perforatum*, 0,077% în *H. maculatum*, 0,103% în *H. tetrapterum* și 0,67% în *H. hirsutum*.

Considerând hiperforina ca unul dintre cele mai importante principii active ale produsului *Hyperici herba* și conținutul foarte scăzut la celelalte specii (de la 100-10 ori mai redus), se justifică utilizarea în fitoterapie, doar a speciei *H. perforatum*.

*Received: 20.07.2010; Accepted: 1.10.2010*