https://doi.org/10.31925/farmacia.2022.6.6 **ORIGINAL ARTICLE**

COMPARATIVE PHYTOCHEMICAL PROFILE OF *HYPERICUM PERFORATUM* AND *HYPERICUM HIRSUTUM* (*HYPERICACEAE*)

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Manuscript received: October 2022

Abstract

The present research focused on comparative phytochemical analysis of *Hypericum perforatum* and *H. hirsutum* aerial parts harvested from different areas of Transylvania (Romania). Total flavonoids and total hypericins for several samples of natural products were determined by spectrophotometric methods, while the identification and quantitative analysis of polyphenolic compounds by HPLC/UV/MS, and hypericin and hyperforin by LC/MS methods. The content in bioactive compounds was higher in *H. perforatum* compared with *H. hirsutum*: flavonoids (3.88 - 5.19% and 1.92 - 3.58%, respectively), and total hypericins (0.42 - 0.65% and 0.13 - 0.29% respectively). Major differences between the two species were observed: rutin and hyperoside were the main polyphenols in *H. perforatum* extract, while in *H. hirsutum* isoquercitrin and hyperoside were predominants, and rutin was found only in traces. Significantly higher amounts of hyperforin and hypericin were determined by LC/MS in *H. perforatum* extract.

Rezumat

Obiectivul studiului a fost analiza fitochimică a părţilor aeriene de *Hypericum perforatum* şi *H. hirsutum* recoltate din zone diferite din Transilvania (România). Pentru mai multe probe s-au determinat flavonoidele totale şi hipericinele totale prin metode spectrofotometrice, identificarea şi cuantificarea compuşilor polifenolici s-au efectuat prin HPLC/UV/MS, iar a hipericinei şi hiperforinei prin metode LC/MS. Conţinutul în compuşi bioactivi a fost mai mare în extractele de *H. perforatum* faţă de cele de *H. hirsutum*: 3,88 - 5,19% şi respectiv 1,92 - 3,58% flavonoide, şi 0,42 - 0,65% şi respectiv 0,13 - 0,29% hipericine totale. S-au observat diferenţe majore între speciile analizate: rutozida şi hiperozida au fost compuşii polifenolici majoritari din extractul de *H. perforatum*, iar isoquercitrozida şi hiperozida în cel de *H. hirsutum*, iar rutozida a fost detectată doar în urme. Concentraţiile de hipericină şi hiperforină determinate prin LC/MS au fost semnificativ mai mari în extractul de *H. perforatum*.

Keywords: *Hypericum sp.*, polyphenols, flavonoids, hypericin, hyperforin

Introduction

Hypericum species L. (*Hypericaceae*) are well-known for their various medicinal properties due to the presence of active compounds, such as: hypericin, pseudohypericin (naphtodianthrones); hyperforin, adhyperforin (phloroglucinol derivatives); hyperoside, rutin, quercetin, quercitrin (flavonoids); essential oil; tannins [8]. Twelve *Hypericum sp*. are mentioned in the Romanian spontaneous flora, while worldwide there are more than 480, known as healing herbs due to their therapeutic properties [5, 15, 16]. The most important species of the genus is considered *H. perforatum* L. (St. John's wort), which has been used in the treatment of mild to moderate depression for its antidepressant effects, and also as antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant or externally to treat skin wounds, eczema, burns [1, 8, 14]. Several studies indicated the variations in quality and quantity of active compounds, with significant differences between species, between populations from different areas, and between different ontogenetic phases of the same individual [5, 14]. Several controlled clinical studies showed the effectiveness of *H. perforatum* extracts for treatment of symptoms in mild depressive disorders, anxiety disorders, sleep disorder and seasonal affective disorder [12, 13]. The pharmacological properties are the result of the additive, synergistic and partly antagonistic effects of numerous bioactive compounds, especially naphtodianthrones and phloroglucinols, whilst the flavonoids increase the bioavailability [9]. Nevertheless, limited information concerning the phytochemistry of some Romanian *Hypericum species* is known. In our previous research on *H. perforatum* and *H. maculatum* aerial parts alcoholic extracts, similar antitumour and antidepressant activities were observed [6, 10, 11], therefore, other species have similar potential as St. John's wort.

In order to enhance the medicinal value of other *Hypericum sp*. from Romania, the aim of our study was to perform a comparative investigation and

quantification of the main active principles from *Hypericum perforatum* and *H. hirsutum* aerial parts.

Materials and Methods

Plant material: several samples of *Hypericum perforatum* and *H. hirsutum* were collected at the full flowering stage from Transylvania (Romania), dried at room temperature, and then ground. Three samples of *H. hirsutum* aerial parts were harvested from different areas: sample 1 – Cluj County (46°77'N, 23°96'E), sample 2 – Cluj County (46°76'N, 24°02'E); sample 3 – Cluj County (46°76'N, 24°01'E); four samples of *H. perforatum* were: sample 4 – Bihor County (47°31'N, 22°33'E), sample 5 – Cluj County (46°77'N, 23°96'E), sample 6 – Cluj County (46°76'N, 24°02'E) and sample 7 – Cluj County (46°76'N, 24°01'E).

The plant material was kindly provided and identified by Prof. PhD. Mircea Tămaş, Pharmaceutical Botany Department, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. All voucher specimens are deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, UMF "Iuliu Haţieganu" Cluj-Napoca, Romania (voucher no. H52-H58).

Extracts preparation: each vegetal product (vp) was reduced to a powder and then extracted with different solvents. Methanol extracts were obtained using 0.5 g vegetal product and 50 mL methanol for 30 min on a water bath at 60ºC [7, 18]; the ethanolic extracts were obtained using 50% ethanol on a water bath, for 30 min [21]. Further assays were performed on methanol extracts (quantitative analysis of total hypericins, HPLC analysis of polyphenols, LC-MS analysis of hypericin and hyperforin), whilst ethanolic extracts were used for the determination of total flavonoids.

The quantitative determinations of flavonoids were made by spectrophotometric method and expressed in rutin (%, g/100 g vp) [21]. The content in total hypericins determined by a spectrophotometric method was expressed in hypericin (%, g/100 g vp) [20].

HPLC analysis of polyphenols

The analysis was conducted using an Agilent 1100 HPLC Series system equipped with degasser, binary gradient pump, column thermostat, autosampler. The LC system was coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap VL). The analysis was performed in the previously described conditions for the identification and quantitative determination of polyphenolic compounds [17, 19]. Standards: 18 polyphenolic compounds (caftaric, gentisic, caffeic, chlorogenic, *p*-coumaric, ferulic, sinapic acids, hyperoside, isoquercitrin, rutin, myricetin, fisetin, quercitrin, quercetin, patuletin, luteolin, kaempferol, apigenin). Calibration curves with good linearity (R^2 > 0.999), in the 0.5 - 50 µg/mL range were employed [17, 19].

LC-MS analysis of hyperforin

The analysis was performed using the Agilent 1100 HPLC Series system coupled with an Agilent 1100 mass spectrometer and hyperforin as standard, in the experimental conditions formerly reported. Briefly, a Zorbax SB-C18 100 mm × 3.0 mm i.d., 3.5 µm column was employed. The mobile phase was ammonium acetate 1 mM and acetonitrile, 35/65 (v/v), flow rate 1 mL/min. Detection was made by MS, the apparatus was set to record the compound with *m/z* 535.4, which is specific to deprotonated hyperforin. The mass spectrometer operated in negative mode, electrospray ion source and nitrogen as nebulising and dry gas. The nebuliser was set at 60 psi and the dry gas flow was 12 L/min at 350ºC. The retention time of hyperforin in above described conditions was 1.4 min [3].

LC-MS analysis of hypericin

The determination was performed using the LC system coupled with MS. The separation of hypericin was made using a Zorbax SB-C18 100 mm × 3.0 mm i.d., 3.5 µm column. The mobile phase consisted in ammonium acetate 1 mM and acetonitrile, 50/50 (v/v), flow rate 1 mL/min. Detection was made by MS, the apparatus recorded the compound with *m/z* 503 (deprotonated hypericin). The mass spectrometer operated in negative mode, electrospray ion source and nitrogen as nebulising and dry gas. The nebuliser was set at 60 psi and the dry gas flow was 12 L/min at 325°C. The retention time of hypericin in above described conditions was 1.5 min.

Results and Discussion

The content in main active compounds was determined for all samples of vegetal product: *H. hirsutum* aerial parts contain flavonoids (2.45 - 3.58%) and hypericins (0.13 - 0.29%), whilst *H. perforatum* aerial parts contain higher levels of both flavonoids (4.2 - 5.19%) and hypericins (0.41 - 0.65%) (Table I). Concerning the aerial parts of *H. hirsutum*, the highest amounts of flavonoids and hypericins were determined in sample 3 (3.58% flavonoids, 0.29% hypericins), whereas for *H. perforatum* the sample 4 was richer in flavonoids (5.19%) and sample 6 was richer in hypericins (0.65%). The observed differences could be correlated with variations in climatic conditions and soil type, which might influence the biosynthesis of natural compounds in both species. In order to comply with the quality standards required for *Hyperici herba*, the vegetal product must contain not less than 0.08% of total hypericins [20]. Therefore, all analysedsamples of *H. perforatum* and *H. hirsutum* collected from different locations of Transylvania can be considered high-quality products, as they meet the demands required by European Pharmacopoeia.

Table I

Quantitative determinations of active compounds

Sample No. (aerial parts)	Flavonoids (g rutin/100 g vp)	Total Hypericins (g hypericin/100 g vp)
H. hirsutum sample 1	2.45 ± 0.04	0.13 ± 0.02
H. hirsutum sample 2	2.92 ± 0.04	0.28 ± 0.01
H. hirsutum sample 3	3.58 ± 0.06	0.29 ± 0.03
H. perforatum sample 4	5.19 ± 0.03	0.44 ± 0.02
H. perforatum sample 5	4.51 ± 0.05	0.42 ± 0.04
H. perforatum sample 6	4.42 ± 0.03	0.65 ± 0.02
H. perforatum sample 7	4.20 ± 0.05	0.41 ± 0.04

All results represent the mean value of three determinations (± SD).

Regarding the results obtained in previous studies on Romanian *H. perforatum*, other authors found 1.02 - 1.04% flavonoids and 0.4 - 0.42% hypericins [2]. Our analysis showed better results for total flavonoids in all *H. perforatum* samples collected from Transylvania, Romania (about four times more). The concentrations of identified compounds using the HPLC method are shown in Table II.

Table II

The concentrations of identified compounds by HPLC (mg/100g vp)

R_T ± SD	Polyphenolic compound (no.)	*H. hirsutum* extract ± SD	Polyphenolic compound (no.)	*H. perforatum* extract ± SD
9.2 ± 0.04	*p*-Coumaric Acid (3)	2.00 ± 0.06	*p*-Coumaric Acid (2)	2.00 ± 0.05
12.4 ± 0.08	Ferulic Acid (4)	1.53 ± 0.03	Ferulic Acid (3)	3.55 ± 0.06
19.0 ± 0.05	Hyperoside (5)	178.67 ± 0.53	Hyperoside (4)	351.30 ± 3.18
19.9 ± 0.12	Isoquercitrin (6)	173.02 ± 0.49	Isoquercitrin (5)	148.36 ± 1.05
20.4 ± 0.09	Rutin (7)	10.05 ± 0.07	Rutin (6)	637.99 ± 3.77
21.1 ± 0.10	Myricetin (8)	3.69 ± 0.05	Myricetin	-
23.3 ± 0.13	Quercitrin (9)	5.52 ± 0.06	Quercitrin (7)	89.65 ± 0.52
26.8 ± 0.12	Quercetin (10)	9.44 ± 0.07	Quercetin (8)	23.76 ± 0.18
29.2 ± 0.09	Luteolin (11)	1.91 ± 0.03	Luteolin	-

All results represent the mean value of three determinations (± SD).

The extracts from both species contain two phenol-carboxylic acids (*p*-coumaric and ferulic acids) and five flavonoids (hyperoside, isoquercitrin, rutin, quercitrin and quercetin), whereas myricetin and luteolin were found only in *H. hirsutum* extract. Rutin was the major flavonoid in *H. perforatum* extract (637.99 mg/100 g vp), followed by hyperoside (351.30 mg/100 g vp) and isoquercitrin (148.36 mg/100 g vp). *H. hirsutum* extract contains mainly hyperoside (178.67 mg/100 g vp) and isoquercitrin (173.02 mg/100 g vp). A higher content of each polyphenolic compound in *H. perforatum* extract when compared with *H. hirsutum* was determined, except for isoquercitrin (148.36 mg/100 g vp, and respectively 173.02 mg/100 g vp). The most important difference between the species was determined regarding the rutin content, over 60 times higher in *H. perforatum* (637.99 mg/100 g vp) then in *H. hirsutum* (10.05 mg/100 g vp).

Hypericin and hyperforin are active principles very important for therapeutic properties of *Hypericum species*, especially for antidepressant activity. The highest amounts of total hypericins were found in sample 3 (for *H. hirsutum*) and in sample 6 (for *H. perforatum*), therefore the presented results are for those samples (Table III).

Table III

The content in hyperforin and hypericin by LC-MS (mg/100 g vp)

Sample No. (aerial parts)	Hyperforin (mg/100 g vp)	Hypericin (mg/100 g vp)
H. hirsutum sample 3	4.99 ± 0.18	22.68 ± 0.42
H. perforatum sample 6	931.7 ± 2.02	116.62 ± 0.99

All results represent the mean value of three determinations (± SD).

Hyperforin was identified in all samples, which is in agreement with the data obtained by Kusari *et al*. on *Hypericum sp*. collected from Slovakia and India [5]. Our samples of *H. perforatum* collected from Transylvania, Romania, contain higher amounts of hyperforin (931.7 mg/100 g) then the species from India (622.4 mg/100 g) [5]. Furthermore, in this study we observed that *H. perforatum* extracts contain hyperforin and hypericin in significantly higher contents then *H. hirsutum* (4.99 mg/100 g and 22.68 mg/100 g respectively).

Despite the fact that the concentrations of active principles are lower in *H. hirsutum* aerial parts, they could represent valuable products as they comply

with the quality provisions of European Pharmacopoeia regarding the total hypericins content.

Conclusions

The aerial parts of *Hypericum species* from Transylvania, Romania are important medicinal raw material, notably *H. perforatum*. The obtained results allow a phytochemical characterisation of *H. hirsutum* from Romania, which is less studied. Even if *H. hirsutum* contains lower amounts of natural compounds then *H. perforatum*, this species could be used to obtain the collective drug *Hyperici herba* and standardized products recommended in biliary disorders or for healing wounds.

Conflict of interest

The authors declare no conflict of interest.

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