

PHYTOCHEMICAL ANALYSIS OF *HYPERICUM MACULATUM* IN ORDER TO OBTAIN STANDARDIZED EXTRACTS

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Abstract

The aim of this study was to perform a phytochemical analysis of *Hypericum maculatum* aerial parts harvested from different areas of Transylvania (Romania) and to obtain a dry standardized and concentrated extract which can be easily used to prepare tablets or capsules. Quantitative analyses of total flavonoids and hypericins for five samples of natural product were performed by spectrophotometric methods, whilst the identification and quantitation of polyphenolic compounds by HPLC/MS. The concentrations of flavonoids (3.7 - 5.5%) and total hypericins (0.12 - 0.74%) were determined. Caftaric acid, chlorogenic acid, hyperoside, isoquercitrin, rutin, quercitrin and quercetin were identified and quantified by HPLC/MS. The physicochemical characterization of the dry extract showed a similar content in active compounds as the ethanolic extract from aerial parts.

Rezumat

Obiectivul studiului a constat în analiza fitochimică a părților aeriene de *Hypericum maculatum* recoltate din diferite zone ale Transilvaniei (România) și obținerea unui extract uscat standardizat și concentrat care ar putea fi utilizat pentru formularea de comprimate sau capsule. Determinările cantitative ale flavonoidelor și hipericinelor totale au fost efectuate prin metode spectrofotometrice pe cinci probe de produs vegetal, iar identificarea și dozarea polifenolilor, prin HPLC/MS. Produsul vegetal conține flavonoide (3,7 - 5,5%) și hipericine totale (0,12 - 0,74%). Acidul caftaric, acidul clorogenic, hiperozida, isoquercitrozida, rutozida, quercitrozida și quercetolul s-au determinat prin analiză HPLC/MS. În urma caracterizării fizico-chimice a extractului uscat s-a observat că acesta conține principii active în concentrații similare cu cele din extractul etanolic obținut din produsul vegetal.

Keywords: *Hypericum maculatum*, polyphenolic compounds, hypericins, HPLC-MS

Introduction

The genus *Hypericum* L. (*Hypericaceae*) is represented by more than 480 species, 12 are mentioned in Romanian spontaneous flora; *H. perforatum* L. and *H. maculatum* Crantz. are most widespread [3, 14, 16]. The main constituents of *Hypericum* sp. are naphthodiantrones (hypericin, pseudohypericin), flavonoids (hyperoside, rutin, quercitrin), tannins, acylphloroglucinols (hyperforin, adhyperforin), essential oils [9].

Significant differences between various species and between different populations of the same species from different areas were observed [13]. The extracts of *Hypericum* sp. have shown to possess significant antioxidant, antimicrobial and wound healing, analgesic, anti-inflammatory properties [9]. Hyperoside is a major flavonol glycoside found in *Hypericum* sp. with antioxidant, anticancer, anti-inflammatory, cardio-protective effects [6]. *H. perforatum* is used for the treatment of anxiety and of mild and moderate depression. Various clinical data have demonstrated that active compounds act by

mechanisms similar with tricyclic anti-depressants or serotonin reuptake inhibitors [2, 9]. Hypericins and hyperforins are mainly responsible for the antidepressant activity and the flavonoid glycosides increase the pharmacological properties of other active compounds [10].

Our previous research on *H. perforatum* and *H. maculatum* harvested from Romania showed similar antidepressant and antitumor effects of aerial parts alcoholic extracts [7, 11, 12]. Because in both Romanian and European Pharmacopoeias only *H. perforatum* L. is mentioned [18], the aim of our study was to obtain and characterize standardized *H. maculatum* extracts in order to improve the medicinal use of this indigenous species.

Materials and Methods

Plant material: the plants were harvested from wild populations from Transylvania at full flowering stage. Five samples of *H. maculatum* aerial parts were collected from different areas: sample 1- Cluj County (area 1: 46° 45' N, 22° 47' E); sample 2-

Braşov County (area 1: 45° 30' N, 25° 13' E); sample 3- Cluj County (area 2: 46° 47' N, 22° 42' E), sample 4- Cluj County (area 3: 46° 57' N, 22° 48' E), sample 5- Braşov County (area 2: 45° 28' N, 25° 15' E). All voucher specimens are deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, UMF "Iuliu Haţieganu" Cluj-Napoca (voucher no. 32-36).

Extracts preparation: the natural product (np) was air-dried, reduced to a powder and then extracted with different solvents. The methanolic extract was obtained using 0.5 g np and 50 mL methanol for 30 min on a water bath at 60°C [8]; the tincture was prepared from 10 g np and 100 g ethanol 70% at room temperature; the fluid extract was prepared by the repercolation method, using 70% ethanol as a solvent [19]. The dry extract was obtained from the fluid one, using the fluid bed drying method and Aeromatic Strea 1 apparatus (GEA, Switzerland). The fluid extract from aerial parts of *H. maculatum* was adsorbed on a powder mixture containing 30% lactose and 70% cellulose, so that 1 g dry extract corresponded to 3 g np [15].

The quantitative determinations of flavonoids were performed by a spectrophotometric method [19] and expressed in rutin (% g/100 g np).

The content in total hypericins determined by a spectrophotometric method was expressed in hypericin (% g/100 g np) [18].

HPLC analysis of polyphenolic compounds

The experiment was carried out using an Agilent 1100 HPLC Series system equipped with degasser, binary gradient pump, column thermostat, auto-sampler. The HPLC system was coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion

Trap VL). For the identification and quantification of polyphenols, the analysis was performed in previously described conditions [1, 17]. 18 polyphenolic compounds (caftaric, gentisic, caffeic, chlorogenic, *p*-coumaric, ferulic, sinapic acids, hyperoside, isoquercitrin, rutin, myricetin, fisetin, quercitrin, quercetin, patuletin, luteolin, apigenin, kaempferol) were used as standards. Calibration curves in the 0.5 - 50 µg/mL concentration range with good linearity ($R^2 > 0.999$) for a five point plot were employed [1, 17].

Results and Discussion

The concentrations of flavonoids (3.7 - 5.5%) and hypericins (0.12 - 0.74%) for all samples of np were determined (Table I). The climate conditions can induce variations in the biosynthesis of active compounds. The samples harvested from Cluj County contained flavonoids (3.7 - 4.44%) and total hypericins (0.48 - 0.74%). The richest sample in flavonoids was no. 5 (5.5%), whereas no. 4 contained more hypericins (0.74%). Considering the quality standards required by the European Pharmacopoeia (*H. perforatum* L. must contain not less than 0.08% of total hypericins), all samples of *H. maculatum* harvested from different areas of Transylvania are high-quality natural products. The extracts (methanolic, fluid ethanolic, dry extracts, tincture) prepared using sample no. 5 (Şirnea) showed close related content in bioactive substances with the natural product (4.96 - 5.18% flavonoids, 0.54 - 0.63% hypericins). All results represent the mean value of three determinations.

Table I
Quantitative determinations of active compounds (\pm SD)

Sample No. (aerial parts)	Flavonoids (g rutin/100 g np)	Hypericins (g hypericin/100 g np)
1	3.88 \pm 0.04	0.48 \pm 0.02
2	4.55 \pm 0.06	0.12 \pm 0.01
3	4.44 \pm 0.05	0.63 \pm 0.03
4	3.70 \pm 0.03	0.74 \pm 0.02
5	5.50 \pm 0.05	0.66 \pm 0.04
Extracts (sample no.5)		
Methanolic extract	5.18 \pm 0.04	0.63 \pm 0.03
Fluid ethanolic extract	5.09 \pm 0.05	0.62 \pm 0.05
Dry extract	5.04 \pm 0.03	0.62 \pm 0.04
Tincture	4.96 \pm 0.02	0.54 \pm 0.02

The dry extract was standardized in flavonoids (5.04%) and hypericins (0.62%). The sample no. 5 contained the highest amount of flavonoids; therefore the HPLC analysis was performed on this natural product (Figure 1).

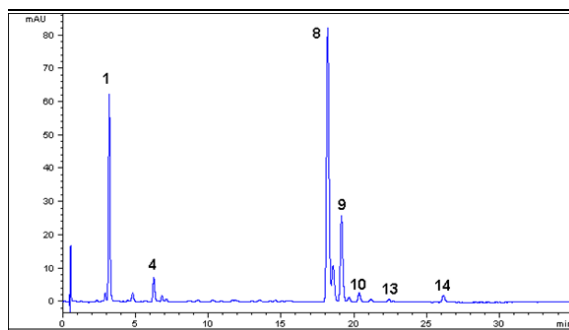


Figure 1.

HPLC chromatogram of *H. maculatum* methanolic extract (sample no. 5)

By HPLC/MS there were identified and quantified two phenol carboxylic acids (caftaric acid, chlorogenic acid), four flavonol glycosides (hyperoside, isoquercitrin, rutin, quercitrin) and one flavonol (quercetin) (Table II).

The major flavonoid was hyperoside, which is in agreement with other studies on the plant harvested from Serbia (661 mg/100 g) [13]. Previous researches on *H. perforatum* from Romania found 1.02 - 1.04% flavonoids and 0.4 - 0.42% hypericins [4], thus *H. maculatum* might be a valuable alternative with higher contents in bioactive substances, especially flavonoids.

Table II

Quantitative determinations of polyphenols by HPLC (mg/100 g np) in *H. maculatum* methanolic extract (sample no. 5)

Peak no.	Compound	R _T ± SD	Concentration (mg/100 g np)
1	Caftaric acid	3.54 ± 0.05	381.8 ± 1.77
4	Chlorogenic acid	6.43 ± 0.05	27.15 ± 0.19
8	Hyperoside	19.32 ± 0.12	545.14 ± 2.96
9	Isoquercitrin	20.29 ± 0.10	244.59 ± 1.13
10	Rutin	20.76 ± 0.15	11.00 ± 0.1
13	Quercitrin	23.64 ± 0.13	8.44 ± 0.05
14	Quercetin	27.55 ± 0.15	9.02 ± 0.07

The physicochemical characterization of the dried extract obtained from aerial parts of *H. maculatum* was performed and the results are presented in Table III. Both fluid extract and the dried one obtained by bed drying method showed close related

contents in flavonoids (5.09%, and respectively 5.04%) and the same amount of hypericins (0.62%). The dried extract can be considered a good quality product and an alternative way to increase the compliance to treatment.

Table III

The physicochemical characterization of dried *H. maculatum* extract (sample no.5)

Characteristic	Parameter
Aspect	Uniform powder
Colour	Yellow-green
Smell	Characteristic aromatic
Density	0.46 g/cm ³
Loss on drying	3.75%
Content in flavonoids	5.04%
Content in hypericins	0.62%
Storage	In dry place, protected from humidity, light, heat
Shelf life	2 years

Conclusions

H. maculatum represents an important source of flavonoids and hypericins with similar therapeutical properties as *H. perforatum*.

The obtained standardized and concentrated *H. maculatum* extract could be easily formulated as tablets or capsules to treat several diseases, like biliary conditions and mild depression. The good correlation between dose and effects allows an increase in therapeutic use of quality herbal medicines.

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