Mar. Üniv. Ecz. Der.,
11 (1 - 2), 343 - 350 (1995)

J. Pharm. Univ. Mar.,
11 (1 - 2), 343 - 350 (1995)

HYPERICINS IN *HYPERICUM* SPECIES

Gerassim M. KITANOV*

ABSTRACT

Hypericins as antidepressant, antiviral and photodynamically active compounds, also having chemotaxonomic importance are well known. Their occurrence in over 20 *Hypericum* species was investigated by TLC method. Quantitative determination of their contents was performed spectrophotometrically. It has been found that the species from the primitive sections *Ascyreia, Androsaemum, Roscyna* and *Spachium* do not contain hypericins, whereas they occur in fairly - large amounts in advanced sections *Drosocarpium* and *Hypericum*. In most species the content of pseudohypericin exceeds that of hypericin. The total quantity of hypericins in the studied species varies widely: from 0.012 % (*H. polyphyllum*) to 0.30 % (*H. barbatum*). Growth dynamic studies on the medicinally importance species *H. perforatum* and *H. maculatum* establish their maximum content during flowering period. Hypericins content in different regional samples vary from 0.07 - 0.17 % (*H. perforatum*) & 0.02 - 0.11 % (*H. maculatum*) .

KEY WORDS

hypericin, pseudohypericin, *Hypericum,* quantitative determination, dynamics of accumulation

* Department of Pharmacognosy, Faculty of Pharmacy, Dunav str. 2, 1000 Sofia, BULGARIA.

INTRODUCTION

Hypericin and pseudohypericin, naturally occurring red pigments, have been very intensively regarded in several aspects. They possess photodynamic, anti depressive and antiviral activities. Many antidepressants containing hypericins are already known (Phytogran, Seda grandelat, Echtronerval, Hyperforat, Psychotonin M, Neuropas, Sedariston, Cesradyston, Neuroplant etc.)/1,2/. These compounds inhibit the propagation of Friend leukaemia virus, Vesicular stomatitis virus, Influenza virus, Herpes simplex virus types I & II, and Equine infection anaemia virus /3-5/. On the other hand, hypericins are also chemotaxonomically important for infrageneric classification of *Hypericum* /6/.

22 species of *Hypericum*, which belong to 11 sections are widespread in Bulgaria. Most of them have not yet been investigated for the content of hypericins. The most widespread species also having the highest therapeutic applications is *H. perforatum*. *H. maculatum* is also used in traditional medicine as equivalent to the official drug *H. perforatum* and recently has been included in some Pharmacopoeias /7,8/.

The present study was aimed : to investigate the presence of hypericin and pseudohypericin in some *Hypericum* species; to quantitatively determine the hypericins' content in these species; to study the dynamics of accumulation of hypericins in *H. perforatum* and *H. maculatum*; and to determine the content of hypericins in the samples of *H. perforatum* and *H. maculatum* collected from different places in Bulgaria.

MATERIALS AND METHODS

The plant material was collected from the wildly growing species of *Hypericum* from their native habitat. The entire terrestrial part of the plant was collected during the period of its full bloom. For the quantitative determination of the hypericins, the material was collected from five-six plants from various places of growth in the same region. For the study of the dynamics of accumulation of the hypericins in *H. perforatum* and *H. maculatum* two places were chosen for the collection situate in the Vitosha mountain near the city of Sofia. The material was collected twice monthly from June to October. The samples of the two species were also collected from different floral regions of Bulgaria during the summer season for the last few years. The material was air dried in shade and milled to particle size 1 mm.

About 0.5 g of the air dried herb was defatted with chloroform and the hypericins were extracted with ethyl acetate. After concentration , the ethyl acetate extracts were chromatographed by TLC on Kieselgel G Merck plates in the solvent system ethyl acetate - formic acid (50:6). The chromatograms were observed in the UV light at 360 nm.

The spectroscopic determinations of hypericins were conducted according to the method described earlier /9/. From each of the samples 3 determinatons were made and the mean value was calculated.

RESULTS

The results of the chromatographic investigations and quantitative determination of the hypericins present in 26 species of *Hypericums* belonging to 12 sections are presented in the Table 1.

TABLE 1

The presence of hypericin (hp) and pseudohypericin (pshp) and their total content (%) in different *Hypericum* species

Sections/ Species	hp	pshp	%
I. *ASCYREIA* Choisy			
1. *H. calycinum* L.	-	-	0.000
2. *H. patulum* Thunb.*	-	-	0.000
II. *ANDROSAEMUM* (Duhamel) Godron			
3. *H. androsaemum* L.	-	-	0.000
III. *ROSCYNA* (Spach) R. Keller			
4. *H. ascyron* L.**	-	-	0.000
IV. *HYPERICUM*			
5. *H. maculatum* Crantz *var. maculatum*	+	+	0.058
5a. *H.maculatum* Crantz *var. immaculatum*	+	+	0.023
6. *H. tetrapterum* Fries	+	+	0.052
7. *H. perforatum* L.	+	+	0.166
8. *H. attenuatum* Choisy**	+	+	ns
9. *H. elegans* Stephan	+	+	0.104
V. *OLYMPIA* (Spach) Nyman			
10. *H. olympicum* L.	+	+	0.015
11. *H. polyphyllum* Boiss. et Ball.***	ns	ns	0.012
VI. *CAMPYLOPUS* Boiss.,			
12. *H. cerastoides* (Spach) N. Robson	+	+	0.029
VII. *DROSOCARPIUM* Spach			
13. *H. montbretii* Spach	+	+	0,174

14. *H. umbellatum* A. Kerner	+	+	0,063
15. *H. richerii* Vill.	+	+	0,134
16. *H. rochelii* Griseb. et Schenk	+	+	0,232
17. *H. boissieri* Petr.	+	+	ns
18. *H. barbatum* Jacq.	+	+	0,306
19. *H. rumeliacum* Boiss.	+	+	0,263
VIII. *OLYGOSTEMA* (Boiss.) Stef.			
20. *H.aucheri* Jaub. et Spach	+	+	0,031
IX. *THASIA* Boiss.			
21. *H. thasium* Griseb.	+	+	0.124
X. *TAENIOCARPIUM* Jaub. et Spach			
22. *H. hirsutum* L.	+	-	0,043
23. *H. linarioides* Bosse	+?	+	ns
XI. *ADENOSEPALUM* Spach			
24. *H. degenii* Bornm.	+	+	0,066
25. *H. montanum* L.***	ns	ns	0,040
XII. *SPACHIUM* (R. Keller) N. Robson			
26. *H. japonicum* Thunb. ****	-	-	0.000

ns - Not studied

* Garden cultivated

** Collected from Siberia

*** Cultivated in and collected from Ukraine

**** Collected from Vietnam

As is evident from the Figure 1, the highest content of hypericins was found to be during the begining of the blooming period. (second half of June for *H. perforatum* and the end of July for *H. maculatum*) and is maintained approximately up to the same level during the entire period of bloom. After the flowering period the content of hypericins gradually decreases and during the ripening of the fruit in September it is lower than 0.05% /Fig.1/.

The limits of hypericins' content in the samples of *H. perforatum* and *H. maculatum* collected from different regions of Bulgaria are shown in Table 2.

CONCLUSIONS

The *Hypericum* species from primitive sections *Ascyreia, Androsaemum, Roscyna* and *Spachium* /6/ do not contain hypericins despite their occurrence in fairly large amounts in advanced sections *Drosocarpium* and *Hypericum*. In all species containing hypericins both hypericin and pseudohypericin are found, excluding *H. hirsutum* in which only hypericin is present. In most species the content of pseudohypericin exceeds that of hypericin.

The total quantity of hypericins in the studied species varies widely, i.e. from 0.012% (*H. polyphyllum*) to 0.306% (*H. barbatum*).

Growth-dynamics studies on the medicinally important species *H. perforatum* and *H. maculatum* establish maximum hypericins content during the flowering period which gradually decreases up to autumn.

The quantity of hypericins in *H. perforatum* drastically varies in the different samples with respect to different factors (0.070-0.170%), but all samples satisfy the requirements of DAC 1979 (not less than 0.05%) /9/. The level of hypericins in *H. maculatum var. maculatum* is lower (0.055-0.110%) than that of *H. perforatum*, but also satisfy the requirements of DAC 1979, whereas the content of these compounds

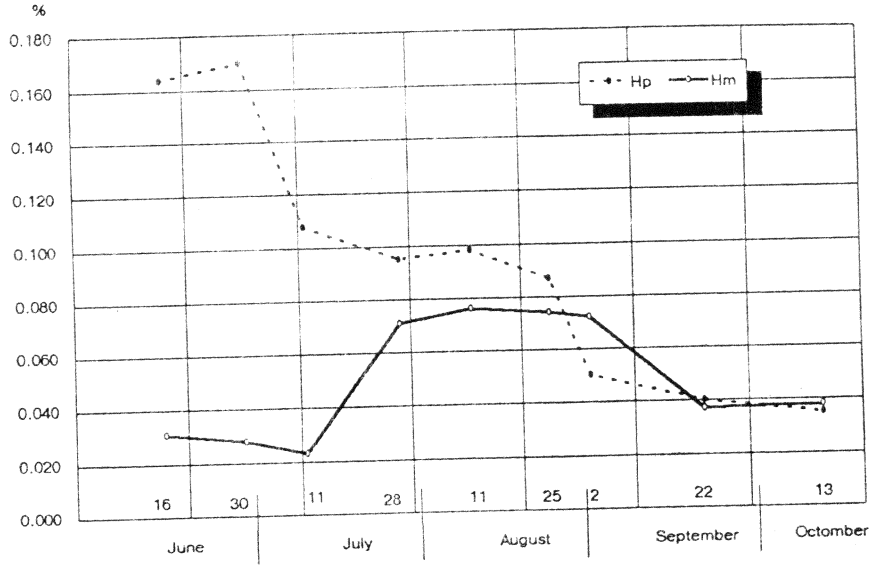

Fig. 1: Dynamics of the hypericins' accumulation (%) during the vegetation of *H. perforatum* (Hp) and *H. maculatum var. maculatum* (Hm)

TABLE 2

The content of hypericins (%) in the samples of *H. perforatum.*, *H. maculatum var maculatum* and *H. maculatum var. immaculatum*, collected from different places of Bulgaria

Species	Samples	Places	Content (%)
H. perforatum	28	28	0.070-0.170
	(One sample collected on 12.10.90 gave 0.045%)		
	(One sample collected from Vitosha gave 0.26%!)		
H. maculatum	5	5	0.055-0.110
var. maculatum	(One sample collected on 12.10.90 gave 0.05%)		
H. maculatum	8	8	0.019-0.047
var. immaculatum			

in *H. maculatum var immaculatum* is the lowest (0.019-0.047%) and cannot be equated to that of the official *H. perforatum*.

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