

# Morphological and Chemical Evaluation on *Hypericum perforatum* and *H. maculatum* in Lithuania

Jolita Radušienė and Edita Bagdonaitė  
Institute of Botany  
LT-2021 Vilnius, Lithuania

Saulius Kazlauskas  
Kaunas University of Medicine  
LT-3000, Kaunas, Lithuania

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## Abstract

An increasing demand for *Hyperici herba* has caused the evaluation of this genus in all Europe. *Hypericum perforatum* L. and *H. maculatum* Cranz are common species in Lithuania. The aim of the investigations was to examine indigenous populations and field accessions of these species in respect to morphological characters and chemical constituents. The results of evaluation revealed high morphological diversity in both species. The t-test comparison showed the difference of characters from wild and field. The content of flavonoids and hypericin varied highly in species, different accessions, and parts of the plant. Significant differences were detected in the mean concentrations of quercetin, hypericin, rutin and hyperoside+isoquercetrin in flowers and leaves of both species. The flavonoid content appeared to be more constant character in both species. The poorer growth and lower mean concentrations of secondary metabolites were discovered in *H. maculatum*. The germplasm of *Hypericum* may be a potential source of genetic variation to allow selecting the valuable material for breeding.

## INTRODUCTION

Recently, *Hypericum* L. has become one of the most worldwide-evaluated genres. The big interest in the plant has been caused by its supposedly photodynamic, antidepressant, and antiretroviral activities (Bombardelli and Morazzoni, 1995; Gaedcke, 1997, Vitiello, 1999). Hölz and Ostrowski (1987) indicated that flavonoids of *H. perforatum* had a sedative effect. Hyperforin has been recently found to be most relevant to the antidepressant activity of *Hypericum* (Chatterjee et al., 1998; Laakman et al., 1998). Hypericin shows a significant antiviral and antiretroviral activity (Vlietinck et al., 1998).

*H. perforatum* L. and *H. maculatum* Cranz are common species in Lithuania. In folk medicine *H. perforatum* is known as a remedy against 99 diseases. Natural sources of raw material of *H. perforatum* are sufficient, however, drug manufacturers tend to utilize plants under controlled conditions rather than gathered from the wild. Only one Russian variety 'Zolotodolinskaja' has been grown in Lithuania so far.

The objective of this study was to assess morphological and chemical inter-population variability in wild populations and field accessions of *Hypericum*, and to reveal the most valuable material for further use in breeding.

## MATERIALS AND METHODS

The material of *H. perforatum* (from 12 sites) and *H. maculatum* (from 7 sites) was collected during field trips in 1999 in the phase of full flowering. The seeds have been gathered in fall period and sown in the field collection. The evaluation of field accessions has been carried out in 2001 according to the morphometric analysis of phenotype and content of secondary metabolites. The total of 14 morphological and productive characters including height of plants, number of internodes, width and length of inflorescences, dimensions of the leaves, petals, sepals, dry mass of inflorescences, leaves, shoots and raw material have been measured.

The spectrophotometric method was used for determination of total amount (percentage) of flavonoids (Tumanova, 1990). Quantitative analysis of flavonoids and hypericin in the ethanolic extracts of flowers and leaves was carried out by modified

HPLC gradient elution method (Hölzl and Ostrowski, 1987). Compounds were identified by means of pure standards.

Differences among populations and accessions were tested by one-way analysis of variance (ANOVA) at  $\alpha = 0.05$  level. The Sheffe multiple comparison test was employed to identify significantly homogenous groups among populations. The hierarchical cluster analysis dendrogram of the population productivity was constructed based on agglomerative grouping and the single linkage between groups clustering method using squared Euclidean distances.

## RESULTS AND DISCUSSION

### Variability of Morphological and Productivity Characters

The one-way analysis of variance (ANOVA) revealed highly significant differences ( $p < 0.05$ ) among *H. perforatum* populations within all measured characters. Peak values of the F statistic were observed for height of plants, length of sepals, width and length of petals, width of leaves, and length of inflorescences (Table 1). *H. maculatum* populations were highly different in height of plants, number of internodes, length and width of petals (Table 2).

The most important characters for distinguishing the morphological variants appeared to be the dimensions of leaves. The leaf length/width ratio in different populations of *H. perforatum* varied from 2.05 to 3.89. The results indicated three morphological variants of *H. perforatum*: narrow (4:1), intermediate (3:1), and broad (2:1) leaved. Robson (1968) classified *H. perforatum* into three varieties: var. *perforatum* with broad leaves, var. *angustifolium* with narrow leaves, and var. *microphyllum* with small leaves. According to our results the broad-leaved populations dominated in Lithuania. Two varieties could be accepted – var. *perforatum* and var. *angustifolium*.

The populations were homogenous in length and width of petals (CV: 12.01 and 14.01 %, respectively) but more heterogenous in dimensions of sepals (CV: 14.89 and 32.34 %, respectively).

Results of the Scheffé test showed the existence of 2 homogenous groups in populations of *H. maculatum* according to the measurement of leaves within the analyzed data set. The first group was comprised of populations with long and broad leaves, while the other group had short and narrow leaves. The majority of plants had flowers with long petals and short sepals.

The height of plants, number of internodes, width and length of inflorescences, and weight of raw material are the most important parameters when the productivity is estimated. The height of plants was the most variable parameter among investigated populations of *Hypericum*. The mean value of height of both species according to the Scheffé test formed 4 statistically different homogeneous groups. The mean value of height in most populations of *H. perforatum* varied from 45.0 to 50.7 cm, while of *H. maculatum* from 54.0 to 58.8 cm.

The difference between the average number of internodes of *H. perforatum* and *H. maculatum* is not big. However, this parameter varies among species populations very much and makes up 3 homogenous groups: few internodes (up to 16), medium number of internodes (17-19), and many internodes (20 and more). According to the length of inflorescences there were 2 homogeneous subsets in populations of both species. The populations of *H. perforatum* were more homogenous in length of inflorescences than *H. maculatum* (CV: 16.5-36.3 % and 26.5-56.8 %, respectively). According to these data the cutting height of flowering horizon when being harvested could be less than 30 cm. The width of inflorescences among populations of *H. perforatum* and *H. maculatum* varied highly (CV: 21.8-32.1 % and CV: 29.55-74.35 %, respectively).

A hierarchical cluster analysis dendrogram provides evidence for the existence of three groups among the studied *H. perforatum* populations on the basis of productivity characters (Fig. 1). The majority of populations (6) had the lowest herbal mass. Four populations were of mean productivity. The dendrogram manifested the distinct character

of two populations (99EB02 and 99EB13), which distinguished themselves by the largest mass of plants, their parts and the largest production of raw material. The productivity of *H. maculatum* was twice as small as *H. perforatum*.

The higher heterogeneity in many of characters was experienced in wild populations than in field accessions. The characterization of *H. perforatum* wild populations and field accessions revealed significant differences within all traits. The t-test comparison showed that the difference of all characters from wild populations and field accessions was statistically significant (Table 1). Mean values of all characters were greater in plants from field than those collected from wild. These differences could be influenced by environmental conditions. Previous investigations in *H. perforatum* demonstrated a wide range of its ecological adaptation scale, occurring on several soil types and in broad selection of plant communities (Radušienė and Bagdonaitė, 2000). The differences of introduced accessions under uniform field conditions may be more related to the genetic factors.

Our results suggest fewer differences in a variety of traits in *H. maculatum* field accessions. Mean values of the plant height, the length of inflorescences, and the weight of stems in field accessions were lower than in wild. Field accessions and wild populations did not differ significantly for width of petals and sepals, number of internodes, dry weight of flowers and leaves (Table 2). The narrower ecological amplitude of this species, which, in turn, makes its introduction more complicated, could explain the poorer growth of *H. maculatum* in field.

### Variability of Chemical Characters

Species of *Hypericum* are characterized by chemical polymorphism. Chemical variability may depend on many factors: environmental conditions, location of growth sites, different genotype, part of plant, the blossoming phase, and etc. (Büter et al., 1998; Walker et al., 2001).

The content of flavonoids and hypericin varies highly in species, different accessions, and parts of plant. Significant differences were detected in the mean concentrations of quercetin, hypericin, rutin and hyperoside+isoquercetrin in flowers and leaves of both species (Table 3). Larger amounts of quercetin, hypericin and quercitrin (only in *H. perforatum*) were found in flowers, while content of rutin and hyperoside+isoquercetrin was higher in leaves. The boxplots represent the distribution of chemical compounds in leaves and flowers of both species in *Hypericum*. The variation of constituents is expressed by displaying minimum-maximum and interquartile ranges relative to the median (Fig. 2) The total amount of flavonoids did not differentiate significantly between two species or between leaves and flowers, though it was an evident variation among accessions (especially in *H. maculatum* leaves). The mean concentrations of rutin and hyperoside+isoquercetrin in flowers, and quercitrin, hypericin as well as rutin in leaves were significantly higher in *H. perforatum* than in *H. maculatum*.

Differences in the content of constituents were found when our results were compared with those of other authors. The amount of total flavonoids in the examined accessions was higher than reported in Hungary (Plúhar, 2000). The values of rutin in *H. perforatum* given by Bombardelli and Morazzoni (1994) (0.28 %), Mártonfi and Repčák (1994) (0.42-0.74 %), and Umeck et al. (1999) (0.00-11.60 mg/g) were lower than our data (0.19-15.02 mg/g in flowers and 3.2-34.71 mg/g in leaves). As for rutin in *H. maculatum* the obtained amounts were very low both in flowers (0.00-0.14 mg/g) and leaves (0.00-0.22 mg/g). Other authors (Umeck et al., 1999) reported higher amount of rutin in flowers (0.15-0.50 mg/g) than obtained by us. Previous investigations of wild populations show higher content of rutin in flowers (0.42-0.77 mg/g) and leaves (0.39-2.43 mg/g) (Radušienė, 2002).

The obtained values of quercitrin and quercetin in our research were lower than those found by Mártonfi and Repčák (1994), who had given the contents from 0.215 to 0.48 % and 1.704-2.412 %, respectively. Their results show lower concentration of hyperoside+isoquercetrin in flowers (1.87-2.98 %) than our research. We observed small

amounts of hypericin in both species. The leaves of three accessions in *H. maculatum* did not contain hypericin. Larger concentration of hypericin was found in *H. perforatum* flowers (0.10-0.81 mg/g), which corresponds with the results of other authors (Hölzl and Ostrowski, 1987; Jensen et al., 1995; Walker et al., 2001).

The comparison of hypericin and hyperoside contents in *H. perforatum* revealed that accessions were rich either in hypericin or in hyperoside. The accessions with high hypericin amount (0.74-0.81 mg/g) contained low concentration of hyperoside (19.78-27.62 mg/g), and vice versa – low hypericin (0.12-0.17 mg/g) and high hyperoside (39.44-44.00 mg/g) content (Fig. 2). The results of chemical investigations in wild populations did not show reliable difference when compared to field accessions (Bagdonaitė et al., 2001).

Regarding flavoglycosides content, Franke et al. (1999) indicated two distinct *H. perforatum* forms: plants with a high content of rutin and hyperoside and plants with a very low content of rutin but with a high content of hyperoside and isoquercitrin. We have observed two accessions with high rutin (12.17-15.02 mg/g) and hyperoside+isoquercitrin (41.05-44.00 mg/g) concentrations, while one accession was distinguished by low amount of rutin (0.19 mg/g) and high content of hyperoside+isoquercitrin (33.43 mg/g).

The discrepancy between our results on the concentration of metabolites and those in other sources could be caused by environmental and genetic factors, mismatch of the blossoming phase. The results, in fact, very much depend on the extraction method, modification of HPLC method, and used equipment.

The material appeared to be phenotypically quite varying therefore needs the further investigations with the use of more elaborated methods. The germplasm of *Hypericum* may be a potential source of genetic variation to allow selecting the valuable material for breeding.

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## **Tables**

Table 1. Summary of the *Hypericum perforatum* characters and the differentiation of populations and field accessions according to each character by ANOVA Fisher's **F** and Student's **t** criterion.

Characters	Populations		Field accessions		t
	M max-M min	F	M max-M min	F	
Height of plant, cm	39.50-81.64	49.44	48.90-71.27	15.86	-2.62
Number of internodes	14.95-22.87	16.95	16.67-20.70	5.90	3.16
Length of inflorescences, cm	12.80-29.00	22.15	19.73-35.00	7.34	-6.35
Width of inflorescences, cm	7.15 - 14.65	11.84	11.80-17.50	4.03	-11.52
Length of petals, mm	8.03 - 10.60	16.50	8.70 - 13.27	24.99	-6.36
Width of petals, mm	3.50 - 5.16	16.82	5.15 - 7.13	12.39	-17.68
Length of sepals, mm	2.75 - 5.77	39.31	5.80 - 7.00	3.27	-20.32
Width of sepals, mm	1.05 - 1.56	3.47	1.30 - 2.07	3.33	-7.55
Length of leaves, mm	12.67-20.71	15.53	17.13-27.80	43.06	-21.55
Width of leaves, mm	4.17 - 9.53	29.81	7.49 - 10.13	13.22	-17.94
Dry weight of flowers, g	0.65 - 1.17	2.28	0.82 - 1.92	3.56	-6.25
Dry weight leaves, g	0.28 - 1.08	9.23	0.94 - 2.30	4.80	-13.83
Dry weight of stems	0.98 - 3.41	12.79	2.58 - 6.45	6.09	-10.95
Weight of raw material, g	1.58 - 2.97	2.69	1.89 - 5.35	4.35	-3.25

P< 0.05; M max-M min – range of mean characters among populations and accessions.

Table 2. Summary of the *Hypericum maculatum* characters and the differentiation of populations and field accessions according to each character by ANOVA Fisher's F and Student's t criterion.

Characters	Populations		Field accessions		t
	M max-M min	F	M max-M min	F	
Height of plant, cm	44.27-65.75	29.29	33.40-43.10	6.44	16.21
Number of internodes	15.87-21.40	36.74	16.60-22.60	3.05	-1.46
Length of inflorescences, cm	14.87-24.45	6.21	7.07-18.90	4.78	2.49
Width of inflorescences, cm	4.53-7.75	4.81	3.72-8.50	3.70	-2.29
Length of petals, mm	7.44-10.03	19.31	6.80-7.80	2.22	10.27
Width of petals, mm	4.00-5.20	12.91	4.10-4.87	2.07	0.60
Length of sepals, mm	3.27-4.10	6.03	3.30-4.50	4.21	-2.28
Width of sepals, mm	1.50-2.25	8.25	1.70-2.30	2.58	-0.44
Length of leaves, mm	16.27-21.64	9.36	18.10-27.90	9.71	-6.21
Width of leaves, mm	8.47-10.45	5.84	9.90-14.80	7.26	-9.98
Dry weight of flowers, g	0.13-0.51	15.61	0.09-0.54	3.36	-1.18
Dry weight leaves, g	0.36-0.81	10.29	0.290-0.75-	3.53	-1.67
Dry weight of stems	0.75-1.44	6.95	0.40-0.99	3.19	2.93
Weight of raw material, g	0.59-1.48	13.30	1.22-1.87	0.92	-3.06

P < 0.05; M max-M min – range of mean characters among populations and accessions.

Table 3. Concentrations of constituents in flowers and leaves of *Hypericum perforatum* and *H. maculatum* field accessions.

Constituents	Flowers				t
	<i>H. perforatum</i>		<i>H. maculatum</i>		
	M	Range	M	Range	
Total flavonoids (%)	5.11	4.01 - 6.87	5.39	4.45-6.37	-0.73
Rutin, mg/g	9.67	0.19-15.02	0.03	0.00-0.14	5.90
Hyperoside+ isoquercetrin, mg/g	32.10	19.78-44.00	25.53	20.08-31.13	2.35
Quercetrin, mg/g	0.67	0.05 - 1.26	0.51	0.03-0.85	1.03
Quercetin, mg/g	1.78	0.16 - 2.83	2.02	0.82-2.67	-0.71
Hypericin, mg/g	0.33	0.10 - 0.81	0.34	0.09-0.56	-0.03
	Leaves				
Total flavonoids (%)	5.54	4.29 - 6.60	4.76	3.49-6.53	1.824
Rutin, mg/g	24.40	3.2 -34.71	0.10	0.00-0.22	7.88
Hyperoside+ isoquercetrin, mg/g	35.09	22.86 43.52	30.50	25.36-35.42	1.92
Quercetrin, mg/g	0.68	0.00 - 4.77	0.25	0.00-0.64	0.78
Quercetin, mg/g	0.75	0.05 - 1.63	0.83	0.48-1.26	-0.39
Hypericin, mg/g	0.05	0.01 - 0.10	0.02	0.00-0.04	2.95

## Figures

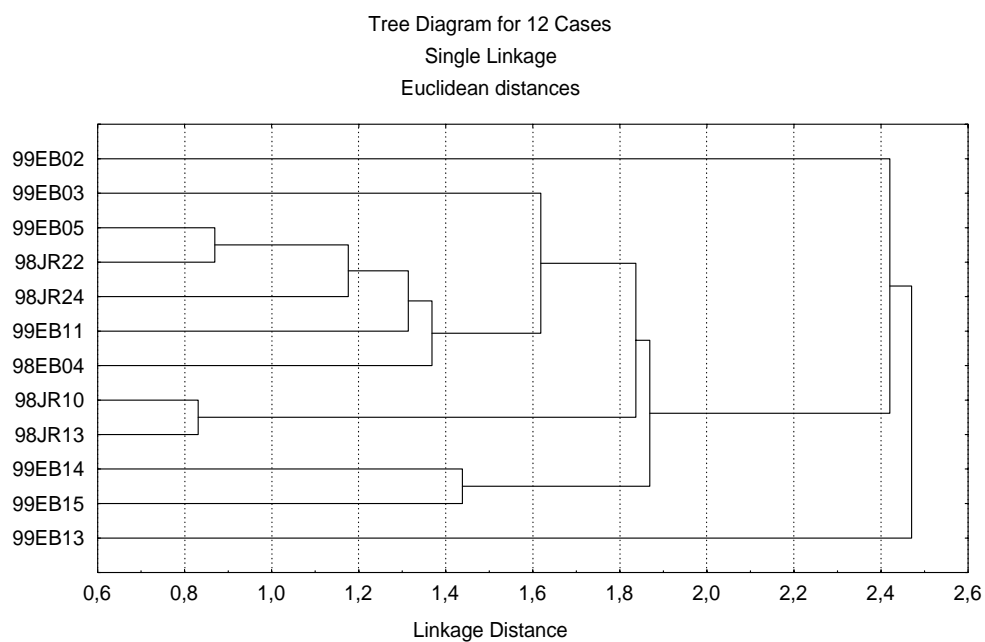


Fig. 1. The cluster analysis dendrogram of *Hypericum perforatum* populations based on their productivity.  
1 – *H. perforatum* flowers, 2 – *H. perforatum* leaves, 3 – *H. maculatum* flowers,  
4 – *H. maculatum* leaves.

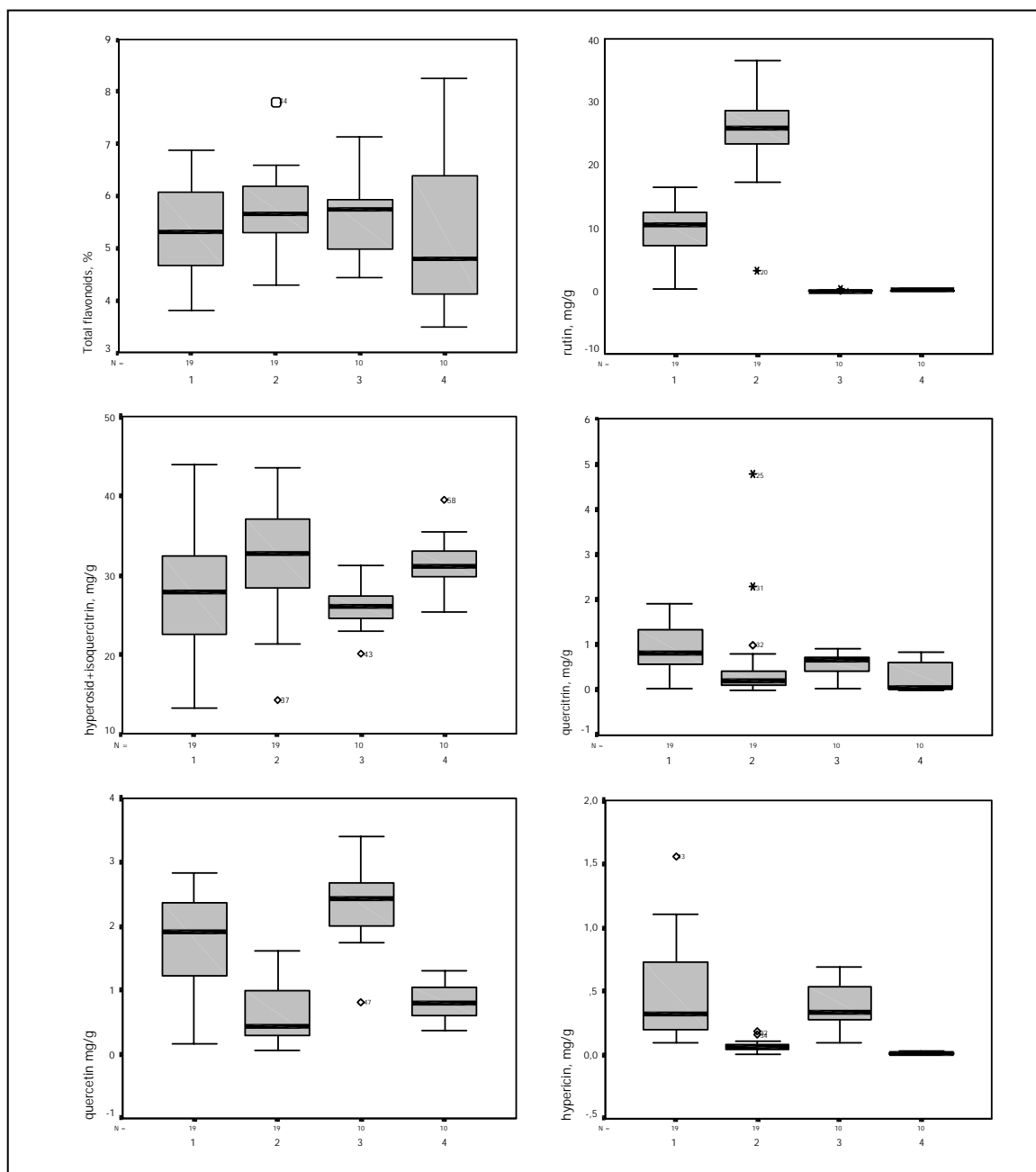


Fig. 2. Boxplots of chemical constituents in *Hypericum perforatum* and *H. maculatum* flowers and leaves. The length of each “box” denotes the interquartile range, the horizontal line inside the box represents the median value, and “whiskers” show the maximum and minimum concentrations of chemical compounds inside the group.