Genetic Diversity of Selected Medicinal Plants in Protected Landscape Areas in the Czech Republic

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Abstract: *Agrimonia eupatoria*, *Betonica officinalis*, *Hypericum perforatum*, *Plantago media*, *Salvia pratensis* and *S. verticillata* were selected as subjects for a research project studying the genetic diversity of wild medicinal plants in the Czech Republic. Some plants (both in the vegetative form and as seeds) from 26 localities at 5 protected landscape areas around the Czech Republic were transferred to the field nursery in Olomouc; there the contents of the active compounds (essential oil, tannins, hypericin and hyperforin, mucus) were evaluated. The large number of results provides a good opportunity to evaluate the quality of natural medicinal plants for pharmaceutical purposes; with regard to its origin, and to selecting starting materials for both breeding and the production of drugs.

Keywords: active substances content; medicinal plants; protected landscape area

The pharmaceutical utilization of medicinal and aromatic plants (MAPs) is strictly connected with the content of their active substances, which in turn depends on their genetic potential and the climatic conditions. Various populations of MAPs make stocks for pharmaceutical drugs with differential quality; and knowledge about its variability and quality is necessary for thoughtful use, conservation, and/or protection of genetic diversity. MAPs are also important and highly desirable components of natural flowering meadows; and for its conservation and re-cultivation of landscapes artificial seed mixtures are produced. However before the distribution of uniform seeds throughout the country, it is necessary to study the variability and biodiversity of the perspective genus.

In order to describe the degree of biodiversity of populations in a studied genus, very variable natural localities were chosen in collaboration with protected Czech landscape areas. *Agrimonia*

Table 1. Protected landscape areas and localities chosen for the experimental studies

Protected landscape area		No. and name of localities
A – Bílé Karpaty Mts	3	Suchovské mlýny, Březová, Brumov-Bylnice
B – České středohoří	3	Hradiště-Mentaurov, Babinské louky, Radobýl
C – Jizerské hory Mts	4	Podhora, Libíč, Rydvaltice, Záskalí
D – Moravský kras	10	Vilémovice sad, Vilémovice louky, Macošská stráň, Balcarka, Jedovnice rybník, Hájovna sad, Křtiny lem, Březina lom, Hádecká planina, Blansek
E – Šumava Mts	6	Vinice-Kašperské Hory, U kapličky, Nebe-Kašperské Hory, Karlina pila, Velký Bor, Dobrá-Stožec

Table 2. Content of active substances in the evaluated medicinal plants populations

Plant species	Evaluated characteristic	Protected landscape area										Average ± SD
		České středohoří		Jizerské hory Mts		Moravský kras		Bílé Karpaty Mts		Šumava Mts		
		No. loc.	content (min–max)	No. loc.	content (min–max)	No. loc.	content (min–max)	No. loc.	content (min–max)	No. loc.	content (min–max)	
Hypericum perforatum	hypericin content* (mg/g)	3	0.72–2.44	3	0.38–2.11	9	0.28–3.86	2	0.62–2.05	3	0.42–1.89	1.26 ± 0.73
	hyperforin content* (mg/g)		56.98–170.24		32.84–158.20		36.78–250.10		38.39–155.70		40.81–161.25	106.38 ± 37.40
Salvia pratensis	essential oil content** (%)	3	0.008–0.015	3	0.011–0.067	3	0.011–0.033	3	0.018–0.050	0		0.019 ± 0.014
Salvia verticillata	essential oil content** (%)	1	0.028–0.072	1	0.038–0.086	2	0.025–0.056	3	0.020–0.072	0		0.042 ± 0.013
Plantago lanceolata	content of mucus***	3	8.0–11.5	2	8.0–10.5	7	7.8–10.6	3	8.0–11.0	1	9.0–10.5	9.5 ± 0.81
	tannins content* (mg/g)	3	17.7–39.6	2	18.6–42.5	7	16.3–45.6	3	18.2–41.9	1	22.8–39.7	30.12 ± 9.230
Betonica officinalis	tannins content* (mg/g)	1	16.7–21.1	0		4	17.9–28.3	1	25.1–30.5	4	18.9–33.5	23.58 ± 4.056
Agrimonia eupatoria	tannins content* (mg/g)	3	30.5–63.9	3	29.6–54.8	1	41.7–56.0	3	35.7–56.8	0		45.718 ± 7.699

No. loc. – No. of evaluated localities; *average of 2 repetitions in years 2006 and 2007; **average of a minimum of 7 repetitions in years 2006 and 2007; ***content of mucus = No. of swelling; average of 3 repetitions in years 2004, 2005, and 2007; SD – standard deviation

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eupatoria, *Betonica officinalis*, *Hypericum perforatum*, *Plantago media*, *Salvia pratensis*, and *S. verticillata* were selected as the subjects of the research, and were transferred to the field nursery in Olomouc. In total, 26 localities at 5 protected landscape areas within the Czech Republic (Table 1) were chosen for this study; however, all six chosen plant species were not present in all of localities evaluated.

In 2004, the maternal plants (10 plants per locality, together with a ball of soil) and seed samples (in amounts that did not threaten the original population = 1 to 10 g) were collected in natural localities, and used for establishing an *ex situ* collection in Olomouc.

The contents of active compounds (essential oil, total polyphenols, hypericin and hyperforin, mucus) were evaluated according to standard laboratory methods. The quantity of the essential oil was measured gravimetrically by hydro distillation. The content of total polyphenols was measured by spectroscopy, and the content of mucus was evaluated by its swelling ability (all Pharmacopoeia Bohemica, ČsL 4 1987). Qualitative analyses of the essential oils were made by GC, and the content of hypericin and hyperforin in *Hypericum perforatum* was analysed by HPLC. At least two measurement repetitions were used for each sample and method. The statistical evaluations of the data obtained were done by ANOVA, one-factor analysis of variance with the level of importance ($\alpha = 0.05$, and 0.01, resp.).

Large differences between the original localities (climatic and soil conditions, etc.) usually influenced the content of the active compounds in medicinal plants from these localities. The transfer of the evaluated plants from their original localities to similar environments in the field nursery in Olomouc obviously brought changes in their habit and the content of active substances. This simple fact made it possible to compare these characteristics in plants from different localities and protected landscape areas, independent of the conditions and stresses of its genetic background.

Results of the contents of active substances in the evaluated plants are only presented here in a basic overview (Table 2). Large differences were found, for example, in *Hypericum perforatum*, where the content of hyperforin ranged between 32.84 and 250.10 mg/g. The most interesting results were found in the quality of the essential oil in *Salvia pratensis*; in the plants from the České Středohoří-Radobýl locality, a very high content of linalool was found (43.3%), compared to the plants from other localities (0.0–10.7%). On the other hand, a very low content of caryophylene (53.8%) was found in this sample, compared to other samples (86.4–97.0%); this indicates that the population from this locality is very different from the others. Big differences in the quality of the essential oils were also found between populations of *Salvia verticillata* (mainly in the content of caryophylene, humulene, and β-pinene).

Statistically significant and highly significant differences in the contents of active substances were shown between populations from chosen natural localities and protected landscape areas. Statistically significant differences, unfortunately, were not probed between the individual plants from each locality, because only mixed samples from each group of plants were tested. The large totality of the results gives a good opportunity to evaluate the quality of natural medicinal plants for pharmaceutical purposes. It enables the selection of suitable starting materials for breeding and the production of drugs.

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