Variability of the content and quality of some active substances within *Achillea millefolium* complex

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ABSTRACT: The present paper compares the production of active substances in different subspecies of *Achillea millefolium* complex growing in the Czech Republic. Representatives of 7 subspecies of *A. millefolium* complex (*A. setacea* Waldst. et Kit., *A. asplenifolia* Vent., *A. pratensis* Saukel et Langer, *A. collina* Rchb., *A. styriaca* Saukel et Langer subsp. *bohemica*, *A. millefolium* subsp. *millefolium*, *A. pannonica* Scheele) and several natural hybrids of *Achillea* L. were collected from 75 natural habitats in different parts of the Czech Republic. Plants were cultivated to investigate plant growth and contents of some effective compounds during 1997–1999. Development of plants was divided into 7 typical phenological phases. From the point of view of active substance production, the stage of early flowering was found as the most suitable phase for *Achillea* (yarrow) harvest. Air-dried powdered flowering heads were analysed for essential oil, tannin and flavonoid content. The essential oil content was obtained by hydro-distillation; the composition of essential oil was analysed by means of gas-liquid chromatography. The total essential oil content of the examined yarrow species ranged between 0.05% and 0.88% of dry matter; ecotypes of *A. collina* and some of its hybrids showed the best results with the highest content of deep blue essential oil. Content of tannins was determined according to PhBs IV; flavonoids were expressed as an apigenin content by an internal method of pharmaceutical company IVAX ČR, a. s., Opava. The total flavonoid content was in the range of 1.37–3.97%; the content of tannins ranged from 0.02 to 0.64%. The highest content of flavonoids was determined in the sample of *A. styriaca* subsp. *bohemica* (3.97%); the highest content of tannins was found in an *A. asplenifolia* sample (0.64%).

Keywords: *A. millefolium* complex; *A. setacea*; *A. asplenifolia*; *A. pratensis*; *A. collina*; *A. styriaca* subsp. *bohemica*; *A. millefolium* subsp. *millefolium*; *A. pannonica*; essential oil; hydro-distillation; chamazulene; tannins; flavonoids

Taxa of the widespread temperate *Achillea millefolium* complex (*A. millefolium* agg.) (*Asteraceae*), mostly scarcely separable ones with broad morphological, cytological and chemical diversity, are usually divided into several subspecies with diploid ($2n = 18$) to octaploid ($2n = 72$) forms (Greger, Werner 1990). Flowering tops and mainly their inflorescences are a rich source of active substances (essential oils, sesquiterpene lactones, flavonoids, tannins, etc.). Infusions of *Herba seu Flos Millefolii* are frequently medicinally used for their anti-inflammatory, antiphlogistic and spasmolytic properties (Jurenitsch 1992; Kastner et al. 1993; Michler, Arnold 1999).

Variability of the content of active substances and their composition during ontogenesis is a well known fact (Černaj et al. 1983). The *Achillea* complex is also characterised by a breakdown of crossing barriers at higher polyploid levels (Dąbrowska 1982; Vetter et al. 1996), so new hybrids and diverse transitional forms occur very often. That is why the quality of *Achillea* drug originating from wild habitats is quite non-homogeneous regarding active substance levels. As Pharmacopoeia Bohemica (1997) requests only a minimal content of essential oils (0.2%) and no determination of *Achillea* subspecies, so contemporary demand for yarrow is still provided by collecting herbs from natural stands. Agricultural production of yarrow would satisfy the market demands for high drug quality and quantity as well as protect devastation of nature and extinction of some subspecies from natural biocoenosis. Cultivation however, requires valuable and standardised chemotaxa, later on, cultivars. To find the best ecotypes for introduction into field cultivation an experiment was set up to investigate various taxa of the genus *Achillea* L. growing in the Czech Republic.

The present paper compares the plant development and production of essential oils, tannins and flavonoids in different subspecies of *A. millefolium* complex.

MATERIAL AND METHODS

Different subspecies of *A. millefolium* complex (*A. setacea* Waldst. et Kit., *A. asplenifolia* Vent., *A. pratensis* Saukel et Langer, *A. collina* Rchb., *A. styriaca* Saukel et Langer subsp. *bohemica*, *A. millefolium* subsp. *millefolium*, *A. pannonica* Scheele), 3 items of closely related

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A. distans and several natural hybrids of *Achillea* L. were collected from natural habitats in different parts of the Czech Republic in 1994–1996. *Achillea* plants were first grown at the Centre of Medicinal Herbs of Masaryk University at Brno; their chromosome numbers were assessed and plants were determined appropriately. From autumn 1996 to 1999, 100 representatives coming from 75 localities were cultivated in experimental fields, Faculty of Horticulture of Mendel University of Agriculture and Forestry, Lednice (164 m above sea level, mean temperature 9.1°C).

Since 1997, the onset of phenological phases was observed in about 2 weeks intervals. Contents of essential oils, tannins and flavonoids were determined in the full flowering stage. Considering the results achieved during observations in 1997 and/or 1998, plants from 19 (7) ecotypes with the highest content of active constituents and some natural hybrids were selected for an active collection of gene pool and other investigations. The onset of phenological phases and dynamics of active substances content were assessed in this collection. The flowering tops of plants were harvested in three different developmental stages (early flowering, full flowering, over-blooming stage), spread out on thin layers and air-dried in dark room.

The essential oil content was obtained by hydrodistillation using Clevenger apparatus; it was carried out according to the prescribed method for content – 'determination' No. 4.6.15. of PhBs IV. (30 g of dried powdered drug was distilled for 4 hours with 400 ml of water). The content of essential oil was determined gravimetrically; the quality of essential oils was roughly evaluated by adjusted STAHL (1953) colour scale (comparison of essential oil colour and colour scale) (Table 1). The composition of essential oil was also analysed by means of gas-liquid chromatography [HEWLETT PACKARD 5890 Series II, FID and a capillary column HP-INNOVAX (60 m × 0.53 mm i.d., film thickness 1.0 μm)] in a laboratory of Genebank in Olomouc. The oven temperature was programmed from 100°C to 150°C at 3°C/min, then 2°C/min to 200°C and then 15 min isothermally. The identity of components was assigned by comparison of their retention times with corresponding data of reference oil components.

Table 1. Colour scale for evaluation of essential oil quality

Essential oil colour	Chamazulene content
Colourless	none
Pale blue	none
Green blue	traces
Light blue	low content
Blue	medium content
Dark blue	high content

Content of tannins was determined according to PhBs IV; flavonoids were expressed as an apigenin content by an internal method (unpublished) of pharmaceutical company IVAX ČR, a. s., Opava.

Analysis of variance was carried out for all obtained results by Unistat; significant differences between variants were proved by Tukey's test (Tukey's-HSD).

RESULTS AND DISCUSSION

The collected plants establish a basis of gene pool of the genus *Achillea* L. (the gene pool was realised in a vegetative way). According to the realised observation of plants, the *Achillea* growth was divided into these 7 developmental stages: rosette leaves, elongation growth, budding, early flowering, full flowering, over-blooming and seed ripening (Table 2).

From the point of view of active substance production, the stage of early flowering was found to be the most suitable phase for *Achillea* harvest. However, because of the lack of plant material, analyses could be carried out only at 3 developmental stages (early flowering, full flowering and over-blooming). Individual subspecies differed considerably in the onset of early flowering phase as well as other phases. As DANIHELKA (2000) considers the knowledge of yarrow phenological phases as a very important taxonomic factor, these findings could be used for better identification of plants in wild habitats with concurrent occurrence of more yarrow subspecies.

As for the content and quality of essential oils, significant differences were found between the analysed subspecies. Total content of essential oils ranged from

Table 2. A proposal for phenological phase description of yarrow plants

	Developmental stage	Description
1	Rosette leaves	rosette leaves developed, stalks undeveloped
2	Elongation growth	stalks about 200 mm high, flower buds undeveloped
3	Budding	flower buds developed, ray and tubular flowers still hidden in buds
4	Early flowering	inflorescences partly developed, ray flowers visible, leaves well developed
5	Full flowering	inflorescences fully developed, ray and tubular flowers flowering
6	Over-blooming	inflorescences partly deflorated, ray and tubular flowers getting brown, involucral bracts losing green colour, leaves still green
7	Seed ripening	ray and tubular flowers deflorated, fully brown as well as involucral bracts fully brown, leaves decreasing

Table 3. Content and quality of essential oil determined in *A. millefolium* agg. at different phenological phases

Subspecies	No.	2*n*	Essential oils content (%)													
			1997		1998						1999					
			II	colour	I	colour	II	colour	III	colour	I	colour	II	colour	III	colour
Achillea asplenifolia	11	18	0.13	DB	0.26	DB	0.18	DB	**	–	0.30	DB	0.22	DB	0.24	DB
	23	18	0.22	DB	0.22	DB	0.18	DB	**	–	0.44	DB	0.33	DB	0.30	DB
Achillea setacea	162	18	*	–	**	–	0.55	B	**	–	**	–	0.45	PB	0.27	PB
	166	18	0.22	PB	0.44	LB	0.33	LB	**	–	0.62	PB	0.53	PB	0.27	PB
	168	18	*	–	**	–	0.55	B	**	–	0.77	PB	0.55	PB	**	–
Achillea collina	6	36	0.35	DB	0.55	DB	0.17	DB	0.33	DB	0.44	DB	0.55	DB	0.44	DB
	25	36	0.53	DB	0.65	DB	0.79	DB	0.26	DB	0.80	DB	0.79	DB	0.66	DB
	40	36	0.35	DB	0.35	DB	0.17	DB	0.26	DB	0.33	DB	0.27	DB	0.18	DB
	101	36	0.40	DB	0.44	DB	0.44	DB	**	–	0.62	DB	0.45	DB	0.22	DB
Achillea pratensis	46	36	0.13	B	0.22	B	0.09	LB	0.11	PB	0.44	PB	**	–	0.14	B
	50	36	0.13	PB	0.22	PB	0.09	LB	0.11	B	0.22	B	0.18	B	0.18	LB
	86	36	0.15	PB	0.22	B	0.09	B	0.11	B	0.05	B	0.06	B	0.06	LB
	213	36	*	–	**	–	0.35	B	**	–	0.11	B	0.18	B	0.18	B
Achillea styriaca subsp. *bohemica*	137	36	0.44	LB	0.66	B	0.35	B	0.44	B	**	–	**	–	**	–
	182	36	*	–	**	–	0.53	B	**	–	0.44	B	0.55	B	0.44	B
Achillea collina × *pratense*	19	36	0.49	DB	0.22	DB	0.18	DB	0.26	DB	0.27	DB	0.35	DB	0.18	DB
	67	36	0.26	DB	0.44	DB	0.35	DB	0.26	DB	0.56	DB	0.55	DB	0.36	DB
	210	36	*	–	**	–	0.44	DB	**	–	0.88	DB	0.79	DB	**	–
	223	36	*	–	**	–	0.26	B	**	–	0.45	B	0.53	B	0.66	B
Achillea collina × *styriaca*	186	36	*	–	**	–	0.44	DB	**	–	0.44	DB	0.44	DB	0.47	DB
Achillea collina × *millefolium*	159	45	0.35	LB	0.35	LB	0.35	LB	0.26	PB	0.53	B	0.35	B	0.35	B
Achillea pratensis × *millefolium*	151	54	0.27	GB	0.44	PB	0.26	PB	0.18	PB	0.33	B	0.26	B	0.26	B
Achillea millefolium subsp. *millefolium*	63	54	0.40	LB	0.33	B	0.09	B	0.26	B	0.47	PB	0.44	PB	0.44	PB
Achillea millefolium × *pannonica*	118	63	0.18	GB	0.11	PB	0.09	PB	0.26	PB	0.44	PB	0.44	PB	0.27	PB
Achillea pannonica	41	72	0.27	LB	0.26	LB	0.22	LB	0.18	PB	0.62	B	0.53	B	0.35	B
	120	72	0.42	LB	0.55	LB	0.26	LB	0.35	LB	0.71	PB	0.62	PB	0.35	PB

* not in active collection in 1997, ** not enough flowers for analysis

Developmental stage: I – early flowering; II – full flowering; III – over-blooming stage

Colour of essential oil: DB – deep blue, B – blue, LB – light blue, GB – green blue, PB – pale blue

Table 4. Content of flavonoids and tannins determined in *A. millefolium* agg. at different phenological phases

Subspecies	No.	2*n*	Flavonoid content (%)							Tannin content (%)						
			1997	1998			1999			1997	1998			1999		
			II	I	II	III	I	II	III	II	I	II	III	I	II	III
Achillea asplenifolia	11	18	**	1.99	2.13	1.79	2.38	2.63	2.02	**	0.42	0.37	0.44	0.51	0.54	0.58
	23	18	2.00	2.24	1.95	1.60	3.11	2.50	2.26	0.18	0.33	0.36	0.36	0.43	0.43	0.52
Achillea setacea	162	18	*	**	2.21	**	**	2.72	2.27	*	0.02	0.03	0.13	**	0.18	0.26
	166	18	1.87	2.29	1.99	1.59	2.75	2.84	2.21	0.06	0.16	0.19	0.20	0.16	0.12	0.30
	168	18	*	**	2.04	**	3.26	2.84	1.99	*	0.02	0.03	0.13	0.19	0.12	0.16
Achillea collina	6	36	2.12	2.08	1.94	1.64	2.67	2.39	2.17	0.12	0.33	0.35	0.29	0.22	0.32	0.36
	25	36	2.70	2.93	2.72	2.10	3.04	3.04	2.97	0.07	0.28	0.26	0.28	0.22	0.27	0.26
	40	36	**	2.04	2.02	1.41	2.50	1.93	1.76	**	0.30	0.27	0.32	0.46	0.38	0.42
	101	36	2.36	2.51	2.39	2.06	3.26	2.69	2.42	0.08	0.55	0.47	0.40	0.43	0.33	0.33
Achillea pratensis	46	36	2.23	2.13	1.96	1.74	2.08	2.03	2.09	0.05	0.44	0.50	0.62	0.44	0.46	**
	50	36	2.90	2.56	2.57	1.82	2.58	2.35	2.02	0.12	0.41	0.33	0.43	0.53	0.50	0.55
	86	36	2.00	2.87	2.19	1.53	2.75	2.82	2.54	0.11	0.49	0.42	0.45	0.36	0.32	0.34
Achillea styriaca subsp. *bohemica*	213	36	*	**	2.46	**	3.97	2.85	2.63	*	0.05	0.08	0.42	0.40	0.55	0.64
	137	36	2.94	3.27	2.94	2.58	**	**	**	0.12	0.52	0.35	0.36	0.50	0.42	0.52
	182	36	*	**	2.48	**	2.83	2.84	2.20	*	0.04	0.06	0.29	0.39	0.40	0.38
Achillea collina × *pratensis*	19	36	**	3.16	2.43	1.69	2.62	2.57	2.23	**	0.35	0.50	0.28	0.38	0.38	0.52
	67	36	2.21	2.56	2.38	1.63	2.84	2.61	2.20	0.10	0.34	0.35	0.37	0.32	0.23	0.24
	210	36	*	**	2.08	**	2.48	2.32	1.99	*	0.04	0.06	0.33	0.26	0.36	0.47
	223	36	*	**	2.22	**	2.70	2.45	1.79	*	0.02	0.04	0.19	0.40	0.34	0.35
Achillea collina × *styriaca*	186	36	*	**	2.19	**	2.88	2.49	**	*	0.03	0.05	0.03	0.32	0.33	**
Achillea collina × *millefolium*	159	45	2.62	2.67	2.55	1.82	3.08	2.87	2.20	0.11	0.36	0.48	0.46	0.30	0.32	0.39
Achillea pratensis × *millefolium*	151	54	2.35	2.25	2.22	2.02	2.85	2.51	1.89	0.09	0.46	0.49	0.39	0.47	0.41	0.44
Achillea millefolium subsp. *millefolium*	63	54	**	2.82	2.82	2.47	3.06	2.91	2.56	**	0.41	0.45	0.36	0.29	0.22	0.36
Achillea millefolium × *pannonica*	118	63	1.58	1.63	1.60	1.37	2.33	2.30	1.97	0.09	0.36	0.35	0.39	0.43	0.40	0.44
Achillea pannonica	41	72	1.72	1.99	1.85	1.39	1.98	2.06	1.80	0.10	0.47	0.39	0.40	0.43	0.33	0.38
	120	72	2.03	1.91	1.92	1.83	2.49	2.06	1.63	0.08	0.48	0.41	0.43	0.47	0.34	0.54

* not in active collection in 1997, ** not enough flowers for analysis
Developmental stage: I – early flowering; II – full flowering; III – over-blooming stage

Table 5. Significance of differences in essential oil content between individual items of *A. millefolium* complex

Item No.	+	PRAT			ASP		COL	COL × PRA	MIL × PAN	PRA × MIL	PAN	MIL	COL × MIL
		86	50	46	213	11	23	43	19	118	151	41	63	159
COL × MIL	159	*												
STYR	137	**												
STYR	182	**	*											
COL × STY	186	**												
PAN	120	**	**	*										
SET	166	**												
SET	168	**	**	**	**	**	*	*	*	**	*			
COL	6	**												
COL	101	**	*											
COL	25	**	**	**	**	**	**	**	**	**	**	**		*
COL × PRA	67	**												
COL × PRA	223	**	*											
COL × PRA	210	**	**	**	**	**	**	**	**	**	*	*		*

*significant difference, **highly significant difference

+ ASP – *A. asplenifolia*, SET – *A. setacea*, COL – *A. collina*, PRAT – *A. pratensis*, STYR – *A. styriaca* subs. *bohemica*, MIL – *A. millefolium*, PAN – *A. pannonica*, COL × PRA – *A. collina* × *pratense*, COL × STY – *A. collina* × *styriaca*, COL × MIL – *A. collina* × *millefolium*, PRA × MIL – *A. pratensis* × *millefolium*, MIL × PAN – *A. millefolium* × *pannonica*

0.05% in *A. pratensis* sample to 0.88% in *A. collina* × *pratense* sample (Table 3). The subspecies *A. collina* × *pratense, A. collina* and *A. styriaca* contained significantly different (higher) amounts of essential oils than *A. pratensis* and *A. millefolium* (Table 5). The colour of essential oil, closely related to its composition (particularly with chamazulene content) (RUMIŃSKA 1983), varied from pale blue colour to deep blue colour in the observed subspecies. The more blue the colour, the higher the amount of chamazulene (the most important compound) in the essential oil. The deep blue colour, which indicates a high content of chamazulene in essential oil, was typical only of essential oils of *A. asplenifolia, A. collina* and *A. collina* × *pratense.* Other items of yarrow contained essential oil with little or hardly any amount of chamazulene, as it is possible to deduce from their essential oil colours (Table 3).

Using GC the following compounds were further identified in the essential oil of most of the analysed samples: α-pinene, sabinene, β-pinene, myrcene, α-terpinene, limonene, 1,8-cineole, γ-terpinene, α- and β-thujone, camphor, caryophyllene, borneol and bisabolol. In spite of the well-known fact that individual subspecies differ in the composition of their essential oils, hardly any differences were found between their chromatograms. It is in good agreement with e.g. SCHULZ, ALBROSCHIET (1988), who confirmed that retention times are not reliable enough for qualitative evaluation of essential oils in general. That is why the quality of essential oil was further evaluated only by assessment of its colour according to STAHL (1953) scale of colours.

Like in ČERNAJ et al. (1983) it was shown also in this experiment that the highest content of essential oils was obtained mostly from samples at the stage of early flowering and the lowest mostly from samples at the over-blooming stage. Total content and also the colour of essential oil were found out relatively stable during the experimental years. The highest content of deep blue essential oil was determined in *A. collina* and *A. collina* × *pratense* samples at the stage of early flowering. Such characteristically coloured oil was also found out in samples of diploid *A. asplenifolia,* but its amount was lower there. The other diploid, *A. setacea* yielded light blue essential oil. The fact that *A. collina* and *A. asplenifolia* are considered as proazulene containing species (KASTNER et al. 1992, 1993) is generally known, but data concerning the chemical composition of *A. collina* natural hybrids are still missing. This experiment showed that hybrids of *A. collina* with other *Achillea* tetraploids resulted in plants with dark blue essential

Table 6. Significance of differences in flavonoid content between individual items of *A. millefolium* complex

Item No. +	MIL × PAN	PAN		COL	PRAT
	118	41	120	43	46
MIL 63	**	**		*	
COL 25	**	**	*	*	
STYR 137	**	**	*	*	
PRAT 213	**	**	*	**	*

*significant difference, **highly significant difference, + explanation of abbreviations see Table 4

Table 7. Significance of differences in tannin content between individual items of *A. millefolium* complex

Item No. +	SET			COL × STYR
	168	162	166	186
PAN 120	*			
PRAT × MIL 151	*			
STYR 137	*			
COL × PRAT 19	*			
PRAT 50	*			
PRAT 46	*			
ASP 11	**	**	*	*

*significant difference, ** highly significant difference, + explanation of abbreviations see Table 4

oil (*A. collina* × *pratense, A. collina* × *styriaca*) even though these tetraploids are so called proazulene-less ones with only light blue essential oil (*A. pratensis, A. styriaca*). Hybrids of *A. collina* with hexaploid *A. millefolium* subsp. *millefolium* (*A. collina* × *millefolium*) showed light blue or blue coloured oil as well as *A. millefolium* subsp. *millefolium* and its hybrid (*A. millefolium* × *pannonica*). Almost colourless oil with no content of chamazulene was obtained from a drug of *A. millefolium* × *pratensis*. From medium to high content of essential oil was obtained in *A. styriaca* subsp. *bohemica* and in *A. pannonica* samples; however these oils were also light blue or blue coloured (low or medium chamazulene content). The lowest content of essential oil was found out almost in all cases in samples of *A. pratensis* with chamazulene at a trace to medium amount.

Concerning the analysis of flavonoids and tannins, it was proved that the content of flavonoids decreased during development of blooms while dynamics of tannin content was found irregular **(**Table 4**).** The total flavonoid content was obtained in the range of 1.37–3.97%; the content of tannins ranged from 0.02 to 0.64%. The highest content of flavonoids was found in samples of *A. styriaca* subsp. *bohemica* at the stage of early flowering in 1997 and 1998 and in the drug of *A. pratensis* also at the early flowering stage in 1999. These subspecies together with *A. collina* and *A. millefolium* differed significantly from the others, particularly from *A. pannonica* and *A. millefolium* × *pannonica* in the lowest content of flavonoids (*A. millefolium* × *pannonica* samples at all developmental stages in 1997 and 1998 and *A. pannonica* sample in 1999) (Table 6).

As for tannins, the highest content was found in samples of *A. pratensis* at the over-blooming stage (except the year 1997, when *A. asplenifolia* sample had the highest tannin content); the lowest content of tannins was observed in samples of *A. setacea* at all developmental stages of flowering heads. *A. setacea* and *A. collina* × *styriaca* contained significantly different (lower) amounts of tannins than the other subspecies (Table 7).

The experiment has shown that the contents of essential oil, flavonoids and tannins vary during flower ontogenesis as well as that subspecies and ecotypes differ considerably within *A. millefolium* complex. The best ecotypes of *A. collina* and *A. collina* × *pratense* (with the highest content of dark blue essential oil) can be recommended for further experiments, breeding or for introduction into field cultivation. The results concerning natural hybrids of yarrow have not been found in the Czech Republic yet. The phenological phase description could serve as an additional tool for the identification of some yarrow subspecies. The collection of other plants from natural habitats and analyses of their active substances are worth to be continued.

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Variabilita obsahu a kvalita účinných látek jednotlivých subspecií komplexního druhu *Achillea millefolium* L.

ABSTRAKT: Práce se zabývá hodnocením vývoje rostlin a porovnáním produkce účinných látek u jednotlivých subspecií komplexního druhu *Achillea millefolium*. Zástupci sedmi subspecií komplexního druhu *A. millefolium* (*A. setacea* Waldst. et Kit., *A. asplenifolia* Vent., *A. pratensis* Saukel et Langer, *A. collina* Rchb., *A. styriaca* Saukel et Langer subsp. *bohemica*, *A. millefolium* subsp. *millefolium*, *A. pannonica* Scheele) a několik přirozených kříženců řebříčku bylo získáno ze 75 různých lokalit v České republice. V letech 1997–1999 byl na pozemcích Zahradnické fakulty v Lednici Mendelovy zemědělské a lesnické univerzity v Brně sledován růst rostlin jednotlivých subspecií a na základě pozorování byl jejich vývoj rozdělen do sedmi fenologických fází. Stadium nakvétání bylo vybráno jako nejvhodnější pro sklizeň řebříčku. Droga byla analyzována na obsah silice, třísloviny a flavonoidů. Množství silic v droze bylo určeno destilací vodní parou; složení silice bylo stanoveno na plynovém chromatografu. Zjištěný obsah silic se pohyboval v rozmezí 0,05–0,88 % (přepočteno na sušinu); vzorek *A. collina* × *pratense* obsahoval nejvyšší množství silice tmavě modré barvy. Obsah třísloviny byl stanoven podle ČsL IV.; obsah flavonoidů byl vyjádřen jako apigenin podle interní normy farmaceutické firmy IVAX ČR, a. s., Opava. Obsah třísloviny se pohyboval v rozmezí 0,02 až 0,64 %; nejvyšší obsah byl analyzován ve vzorku *A. asplenifolia* (0,64 %). Celkové množství flavonoidů bylo stanoveno v rozmezí 1,37–3,97 %; nejvyšší obsah byl zjištěn ve vzorku *A. styriaca* subsp. *bohemica* (3,97 %).

Klíčová slova: *A. millefolium* complex; *A. setacea*; *A. asplenifolia*; *A. pratensis*; *A. collina*; *A. styriaca* subsp. *bohemica*; *A. millefolium* subsp. *millefolium*; *A. pannonica*; silice; destilace vodní parou, chamazulen; třísloviny; flavonoidy

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