Variability of the content of active substances during *Achillea collina* Rchb. Alba ontogenesis

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ABSTRACT: The fluctuation of essential oil, flavonoid and tannin content in *Achillea collina* Rchb. Alba (*flos* and *herba millefolii*) during ontogenesis was studied in this paper. Ten developmental stages of plants – from the beginning of flower differentiation to ripe seed stage – were tested for 3 years. The essential oil content was found to range between 0.4 and 1.6% of dry material and it was statistically significantly (one-factor analysis of variance; $\alpha = 0.05$) influenced by developmental stage, harvested part of the plant and also by seasonal climatic conditions. The flavonoid content was influenced only by developmental stage and not by the type of yarrow drug or season. Its values ranged from 0.7 to 6.0% of dry material and it had a clear decreasing tendency during the plant ripening. Compared to flavonoids tannin production showed a reverse trend in yarrow. Their content was 0.3–1.0% and it increased during seed maturing. However differences between tannin contents during yarrow ontogenesis are not so dramatic and the influence of harvested part and season is also disputable. Comparison of the content of active substances in the drug from one- and six-years old yarrow plants did not indicate any differences due to age. Developmental stage V, beginning of flowering, can be recommended to producers as an optimum harvest stage because in this period the drug has the highest essential oil content in relation to plant mass yield and also to flavonoid and tannin contents.

Keywords: *Achillea collina*; essential oils; flavonoids; tannins; variability; ontogenesis

The fluctuation of content of active substances in medicinal plants during ontogenesis is a well-known fact. Collecting of plants for pharmaceutical use should be practised in the stage of development with optimal content and composition of their active substances.

Achillea collina Rchb., and especially the Alba variety, is the best species of *Achillea millefolium* s.l. complex (yarrow) used in pharmaceutics because of high essential oil yield and the presence of chamazulene in the oil. Not only essential oil is of therapeutic importance but also flavonoids and tannins play a key role in the curative action of yarrow.

The dynamics of essential oil production depends on ontogenesis and morphological part of the plant and this fact was investigated in yarrow for example by Rumiňska (1970), Černaj et al. (1983) and Figueiredo et al. (1992). Information about flavonoid and tannin content variability during yarrow ontogenesis is currently missing.

The present paper deals with the variability of contents of essential oils, flavonoids and tannins and their correlations in *Achillea collina* Rchb. Alba during plant ontogenesis.

MATERIAL AND METHODS

Plant material

The *Achillea collina* Rchb. Alba plants cultivated at Faculty of Horticulture in Lednice of Mendel University of Agriculture and Forestry in Brno were used in this experiment. Alba variety was improved by Faculty of Natural Sciences, Pavel Jozef Šafárik University in Košice (Slovak Republic) in 1992 and according to declaration it contains 0.4% of essential oils and 26% of proazulenes (Osvědčení 1992). The cultivation of yarrow is usually recommended to last 3–4 years but the plants used for the main part of this experiment were already 4 years old at the beginning of experiment. Their cultivation started in 1996 and to compare the content of active substances and its variability during ontogenesis in the cultures of different age another planting was founded in 2001.

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Fig. 1. Ten different developmental stages of plants

The two types of plant aerial parts were analyzed: "*flos millefolii*" – flowers with stems about 5 cm long – and "*herba millefolii*" – flowering tops with stems about 30 cm long. Plant material was dried up under laboratory temperature conditions and ground with a laboratory grinder.

The content of active substances was determined in the following 10 developmental stages of plants (Fig. 1):

I beginning of flower-bud differentiation,
II flower buds are partly developed, green, flower top is covered by compact hairs,
III flower buds developed, green; ray and tubular flowers both undistinguished, hidden in buds,
IV flower buds developed; ray flowers start to jet out from the buds, tubular flowers still hidden in buds,
V inflorescences partly developed; tubular and ray flowers flowering at < 50% of inflorescences,
VI inflorescences partly developed; tubular and ray flowers flowering at > 50% of inflorescences,
VII fully developed inflorescences; tubular and ray flowers flowering at 100% of inflorescences,
VIII shedding of blossoms; tubular and ray flowers getting gradually brown; leaves still remain green,
IX inflorescences almost brown; seed formation; leaves decreasing, getting brown,
X inflorescences and leaves completely brown and almost dry; ripe seeds.

Essential oils

The essential oil was obtained by steam distillation. Dried powdered drug (30 g) was distilled with 500 ml water for 3 hours. The water distillation method with decalin ($C_{10}H_{18}$; 0.1 ml) according to the Pharmacopoeia Bohemoslovenica IV (ČsL 1987) was used. The content of essential oil was determined gravimetrically.

Tannins

The tannin content was determined by a method based on the colour reaction of tannins with ammonium phosphowolframate and phosphomolybdate and photometric measurement of the colour (ČsL 4, 1987). 2,000 g of ground plant material was boiled in distilled water under a reverse cooler. Tannins are a precipitate of ammonia solution of sodium acetate and the precipitate is separated on a centrifuge. Clear precipitate is dissolved in 5% sulphuric acid and a small part of this solution is mixed with Folin-Ciocalteu reagent and sodium carbonate. The intensity of the yellow-green solution colour is measured after 1 hour at 720 nm against the blank sample of distilled water with Folin-Ciocalteu reagent and sodium carbonate. The tannin content is read off the calibration curve. The calibration curve was constructed from 0.5; 1.0 and 2.0% tannin solution in distilled water.

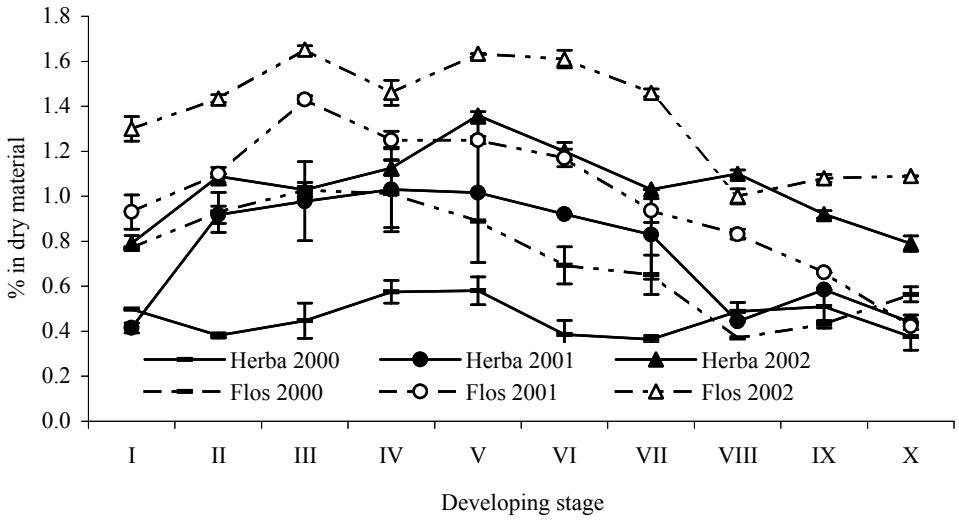

Fig. 2. Essential oil content (%) in *Achillea collina* Rchb. Alba

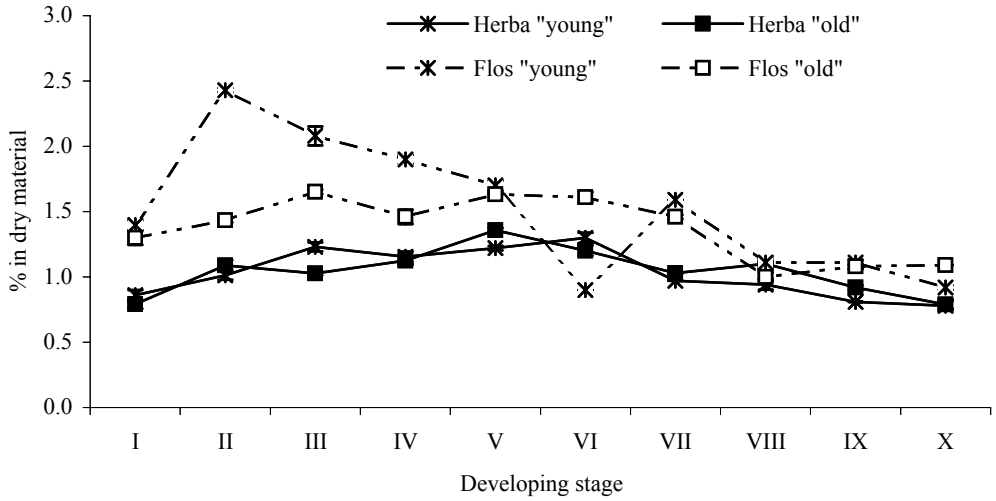

Fig. 3. Comparison of essential oil content (%) in 1 and 6 years old plants

Flavonoids

The flavonoid content was determined like apigenin content by an internal method of the firm UNIGEO, a. s., Ostrava. 0.400 g of powdered plant material is shaken with 50 ml of 60% ethanol for 20 hours; 1.00 ml of the extract is diluted to 50 ml of 60% ethanol and mixed. The absorbance of solution is measured at 336 nm against blank sample of 60% ethanol. The flavonoid content is expressed as apigenin (X [%]) after recalculation:

$$X = \frac{Avz \times 2{,}500}{q \times 785} \quad (\%)$$

where: Avz – absorbance of drug sample,
q – weight of plant material (g),
785 – specific absorption constant of apigenin.

Recalculation of contents per dry matter

All data on the active substance content in yarrow drug are recalculated per plant dry matter. Plant material (air-dried under laboratory conditions) is heated in an oven at 105°C for 4 hours – after this manipulation the weight of plant mass remains constant (according to ČsL 4, 1987). This dry matter makes about 80–90% of yarrow air-dried material.

Statistical evaluation

Each measurement was made in two parallel replications and one-factor analysis of variance at the significance level $\alpha = 0.05$ was used for statistical evaluation of data.

RESULTS AND DISCUSSION

Content of essential oils

As the first result of this experiment it is necessary to say that the essential oil content in *Achillea collina* Rchb. Alba is very high compared to the other yarrow species (Špinarová, Petříková 2003). It exceeds the value minimally 0.4% at all investigated developmental stages independently of the harvested part and year of harvesting. But the essential oil con-

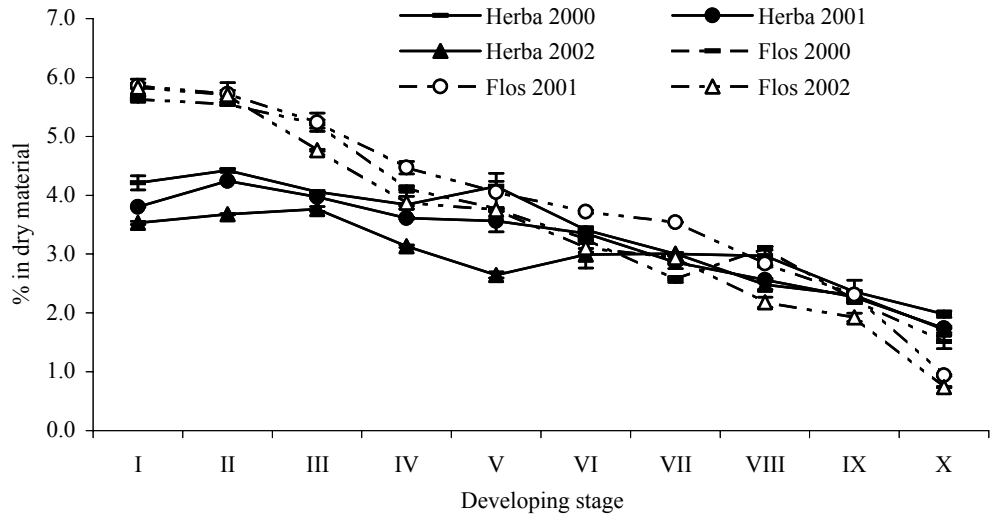

Fig. 4. Flavonoid content (%) in *Achillea collina* Rchb. Alba

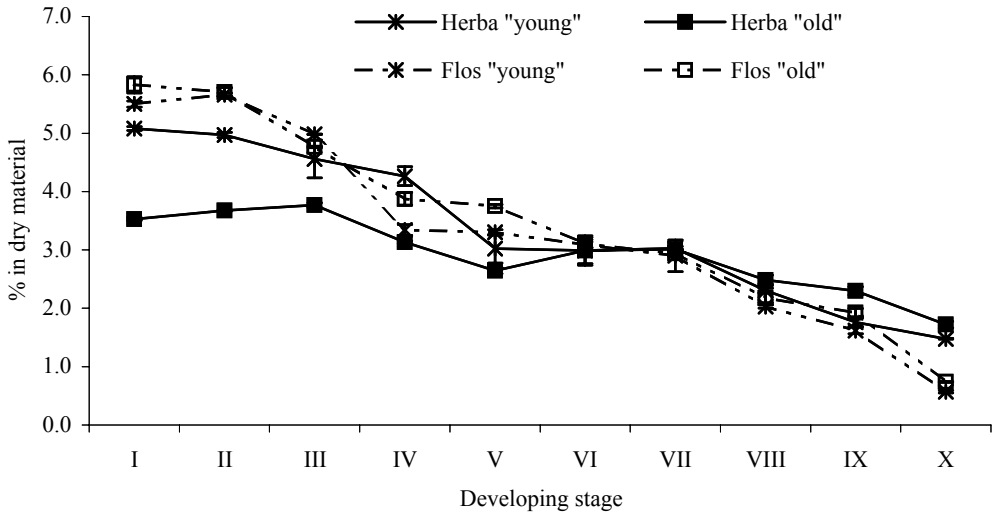

Fig. 5. Comparison of flavonoid content (%) in 1 and 6 years old plants

tent in this yarrow variety is broadly much higher – it can also exceed 1.6%, which is the value several times higher than the limits set by Czech pharmacopoeia – 0.2% (ČL 2002).

Detailed evaluation of essential oil content brought some surprising information compared to literature. According to Černaj et al. (1983) the highest amount of essential oil is produced in yarrow aboveground parts at the stage of fully developed inflorescences while according to our results it is earlier. In the case of *millefolii herba* it was at stage IV–V, it means fully developed flower bud or beginning of flower bud opening. In the case of *millefolii flos* the highest amount of essential oil was found even at stage III, when buds are developed but they are not going to open themselves yet.

Statistically significant differences were found between the results of all variants. They were proved between essential oil contents of individual developmental stages, between harvested parts (*flos millefolii* had significantly higher content of essential oils than *millefolii herba*) and the influence of the season (climatic conditions during planting) was also significantly. Detailed data on essential oil content in *Achillea collina* Rchb. Alba are shown in Fig. 2.

The comparison of essential oil contents in yarrow drug that came from the "old" and "young" planting (this fields has been found in 1996 and 2001, essential oil analysis was done in 2002) also provided interesting information. It was observed that both "young" and "old" yarrow plants were able to synthesize essential oil at the same level – no significant difference was found between essential oil content in plants 1 and 6 years old (Fig. 3).

Content of flavonoids

Brief information about flavonoid content during three developmental stages of yarrow was presented by Špinarová and Petříková (2003). The flavonoid content in some wild *Achillea collina* plants at stages "early flowering", "full flowering" and "over-bloom-

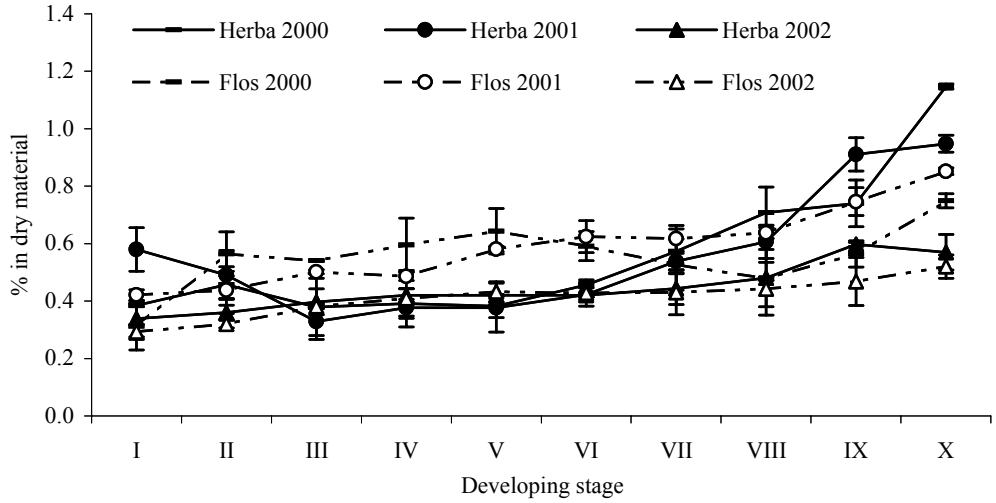

Fig. 6. Tannin content (%) in *Achillea collina* Rchb. Alba

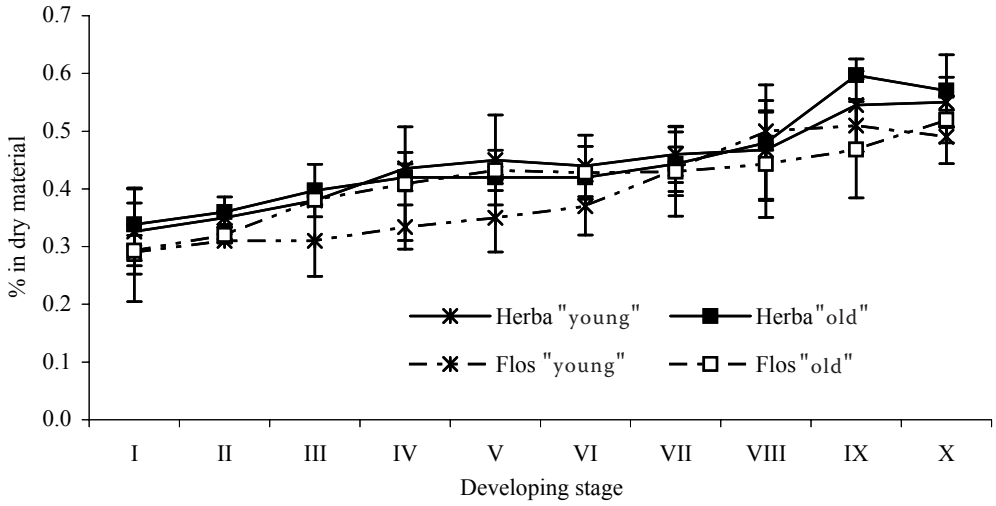

Fig. 7. Comparison of tannin content (%) in 1 and 6 years old plants

ing" was established to amount to about 1.5–2.5% in dry material with a slightly decreasing trend towards a higher flowering stage. Our results confirm and specify this data. The flavonoid content (as well as essential oil content) in *Achillea collina* Rchb. Alba was significantly higher compared to wild yarrow. At the stage of full flowering the flavonoid content amounted to about 3% of dry material. During all ten evaluated stages the yarrow flavonoid content ranged between 0.7–6.0% in dry material and the dynamics of its production had a clear decreasing trend during plant development. The highest flavonoid content was found at stage I – beginning of flower differentiation and the lowest flavonoid content was observed in stage X – ripe seeds. Statistically significant differences in this characteristic were found between yarrow developmental stages, but no differences were proved between harvested parts of the plant and seasons (Fig. 4). The influence of yarrow planting age was not observed either (Fig. 5).

Content of tannins

The content of *A. collina* tannins was also mentioned in Špinarová and Petříková (2003). 0.1–0.6% of tannins was found in yarrow dry drug at stages "early flowering", "full flowering" and "overblooming" and we did not find any changes in this characteristic according to development of yarrow drug. Detailed analysis of tannin content in *Achillea collina* Rchb. Alba showed the following data. Flavonoid content was established on the level 0.3–1.0% during all developmental stages of the drug (Fig. 6). A slightly increasing tendency was found at later developmental stages and no statistically significant difference was found between the harvested parts of yarrow. The influence of the season and developmental stage on tannin content in yarrow is not so unfortunately clear. Differences between seasons were found only in 2002 and only in the case of *flos millefolii* drug. On the contrary, statistically significant differences between developmental stages of the drug were found only in the drug *herba millefolii*. No influence of yarrow plant age was proved (Fig. 7).

CONCLUSION

Essential oils and as well as flavonoids and tannins as co-active substances of yarrow drug in *Achillea collina* Rchb. Alba showed clear dynamics interrelated with yarrow plant ontogenesis. The curve of essential oil content has a slight sinusoid shape – from 0.5–1.3% at the beginning of flower differentiation to maximum 0.6–1.6% at the stage of flowering beginning and back to 0.4–1.1% at the stage of ripe seeds. The curves of flavonoid and also tannin contents have a linear shape. During yarrow ontogenesis the flavonoid content increases (from 3.5 to 6.0% in dry material) and tannins also increase (from 0.3 to 1.1% in dry material).

In contrast with the commonly used rule for yarrow drug collecting (at the stage of full flowering) for an effective use of yarrow plant potential it is necessary to recommend an earlier stage of flowering. According to our results developmental stage V – beginning of flowering is optimum for harvesting because in this period the essential oil content is highest in relation to plant mass yield and also flavonoid and tannin content.

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Variabilita obsahu účinných látek v průběhu ontogeneze *Achillea collina* Rchb. Alba

ABSTRAKT: Předmětem práce je studium dynamiky obsahu silic, flavonoidů a tříslovin v řebříčkové droze *flos* a *herba millefolii* v průběhu ontogeneze řebříčku chlumního (*Achillea collina* Rchb. Alba). Obsah jeho účinných látek byl po tři roky zkoumán v deseti vývojových stadiích rostlin – od počátku diferenciace květenství až po stadium zralých semen. Bylo zjištěno, že obsah silic se u této drogy pohybuje v rozmezí 0,4–1,6 % suché hmoty a je statisticky průkazně (jednofaktorová analýza variance; α = 0,05) ovlivňován vývojovým stadiem rostlin, sklizňovou částí i klimatickými vlivy v průběhu pěstování. Obsah flavonoidů je naproti tomu ovlivněn pouze vývojovým stadiem rostlin a pohybuje se mezi 0,7–6,0 % suché hmoty bez ohledu na typ drogy. Obsah flavonoidů vykazuje u řebříčku ve směru vývoje rostlin jasný negativní trend. Změna obsahu tříslovin má naproti tomu zcela opačnou tendenci – v průběhu ontogeneze jejich množství v droze stoupá z 0,3 až na 1 % suché hmoty. Ovlivnění jejich výtěžnosti z řebříčkové drogy výběrem sběrové části nebo klimatickými podmínkami při pěstování je diskutabilní. Při srovnání obsahu účinných látek v droze z jednoletého a šestiletého řebříčkového porostu bylo zjištěno, že stáří rostlin jejich množství neovlivňuje. Jako optimální sklizňové stadium je pro pěstitele a zpracovatele řebříčkové drogy vzhledem k obsahu terapeuticky aktivních látek možné doporučit stadium začátku kvetení – v této době obsahuje droga nejvyšší obsah silic s ohledem na výtěžnost hmoty a také na obsah doprovodných účinných látek – flavonoidů a tříslovin.

Klíčová slova: *Achillea collina*; silice; flavonoidy; třísloviny; variabilita; ontogeneze

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