Accumulation of flavonoid compounds in flowering shoots of *Achillea collina* Becker ex. Rchb. Alba during flower development

K. Karlová

Department of Gene Bank, Research Institute of Crop Production Prague-Ruzyně, Workplace in Olomouc, Czech Republic

ABSTRACT: The objective of this paper was a screening of flavonoid content in *Achillea collina* Becker ex. Rchb. Alba flowering tops (*Herba millefolii*) and flowers (*Flos millefolii*). Ten developmental stages of plant (flowers and tops separately) – from the beginning of flower differentiation to ripe seed stage – were studied. The methanol extract of plant material was used for HPLC analysis. The flavones apigenin, luteolin, and their 7-O-glucosides were found as the main flavonoid constituents in all developmental stages of both flower and top drug. Apigenin and apigenin-7-O-glucoside contents have a similar accumulation tendency during ontogenesis – their contents fluently increased until the full flower phase and then they decreased. Maximal apigenin and apigenin-7-O-glucoside content was established to range between 0.6 and 0.7 mg/g in *millefolii herba* and 0.9 and 1.3 mg/g in *millefolii flos*. The luteolin content has the same tendency and also quantity as apigenin and the highest value at the full flowering stage was found even stronger. On the other hand, luteolin-7-O-glucoside has the highest content at the stage of small flower bud (about 1.0 mg/g). Its amount decreased later on and it increased to the second maximum at the full flower phase.

Keywords: *Achillea collina*; flavonoids; apigenin; luteolin; HPLC; ontogenesis

The yarrow (*Achillea* L.) is one of the most asked stocks of pharmaceutic, cosmetic and food industries. Its effects on the human organism are antispasmodic, amarum, stomachic, carminative and cholagogum. The main effective substance of yarrow drug is essential oil but the antispasmodic activity is due to flavonoid content. The antioxidant activity of flavonoids is also indisputable (Bors et al. 1990; Rice-Evans et al. 1997; Pulido et al. 2000). Their presence and efficiency in some *Achillea* L. species were demonstrated already in 1961 (Hörhammer in Guédon et al. 1993) and later on confirmed by many authors (Valant-Vetschera 1987; Schultz, Albroscheit 1988).

Problems of yarrow flavonoid content have already been discussed in literature several times but information about flavonoids of *Achillea collina* Becker ex. Rchb. Alba, one of the most pharmaceutically effective taxa of *Achillea* genus, is still missing. It is commonly known that above all flavones (luteolin, apigenin and their derivates) are present in yarrow plants. Information about their amounts unfortunately differs very much (Chandler et al. 1982; Franzén 1988) and the dynamics of their formation during plant ontogenesis has not been solved until now at all. The very fact, exact information about effective compound formation, is important for producers and processing industry to optimise the harvesting period of pharmaceutical drugs. The aim of this work is to carry out primary study of flavone content variability in *Achillea collina* Becker ex. Rchb. Alba tops and flower heads during plant ontogenesis.

MATERIAL AND METHODS

Plant material

Achillea collina Rchb. Alba plants cultivated at the Faculty of Horticulture in Lednice of Mendel University of Agriculture and Forestry Brno were used in our experiment. Description of plant mate-

Supported by the Faculty of Horticulture in Lednice of Mendel University of Agriculture and Forestry Brno, Research Plan MSMT No. 435100002.

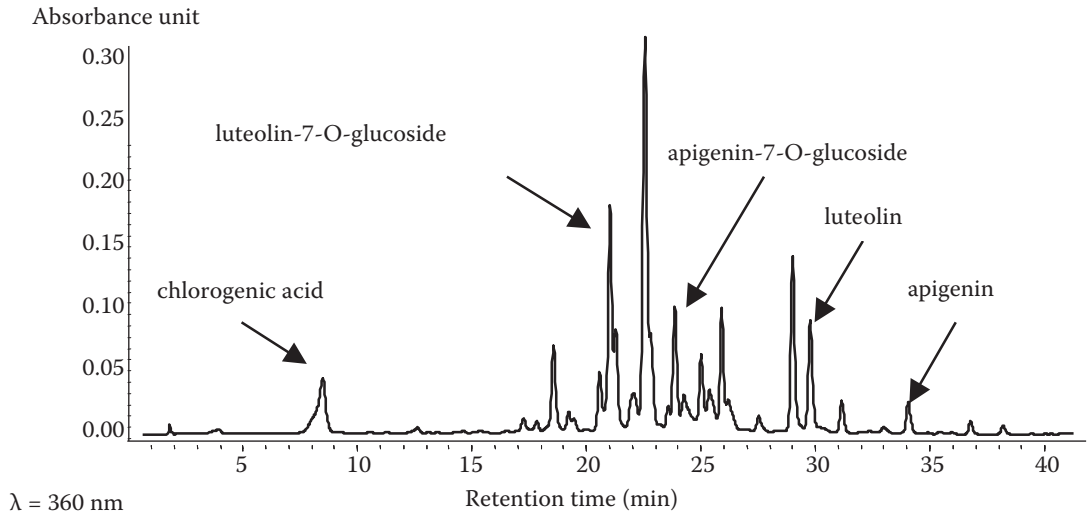

Fig. 1. Typical HPLC chromatogram of MeOH extract of yarrow

rial, harvesting details and also determination of 10 developmental stages of plants (stage I = beginning of flower-bud differentiation; stage VII = fully developed inflorescences; tubular and ray flowers flowering at 100% of inflorescences; stage X = inflorescences and leaves are completely brown and almost dry; ripe seeds) were presented in a previous article (Karlová, Petříková 2005).

Extraction, HPLC analysis and recalculation per dry matter

The content of flavonoids was determined in their glycoside form and therefore nonhydrolysed plant extracts were prepared: 0.25 g of ground drug was extracted in UV bath in 1.875 ml of 80% MeOH for 15 min and the extract was filtrated through a 0.2 μm PTFE filter.

MeOH extracts of flavonoids were analysed by Waters 600E HPLC system with reverse column Nova-Pak C_{18} (3.9 × 150 mm, inside diameter 4 μm) (Waters Chromatography, Milford, MA). The mobile phase acetonitrile – 0.1% TFA (trifluoroacetic acid) was used for flavonoid separation in the column together with a gradient program: linear 5–25% per 30 min, 25–30%/5 min and 30–50%/2 min, and washing by 50% acetonitrile in 0.1% TFA for another 3 min; flow rate 1.0 ml/min. The spectra of flavonoid compounds coming through the column were on-line screened by Waters TM996 PAD detector at the wavelength range 190–900 nm. Peak purity, identification, integration and calibration of flavonoids were proved by Waters Millenium^{32} software, version 3.05.01 and commercial available flavonoid standards (Apin Chemicals, Ltd., Abingdon, Oxon, UK).

All data on flavonoid contents in the 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 Československý lékopis 1987). This dry matter makes about 80–90% of the yarrow air-dried material.

Statistical evaluation

Each measurement was done in two parallel replications and one-factor analysis of variance at the significance level α = 0.05 (in Excel software environment) was used for statistical evaluation of data.

Table 1. Maximal contents of apigenin, luteolin and their 7-O-glucosides in *Achillea collina* Becker ex. Rchb. Alba

Flavonoid	Maximal content (mg/g)		In developmental stage	
	Herba mill.	*Flos mill.*	*Herba mill.*	*Flos mill.*
Luteolin-7-O-glucoside	0.923 ± 0.001	1.096 ± 0.147	II	I
Luteolin	0.613 ± 0.054	0.999 ± 0.047	VII	VII
Apigenin-7-O-glucoside	0.656 ± 0.043	1.295 ± 0.054	VII	VII
Apigenin	0.601 ± 0.049	0.852 ± 0.020	VII	VII

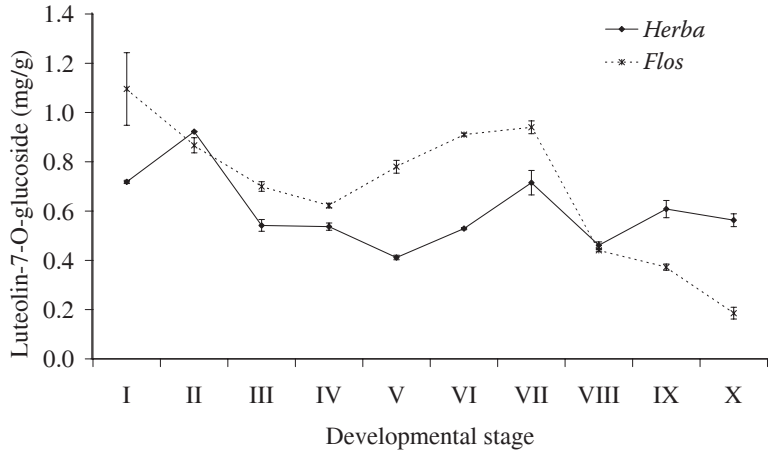

Fig. 2. Content of luteolin-7-O-glucoside in *Achillea collina* Becker ex. Rchb. Alba

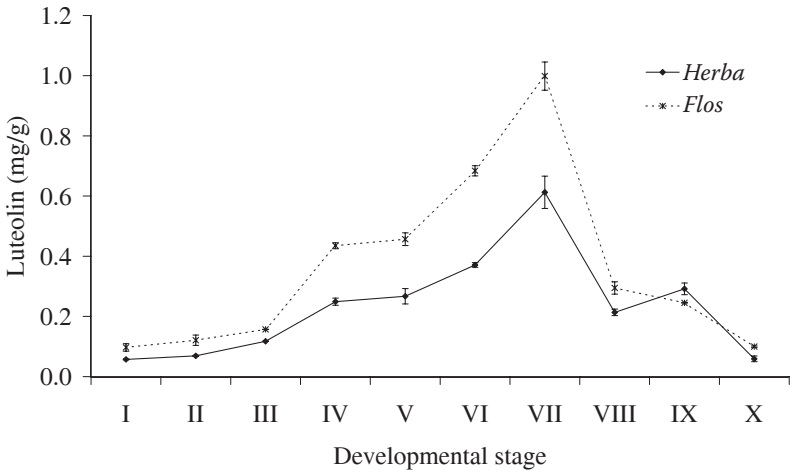

Fig. 3. Content of luteolin in *Achillea collina* Becker ex. Rchb. Alba

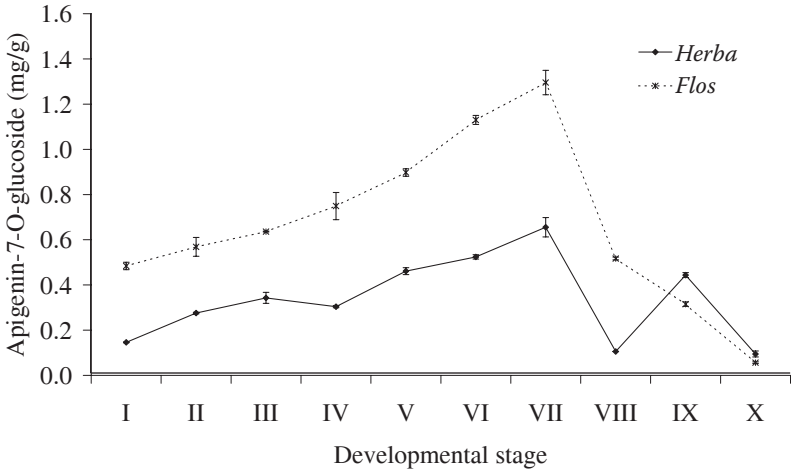

Fig. 4. Content of apigenin-7-O-glucoside in *Achillea collina* Becker ex. Rchb. Alba

RESULTS AND DISCUSSION

The results obtained by HPLC analysis of *Achillea collina* Rchb. Alba confirmed that flavones and their derivatives are the major flavonoids in both *herba* and *flos millefolii* yarrow drug. The flavones apigenin, luteolin and their -7-O-glucosides are visible on HPLC chromatograms as ones of the highest peaks (Fig. 1).

From the aspect of dynamics of the formation of these major flavonoids it is interesting that flavonoids are generally synthesised by the plants in a very similar way like the essential oils (Černaj et al. 1983) – the flavonoid amount increases from the stage of flower differentiation continuously until the stage of full flowering, when the maximal flavonoid content is reached. This maximal content is about 0.6–0.7 mg/g of dry plant material in *millefolii herba*

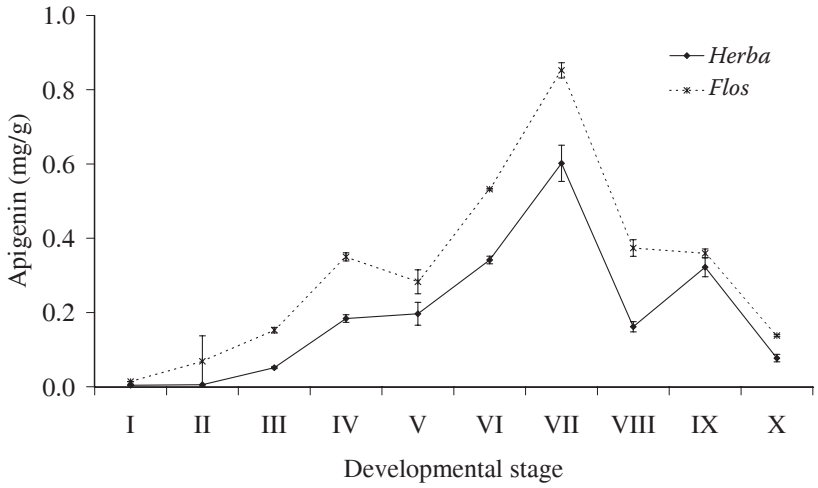

Fig. 5. Content of apigenin in *Achillea collina* Becker ex. Rchb. Alba

and 0.9–1.3 mg/g in *millefolii flos* (Table 1). During the fading away and seed ripening stage the flavonoid content decreases. There is only one exception in flavonoids on which this experiment was focused – it is luteolin-7-O-glucoside, which reached the absolute maximum content at the beginning of flower differentiation. In the full flowering phase luteolin-7-O-glucoside content has the second maximum (0.715 and 0.940 mg/g in *herba* and *flos millefolii*) but it does not reach the first one (0.923 and 1.096 mg/g in *herba* and *flos millefolii*). All four evaluated flavonoids showed statistically significant differences in their contents between the individual developmental stages of yarrow.

Differences in the contents of *herba* and *flos millefolii* flavonoids are not due to the presence of individual substances but only to their amount (Figs. 2 to 5). However, a statistically significant difference in the contents of *herba* and *flos millefolii* flavonoids was improved only with apigenin-7-O-glucoside.

Comparing the ratio of simple and derived flavonoid forms it was found that apigenin and apigenin-7-O-glucoside were very well balanced whereas the luteolin-7-O-glucoside content prevailed over luteolin. This difference was larger mainly at the beginning of flowering and in the seed ripening period. Unfortunately, luteolin preference as an enzymatic flavone glycolysis substrate has not been proved until now.

Some tested yarrow drugs contained also a minor amount of rutin (quercetin-3-O-rutinoside). Rutin traces were found in yarrow HPLC chromatograms independently of both the harvested part and the developmental stage of plants. The presence of quercetin and kaempferol, which are sometimes also alluding to be found in yarrow according to literature (Valant 1978; Krenn 1998), was not proved.

CONCLUSION

The flavones apigenin, luteolin, and their 7-O-glucosides were found to be the main flavonoid constituents in all developmental stages of both flower and top drug. All four evaluated flavonoids showed statistically significant differences in their contents between individual developmental stages of yarrow and their accumulation tendency was also different. The maximal apigenin and apigenin-7-O-glucoside content was determined to range between 0.6 and 0.7 mg/g in *millefolii herba* and between 0.9 and 1.3 mg/g in *millefolii flos*. The luteolin maximal content was found to be 0.613 mg/g in *millefolii herba* and 0.999 mg/g in *millefolii flos*, and the luteolin-7-O-glucoside maximal content was about 1.0 mg/g in both evaluated types of yarrow drug.

References

BORS W., HELLER W., SARAN H., 1990. Flavonoids as antioxidants: determination of radical scavenging efficiencies. Methods in Enzymology, *299*: 15–27.

ČERNAJ P., LIPTÁKOVÁ H., MOHR G., REPČÁK M., HONČARIV R., 1983. Variability of the content and composition of essential oil during ontogenesis of *Achillea collina* Becker. Herba Hungarica, *22*: 21–26.

Československý lékopis 1987. IV. vydání, 1. svazek. Praha, Avicenum.

CHANDLER R.F., HOOPER S.N., HARVEY M.J., 1982. Etnobotany and phytochemistry of yarrow, *Achillea millefolium*, Compositae. Economic Botany, *36*: 203–223.

FRANZÉN R., 1988. Flavonoid diversification in the *Achillea ageratifolia* and *A. clavennae* groups (*Asteraceae*). American Journal of Botany, *75*: 1640–1654.

GUÉDEON D., ABBE P., LAMAISON J.L., 1993. Leaf and flower head flavonoids of *Achillea millefolium* L. subspecies. Biochemical Systematics and Ecology, *21*: 607–611.

KARLOVÁ K., PETŘÍKOVÁ K., 2005. Variability of the content of active substances during *Achillea collina* Rchb. Alba ontogenesis. Horticultural Science (Prague), *32*: 17–22.

KRENN L., 1998. Flavonoide verschieneder Schafgarben-Taxa. In: Workshop *Achillea* – Botanik, Inhaltsstoffe, Analytik und Wirkung in Drogenreport, *11*: 29–34.

PULIDO R., BRAVO L., SAURA-CALIXTO F., 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry, *48*: 3396–3402.

RICE-EVANS C.A., MILLER N.J., PAGANGA G., 1997. Antioxidant properties of phenolic compounds. Trends in Plant Sciences, *2*: 152–159.

SCHULTZ H., ALBROSCHEIT G., 1988. High-performance liquid chromatographic characterization of some medical plant extracts used in cosmetic formulas. Journal of Chromatography, *442*: 353–361.

VALANT K., 1978. Charakteristische Flavonoidglykoside und verwandtschaftliche Gliederung der Gattung *Achillea*. Naturwissenschaften, *65*: 437–438.

VALANT-VETSCHERA K.M., 1987. Flavonoid aglycoside accumulation trends of *Achillea nobilis* L. and related species. Biochemical Systematic and Ecology, *15*: 45–52.

Received for publication March 7, 2006
Accepted after corrections April 18, 2006

Obsah flavonoidních látek v kvetoucích výhonech *Achillea collina* Becker ex. Rchb. Alba v průběhu ontogeneze

ABSTRAKT: Tématem práce bylo hodnocení obsahu hlavních flavonoidů v květenstvích a kvetoucí nati řebříčku chlumního (*Achillea collina* Becker ex. Rchb. Alba) v průběhu ontogeneze. Obsah těchto látek byl zkoumán v deseti vývojových stadiích rostlin – od počátku diferenciace květenství až po stadium zralých semen. Pro HPLC analýzu byl použit metanolový extrakt rostlinného materiálu. Bylo zjištěno, že mezi hlavní látky flavonoidní povahy patří u obou typů řebříčkové drogy ve všech vývojových stadiích apigenin, luteolin a jejich -7-O-glukosidy. Tvorba a akumulace apigeninu a apigenin-7-O-glukosidu má u řebříčku podobnou tendenci – jejich obsah se s vývojem nadzemních částí rostlin plynule zvyšuje až do stadia plného květu, kde nastává zlom a následuje plynulý pokles. Maximální obsah apigeninu a apigenin-7-O-glukosidu byl zjištěn v rozmezí 0,6–0,7 mg/g v nati a 0,9–1,3 mg/g v květenstvích. Změny obsahu luteolinu mají u řebříčku shodný trend jako u apigeninu, pouze jeho maximální dosažené množství je ve stadiu plného květu lehce vyšší. Luteolin-7-O-glukosid v řebříčku na druhé straně dosahuje nejvyššího obsahu ve stadiu malých zelených poupat (kolem 1,0 mg/g) – při dalším vývoji poupat a nakvétání se až do stadia plného květu jeho obsah snižuje a pak opět stoupá.

Klíčová slova: *Achillea collina*; flavonoidy; apigenin; luteolin; HPLC; ontogeneze

Corresponding author:

Ing. Kateřina Karlová, Ph.D., Výzkumný ústav rostlinné výroby Praha-Ruzyně, Oddělení genové banky, pracoviště Olomouc, Šlechtitelů 11, 783 71 Olomouc-Holice, Česká republika
tel.: + 420 585 209 966, fax: + 420 585 209 969, e-mail: Karlova@genobanka.cz