

Acta fytotechnica et zootechnica Special Number  
Nitra, Slovaca Universitas Agriculturae Nitriae, 2011, s. 33–35

## THE COMPARISON OF THE CONTENT OF ESSENTIAL OIL AND FLAVONOIDS IN SELECTED SPECIES OF GENUS *ACHILLEA MILLEFOLIUM* AGG. CULTIVATED IN CONVENTIONAL AND ORGANIC WAY

## POROVNANIE OBSAHU SILICE A FLAVONOIDOV VYBRANÝCH DRUHOV RODU *ACHILLEA MILLEFOLIUM* AGG. PESTOVANÝCH KONVENČNÝM A EKOLOGICKÝM SPÔSOBOM

Lubomír REHUŠ, Jarmila NEUGEBAUEROVÁ

Mendel University in Brno, Czech Republic

The content of essential oil and flavonoids in 16 taxons of the *Achillea millefolium* agg. group growing in conventional and ecological system was evaluated. at the Mendel University in Brno, Faculty of Horticulture in Lednice. The evaluation includes the species *Achillea millefolium*, *A. collina* and *A. pannonica*. Dried aerial part harvested in full blossom was the material used for the evaluation. The amount of essential oil was determined by distillation according to Czech Pharmacopoeia (2002). The content of flavonoids was determined according to the methods of Unigeo Company, by the absorption spectrophotometry method.

**Key words:** essential oil, flavonoids, apigenin, *Achillea millefolium* agg., yarrow, organic farming, conventional agriculture

The genus *Achillea* consists of more than 120 perennial herb species being widespread in the Northern Hemisphere. The most important species belong to the group *Millefolium*. The group is a polyploidy complex ( $n = 9$ ) with species from the diploid to the octoploid level (Nemeth, 2005). The group *Achillea millefolium* agg. s.l. is represented in central Europe by seven species – *Achillea setacea* W. et K., *Achillea asplenifolia* VENT., *Achillea roseoalba* EHREND., *Achillea collina* HEIMERL., *Achillea pratensis* SAUKEL et LÄNGER, *Achillea millefolium* L. and *Achillea pannonica* SHEELE (Danihelka, 2004).

*Achillea millefolium* ( $2n = 54$ ) is a perennial, sparsely villous till napless, green plant. Stems are erect 20 – 90 (-100) cm tall. Leaves alternate, petiolate, lower cauline leaves petiolate rarely sessile, 2 – 3 times pinnatisect, primary segments usually ovate to nearly ovate. Capitula arranged in usually dense (1-) 2 – 10 (-12) cm long and (2.2-) 2.5 – 10.5 (-11.5) cm wide corymbs. Ligules white, pink, rarely dark pink. Flowering from June to September.

*Achillea collina* ( $2n = 36$ ) perennial, densely villous, green or greyish plants. Stems are erect (10-) 20 – 90 (-95) cm tall. Leaves alternate, shortly petiolate or petiolate lower cauline leaves sessile usually amplexicaul, 2 – 3 times pinnatisect, primary segments usually ovate to nearly ovate, 3 – 9 mm long and (1.8-) 2.0 – 6.0 mm wide. Capitula arranged in usually dense sometimes rare (1.7-) 2.1 – 7.0 (-8.0) cm long and (1.9-) 2.8 – 6.5 (-7.0) cm wide corymbs. Ligules white, very rarely pink (0.8-) 1.1 – 1.8 (-2.0) mm long and (0.8-) 1.1 – 2.4 (-2.6) mm wide. Flowering from June to September (Danihelka, 2004).

*Achillea pannonica* ( $2n = 72$ ) is perennial, densely villous, often greyish green plant. Stems are erect (9-) 20 – 75 (-100) cm tall. Leaves alternate, shortly petiolate, lower cauline leaves very shortly petiolate or sessile, 2 – 3 times pinnatisect, primary segments usually ovate in outline (2-) 6 – 13 mm long and 1.5 – 4.0 (-9.0) mm wide. Capitula arranged in usually dense, 1.8 – 6.8 (-10.3) cm long and (1.5-) 2.1 – 6.6 (-9.0) cm wide corymbs. Ligules always white (4-) 5 (-6), three-lobate (1.0-) 1.1 – 1.7 (-2.1) mm long and (0.9-) 1.4 – 2.4 (-2.6) mm wide when dry. Flowering from late May to September (Danihelka, 2001).

*Achillea* has been used in ethnopharmacology for thousands years. According to the literature, pharmacological effects are mainly caused by essential oils, proazulene (chamazulene), sesquiterpene lactones and flavonoids. A wide range of use of this genus can be explained by the variety and complexity of present substances. It is generally considered to be a plant with strong inflammatory and bacteriostatic effects and can be used with gastrointestinal diseases, problems with liver and gall bladder, for treating haemorrhage, menstrual cramps and cold. Apart from pharmaceutical use, it is also used in cosmetics, liqueur production and distilleries. Figueiredo (1995) states its use as a larvicide and insecticide and its inhibitive effects on germination of seeds of other plant species are also known.

The highest content of essential oil can be found in plants just before and after flowering. Its content ranges from 0.1 to 1.0 % depending on species, genotype and ecological factors. Pharmacopoeia Europea IV. states minimum content of essential oil in flowering parts of plants 2 ml kg<sup>-1</sup>. According to Czech Pharmacopoeia (Český lékopis, 2002), the content of chamazulene should not be lower than 0.02 % in dried flowers. The widest range of substances was found in species *A. millefolium*, *A. pannonica* and *A. collina* (Radulovic et al., 2007).

The minimum request for the content of flavonoids in the drug *Millefolii herba* is not stated in Pharmacopoeia Europea IV. The authors Nemeth and Bernath (2008) state 0.60 % content of flavonoids and 1.48 % of phenolic acids. Candan et al. (2003) states that the essential oil extracted from the genus *Achillea millefolium* subsp. *millefolium* has higher antioxidant ability than ascorbic acid and curcumin. Flavonoids in the genus *Achillea* occur in the form of mono and diglycosides of apigenin, luteolin and quercetin. The content of flavonoids in plants stated as apigenin ranges in *Millefolii herba* from 1.5 to 4 %, calculated to the constant content of dry substance (Špinarová and Petříková, 2003). Figures ranging from 1.38 % to 3.97 % were measured in the collection of the genus *Achillea* in Lednice (Špinarová, 2002). Flavonoids have a polar structure thanks to which they are well soluble in water and

alcohol and thus can be applied easily and effectively in the form of tea or tinctures.

## Material and methods

An experimental land for conventional and ecological cultivation is situated in the area of the Faculty of Horticulture, Mendel University in Lednice. Species from the group *Achillea millefolium* agg., especially *A. collina* J. HEIMERL *A. millefolium* L. and *A. pannonica* SCHEELE were selected for the experiment. They are situated in a gene pool collection of the Faculty of Horticulture in Lednice. The care of plants resides in weeding and herbicide spraying with 0.3 % Targa Super. The seeds for ecological variant were gained from the sources of the faculty as well as from Planta naturalis Company and precultivated in a greenhouse. The planting on the land was carried out on 29 May 2010. Six taxons were planted in total: *A. collina* J. HEIMERL, *A. millefolium* L. and *A. pannonica* SHEELE., *Achillea millefolium* agg. Nr. 4 – 40, *Achillea millefolium* agg. Nr. 2 – 41, *Achillea millefolium* agg. Nr. 3 – 81, one plot of land with the size 2 x 2 m for each taxon, the spacing of planting is 0.4 x 0.4 m. Three repetitions were planted from every item. The land is arranged by means of the method of Latin square. The care resided in manual weeding.

The material was collected manually during full blossom in the period from 15 June to 30 September 2010. It was dried in bunches at maximum temperature of 35 °C by means of natural heat in airy place. After drying, it was stored in paper bags until laboratory assessment was carried out. Before the assessment itself, the dried drug was ground in a laboratory grinder with the size of mesh 2.0 mm. The quantitative content of essential oil was determined by means of distillation by water steam (3 h) according to the methods stated in Czech Pharmacopoeia (2002). The content of flavonoids was determined according to the methods of Unigeo Company and the measurement was carried out by means of the method of absorption spectrophotometry on the machine Jenway Model 6100 at the wavelength 336 nm.

## Results and discussion

According to Czech Pharmacopoeia, the minimum content of essential oil for pharmaceutical use of the drug *Millefolii herba* should be 2.0 ml kg<sup>-1</sup>. This limit was reached in 3 out of 10 assessed items in the experiment in 2010. The highest amount of essential oil was found in the item *Achillea collina* Nr. 319 from conventional way of cultivation (3.61 ml kg<sup>-1</sup>). The second highest content was found in *A. millefolium* Nr. 288 (3.06

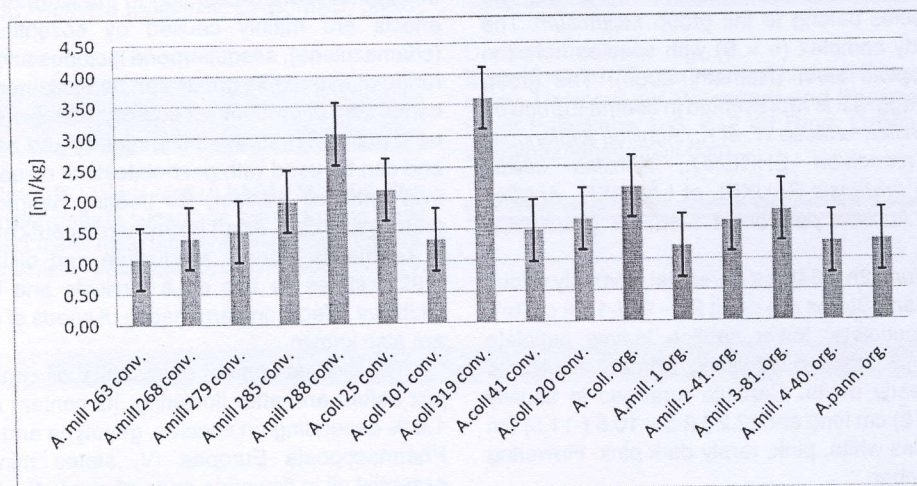


Figure 1 Average content of essential oil

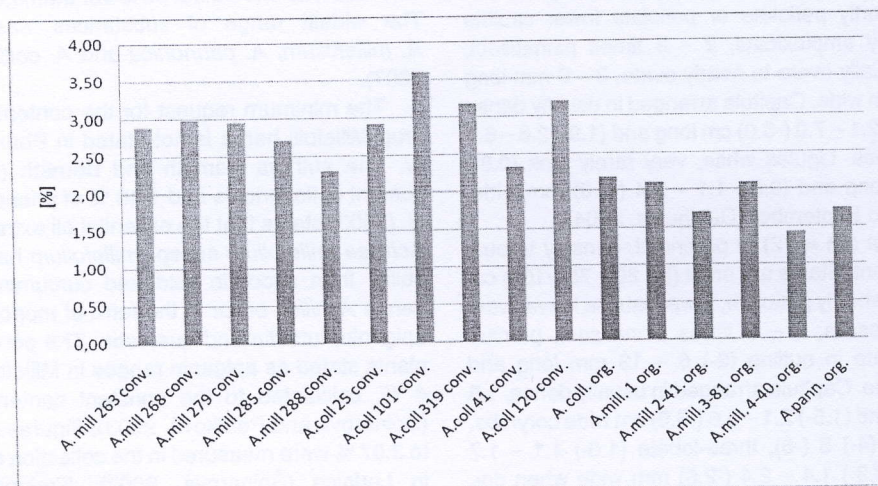


Figure 2 Average content of flavonoids

ml kg<sup>-1</sup>). Apart from these two items, *A. collina* Nr. 25 (2.15 ml kg<sup>-1</sup>) was also up to the standard. From ecologically cultivated yarrows, the item *A. collina* (2.16 ml kg<sup>-1</sup>) reached the standard limit. An average content of essential oil from conventional cultivation was 1.92 ml kg<sup>-1</sup> and from ecological 1.56 ml kg<sup>-1</sup>. Content of essential oil is shown in Figure 1.

Czech Pharmacopoeia does not determine specific request for the content of flavonoids in *Millefolii herba*. The content of flavonoids in plants stated as apigenin ranges in yarrow drug from 1.5 to 4 % calculated to the constant content of dry substance (Špinarová and Petříková, 2003). Benetis (2008) states the content of apigenin 2.81 %. The average contents are shown in the Figure 2. The highest content was found in items from conventional cultivation *A. collina* Nr. 101 – 3.59 % and *A. collina* Nr. 319 – 3.17 % and *A. collina* Nr. 120 – 3.20 %. From plants cultivated in ecological way, the highest content was found in *A. collina* – 2.17 %, *A. millefolium* agg. 1 – 2.10 % and *A. millefolium* agg. 3 – 81 – 2.09 %. Lower crop in the first year of cultivation is stated in literature. This fact probably influenced the amount of crop of essential oil and flavonoids in ecologically cultivated yarrows.

### Conclusions

When evaluating the content of essential oil and flavonoids, we assumed that ecologically cultivated healing plants have higher content of effective substances than those cultivated conventionally. Vildová (2006) observed quantitative as well as qualitative parameters of essential oil and flavonoids in the species *Matricaria recutita* L., which has shown higher content of essential oil and flavonoids in ecologically cultivated plants. Statistically significant differences in items *A. collina* 319 and *A. millefolium* 288 were found by means of experiment with representatives of the group *Achillea millefolium* agg. Their content of essential oil reached minimum limits defined by Czech Pharmacopoeia (2002). This level was reached only by *A. collina* from ecologically cultivated items. Differences in other items were not statistically provable.

The content of flavonoids in the drug *Millefolii herba* was also higher in conventionally cultivated yarrows. The highest content was in the item *A. collina* Nr. 101 – 3.59 % and *A. collina* Nr. 319 – 3.17 % and *A. collina* Nr. 120 – 3.20 %, however, the content was lower in ecologically cultivated plants *A. collina* – 2.17 %, *A. millefolium* 1 – 2.10 % and *A. millefolium* 3 – 81 – 2.09 %. All of these figures are comparable with figures stated in literature.

### Súhrn

Cieľom pokusu bolo hodnotenie kvantitatívneho obsahu silíc a flavonoidov v 16 taxónoch rodu *Achillea* pestovaného v konvenčnom a ekologickom systéme hospodárenia na MZU v Brne, Fakulta záhradníctva v Lednici. Z pokusu je zrejme, že ekologický systém nepreukázal vyššie úrody pri žiadnej zo stanovovaných zložiek, čo je pravdepodobne zapríčinené rôznym vekom porovnávaných položiek, a preto možno predpokladať vyššie hodnoty pri ekologicky pestovaných rebríčkoch v nasledujúcej vegetačnej sezóne.

**Kľúčové slová:** silice, flavonoidy, apigenin, rebríček, *Achillea*, ekologické poľnohospodárstvo, konvenčné poľnohospodárstvo

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### Contact address:

Ing. Lubomír Rehuš, Mendel University in Brno, Faculty of Horticulture, Department of Floriculture and Vegetable Crop Growing, Valtická 337, 691 44 Lednice, e-mail: xrehus@node.mendelu.cz

Ing. Jarmila Neugebauerová, Ph.D. Mendel University in Brno, Faculty of Horticulture, Department of Floriculture and Vegetable Crop Growing, Valtická 337, 691 44 Lednice, e-mail: neugebj@zf.mendelu.cz