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Original Article

Comparison of flavonoid profiles of cultivated plants of *Achillea asplenifolia*, *Achillea collina* and cultivar "Proa"

Milena Nikolova[1*], Antonina Vitkova[1], Malina Delcheva[1], Aglika Edreva[2], Emiliya Gesheva[2]

[1]Department of Applied Botany, Institute of Biodiversity and Ecosystem Research 23, Acad. G. Bonchev, str., 1113 Sofia, Bulgaria
[2]Institute of Plant Physiology and Genetics, 1113 Sofia, Bulgaria

** E-mail: milena_n@bio.bas.bg*

***Abstract*:**
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Flavonoid aglycone profiles of cultivated plants of *Achillea collina*, *A. asplenifolia* and cultivar "Proa" presented with different origins were compared. The evaluation was undertaken to determine intraspecific variability of flavonoid profiles when the plants are grown in the uniform conditions. Eight flavonoid aglycones were detected by thin layer chromatography and comparison with known compounds. Highly methylated quercetagetin derivatives predominate in the exudates. The flavonoid profiles of *A. colina* consist mainly of quercetagetin 3,6,4'-trimethyl ether (centaureidin), 6-hydroxykaempferol 3,6,4'-trimethyl ether and quercetagetin 3,6,7,4'-tetramethyl ether (casticin) while the profiles of *A. asplenifolia* of quercetagetin 3,6,7,3',4'-pentamethyl ether (artemetin), casticin and scutellarein 6,4'-dimethyl ether. In the profiles of *A. asplenifolia* quercetagetin 3,6,4'-trimethyl ether was not found in the extracts of one of the two origins. The extracts of cultivar exhibited a less diversified flavonoid profile with main component centaureidin. No significant difference was found in the flavonoid profiles of the extracts of the both origins of the cultivar "Proa" and *A. collina*.

Key words: Chemotaxonomy, external flavonoids, intraspecific variability, TLC.

Introduction

The species of *Achillea millefolium* group are valuable plants with wide application in the phytotherapy and cosmetics. Six *Achille*a species of the group occur in Bulgaria - *A. collina* J. Becker ex Reichenb, *A. millefolium* L., *A. setaceae* Waldst. et Kit., *A. asplenifolia* Vent., *A. pannonica* Scheele, *A. distans* Waldst. et Kit. ex Wild. As an extension of previous study of *Achillea* genus in Bulgaria (Saukel et al., 2003, Vitkova et al., 2005) in the present research we compare the profiles of surface flavonoid aglycones of *A. collina*, *A. asplenifolia* and cultivar "Proa". The most widespread species is tetraploid *A. collina* (4x), which probably has a hybrid origin of *A. setacea* (2x) and *A. asplenifolia* (2x). It is spread from the sea level up to 1800 m altitude, grows both in xerothermal and the mesophilic places. Recently diploid *Achillea asplenifolia* was established for Bulgaria in herbaceous habitats in antropogenic influenced phytocenoses from 500 to 600 m a.s.l. Only two populations have been detected in the territory of the county – near the village of Opizvet and village Katina, Sofia region. The plants of the last population characterized by a combination of characteristics of *A. asplenifolia* and. *A. roseoalba* (Vitkova et al., 2005). Cultivar *Achillea collina*

"Proa" represents a tetraploid, rich in proazulenes (Kastner et al., 1992). The examined in the present study species have been objects of phytochemical analysis regarding different secondary metabolites – sesquiterpene lactones, flavonoids, essential oils (Konakchiev et al., 2005, Todorova et al., 2005, Trendafilova et al., 2006, Trendafilova et al., 2007). The species of *Achillea* including *A. asplenifolia* and *A. collina* have been extensively studied for content of surface flavonoids (Valant-Vetschera & Wollenweber 1988, Ivancheva & Stancheva, 1996, Valant-Vetschera & Wollenweber 1996, Valant-Vetschera & Wollenweber, 2001a). External flavonoids attract attention except for their usefulness in taxonomic studies (Wollenweber & Schneider, 2000, Valant-Vetschera & Wollenweber 2001b, Valant-Vetschera et al., 2003) but also with their ecological role (Onyilagha & Grotewold, 2004).

The aim of present study was to compare flavonoid aglycone profiles of cultivated plants of *Achillea collina*, *A. asplenifolia* and cultivar "Proa"presented with different origins. The evaluation was undertaken to determine intraspecific variability of flavonoid profiles when the plants are grown in the uniform conditions.

Material and methods

Plant material. The plant material included two wild species (*Achillea collina* and *A. asplenifolia*) and cultivar "Proa". Each species was presented by two populations. Seeds of both species were collected from their natural habitats. The material of *Achillea collina* were collected from Vitosha Mountain and village Gorni Lozen, Sofia region and those of *A. asplenifolia* from village Bezden, Sofia region and village Katina, Sofia region. The seeds of cultivar Proa have German and Poland origin. Seedlings were produced in a greenhouse and then transferred to the experimental field of the Institute of Plant Physiology and Genetics, near Sofia, 570 m a.s.l., in 2009. All samples are collected at full flowering stage.

Preparation of acetone exudates. Air-dried, but not ground (1g) plant material was briefly (2-3 min) rinsed with acetone at room temperature to dissolve the lipophilic components accumulated on the surface. The obtained acetone filtrate was then dried using a rotary-evaporator to give a crude extract which was suspended in MeOH and then subjected on TLC.

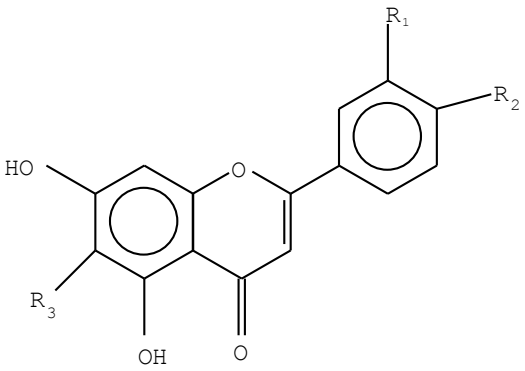
Thin layer chromatographic analysis. The acetone exudates were screened for surface flavonoids by TLC analysis. Three TLC sorbents and several mobile phases were used for the analysis of the flavonoid exudates. Toluene-dioxan-acetic acid (95:25:4, v/v/v) was applied for the development of the aglycones mixture on silica gel plates Kiselgel 60 F_{254} (10x20 cm, 0.2 mm layer). Toluene-methylethylketone-methanol (60:25:15, v/v/v); toluene-petrol ether-methylethylketone-methanol (60:30:10:5, v/v/v/v) and toluene-methylethylketone-methanol (30:20:15, v/v/v) were used for DC-Alufolien Polyamid 11 F_{254} plates (10x20 cm, 0.15 mm layer). Acetic acid–water (30:70, v/v) was used for cellulose plates DC-Alufolien Cellulose 5552 (10x20 cm, 0.1 mm layer). Chromatograms were viewed under UV light before and after spraying with ‘‘Natural product reagent A”, 1% solution of diphenylboric acid 2-aminoethyl ester complex in methanol. The identification of the compounds was achieved by co-chromatography with authentic markers obtained from Prof. Eckhard Wollenweber.

Results and discussion

Cultivated plants of *A. collina*, *A. asplenifolia* and cultivar "Proa" were analyzed for their profiles of surface flavonoid aglycones. Four individuals from each origin were studied. Flavonoid profile of each individual was examined inflorescences and stem areas (leaves and stem) separately. Total 48 extracts were comparatively analyzed for content of surface flavonoid aglycones. Eight flavonoid aglycones were identified by thin layer chromatography and comparison with known compounds (Fig. 1). Polymethoxy derivatives of 6-hydroxyflavonol and 6-hydroxyflavone in various combinations predominate in the exudates (Table 1). The simple flavonoids (apigenin and luteolin) are rather accumulated in the inflorescences than in the exudates of stem areas. The extracts of cultivar "Proa“ exhibited a less diversified flavonoid profile with the main flavonoid quercetagetin 3,6,4'-trimethyl ether (**5**) in all studied parts. Quercetagetin 3,6,7,4’-tetramethyl ether (**6**) was detected in the stem areas in trace.

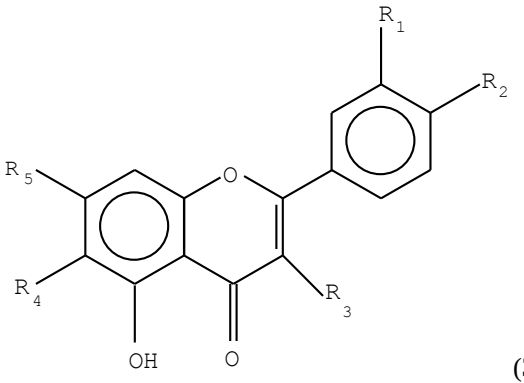
The main flavonoid of the extracts of *A. colina* was quercetagetin 3,6,4'-trimethyl ether (centaureidin) (**5**). This result indicates the closeness between cultivar Proa and *A. colina.* Additionally of the extracts of *A. collina* 6-hydroxykaempferol 3,6,4'-trimethyl ether (**3**), quercetagetin 3,6,7,3',4'-pentamethyl ether (**7**) and scutellarein 6,4'-dimethyl ether (**8**) were detected. The flavonoid quercetagetin 3,6,7,4’-tetramethyl ether (**6**) that detected in trace amounts in the cultivar while in the extracts of *A. collina* is presented in large quantity. Flavonoid

profiles of *A. asplenifolia* consisted predominantly of quercetagetin 3,6,7,4'-tetramethyl ether (casticin) (**6**), quercetagetin 3,6,7,3',4'-pentamethyl ether (artemetin) (**7**) and scutellarein 6,4'-dimethyl ether (**8**). The identified compounds are in accordance with the data previously reported for these species (Valant-Vetscheta & Wollenweber, 1988, Trendafilova et al., 2007).



(**1,2,8**)

R_1=H, R_2=OH apigenin (**1**)
R_1=OH, R_2=OH luteolin (**2**)
R_1=H, R_2=OCH_3, R_3=OCH_3
scutellarein 6,4'-dimethyl ethers (**8**)



(**3-7**)

R_1=H, R_2=OCH_3,R_3=OCH_3, R_4=OCH_3, R_5=OH
6-hydroxykaempferol 3,6,4'-trimethyl ether (**3**)
R_1=OH, R_2=OCH_3,R_3=OCH_3, R_4=OCH_3, R_5=OCH_3
quercetagetin 3,6,7-trimethyl ether (**4**)
R_1=OH, R_2=OH,R_3=OCH_3, R_4=OCH_3, R_5=OCH_3
quercetagetin 3,6,4'-trimethyl ether (**5**)
R_1=OH, R_2=OCH_3,R_3=OCH_3, R_4=OCH_3, R_5= OCH_3
quercetagetin 3,6,7,4'-tetramethyl ether (**6**)
R_1= OCH_3, R_2=OCH_3,R_3=OCH_3, R_4=OCH_3, R_5= OCH_3
quercetagetin 3,6,7,3',4'-pentamethyl ether(**7**)

Fig. 1. Structures of the identified flavonoid aglycones (**1-8**)

Individual variability of the flavonoid composition was observed regarding flavonoids that are presented in lesser amounts. No significant differences in the flavonoid profiles of the extracts of the both origins of cultivar Proa. Quantitative differences were observed in the accumulation of flavonoids in the profiles of *A. collina* and *A. asplenifolia* from different origins. Furthermore in the profiles of *A. asplenifolia* from the population of village Katina quercetagetin 3,6,4'-trimethyl ether was not detected as well as the flavonoid quercetagetin 3,6,7-trimethyl ether which was abundant in the same population was found in trace in the individuals of the population of village Bezden. It is possible that these quality differences of flavonoid profiles are due to large quantitative differences that TLC analysis recognizes as qualitative. Also must be taken into account that there are data that the population of v. Katina showed combined features of *A. asplenifolia* and *A. roseoalba* (Vitkova et al., 2005). The results of flavonoid variability of A. *asplenifolia* were consistent with conclusions of Dagnon et al., 2011. The authors reported on the basis of essential oils pattern high index of similarity (94.5%) for two populations of *A. collina* and less similar (82.3%) for *A. asplenifolia* populations. More data are needed to establish whether the plants of this population deserve an independent taxonomic rank.

Conclusion

In conclusion the comparative analysis of flavonoid profiles of examined species showed that *A. asplenifolia* has more variable flavonoid composition in comparison to *A. colina* and cultivar "Proa". The extracts of the last showed the most constant and simple flavonoid profile. The results obtained in this study indicate that surface flavonoids are a reliable taxonomic feature in the target species.

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Table1. Flavonoid aglycones in the samples of *Achillea* species

Samples	Flavonoid aglycones							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Cultivar Proa, origin Germany								
inflorescences 1	X	X			XXX			
inflorescences 2	tr	X			XXX			
inflorescences 3	X	X			XXX			
inflorescences 4	X	X			XX			
stem areas 1					XXX	tr		
stem areas 2					XXX	tr		
stem areas 3					XXX	tr		
stem areas 4					XX	tr		
Cultivar Proa, origin Poland								
inflorescences 1	XX	XX			XXX			
inflorescences 2	tr	tr			XXX			
inflorescences 3	XX	XX			XX			
inflorescences 4	XX	XX			XXX			
stem areas 1	tr				XXX			
stem areas 2	tr				XXX			
stem areas 3	X	X			XX			
stem areas 4	X	X			XXX			
***A.collina,* origin Gorni Lozen, Sofia region**								
inflorescences 1	XX	X	X		X	X		
inflorescences 2	XX	XX	X		XX	X		
inflorescences 3	X	X	X		X	tr		
inflorescences 4	XX	XX			X	X	tr	tr
stem areas 1			X		X	X		
stem areas 2			X		XX	X		
stem areas 3	tr	tr	X		XX	X		
stem areas 4			X		XX	X	tr	tr
***A.collina,* origin Vitosha Mountain**								
inflorescences 1	XX	XX	tr		tr		X	X
inflorescences 2	XX	XX	tr		tr		tr	tr
inflorescences 3	XX	XX	tr		XX	X	X	X
inflorescences 4	XX	XX	tr		XX	tr	tr	tr
stem areas 1	X		tr		XX	X	X	X
stem areas 2	X		tr		XXX	X	X	X
stem areas 3	X		tr		XX	X	X	X
stem areas 4	X		X		XXX	X	tr	tr
***A. asplenifolia,* origin Bezden, Sofia region**								
inflorescences 1	tr	X		tr	X	tr	XX	XX
inflorescences 2	tr	X			X	tr	XX	XX
inflorescences 3	X	X			XX		XX	XX
inflorescences 4	XX	XX		tr	XX		tr	tr
stem areas 1					X	X	XX	XX
stem areas 2					tr	X	XX	XX
stem areas 3					X	X	tr	tr
stem areas 4				tr	XX	X	XX	XX
***A. asplenifolia,* origin Katina, Sofia region**								
inflorescences 1	XX	XX		XX		X	XX	XX
inflorescences 2	X	X		tr		X	XX	XX
inflorescences 3	X	X		X		X	XX	XX
inflorescences 4	XX	XX		tr		X	XX	XX
stem areas 1				XX		X	XX	XX
stem areas 2				X		X	XX	XX
stem areas 3				X		X	XX	XX
stem areas 4				X		X	XX	XX

Legend: apigenin (**1**), Luteolin (**2**), 6-hydroxykaempferol 3,6,4'-trimethyl ethers (**3**), quercetagetin 3,6,7-trimethyl ethers (**4**), quercetagetin 3,6,4'-trimethyl ethers (**5**), quercetagetin 3,6,7,4'-tetramethyl ethers (**6**) quercetagetin-3,6,7,3',4'-pentamethyl ethers (**7**), scutellarein 6,4'-dimethyl ethers (**8**), tr. – trace

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