

Determination of apigenin content in some Bulgarian *Veronica* species

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Abstract:

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Thirteen Bulgarian species of *Veronica* L. - *V. bellidioides* L., *V. kellereri* Deg. et Urum., *V. prostrata* L., *V. austriaca* L., *V. chamaedrys* L., *V. vindobonensis* (M.Fischer) M. Fischer, *V. urticifolia* Jacq., *V. officinalis* L., *V. beccabunga* L., *V. serpyllifolia* L., *V. polita* Fries, *V. hederifolia* L., *V. persica* Poir have been analysed for the flavonoid aglycone composition as well as for apigenin content in their leaf exudates. Their general flavonoid compounds are derivatives of flavones - apigenin, luteolin, chrysoeriol. The apigenin was found to be the main constituent in leaf exudates of all species. Apigenin content was quantified by TLC-densitometry. The alpine species *V. kellereri* showed the highest apigenin content. The variation of flavonoid content were examined in populations, which inhabit different ecological conditions. It was found that the populations at the alpine regions have the highest flavonoid content.

Key words: *Veronica* spp., flavonoid aglycones, apigenin

Introduction

Scrophulariaceae have so far only rarely been reported to exhibit externally accumulated flavonoid aglycones. The presence of exudate flavonoids has been reported for species belonging to the genera *Antirrhinum*, *Asaria*, *Calceolaria*, *Mimulus*, *Odonites*, *Diplacus* (Wollenweber et al., 2000). Genus *Veronica* L. is represented in the Bulgarian flora by 36 species (Peev, 1995). This are perennial herbaceous plants, occurring in various ecological conditions. A leaf survey of *V. chamaedrys*, *V. vindobonensis*, *V. polita* and *V. persica* has shown that apigenin is the common flavone (Nikolova et al., 2001, 2002). The present study is a comparative analysis of the leaf exudate flavonoids of 13 Bulgarian *Veronica* species which grow in different habitats - arid, alpine - *V. kellereri* and *V. bellidioides*; wet, shady - *V. urticifolia*; wet - *V. beccabunga*, settlements - *V. polita*, *V. persica*, *V. hederifolia*. We investigated

apigenin content in *V. chamaedrys*, *V. vindobonensis*, *V. officinalis*, which are widely distributed at different altitude. The results provide more information in the flavonoid aglycones variation relation to environmental conditions.

Materials and Methods

Plant material

Aerial parts were collected from flowering plant. Voucher specimen were deposited in the Herbarium at the Institute of Botany (SOM). The list of species analysed is presented along with **Table 1**.

Quantification of apigenin

Samples (two grams) of air-dried plants were rinsed with 20 ml acetone for 2 min. After evaporation of acetone the dried extracts was

Table 1. Investigated samples of *Veronica* species

Taxon	No of population s samples	SOM	Origin, basic rock, m.a.s.l.
I. Section <i>Veronicastrum</i> Benth			
<i>Veronica bellidioides</i> L.	No 1	155142	Vitosha, Black top, Si, 2290 m.
<i>Veronica kellereri</i> Deg. et Urum.	No 1	Co500	Pirin, Ca, 1900 m.
II. Section <i>Veronica</i>			
<i>Veronica prostrata</i> L.	No 1	Co527	Belidie Han, Ca, 800 m.
<i>Veronica austriaca</i>	No 1	Co581	Vitosha, around lift station, Si, 1400 m,
<i>Veronica chamaedrys</i> L.	No 1	Co473	Pirin, Predela, Si, 1000 m.
	No 2	Co474	Pirin, Bansko, Ca, 1100 m.
	No 3	Co477	Pirin, Ca, 1500 m.
	No 4	Co475	Pirin, Shiligarinica, Ca, 1700 m.
	No 5	Co535	Pirin, h. "Vihren", Ca, 1950 m.
	No 6	Co458	Pirin, Ca, 2300 m.
<i>Veronica vindobonensis</i> (M. Fischer) M. Fischer	No 1	Co468	Shkorpilovtsi, around Black Sea, Si, 60 m.
	No 2	Co493	Brashlyan, Strandja, 350 m.
	No 3	Co544	around Kableshkovo, East Stara Planina, 400 m.
	No 4	Co540	Vitosha north-western slope, Si, 1000 m
	No 5	Co480	Vitosha h. "Planinets", Si, 1440 m.
	No 6	Co457	Vitosha, Black top, Si, 2290 m.
<i>Veronica urticifolia</i> Jacq.	No 1	155150	Ribaritsa, 650 m.
<i>Veronica officinalis</i> L.	No 1	Co497	Lozenska mountain, 800 m
	No 2	Co499	Vitosha, h. "Aleko", Si, 1800 m.
	No 3	Co503	Rila, h. "Musala", Si, 2300 m.
III. Section <i>Beccabunga</i> Griseb.			
<i>Veronica beccabunga</i> L.	No 1	155149	Ribaritsa, 650 m.
IV. Section <i>Alzinebe</i> (Griseb.) Lehman			
<i>Veronica serpyllifolia</i> L.	No 1	Co579	Ljulin mountain, 700 m., Si,
<i>Veronica polita</i> Fries	No 1	Co554	Sofia, 600 m., Si
<i>Veronica persica</i> Poir	No 1	155144	Sofia, 600 m., Si
<i>Veronica hederifolia</i> L.	No 1	Co546	Sofia, 600 m., Si

dissolved in 250 µl methanol and 60 µl was spotted on Merck silica gell TLC plate 60 F₂₅₄, 0.2 mm layer and were developed with system: toluene/dioxan/acetic acid (95:25:4). The spots of aglycones were detected after spraying with Naturstoffreagenz A and were snapped under UV light = 336 nm with digital camera. The images were processed with Quany Scan ® Biosoft software. The apigenin content were calculated from the integration of spots corresponding to three different quantities of the standard aglycone and three spots of solutions of unknown concentrations. Calibration graphs were established with standard methanol solutions of apigenin.

TLC analysis on the acetone exudates of *Veronica* species

Air-dried plant material (50 gr.) was briefly rinsed with acetone to dissolve the exudate material. The concentrated materials were defatted and passed over Sephadex LH-20 eluted with MeOH. Comparative TLC with authentic substances allowed the identification of most of the flavonoid present. Exudates were monitored and comparisons with markers were done by TLC on silica gel with toluene-dioxane-HoAc. For TLC on polyamide DC-11 we used toluene-petrol₁₀₀₋₁₉₀-MeCoEt - 60:20:15. Chromatograms were viewed before and after spraying with Naturstoffreagenz A.

Results and Discussion

Apigenin is a common flavonoid aglycone of all investigated *Veronica* species. Its variation among different species and populations of one species are present at **Table 2** and **Fig. 1**. The results show that *V. kellereri* and populations of *V. officinalis*, *V. chamaedrys*, *V. vindobonensis* which grow at the alpine regions have the highest apigenin content. Such results were reported consideration in the early work Shibata, (1915) on flavonoid content of alpine plants which suggest that their concentrations increased with altitude (Larson, 1988). Probably this high concentration is related to the extreme condition typical for alpine regions - high UV-B radiation, low moisture, large twenty-four hour temperature difference. Naturally occurring UV-B radiation along elevation gradient increases by 20-25 % from sea level to the 3000 m in the Bulgarian mountain (Lingova, 1995). There are many reports on the positive relationship between accumulation of flavonoids and UV-B radiation (Richard, 1978; Tevini et al., 1991; Flint et al., 1985; Lios, 1994; Markham, et al., 1998) arid conditions (Wollenweber, 1990; Kuzmanov, et al. 1997) and other abiotic stress conditions (Ravn et al., 1998).

Table 2. Apigenin content in samples of *Veronica* populations

Taxon	Number of populations samples	apigenin [$\mu\text{g}\cdot\text{g}^{-1}$]
<i>V. bellidioides</i>	No 1	0.27
<i>V. kellereri</i>	No 1	1.90
<i>V. prostrata</i>	No 1	0.48
<i>V. austriaca</i>	No 1	0.09
<i>V. chamaedrys</i>	No 1	0.25
	No 2	0.20
	No 3	0.15
	No 4	0.16
	No 5	1.90
	No 6	1.70
<i>V. vindobonensis</i>	No 1	0.69
	No 2	1.80
	No 3	0.45
	No 4	0.30
	No 5	0.02
	No 6	0.84
<i>V. urticifolia</i>	No 1	0.23
<i>V. officinalis</i>	No 1	0.09
	No 2	0.14
	No 3	0.72
<i>V. becabunga</i>	No 1	0.50
<i>V. serpyllifolia</i>	No 1	0.14
<i>V. polita</i>	No 1	0.30
<i>V. persica</i>	No 1	0.81
<i>V. hederifolia</i>	No 1	0.28

The populaion (sample No 2) of *V. vindobonensis* from Strandja has also high apigenin concentration. This fact probably is connected with influence of the Mediterranean climate in this region. Habitats in Mediterranean are described as a suitable for accumulation of externally desposited flavonod aglycones (Wollenweber and Dietz, 1981, Wollenweber, 1990).

The study of acetone exudates from *Veronica* showed that the most common aglycones are - apigenin, luteolin, chrisoeriol. 6-metoxy derivatives were found in *V. polita*, *V. persica* and some population of *V. vidobonensis* (Nikolova, et al., 2001, 2002) (**Table 3** and **Fig. 2**).

Table 3. Flavonoid aglycones in the *Veronica* species

Taxon	A	B	C	D	E
<i>V. bellidioides</i>	*				
<i>V. kellereri</i>	*	*			
<i>V. prostrata</i>	*				
<i>V. austriaca</i>	*				
<i>V. chamaedrys</i>	*	*	*		
<i>V. vindobonensis</i>	*	*	*		*!
<i>V. urticifolia</i>	*	*			
<i>V. officinalis</i>	*	*			
<i>V. becabunga</i>	*				
<i>V. serpyllifolia</i>	*				
<i>V. polita</i>	*			*	
<i>V. persica</i>	*	*		*	
<i>V. hederifolia</i>	*				

Legend: A - apigenin; B- luteolin; C- chrisoeriol; D - 6-OH luteolin 6,7,3'- trimethyl ether, E- scutellarein 6,4' - dimethyl ether, *! - only in the samples No1 and No2

Conclusions

Apigenin is a common flavonoid aglycone in all investigated *Veronica* species.

The proposed method of apigenin densitometric determination is simple, sensitive and can be used for routine assay of externally flavonoid aglycones in plants.

The species *V. kellereri* has the highest apigenin content. The higher altitude populations of *V. officinalis*, *V. chamaedrys*, *V. vindobonensis* have greater apigenin content in comparison to plant at low altitudinal site.

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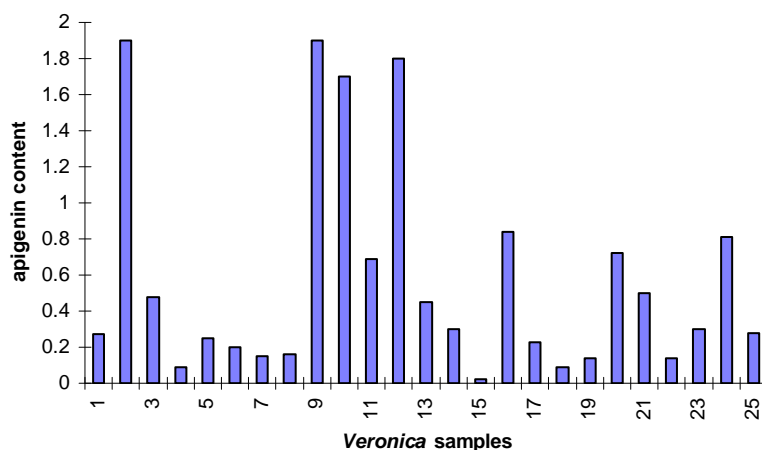
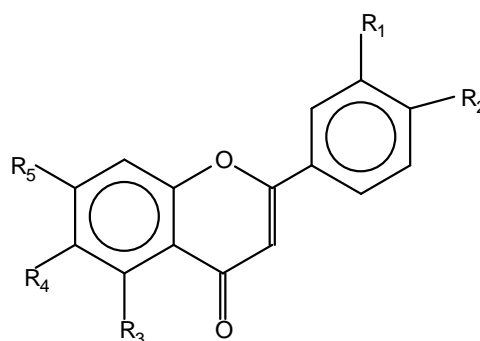


Fig. 1. Apigenin content in samples of *Veronica* populations

1. *V. bellidioides*; 2. *V. kellereri*; 3. *V. prostrata*; 4. *V. austriaca*; 5. *V. chamaedrys* № 1; 6. *V. chamaedrys* № 2; 7. *V. chamaedrys* № 3; 8. *V. chamaedrys* № 4; 9. *V. chamaedrys* № 5; 10. *V. chamaedrys* № 6; 11. *V. vindobonensis* № 1; 12. *V. vindobonensis* № 2; 13. *V. vindobonensis* № 3; 14. *V. vindobonensis* № 4; 15. *V. vindobonensis* № 5; 16. *V. vindobonensis* № 6; 17. *V. urticifolia*; 18. *V. officinalis* № 1; 19. *V. officinalis* № 2; 20. *V. officinalis* № 3; 21. *V. becabunga*; 22. *V. serpyllifolia*; 23. *V. polita*; 24. *V. persica*; 25. *V. hederifolia*



apigenin
 scutellarein 6,4'-dimethyl ether
 luteolin
 chrysoeriol
 6-OH luteolin 6,7,3' - trimethyl ether

$R_1=R_4=H, R_2=R_4=R_5=OH$
 $R_1=H, R_2=R_4=OCH_3, R_3=R_5=OH$
 $R_1=R_2=R_3=R_5=OH, R_4=H$
 $R_1=OCH_3, R_2=R_3=R_5=OH, R_4=H$
 $R_1=R_4=R_5=OCH_3, R_2=R_3=OH$

Fig. 2. Flavonoid aglycones in *Veronica* species

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Резюме

Съдържание на апигенин във видове от род *Veronica* L.

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Съдържанието на флавоноидни агликонни е анализирано в 13 вида от род *Veronica*, разпространени в България - *V. bellidioides* L., *V. kellereri* Deg. et Urum., *V. prostrata* L., *V. austriaca* L., *V. chamaedrys* L., *V. vindobonensis* (M.Fischer) M. Fischer, *V. urticifolia* Jacq., *V. officinalis* L., *V. beccabunga* L., *V. serpyllifolia* L., *V. polita* Fries, *V. hederifolia* L., *V. persica* Poir. Установените флавоноидни агликонни са производни на флавонолите - лутеолин, апигенин, хризоеириол. Основният агликон доказан в ацетонови ексудати на всички изследвани видове е апигенин. Неговото количество е определено чрез ТСХ денситометрия. Алпийският вид *V. kellereri* е с най-високо съдържание на апигенин. Колебанията в съдържанието на апигенин са анализирани в популации на един вид, развиващи се при различни екологични условия. Най-високо съдържание на апигенин е установено в популациите развиващи се във високопланински условия и в популацията на района на Странджа. Използваният метод за количествен анализ на апигенин е лесно изпълним, чувствителен и подходящ за определяне съдържанието на флавоноидни агликонни в ацетонови ексудати от растения.