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Iridoid and flavonoid patterns of the genus *Veronica* sect. *Alsinebe* subsect. *Agrestis* (Benth.) Stroh (Lamiales) and their systematic significance

Shahryar Saeidi Mehrvarz[1], Nosrat O. Mahmoodi [2], Roshanak Asadian[3] and Gholamreza Bakhshi Khaniki[3]

[1]*Deparment of Biology, Faculty of Science, The university of Guilan, Rasht, Iran*
[2]*Deparment of Chemistry, Faculty of Science, The university of Guilan, Rasht, Iran*
[3]*Department of Biology, Faculty of Science, Payam noor University of Tehran, Tehran, Iran*

[1]Correspoding Author: Email: saeidimz@guilan.ac.ir

Abstract

Distribution of two iridoid and 6 flavonoid compounds in four *Veronica* sect. *Alsinebe* subsect. *Agrestis* species (23 samples) from Iranian natural populations was investigated. *Veronica francispetae* and *V. siaretensis* were studied for these compounds for the first time. The iridoid and flavonoid patterns showed a good correlation with other chemical and morphological features of these taxa. The studied species are closest together according to the flavonoid patterns: species containing quercetin derivatives are *V. persica, V. polita* and species containing quercetin are *V. francispetae*, *V. siaretensis.*

Keywords: *Veronica;* iridoid; flavonoid; chemosystematic; Iran.

Abbreviations: F-Flavonoid; Fl-at flowering; Fr- at fruitification; I- iridoid

Introduction

The genus *Veronica* L. comprises 184 (Elenevskii, 1978) to about 300 (Willis, 1980) species distributed mainly in northern hemisphere. *Veronica* sect. *Alsinebe* as defined by Römpp (1928) is the largest section of this genus. According to Römpp (1928) this section is divided into subsections; *Acinifolia, Agrestis, Biloba, Megasperma, Microsperma, Pellidosperma* and *Serpyllifolia*. The studied taxa are *V. persica, V. polita*, *V. francispetae* and *V. siaretensis, which* belong to subsect. *Agrestis.* However, in most taxonomic schemes *Agrestis* is considered as a subsection of genus *Veronica* (Fischer and Peev, 1995; Albach et al. 2004). The earlier studies on this subsection describe the macro-morphological features of the species (Fischer, 1981; Juan et al., 1997). The later works report data of pollen morphological characters (Hong, 1984; Fernandez et al. 1997; Saeidi & Zarrei, 2006), seed characters (Juan et al., 1994; Saeidi et al., 2001b), chromosome counts (Ferakova, 1976; Fischer, 1981; Aryavand, 1987; Ghaffari, 1987; Fernandez et al. 1997; Saeidi & Kharabian, 2005) and chemical characters. Chemical characters such as flavonoid (Grayer-Barkmeijer, 1978; Peev, 1982) and iridoid glucosides (Grayer-Barkmeijer, 1973; Lahloub, 1991, 1992; Taskova et al., 2002) were used in the chemosystematic studies of *Veronica*. A little number of reports has been issued yet about the identification

Table 1. **Studied *Veronica* samples for iridoids and flavonoids and their voucher numbers**

Species	Voucher	The number of studied samples	Phenophase
V. francispetae M. A. Fischer	Mozaffarian 41032	3	Fl
	Saeidi 24025	3	Fl
V. persica Poir.	Saeidi 24092	3	Fr
	Jamzad & Asri 71766	2	Fl
V. polita Fries	Assadi & Massoumi 55325	4	Fl
	Saeidi 1315	3	Fr
V. siaretensis Lehmann	Saeidi 42206	2	Fl
	Saeidi & Kaviani 1248	3	Fr

and biological activity of iridoids and flavonoids, isolated from other plant species. For example quercetin and catalpol are known to possess antioxidant (Chiang et al. 2004), and appreciable antibacterial activities (Rombout and Links, 1956), respectively. Therefore *Veronica species* can be used as medicinal plants for their chemical compounds. In this paper, based on analysis of eight chemical markers in four *Veronica* species, we discuss the taxonomic significance of iridoids and flavonoids at species level.

Materials and Methods

General Procedures

Stems and leaves of four species belonging to *Veronica (V. persica, V. polita, V. siaretensis, V. francispetae)* were collected and have been analyzed for their phenolic compounds. Voucher specimens (Table.1) were deposited in the Herbarium of the University of Guilan, Iran. Powdered arial parts (300 gr) were extracted with methanolic solvent. Compounds were repeatedly purified by thin layer chromatography, until the absorption properties became constant. Elution process was carried out in 95% methanol and applied (spotted) to the plate, and then run in BAW (n- Butanol: Acetic acid: Water) 4:1:5 and 15% Acetic acid separately. Finally, the solution was filtered and allowed to be concentrated, and directly used for spectral analyses. The flavonoid compounds were separated on GC-MS. The isolated compounds were identified by the IR, NMR, GC-MS, TLC and UV spectra in comparing with standards.
In the cases where only small herbarium samples were available, individual glycosides were solely identified by their characteristic signals and compared with authentic spectra. All GC and Mass spectra were obtained from a GC-MS Agilent Technologies QP-5973N MSD instrument. All ^{1}HNMR data were recorded in $CDCl_3$, CD_3COCD_3 or DMSD-d_6 using a Bruker Avance 500-MHz spectrometer.
Chemical shifts are reported in ppm (δ) using TMS as internal reference. IR spectra were obtained on a Shimadzu IR-470. The UV-Vis spectra were recorded on a Shimadzu UV-2100. Chemicals were purchased from Fluka, Merck, and Aldrich.

Results and Discussion

The constant and characteristic iridoid and flavonoid profiles of the studied species allow their use in analyzing some taxonomic problems at specific level. Eight compounds (two iridoids and six flavonoids) in four species of *Veronica* were isolated and identified by spectral methods using authentic reference compounds. *Veronica francispetae* and *V. siaretensis* were analyzed for iridoids and flavonoid for the first time. A thin layer chromatography analysis was performed and the distribution of eight compounds in a total of 23 samples from four *Veronica* species is summarized in Table 2. Plant samples from three localities of each taxon, when possible from habitats with different ambient conditions, were studied. The analysis showed qualitatively constant iridoid patterns of the species, which were not influenced by environmental conditions and phenophase. Only negligible quantitative changes were registered, which confirm the value of iridoids as important and reliable taxonomic markers for the genus *Veronica.* We focused at higher taxonomic levels (section).

Table2. **Iridoid and flavonoid patterns of the studied *Veronica* sect. *Alsinebe* subsect. *Agrestis* (Benth.) Stroh**

Taxon	Iridoid and flavonoids compounds[a]							
	1(I)	2 (F)	3(F)	4 (F)	5(F)	6(F)	7(F)	8(I)
V. francispetae M. A. Fischer	*		*	*	*	*	*	
V. siaretensis Lehmann	*		*	*	*	*	*	
V. polita Fries	*	*		*	*	*	*	
V. persica Poir.	*	*		*	*	*	*	*

[a] Iridoid and flavonoids compounds; Catalpol (1), quercetin (3′, 4′, 5, 7-tetramethyl ether) (2), quercetin (3), Metoxy flavone (4), Kamepferol-7-O-B-D- Gluco pyranoside (5), Hydroxy flavone (6), Apiginin 7-O-neohesperidoside (7), 6-O-isovanilloyl catalpol (8).

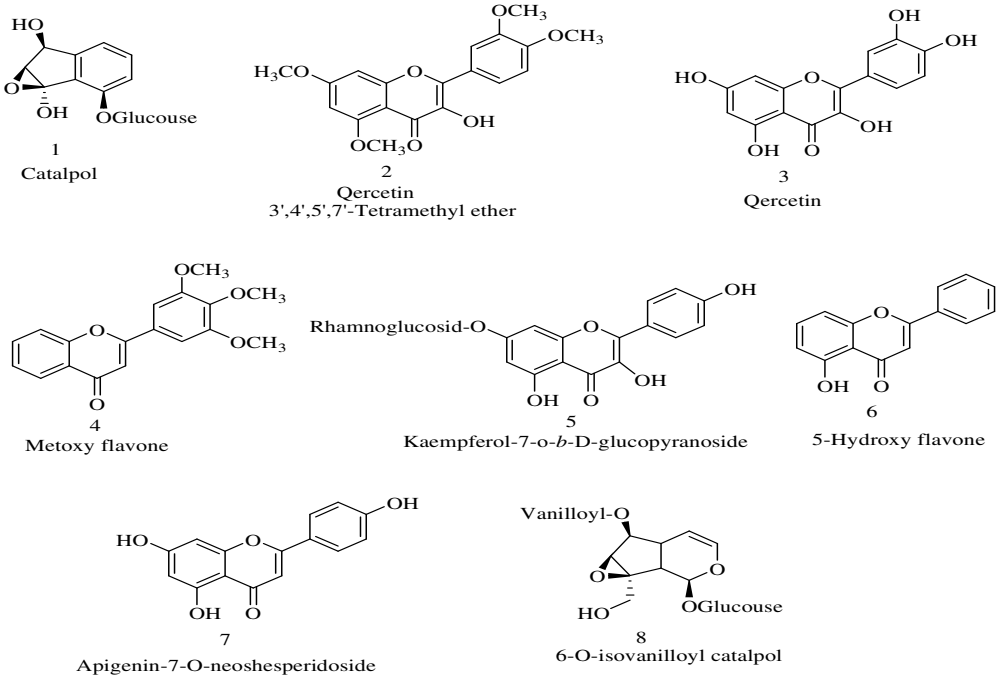
F-Flavonoid; I- iridoid.

6- Hydroxy and 8- Hydroxy flavone glucosides are found together only in *V. persica*, which suggests an allopolyploid origin of the species (Tomas-Barberan et al., 1988).

Based on the iridoid and flavonoid patterns of the studied species, we could outline the following evolutionary summary of subsect. *Agrestis:*

- Species, in which quercetin (**3**) is a precursor of quercetin derivatives as quercetin (3′, 4′, 5, 7-tetramethyl ether) (**2**): *V. persica* and *V. polita.*
- Species, in which catalpol (**1**) is a precursor of catalpol derivatives as 6-O-isovanilloyl catalpol (**8**): *V. persica.*
- Species, which synthesize quercetin (**3**): *V. francispetae* and *V. siaretensis.*

Fig 1. **Different compounds in *Veronica* species**



The main component in these two species was quercetin (**3**) accompanied with traces of other flavonoid and catalpol (**1**). According to Elenevskii (1978) *V. siaretensis* is probably a hybrid between *V. persica* and *V. polita*, while this species has been considered as an independent taxon based on morphological features (Fischer, 1981), fruit anatomy (Saeidi et al. 2001a) and pollen characters (Saeidi and Kharabian, 2005). Our results indicate *V. siaretensis* is separated from its allies based on iridoid and flavonoid patterns (Table 2). These species contained mainly quercetin, while *V. persica* and *V. polita* possessed quercetin (3′, 4′, 5, 7-tetrametyl eter) (**2**). Chemical variation is not so pronounced within all the studied species of this subsection. *V. persica* and *V. polita* are generally related in their morphology, however, *V. persica* can be distinguished from *V. polita* due to the occurrence of 6-O-isovanilloyl catalpol. *V. persica* is an aggressive tetraploid species. Different opinions exist regarding its origin as an autopolyploid from *V. polita* (Peev, 1978).

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