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Acylated flavonoids and phenol glycosides from *Veronica thymoides* subsp. *pseudocinerea*

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Abstract

A new acylated flavone glucoside, 3′-hydroxyscutellarein 7-*O*-(6″-*O*-protocatechuoyl)-β-glucopyranoside (**1**), and a new phenol glucoside, 3,5-dihydroxyphenethyl alcohol 3-*O*-β-glucopyranoside (**6**) were isolated from *Veronica thymoides* subsp. *pseudocinerea* together with seven known flavone, phenol and lignan glycosides; 3′-hydroxyscutellarein 7-*O*-(6″-*O*-*trans*-feruloyl)-β-glucopyranoside (**2**), 3′-hydroxy, 6-*O*-methylscutellarein 7-*O*-β-glucopyranoside (**3**), luteolin 7-*O*-β-glucopyranoside (**4**), isoscutellarein 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl (1‴ → 2″)-β-glucopyranoside (**5**), 3,4-dihydroxyphenethyl alcohol 8-*O*-β-glucopyranoside (**7**), benzyl alcohol 7-*O*-β-xylopyranosyl (1″→ 2′)-β-glucopyranoside (**8**), and (+)-syringaresinol 4′-*O*-β-glucopyranoside (**9**). Compounds **2**, **3** and **7**–**9** were reported for the first time in the genus *Veronica*. The structures of the isolates were determined by means of spectroscopic (UV, IR, 1D and 2D NMR, HR ESI-MS) methods. Isolated compounds (**1**–**7**) exhibited potent radical scavenging activity against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

Keywords: *Veronica thymoides* subsp. *pseudocinerea*; Scrophulariaceae; Acylated flavone glycosides; Phenolic glycosides; Lignan glucoside; Free radical scavenging activity; DPPH

1. Introduction

The genus *Veronica* (Scrophulariaceae) is represented in Turkish Flora by 79 species, 26 of which are endemic (Davis, 1978). Some of the *Veronica* species are used as diuretic, for wound healing and against rheumatic pains in traditional medicine (Baytop, 1984; Fujita et al., 1995). In addition, several *Veronica* species are used to treat cancer, influenza, hemoptysis, laryngopharyngitis, hernia, cough and respiratory diseases and are also used as an expectorant and antiscorbutic (Tomassini et al., 1995; Su et al., 1999; Graham et al., 2000). Earlier investigations performed on *Veronica* species resulted in the isolation of mainly iridoid glucosides, especially benzoic and cinnamic acid esters of catalpol, some phenylethanoid and flavonoid glycosides (Chari et al., 1981; Harput, 2002; Harput et al., 2002a,b, 2003; Saracoglu et al., 2002; Taskova et al., 1998, 2002). Previously, a large variety of flavone aglycones such as luteolin, apigenin, chrysoeriol, scutellarein and isoscutellarein were reported from *Veronica* species. Glycosilation of these aglycones was usually seen at 7th or 5th position and the acylation of the sugars was another characteristic feature of some of the glycosides (Grayer-Barkmeijer, 1978; Chari et al., 1981; Peev, 1982; Wang et al., 1995; Harput, 2002; Albach et al., 2003). The acylated flavone glycosides were reported as chemosystematic markers in the genus *Veronica* (Grayer-Barkmeijer, 1978; Albach et al., 2003).

In a continuation of our studies on the bioactive constituents of *Veronica* species, we have further studied on an endemic *Veronica* species, *Veronica thymoides* subsp. *pseudocinerea*. This study was resulted with the isolation of several flavone and phenol gycosides, a lignan glucoside, iridoid glucosides and steroidal saponins. Here we have reported the isolation and the structure

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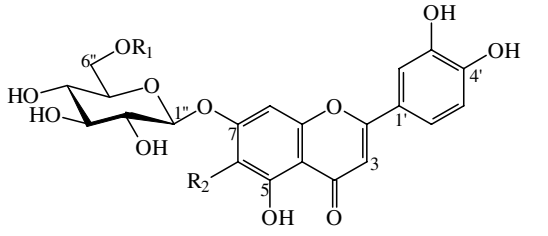
elucidation as well as the free radical scavenging activities of a new acylated flavone and a new phenol glucosides together with seven known flavone, phenol and lignan glycosides from the aerial parts of the titled plant (Figs. 1–3, Table 2). The first occurence of 3′-hydroxyscutellarein 7-*O*-(6″-*O*-*trans*-feruloyl)-β-glucopyranoside, 6-methoxyflavone, simple phenol and lignan glycosides in the genus *Veronica* has also been reported.

2. Results and discussion

The methanol extract of *V. thymoides* subsp. *pseudocinerea* was suspended in water and partitioned with petroleum ether. The water fraction of the methanol extract was subjected to polyamide column chromatography to afford five main fractions. Repeated column chromatography (RP, silica gel and sephadex LH-20) of the fractions which eluted with water, 50% methanol and methanol from the polyamide column, resulted in the isolation of nine compounds (**1**–**9**) in pure form. Compound **1** was isolated as a pale yellow amorphous powder with negative optical rotation ($[\alpha]_D^{23}$ −199.3°, $c = 0.43$; pyridine). The HR ESI-MS of **1** exhibited a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 623.1025 suggesting the molecular formula $C_{28}H_{24}O_{15}$ which was confirmed by the observation of a methylene, thirteen methine and fourteen quaternary carbon resonances in its ^{13}C NMR and DEPT spectra (Table 1). It exhibited UV and IR absorptions confirming its polyphenolic nature. Its UV spectrum showed λ_{max} at 221, 259, 285 and 344 nm which was characteristic for 5,7,3′,4′-tetrahydroxyflavones and the presence of a short wavelength band at 285 nm suggested that the C-6 position might be hydroxylated (Harborne and Williams, 1971). Analysis of the aromatic region of the ^1H NMR spectrum of **1** (Table 1) confirmed that the compound **1** was a 3′-hydroxyscutellarein derivative (Iinuma et al., 1980; Chari et al., 1981; Harput, 2002; Albach et al., 2003), with characteristic resonances for H-3 at δ 6.54 (1H, *s*, δ_C 103.55 by HMQC), H-8 at δ 6.83 (1H, *s*, δ_C 95.49), H-2′ at δ 7.27 (1H, *d*, $J = 2.1$ Hz), H-5′ at δ 6.82 (1H, *d*, $J = 8.5$ Hz) and H-6′ at δ 7.21 (1H, *dd*, $J = 8.5, 2.1$ Hz). The ^{13}C resonance of C-6 at δ_C 131.91 was consistent with a hydroxyl group at this position. This suggestion was confirmed by the HMBC correlation between H-8 (δ 6.83) and C-6 (δ_C 131.91, C) (Fig. 2). The remaining resonances in the aromatic region were those of an protocatechuoyl group with an ABX system at δ 7.34 (1H, *d*, $J = 2.4$ Hz, H-2‴), δ 6.51 (1H, *d*, $J = 7.9$ Hz, H-5‴) and δ 7.36 (1H, *dd*, $J = 8.2, 2.1$ Hz, H-6‴). The structure of the acyl group was confirmed by complete assignment of its ^1H and ^{13}C resonances from COSY, HMQC and HMBC data (Table 1, Fig. 2). The final group of resonances in the ^1H NMR spectrum of **1** were those of a sugar with an anomeric proton at δ 5.12 (1H, *d*, $J = 7.6$ Hz). The HMBC correlation observed between this anomeric proton and C-7 (δ_C 152.52), confirmed that the sugar was attached to C-7 of the aglycone. The complete structure of the sugar unit was elucidated by using COSY and HMQC experiments and the coupling



	R_1	R_2
1	protocatechuoyl	OH
2	*trans*-feruloyl	OH
3	H	OCH_3
4	H	H

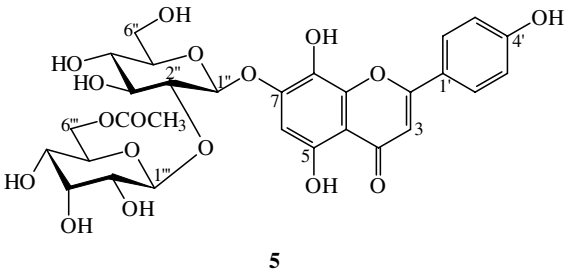


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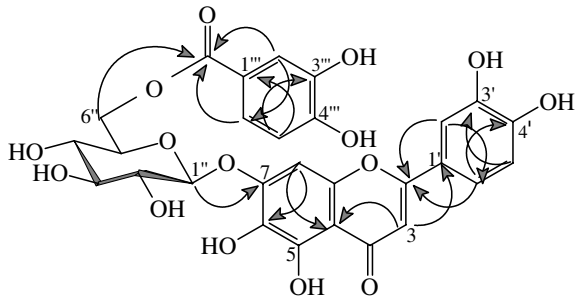
Fig. 1. Flavone glycosides from *Veronica thymoides* subsp. *pseudocinerea*.



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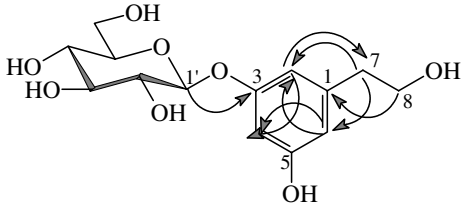

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Fig. 2. Selected HMBC correlations for compounds **1** and **6**. Arrows point from H to C.

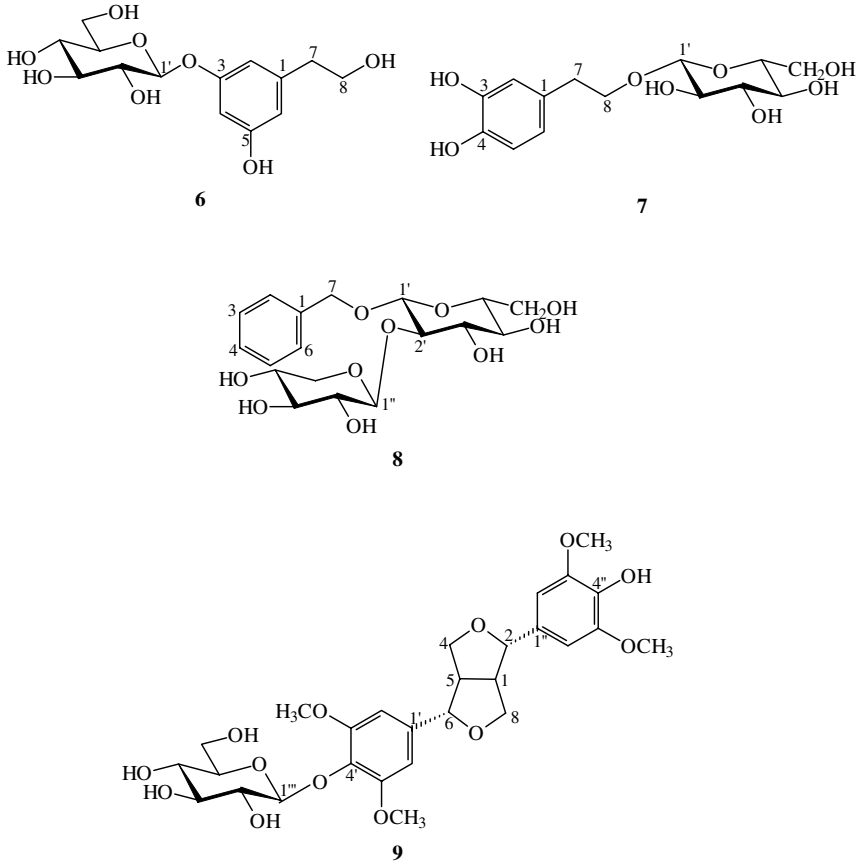

Fig. 3. Phenol and lignan glycosides from *Veronica thymoides* subsp. *pseudocinerea*.

constant of anomeric proton as a β-glucopyranose. Of particular interest were the downfield-shifted resonances of 6″-CH_2 (δ 4.76 and 4.37) and C-6″ (δ_C 64.77) suggested that the protocatechuoyl moiety was placed at C-6″. The position of sugar residue and protocatechuoyl moiety were unambiguously determined by the HMBC experiment (Fig. 2). Consequently the structure of compound **1** was established as 3′-hydroxyscutellarein 7-*O*-(6″-*O*-protocatechuoyl)-β-glucopyranoside [= 6-hydroxyluteolin 7-*O*-(6″-*O*-protocatechuoyl)β-glucopyranoside], a new flavone glucoside, which was isolated for the first time from the nature.

The structures of compounds **2-5** were elucidated by UV, IR, 1D and 2D NMR, HR ESI-MS, FAB-MS experiments and comparison with literature data as 3′-hydroxyscutellarein 7-*O*-(6″-*O*-*trans*-feruloyl)-β-glucopyranoside [= 6-hydroxyluteolin 7-*O*-(6″-*O*-*trans*-feruloyl)-β-glucopyranoside] (**2**) (Kawabata et al., 2003), 3′-hydroxy, 6-*O*-methylscutellarein 7-*O*-β-glucopyranoside (= 6-*O*-methylluteolin 7-*O*-β-glucopyranoside = nepetin 7-*O*-β-glucopyranoside) (**3**) (Wolf and Denford, 1984; Merfort, 1988), luteolin 7-*O*-β- glucopyranoside (**4**) (El-Negoumy et al., 1986; Feeny et al., 1988), isoscutellarein 7-*O*-(6‴-*O*-acetyl)-β-allopyranosyl (1‴ → 2″)-β-glucopyranoside (**5**) (Lenherr et al., 1984; Lenherr and Mabry, 1987; Wang et al., 1995; Harput, 2002; Albach et al., 2003). This is the first report for the isolation of compounds **2** and **3** from a *Veronica* species. On the other hand, the ^{13}C NMR data of compound **2** was reported for the first time in this study.

Compound **6** was isolated as an amorphous powder with negative optical rotation ($[\alpha]_D^{23}$ −33.5°, $c = 0.18$; MeOH). The HR ESI-MS of **6** exhibited a pseudomolecular ion peak $[M + Na]^+$ at *m/z* 339.1039 suggesting the molecular formula $C_{14}H_{20}O_8$ which was confirmed by the observation of three methylene, eight methine and three quaternary carbon resonances in its ^{13}C NMR and DEPT spectra (Table 1). Its ^1H NMR spectrum displayed three *meta*-coupled aromatic protons at δ 7.07 (1H, *d*, $J = 1.8$ Hz, H-2), 6.76 (1H, *s*, H-4) and 6.77 (1H, *d*, $J = 1.8$ Hz, H-6), two methylenes [benzylic methylene protons at δ 2.71 (2H, t, $J = 7.3$ Hz, H-7), hydroxymethylene protons at δ 3.70 (2H, H-8)] which were coupled with each other in COSY. The corresponding ^{13}C resonances were obtained from HMQC spectrum and the aglycone of **6** was found to be as a 3,5-dihydroxyphenethyl alcohol (Fig. 3). An anomeric proton at δ 4.74 (1H, *d*, $J = 7.6$ Hz, H-1′) and the signals in the region of δ 3.40–3.89 suggested the presence of a glucose unit. The β-anomeric configuration of the glucose was

Table 1
^{13}C and ^{1}H NMR spectral data for compounds **1**, **2**, and **6** (CD_3OD; ^{13}C: 125 MHz, ^{1}H: 500 MHz)[a]

C/H	**1**			**2**			**6**		
	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (Hz)	δ_C (ppm)	δ_H (ppm)	J (Hz)
1							132.08		
2	167.02			166.57			119.63	7.07 *d*	1.8
3	103.55	6.54 *s*		103.21	6.35 *s*		146.80		
4	184.41			184.26			116.91	6.76 *s*	
5	148.10			148.99			147.00		
6	131.91			131.80			125.19	6.77 *d*	1.8
7	152.52			152.47			39.52	2.71 *t*	7.3
8	95.49	6.83 *s*		95.33	6.79 *s*		64.32	3.70[b]	
9	151.00			151.02					
10	107.55			107.43					
1′	123.68			123.45					
2′	114.36	7.27 *d*	2.1	114.12	7.23 *br s*				
3′	146.89			146.86					
4′	151.31			151.13					
5′	116.78	6.82 *d*	8.5	116.65	6.80 *d*	8.5			
6′	120.54	7.21 *dd*	8.5, 2.1	120.32	7.21 *dd*	8.0, 2.1			
Glc									
1″	102.08	5.12 *d*	7.6	101.74	5.09 *d*	7.6	104.45	4.74 *d*	7.6
2″	74.61	3.64 *t*	9.1	74.52	3.63 *dd*	9.1, 7.0	74.90	3.47 *dd*	9.1, 7.9
3″	77.41	3.59 *t*	9.1	77.51	3.58 *t*	9.1	78.31	3.46[b]	
4″	71.99	3.52 *t*	9.1	72.61	3.43 *t*	9.2	71.38	3.40[b]	
5″	75.78	3.88 *m*		75.60	3.88 *m*		77.65	3.40[b]	
6″	64.77	4.76 *dd*	11.9, 2.4	64.77	4.75 *dd*	11.9, 2.4	62.49	3.89 *dd*	11.9, 2.0
		4.37 *dd*	12.2, 6.7		4.27 *dd*	11.9, 8.5		3.70[b]	
Acyl									
1‴	122.36			127.33					
2‴	117.44	7.34 *d*	2.4	111.21	6.71 *d*	1.8			
3‴	146.13			148.00					
4‴	151.73			150.42					
5‴	115.87	6.51 *d*	7.9	116.27	6.49 *d*	8.2			
6‴	123.61	7.36 *dd*	8.2, 2.1	123.73	6.55 *dd*	8.2, 1.8			
α				114.88	6.21 *d*	15.8			
β				147.54	7.43 *d*	15.8			
C=O	168.23			168.91					
OCH_3				56.05	3.63 *s*				

[a] The ^{13}C and ^{1}H assignments were based on DEPT and 2D NMR (^{1}H, ^{1}H-COSY, ^{1}H, ^{13}C-HMQC and ^{1}H, ^{13}C-HMBC) experiments.
[b] Signal patterns are unclear due to overlapping.

judged based on the large $^{3}J_{\text{H-1, H-2}}$ coupling constant of the anomeric proton (δ 4.74, *d*, $J = 7.6$ Hz). An HMBC cross-peak between H-1′ (δ 4.74) and C-3 (δ 146.80) showed that the β-glucopyranose unit was attached to the C-3 position of the aglycone (Fig. 2). The complete structure of **6** was determined by 1D and 2D NMR spectroscopy as the 3,5-dihydroxyphenethyl alcohol 3-*O*-β-glucopyranoside, a new phenol glucoside, which was isolated for the first time from the nature.

Compounds **7**–**9** were identified by the comparison of their spectral data with those reported in the literature as 3,4-dihydroxyphenethyl alcohol 8-*O*-β-glucopyranoside (**7**) (Shimomura et al., 1987), benzyl alcohol 7-*O*-β-xylopyranosyl (1″→ 2′)-β-glucopyranoside (**8**) (Kamel et al., 2000) and (+)-syringaresinol 4′-*O*-β-glucopyranoside (**9**) (Deyama et al., 1985), respectively.

Although the acylated flavone glycosides are common compounds in *Veronica* species, there are no reports on the isolation of simple phenol and lignan glycosides. This is the first report of the isolation of compounds **7**–**9** in the genus *Veronica*.

In our previous studies on *Veronica* species, we have reported that the antioxidant, anti-inflammatory and cytotoxic activities of the plant extracts and the isolated compounds (Harput et al., 2002b,c; Saracoglu et al., 2002). On the basis of these results we determined the free radical scavenging activity of isolated compounds (**1**–**9**) using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging system by comparing 3-*tert*-butyl-4-hydroxy-anisole (BHA), widely used synthetic antioxidant and *dl*-α-tocopherol (α-TOC), commonly used natural antioxidant. Compound **8** did not show any scavenging activity against DPPH radical while the scavenge % of compound **9** was not significant. As shown on Table 2, the DPPH radical scavenging activities of compounds **1**–**7** were more than that of BHA and

Table 2
Free radical scavenging activity of compounds **1**–**7**, BHA and α-TOC on DPPH radical (1.5×10^{-5}M)[a]

Compounds	Scavenge (%)			
	10 μM	2.5 μM	0.5 μM	0.1 μM
1	50.0	46.8	36.4	3.9
2	49.1	43.8	17.0	
3	38.1	31.5	3.5	
4	38.0	31.5	3.5	
5	40.5	6.5		
6	41.4	43.7	14.4	2.3
7	41.4	43.7	14.4	2.3
BHA	23.0	5.0		
α-TOC	41.1	15.5	0.2	

Blank, in the absence of sample; BHA, 3-*tert*-butyl-4-hydroxyanisole; α-TOC, *dl*-α-tocopherol.

[a] Each value is the average of duplicate determinations. Inhibitory ratio of each compound is expressed as follow: scavenge $\% = 100 \times [(Abs_{blank} - Abs_{sample})/Abs_{blank}]$.

more than or comparable to that of α-TOC in the concentration of 10 μM. On the other hand, the same compounds were found to be more potent than BHA and α-TOC in the concentration of 2.5 μM. 3′-Hydroxyscutellarein 7-*O*-(6″-*O*-protocatechuoyl)-β-glucopyranoside (**1**) and 3′-hydroxyscutellarein 7-*O*-(6″-*O*-*trans*-feruloyl)-β-glucopyranoside (**2**) exhibited the strongest free radical scavenging activity. The radical scavenging effects of anti-oxidants on DPPH radical are thought to be due to their hydrogen donating ability (Hatano et al., 1989). Compounds **1**–**7** contain free phenolic hydroxyl groups in their structures, and phenolic hydroxyls have been recognized to function as electron or hydrogen donors. Thus, the DPPH radical scavenging activity of these compounds may be mostly related to their phenolic hydroxyl groups and it may play an important role in the actions of the *Veronica* species, and partly explain to the usage of them in traditional medicine.

3. Experimental

3.1. General

The UV (MeOH, λ_{max}, nm) and IR (KBr, ν_{max}, cm^{-1}) spectra were recorded on a Shimadzu UV-240 and Perkin–Elmer 2000 FTIR spectrophotometers, respectively. NMR measurements were performed on a JEOL JNM-A 500 spectrometer in methanol-d_4 (^{1}H: 500 MHz; ^{13}C: 125 MHz). Chemical shifts were given in ppm with tetramethylsilane (TMS) as an internal standard. FAB-MS was recorded in a NBA matrix in the positive ion mode on a JEOL JMS-DX300 spectrometer. HR ESI-MS was measured in ESI positive ion mode on a Bruker Daltonics APEX III spectrometer. Optical rotations were measured on JASCO DIP 140 digital spectrometer using a sodium lamp operating at 589 nm.

Column chromatography was carried out on silica gel (Merck, Kieselgel 60, 60-230 mesh), polyamide (Fluka, 50–160 μm) and sephadex LH-20 (Pharmacia). Medium pressure liquid chromatography (MPLC) was realized on Labomatic (18.5×352 mm) and Büchi (25×460 mm) glass columns filled with Li Chroprep RP-18 (Merck) using Lewa M5 peristaltic and Büchi B-684 pumps. Thin layer chromatography (TLC) was conducted on pre-coated, commercial silica gel (Merck, $60F_{254}$) plates with $CHCl_3$–MeOH–H_2O (61:32:7, 70:30:3, 80:20:2) as a developing solvent system. Compounds **1**–**9** were detected by UV fluorescence and/or spraying with 1% vanillin/H_2SO_4, followed by heating at 100 °C for 5 min. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was obtained from Aldrich Chem. Co. (Milwaukee, WI, USA). 3-*Tert*-butyl-4-hydroxyanisole (BHA) and *dl*-α-tocopherol (α-TOC), were purchesed from Nacalai Tesque Co. (Kyoto, Japan).

3.2. Plant material

Veronica thymoides P.H. Davis subsp. *pseudocinerea* M.A.Fischer (Scrophulariaceae) was collected from Amasya, Merzifon, Tavsandagi, in July, 2002. A voucher specimen has been deposited in the Herbarium of Faculty of Pharmacy, Hacettepe University (HUEF 02017).

3.3. Extraction and isolation

The air dried aerial parts of *V. thymoides* subsp. *pseudocinerea* (312 g) were extracted with MeOH at 40 °C for 12 h (2×, 2 l). The combined extracts were evaporated under vacuum to give 59 g of crude extract. The MeOH extract was dissolved in H_2O (0.1 l). H_2O-insoluble material was removed by filtration. The filtrate was partitioned with petroleum ether (6×, 100 ml), and the petroleum ether fraction was discarded. The water fraction was lyophilized to yield 45 g dry weight. The water fraction was dissolved in 50 ml of H_2O and subjected to polyamide column chromatography eluted with H_2O, followed by increasing concentrations of MeOH to give five fractions: Frs. A–E. [Fr. A (H_2O), 27.0 g; Fr. B (25% MeOH), 3.50 g; Fr. C (50% MeOH), 3.50 g; Fr. D (75% MeOH), 0.45 g; Fr. E (MeOH), 0.20 g].

The fraction eluted with H_2O from the polyamide column (Fr. A) was applied to a series of column chromatographies to yield compounds **6**–**9**. An aliquot of Fr. A (2.0 g) was applied to Medium Pressure Liquid Chromatography (MPLC) by using reversed-phase column. Eluting with increasing amounts of MeOH in H_2O ($5 \rightarrow 100$ %) yielded three main fractions: Frs. A1–A3. Fr. A1 (81.0 mg) was rich in compounds **6**, **7** and was rechromatographed over MPLC yielded compounds **6** (3.0 mg) and **7** (10.0 mg) in pure forms. Fr. A2 was found to contain compound **8**. Chromatography of Fr.

A2 (51.0 mg) over silica gel by stepwise elution with $CHCl_3$–MeOH (90:10 → 50:50) gave crude compound **8** (28.0 mg). Silica gel column chromatography of Fr. A3 (130 mg), eluting with $CHCl_3$–MeOH (100:0 → 90:10), resulted to the isolation of compound **9** (6.0 mg). The fraction eluted with 50% MeOH from the polyamide column (Fr. C) was rich in compounds **3**–**5**. An aliquot of Fr. C (1.9 g) was applied to medium pressure liquid chromatography (MPLC) by using reversed-phase column. Eluting with increasing amounts of MeOH in H_2O (5 → 100 %) yielded six main fractions: Frs. C1–C6. Fr. C1 (60 mg) was applied to sephadex LH-20 eluting with MeOH yielded compounds **3** (2.6 mg) and **4** (3.0 mg). Fr. C2 (70 mg) was chromatographed over silica gel by stepwise elution with $CHCl_3$–MeOH (95:5 → 80:20) and then was purified by sephadex (MeOH) to yield compound **5** (4.0 mg). Chromatography of the fraction eluted with MeOH from the polyamide column (Fr. E) over MPLC and then purification by sephadex LH-20 with MeOH gave compounds **1** (5.0 mg) and **2** (6.5 mg).

The other fractions of the polyamide column (Frs. B and D) were rich in iridoid glucosides and steroidal saponins. Therefore, isolation and structure elucidation studies on these fractions have still been continuing.

*3.4. 3′-Hydroxyscutellarein 7-O-(6″-O-protocatechuoyl)-β-glucopyranoside (**1**)*

Pale yellow amorphous powder; $[\alpha]_D^{23}$ −199.3°(c = 0.43, pyridine); UV (MeOH) λ_{max} nm: 221, 259, 285, 344; IR (KBr) ν_{max} cm^{-1}: 3404, 1714, 1663, 1604, 1516; ^1H NMR and ^{13}C NMR: see Table 1; HR ESI-MS *m/z*: 623.1025 $[M + Na]^+$.

*3.5. 3′-Hydroxyscutellarein 7-O-(6″-O-trans-feruloyl)-β-glucopyranoside (**2**)*

Pale yellow amorphous powder; $[\alpha]_D^{23}$ −151.2° (c = 0.16, MeOH); UV (MeOH) λ_{max} nm: 216, 287, 337; IR (KBr), ν_{max} cm^{-1}: 3355, 1700, 1663, 1605, 1518; ^1H NMR and ^{13}C NMR: see Table 1; HR ESI-MS *m/z*: 663.1342 $[M + Na]^+$.

*3.6. 3,5-Dihydroxyphenethyl alcohol 3-O-β-glucopyranoside (**6**)*

Amorphous powder; $[\alpha]_D^{23}$ −33.5°(c = 0.18, MeOH); UV (MeOH) λ_{max} nm: 235, 266; IR (KBr), ν_{max} cm^{-1}: 3425, 1607, 1523; ^1H NMR and ^{13}C NMR: see Table 1. HR ESI-MS *m/z*: 339.1039 $[M + Na]^+$.

3.7. DPPH free radical scavenging activity

The free radical scavenging effect of the isolated compounds **1**–**9** was assessed by the decoloration of ethanol solution of DPPH spectroscopically; BHA and *dl*-α-tocopherol were used as standards (Table 2) (Hatano et al., 1989; Harput et al., 2002b,c; Saracoglu et al., 2002). Each EtOH solution (100 μl) of compounds **1**–**9** at various concentrations (10–0.1 μM) was added to 1.5×10^{-5} M DPPH/EtOH solution. The reaction mixture was shaken vigorously and the absorbance of remaining DPPH was measured at 520 nm after 30 min. The radical scavenging activity was determined by comparing the absorbance with that of a blank (100%) containing only DPPH and solvent.

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