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RESEARCH ARTICLE

Iridoid content and biological activities of *Veronica cuneifolia* subsp. *cuneifolia* and *V. cymbalaria*

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Abstract

Context: The genus *Veronica* L. (Plantaginaceae) is represented by 79 species, 26 of which are endemic in Turkey. Some *Veronica* species are used for the treatment of different inflammatory diseases and cancer in traditional medicine. In addition, chemotaxonomy of the genus is important for the reclassification of the family Plantaginaceae after different phylogenetic studies.

Objective: *Veronica cuneifolia* subsp. *cuneifolia* D. Don and *V. cymbalaria* Bodard were studied from the view point of iridoid glucosides which are known as chemotaxonomical markers for this genus. Radical scavenging and cytotoxic activities of the extracts were also determined in this study.

Material and methods: Major compounds, isolated from iridoid fractions of *V. cuneifolia* subsp. *cuneifolia* were used as the standard compounds for HPLC after determination of their structures, and investigated for their presence in iridoid fractions of *V. cymbalaria*. Additionally, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and SO radical scavenging and cytotoxic activities against three cancer and a noncancerous cell lines of both extract were also tested using the MTT method.

Results: While aucubin, catalpol, verproside, amphicoside, verminoside, and veronicoside were obtained from *V. cuneifolia* subsp. *cuneifolia*, two more iridoid glucosides, 6-O-veratroylcatalposide and 6-O-isovanilloylcatalpol, were isolated from *V. cymbalaria*. Comparing both species, *V. cuneifolia* subsp. *cuneifolia* showed stronger radical scavenging and cytotoxic activities than *V. cymbalaria*.

Discussion: Our results demonstrated that the iridoid contents of both species were very close to each other confirming to the chemotaxonomic studies on *Veronica* species and their different bioactivity range make the plants interesting from the view point of natural drug discovery research.

Keywords: *Veronica* species; iridoid glucosides; cytotoxic activity; free radical scavenging activity

Introduction

The genus *Veronica* L. has traditionally been considered a member of Scrophulariaceae family. However, recent extensive molecular investigations of this and related families have demonstrated that traditional Scrophulariaceae is polyphyletic (Olmstead & Reeves, 1995; Olmstead et al., 2001; Oxelman et al., 2005). As a consequence of these results, the genus *Veronica* has been transferred to the Plantaginaceae (Albach et al., 2005; Olmstead, 2005). Recently, it has been shown that the iridoid glucosides aucubin, catalpol, and 6-O-catalpol

esters are characteristic for this genus and chemotaxonomic research concerning iridoid content of the genus have great importance for the reclassification of the close genera (Harput et al., 2002a, 2002b; Taskova et al., 2004; Jensen et al., 2005).

The genus *Veronica* is represented by 79 species, 26 of which are endemic in Turkish flora (Fisher, 1978). Some *Veronica* species are used as diuretics, for wound healing and against rheumatic pains in traditional medicine in Turkey (Baytop, 1984; Fujita et al., 1995). In addition, several *Veronica* species are used to treat cancer, influenza,

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hemoptysis, laryngopharyngitis, hernia, cough, and respiratory diseases, and are also used as an expectorant and antiscorbutic (Tomassini et al., 1995; Su et al., 1999; Grahame et al., 2000). Earlier investigations performed on *Veronica* species resulted in the isolation mainly of iridoid glucosides, especially benzoic and cinnamic acid esters of catalpol, some phenylethanoid, and flavonoid glycosides (Chari et al., 1981; Taskova et al., 1998; Harput et al., 2002a, 2002b; Saracoglu et al., 2002; Jensen et al., 2005). In this study, *Veronica cuneifolia* subsp. *cuneifolia* D. Don and *V. cymbalaria* Bodard were investigated from the view point of iridoid glucosides together with their radical scavenging and cytotoxic activities.

Material and methods

Plant material

Veronica cuneifolia subsp. *cuneifolia* D. Don from Antalya, Akseki, and *V. cymbalaria* Bodard was collected from Şanlıurfa, Turkey in June, 1999 and 2006, respectively. Both plants were identified by Prof. Dr. H. Duman from Gazi University, Ankara, Turkey. A voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 06006; HUEF 99131).

General

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), ascorbic acid were obtained from Sigma-Aldrich Chem Co (St. Louis, MO). 3-*t*-butyl-4-Hydroxyanisole (BHA) was purchased from Nacalai Tesque Co. (Kyoto, Japan). Fetal bovine serum (FBS) and minimal essential medium with Earl's salts (MEM-EARLE) with nonessential amino acids and antibiotics (penicillin and streptomycin) were purchased from Biochrom AG (Berlin, Germany). Trypsin (1:250) was obtained from Biochrom KG (Berlin, Germany). Media were supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Hep-2 (human larynx epidermoid carcinoma), RD (human rhabdomyosarcoma), L-20B (transgenic murine cells) and VERO (African Green Monkey kidney cells) were provided by Refik Saydam Hygiene Center, Virology Laboratory, Ankara, Turkey.

Preparation of the aqueous extract and isolation of the compounds from *V. cuneifolia* subsp. *cuneifolia*

The air-dried aerial parts of the plant (30 g) were extracted with methanol (MeOH) at 40°C for 12 h (3 × 300 mL). The MeOH phases were evaporated under vacuum to give MeOH extract (6.31 g). MeOH extracts was dissolved in water and partitioned with petroleum ether to remove chlorophylls. The water fraction was lyophilized to yield 5.97 g dry weight. Water extract was tested for cytotoxic and radical scavenger activities. Remaining extract was subjected to polyamide column chromatography eluted with H₂O, followed by increasing concentrations of MeOH to give five main fractions: MeOH 0% A; MeOH 25% B;

MeOH 50% C; MeOH 75% D; MeOH 100% E. Fr. A consisted of 6 subfractions. VLC of Fr. A2 using Lichroprep C18, eluting with increasing concentration of MeOH (0% → 100%), gave compounds **1** and **2** in pure form. Similarly, VLC of Fr. A5 in same conditions was resulted to the isolation of **3**, **4** and **5**, purely. Fr. C consisted of three subfractions and Sephadex column chromatography of Fr. C1 using MeOH gave compound **6** in pure form. Compound **7** was determined from the HPLC chromatograms comparing its retention time with that of authentic sample.

Preparation of the aqueous extract and the isolation of compounds from *V. cymbalaria*

Dried aerial parts of *V. cymbalaria* (10 g) were extracted with MeOH. Five main polyamide fractions were obtained from the aqueous extract (3.75 g) of the plant as the same conditions of *V. cuneifolia* subsp. *cuneifolia*. Iridoid fraction of *V. cymbalaria* was subjected to analytical RP-HPLC analysis to determine iridoid composition of the plant using compounds **1–7** as standard compounds. As a result of silica gel column chromatography of Fr. A3 which was determined to contain different structures, compounds **8** and **9** were isolated using CHCl₃:MeOH (100:0 → 85:15) as solvent system.

DPPH radical scavenging effect

The DPPH radical scavenging effect of the aqueous extract of *V. cuneifolia* subsp. *cuneifolia* and *V. cymbalaria* were assessed by the decoloration of MeOH solution of DPPH spectroscopically; butyl-4-hydroxyanisole (BHA) and ascorbic acid were used as reference compounds. MeOH solution (100 µL) of the samples at various concentrations were added to DPPH/MeOH (1 mM) 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 blank (100%) containing only DPPH and solvent. All the analyses were done in 3 replicates (Hatano et al., 1989; Harput et al., 2002a; Kostadinova et al., 2007; Jensen et al., 2010).

Superoxide radical scavenging effect by alkaline DMSO method

The method of Elizabeth and Rao (1990) was used for the detection of superoxide radical scavenging activity of the extract with slight modification. Briefly, a superoxide radical was generated in a nonenzymatic system. The reaction mixture containing 10 µL of NBT (1 mg/mL solution in DMSO) and 30 µL of the extract or reference compounds were dissolved in DMSO. Alkaline DMSO (100 µL of a 1 mL DMSO containing, 5 mM NaOH in 0.1 mL water) was added to give a final volume of 140 µL and the absorbance was measured at 560 nm using microplate reader (Elizabeth & Rao, 1990; Srinivasan et al., 2007; Jensen et al., 2010).

Cytotoxic activity

Cell suspensions (0.1 mL) of cancer cells were seeded into 96-multiwell plates at the concentration of 2×10^5

cells/mL for Hep-2, 1×10^5 cells/mL for RD, L-20B and VERO cells and cultured for 24 h in cell culture media in a humidified 5% CO₂ incubator at 37°C. Cells were incubated with various concentrations of the test samples for 48 h (800, 400, 200, 100, 50, 20, 10 µg/mL). After incubation, the cells were washed and replaced by fresh medium. MTT solution (10 µL of 5 mg/mL in PBS) was added and incubated for 4 h. Following the incubation, 100 µL of 10% SDS (sodium dodecyl sulfate) was added to each well to dissolve formed formazan crystals. The absorbance was measured at 577/655 nm using microplate reader (Mossman 1983; Harput et al., 2002a).

Results

The MeOH extract of *V. cuneifolia* subsp. *cuneifolia* was suspended in water and partitioned with petroleum ether. The water fraction of the MeOH extract was subjected to polyamide column chromatography to afford five main fractions. Repeated column chromatography (RP, silica gel, Sephadex LH-20) of the fractions which eluted with water from the polyamide column, resulted in the isolation of 6 compounds (**1-6**) in pure form and a minor iridoid glucoside (**7**) was determined by HPLC chromatograms comparing its retention time and UV spectrum with that of authentic sample. After structure determination of the isolated compounds, they were used as standard compounds for iridoid fractions of *V. cymbalaria*. Two more compounds (**8** and **9**) found in addition to 7 compounds from the HPLC analysis of *V. cymbalaria* and isolated from *V. cymbalaria* using different chromatographic techniques (Figure 1).

Compounds **1-6** were isolated as colorless amorphous compounds. Their TLC characteristics, UV and IR spectra were suggested iridoid structures for these compounds. Absorption band at 202 nm in their UV spectra was

characteristic for their enol-ether systems and indicated to the presence of nonsubstituted C-4 position for the compounds. The HR ESI-MS of **1** exhibited a pseudomolecular ion peak [M+Na]⁺ and [2M+Na]⁺ at *m/z* 369.1168 and 715.2454 suggesting the molecular formula C₁₅H₂₂O₉, which was confirmed by the observation of two methylene (CH₂), 12 methyn (CH) and a quaternary carbon resonances in its ¹³C and DEPT spectra.

Anomeric proton and carbon signal at δ_H 4.67 (d, *J*=7.9 Hz, H-1') and δ_C 99.98 (CH, C-1') together with other signals in the region of δ_H 3.21-3.85 indicated the presence of glucose unit (Tables 1-2). β-Anomeric configuration of the glucose was judged based on the large ³*J*_{H-1,H-2} coupling constant of the anomeric proton (*J*=7.9 Hz). When 6 glucose signals removed from total C signals in ¹³C NMR spectra, remaining 9 C signals suggested to the cyclopentan-pyran iridoid skeleton for the aglycon. H-1 acetal signal at δ_H 4.95 (1H, d, *J*=7.3 Hz) correlated to δ_H 2.89 (1H, t, *J*=7.5 Hz, H-9) and H-9 proton signal at δ_H 2.89 correlated to δ_H 2.65 (1H, m, H-5) in COSY spectrum. COSY correlations between H-5 (δ_H 2.65) to δ_H 5.09 (1H, dd, *J*=6.1/4.0 Hz, H-4) and 4.43 (1H, m, H-6); H-4 (δ_H 5.09) to δ_H 6.30 (1H, dd, *J*=6.2/2.0 Hz, H-3) confirmed the presence of double bond between C-3 and C-4. Additional COSY correlations from H-6 (δ_H 4.43) proton signal to δ_H 5.76 (1H, t, *J*=1.5 Hz, H-7) and triplet H-9 signal indicated the quaternary C-8 signal and second double bond between C-7 and C-8. The position of hydroxymethyl group at C-8 was confirmed from the HMBC correlation of H₂-10/C-8, H₂-10/C-7 and H₁-10/C-9. In addition, H-1'/C-1 correlation in HMBC spectrum showed us to a β-glucose unit at C-1 position. From these results structure of compound **1** was determined as aucubin and confirmed with the comparison of its spectral data with the previously published data for aucubin (Akdemir & Calis, 1991). Similar UV, IR, ¹H and ¹³C NMR spectra of

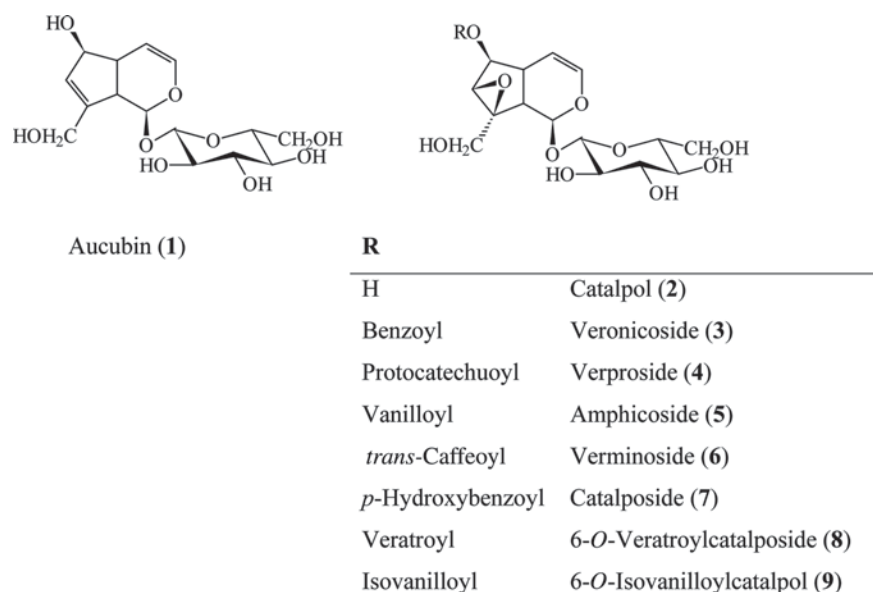


Figure 1. Structures of compounds **1-9** isolated from *V. cuneifolia* subsp. *cuneifolia* and *V. cymbalaria*. (See colour version of this figure online at www.informahealthcare.com/phb)

Table 1. ^{13}C NMR data of isolated compounds (CD_3OD ; 125 MHz).

C/H atom	δ_{C} ppm (1)	δ_{C} ppm (2)	δ_{C} ppm (3)	δ_{C} ppm (4)	δ_{C} ppm (5)	δ_{C} ppm (6)	δ_{C} ppm (8)	δ_{C} ppm (9)
Aglycone								
1	97.78	95.26	95.15	95.06	95.08	95.14	95.08	95.09
3	141.63	141.78	142.57	142.42	142.41	142.44	142.46	142.43
4	105.76	103.99	102.89	102.96	102.95	102.99	102.91	102.97
5	46.34	39.08	36.85	36.76	36.77	36.83	36.78	36.79
6	82.90	79.57	82.16	81.55	81.69	81.37	81.86	81.71
7	130.30	62.52	60.24	60.28	60.30	60.33	60.26	60.31
8	148.07	66.18	66.97	66.82	66.87	66.88	66.90	66.86
9	47.99	43.55	43.30	43.17	43.27	43.20	43.23	43.25
10	61.46	61.58	61.34	61.31	61.29	61.36	61.29	61.31
Glucose								
1'	99.98	99.68	99.79	99.67	99.70	99.77	99.72	99.73
2'	74.97	74.82	74.94	74.84	74.85	74.92	74.86	74.89
3'	77.95	77.67	77.80	77.67	77.71	77.78	77.72	77.74
4'	71.62	71.74	71.88	71.77	71.78	71.85	71.79	71.82
5'	78.33	78.59	78.75	78.66	78.66	78.72	78.66	78.69
6'	62.71	62.89	63.03	62.94	62.94	63.00	62.96	62.96
Acyl								
1''			131.07	122.12	121.97	127.68	123.27	122.50
2''			130.71	117.53	113.65	115.25	113.46	116.10
3''			129.73	146.25	148.86	149.88	150.27	147.60
4''			134.59	152.01	153.33	146.93	155.14	153.55
5''			129.73	115.93	116.04	118.61	111.98	111.50
6''			130.71	123.87	125.33	123.19	125.13	124.90
C=O			167.92	167.99	167.88	169.00	167.69	167.80
OCH_3					56.47		56.50	
OCH_3							56.52	56.47
α						114.57		
β						147.67		

The assignments are based on DEPT, COSY, HMQC, and HMBC experiments.

compound **2** with **1** suggested to 4 nonsubstituted iridoid glucoside structure for **2**. Its pseudomolecular ion peaks at 385.1110 $[\text{M} + \text{Na}]^+$ and 747.2344 $[2\text{M} + \text{Na}]^+$ in HR ESI-MS spectrum and C signals in ^{13}C NMR and DEPT spectra suggested to molecular formula of **2** as $\text{C}_{15}\text{H}_{22}\text{O}_{10}$. ^1H NMR and ^{13}C NMR spectra of compound **1** and **2** were found to be similar to each other. COSY correlations of δ_{H} 3.90 (1H, H-6) to oxymethyn at δ_{H} 3.44 (1H, d, $J=0.9$ Hz, H-7), and no other correlations of H-7 make the main difference of two compounds. Two oxymethyn carbon signals at δ_{C} 79.57 and 62.52 for C-6 and C-7 position in ^{13}C -NMR spectrum and comparison of these data with catalpol and catalpol derivatives confirmed the presence of epoxide function between C-7 and C-8 (Bianco et al., 1983). As no difference was observed other than epoxide function, the structure of compound **2** was determined as catalpol with the comparison of its spectral data with previously published articles (Chaudhuri & Sticher, 1981; Akdemir & Calis, 1991; Ozipek et al., 2000).

The UV and IR spectra of compounds **3-6** were similar to those of compound **1** and **2**. However, the signals at 233 and 274 nm in their UV spectra for aromatic ring and IR absorption bands at 3410 cm^{-1} for hydroxyl, 1640 cm^{-1} for olephinic double bonds, ester carbonyl

at 1705 cm^{-1} and aromatic signals at $1515\text{--}1600\text{ cm}^{-1}$ indicated to aromatic esterification for these compounds. C-1 monoglucosidic catalpol structure for compounds **3-6** was confirmed from the comparison of their spectral data with compound **2**, catalpol. The difference of compounds **3-6** to catalpol was found from the aromatic rings and ester carbonyl signals. Of particular interest were downfield-shifted resonances of C-6 and H-6 (δ_{H} 5.03–5.17, H-6 and δ_{C} 81.37–82.12, C-6) suggested that esterification was placed at C-6. The position of ester moiety was unambiguously determined by HMBC experiment from the correlations between H-6 and carbonyl (C=O) resonances. Detailed examination of aromatic resonances of compounds **3-6** from COSY and HMQC experiments led to the finding that esterified acids were benzoic, protocatechuic, vanillic, and *trans*-caffeic acids, HMBC correlations and comparison of previously published data for compounds **3-6** were confirmed their structures as benzoylcatalpol (veronicoside [**3**]), protocatechuoylcatalpol (verprosine [**4**]), vanilloylcatalpol (amphicoside [**5**]), *trans*-caffeoylcatalpol (verminoside [**6**]; Sticher & Afifi-Yazar, 1979a, 1979b; Afifi-Yazar & Sticher, 1980; Lahloub, 1983). In addition to these compounds, one

Table 2. ^1H NMR data of isolated compounds (CD_3OD ; 500 MHz).

[illegible]

The assignments are based on DEPT, COSY, HMQC, and HMBC experiments. 3J values can not be determined due to overlapping of the signals.

more compound was observed in the HPLC chromatogram of the polyamide fractions. It was determined as *p*-hydroxybenzoylcatalpol (catalposide [7]) from its HPLC chromatograms comparing time and UV spectra with that of authentic sample which was previously isolated from *Veronica* species (Figure 1).

After structure determination of compounds **1–7**, they were used as standard compounds for HPLC analysis of iridoid fractions of *V. cymbalaria*. Two more compounds were detected in addition to 7 compounds from the HPLC analysis of *V. cymbalaria* in 40% MeOH:(H₂O + 1% H₃PO₄) system. These two compounds (**8** and **9**) were isolated from *V. cymbalaria* using different chromatographic techniques. As a result of detailed examination of UV, IR, and NMR spectra of these compounds (Tables 1 and 2) and comparison of their spectral data with those of compounds **1–6**, their structures were determined as 6-*O*-veratroylcatalposide (**8**; Ozipek et al., 2000) and 6-*O*-isovanilloylcatalpol (**9**; Figure 1; Sticher & Afifi-Yazar, 1979b). Although catalpol derivatives are well known compounds for genus *Veronica*, their NMR data has not been available in recent publications. Detailed ¹H and ¹³C NMR data of aucubin and catalpol derivatives in Tables 1 and 2 given by us could be considered to be helpful for the researcher working with these iridoid glucosides.

In addition to the phytochemical studies, the aqueous extracts of *V. cuneifolia* subsp. *cuneifolia* and *V. cymbalaria* were evaluated for their radical scavenging and cytotoxic activities. Cytotoxic activity of the aqueous extracts was tested against three different cancer cells and one non-cancerous cell line. 2 × 10⁵ cells/mL cells for Hep-2, 1 × 10⁵ cells/mL for L-20B, RD and

VERO cell lines were used for the assay and incubated for 48 h with various concentrations of the extracts. After incubation, viability was determined by the MTT method. Both extracts showed cytotoxic activity against Hep-2, RD, L-20B and non-cancerous VERO cell lines. Maximum effect was observed in 800 µg/mL concentration of the extracts for three of the cancer cell lines (Figure 2). While *V. cuneifolia* subsp. *cuneifolia* showed higher cytotoxicity than *V. cymbalaria*, their cytotoxicity against VERO cell line found to be lower than tested cancer cells (Table 3). Although, both extracts showed moderate cytotoxic activity against Hep-2, RD and L-20B cell lines, different activity range against different cells indicated the selective effect of the extracts.

Similarly, DPPH and SO radical scavenging activities of the aqueous extracts and the reference compounds were tested and *V. cuneifolia* subsp. *cuneifolia* was found to show higher scavenging activity than *V. cymbalaria* against tested radicals. However, both extracts showed very low scavenging activity comparing to reference compounds, BHA and AA (IC₅₀ < 10 µg/mL; Table 3). Different cytotoxic activity against cancer cell lines and scavenging activity of *V. cuneifolia* subsp. *cuneifolia* led us to focus on phytochemical and biological studies on *V. cuneifolia* subsp. *cuneifolia*. In our previous research, *V. cymbalaria*, *V. hederifolia*, *V. pectinata* var. *glandulosa*, *V. persica*, and *V. polita* were found to be cytotoxic against KB epidermoid carcinoma and B16 melanoma cell lines. Similar cytotoxic activity of *V. cymbalaria* against KB, B16, Hep-2, RD, and L20B cell lines indicated a wide cytotoxic activity spectrum for *Veronica* species.

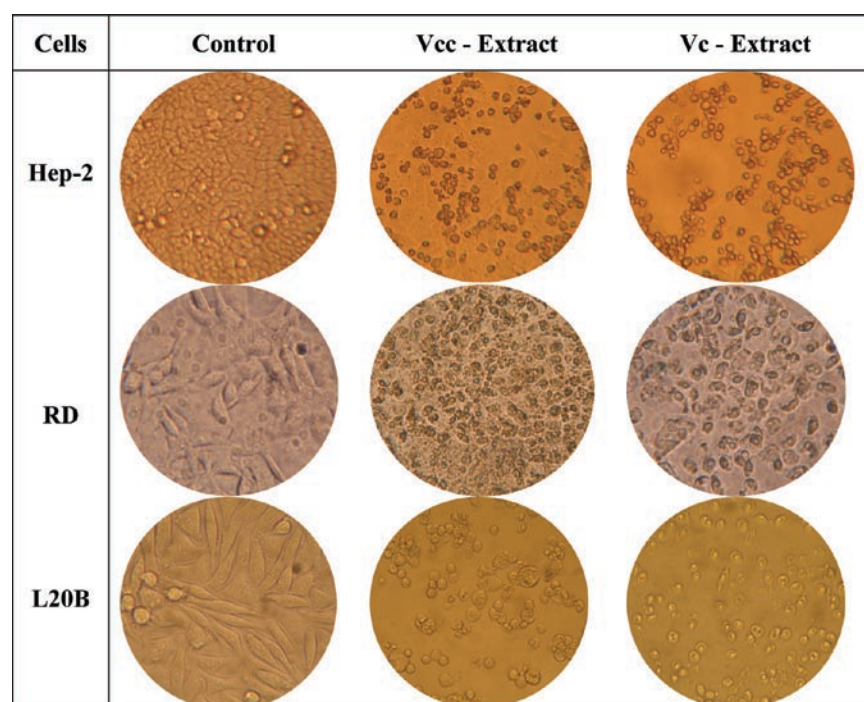


Figure 2. Cytotoxic activity of *V. cuneifolia* subsp. *cuneifolia* (Vcc) and *V. cymbalaria* (Vc) aqueous extract (800 µg/mL) against different cancer cell lines using the MTT assay.

Table 3. Cytotoxic and radical scavenging activities of *V. cuneifolia* subsp. *cuneifolia* (Vcc) and *V. cymbalaria* (Vc).

	IC ₅₀ (µg/mL) ^a	
	Vcc	Vc
Hep-2	389.0	472.3
RD	250.4	546.5
L-20B	262.7	435.1
VERO	410.9	696.8
	IC ₅₀ (µg/mL) ^b	
DPPH	32.9	153.6
SO	214.5	424.4

^aIC₅₀ value against each cancer cell line, which was defined as a concentration (µg/mL) that caused 50% inhibition of cell growth *in vitro*.

^bIC₅₀ value against each radical, which was defined as a concentration (µg/mL) that suppressed 50% of the oxidation extent.

Discussion

In our continuous research on the genus *Veronica*, we present here radical scavenging and cytotoxic activities of the aqueous extracts of *V. cuneifolia* subsp. *cuneifolia* and *V. cymbalaria* together with their iridoid contents for the first time in this study. While aucubin, catalpol, verminoside, verproside, amphicoside, and catalposide were obtained from *V. cuneifolia* subsp. *cuneifolia*, two more iridoid glucosides, 6-*O*-veratroylcatalposide and 6-*O*-isovanilloylcatalpol were isolated from *V. cymbalaria*. Comparing both species, *V. cuneifolia* subsp. *cuneifolia* showed stronger radical scavenging and cytotoxic activities than *V. cymbalaria* for all tested concentrations. Cytotoxic activities of aucubin, catalpol, and catalpol derivatives isolated from *Veronica* species were also determined against Hep-2, RD, and L-20B cell lines, previously (Saracoglu et al., 2008; Saracoglu & Harput-Hudaverdi, 2009). Verminoside (**6**), the *trans*-caffeic acid ester of catalpol, showed very strong cytotoxicity against tested cell lines. While cytotoxic activity of amphicoside (**5**) and veronicoside (**3**) found lower than that of verminoside, 6-*O*-veratroylcatalposide and verproside were found to have cytostatic activity. Catalpol and aucubin did not show any cytotoxic activity against three of the tested cell lines (Saracoglu et al., 2008; Saracoglu & Harput-Hudaverdi, 2009). Our previous results are in good agreement with present results and could be good explanation of the cytotoxic activity of *V. cuneifolia* subsp. *cuneifolia* and *V. cymbalaria* extracts. However, our results were also evaluated for the chemotaxonomic research on the genus *Veronica* and iridoid glucosides were aimed to research in this study because of their chemotaxonomic importance for the reclassification of the Plantaginaceae family (Jensen et al., 2005). According to Taskova et al. (1998, 2004) the genus *Veronica* consists of four subsections: Chamaedrys, Alsinebe, Beccabunga, and Veronicastrum and 4-substituted iridoid glucosides were not isolated from the subsections Chamaedrys (A1-2) and Alsinebe (F-G) up to now. Our results are in

good correlation with these results with the absence of 4-substituted iridoid glucosides in *V. cuneifolia* subsp. *cuneifolia* (subsection Chamaedrys) and *V. cymbalaria* (subsection Alsinebe).

Conclusion

The results of this study give an insight to the chemotaxonomic and bioactivity research on the genus *Veronica* which is widely distributed and used for the treatment of different diseases in Turkey and the world. These species together with other *Veronica* species from different subsections should be investigated in detail both for their chemical constituents and different bioactivities for the future studies.

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Declaration of interest

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