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Original Article

COMPARATIVE HPLC DETERMINATION OF IRIDOID CONTENTS IN *VERONICA CUNEIFOLIA* SUBSP. *CUNEIFOLIA* AND *V. CYMBALARIA*

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Abstract

In our continued chemotaxonomic investigations on water soluble compounds of Veronica species, two additional species, Veronica cuneifolia subsp. cuneifolia and V. cymbalaria were compared on the basis of their major components in iridoid fractions. Iridoid glucosides which are known as chemotaxonomical marker for this genus are investigated by HPLC-DAD system using different solvent systems. Seven iridoid glucosides; aucubin, catalpol, veronicoside, verproside, amphycoside, verminoside and catalposide were identified for both species. In addition, 2 more iridoid glucosides, 6-O-veratroylcatalposide and 6-O-isovanilloylcatalpol were determined from V. cymbalaria and isolated from its iridoid fractions. The presence of two rare iridoid glucosides in V. cymbalaria makes the plant interesting for the reclassification of Veronica species.

Keywords: *Veronica species, iridoid glucosides, catalpol derivatives, HPLC*

Veronica cuneifolia subsp. *cuneifolia* ve *V. cymbalaria*'nın İridoit İçeriklerinin YPSK ile Karşılaştırılması

Veronica türlerinin suda çözünen bileşenleri üzerinde yapmakta olduğumuz çalışmaların devamında, Veronica cuneifolia subsp. cuneifolia ve V. cymbalaria, iridoit fraksiyonlarında bulunan major iridoit glukozitleri yönünden karşılaştırılmıştır. Bu cins için kemotaksonomik açıdan önemli olan iridoid glukozitleri farklı solvan sistemleri kullanılarak YPSK-DAD metodu ile incelenmiştir. Her iki bitkide de okubin, katalpol, veronikozit, verprozit, amfikozit, verminozit ve katalpozit olmak üzere toplam 7 bileşik belirlenmiştir. Buna ilave olarak, V. cymbalaria'da 2 farklı iridoit glukoziti, 6-O-veratroil katalpozit ve 6-O-izovanilloilkatalpol tespit edilmiş ve izolasyonları gerçekleştirilmiştir. Nadir rastlanan bu iki bileşiğin V. cymbalaria'da bulunması bu bitkiyi Veronica türlerinin yeniden sınıflandırılmasında ilginç bir tür olarak ortaya koymaktadır.

Anahtar Kelimeler: *Veronica türleri, iridoit glukozitleri, katalpol türevleri, YPSK*

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INTRODUCTION

Veronica L. (Plantaginaceae) is a large and almost cosmopolitan genus of annual and perennial herbs located in Central/ Southern Europe and Turkey. It is represented by 79 species, 26 of which are endemic in Turkish Flora (1). It 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 (2-4). As a consequence of these results, the genus *Veronica* has been transferred to Plantaginaceae family (5-6). The phytochemistry of the genus has been studied extensively with many species surveyed for their iridoid and flavonoid constituents. Iridoids, mainly aucubin, catalpol and 6-*O*-catalpol esters are characteristic for this genus and chemotaxonomic researches concerning iridoid content of the genus are important for the reclassification of the close genera (7-10).

In addition to chemotaxonomic importance of the genus, some of the *Veronica* species are used as diuretic, for wound healing and against rheumatic pains in traditional Turkish medicine (11-12). 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 (13-15). Concerning the chemotaxonomic and pharmacological importance of the genus, *Veronica cuneifolia* subsp. *cuneifolia* and *V. cymbalaria* were investigated from the view point of iridoid glucosides using HPLC-DAD system to find any difference in their iridoid composition.

EXPERIMENTAL

Plant Material

Veronica cuneifolia subsp. *cuneifolia* D. Don from Antalya, Akseki and *V. cymbalaria* Bodard was collected from Şanlıurfa, Turkey. Voucher specimen has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 06006; HUEF 99131).

HPLC Apparatus and Conditions

HPLC investigations were performed on a Dionex HPLC instrument consisting of a P680 HPLC pump, Dionex ASI-100 autosampler and Dionex Photodiod Array Detector. The column was Hichrom-Nucleosil 100-5 C_{18} (5 μm, 250 mm X 4.6 mm) and column temperature was maintained at 27 °C. 10 μl injection volume and 1 ml/min flow rate were used for the each experiment. Chromatographic grade double distilled water; HPLC grade methanol and analytical grade trifluoro asetic acid were used for the HPLC analysis. Samples were passed through 0.45 μm filter and injected into the HPLC system.

General

Column chromatography was carried out on silica gel (Merck, Kieselgel 60, 60-230 mesh), polyamide (Fluka,50–160 lm) and Sephadex LH-20 (Pharmacia). Thin layer chromatography (TLC) was conducted on pre-coated, commercial silica gel (Merck, 60F254) 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.

Isolation of Authentic Samples

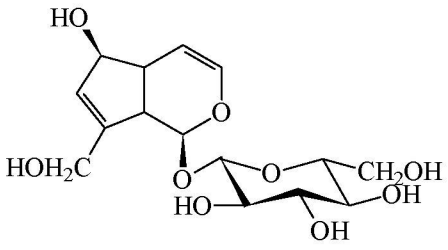
Isolation and structure elucidation of aucubin, catalpol, veronicoside, verproside, amphycoside, verminoside and catalposide were previously reported (16,17).

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

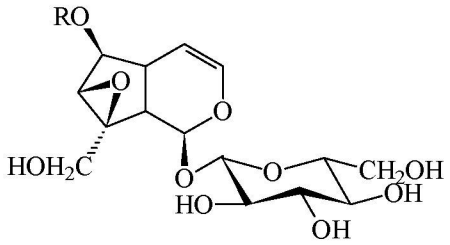
Dried aerial parts of *V. cymbalaria* (10 g) were extracted with MeOH. Five main polymide fractions were obtained from the water extract (3.75 g) of the plant with the same conditions of *V. cuneifolia* subsp. *cuneifolia*. Iridoid fractions of *V. cymbalaria* was subjected to analytical RP-HPLC analysis 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, compound **8** and **9** were isolated using $CHCl_3$:MeOH (100:0→85:15) as a solvent system (16,17).

RESULTS AND DISCUSSION

In this study, *V. cuneifolia* subsp. *cuneifolia* and *V. cymbalaria* were compared on the basis of their major components in the iridoid fraction by HPLC for the first time. The methanol extract of *V. cuneifolia* subsp. *cuneifolia* 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, Sephadex LH-20) of the fractions (Fr. A_1-A_6) which eluted with water from the polyamide column, resulted in the isolation of 6 compounds [**1-6**] in pure form. Their structures were determined as aucubin [**1**], catalpol [2], veronicoside [**3**], verproside [**4**], amphycoside [**5**], verminoside [**6**] using different spectral methods (Fig. 1) [UV, IR, 1D NMR, 2D NMR (COSY, HMQC, HMBC), FAB-MS and HR ESI-MS] (16-22). In addition to these compounds, a minor iridoid glucoside [**7**] was determined from the HPLC chromatograms comparing its retention time and UV spectrum with that of authentic sample which is previously isolated from *V. persica* (10) (Fig. 2-3).



Aucubin (**1**)



R	
H	Catalpol (**2**)
Benzoyl	Veronicoside (**3**)
Protocatechuoyl	Verproside (**4**)
Vanilloyl	Amphycoside (**5, VY-2a**)
Trans-caffeoyl	Verminoside (**6**)
p-Hydroxybenzoyl	Catalposide (**7**)
Veratroyl	6-*O*-Veratroyl catalposide (**8, VY-1**)
Isovanilloyl	6-*O*-Isovanilloyl catalpol (**9, VY-2b**)

Figure 1. Structures of the compounds **1-9** determined from *V. cuneifolia* subsp. *cuneifolia* and *V. cymbalaria*.

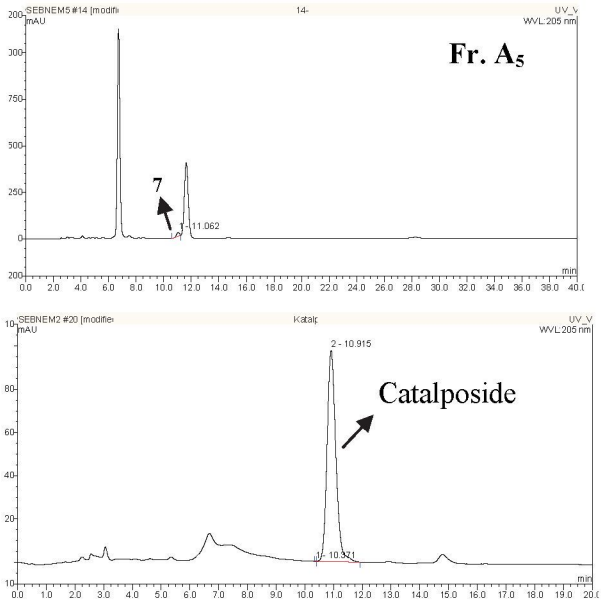


Figure 2. HPLC chromatogram of Fr. A_5 and catalposide [%40 MeOH- (H_2O+ 1% H_3PO_4)].

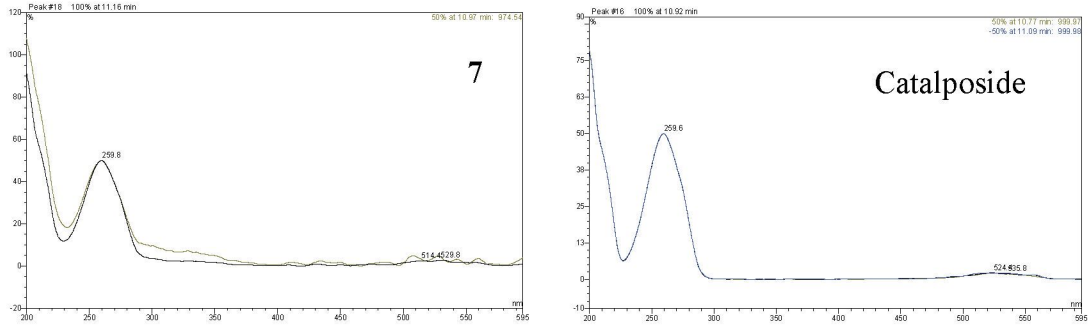


Figure 3. UV spectra of compound **7** and catalposide.

After structure determination of the isolated compounds [1-7], they were used as authentic samples for HPLC studies on iridoid fractions of *V. cymbalaria.* Each compound was investigated for their presence in *V. cymbalaria* separately and compounds **1-7** were observed in HPLC chromatograms of iridoid fractions of *V. cymbalaria.* Their presence was confirmed with the comparison of their UV spectra with that of standard compounds. Comparison of the water extract of both plants was shown in figure 4A and B.

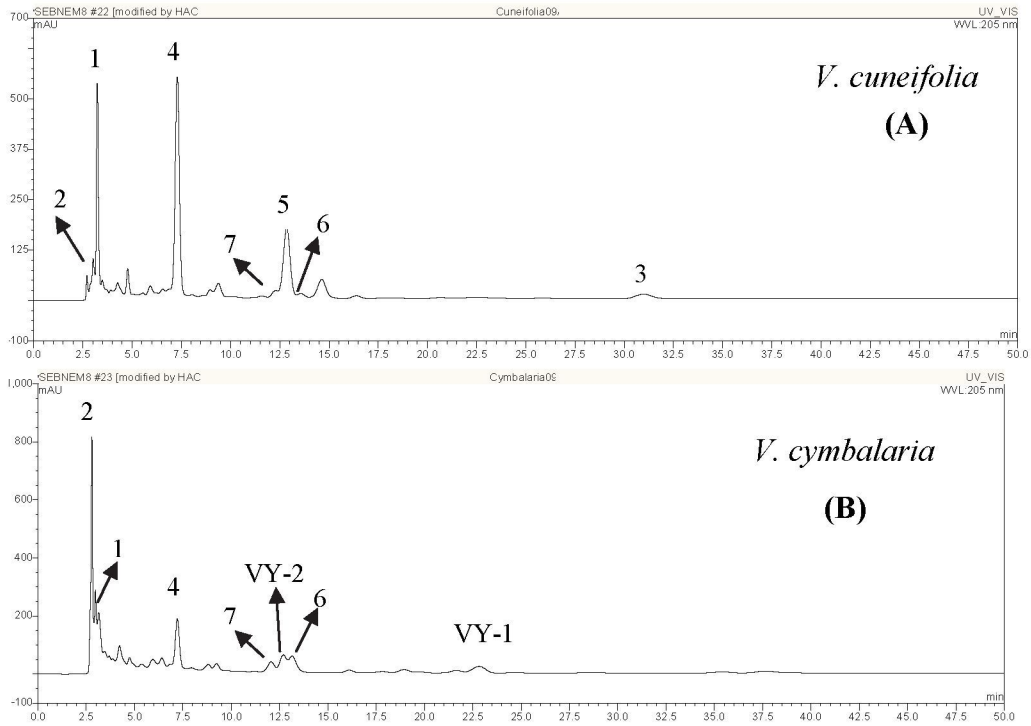


Figure 4. HPLC chromatograms of water extracts of *V. cuneifolia* subsp. *cuneifolia* **(A)** and *V. cymbalaria* **(B)** [%40 MeOH- (H_2O+ 1% H_3PO_4)].

Two different compounds (VY-1 and VY-2) were determined in HPLC chromatograms of *V. cymbalaria* iridoid fractions different from *V. cuneifolia* subsp. *cuneifolia*. These two compounds were isolated from *V. cymbalaria* using different chromatographic techniques and structure of VY-1 was determined as 6-*O*-veratroylcatalposide from the comparison of its NMR spectral data with that of published paper (16, 23). On the other hand, detailed examination of HPLC chromatogram and NMR spectra of VY-2 was indicated that VY-2 was a mixture of VY-2a and VY-2b (Fig. 5). Comparison of HPLC chromatogram of VY-2 in 30% MeOH-(H_2O+1% H_3PO_4) solvent system was indicated that VY-2a is amphicoside (5) (Fig. 5-6). Structure of VY-2b was determined as 6-*O*-isovanilloylcatalpol from the separation of its NMR signals from that of amphicoside (16, 22).

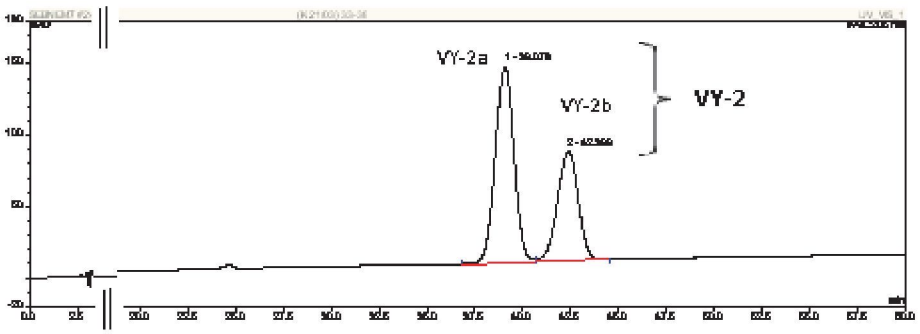


Figure 5. HPLC chromatogram of VY-2 [30% MeOH- (H_2O+ 1% H_3PO_4)].

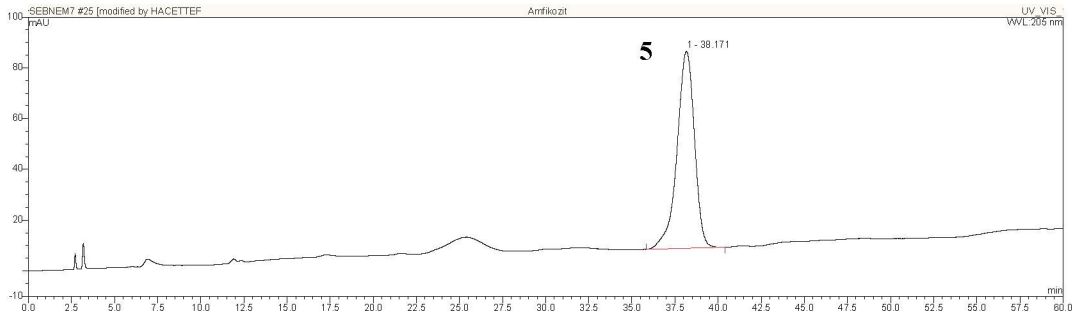


Figure 6. HPLC Chromatogram of amphicoside **(5)** [30% MeOH- (H_2O+ 1% H_3PO_4)].

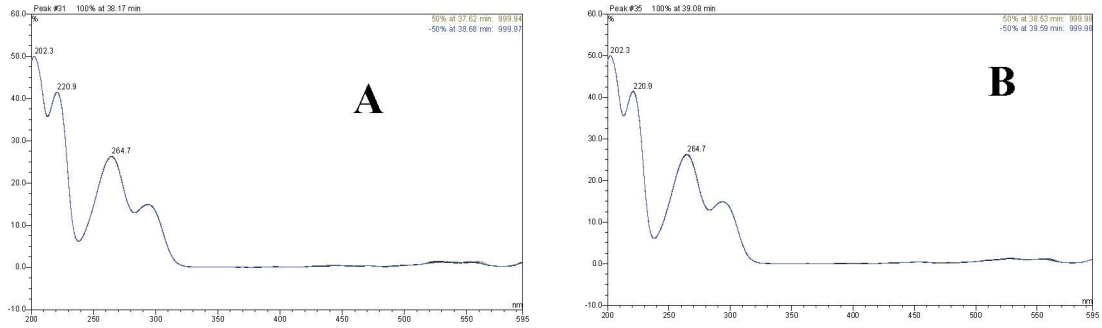


Figure 7. UV spectra of amphicoside **(5)** **(A)** and **VY-2a** **(B)**.

Consequently, aucubin, catalpol, verproside, amphycoside, verminoside and catalposide from *V. cuneifolia* subsp. *cuneifolia* and 2 more iridoid glucosides, 6-*O*-veratroylcatalposide and 6-*O*-isovanilloylcatalpol were determined from *V. cymbalaria*. According to Taskova et al. (7), the genus *Veronica* is composed of 4 sections: Chamaedrys, Alsinebe, Becabunga and Veronicastrum. While *V. cuneifolia* subsp. *cuneifolia* is included in section Chamaedrys, *V. cymbalaria* is included section Alsinebe. Iridioid profiles of these two sections are very close to each other (7,24,25). Similar iridoid contents of tested species are supported to this information. On the other hand, the presence of rare catalpol derivatives 6-*O*-veratroyl catalposide **(8)** and 6-*O*-isovanilloil catalpol **(9)** in *V. cymbalaria* make the plant interesting for chemotaxonomic researches of genus *Veronica*.

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