

## LC /MS ANALYSIS OF AUCUBIN AND CATALPOL OF SOME VERONICA SPECIES

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### Abstract

A comparative study was performed on 12 species of the genus *Veronica* L. (*Plantaginaceae* sensu APG 2003, formerly *Scrophulariaceae*) namely *Veronica persica*, *V. bachofenii*, *V. urticifolia*, *V. agrestis*, *V. austriaca*, *V. chamaedrys*, *V. officinalis*, *V. orchidea*, *V. spicata*, *V. beccabunga*, *V. hederifolia*, *V. prostrata*. In these species we have determined the aucubin and catalpol content by using a HPLC analysis with mass spectrometry detection. The content of aucubin is varying from 799.8 µg% in *Veronica persica* to 43.4 µg% in *Veronica beccabunga*. The highest content of catalpol is found in *Veronica spicata* (318.8 µg%) and the lowest in *Veronica beccabunga* (27.6 µg%), respectively.

### Rezumat

S-a efectuat un studiu comparativ pentru 12 specii ale genului *Veronica* L. (*Plantaginaceae* după APG 2003, înainte *Scrophulariaceae*): *Veronica persica*, *V. bachofenii*, *V. urticifolia*, *V. agrestis*, *V. austriaca*, *V. chamaedrys*, *V. officinalis*, *V. orchidea*, *V. spicata*, *V. beccabunga*, *V. hederifolia*, *V. prostrata*. Scopul lucrării a fost determinarea cantitativă prin analiză HPLC cuplată cu spectrometrie de masă a aucubinei și catalpolului. Conținutul de aucubină variază în speciile cercetate de la 799,8 µg% pentru *Veronica persica*, la 43,4 µg% pentru *Veronica beccabunga*. Conținutul de catalpol variază de la 318,8 µg% pentru *Veronica spicata* la 27,6 µg% pentru *Veronica beccabunga*.

**Keywords:** aucubin, catalpol, *Veronica* genus, HPLC/MS

### Introduction

The genus *Veronica* L. (*Plantaginaceae* sensu APG 2003, formerly *Scrophulariaceae*) [1, 2] is characterized by the presence of iridoid glucosides [15]. Iridoid glucosides are important natural products [3, 11]. Many of them are of interest due to their pharmacological activity as well as their importance in chemotaxonomy. They have been used as chemotaxonomic markers at different taxonomic levels [15].

Iridoids represent a large group of cyclopenta[c]pyran monoterpenoids that occur wide-spread in nature, mainly in dicotyledonous plant families like *Apocynaceae*, *Scrophulariaceae*, *Diervillaceae*, *Lamiaceae*, *Loganiaceae* and *Rubiaceae*. Recently, more extensive studies revealed that iridoids exhibit a wide range of bioactivities, such as neuroprotective, antiinflammatory and immunomodulator, hepatoprotective and cardioprotective effects. Citostatic, antioxidant, antimicrobial, hypoglycaemic, hypolipidemic, choleric, antispasmodic and purgative properties were also reported [16].

Bioactivities like antibacterial, anticoagulant, antifungal and antiprotozoal are highlighted [7].

The most spread iridoid glucosides in the *Veronica* genus are aucubin and catalpol, both of them belonging to the chromogenes iridoids [14]. Aucubin is an active component that has shown wide pharmacological activities including hepatoprotective, antitoxic, anti-inflammatory, antioxidant, antiaging, antiosteoporosis and neurotrophic and should be further researched and used in therapy [8].

In the Romanian spontaneous flora the *Veronica* genus is represented by 41 species and 3 hybrids grouped in 5 subgenus [12]. Of the various *Veronica* species the most important is *Veronica officinalis* [9] used in phytotherapy [4, 5]. We have studied the content in aucubin and catalpol for 12 species of *Veronica* genus: *Veronica persica*, *V. bachofenii*, *V. urticifolia*, *V. agrestis*, *V. austriaca*, *V. chamaedrys*, *V. officinalis*, *V. orchidea*, *V. spicata*, *V. beccabunga*, *V. hederifolia*, *V. prostrata* representative for all the 5 subgenus of *Veronica*.

The aim of our work was to compare the content of aucubin and catalpol for the 12 species of *Veronica* genus in order to establish their profile for *Veronica officinalis* comparatively with the other members of *Veronica* genus.

### Materials and methods

*Plant material:* The plant material consisting of herba (aerial parts) of these species of *Veronica* genus was collected in Cluj County. Plant materials were dried at room temperature. Voucher specimens were deposited in the Herbarium of Pharmaceutical

Botany Department, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy [6].

*Standards:* Aucubin from Roth (Germany) and catalpol from Merck (Germany).

*Chemicals and Solvents:* All chemicals and solvents were of analytical – reagent grade.

*Apparatus and Software:* LC analysis was performed using a 1100 Series LC system (Agilent Technologies, Darmstadt, Germany) consisting of a binary pump, degasser, autosampler, UV detector and column thermostat. The LC system was coupled to an Agilent mass detector VL ion trap detector (Bruker Daltonik, GmbH, Brehmen Germany). The detection of aucubin and catalpol was performed on both UV and MS mode (fig. 1). Chromatographic and mass spectrometric data acquisition were performed using Chemstation software (Agilent Technologies) version B.01.03, and LC/MSD Trap Control (Bruker Daltonik) Version 5.3, while data processing was performed using LC/MSD Quant Analysis (Bruker Daltonik), Version 1.7 [10].

*Chromatographic Conditions:* Chromatographic separation was achieved on an Atlantis HILIC analytical column (100 mm x 3.0 mm i.d., 3.5  $\mu$ m; Waters Corporation) preceded by a 0.2  $\mu$ m online filter (Agilent Technologies). The mobile phase was water and acetonitrile (ACN) elution in gradient after the following program: start with 95% ACN, at 2.3 min 81.1 ACN, at 2.31 min 10% ACN, at 2.7 min 10% ACN, at 2.71 min 95% ACN. The mobile phase was delivered at a flow rate of 1 mL/min, the autosampler injection volume was 2  $\mu$ L and the column temperature was 40°C. Afterwards it was added 1% acetic acid in water and sodium acetate 200  $\mu$ M at a flow rate of 0.07mL/min.

The mass spectrometer was operated in the electrospray ionization (ESI) positive ionization mode, and was set for isolation of sodium ions of aucubin and catalpol, with  $m/z$  369 and 385 respectively (single ion monitoring mode). The mass spectrometric parameters were as follows: nebulizer gas, nitrogen at 60 psi; dry gas, nitrogen at 12 L/min; dry gas temperature 300°C; capillary voltage, 4000V.

The calibration curve for aucubin was achieved in the concentration range of 35.8-1431 ng/mL, and for catalpol in the concentration range of 40-1627 ng/mL. All the standard solutions were prepared in acetonitrile (fig. 2).

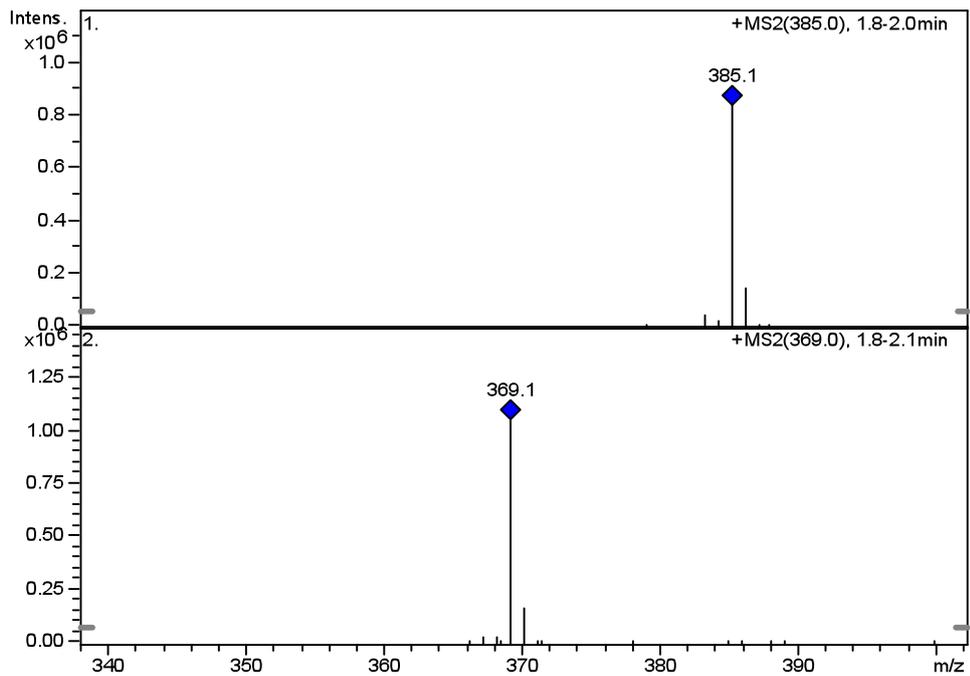


Fig. 1. ESI-MS spectra of catalpol (upper image) and aucubin (lower image)

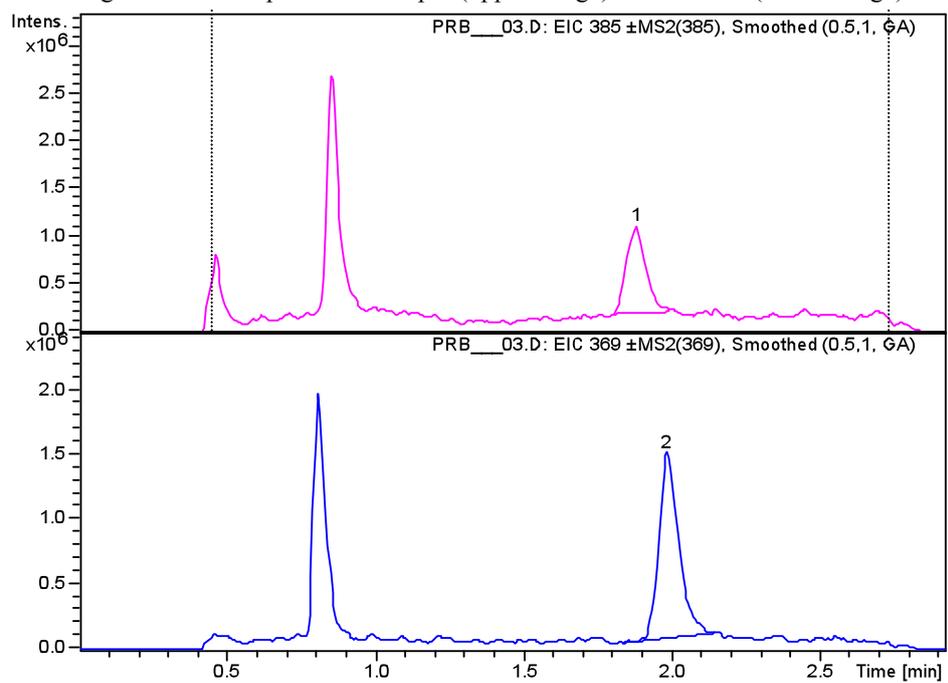


Figure 2

Chromatograms of catalpol and aucubin by MS detection  
(upper image,  $t_R=1.90$  min, lower image,  $t_R=1.95$  min)

*Sample preparation:* 1.25 g of plant material (aerial parts dried at room temperature and ground) were extracted with 25 mL methanol at room temperature for 24 hours. After filtration the solution was concentrated to 2 mL in vacuum at 40°C and filtered through a 0.45 µm filter. The samples were diluted 1/10 before injection. The results obtained are presented in table I.

## Results and discussion

**Table I**  
Results of LC/MS determination of aucubin and catalpol

	Aucubin µg%	Catalpol µg%
<i>Veronica persica</i>	799.8	138.4
<i>Veronica bachofenii</i>	120.4	154
<i>Veronica urticifolia</i>	666.4	99.6
<i>Veronica agrestis</i>	301.2	63.4
<i>Veronica austriaca</i>	460.2	147.2
<i>Veronica chamaedrys</i>	328.6	144.4
<i>Veronica officinalis</i>	107.4	232.2
<i>Veronica orchidea</i>	268.2	296.2
<i>Veronica spicata</i>	120.4	318.8
<i>Veronica beccabunga</i>	43.4	27.6
<i>Veronica hederifolia</i>	432.8	93
<i>Veronica prostrata</i>	377.6	170.8

The content of aucubin is varying from 799.8 µg% in *Veronica persica* to 43.4 µg% in *Veronica beccabunga*. The content of catalpol is the highest in *Veronica spicata* (318.8 µg%) and the lowest in *Veronica beccabunga* (27.6 µg%). *Veronica beccabunga* (13) is a plant from marsh and it has a lot of aerenchyma in its aerial parts that can explain the low content in iridoids. For *Veronica officinalis* the content of aucubin is 107.4 µg% and for catalpol is 232.2 µg%, fact that situated this species among the species with a medium content of iridoids so its pharmacological activities can be explained by the phytocomplex content.

## Conclusions

This is the first reported method for the analysis of aucubin and catalpol contents for the *Veronica* genus of the Romanian wild flora. The contents in aucubin and catalpol for *Veronica officinalis* is 107.4 µg% and 232.2 µg% respectively.

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