

BRIEF COMMUNICATIONS

HPLC ANALYSIS OF SALICYLIC ACID DERIVATIVES FROM *Viola* SPECIES

A. Toiu*, L. Vlase, I. Oniga, and M. Tamas

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Viola genus contains many species, native to Europe, North Africa, and temperate regions of Asia. Wild pansy (*Viola tricolor* L.) is very widespread in Romania's spontaneous flora. The aerial parts are used in traditional medicine to treat skin conditions, bronchitis, cystitis, and rheumatism. The anti-inflammatory, expectorant, and diuretic properties are due to the presence of the following active principles: saponins, flavonoids, mucilages, salicylic acid derivatives, coumarins, and carotenoids [1-3]. *Viola arvensis* Murray and *Viola declinata* Waldst. et Kit. are closely related to *V. tricolor* L., but their chemical composition is less studied [4, 5]. The cyclotides were recently identified in many *Viola* species, and some biological activities (including cytotoxic, anti-HIV, antimicrobial) were demonstrated [6-8].

The anti-inflammatory activity is related to depurative and anti-allergic effects because some skin conditions can be caused by inflammation. The anti-inflammatory properties are ascribed to salicylic derivatives and rutin, and can be enhanced by saponins [9].

We have initiated a comparative phytochemical study and we have analyzed the polyphenolic compounds (flavonoids, polyphenol carboxylic acids, anthocyanins, proanthocyanins) from three *Viola* species by TLC, HPLC, and spectrophotometric methods [10]. In this work we performed a comparative HPLC study of salicylic acid derivatives from three *Viola* species from Romania, these compounds being involved in the anti-inflammatory activity of wild pansy.

We have identified and measured by HPLC salicylic acid, before and after acid hydrolysis in ethanolic extracts of dried aerial parts of *Viola tricolor* L., *V. arvensis* Murray, and *V. declinata* Waldst. et Kit. We present the concentrations of salicylic acid (mg/100 g vegetal product) for the three *Viola* species in Table 1.

Two grams of powdered vegetal product was extracted with 70% ethanol for 30 min on a water bath at 80°C. After extraction the mixtures were centrifuged at 4000 rpm. In order to study the presence of salicylic acid after hydrolysis, we mixed these solutions together with 2 M hydrochloric acid, and the solutions were heated at 80°C for 60 min on the water bath. The mixtures were centrifuged at 4000 rpm. The solutions were diluted with distilled water in a 10 mL volumetric flask and filtered through a 0.45 µm filter before injection.

We used an Agilent 1100 HPLC Series (Agilent, USA) equipped with a degasser G1322A, HP 1100 Series binary pump, and a Zorbax SB-C18 reversed-phase analytical column 100 mm × 3.0 mm i.d., 3.5 µm particle (Agilent technologies, USA), and we operated at 45°C. The mobile phase was a binary gradient: distilled water with 0.1% (v/v) 85% orthophosphoric acid and acetonitrile. The linear gradient started at 5% acetonitrile for 2 min, followed by isocratic elution with 25% acetonitrile over the next 3 min. The flow rate was 1 mL/min and the injection volume was 10 µL.

The fluorescence detector operated at 310 nm excitation wavelength and 450 nm emission wavelength. Salicylic acid was identified by an external standard method and by comparison of its retention time with that of the standard under the same chromatographic conditions and quantified by the external standard method.

Salicylic acid (Sigma, Germany) was used as the standard in order to perform quantitative determinations in distilled water solutions with concentrations between 68-21960 ng/mL. The calibration curve of salicylic acid standard was linear between 68.625-21960 ng/mL.

Faculty of Pharmacy, University of Medicine and Pharmacy „Iuliu Hatieganu”, 13 Emil Isac Street, Cluj-Napoca, 400023, Romania, fax: 0040264595454, e-mail: ancamaria_toiu@yahoo.com. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 284-285, May-June, 2008. Original article submitted January 22, 2007.

TABLE 1. Concentration of Salicylic Acid (mg/100 g Vegetal Product) for Aerial Parts of *Viola tricolor*, *V. arvensis* and *V. declinata*

| Species | Concentration, mg/100 g | |
|---------------------|-------------------------|------------------|
| | Before hydrolysis | After hydrolysis |
| <i>V. tricolor</i> | 112.23 | 112.27 |
| <i>V. arvensis</i> | 112.21 | 116.12 |
| <i>V. declinata</i> | 123.51 | 123.98 |

We have analyzed for the first time salicylic acid derivatives from *V. declinata* and we completed the literature data with the new data, concerning qualitative and quantitative determinations of salicylic acid by HPLC study. Our phytochemical study showed small differences between the three *Viola* species, especially quantitative ones. The results allow a better characterization of some vegetal products in order to justify their therapeutic uses.

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