

PHARMACOGNOSTIC RESEARCH ON *VIOLA DECLINATA* WALDST. ET KIT. (VIOLEACEAE)

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Abstract

In order to continue our previous studies on some *Viola species* from Romania, we have analysed for the first time some active principles from aerial parts of *V. declinata* Waldst. et Kit. We have performed gravimetric methods for saponins and mucilages, and spectrophotometric determinations and HPLC analysis of carotenoids. We established the content in saponins (4.40%), mucilages (10.26%), and total carotenoids (8.45 mg/100g vegetal product, expressed in β -carotene). In fresh aerial parts we have determined by HPLC: violaxanthin (281 μ g/100g), antheraxanthin (283 μ g/100g), lutein (896 μ g/100g), zeaxanthin (479 μ g/100g), β -carotene 5,6-epoxide (48 μ g/100g), β -carotene (546 μ g/100g), and 9Z- β -carotene (76 μ g/100g). Our results showed the presence of the same active principles in *Viola declinata* and *V. tricolor*, the differences being only quantitative.

Rezumat

În continuarea studiilor noastre asupra unor specii de *Viola* din România, am analizat pentru prima dată unele principii active din părțile aeriene de *V. declinata* Waldst. et Kit. Am utilizat metode gravimetrice pentru dozarea saponinelor și mucilagiilor și metode spectrofotometrice și cromatografice (HPLC) pentru analiza carotenoidelor. Concentrația saponinelor a fost de 4,40%, cea de mucilagii de 10,26%, iar cea a carotenoidelor totale de 8,45 mg/100g produs vegetal, exprimată în β -caroten. În părțile aeriene proaspete am determinat prin HPLC: violaxantina (281 μ g/100g), anteraxantina (283 μ g/100g), luteina (896 μ g/100g), zeaxantina (479 μ g/100g), β -caroten 5,6-epoxidul (48 μ g/100g), β -carotenul (546 μ g/100g) și 9Z- β -carotenul (76 μ g/100g). Rezultatele au arătat prezența acelorași grupe de principii active în speciile *Viola declinata* și *V. tricolor*, diferențele fiind doar cantitative.

Keywords : *Viola declinata*; saponins; mucilages; carotenoids

Introduction

Viola tricolor L. (wild pansy) is very spread in spontaneous flora of Romania and it is considered one of the most important medicinal plants within its family [9]. The aerial parts are used in traditional medicine to treat various skin conditions, bronchitis, rheumatism. Their anti-inflammatory, expectorant and diuretic properties are due to the presence of saponins, flavonoids, mucilages, salicylic derivatives, carotenoids. Anti-inflammatory

properties were demonstrated and they are ascribed to salicylic derivatives, rutin and can be enhanced by saponins [7,11,14,15].

V. declinata is closely related to *V. tricolor* (they belong to the *Viola* sp. with un-cordate leaves and un-straight stipels), but it's a Carpathian-Balkan flowering element, and the chemical composition has been less studied than the one of wild pansy. *V. declinatae herba* contains flavonoids (1.454-1.495%, expressed in rutoside), polyphenol carboxylic acids, anthocyanins, proanthocyanins [12,13].

Knowing the use of *Viola tricolor* in traditional medicine as diuretic and depurative plant (that can be due to saponins and flavonoids), the aim of this study was to complete the pharmacognostic data on *V. declinata* with the analyses on saponins, mucilages and carotenoids.

Materials and methods

We have analysed the aerial parts of *V. declinata* Waldst. et Kit. (*Violae declinatae herba*) harvested from spontaneous flora of Alba county, Romania, in July 2006. For HPLC study of carotenoids we used fresh aerial parts, whereas for total carotenoids, saponins and mucilages content, dried and powdered aerial parts were used.

Preliminary study was performed by a qualitative screening on *Violae declinatae herba*. Separation of the most important groups of active principles was made by successive and selective extraction of the vegetal product with solvents of different polarity (chloroform, methanol, water) [3].

Spectrophotometric determinations were made using a spectrophotometer UV-VIS JASCO V-530.

The quantitative analysis of carotenoids was made using the method described by Britton et al. [2] and the results were expressed in β -carotene (mg/100g). The concentration of total carotenoids was calculated using the formula:

$$\text{mg carotenoids/100 g} = A \times V \times 1000 / (A_{1\text{cm}}^{1\%} \times G)$$

where: A=the absorbance of solution at 450 nm, V=the volume (=50 mL), $A_{1\text{cm}}^{1\%}$ = the specific absorbance of β -carotene in hexane, at $\lambda=450$ nm (=2500), G=vegetal product mass (g).

The isolation of saponins: 30g of vegetal product was extracted with methanol, three times, on a water bath, to reflux, at 60°C. The methanolic solutions were concentrated to rotavapor at reduced pressure, to obtain a liquid of syrupy consistency. This solution was poured over acetone, when the saponins precipitated [8]. The vegetal product obtained after the extraction of saponins was dried and then was extracted with water, three times. The solution was concentrated to one fourth of the initial volume, and then was

poured over an ethanolic solution (acidulated 1% with acetic acid) [4, 10]. The precipitate was separated by filtration, its weight was determined and was reported at 100g vegetal product. The haemolytic index of vegetal product and the haemolytic index of isolated saponin were determined [1].

HPLC-PDA analysis of carotenoids [5] was performed with an HPLC system Agilent 1100 Series, a LiChrosorb RP18 (250 x 4.6 mm) analytical column, operated at 35°C. The mobile phase was a mixture MeCN-H₂O (9:1), and ethyl acetate. Samples preparation: 5g of fresh powdered vegetal product was extracted with acetone and diethylether. Detection: detector VIS at 450 nm. Authentic reference carotenoids (neoxanthin, violaxanthin, antheraxanthin, lutein, zeaxanthin, α -cryptoxanthin, β -carotene 5,6-epoxide, β -carotene and 9Z- β -carotene) were purchased from Hoffman – La Roche.

Results and discussion

Phytochemical screening conducted on the vegetal product led to the identification of terpenoids, carotenoids, fatty acids (in chloroform solution), of tannins, triterpenic saponins, flavonoids (in methanol solution), and polysaccharides, aminoacids, carbohydrates (in water solution).

The HPLC chromatogram of carotenoids from the aerial parts of *Viola declinata* is presented in figure 1. The identified carotenoids and their concentrations ($\mu\text{g}/100\text{g}$) are presented in table I.

By HPLC we identified lutein as the major carotenoidic compound (896 $\mu\text{g}/100\text{g}$). By spectrophotometric method we observed that *Viola declinatae herba* contains a small amount of total carotenoids (8.45 mg /100g, expressed in β -carotene) (table II).

Saponins and mucilages are the main active principles, in concentration of 4.40%, and respectively 10.26% (table II).

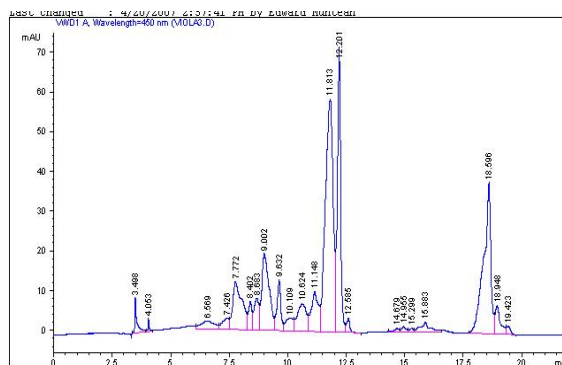


Figure 1
HPLC chromatogram of carotenoids from *Viola declinata*

Table IThe concentrations ($\mu\text{g}/100\text{g}$) of identified carotenoids by HPLC

Compound	Carotenoids ($\mu\text{g}/100\text{g}$)
Neoxanthin	traces
Violaxanthin	281
Antheraxanthin	283
Lutein	896
Zeaxanthin	479
α -cryptoxanthin	traces
β -carotene 5,6-epoxide	48
β -carotene	546
9Z- β -carotene	76

Table II

The concentrations of total carotenoids, saponins and mucilages

Compounds	Concentration
Total carotenoids ($\text{mg}/100\text{g}$, expressed in β -carotene)	8.45
Saponins (%)	4.40
Mucilages (%)	10.24

Previous studies on *Viola sp.*, showed that *V. tricolor* contains high amount of saponins (5.2%) [8], mucilages (10%) [15], and flavonoids (1.81-1.99%, expressed in rutoside) [12]. Our results showed that the chemical composition of *Viola declinatae herba* is very similar to that of *V. tricoloris herba* [8,12,13,14], there are only quantitatively differences of the most important groups of active principles.

Comparing with data from literature, the concentration of isolated saponins from aerial parts of *V. declinata* is very close to or smaller than the concentration of these active principles in some vegetal products used in therapy for content in saponins (*Primulae radix* 5-10%, *Liquiritiae radix* 3-12%, *Hippocastani semen* 10%) [6].

The haemolytic index of *Viola declinatae herba* was 66.6, and the haemolytic index of isolated saponin was 4000. In the same experimental conditions, we obtained for standard Merck saponin a haemolytic index of 50000, so the vegetal product can be used in therapy without risk of toxicity.

Comparing with other species used in therapy for their content in carotenoids (*Calendulae flos*, *Croci stigma*, *Tagetes flos*) [6], *V. declinatae herba* contains small amounts of these active principles.

The high content in mucilages of *V. declinatae herba* may recommend the use in therapy, like other vegetal products rich in mucilages (*Althaeae folium* 9.8%, *Althaeae radix* 30%, *Malvae folium* 8.2%) [4].

Conclusions

We analysed for the first time the carotenoids from *Viola declinatae herba* by HPLC and spectrophotometric method, and we measured the concentration of 7 carotenoidic pigments.

We determined a high amount of saponins and mucilages in aerial parts of *Viola declinata*.

Because there are small differences between the chemical composition of *V. tricolor* and *V. declinata*, the two species can be used together in therapy, for the same pharmacological properties.

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