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QUALITATIVE ANALYSIS OF FLAVONIC GLYCOSIDES AND PHENYLPROPANIC DERIVATIVES FROM *RHODODENDRON KOTSCHYI* SPECIE

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Abstract

Using thin layer chromatography followed by photodensitometric analysis the flavonic glycosides and phenylpropanic derivatives from the leafs and the flowers of Rhododendron kotschyi specie were analysed. The results indicate the presence of two flavonic glycosides, hyperoside and quercitroside, and of two phenylpropanic derivatives, chlorogenic acid and caffeic acid, respectively.
***Keywords**: flavonic glycosides, Rhododendron kotschyi, thin layer chromatography.*

Introduction

This study focuses on the qualitative analysis of flavonic glycosides and phenylpropanic derivatives from leafs and flowers of *Rhododendron kotschyi* specie.

Rhododendron kotschyi belongs to the *Rhododendroideae* subfamily, the *Ferruginea* subsection of the *Ericaceae* family, popularly known as rose bay. It is a bush that vegetates in inferior alpine storey, where it also forms charateristic associations, being a Dacian-Balkanic endemism spread in the Eastern Carpathians, absent in the Western Europe mountains (Anonymous 1965).

Vegetal material harvested in June (flowers and leafs) was used in this study. After being dried at room temperature, the vegetal products were pulverised.

Experimentals

*Chromatographic analysis of flavonic glycosides and phenylpropanic derivatives***:** Qualitative analysis of flavonic glycosides

and phenylpropanic derivatives was performed simultaneously. Each methanolic product 5% was applied on a thin plate, in the same system of solvents (mobile phase).

1g of vegetal product powder (leafs and flowers *Rhododendron*) was subjected to extraction in the presence of 20 ml methanol. The operation was carried out in Erlenmayer flaks with a funnel, on a water bath, up to the boiling point of the solvent. As soon as the solvent reached the boiling point, the heating was stopped, the solution was cooled to room temperature, the obtained extracts were filtered through Whatmann 0.45 μm and then topped up to a volume of 20 ml. This way, the 5% extracts of the two vegetal products were obtained, leaf extract of *Rhododendron kotschyi (Rh. lf.)* and flower extract of *Rhododendron kotschyi (Rh. fl).* Chromatography was performed at the same time on standard samples (Merck) of glycosides and phenylpropanic derivatives as follows: luteolin 7-O-glucosid; rutoside, quercitroside, hyperoside, caffeic acid, chlorogenic acid, methanolic solutions 0.1%).

Chromatographic analysis of these extracts was carried out using Kiselgel plates (Merck) 60 F_{254}, 10x15 cm size, with 0.25 mm thickness of the layer. Extract or standards (Merck) spots were applied at 1 cm width, a volume of 2x10 μl for each spot. The sequence of the spot deposition was: Rh. lf. (1), Rh. fl. (2), luteolin 7-O glucoside (3), cafeic acid şi chlorogenic acid (overlayed) in the same spot (4), rutoside and quercitroside (overlayed) in the same spot (5) and hyperoside (6). The numbers of the spot there are in the brackets and there are corresponding with the number from densitogram (figure 1).

For developing, the following system was used: Glacial acid acetic: Anhydrous formic acid: Water: Ethyl Acetate (7 : 7 : 14 : 72) (Stalh 1969).

The developing of the plate was achieved over a distance of approximately 10 cm. After developing, the plate was dried with hot air, and then subjected to successive revelation with a type and b type reactive, as follows:

a) NEU (2-Aminoethyl diphenylborate 1% in methanol)
b) PEG 4000 (polyethylenglycol 1% in methanol)

The examination of the plate was achieved in the presence of UV light at 365nm before and after revelation with NEU+PEG (York 1990).

The use of the two reactive for revelation of polyphenolic leads to yellow, yellow-orange fluorescent spots in the case of flavonodic compounds (flavonic glycosides) and blue fluorescence in the case of phenylpropanic derivatives (Wagner 1984).

Photodensitometric analysis of glycosides and phenylpropanic derivatives: After performing thin layer chromatographic analysis of the methanolic extracts 5% obtained from the two vegetal products and analysis of the data, semicantitative analysis of the components from extracts, photodensitometrically, by using a densitometer DESAGA CD 60 was carried out.

Results and Discussions

For the reference samples used, R_f values were calculated (table 1) and some of them could be identified in the tested vegetal products, following colour and R_f values comparisons. In addition to the identified compounds using the reference samples, unidentified products are present, as much in flavonic glycosides class as in phenylpropanic derivatives class.

Rhododendron leafs contains: hyperoside, chlorogenic acid, quercitroside, caffeic acid, and one unidentified compounds from flavonic glycoside family ($R_f = 0.47$).

Rhododendron flowers contains: hyperoside, chlorogenic acid, quercitroside, caffeic acid, and two unidentified compounds from flavonic glycoside family ($R_f = 0.47$ and $R_f = 0.81$).

The tests for the detection of flavonic glycosites and phenylpropanic derivatives products in flowers and leafs of *Rhododendron kotschyi,* revealed very similar compositions in the two samples.

At the same time, the analysis of the chromatogram shows a large presence of yellow orange fluorescence over the number spots of all extracts analysed.

Using photo densitometry, the methanolic extracts of the vegetal products were analysed following the amount of chlorogenic acid, Caffeic acid, hyperoside, quercitroside, rutoside, luteolin 7-O-glucoside, as well as the presence of other derivatives.

Table 1. Spot characterisation of the chromatogram (upon colour and R_f values)

No.	Product name	R_f	Spot colour	Extract presence
1.	Hyperoside	0.42	Orange-Red	Rh. lf. Rh. fl.
2.	Chlorogenic acid	0.43	Blue-Green	Rh. fl. Rh. lf.
3.	Unidentified compound	0.47	Orange-Red	Rh. fl. Rh. lf.
4.	Quercitroside	0.67	Orange	Rh. fl. Rh. lf.
5.	Unidentified compound	0.81	Yellow	Rh. fl
6.	Caffeic acid	0.94	Blue	Rh. fl. Rh. lf.

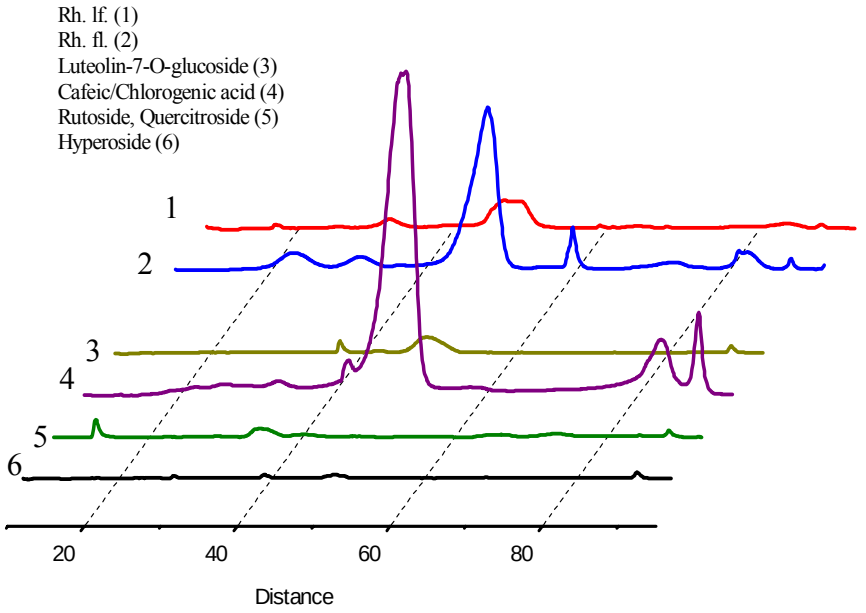

Fig. 1. Densitogram of the samples

Out of the analysed products, the richest in phenylpropanic derivatives is the *Rhododendron* leaf extracts and the richest in flavonic glycosides is the *Rhododendron* flower extract.

The interpretation of the densitograms led to the following statements:

- In *Rh.kotschyi* leafs, chlorogenic acid represents the major percentage of spots 45.4%, followed by quercitroside 30.7% and hyperoside 11.4%. Caffeic acid is found in a percentage of 8.6%.
- In *Rh.kotschyi* flowers, quercitroside has the highest percentage 73.4%, the rest of the components, identical to the ones found in leafs are found in less than 6%.

Table 2. Densitometric evaluation of the analysed samples

Sample No.	Sample name	Compound name	R_f	%
1.	Rh. lf.	Hyperoside Chlorogenic Quercitroside Caffeic acid	0.42 0.43 0.67 0.94	11.4 45.4 30.7 8.6
2.	Rh. fl.	Hyperoside Chlorogenic Quercitroside Caffeic acid	0.42 0.43 0.67 0.94	5.4 5.7 73.4 5.1
3.	Luteolin 7-O-glucoside	-	0.44	64.6
4.	Chlorogenic acid Caffeic acid	- -	0.43 0.94	50.1 16.6
5.	Rutoside Quercitroside	- -	0.23 0.67	50.4 43.2
6.	Hyperoside	-	0.42	76.5

Comparing this result with the content in flavonoids of other species we can affirm that *Rh. kotschyi* is rich in flavonoids, this being considered a natural source of flavonoids (Tamas 1973).

Flavonic glycosides are found as much in leafs, as in the flowers of *Rh.kotschyi*, their abundance being assessed through thin layer chromatography and photo densitometry. The presence of this compound in *Rh.kotschyi* flowers has never been reported as detected,

being signalled the occurrence of the pentacyclic triterpenes, friedelin and the epimeric 3-friedelanols (Chandler 1979).

Conclusions

According to the photodensitometric analyses performed, quercitroside is the major component in flowers, and hyperoside is the major component in leafs. Together with the flavonoidic compounds, by using this technique, the presence of phenylpropanonic derivatives in both exctracts was evidenced.

Chlorogenic and caffeic acids are present in *Rh.kotschyi* specie in both vegetal products studied (leafs and flowers).

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