

Chromatographic Analysis of the Flavonoids from *Robinia Pseudoacacia* Species

DANIELA CĂLINA¹, NELI KINGA OLAH², EMILIA PĂTRU³, ANCA DOCEA¹, H. POPESCU⁴, MARIA-VIORICA BUBULICA¹

¹University of Medicine and Pharmacy of Craiova, Faculty of Pharmacy

²Department of Research & Development, PlantExtrakt, Laboratories of Phytotherapy and Homeopathy

³University of Medicine and Pharmacy of Craiova, Faculty of Medicine

⁴University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj, Faculty of Pharmacy

ABSTRACT: The purpose of this study was the qualitative and quantitative analysis of flavonoids from *Robinia pseudoacacia* using two different techniques of analysis: Thin Layer Chromatography (TLC) and TLC coupled with photo-densitometry. The results obtained by chromatographic analysis showed a higher concentration of flavonoids in flowers than in leaves. The flowers harvested in the plains have a higher concentration of hyperoside (0.9 mg/mL) compared with the flowers collected from the hills (0.54 mg/mL). The leaves are richer in rutoside (0.98 mg/mL) compared with the flowers.

KEYWORDS: TLC, TLC-photodensimetry, flavonoids, *Robinia pseudoacacia*

Introduction

Robinia pseudoacacia (Black locust) is the main honey-bearing tree that grows spontaneously in Romania, especially in the plains and hills. It is native to North America. In Romanian ethno-pharmacology only flowers are used for their alkalizing, antispasmodics, anti-tussives, sedatives effect, other parts of the plant especially bark and seeds are toxic [1-3].

Flavonoids with phenyl-propane compounds and tannins are part of complex category of polyphenols [4]. They are a valuable group of plant origin compounds of great interest in phytotherapy and pharmacology [5-6]. In the chemical composition of *Robinia pseudoacacia* L. species have been cited in the literature the presence of the following flavonoids: robinin (kaempferol-3-O-ramnozyl-galactozil-7-ramnozid) and acacetin-7-O-rutosid, apigenin, diosmetin, luteolin but also secundiflorol, mucronulatol, isomucronulatol and isovestitol [7]. Our previous studies on the chemical composition revealed the presence of several bioactive compounds such as phytosterols, which are present in the flowers, and other lipidic compounds as those from the seeds of sunflower [8-9].

The purpose of this study was the qualitative and quantitative analysis of the flavonoids in different plant parts specifically in flowers, leaves, bark and seeds of *Robinia pseudoacacia* using thin layer chromatography coupled with photo-densitometry. This is a simple and rapid method, applied for separation, identification and quantitative determination of chemical

compounds with the main advantage that allows the possibility to investigate one or more components without their previous isolation and purification [10-11].

Material and Methods

Plant material

Herbal product was harvested in May-June (flowers, leaves) and October (seeds and bark) from two different geographical areas: plain (Craiova, Dolj county) and Hill (Râmnicu Vâlcea, Vâlcea County). Herbal product was drying immediately after harvest at ambient temperature and then it was sprayed.

Extract preparation

Methanolic flowers extracts: 1 g powdered herbal material was wetted with 5 mL methanol. After 2-3 minutes was added 10 mL methanol and stirring at approx. 1000 rpm at 50° for 30 minutes. After cooling, herbal material was filtered and the filter and herbal material was washed with methanol up to 10 mL extract.

Methanolic leave, bark and seed extracts were prepared similarly to flowers extracts. Extracts were noted as follows: R1 - *Robinia pseudoacacia* seeds extracts from plain area (Craiova), R2 - *Robinia pseudoacacia* seeds extracts from hill area (Râmnicu Vâlcea), R3 - *Robinia pseudoacacia* flowers extracts from plain area (Craiova), R4 - *Robinia pseudoacacia* flowers extracts from hill area (Râmnicu Vâlcea), R5 - *Robinia pseudoacacia* leaves extract from the plain area (Craiova), R6 - *Robinia pseudoacacia* bark extract from plain area (Craiova).

Thin layer chromatography (TLC) was performed under the following conditions:

- stationary phase: silica gel G60 F254-precoated TLC plates (Merck); mobile phase: ethyl acetate–ethylmethyl ketone–formic acid–water (50:30:10:10, in volumes); solution to analyze: 20% methanolic extractive solutions from the different parts of *Robinia pseudoacacia* (R1- R6); standard solutions: rutoside 1,22 mg/mL (Roth) and hyperoside 1,1 mg/mL (Merck; Darmstadt, Germany) methanol solutions; stationary phase: silica gel G60 F254-precoated TLC plates (Merck); mobile phase: ethyl acetate–ethylmethyl ketone–formic acid–water (50:30:10:10, in volumes); about 5–10 μ L of the sample and reference compounds have been applied on the plate as 10 mm bands; migration distance: 15 cm; migration time: 40 min; detection: UV light (254 nm) and natural products reagent (NP/PEG), in fluorescence.

For flavonoid compounds when using the two revealing reagents, we obtained yellow, yellow – green and yellow – orange spots.

TLC coupled with photo-densitometry

The thin layer chromatography has been accomplished by using the above mentioned experimental conditions. In TLC coupled with photo- densitometry, the chromatographic plate was scanned with a Desaga CD60 photo-densitometer scanner after spraying with iron chloride (anisaldehyde). The photo - densitometer parameters: in reflection mode, deuterium lamp, $\lambda = 254$ nm, minimum area read: 100. "In situ" UV-VIS spectra for the main flavonoids were obtained using the photo-densitometer. Device parameters are: deuterium and tungsten lamp, wavelength 254 nm, wavelength interval for UV–VIS spectra in situ 200–500 nm, slit width 0.2 mm, repetition four times/position.

Results and Discussions

Flavonoids are a class of compounds which possess high antioxidant properties [12]. Flavonoid spots appear at λ 254 nm in UV light as dark spots [13] and after the revelation with NP / PEG reagent acquire yellow-green fluorescence.

By comparing the R_f – sized, color and intensity of spots was possible to identify the following components: hyperoside and acacetin-7-O-rutoside (Figure 1). The other components separate on chromatographic plate could not be identified if they belong to flavonoids class. The

results led to the identification of flavonoids in flowers (R3, R4) and leaves (R5), less in bark (R6). Their absence was observed in the seeds (R1, R2). Separation of rutoside and hyperoside was made at the following R_f values: 0.13, respectively 0.35. The R_f values obtained are presented in Table 1.

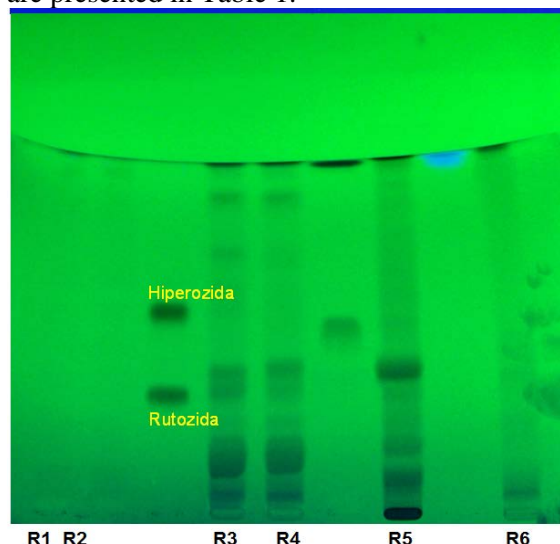


Figure 1. TLC-chromatogram in UV (254 nm) for the samples of *Robinia pseudoacacia* (R1-R6) and for the standard solutions (hyperoside, rutoside)

Table 1. R_f values for the analyzed flavonosides and for the reference compounds

Standards	R_f values	
	hyperoside 0,13	rutoside 0,35
Samples	R_f values	
R1	-	-
R2	-	-
R3	0,32	0,36
R4	0,33	0,38
R5	0,15	0,36
R6	-	-

After the photo-densitographic evaluation was made, some densitograms showed the presence of flavonoids in different concentrations. Figure 2 (a-d) contains the overlapped TLC-densitograms for the *Robinia pseudoacacia* methanolic extracts from flowers (R3, R4), leaves (R5) and bark (R6) with the standards (UV 254 nm). The analysis of methanolic flowers extracts revealed that the flowers collected from the plain area have higher flavonoid content than those harvested from the hill.

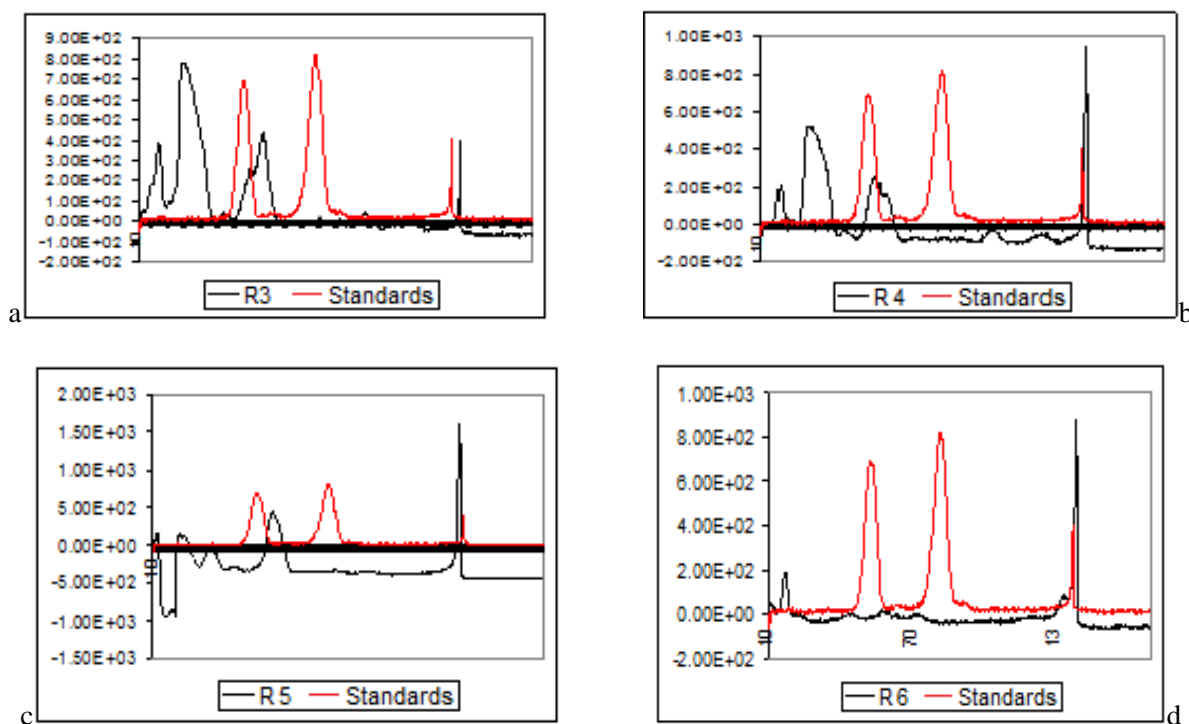


Figure 2. TLC-densitograms in UV (254 nm) for the samples of different parts of *Robinia pseudoacacia*: a – R3, b – R4, c – R5, d – R6

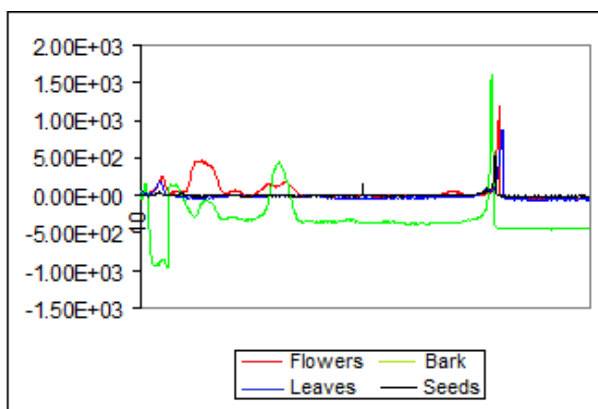


Figure 3. The densitograms of flower, leaves, bark and seeds extracts of *Robinia pseudoacacia*

In Figure 3 are presented the overlapped densitograms of methanolic extracts from flowers, leaves, bark and seeds of *Robinia pseudoacacia*.

From overlapping densitograms of the four vegetative organs of the plant it was found that flavonoids are mainly present in flowers and leaves, but in bark and seeds are in insignificant amounts.

For quantitative determinations, five points calibration curves were built, under the same chromatographic and densitometric conditions. We obtained equations with good correlation coefficients. In Table 2 the data of the calibration curve for rutoside are shown.

Table 2. The data for the calibration curve, expressed in rutoside

Concentration (mg/mL)	Found concentration (mg/mL)	Area	Acuracy (%)
0.091089	0.1	371.5	91.08937
0.149145	0.15	585.1	99.43031
0.301896	0.3	1147.1	100.632
0.61498	0.6	2299	102.4967
1.192878	1.2	4425.2	99.40648
Coefficient X = 3679.2			
Intercept = + 36.364			
Correlation coefficient $R^2 = 0.9996$			
Average = 98.61098 %			
Standard deviation (SD) =4.389263			

With DESAGA CD₆₀ photo-densitometer was performed UV-VIS spectra on 200-500 nm for rutoside and hyperoside. Maxima of absorption were at 260 respectively 350 nm for hyperoside, and at 210, 320 respectively 410 nm for rutoside.

After analyzing the results we could determinate that flavonoids from flowers and leaves of *Robinia pseudoacacia* have similar structures with hyperoside and rutoside.

The results showed a higher concentration of flavonoids in flowers than in leaves. It is known that flowers are consumed in various foods so

Robinia pseudoacacia may become a valuable source of antioxidants in human nutrition. The flowers have the highest content of flavonoids, ranging from 0.15 to 0.9 mg / ml. The flowers harvested in the plains have a higher concentration of hyperoside (0.9 mg / ml) compared with the flowers taken from the hill (0.54 mg / ml). The leaves are rich in rutoside (0.98 mg / ml) compared with the flowers (Table 3). In the seeds and bark are insignificant amounts of flavonoids. The results are comparable to other results in the literature for other herbs [14].

Table 3. Concentration of hyperoside and rutoside within the analyzed extracts

Samples	Area		Methanolic extract (mg/ml)	
	hyperoside	rutoside	hyperoside	rutoside
<i>Robinia ps.</i> flowers, plain area (Craiova)	3363,5	676,9	0,9	0,17
<i>Robinia ps.</i> flowers, hill area (Râmnicu-Vâlcea)	2033,3	598,0	0,54	0,15
<i>Robinia ps.</i> leaves	652,9	3626,8	0,17	0,98

In Figure 4 is shown the variation of flavonoids concentrations in the studied samples and quantity in plant.

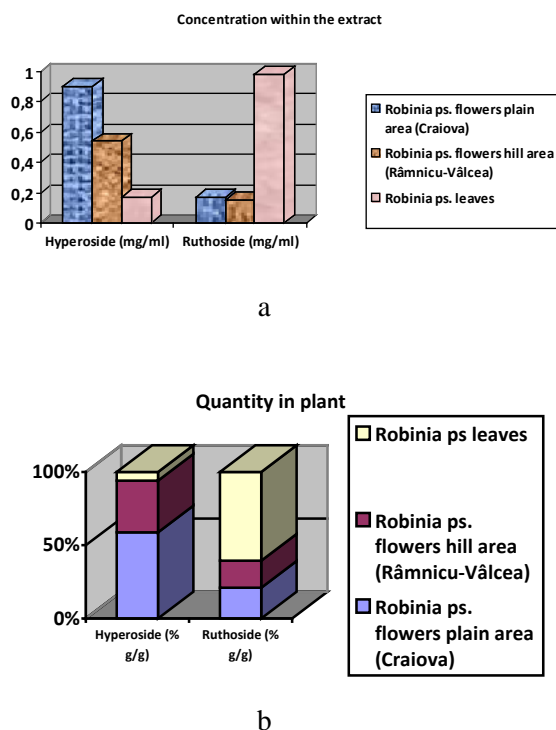


Figure 4. The variation of hyperoside and rutoside concentration: a - in methanolic extracts, b - in plant

Conclusion

The qualitative and quantitative analysis of flavonoids compounds was made using two chromatographic methods: Thin Layer Chromatography (TLC) and TLC coupled with photo-densitometry. It was investigated the presence of hyperoside and rutoside in methanolic extracts obtained from different parts of the plant: flowers from plains and hill area, leaves, seeds and bark. The identification was made by comparing the spots R_f values with the R_f values for rutoside and hyperoside. TLC coupled with photo-densitometry allowed the quantitative determination of hyperoside and rutoside. This concentration varies with the analyzed plant part. Thus, we conclude that the methods of analysis used demonstrates that *Robinia pseudoacacia* flowers and leaves have the highest concentration of flavonoids, compounds with pharmacological benefic potential (heard protective, reducing low-density lipoproteins, anti-ulcerous and liver protective, antimicrobial) and relatively low toxicity.

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Corresponding Author: Daniela Calina, PhD, University Lecturer, 2-4 Petru Rares Street, 200638 Craiova, Romania, e-mail calinadaniela@gmail.com, tel/fax: 0040251533713