PRELIMINARY RESEARCHES REGARDING THE FLAVONOSIDES CONTENT OF SOME *EPILOBIUM* SPECIES (ONAGRACEAE)

BEJENARU Ludovic Everard, OLAH Neli, MOGOŞANU George Dan, BEJENARU Cornelia, POPESCU Honorius

Abstract. The preliminary qualitative and quantitative determination of flavonosides in the aerial parts of *Epilobium* species from the Romanian flora (*Epilobium angustifolium, E. collinum, E. hirsutum, E. montanum,* and *E. parviflorum*) has been performed by thin layer chromatography (TLC)-densitometry.

Keywords: *Epilobium* sp., flavonosides, TLC-densitometry.

Rezumat. Cercetări preliminare privind conţinutul în flavonozide al unor specii de *Epilobium* (Onagraceae). Lucrarea cuprinde cercetări preliminare, prin cromatografie în strat subţire (CSS) cuplată cu fotodensitometrie, privind identificarea şi determinarea cantitativă a flavonozidelor din părţile aeriene ale unor specii de *Epilobium* recoltate din flora României (*Epilobium angustifolium, E. collinum, E. hirsutum, E. montanum* şi *E. parviflorum).*

Cuvinte cheie: *Epilobium* sp., flavonozide, CSS-fotodensitometrie.

INTRODUCTION

The aerial parts, and sometimes the underground parts of some *Epilobium* species (Onagraceae) are used in Romanian ethnopharmacology for their medicinal properties: emollient, demulcent, astringent, anti-haemorrhage, anti-inflammatory, diuretic, cytostatic, antibacterial, cicatrising (CIOCÂRLAN, 2000; CIULEI et al., 1993; PÂRVU, 2005).

Flavonosides, tannins, triterpene acids, coumarins, phenylpropane derivatives, amino acids, mucilages, and fatty acids have been previously isolated from the aerial parts of *Epilobium* species (BARRETT, 2004; BEJENARU et al., 2009; BRUNETON, 1993; CIULEI et al., 1993; DUKE et al., 2002; PÂRVU, 2005).

In this paper, the preliminary qualitative and quantitative determination of flavonosides in the aerial parts of five *Epilobium* species (*E. angustifolium* L. sin. *Chamaenerion angustifolium* (L.) SCOP., *E. collinum* C.C. GMELIN, *E. hirsutum* L., *E. montanum* L., and *E. parviflorum* SCHREBER) has been performed by TLC-densitometry.

MATERIAL AND METHODS

Plant material

The raw material has been collected in July–August 2004 as follows: *E. hirsutum* and *E. parviflorum* from Craiova (Dolj County), *E. collinum* from Lainici (Gorj County), *E. angustifolium* and *E. montanum* from Rânca (Gorj County). Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy of Craiova.

Extraction

Samples of accurately weighed, air-dried, and powdered aerial parts of five *Epilobium* species were extracted with methanol (1:5) under stirring (50^0C) and mixing 30 minutes at approximately 1000 rotations/min. The extractive solutions were filtered and then stored in dark bottles in the refrigerator until use. The methanolic extracts were identified as: E_1 – *Epilobii angustifolii herba*; E_2 – *E. collini herba*; E_3 – *E. hirsuti herba*; E_4 – *E. montani herba*; E_5 – *E. parviflori herba*.

Reagents and solvents

All of the analytical grade solvents and reagents were purchased from Merck (Darmstadt, Germany).

TLC experimental conditions

TLC experimental conditions include: stationary phase, silica gel G60 F_{254}-precoated TLC plates (Merck); mobile phase, ethyl acetate–ethylmethyl ketone–formic acid–water (50:30:10:10, in volumes); samples, 20% methanol extractive solutions from the aerial parts of *Epilobium* species (E_1–E_5); references, rutin (Roth) 1.22 mg/mL and hyperoside 1.10 mg/mL methanol solutions; about 5–10 µL of the sample and reference compounds have been applied on the plate as 10 mm bands; running distance, 15 cm; detection, UV light (254 nm) and natural products reagent (NP/PEG), in fluorescence; densitometry, Desaga CD60 scanner, wavelength 254 nm, wavelength interval for UV–VIS spectra *in situ* 200–500 nm, slit width 0.2 mm, repetition four times/position [7–10, 12] (GÂRD et al., 2009a, 2009b; GOCAN & CÂMPAN, 2004; JORK et al., 2005; WAGNER & BLADT, 1996).

RESULTS AND DISCUSSIONS

Figure 1 shows the TLC-chromatogram for the methanolic extracts from the aerial parts of the *Epilobium* species. Figure 2 (a-e) contains the TLC-densitograms for the *Epilobium* extracts (E_1-E_5). The quantitative determination of flavonosides has been performed using the calibration curve for rutin, obtained in the same chromatographic and densitometric conditions, with equation and correlation quotient (Fig. 3).

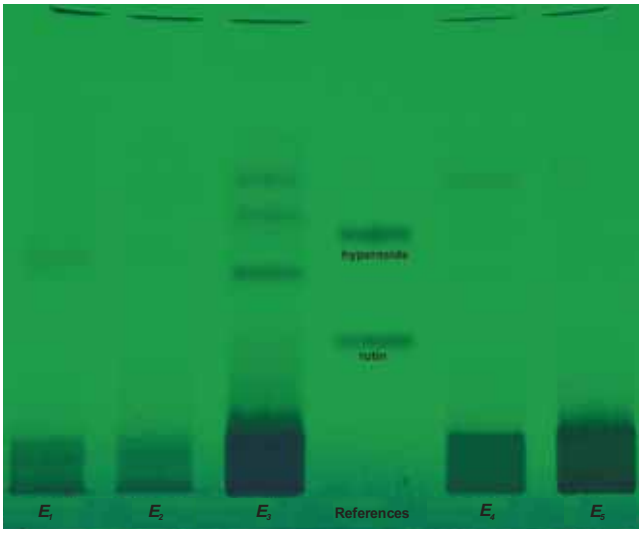

Figure 1. TLC-chromatogram in UV (254 nm) for the samples of *Epilobii herba* (E_1–E_5) and for the references (rutin and hyperoside).
Figura 1. Cromatograma CSS în UV (254 nm) pentru probele de *Epilobii herba* (E_1–E_5) şi pentru standarde (rutozidă şi hiperozidă).

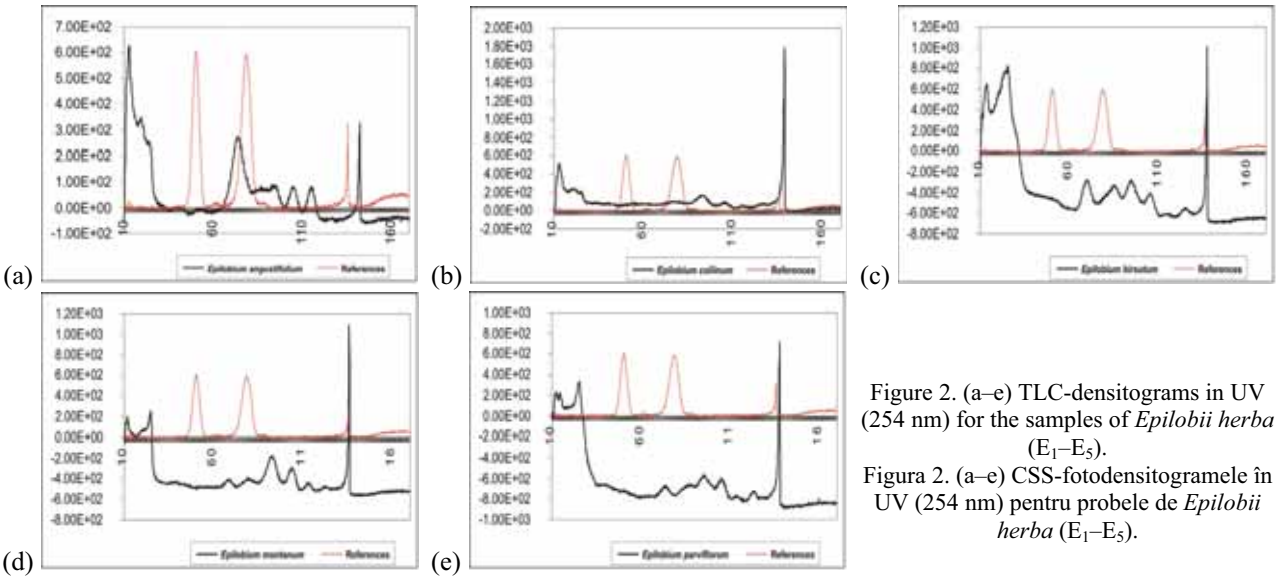

Figure 2. (a–e) TLC-densitograms in UV (254 nm) for the samples of *Epilobii herba* (E_1–E_5).
Figura 2. (a–e) CSS-fotodensitogramele în UV (254 nm) pentru probele de *Epilobii herba* (E_1–E_5).

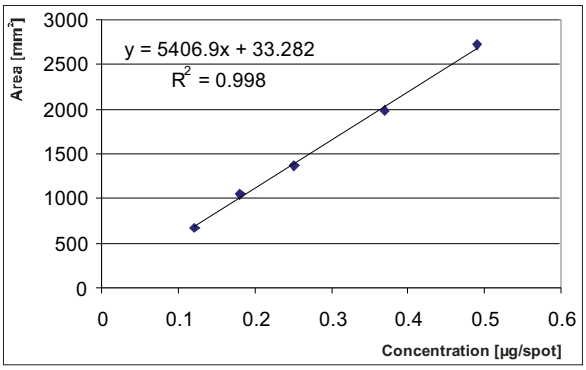

Figure 3. Rutin-calibration curve for flavonosides determination by TLC-densitometry.
Figura 3. Curba de calibrare în rutozidă, pentru determinarea flavonozidelor prin CSS-fotodensitometrie.

For the qualitative analysis of the methanolic extracts, the presence of some flavonosides with chromatographic characteristics closed to rutin and hyperoside was observed. The main flavonosides (1–4) showed yellow to orange fluorescence after spraying with NP/PEG reagent. R_f values for the analyzed flavonosides from the methanolic extracts E_1–E_5, and for the reference compounds are shown in Table 1.

Table 1. R_f values for the analyzed flavonosides 1–4, and for the reference compounds.
Tabel 1. Valorile R_f pentru flavonozidele analizate (1–4) şi pentru standarde.

Species (methanolic extract)	R_f values			
	Flavonoside 1	***Flavonoside 2***	***Flavonoside 3***	***Flavonoside 4***
E. angustifolium	0.46	0.56	0.61	0.79
E. collinum	–	–	0.61	–
E. hirsutum	0.45	0.56	0.65	0.74
E. montanum	–	–	0.64	0.77
E. parviflorum	0.47	0.58	0.64	0.73
	rutin – 0.29; hyperoside – 0.52			

Regarding the R_f values, obtained after the analysis of chromatograms and densitograms, we could observe the separation of four flavonosides (1-4) in high amounts in the aerial parts of *E. angustifolium*, *E. hirsutum* and *E. parviflorum*. In the aerial parts of *E. montanum*, only two flavonosides (R_f 0.64, respectively 0.77) were found in high amounts. The aerial parts of *E. collinum* contain high amounts of only one flavonoside (R_f 0.61). The absorption maximum values in UV for the analyzed flavonosides compared to the references are shown in Table 2.

Table 2. The absorption maximum values in UV for the analyzed flavonosides and for the references.
Tabel 2. Maximele de absorbţie în UV pentru flavonozidele analizate şi pentru standarde.

Species	Compound	Absorption maximum values [nm]
E. angustifolium	Flavonoside 1	260; 360
E. hirsutum	Flavonoside 1	270; 310; 380
E. parviflorum	Flavonoside 3	260; 360
	Rutin	260; 360
	Hyperoside	260; 365

From the experimental data, it could be established that the flavonoside 1 from *E. angustifolium* and flavonoside 3 from *E. parviflorum* have a similar structure with rutin and hyperoside, and probably they are quercetin-based glycosides. The quantitative determination of flavonosides was performed, in the first step, through the integration of densitograms with the densitometer programme, taking into account the ratio between the area of the separated compounds and the total area of the analyzed sample. Therefore, the content of flavonosides in the methanolic extracts of five *Epilobium* species was established (Table 3, Fig. 4).

Table 3. The content of flavonosides (1–4) in the methanolic extract of five *Epilobium* species.
Tabel 3. Conţinutul de flavonozide (1–4) în extractul metanolic de la cinci specii de *Epilobium*.

Species	Content in the methanolic extract [%]			
	Flavonoside 1	***Flavonoside 2***	***Flavonoside 3***	***Flavonoside 4***
E. angustifolium	15.70	5.65	5.30	5.27
E. collinum	–	–	3.71	–
E. hirsutum	6.38	8.03	5.45	2.42
E. montanum	–	–	22.89	10.02
E. parviflorum	2.24	3.96	3.93	4.62

Starting from the rutin-calibration curve, the content of flavonosides, expressed in rutin, in the methanolic extracts, respectively in the aerial parts of five *Epilobium* species, was determined (Table 4, Fig. 5). High amounts of flavonosides 3 and 4 were determined in the aerial parts of *E. montanum*. High levels of flavonosides 1 and 2 were found in the aerial parts of *E. angustifolium* and *E. hirsutum*. Closer values of flavonosides (1–4) were determined in the aerial parts of *E. parviflorum*.

Table 4. Content of flavonosides (1–4), expressed in rutin, in the methanolic extract and in the aerial part of *Epilobium* species.
Tabel 4. Conţinutul de flavonozide (1–4), exprimat în rutozidă, în extractul metanolic şi în partea aeriană a speciilor de *Epilobium*.

Species	Flavonoside 1	Flavonoside 2	Flavonoside 3	Flavonoside 4
	Content			
	Methanolic extract [mg/mL] / Aerial parts [% g/g]			
E. angustifolium	0.23 / 0.12	0.08 / 0.04	0.07 / 0.04	0.07 / 0.04
E. collinum	–	–	0.04 / 0.02	–
E. hirsutum	0.19 / 0.10	0.25 / 0.13	0.16 / 0.08	0.07 / 0.04
E. montanum	–	–	0.31 / 0.16	0.13 / 0.07
E. parviflorum	0.05 / 0.03	0.08 / 0.04	0.08 / 0.04	0.10 / 0.05

Flavonosides analysis is one-step closer to establishing a chromatographic imprint and a qualitative database for the differentiation of the *Epilobium* species from the Romanian flora.

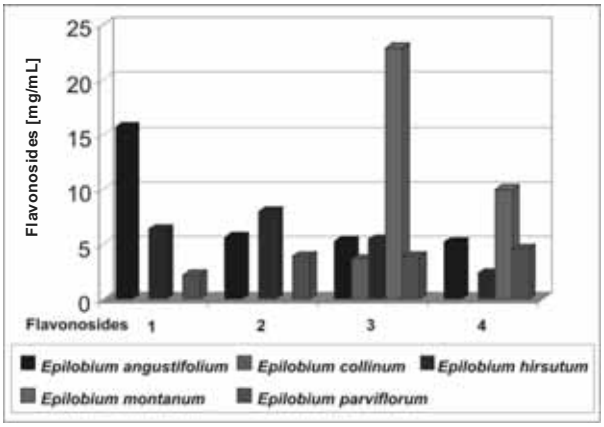

Figure 4. Content of flavonosides (1–4) in the methanolic extract of the *Epilobium* species.
Figura 4. Conținutul de flavonozide (1–4) în extractul metanolic din speciile de *Epilobium*.

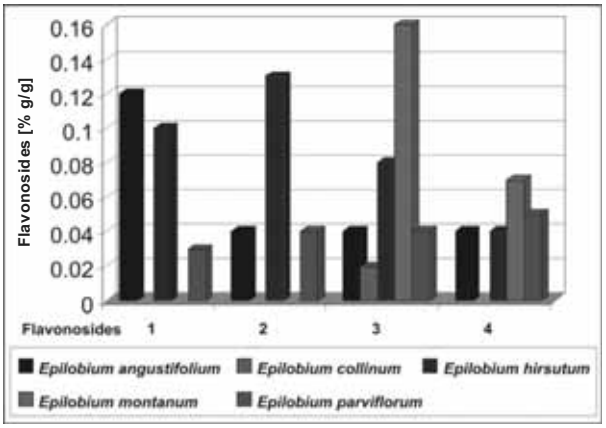

Figure 5. Content of flavonosides (1–4), expressed in rutin, in the aerial part of five *Epilobium* species.
Figura 5. Conținutul de flavonozide (1–4), exprimat în rutozidă, în partea aeriană a speciilor de *Epilobium*.

CONCLUSIONS

The preliminary qualitative and quantitative determination of four flavonosides in the aerial parts of five *Epilobium* species from the Romanian flora was performed by TLC-densitometry. High amounts of each of the flavonosides have been found in the aerial parts of *E. angustifolium*, *E. hirsutum*, and *E. parviflorum*. In the aerial parts of *E. montanum*, only two flavonosides (R_f 0.64, respectively 0.77) were found in high amounts. The aerial parts of *E. collinum* contain high amounts of only one flavonoside (R_f 0.61).

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Bejenaru Ludovic Everard, Mogoşanu George Dan,
Bejenaru Cornelia, Popescu Honorius
Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova
2–4 Petru Rareş Street, 200349 Craiova, Romania
E-mail: ludovic.bejenaru@catena.ro, mogosanu2006@yahoo.com

Olah Neli
Plant Extrakt, Laboratories of Phytotherapy
and Homeopathy 407059 Rădaia, Cluj, Romania
E-mail: olahdr@aol.com

Received: April 30, 2010
Accepted: August 30, 2010