Original Paper

Researches on the preparation and characterization of some tinctures from *Silene albae herba* and *Silene pendulae herba*

ŢÎRCOMNICU VIORELA (1), GÎRD CERASELA ELENA (2), RADU S. (1)

Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova; (2) Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest

ABSTRACT Starting from the varied chemical composition of *Silene* species (*Caryophyllaceae* family), the pharmacological utilizations (homeopathy) and the cumulative toxicity for a long time administration, were performed researches concerning the preparation and characterization of tinctures from aerial parts of *S. alba* and *S. pendula* species. Tinctures were obtained, according to the Romanian Pharmacopoeia Xth edition, by percolation and characterized from physico-chemical point of view: color, taste, smell, relative density, refractive index, and quality conditions — content in iron and heavy metals, alcohol concentration and evaporation residue. Qualitative and quantitative analyses of tinctures were making for the flavonosids, using thin layer-chromatography, respectively VIS spectrophotometry.

KEY WORDS Silene albae herba, Silene pendulae herba, tinctures, flavonosids.

Introduction

Text Silene alba (Miller) E.H.L. Krause sin. Melandrium album (Miller) Garcke, White Campion, is native to most of Europe, Western Asia and North Africa. It is an herbaceous annual, biennial or short-lived perennial plant, growing in most open habitats, particularly wasteland and fields, preferring sunny areas [1].

* Silene pendula L., Nodding Catchfly, is an herbaceous annual species, growing in Southern Mediterranean region, Southern Russia, Caucasus, Turkey. In the Romanian' flora, is a cultivated plant, in gardens, flowering in March to April [1].

In the specialty papers, there are incomplete data on the chemical composition of *Silene* species, as follows: flavonosides (apigenin- and luteolin-O,C-glycosides), phytoecdysteroids (ecdysone, 2-deoxy-20-hydroxyecdysone), triterpenoid saponins (gypsogenin and quillaic acid sapogenins), and polyphenolic acids [2–9].

The purpose of this paper is the preparation and physico-chemical characterization of some tinctures from the aerial parts of *S. alba* and *S. pendula* species.

Material and Methods

Plant material

From the *S. alba* and *S. pendula* species, the aerial parts were collected at the flowering, in April 2010, from the Botanical Garden of The University of Craiova, Dolj County. Voucher specimens are deposited in the Herbarium of the University of Medicine and Pharmacy of Craiova.

Preparation of tinctures

The tinctures were obtained by percolation, according to the Romanian Pharmacopoeia Xth edition, in a ratio of vegetal product / extraction solvent (70° ethanol) 1: 5. The 20% tinctures were filtered and then stored in dark bottles in the refrigerator, until use [10, 11].

Reagents and solvents

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

Organoleptic characterization

Tinctures are clear liquids, colorful. A slight sediment may form on standing and that is acceptable as long as the composition is not changed significantly [10].

Determination of relative density

Determination of relative density of the two tinctures was performed using the pycnometer, the fourth decimal precision [10].

Determination of refractive index

Refractive index determination was made by Abbé refractometer [10].

Quality conditions

The quality conditions were established according to Romanian Pharmacopoeia [10]: iron – up to 0.001%; heavy metals – up to 0.001%; alcohol concentration; evaporation residue.

Identification of flavonosides

Thin-layer chromatography (TLC) can separate and identify, using appropriate standards, a series of flavonosides, their aglycones or polyphenolic acids (caffeic and chlorogenic acids) [12–19]:

- Stationary phase: silica gel Merck, 10×10 cm plates;
- Mobile phase: ethyl acetate—water—formic acid—acetic acid (72:14:7:7);
- Samples: 20% tinctures of *Silene albae herba* and *Silene pendulae herba*;
- Standards (s): rutoside, hyperoside, apigenol-7-neohesperidoside, quercitrin, luteol-7-glucoside, apigenol-7-glucoside, chlorogenic acid, caffeic acid (0.1% methanolic solutions);
- The amount applied to the starting line: 20 μ l of samples solutions, 10 μ l of references solutions spots applied are linear (band) width of 1 cm;
 - Migration distance: 12 cm;
- Revelator: NEU/PEG reagent, followed by examination of plates in UV light, λ 365 nm.

Quantitative analysis of flavonosides

Quantitative determination of flavonosides was made using spectrophotometric method, through the reaction with aluminum chloride, according to the Romanian Pharmacopoeia. The standard curve was obtained using appropriate extinction values of rutoside solutions [10].

Results and Discussion

Results of physico-chemical characterization of the two *Silene herba* tinctures are shown below (Figures 1 and 2, Tables 1–3).

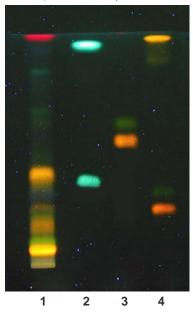


Figure 1 – TLC chromatogram of Silene albae herba tincture (1). Standards, bottom-up: 2 – chlorogenic acid, caffeic acid; 3 – luteol-7-glucoside, apigenol-7-glucoside; 4 – rutoside, hyperoside, apigenol-7-neohesperidoside, quercitrin.

Table 1 – Results of TLC analysis of Silene albae herba tincture

Sample R _f Color Fluorescence (VIS) (UV)	Comments
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1.	0.07	yellow	yellow-orange	flavonosid
	0.10	yellow	yellow-green	flavonosid
	0.18	yellow	yellow-orange	flavonosid
	0.25	yellow	yellow-orange	probably rutoside
	0.40	yellow	yellow-orange	flavonosid
2.	0.37	-	blue	chlorogenic acid (s)
	0.95	-	blue	caffeic acid (s)
3.	0.54	yellow	yellow-orange	luteol-7-glucoside (s)
	0.61	yellow	yellow-green	apigenol-7-glucoside (s)
4.	0.25	yellow	yellow-orange	rutoside (s)
	0.30	yellow	yellow-green	hyperoside (s)
	0.89	yellow	yellow-orange	apigenol-7- neohesperidoside (s)
	0.97	yellow	yellow-orange	quercitrin (s)



Figure 2 – TLC chromatogram of Silene pendulae herba tincture (1). Standards, bottom-up:
2 – rutoside, hyperoside, apigenol-7neohesperidoside, quercitrin; chlorogenic acid, caffeic acid; 3 – luteol-7-glucoside, apigenol-7-glucoside.

Table 2 – Results of TLC analysis of Silene pendulae herba tincture

Sample	R_{f}	Color (VIS)	Fluorescence (UV)	Comments
1.	0.36	yellow	yellow-orange	probably rutoside
	0.43	yellow	yellow-green	probably hyperoside
	0.61	yellow	yellow-orange	probably luteol-7- glucoside
	0.69	yellow	yellow-green	probably apigenol-7- glucoside
2.	0.36	yellow	yellow-orange	rutoside (s)
	0.43	yellow	yellow-green	hyperoside (s)
	0.58	yellow	yellow-orange	apigenol-7- neohesperidoside (s)
	0.98	yellow	yellow-orange	quercitrin (s)
3.	0.61	yellow	yellow-orange	luteol-7-glucoside (s)
	0.69	yellow	yellow-green	apigenol-7-glucoside (s)

The tincture from *Silene albae herba* is possible to contain rutoside.

The tincture from *Silene pendulae herba* is possible to contain rutoside, hyperoside, luteol-7-glucoside, and apigenol-7-glucoside.

Table 3 – Physico-chemical characterization of tinctures from Silene albae herba and Silene pendulae herba

Physico-chemical		Tinctura Silene
characterization	albae herba	pendulae herba
Aspect	clear liquid	clear liquid
Color	yellow-green	yellow-green
Smell	typically, low	typically, low
	aromatic	aromatic
Taste	slightly bitter,	slightly bitter,
Taste	burning	burning
Relative density	0.9625	0.9640
Refractive index	1.3693	1.3675
Iron [%]	_	_
Heavy metals [%]	_	-
Alcohol content [% m/m]	68.75	68.35
Evaporation residue [%]	5.15	5.85
Qualitative analysis (TLC)	flavonosids	flavonosids
Quantitative spectro-		
photometric analysis [mg rutoside / 100 mL tincture]	2.65	3.42

Conclusions

- 1. Preparation and characterization of some tinctures from *Silene herba* have as a starting point the chemical composition, the medicinal uses, and the potential toxicity of *Silene* species.
- 2. Tinctures were obtained by percolation, and characterized from physico-chemical point of view
- 3. Qualitative and quantitative analyses of the two tinctures were making for the flavonosides.
- 4. Silene albae herba tincture is possible to contain rutoside.
- 5. Silene pendulae herba tincture is possible to contain rutoside, hyperoside, luteol-7-glucoside, and apigenol-7-glucoside.

References

- Ciocârlan V (2000) Flora ilustrată a României. Pteridophyta et Spermatophyta, Ed. Ceres, Bucureşti, 224.
- Heinsbroek R, van Brederode J, van Nigtevecht G, Maas J, Kamsteeg J, Besson E, Chopin J – (1980) The 2"-O-glucosylation of vitexin and isovitexin in petals of Silene alba is catalysed by two different enzymes, Phytochemistry, 19(9):1935–1937.
- Hegnauer R (1989) Chemotaxonomie der Pflanzen, Band 8, Birkhäuser Verlag, Basel–Boston–Berlin, 1989, 215–220.
- Báthori M (1998) Purification and characterization of plant ecdysteroids of *Silene* species, TrAC Trends Anal Chem, 17(6):372–383.
- Meng Y, Whiting P, Zibareva L, Bertho G, Girault JP, Lafont R, Dinan L – (2001) Identification and quantitative analysis of the phytoecdysteroids in Silene species (Caryophyllaceae) by highperformance liquid chromatography: novel ecdysteroids from S. pseudotites, J Chromatogr A, 935(1–2):309–319.
- Zibareva L, Volodin V, Saatov Z, Savchenko T, Whiting P, Lafont R, Dinan L – (2003) Distribution of phytoecdysteroids in the *Caryophyllaceae*, Phytochemistry, 64(2):499–517.

- Simon A, Tóth N, Tóth G, Kele Z, Groska J, Báthori M – (2009) Ecdysteroids from Silene viridiflora, Helvetica Chimica Acta, 92(4):753–761.
- Jia Z, Koike K, Sahu NP, Nikaido T (2002) Triterpenoid saponins from *Caryophyllaceae* family, Studies Nat Prod Chem, 26(7):3–61.
- Larhsini M, Marston A, Hostettmann K (2003) Triterpenoid saponins from the roots of Silene cucubalus, Fitoterapia, 74(3):237–241.
- 10. ***, Farmacopeea Română ediția a X-a, Ed. Medicală, București, 1993, 921–922.
- 11. Popovici Iuliana, Lupuleasa D (1997) Tehnologie farmaceutică, vol. I, Ed. Polirom, Iaşi, 359–389.
- Wagner H, Bladt Sabine (1996) Plant drug analysis. A thin layer chromatography atlas, 2nd edition, Springer Verlag, Berlin–Heidelberg, 125–146, 195–236.
- Gîrd Cerasela Elena, Duţu Ligia Elena, Popescu Maria Lidia, Pavel Mariana – (2005) Farmacognozie, baze practice, vol. I, Ed. Universitară "Carol Davila", Bucureşti, 2005, 145, 235.
- 14. Jork H, Funk W, Wimmer H (1990) Thin-layer chromatography. Reagents and detection methods, Vol. 1a, Weinheim, 277–283.
- 15. Markham KR (1982) Techniques of flavonoid identification, Academic Press, New York, 31.
- 16. Harborne JB, Mabry TJ, Mabry H (1975) The flavonoids, Chapman and Hall, London, 46.
- 17. Farkas L, Gábor M, Kállay F (1985) Flavonoids and bioflavonoids, Akadémiai Kiadó, Budapest, 12.
- Mabry TJ, Markham KR, Thomas MB (1970) The systematic identification of flavonoids, Springer-Verlag, Berlin–Heidelberg–New York, 33.
- Tămaş M, Crişan Gianina, Dulfu C, Purtan Mirela (2002) Studiul comparativ al flavonoidelor din frunzele şi mugurii speciilor indigene de plop, Farmacia, 50(3):78–83.
- Ciocârlan V (2000) Flora ilustrată a României.
 Pteridophyta et Spermatophyta, Ed. Ceres,
 București, 224.
- 21. Heinsbroek R, van Brederode J, van Nigtevecht G, Maas J, Kamsteeg J, Besson E, Chopin J – (1980) The 2"-O-glucosylation of vitexin and isovitexin in petals of *Silene alba* is catalysed by two different enzymes, Phytochemistry, 19(9):1935–1937.
- 22. Hegnauer R (1989) Chemotaxonomie der Pflanzen, Band 8, Birkhäuser Verlag, Basel–Boston–Berlin, 1989, 215–220.
- 23. Báthori M (1998) Purification and characterization of plant ecdysteroids of *Silene* species, TrAC Trends Anal Chem, 17(6):372–383.
- 24. Meng Y, Whiting P, Zibareva L, Bertho G, Girault JP, Lafont R, Dinan L (2001) Identification and quantitative analysis of the phytoecdysteroids in *Silene* species (*Caryophyllaceae*) by high-performance liquid chromatography: novel ecdysteroids from *S. pseudotites*, J Chromatogr A, , 935(1–2):309–319.
- 25. Zibareva L, Volodin V, Saatov Z, Savchenko T, Whiting P, Lafont R, Dinan L (2003) Distribution of phytoecdysteroids in the *Caryophyllaceae*, Phytochemistry, 64(2):499–517.
- 26. Simon A, Tóth N, Tóth G, Kele Z, Groska J, Báthori M (2009) Ecdysteroids from *Silene viridiflora*, Helvetica Chimica Acta, 92(4):753–761.
- 27. Jia Z, Koike K, Sahu NP, Nikaido T (2002) Triterpenoid saponins from *Caryophyllaceae* family, Studies Nat Prod Chem, 26(7):3–61.
- 28. Larhsini M, Marston A, Hostettmann K (2003)

- Triterpenoid saponins from the roots of *Silene cucubalus*, Fitoterapia, 74(3):237–241.
- 29. ***, Farmacopeea Română ediția a X-a, Ed. Medicală, București, 1993, 921–922.
- 30. Popovici Iuliana, Lupuleasa D (1997) Tehnologie farmaceutică, vol. I, Ed. Polirom, Iaşi, 359–389.
- Wagner H, Bladt Sabine (1996) Plant drug analysis. A thin layer chromatography atlas, 2nd edition, Springer Verlag, Berlin–Heidelberg, 125–146, 195–236.
- 32. Gîrd Cerasela Elena, Duţu Ligia Elena, Popescu Maria Lidia, Pavel Mariana (2005) Farmacognozie, baze practice, vol. I, Ed. Universitară "Carol Davila", București, 2005, 145, 235.
- 33. Jork H, Funk W, Wimmer H (1990) Thin-layer chromatography. Reagents and detection methods, Vol. 1a, Weinheim, 277–283.

- 34. Markham KR (1982) Techniques of flavonoid identification, Academic Press, New York, 31.
- 35. Harborne JB, Mabry TJ, Mabry H (1975) The flavonoids, Chapman and Hall, London, 46.
- 36. Farkas L, Gábor M, Kállay F (1985) Flavonoids and bioflavonoids, Akadémiai Kiadó, Budapest, 12.
- 37. Mabry TJ, Markham KR, Thomas MB (1970) The systematic identification of flavonoids, Springer-Verlag, Berlin–Heidelberg–New York, 33.
- 38. Tămaş M, Crişan Gianina, Dulfu C, Purtan Mirela (2002) Studiul comparativ al flavonoidelor din frunzele şi mugurii speciilor indigene de plop, Farmacia, 50(3):78–83.

Corresponding address: Stelian Radu, Professor, Department of Organic Chemistry, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova, 2–4 Petru Rareş Street, 200349 Craiova, Romania; Phone/Fax +40251–523 929, e-mail: sradu@umfcv.ro