

HPTLC EVALUATION OF THE PYRROLIZIDINE ALKALOID SENECTIONINE IN CERTAIN PHYTOCHEMICAL PRODUCTS

OANA CRISTINA ȘEREMET¹, OCTAVIAN TUDOREL OLARU^{*2},
MIHAELA ILIE³, SIMONA NEGREȘ¹, DAN BĂLĂLĂU³

"Carol Davila" University of Medicine and Pharmacy, Faculty of
Pharmacy, 6 Traian Vuia St., Bucharest, Romania

¹Pharmacology and Clinical Pharmacy Department

²Botany and Cellular Biology Department

³Toxicology Department

*corresponding author: octav_olaru2002@yahoo.ro

Abstract

Petasites hybridus (butterbur), *Tussilago farfara* (coltsfoot) and *Senecio vernalis* (spring groundsel) are species traditionally used in phytotherapy that beside the therapeutic compounds, contain toxic pyrrolizidine alkaloids. A HPTLC method was developed using a new mobile phase (chloroform: methanol: ammonia 25%: hexane - 82: 14: 2.6: 20 v/v) that permitted the separation of senecionine, one of most toxic pyrrolizidine alkaloids. Although the literature quotes as being present in all three species, senecionine was identified only in *S. vernalis*. The identity of used vegetal material was confirmed by microscopic and macroscopic examination.

Rezumat

Petasites hybridus (captalan), *Tussilago farfara* (podbal) și *Senecio vernalis* (cruciuliță) sunt specii utilizate tradițional în fitoterapie care, pe lângă principiile active, conțin alcaloizi pirolizidinici toxici. Am dezvoltat o metodă HPTLC folosind o nouă fază mobilă (cloroform: metanol: amoniac 25%: hexan - 82: 14: 2.6: 20 v/v) care a permis separarea senecioninei, unul dintre cei mai toxici alcaloizi pirolizidinici. Cu toate că literatura citează senecionina ca fiind prezentă în toate cele trei specii, aceasta a fost identificată doar în *S. vernalis*. Identitatea materialului vegetal folosit a fost confirmată prin examen macroscopic și microscopic.

Keywords: *Senecio vernalis*; *Petasites hybridus*; *Tussilago farfara*; senecionine; HPTLC

Introduction

Petasites hybridus (butterbur), *Tussilago farfara* (coltsfoot) and *Senecio vernalis* (spring groundsel) are species from Asteraceae family traditionally used in phytotherapy. *Petasites hybridus* root it is known for its spasmolytic, anti-allergic and anti-inflammatory principles and it has been used for spasms of the urogenital and digestive tract, prophylactic treatment of migraine, dysmenorrhoea, asthma and allergic rhinitis [5].

Tussilago farfara leaves are used to relief dry caught and other respiratory diseases symptoms [15]. The aerial parts of *Senecio* species are used under the generic name of *Senecionis herba* for their antidiarrheal, diuretic, diaphoretic, emmenagogue, galactagogue and expectorant properties [8].

These herbs, beside the therapeutic bioactive compounds, contain pyrrolizidine alkaloids (PAs) and their N – oxides. PAs are potentially toxic for the liver and/or lungs in humans and are also carcinogenic for animals. Their hazard arises from the formation of pyrrolic metabolites (dehydroalkaloids) in the liver able to bind to nucleophilic centers in tissues or cross-link DNA, leading to hepatotoxicity and carcinogenicity [13, 14].

According to the literature, the toxic PAs found in the three studied species are senecionine, intergerrimine, retrosine, seneciphylline, jacobine, senkirkine in butterbur roots, senkirkine, senecionine, tussilagine, isotussilagine in coltsfoot leaves and retrorsine, senecionine, senkirkine, senecivernine, integerrimine, seneciphylline, riddelliine, in spring groundsel aerial parts [3, 14]. The quantitative and qualitative composition of these alkaloids varies within species. Senecionine, with a LD₅₀ in male rats of approximately 50 mg/kg b.w., is the most toxic of all cited PAs [1].

Thin Layer Chromatography (TLC) is widely used for the isolation, qualitative analysis and quantitative analysis of the individual compounds from plant extracts that are very complex mixtures of the structurally differentiated chemical compounds [4].

The aim of the paper was to separate and identify senecionine from purified extracts from coltsfoot and butterbur teas and from harvested spring groundsel. Besides, the identity of the three plants was confirmed by macroscopic and microscopic evaluation.

Materials and Methods

Plant Material

Coltsfoot and common butterbur teas (produced by Stef Mar Ltd., Valcea) purchased from retail stores were the source for *P. hybridus* roots and *T. farfara* leaves.

S. vernalis aerial part and roots were harvested in August 2012, from Deva (Hunedoara County), naturally dried and conserved in laboratory conditions. Some fresh pieces of vegetal material were fixed in a alcoholic solution for the microscopic analysis. A voucher specimen

is deposited at the Department of Botany, Faculty of Pharmacy, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

The vegetal material was examined macroscopically and compared with the morphological characters described in the literature.

For the microscopic examination the dried plants were pulverised and clarified with chloral hydrate 80%. Cross sections through the root, stem and leaf (clarified with sodium hypochlorite solution and double coloration with iodine green and alauun-carmin red) of *S. vernalis* were made [11]. The preparations were analyzed at a Labophot 2-Nikon microscope (ocular 10x, ob. 4x, 10x, 40x).

High Performance Thin Layer Chromatography (HPTLC)

Sample Preparation

The dried plants were pulverised (sieve V) in accordance with accepted procedures for vegetal products containing alkaloids [16] and 5 g were refluxed for 2 h with 500 mL of 50% methanol acidified with citric acid to pH 2–3. These conditions enable the extraction of both *N*-oxide and tertiary bases. After filtration, the solutions were reduced to approximately 100 mL and stirred with Zn powder for 3 h at room temperature to convert PA-*N*-oxides to free PAs. The solutions were filtered and partitioned twice with 30 mL chloroform and twice with 30 mL ether in order to remove lipophilic accompanying substances. The aqueous solution was alkalized with 25% ammonia (pH 9–10) and the PAs, as free bases, extracted by 3-fold partition each against 30 mL chloroform. The combined organic layers were evaporated to 1 mL on a concentration evaporator workstation, TurboVap 500 (Caliper, USA) and dried under nitrogen flow at room temperature (Techne Dry-Block DB-3D, Bibby Scientific Inc., England). The residue was redissolved in 0.5 mL methanol.

Standard

Senecionine (GC $\geq$ 95%) was purchased from Carl Roth Germany and a 200 μ g/mL standard solution in methanol was prepared.

Chromatography

TLC evaluation was performed on HPTLC Silica gel 60 F₂₅₄ glass plates, 20 cm x 10 cm (Merk) and TLC Silica gel 60 F₂₅₄ glass plates 20 cm x 20 cm (Merk). The mobile phases used were: chloroform: methanol: ammonia 25% 85: 14: 1 (v/v) (MP I) [9] and chloroform: methanol: ammonia 25%: hexane 82: 14: 2.6: 20 (v/v) (MP II).

The plates were spotted with 5, 7.5, 10, 12.5, 15 and 17.5 μ L of senecionine stock solution in parallel with 5, 10 and 15 μ L of each

sample prepared as described above using a Linomat 5 (Camag, Switzerland), computer assisted, semi-automatic sample spotting device.

The plates were developed with the mobile phases in a developing vertical glass tank with lid, previously saturated with the mobile phases vapors. When the mobile phases front reached about 1 cm from the plate edge, the plates were extracted from the tank, completely dried in air at room temperature (25°C), scanned at $\lambda=220$ nm (UV light), in reflectance mode using a Camag TLC Scanner 3 with WinCats 3.2.1 software. The R_f (retention factor) values were automatically computed on tracks, for each spot. The peak purity was assessed by comparing the UV spectra at peak start, peak middle and peak end positions of the spot in standard and sample test. After scanning the plates were sprayed with Dragendorff reagent.

Results and Discussion

The morphological characters of the vegetal material were the same with the ones quoted by literature [6, 7, 10].

The microscopic examination of the pulverised drugs revealed specific spongy parenchyma, anomocytic stomata, calcium oxalate druses, pluricellular trichomes (*T. farfara*) (Figure 1); parenchyma, suber (*P. hybridus*); semiangular, tricolporate pollen grains with echinate exine, anomocytic stomata, irregular pappus hairs, protoxylem vessels (*S. vernalis*) (Figure 2).

The microscopic examination of the *S. vernalis* cross sections of the root revealed secondary structure with well-developed secondary xylem (Figure 3). The ridged circular stem presents: tangential collenchyma (under the epidermis), angular collenchyma (under the ridges), evident endodermis, collateral vascular bundles with sclerenchyma sheath and above secretory cavity and continuous sclerenchyma formation running round the stem (Figures 4 - 5). The leaf had homogeneous structure, tangential collenchyma, pericycle with cell corners thickened with cellulose, collateral vascular bundle with sclerenchyma sheath, anomocytic stomata and pluricellular glandular trichoma on the surface of the epidermis (Figure 6).

The identified microscopic characters are in accordance with the ones mentioned in literature for all three species [2, 7, 12].

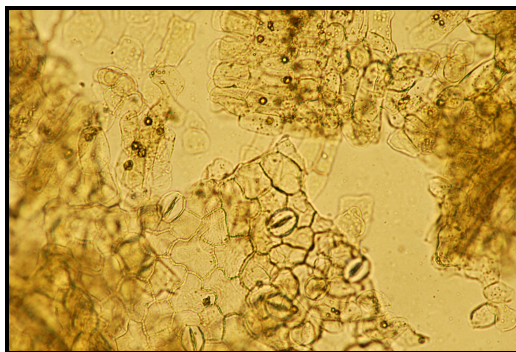


Figure 1
T. farfara - powder (ob. 10x)

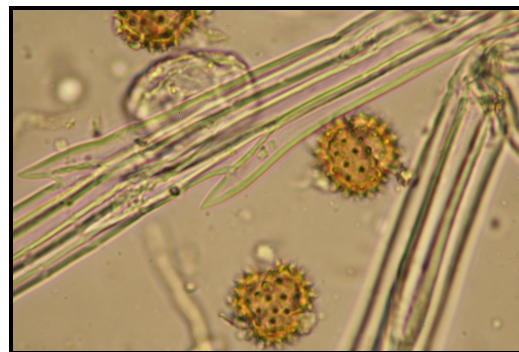


Figure 2
S. vernalis – powder (ob. 40x)

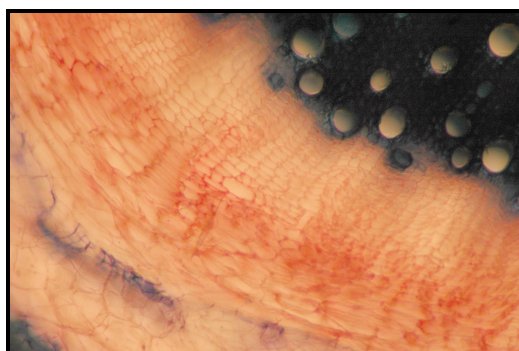


Figure 3
S. vernalis root cross - section (ob. 10x)

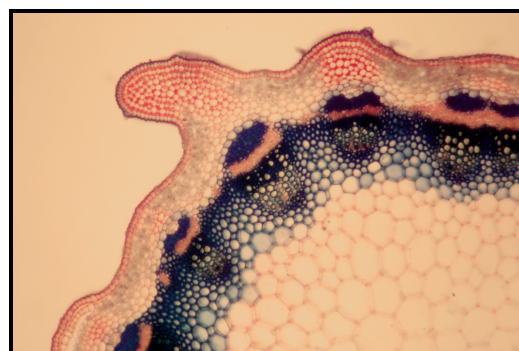


Figure 4
S. vernalis steam cross - section (ob. 4x)

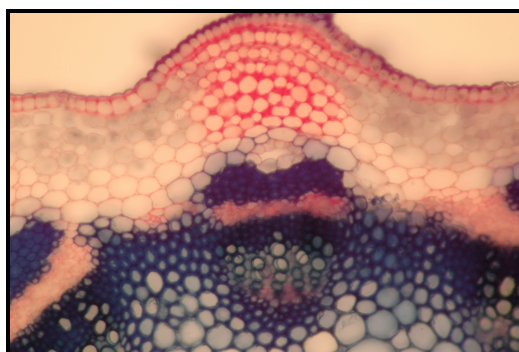


Figure 5
S. vernalis steam cross - section (ob. 10x)



Figure 6
S. vernalis leaf glandular trichoma (ob. 40x)

The use of HPTLC plates (200- μm layer of silica gel, with a particle size of 5 – 6 μm , a narrow particle size distribution) lead to improved separation compared with the conventional TLC plates. The chromatoplates obtained for the two solvent systems showed that only the HPTLC plates developed with MP II enabled a good separation of senecionine from the other components of the extracts (Figure 7).

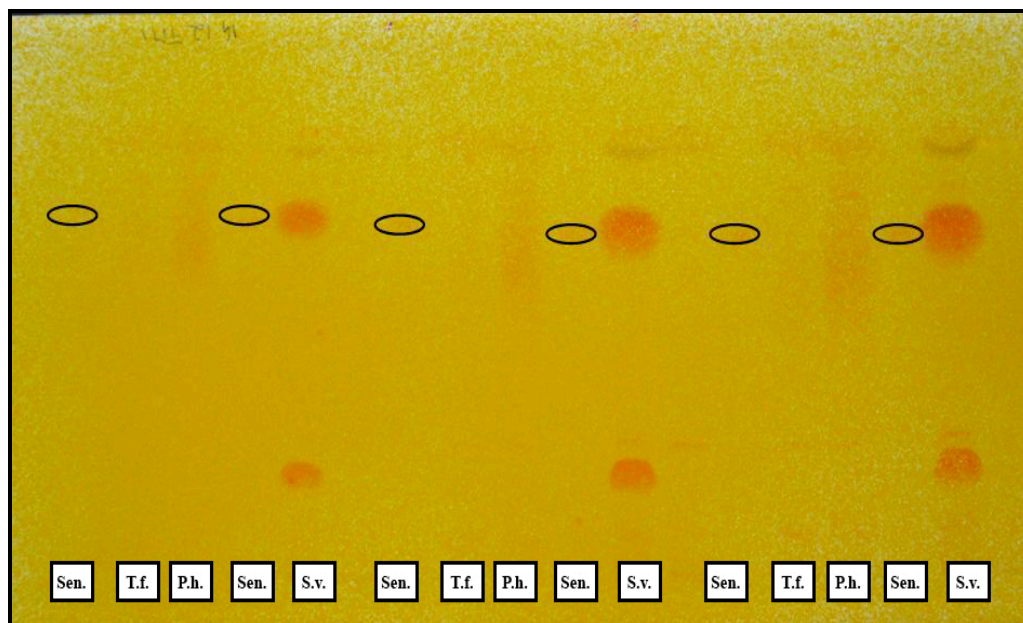


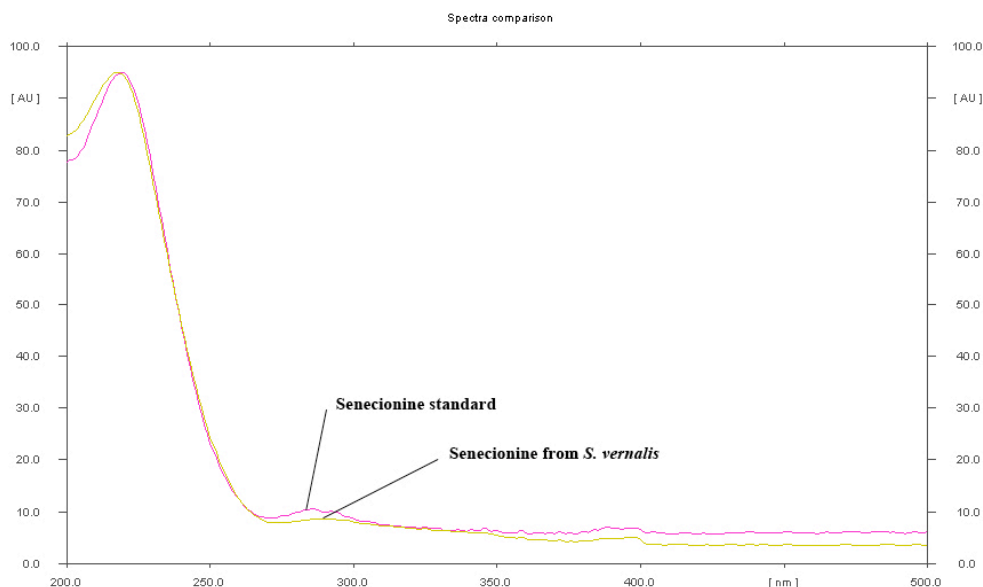
Figure 7

HPTLC plate obtained with MP I and sprayed with Dragendorff reagent (Sen = senecionine; T.f. = *T. farfara* extract; P.h. = *P. hybridus* extract; S.v. = *S. vernalis* extract)

The R_f value obtained for senecionine was 0.79 ± 0.03 , as a mean of the R_f values computed on the peaks for the corresponding tracks. The UV spectra of the standard showed a maximum peak at 220 nm.

Senecionine was found in the *S. vernalis* extract. In *T. farfara* and *P. hybridus* extracts the alkaloid is missing or is under the limit of detection of the method.

As means of identification of senecionine we used the R_f values, the UV spectra (Figure 8) and the color of the spots after spraying with Dragendorff reagent.

**Figure 8**

The compared UV spectrum of senecionine standard and corresponding spots from *S. vernalis*

Peak purity results (obtained by scanning at 220 nm) were satisfactory. The mean correlations factors of the peak start spectrum with the peak centre spectrum were 0.9999, 0.9997 for standard and sample tracks, respectively. The mean correlations factors of the peak centre spectrum with the peak end spectrum were 0.9997 and 0.9985 for standard and sample tracks, respectively.

Conclusion

The identity of butterbur and coltsfoot teas and *S. vernalis herba* was confirmed by microscopic and macroscopic examination.

The use of HPTLC plates and the new mobile phase (chloroform: methanol: ammonia 25%: hexane - 82: 14: 2.6: 20 v/v) lead to good separation of senecionine from other components of the extracts.

Although the literature quotes as being present in all three species, senecionine was identified only in *S. vernalis*.

The new HPTLC method should be used further for the semi-quantitative assay of senecionine in *S. vernalis*.

References

1. Aniszewski T. Alkaloids, Secrets Of Life, Alkaloid chemistry, biological significance, applications and ecological role. Elsevier, Amsterdam, 2007, 167
2. Blazek Z., Kucera M., Suchar A. Atlas Drog, 1957, Vydavatel'stvo Slovenskej Akademie Vied, Bratislava, 109 - 110
3. Chizzola R., Ozelsberger B., Langer T. Variability in chemical constituents in *Petasites hybridus* from Austria. *Biochem.Syst. Ecol.*, 2000, 28, 421-432
4. Dinu M., Popescu M.L., Ancuceanu R., Hovaneț M.V., Ghițulescu G. Contribution to the pharmacognostical and phytobiological study on *Abutilon theophrasti* Medik. (*Malvaceae*), *Farmacia*, 2012, 60 (2), 184 – 193
5. Fliebig B.L., Grozdeva M., Hess S., Hüll M., Danesch U., Bodensieck A., Bauer R. *Petasites hybridus* extracts *in vitro* inhibit COX-2 and PG_{E2} release by direct interaction with the enzyme and by preventing p42/44 MAP kinase activation in rat primary microglial cells, *Planta Med.*, 2005, 71, 12 – 19
6. Gîrd C.E., Duțu L.E., Popescu M.L., Pavel M., Iordache A.T., Tudor I. Bazele teoretice și practice ale analizei farmacognostice, vol. I, Ed. Curtea Veche, București, 2008, 74 - 75
7. Gîrd C.E., Duțu L.E., Popescu M.L., Pavel M., Iordache A.T., Tudor I. Bazele teoretice și practice ale analizei farmacognostice, vol. II, Ed. Curtea Veche, București, 2009, 73 - 74
8. Mogoșanu G.D., Pinteș A., Bejenaru L.E., Bejenaru C., Rău G., Popescu H. HPLC analysis of carotenoids from *Senecio vernalis* and *S. jacobaea* (*Asteraceae*), *Farmacia*, 2009, 57(6), 780 – 786
9. Rizk A.M., Hammouda F.M., Roeder E., Wiedenfeld H., Ismail S. I., Hassan N.M., Hosseiny H.A. Occurrence of pyrrolizidine alkaloids in *Heliotropium bacciferum*. Constituents of plants growing in Qatar XIII. *Scientia Pharmaceutica*, 1988, 56, 105 - 110
10. Sell P., Murrell G. Flora of Great Britain and Ireland, Vol. 4 Campanulaceae – Asteraceae, Cambridge University Press, Cambridge, 2005, 502.
11. Tiță M.G., Lupuleasa D., Mogoșanu G.D. Histo-anatomical researches on the vegetative organs of *Eupatorium cannabinum* species. *Farmacia*, 2012, 60(5), 621- 633
12. Upton R., Gratt A., Jolliffe G., Langer R., Williamson E. American Herbal Pharmacopoeia: Botanical Pharmacognosy - Microscopic Characterization of Botanical Medicines, CRC Press, Boca Raton, 2011, 121, 662-665
13. *** CHMP Public statement on the use of herbal medicinal products containing toxic, unsaturated pyrrolizidine alkaloids. (EMA/HMPC/893108/2011), 2012
14. *** IPCS Environmental Health Criteria 80. Pyrrolizidine Alkaloids, Geneva 1988
15. *** PDR for Herbal Medicines, Third Edition, Thompson PDR, Montvale, 2004, 220 -221
16. *** Farmacopeea Română, ed. a X-a, Ed. Medicală, 1993, 872

Manuscript received: December 13th 2012