ORIGINAL ARTICLE

PHYTOCHEMICAL ANALYSIS OF *FUMARIA OFFICINALIS* L. (*FUMARIACEAE*)

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Abstract

The present study describes the investigation of active compounds from several samples of *Fumaria officinalis L.* (*Fumariaceae*). The identification of the isoquinoline alkaloids (allocryptopine, chelidonine, protopine, bicuculline, sanguinarine, cheleritrine, stylopine and hydrastine) was performed by comparison with reference standards using an HPLC-DAD method, and their quantification by LC-DAD and spectrophotometric methods. The presence of polyphenolic compounds was simultaneously assessed by HPLC. Protopine and sanguinarine were identified in all extracts. The major alkaloids were protopine and chelidonine (258.3 mg/100 g and respectively 94.13 mg/100 g). The spectrophotometric determinations of alkaloids showed minor differences between commercial samples and those harvested from spontaneous flora. The concentration of isoquinoline alkaloids expressed in chelidonine was between 0.69 and 0.76% in all samples. The pattern of phenol carboxylic acids showed the presence of cynarin, chlorogenic, isochlorogenic and ferulic acids. The flavonoids isovitexin, rutin, isoquercitrin and quercitrin were found in all assessed samples of *Fumaria officinalis* aerial parts.

Rezumat

În acest studiu se prezintă analiza compușilor activi din mai multe probe de *Fumaria officinalis L.* (*Fumariaceae*). Identificarea alcaloizilor izochinolinici (allocriptopina, chelidonina, protopina, bicuculina, sanguinarina, cheleritrina, stilopina, hidrastina) a fost realizată printr-o metodă HPLC-DAD, iar determinarea lor cantitativă prin LC-DAD și o metodă spectrofotometrică. Prezența compușilor polifenolici a fost evaluată printr-o metodă HPLC. În toate extractele au fost identificați alcaloizii izochinolinici protopina și sanguinarina. Alcaloizii majoritari au fost protopina și chelidonina (258,3 mg/100 g, respectiv 94,13 mg/100 g). Determinările spectrofotometrice ale alcaloizilor au arătat că între probele comerciale și cele recoltate din flora spontană există diferențe minore. Concentrația alcaloizilor exprimați în chelidonină a fost între 0,69 și 0,76% în toate probele analizate. Dintre acizii polifenolcarboxilici au fost identificați acidul clorogenic, cinarina, acidul izoclorogenic și acidul ferulic, iar dintre flavonoide izovitexina, rutozida, izocvercitrozida și cvercitrozida în toate cele trei probe analizate.

Keywords: *Fumaria officinalis*; isoquinoline alkaloids; HPLC-DAD; polyphenolic compounds

Introduction

The *Fumariaceae* family is very rich in isoquinoline alkaloids, especially of the aporphine, benzophenanthridine, protoberberine and protopine types. Nine *Fumaria* species are mentioned in ethnobotanical data from Romania [1]. The identification of these plants is frequently vague or imprecise due to their highly similar morphological characteristics; therefore the results of chemotaxonomic investigations could be valuable for the systematic evaluation of this genus [7, 9].

F. officinalis (fumitory) is an annual plant. The medicinal parts are represented by the dried aerial parts harvested during flowering. According to the European Pharmacopoeia 8th Ed. (Eur. Ph.), *Fumariae herba* should contain minimum 0.4% isoquinoline alkaloids expressed in protopine [2]. In the traditional medicine, the plant is used as diuretic, laxative, for the management of liver and skin disorders, for treating cystitis, rheumatism, arthritis. Previous phytochemical analysis of *F. officinalis* has shown the presence of isoquinoline alkaloids and polyphenols [6].

This paper describes the identification and quantification of isoquinoline alkaloids by HPLC-DAD and spectrophotometric methods, as well as the analysis of polyphenolic compounds in aerial parts of *F. officinalis* L. The methods are based on previous published method [8, 11], with some modifications. The aim of this work was to bring

new data on the chemical composition of several samples of *F. officinalis* aerial parts.

Materials and Methods

Plant material and preparation of extracts

The aerial parts of *F. officinalis* L. were collected in June 2010 from Luxembourg (sample 1), and commercially available samples of *Fumariae herba* were purchased in Liege (Belgium) from a pharmacy: sample 2 and 3. Sample 1 was authenticated by the co-author Professor Mircea Tamas. Voucher specimens are deposited in the Herbarium, Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy Cluj-Napoca, Romania.

The extract was obtained as previously described [3, 8]. For the quantitative determination of isoquinoline alkaloids by both HPLC and spectrophotometry, the method from *Fumariae herba* monograph was used [2].

General Apparatus and Chromatographic Conditions: an Agilent 1100 HPLC Series system was used (Agilent, Santa Clara, CA, USA) consisting of a degasser, a high pressure Quaternary pump, a Autosampler, a Thermostatic Compartment and a Diode Array Detector.

HPLC-DAD conditions for the analysis of alkaloids

The separation of alkaloids from *F. officinalis* was carried out using the conditions previously described [8].

For all compounds, the limit of quantification was 0.5 µg/mL, and the limit of detection was 0.1 µg/mL. Quantitative determinations were performed using an external standard method [4, 5, 8, 13]. The following standards were used for the isoquinoline alkaloids analysis: protopine, bicuculline, stylopine, chelidonine, allocryptopine, hydrastine, sanguinarine chloride hydrate, cheleritrine chloride.

HPLC-DAD conditions for the analysis of polyphenolic compounds

The separation of polyphenols was carried out using a Hypersil ODS C_{18} column (250 × 4.6 mm i.d., 5 µm particle). Solvents for the preparation of the mobile phases were: I - acetonitrile and II - 0.05% trifluoroacetic acid, pH 2.5. Mobile phases consisted of A 25% of I and 75% of II (v/v); and B 60% of I and 40% of II (v/v). The gradient elution was: 0-1 min 100% II, 1-2 min 97 % II, 3-55 min 60% II, 56 min 100% II. The UV detection was performed at 360 nm [10]. The chromatograms and UV spectra of each separated compound from the tested extracts were compared to those of standards, which allowed the positive identification of compounds, based on spectral match and retention times [10, 13].

Chlorogenic acid, isovitexin, isochlorogenic acid, cynarin, rutin, quercitrin, isoquercitrin, kaempferol, isovitexin, hyperoside, ferulic acid were used as standards for the polyphenolic compounds determination.

Spectrophotometric determinations: the absorbances of the solutions were determined at 570 nm, using a UV-VIS UVIKON 922 spectrophotometer and the results were expressed in chelidonine (g chelidonine / 100 g vegetal product) [2].

Results and Discussion

The identification of isoquinoline alkaloids

A high performance liquid chromatographic (HPLC) method has been developed for the identification of eight isoquinoline alkaloids from natural products. The simultaneous analysis of alkaloids was performed by a single column pass, and the alkaloids from *Fumaria* species extracts eluted in less than 30 min (Table I). We used several columns and gradient elution in order to obtain a better separation of the compounds.

Table I

Retention times (R_T) of isoquinoline alkaloids (min)

Peak no.	Isoquinoline alkaloid	$R_T \pm SD$ (min)
1.	Cheleritrine	18.00 ± 0.05
2.	Hydrastine	21.76 ± 0.03
3.	Bicuculline	22.40 ± 0.04
4.	Protopine	24.91 ± 0.14
5.	Chelidonine	26.19 ± 0.08
6.	Allocryptopine	27.16 ± 0.10
7.	Stylopine	27.31 ± 0.10
8.	Sanguinarine	29.21 ± 0.02

Note: SD, standard deviation.

The chemical composition of some *Fumaria* species has been insufficiently studied in the past. Protopine, allocryptopine and stylopine were previously identified by GC-MS and by HPLC in *F. officinalis* [11, 12]. The present phytochemical research adds new information on the alkaloid composition in several samples of *Fumariae herba*. The presence and amount of bicuculline, sanguinarine, chelidonine are reported.

The the quantitative determination of isoquinoline alkaloids by HPLC

For quantitative determination, extracts were prepared as described in *Fumariae herba* monograph [2]. This method allows a better extraction of isoquinoline alkaloids than the one used for qualitative analysis, some compounds being identified only in these extracts. The HPLC chromatogram of *F. officinalis* sample 3 extract is presented in Figure 1.

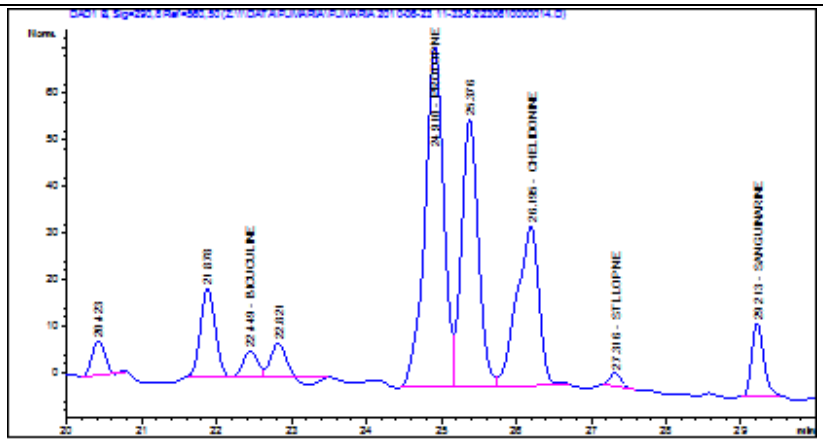

Figure 1.
HPLC chromatogram of alkaloids from *F. officinalis* aerial parts

The amount of individual alkaloids in *F. officinalis* extracts as determined by HPLC-DAD is reported in Table II.

Protopine was the major alkaloid identified in all extracts (123.38 - 258.3 mg/100 g). Although only two compounds were determined in sample 1 collected from spontaneous flora, the highest content in protopine was quantified in this one. Five and respectively six isoquinoline compounds were identified by HPLC in commercial samples 2 and 3. Considering previous work on fumitory, the analysed samples proved richer in these alkaloids [11, 12].

Table II
The isoquinoline alkaloids content of *F. officinalis* aerial parts

Alkaloid	Content (mg/100 g vegetal product)		
	Sample 1	Sample 2	Sample 3
Bicuculline	tr	8.31 ± 0.06	tr
Protopine	258.3 ± 1.98	123.38 ± 1.19	158.82 ± 1.57
Chelidonine	tr	71.88 ± 0.68	94.13 ± 0.89
Stylopine	tr	1.74 ± 0.02	4.12 ± 0.14
Sanguinarine	2.41 ± 0.01	1.41 ± 0.01	5.03 ± 0.04

Note: Values are the mean ± SD (n = 3); (tr) = traces

The pattern of isoquinoline alkaloids shows large differences between different *Fumaria* species previously analysed [8], so these compounds could be used as potential taxonomic markers in order to distinguish the plants. The major alkaloid in five *Fumaria species* was protopine, with the highest amount in *F. parviflora* (288.27 mg/100 g), followed by *F. officinalis* (258.3 mg/100 g) and *F. rostellata* (156.15 mg/100 g). Bicuculline and stylopine were found only in *F. officinalis, F. vaillantii* and *F. parviflora*. Chelidonine was determined only in *F. officinalis* and *F. vaillantii* extracts [8].

The quantitative determination of alkaloids by spectrophotometric method

In order to determine the concentration of total alkaloids in *F. officinalis*, extracts were prepared as described in *Chelidonii herba* monograph [2]. The amount of total alkaloids in *F. officinalis* extracts as determined by a spectrophotometric method is reported in Table III.

Table III
The total isoquinoline alkaloids content of *F. officinalis*

Sample	Concentration % (g chelidonine/100 g vegetal product)
1	0.76 ± 0.06
2	0.70 ± 0.09
3	0.69 ± 0.02

Note: Values are the mean ± SD (n = 3)

The results obtained using spectrophotometric determinations show small differences between the analysed samples, the richest being *Fumariae herba* harvested from spontaneous flora. Considering the recommendation of Eur. Ph. [2] about the quality of a natural product (minimum 0.4% isoquinoline alkaloids), all samples can be used in therapy as they meet the imposed quality standards.

The identification of polyphenolic compounds by HPLC

A high performance liquid chromatographic (HPLC) method has been developed for the determination of phenolic compounds (four phenolic acids, three

quercetin glycosides, and one aglycone) from natural products. The simultaneous analysis of different classes of polyphenols was performed by a single pass column. The polyphenols eluted in less than 45 min (Table IV). The HPLC Chromatogram of *F.officinalis* sample 2 is presented in Figure 2.

Chlorogenic, isochlorogenic and ferulic acids, cynarin, isovitexin, rutin, isoquercitrin and quercitrin were identified in ethanolic extracts of *F. officinalis* samples. Considering the antioxidant properties of *F. officinalis* [6], the determination of the compounds responsible of this effect could be valuable to improve the medicinal uses of *Fumaria* species.

Table IV

Retention times (R_T) of polyphenolic compounds (min)

Peak no.	Polyphenolic Compound	$R_T \pm SD$ (min)
1.	Chlorogenic acid	19.54 ± 0.04
2.	Cynarin	23.77 ± 0.05
3.	Isovitexin	27.21 ± 0.03
4.	Ferulic acid	27.49 ± 0.07
5.	Rutin	28.63 ± 0.09
6.	Isoquercitrin	29.63 ± 0.05
7.	Isochlorogenic acid	31.25 ± 0.08
8.	Quercitrin	32.13 ± 0.03

Note: SD, standard deviation.

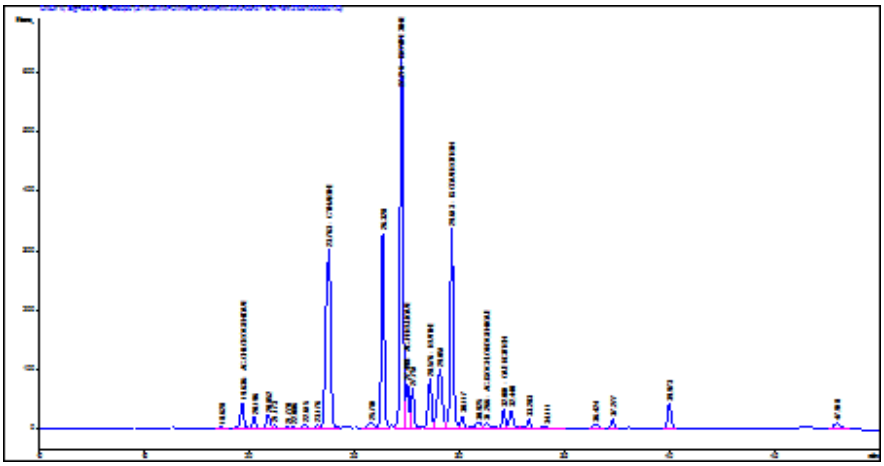

Figure 2.

HPLC chromatogram of polyphenols from *F. officinalis*

Conclusions

We analysed the isoquinoline alkaloids and polyphenolic compounds from *F. officinalis*, and we completed the literature data with new phyto-chemical information concerning the active substances from *Fumaria* species. The simultaneous determination of alkaloids was performed using a rapid, highly accurate and sensitive HPLC method assisted by UV detection. The study showed differences between commercial samples and those harvested from spontaneous flora.

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