PHYTOCHEMICAL ANALYSIS OF FUMARIA OFFICINALIS L. (FUMARIACEAE)

RAMONA PÂLTINEAN1, ANCA TOIU2*, JEAN NOËL WAUTERS3, MICHEL FRÉDÉRICH3, MONIQUÉ TITS3, LUC ANGENOT3, MIRCEA TĂMAS3, GIANINA CRÎŞAN1

1Department of Pharmaceutical Botany, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy, 12 Ion Creanga Street, 400010 Cluj-Napoca, Romania
2Department of Pharmacognosy, Faculty of Pharmacy, "Iuliu Hatieganu" University of Medicine and Pharmacy, 12 Ion Creanga Street, 400010 Cluj-Napoca, Romania
3Laboratory of Pharmacognosy, Center for Interdisciplinary Research on Medicines (CIRM), Faculty of Medicine, University of Liège, 1, Avenue de l’ Hôpital, B-4000- Liège, Belgium

*corresponding author: ancamaria_toiu@yahoo.com

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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, chelerythrine, 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, isouqueritin and querctiritin 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 (allocryptopina, chelidonina, protopina, bicuculina, sanguinarina, cheleritrina, stilopina, hidrstaina) a fost realizată printr-o metodă HPLC-DAD, iar determinarea lor cantitativă prin LC-DAD și o metodă spectrototometrică. Prezența compușilor polifenolici a fost evaluată printr-o metodă HPLC. În toate extractele au fost identificați alcaloizi izochinolinici protopina și sanguinarina. Alcaloizi majoritari au fost protopina și chelidonina (258,3 mg/100 g, respectiv 94,13 mg/100 g). Determinările spectrototometricice 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 chlorogenic, cinarin, acidul isochlorogenic și acidul ferelic, iar dintre flavonoide izovitexina, rutozidă, izouquerzitrozida și quercetirozida 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 quantification determination of isoquinoline alkaloids by both HPLC and spectrophotometry, the method from *Fumaria 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, an 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 C18 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 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.

**Table I**

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Isoquinoline alkaloid</th>
<th>( R_T \pm SD ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Cheleritrine</td>
<td>18.00 ± 0.05</td>
</tr>
<tr>
<td>2.</td>
<td>Hydastine</td>
<td>21.76 ± 0.03</td>
</tr>
<tr>
<td>3.</td>
<td>Bicuculline</td>
<td>22.40 ± 0.04</td>
</tr>
<tr>
<td>4.</td>
<td>Protopine</td>
<td>24.91 ± 0.14</td>
</tr>
<tr>
<td>5.</td>
<td>Chelidonine</td>
<td>26.19 ± 0.08</td>
</tr>
<tr>
<td>6.</td>
<td>Allocryptopine</td>
<td>27.16 ± 0.10</td>
</tr>
<tr>
<td>7.</td>
<td>Stylopine</td>
<td>27.31 ± 0.10</td>
</tr>
<tr>
<td>8.</td>
<td>Sanguinarine</td>
<td>29.21 ± 0.02</td>
</tr>
</tbody>
</table>

Note: SD, standard deviation.

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.
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>
<thead>
<tr>
<th>Alkaloid</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicuculline</td>
<td>tr</td>
<td>8.31 ± 0.06</td>
<td>tr</td>
</tr>
<tr>
<td>Protopine</td>
<td>258.3 ± 1.98</td>
<td>123.38 ± 1.19</td>
<td>158.82 ± 1.57</td>
</tr>
<tr>
<td>Chelidonine</td>
<td>tr</td>
<td>71.88 ± 0.68</td>
<td>94.13 ± 0.89</td>
</tr>
<tr>
<td>Stylopine</td>
<td>tr</td>
<td>1.74 ± 0.02</td>
<td>4.12 ± 0.14</td>
</tr>
<tr>
<td>Sanguinarine</td>
<td>2.41 ± 0.01</td>
<td>1.41 ± 0.01</td>
<td>5.03 ± 0.04</td>
</tr>
</tbody>
</table>

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* 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 *Chelidoniae herba* monograph [2]. The amount of total alkaloids in *F. officinalis* extracts as determined by a spectrophotometric method is reported in Table III.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration % (g chelidonine/100 g vegetal product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.70 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>0.69 ± 0.02</td>
</tr>
</tbody>
</table>

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.

![HPLC Chromatogram of *F. officinalis*](image)

**Table IV**

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Polyphenolic Compound</th>
<th>R&lt;sub&gt;T&lt;/sub&gt; ± SD (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chlorogenic acid</td>
<td>19.54 ± 0.04</td>
</tr>
<tr>
<td>2.</td>
<td>Cynarin</td>
<td>23.77 ± 0.05</td>
</tr>
<tr>
<td>3.</td>
<td>Isovitexin</td>
<td>27.21 ± 0.03</td>
</tr>
<tr>
<td>4.</td>
<td>Ferulic acid</td>
<td>27.49 ± 0.07</td>
</tr>
<tr>
<td>5.</td>
<td>Rutin</td>
<td>28.63 ± 0.09</td>
</tr>
<tr>
<td>6.</td>
<td>Isoquercitrin</td>
<td>29.63 ± 0.05</td>
</tr>
<tr>
<td>7.</td>
<td>Isochlorogenic acid</td>
<td>31.25 ± 0.08</td>
</tr>
<tr>
<td>8.</td>
<td>Quercitrin</td>
<td>32.13 ± 0.03</td>
</tr>
</tbody>
</table>

*Note:* SD, standard deviation.

**Conclusions**

We analysed the isoquinoline alkaloids and polyphenolic compounds from *F. officinalis*, and we completed the literature data with new phytochemical 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.

**Acknowledgements**

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**References**

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