ORIGINAL ARTICLE

HISTO-ANATOMICAL RESEARCHES ON THE VEGETATIVE ORGANS OF FIVE ROMANIAN *FUMARIA* SPECIES

RAMONA PĂLTINEAN, GIANINA CRIŞAN*, GEORGETA BALICA, IRINA IELCIU, CRISTINA ŞTEFĂNESCU, MIRCEA TĂMAŞ

Department of Pharmaceutical Botany, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

**corresponding author: gcrisan@umfcluj.ro*

Manuscript received: February 2014

Abstract

The present investigation has been carried out in order to define the histo-anatomical features of vegetative organs belonging to five *Fumaria* species and to provide a microscopical characterization of the powder obtained from the aerial dried parts of these species. The microscopic characterization of the powder histological elements was made by comparing them with data existing for the officinal species, *Fumaria officinalis* L. The following species were analysed: *Fumaria rostellata* Knaff., *Fumaria parviflora* Lam., *Fumaria vaillantii* Loisel., *Fumaria schleicheri* Soy.Will. and *Fumaria jankae* Hausskn.
The revealed differences regard mainly histo-anatomical structures of the stems. In this view, the aim of the present study was to identify the differences between the five *Fumaria* species and to determine if the number and positioning of the collateral vascular bundles in the angles of the pentagonal-shaped stem are different for each species. Data obtained in the current study can be useful to develop further scientific investigation, and represent a strong evidence to avoid possible confusions between the species of *Fumaria* genus, at the same time being a landmark, the first of this type in our country, for these five species.

Rezumat

Studiul de faţă îşi propune să evidenţieze caracterele histo-anatomice ale organelor vegetative aparţinând unor specii ale genului *Fumaria* şi să realizeze o caracterizare microscopică a elementelor histologice din pulberile obţinute prin măcinarea părţilor aeriene uscate ale acestora. Caracterizarea microscopică a pulberilor s-a realizat prin comparare cu datele existente pentru specia oficinală, *Fumaria officinalis* L. Speciile luate în studiu au fost: *Fumaria rostellata* Knaff., *Fumaria parviflora* Lam., *Fumaria vaillantii* Loisel., *Fumaria schleicheri* Soy.Will. şi *Fumaria jankae* Hausskn. Diferenţele identificate se referă în special la structurile anatomice ale tulpinii. În acest sens, scopul studiului de faţă a fost de a realiza o identificare corectă prin observarea diferenţelor dintre cele cinci specii ale genului *Fumaria* şi de a determina dacă numărul şi poziţia fascicolelor conducătoare colaterale în unghiurile tulpinilor pentamuchiate sunt diferite pentru cele cinci specii. Rezultatele obţinute în cadrul acestui studiu pot constitui bazele unor investigaţii detaliate ale acestor specii şi reprezintă un motiv în plus pentru evitarea eventualelor confuzii între speciile aparţinând genului *Fumaria*, constituind totodată un punct de reper, primul de acest gen realizat în ţara noastră, pentru aceste cinci specii.

Keywords: *Fumaria*, vegetative organs, cross sections, anatomical structure

Introduction

Fumaria genus, known as the "fumitory" or "earth smokes", comprises annual species, which usually grow like weeds in arable land in Western Europe [1, 19]. These taxa are also found in Southern and Eastern parts of Europe, but are more frequently met in sandy and rocky places, *Fumaria* species being herbaceous plants, with branched stems and leaves [5, 15, 16, 18]. The scientific literature describes a single species belonging to this genus, *Fumaria officinalis L.*, which has been used as folk remedy for hepatobiliary and gastrointestinal disorders, as a blood purifier and as an anti-allergic agent [6]. Today, there are phytochemical and pharmacological studies for most *Fumaria* taxa, but only the medicinal vegetal product, *Fumariae herba* (the aerial part of *Fumaria officinalis* L.), shows a monograph in the European Pharmacopoeia, 7th Edition [3]. The first brief description of the anatomical structure of the *Fumaria* genus, made by Metcalfe and Chalk in 1950, concluded that the anatomical features of the *Fumariaceae* species are similar to those of the herbaceous members of the *Papaveraceae* family [9]. Recently, in Argentina were studied the anatomical features for the leaves of two species, *Fumaria officinalis L.* and *Fumaria capreolata L.* [8]. At the same time, in India, several studies described morphological and anatomical characters for the species *Fumaria indica* Hausskn. [7] and *Fumaria vaillantii* Loisel. [14]. The increased risk of confusing the species belonging to *Fumaria* genus is due to similar

morphological characters [11]. In order to avoid possible confusions and to identify possible differences, the aim of the present study was to feature the histological elements of the powder obtained from the aerial parts of the plants and histo-anatomical researches on the vegetative organs of the species. Microscopic characterization for the powder obtained from *Fumaria rostellata* Knaff., *Fumaria parviflora* Lam., *Fumaria vaillantii* Loisel., *Fumaria schleicheri* Soy.Will. and *Fumaria jankae* Hausskn. was made by comparing microscopic analyses of the powder from these species with the officinal powder, *Fumariae herba*, according to the monograph in the European Pharmacopoeia, 7th Edition. We also made a histo-anatomical characterization for these species because no study was carried out in Romania before, on this subject. Regarding the species *Fumaria jankae* Hausskn., *Fumaria parviflora* Lam., *Fumaria rostellata* Knaff. and *Fumaria schleicheri* Soy.Will., no histo-anatomical data was found in the scientific literature. Flora Europaea only mentions the presence of *Fumaria jankae* Hausskn. (hybrid between *Fumaria schleicheri* X *Fumaria rostellata*) taxon in Romania, in Bihor county (Săcuieni), where it was harvested from. Histo-anatomical studies were mainly focused on stem characters, but we present also the root structure and leaf structure, only for one species due to the fact that no difference were identified for these organs in the studied species.

Materials and Methods

The plant material was collected from Western Transylvania (Romania), during the blooming period, in May-June 2009 and 2010 (Table I.).

Table I

Date and harvesting area for the examined taxa

No.	Date	Harvesting area County/Location	Taxa
1.	June-2009	Cluj / Nădăşel	*Fumaria schleicheri*
2.	June-2009	Bistriţa Năsăud / Bistriţa	*Fumaria vaillantii* Loisel.
3.	May-2010	Bihor / Săcuieni	*Fumaria parviflora* Lam.
4.	May-2010	Bihor / Săcuieni	*Fumaria jankae* Hausskn.
5.	May-2010	Sălaj / Vîrşolţ	*Fumaria rostellata* Knaff.

The species were identified by the coauthor, Professor Mircea Tămaş PhD. Voucher specimens were deposited in the Herbarium, at the Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Haţieganu" Cluj-Napoca, Romania.The samples for microscopic identification of powder were dried for 20 days in the dark, at room temperature. The herbaceous part of the five species was grinded to a diameter of 350 µm, in order to obtain fine powder and then subjected to microscopic examination using chloral hydrate solution [3].

The materials for histo-anatomical study were boiled in chloral hydrate 5%, for 10 minutes, then fixed in alcohol: acetic acid (3:1) mixture and preserved at 4ºC. After fixing, the fragments were washed, dehydrated and paraffin-embedded. The 12 µm sections, made using microtome Microtec 4050, were stained using Toluidine Blue 0.2% reagent and preserved in Canada balsam [17]. Histological and anatomical observations were performed with an Olympus CX31 binocular microscope, connected to a digital camera.

Results and Discussion

Identification of the powder material

The microscopic analyses of the powder (X200) showed the presence of upper (Figure 1A) and lower (Figure 1B) epidermis of leaf, fruit fragments (Figure 1C and 1D), fragments of leaf lamina (Figure 1F), the vessels of the stem (Figure 1G and 1H) and pollen grains (Figure 1E). The leaf lamina (Figure 1F) includes polygonal epidermal cells and it is characterized by an upper (Figure 1A) and a lower (Figure 1B) epidermis, which contain epidermal cells and anomocytic stomata [3, 4]. In figure 1D fragments of endocarp, represented by cells with thick and sinuous walls, were identified. Epicarp fragments (Figure 1C) show polygonal cells, with the wall protected by a cuticle [2-4, 9, 13]. Other identified components were pollen grains (Figure 1E), with a diameter between 30-40 µm, and 2 types of xylem vessels: with small (Figure 1G) and large (Figure 1H) diameter, having different types of lignin thickenings. By comparing these results with the ones existing for the vegetal medicinal product, *Fumariae herba,* and using data existing in the scientific literature, no differences were identified for the five species. Thus, microscopic analysis of the powder does not provide data regarding the presence of specific elements and the risk of confusions is elevated.

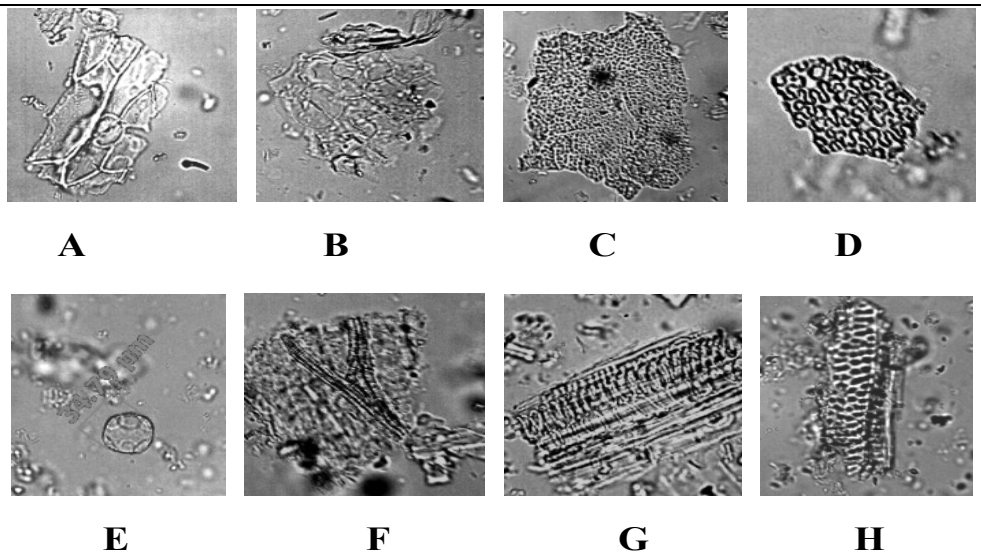

Figure 1.
Powder characteristics of *Fumaria* species (X200)

Histo-anatomical analysis

Cross section of the roots

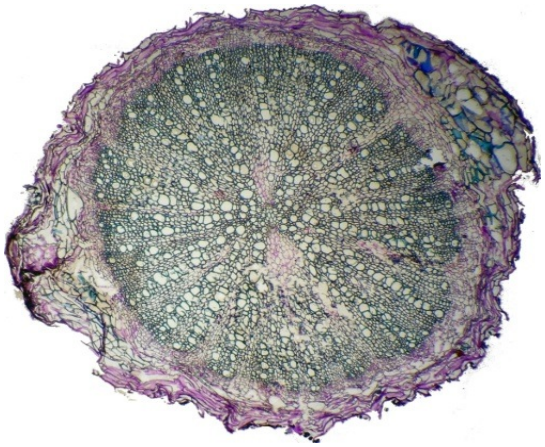

Figure 2.
Cross-section through the root of *Fumaria parviflora* Lam., coloured with Toluidine Blue (general view X400)

The cross-section of the roots (Figure 2) revealed a secondary anatomical structure similar for the five studied species. The microscopic study showed the presence of typical histological zones [10] such as: cork, phellogen, secondary cortex, secondary phloem, cambium, secondary xylem and primary xylem (from the outside to the inside). The cork is composed of tangentially suberified flattened cells that represent a protective barrier for the root. The cells of the phellogen are responsible for the secondary growth and they generate the cork on the exterior side and secondary cortex on the interior. Secondary cortex consists of several layers of cells with thin cellulose walls, coloured in violet with Toluidine Blue reagent. Secondary phloem is wide and circular. Cambial cells are arranged in series, flattened tangentially, generating secondary phloem on the exterior and secondary xylem on the interior. The secondary xylem occupies 1/3 of the cross-section and consists of leading woody vessels, xylem fibres and xylem parenchyma. Cell walls of the fibres and vessels are strongly thickened with lignin, coloured in green with Toluidine Blue. The secondary xylem is crossed regularly in radial direction by the secondary medullary rays cells. In the centre of the cross-section, vessels with smaller diameter, specific to primary xylem, were identified.

Cross section of the stems

The cross-sections of stems belonging to the five species are pentagonal-shaped, five prominences from which two pairs of opposite edges and can be differentiated by their prominence and length. The species *F. vaillantii* (Figure 3A) and *F. parviflora* (Figure 4D) have smoothed edges, even showing mono-symmetry. The species *F. jankae* (Figure 3C) has two prominent and three attenuated edges and the species *F. schleicheri* (Figure 3B) and *F. rostellata* (Figure 4E) have all prominent edges sharpened.

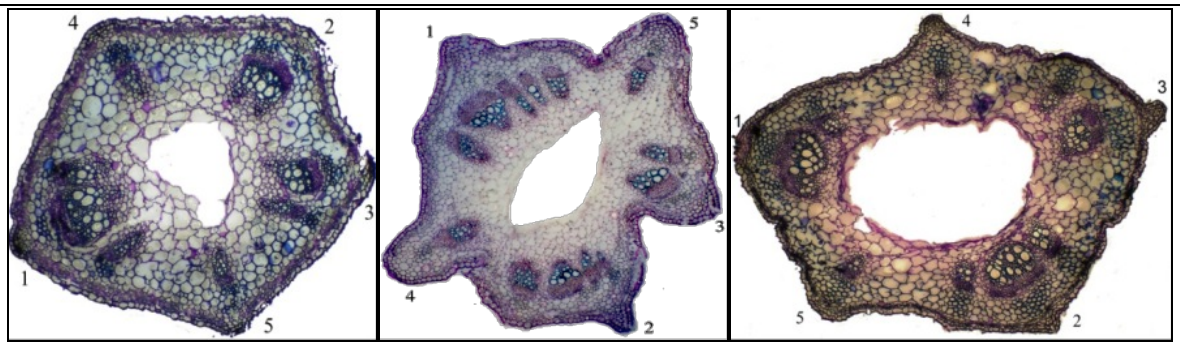

Figure 3.
Cross-section through the stems of *F. vaillantii* (A), *F. schleicheri* (B) and *F. jankae* (C) coloured with Toluidine Blue (general view X400)

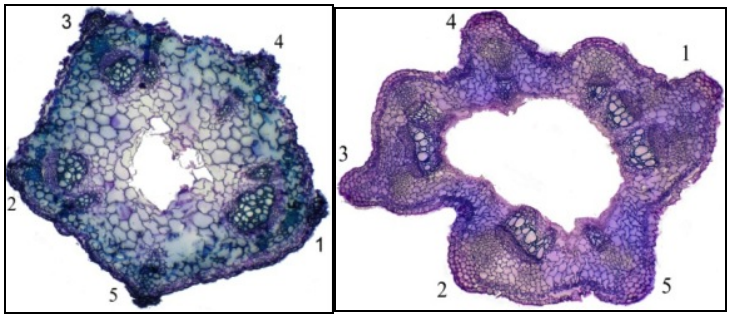

Figure 4.
Cross-section through the stems of *F. parviflora* (D) and *F. rostellata* (E), coloured with Toluidine Blue (general view X400)

The primary anatomical structure of the five species is characterized by the presence of three major areas: epidermis, bark and central cylinder. Under the polygonal cells that form the epidermis, in the 5 corners of the cross section we identified collenchymatic tissue, coloured in violet with Toluidine Blue. Also, in the bark we have identified the circular chlorenchyma, formed by 1-2 layers of cells, tightly joined together and rich in chloroplasts. In the central cylinder are found 5 groups of collateral vascular bundles, arranged orderly, single or double, in each edge. The number of these vascular bundles is different, depending on the species (Table II). Each vascular bundle is protected by a well-developed sclerenchyma arch, coloured in green with Toluidine Blue. The xylem conductive tissue (formed of well-developed vessels and xylem parenchyma tissue) is separated by the phloem conductive tissue (formed of sieved tubes and companion cells) by cambium, coloured in violet with Toluidine Blue. Pith cells are cylindrical, flattened tangentially and delineate inside a central lacuna.

Table II
Distribution of vascular bundles in cross section of the stems

Taxons	Vascular bundles distribution		
	Edge 1	**Edges 2 and 3**	**Edges 4 and 5**
F. vaillantii	3 vascular bundles (Figure 3A-1)	2 vascular bundles on each side (Figure 3A-2 and 3A-3)	1 vascular bundle on each side (Figure 3A-4 and 3A-5)
F. schleicheri	5 vascular bundles (Figure 3B-1)	4 vascular bundles on a side (Figure 3B-2) and 2 vascular bundles on the opposite side (Figure 3B-3)	1 vascular bundle on each side (Figure 3B-4 and 3B-5)
F. jankae	3 vascular bundles (Figure 3C-1)	3 vascular bundles on a side (Figure 3C-2) and 2 vascular bundles on the opposite side (Figure 3C-3)	1 vascular bundle on each side (Figure 3C-4 and 3C-5)
F. parviflora	2 vascular bundles on each side and cannot differentiate a leading edge (Figure 4D-1, 4D-2 and 4D-3)		1 vascular bundle on each side (Figure 4D-4 and 4D-5)
F. rostellata	3 vascular bundles (Figure 4E-1)	1 vascular bundle on a side (Figure 4E-2) and 2 vascular bundles on the opposite side (Figure 4E-3)	1 vascular bundle on each side (Figure 4E-4 and 4E-5)

Cross section of the leaf

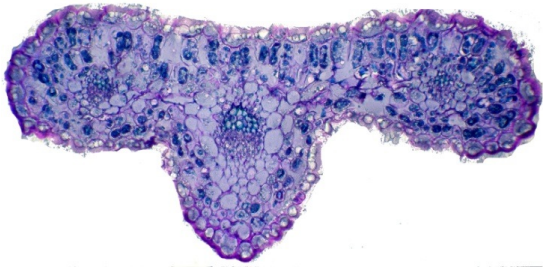

Figure 5.
Cross-section through the leaf of *F. rostellata* (general view X400)

Analysing cross-sections of the 5 taxons' leaves, we noticed that all the species present a lamina with a bifacial structure (Figure 5). Both epidermis are protected by a cuticle and only under the lower epidermis collenchymatic tissue, coloured in violet with Toluidine Blue, can be distinguished. The leaf mesophyll consists of 1 or 2 layers of palisade cells under the superior epidermis and 1 or 2 layers of spongy tissue under the upper epidermis, with large intercellular cavities. The vascular bundle is solitary and surrounded by parenchymatic cells, coloured in violet with Toluidine Blue.

Conclusions

Microscopic observations upon the powder of analyzed species, compared with *Fumariae herba* (from *Fumaria officinalis L.*) and with data from the monograph in the European Pharmacopoeia, 7th Edition, brought a benefit for the identification and authentication of *Fumaria* genus, but do not provide relevant data pointing towards a certain species.

From the histo-anatomical point of view, the differences for the observed species only appear in cross-section of the stem and can be differentiated using prominent edge length, number and disposition of vascular bundles in each edge, which provides specificity of each taxon.

The risk of confusing taxons is nevertheless present and only phytochemical screening can provide relevant data in order to identify each species [12].

Consequently, histo-anatomical and powder analysis of *Fumaria* species are the stages that follow morphological differentiation and represent the basis of the authentication of collective vegetal medicinal products.

References

1. Bruckner C., Clasification of the Carpel Number in *Papaverales*, *Capparales* and *Berberidaceae*. *The Botanical Review*, 2000; 66(2): 157-188.
2. Dinu M., Ancuceanu R., Rădulescu V., Ilieş D.C., Munteanu C., Aramă C., A comparative morphological and chemical study of the seeds from *Trigonella foenum graecum* (L.) and *T. Caerulea* (L.) Ser. Species. *Farmacia*, 2013; 61(6): 1069-1081.
3. EDQM European Pharmacopoeia 7th Edition Druckerei C.H. Beck Press, Germany 2010: 306-311.
4. Eschrich W., Pulver-Atlas der Drogen, Deutscher Apotheker Verlag, Stuttgart, 2004: 122-123.
5. Francis R., The Wild Flower Key, Penguin Books Ltd, Anglia, 2006: 116-119.
6. Goetz P., Ghedira K., Le Jeune R., *Fumaria officinalis* L. (*Fumariaceae*). *Phytotherapie*, 2009; 7: 221- 225.
7. Gupta P.C., Rao Ch V., Morpho-anatomical and physicochemical studies of *Fumaria indica* (Hausskn.) Pugsley. *Asian Pac J. Trop Biomed*, 2012; 2(10): 830-834.
8. Lujan M.C., Bonzani N., Filippa E., Barboza G.E., Las "fumarias" (*Fumaria* spp., *Fumariaceae*) de Uso Etnomédico en Argentina y sus Adulterantes. *Acta. Farm. Bonaerense*, 2004; 23(2): 154-164.
9. Metcalfe C.R., Chalk L., Anatomy of the dicotyledons, Vol I, Clarendon Press, Oxford 1957: 74-79.
10. Olaru O.T., Anghel A.I., Istudor V., Ancuceanu R.V., Dinu M., Contributions to the pharmacognostical and phytobiological study of *Fallopia aubertii* (L. Henry) Holub. (*Polygonaceae*). *Farmacia*, 2013; 61(5): 991-999.
11. Păltinean R., Wauters J.N., Tits M., Frédérich M., Angenot L., Tămaş M., Crişan G., Comparative morphological studies on some species of the genus *Fumaria*. *Farmacia*, 2013; 61(2): 371-377.
12. Păltinean R., Toiu A., Wauters J.N., Frédérich M., Tits M., Angenot L., Tămaş M., Crisan G., Identification and determination of alkaloids in *Fumaria* Species from Romania. *Dig. J. Nanomater Bios*, 2013; 8(2): 817-824.
13. Rahfeld B., Mikroskopischer Farbatlas pflanzlicher Drogen, Spektrum Akademicher Verlag, Heidelberg, 2009: 84-85.
14. Rajopadhye A., Upadhye A.S., Botanical and phytochemical standardization of *Fumaria vaillantii* Loisel. *Indian Journal of Natural Products and Resources*, 2011; 2(3): 369-374.
15. Săvulescu T., Flora Republicii Populare Române, Vol. III, Editura Academiei Republicii Populare Române, Bucureşti, 1955: 217-220.
16. Tămaş M., Botanică farmaceutică, Vol. III, Editura Medicală Univ. „Iuliu Haţieganu" Cluj - Napoca, 1999: 3-50.
17. Tămaş M., Lucrări practice de biologie celulară, Litografia I.M.F. Cluj-Napoca 1976.
18. Toma C., Rugina R., Anatomia plantelor medicinale, Editura Academiei Române, Bucureşti, 1998.
19. Tutin T.G., Burges N.A., Chater A.O., Edmonson J.R., Heywood V.H., Moore D.M., Valentine, D.H., Walters S.M., Webb D.A. (eds) Flora Europaea, Vol. 1 (Psilotaceae to Platanaceae). 2nd Edition, Cambridge University Press, Cambridge 2010: 306-311.