Greener Journal of Biological Sciences
Vol. 4 (4), pp. 103-110, May, 2014
ISSN: 2276-7762; ICV: 5.99

DOI Link: http://doi.org/10.15580/GJBS.2014.4.031014138
http://gjournals.org/GJBS

Comparative Anatomical Study on *Ferula* L. species in NE Iran

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ARTICLE INFO

Article No.: *031014138*
***Type*:** *Research*
DOI: *10.15580/GJBS.2014.4.031014138*

Submitted: *10/03/2014*
Published: *28/05/2014*

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Keywords:
Anatomy, mericarp, Iran, Ferula, sclerenchyma, collenchyma

ABSTRACT

In present research, comparative anatomical study was carried on eight *Ferula* species in NE Iran. The specimens were collected during March-June 2010- 2012. After identification, the cross sections from the base of stem, mature leaves, petioles and mericarps were prepared, and then stained by differential staining. Internal structure of stems, leaves and petioles varied among the species due to the shape and type of collenchymous tissue below the epidermis layer and sclerenchymous strands above the phloem, the shape of vascular bundle, the position and number of secretory ducts and the type of mesophyll. Also, in the mericarps, the number of dorsal rib, vallecular vittae, commissural vittae and wing width changed among the species.

INTRODUCTION

Ferula L. belongs to Apiaceae Lindl. family comprises about 208 species distributed in east of Medittranean region, Central Asia, Afghanistan, Pakistan, Iran, Turkey, China and Italy (Korovin, 1951; Pesmen, 1972; Chamberlain and Rechinger, 1987; Chen and Lio, 1989; Pimenov and Leonov, 2004; Menglan and Watson, 2005). This genus has 32 and 11 species in Iran and NE Iran (Khorasan Razavi Province) respectively (Chamberlain and Rechinger, 1987). The species of *Ferula* in this province belong to four subgenera: *Scorodosma* (Bunge.) Drude., *Merwia* (Fedtsch.) Korov., *Narthex* (Falconer.) Drude and *Peucedanoides* (Boiss.) Korov. "*Ferula* is traditionally classified in tribe Peucedaneae and subtribe Ferulinae (Drude, 1897–1898; Pimenov and Leonov, 1993). The most comprehensive infrageneric classification of *Ferula* is that proposed by Korovin (1939, 1940, 1947); subsequently, it was modified for the Flora of the U.S.S.R (Korovin, 1951). Korovin recognized six subgenera and eight sections, with some of these sections being divided into series or ''grex'' categories. In the revision of *Ferula* for the Kazakhstan flora, Safina and Pimenov (1984) rejected Korovin's subgenera and recognized 12 sections instead. Chamberlain and Rechinger (1987), in their revision of *Ferula* for Flora Iranica, retained only Korovin's subgenera, but refrained from referring four newly described species to any of them. Chamberlain and Rechinger (1987) identified most of the species from flowering material (Cited by: Kurzyan-Mlynik et al., 2008). The previous reports of anatomical studies are related to Anatomical study of the mericarp and root of *F. concaulca* Korov. (Moroz and Ladygina, 1974), Carpology of the species of type subgenus of the genus *Ferula* and some problems of their systematics (Safina and Pimenov, 1990), Mericarp anatomy study in a new species *Ferula hezarlalehzarica* Ajani (Ajani and Ajani, 2008) and Anatomical research of vegetative organs of the *Ferula iliensis,* Krasn & Korov (Akhmetova et al., 2013). Due to a few reports of internal structure of *Ferula* organs, comparative anatomical study was carried on to identify the variation of internal structure of stems, petioles, leaves and mericarps characters among the NE Iran *Ferula* species.

MATERIALS AND METHODS

Ferula species were collected from their localities in NE Iran during May- June 2010- 2012. The specimens were identified using Flora Iranica, Flora of U.S.S.R, Flora of Turkey, Flora of Pakistan, Flora of China (Korovin, 1951; Pesmen, 1972; Nasiri, 1972, Chamberlain and Rechinger, 1987; Menglan and Watson, 2005). For anatomical study, manual cross sections of the base of stem, petioles, mature leaves of *Ferula* were prepared and stained by Carmine and green methyl. Also, cross sections of mature and dried mericarps of above species except *F. flabelliloba* and *F . xylorhachis* were prepared and studied under LM Labomed model CMZ4. The species and their localities were presented in Table 1. The vouchers are deposited in the Islamic Azad University of Mashhad (IAUM) and Ferdowsi University of Mashhad (FUMH) herbaria.

Table 1: The localities of studied *Ferula* species

Species	Locality
F. foetida Regel. (subg. *Scorodosma*)	Bardaskan, Darvaneh village, Ashena & Sparham, 9045 (IAUM); south of Sabzevar, Joharchi &Zangouei, 16237, (FUMH).
F. latisecta Rech. f. & Aell. (subg. *Merwia*)	Kalat, Khour village, 1009 m, Ashena, 9036 (IAUM); west north of Mashhad, Poushan village, Ashena, 9037 (IAUM); Dargaz, Dolatshanlou village, 2300 m, Ashena, 9038, (IAUM); West south of Kalat Naderi Jarf mount. 2100 m, Joharchi and Zangouei, 2395 (FUMH); South of Dargaz, between Rishkhar and Darbandi, Kalate Gourni, Joharchi and Zangouei, 18660 (FUMH).
F. szowitziana DC.	Sarakhs, Bazangan lake, 916 m, Ashena, 9043 (IAUM); Taibad, between Polband and Sad villages, 900 m, Faghihnia and Zangouie, 21885 (FUMH); Torbat Jam, east of Salehabad, between Saghar Cheshme and Garmab Olia, 490 m, Joharchi and Zangouie, 34486 (FUMH); 5 km Bajestan to Gonabad, 1950 m, Faghihnia and Zangouei, 25113 (FUMH); West of Mashhad, Dehbar, 1700m, Joharchi and Mahvan (FUMH).
F. flabelliloba Rech. f. & Aell.	Mashhad, Zoshk village, 1275 m, Ashena, 9048 (IAUM); Chenaran, 3 Km Frizi to Kandelan, 1832 m, Memariani and Zangouie, 36072 (FUMH); Mashhad, Dehbar, 1783 m, Ashena, 9047 (IAUM); Mashhad, Pivejen, Binaloud mount. 2100 m, Faghihnia and Zangouei, 18966 (FUMH).
F. diversivittata Regel. & Schmalh.	West north of Mashhad, Dolatabad village, 1285 m, Ashena, 9033 (IAUM); West north of Torbat Heydarieh, Bezgh, 1500 m, Faghihnia and Zangouei, 28798

(subg. *Narthex*)	(FUMH); South of Dargaz, southern slopes Alahoakbar mount., Joharchi, 43221 (FUMH), Kalat, Khour village, 1009 m, Ashena, 9035 (IAUM); Chenaran, Akhlamad valley, 1550 m, Joharchi and Zangouei, 33536 (FUMH).
F. gummosa Boiss.	15 km Mashhad to Torbat Heydarieh, 1656 m, Ashena, 9031 (IAUM); Gonabad, 2600 m, Ashena, 9032 (IAUM); Quchan, east of Bajgiran, Aslemeh, 1600 m, Joharchi and Zangouei, 32795 (FUMH); Sarakhs, Bazangan lake, 916 m, Ashena, 9030 (IAUM); Torbat Jam, north of Salehabad, Zalou mount, 1300 m, Joharchi and Zangouei, 34577 (FUMH).
F. xylorhachis Rech. f. (subg. *Peucedanoides*)	North west of Mashhad, Dolatabad village, 1285 m, Ashena, 9044 (IAUM); west of Mashhad, Kalate zabetian, Rezaei and Mahvan, 13252 (FUMH).
F. ovina (Boiss.) Boiss.	Gonabad, 2600 m, Ashena, 9042 (IAUM); Fariman, Ghalandarabad, 1700 m, Faghihnia and Zangouei, 17454 (FUMH); north east of Chenaran, Boghmach mount, 1600 m, Hojjat and Zangouei, 32895 (FUMH); Gonabad, north west of Sanva, Nakhou valley, Faghihnia and Zangouei, 28617 (FUMH); Dargaz, between Shekarab and Tandoureh, 2000 m, Joharchi and Zangouei, 29110 (FUMH).

RESULTS AND DISCUSSION

Stem-The results of stem cross section showed, uni-bi seriate epidermis layer, collenchymous bundles, secretory ducts distributed in cortex and central vascular bundles, parenchymous layers, sclerenchymous tissue above the phloem and peripheral and central vascular bundles. Some differences were observed in the shape and type of collenchymous strands below the epidermis layer and sclerenchymous bundles above the phloem, the shape of peripheral and central vascular bundles and the position of secretory ducts (Table 2, Figures 1A- H) . *Leaves* - The cross section of mature leaves of studied species indicated isolateral mesophyll except *F. diversivittata* Rech. f. & Schmalh.*, F. foetida* Regel. and *F. latisecta* Rech. f. & Aell. which were dorsi-ventral (Table 2, Figures 2A- B). *Petiole*- The internal structure of petioles displayed uni- seriate epidermis layer, collenchymous strands, parenchymous tissue, vascular bundles including sclerid bundles above the phloem (Table 2, Figures 3A- H).

Table 2: Anatomical characters of stem, petiole and leaf of studied species of *Ferula* Species

Species	The shape of central vascular bundles	The shape of peripheral vascular bundles	The type of sclerenchymous tissue above the phloem in stem	The shape of petiole outline	The shape of vascular bundles in petiole
F. foetida	Small ovoid	Triangular- subulate	Fiber- sclerid	Tetrahedral	Obovate
F. latisecta	Obovate	Oblong triangular, alternately large and small	-	Triangular	Ovoid
F. szowitziana	Ovoid	Ovoid- triangular alternately large and small	Sclerid	Circular	Broad triangular
F. flabelliloba	Ovoid-circular	Oblong triangular, alternately large and small	Fiber	Triangular	Elliptical-circular
F. diversivittata	Ovoid-elliptical, 2 lobbed	triangular- subulate	-	Triangular	Obovate-circular
F. gummosa	Ovoid-triangular	Oblong triangular, alternately large and small	Fiber	Circular	Obovate-circular
F. xylocharis	Ovoid	Triangular- ovoid	Fiber	circular	Circular
F. ovina	Ovoid	Triangular with unequal size	Fiber	Triangular	Obovate-circular

Table 2 continued

Species	The type of sclerenchymous tissue above the phloem in petiole	The number of collenchymous layers below the epidermis layer	The number of sclerenchymous layers above the phloem in petiole	The type of collenchy-mous bundle	Arrangement of mesophyll
F. gummosa	Cap shaped or broad crescent	7- 8	4- 5	Rectangular	isolateral
F. foetida	Cap shaped or broad crescent	8- 9	3	Broad crescent shaped	Dorsi-ventral
F. szowitziana	Broad crescent shaped	11- 12	2	Circular-rectangular	isolateral
F. ovina	Thin crescent shaped	10- 12	10- 11	rectangular	isolateral
F. diversivittata	Thin crescent shaped	4- 5	14- 15	Broad crescent shaped	Dorsi-ventral
F. latisecta	2 lobbed crescent shaped	4- 5	20- 22	Broad rectangular	Dorsi-ventral
F. flabelliloba	2 lobbed thin cap shaped	5- 6	13- 14	Broad crescent shaped	Isolateral
F. xylocharis	A ring around the vascular bundle	5- 6	6- 7	Broad elliptical	Isolateral

Mericarp- In cross section of six studied species mericarps, the number of dorsal rib, vallecular and commissural vitae and wing width and orientation varied (Table 3, Figures 4A- F).

Table 3: The characters of mericarp anatomy of studied *Ferula* Species

Species	Dorsal rib	Vallecular vittae	Commissural vittae	Wing width
F. gummosa	3	8, oblong elliptical	8, eliptical	1 mm, 3mm, right wing horizontal, left one curved toward down
F. foetida	3	3- 4, oblong elliptical	19, elliptical	3mm, right wing horizontal, left one curved toward down
F. szowitziana	3	19, elliptical	13, oblong elliptical	2-4 mm, right wing horizontal, left one curved toward down
F. ovina	4	7, oblong elliptical	6, oblong elliptical	1 mm, right wing horizontal, left one curved toward down
F. diversivittata	3	5, oblong elliptical	6, oblong elliptical	2 mm, right wing curved toward up, left one curved to down
F. latisecta	4	4, triangular-elliptical	9, oblong elliptical	1.5 mm, horizontal wing

Based on the results of stem internal structure, *F. xylorhachis* Rech. f. differed from another species due to the shape of collenchymous bundles in cortex and sclerenchymous strands above the vascular bundles and the position of secretory ducts. Secretory ducts were observed in the cortex and phloem of the central vascular bundles in all studied species expect *F. szowitziana* DC and *F. xylorhachis*. Also, sclerenchymous tissue above the phloem varied sclerid, fiber and sclerid-fiber except *F. xylorhachis* just had fiber. The shape of sclerenchymous tissue above the phloem changed cap to crescent shaped. Moreover, the shape of vascular bundles varied obovate to triangular in the peripheral vascular bundles and circular to obovate in central vascular bundles. In Akhmetova's et al. report "mechanical tissue is very well developed directly below

the epidermis in the stem of *F. iliensis*. Collenchyma arranged separately portions and collateral vascular bundles including larger bundles alternate with small ones. Sclerenchymous tissue posed directly above the phloem as cap shaped bundles" (Akhmetova et al., 2013). In cross section of the petioles, the outlines of petioles were observed circular, triangular and tetrahedral. The shape of collenchymous tissue below epidermis layer varied rectangular, elliptical to crescent shaped. The shape of sclerenchymous bundles above the phloem were noticed cap- crescent shaped to a complete ring around the vascular bundle in *F. xylorhachis*. The vascular bundles shape ranged ovoid to circular. Furthermore, the mesophyll arrangement of all studied specimens were isolateral except in *F. foetida, F. diversittata*, and *F. latisecta* were dorsi-ventral. Already, Akhmetova et al. showed isolateral mesophyll in *F. ilinesis* (Akhmetova et al., 2013). Cross section of mericarps of studied specimens indicated 3- 4 dorsal ribs, 3-19 and 6- 19 vallecular and commissural vittae respectively. Also, wing width varied 1-4 mm and the tip of wing was horizontal or deviate toward up or down. Safina and Pimenov (1990) mentioned anatomical structure of the fruits of 16 species of Mediterranean *Ferula* species differ considerably in their carpological characters (the number of vascular bundles in the marginal ribs, the peculiarities of mesocarp sclerification, the organization of secretory system) from the middle-asiatic *Ferulas* (Safina and Pimenov 1990). Ajani and Ajani (2008) in comparison of fruit anatomy between two species of *Ferula*, reported dorsal ribs 3–4, elliptic; vallecular vittae (1–)2–3(–4), unequal, elliptic; commissural vittae 10–11, unequal, elliptic or circular; wing without appendix at base of commissural surface, lignified cells extend to end of wing with deviation towards commissural surface in *Ferula hezarlalehzarica* and dorsal ribs 3, valecular vittae 3, commissural vitae 6-8, wing with appendix at base, lignified cells extend to tip of wing with deviation towards dorsal surface in *F. kosopoljanskyi* Korov. Liu et al. (2006) believed "fruit features of taxonomic importance include developmental origin of the wings, carpel shape, presence of vittae, woodiness of the endocarp, position of crystals, and type of carpophores. Korovin (1947) argued that fruit anatomy was of little value for the classification of *Ferula*. So, he used mostly characters of leaves and flowers to delimit infrageneric taxa. In contrast, Safina and Pimenov (1983, 1984, 1990) stressed the importance of fruit characters for circumscribing sections and inferring phylogenetic relationships. In their revision of *Ferula* for Kazakhstan, Safina and Pimenov (1984) described nine basic fruit types, with most sections they recognized being monomorphic with respect to fruit anatomy. In studies of Korovin's subgenera Peucedanoides (Safina and Pimenov, 1983) and *Ferula* (Safina and Pimenov, 1990), they provided evidence suggesting that these subgenera are highly heterogeneous with respect to fruit anatomy, with some of their respective members more similar to those of other subgenera".

"Classification systems of *Ferula* and the taxonomic value of fruit characters Korovin (1947) based his taxonomic treatment of *Ferula* mostly on morphological characters. While acknowledging Koso-Poljansky's and Calestani's contributions of fruit anatomy to umbellifer taxonomy, he regarded these characters as useless at the infrageneric level. Consequently, his descriptions of subgenera focused primarily on characters of habit, inflorescence, leaves, and flowers, and less so on basic fruit features, such as the direction of compression and the number of vallecular vittae" (Cited by: Kurzyan-Mlynik et al., 2008). Kurzyan-Mlynik et al. (2008) believe a general similarity of fruit anatomy is a better predictor of species affinity than any single fruit character as supported by the analysis of character evolution. All fruit characters analyzed are highly homoplastic. Their analysis of the evolution of phenotypic characters in *Ferula* fails to indicate any clear pattern of morphological variation that would be of taxonomic use. In conclusion, anatomical results of vegetative organs and mericarps showed differences between studied species but those could not exactly help to identify species because of characters are overlapping.

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Cite this Article: Ashena F(Aida), Jafari A, Shahrokhabady KN (2014). Comparative Anatomical Study on *Feriula* L. species in NE Iran**.** Greener Journal of Biological Sciences, 4(4): 103-110, http://doi.org/10.15580/GJBS.2014.4.031014138**.**

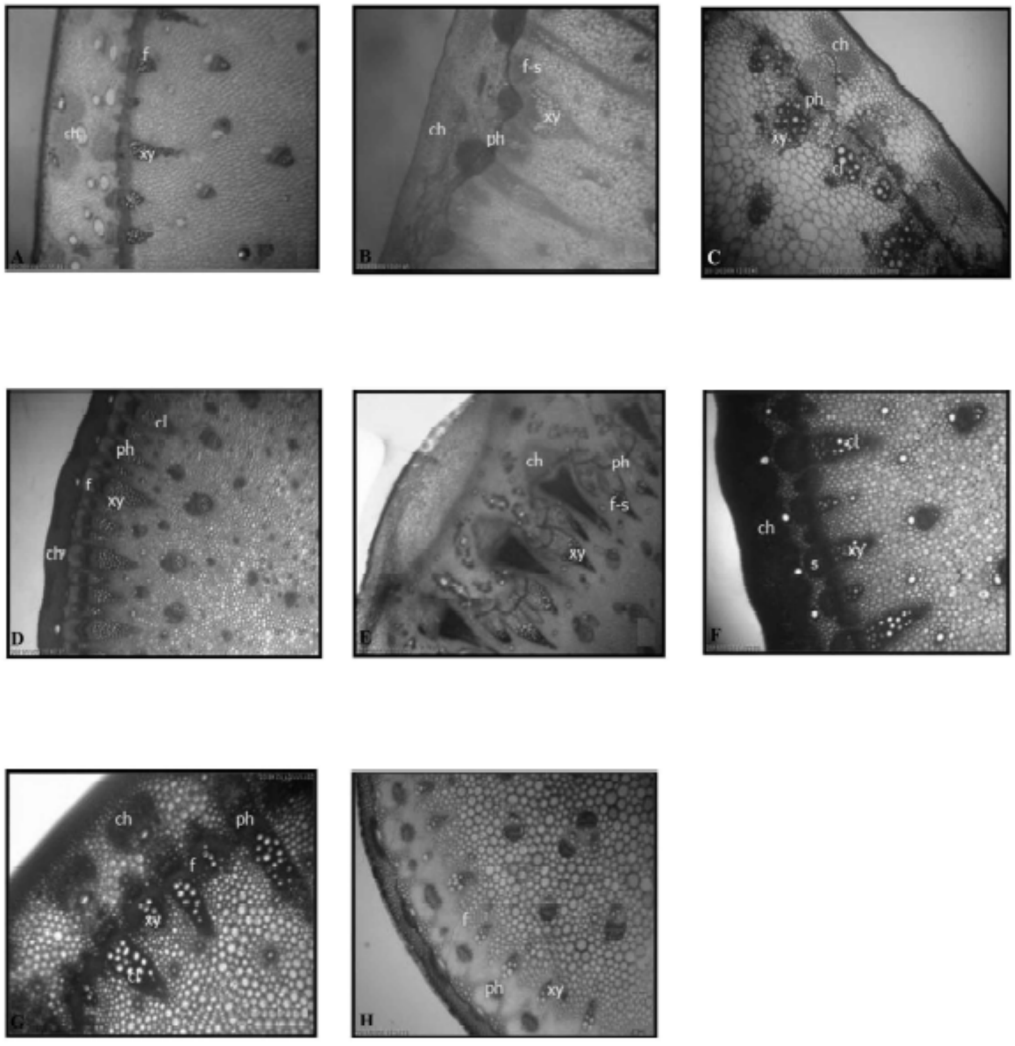
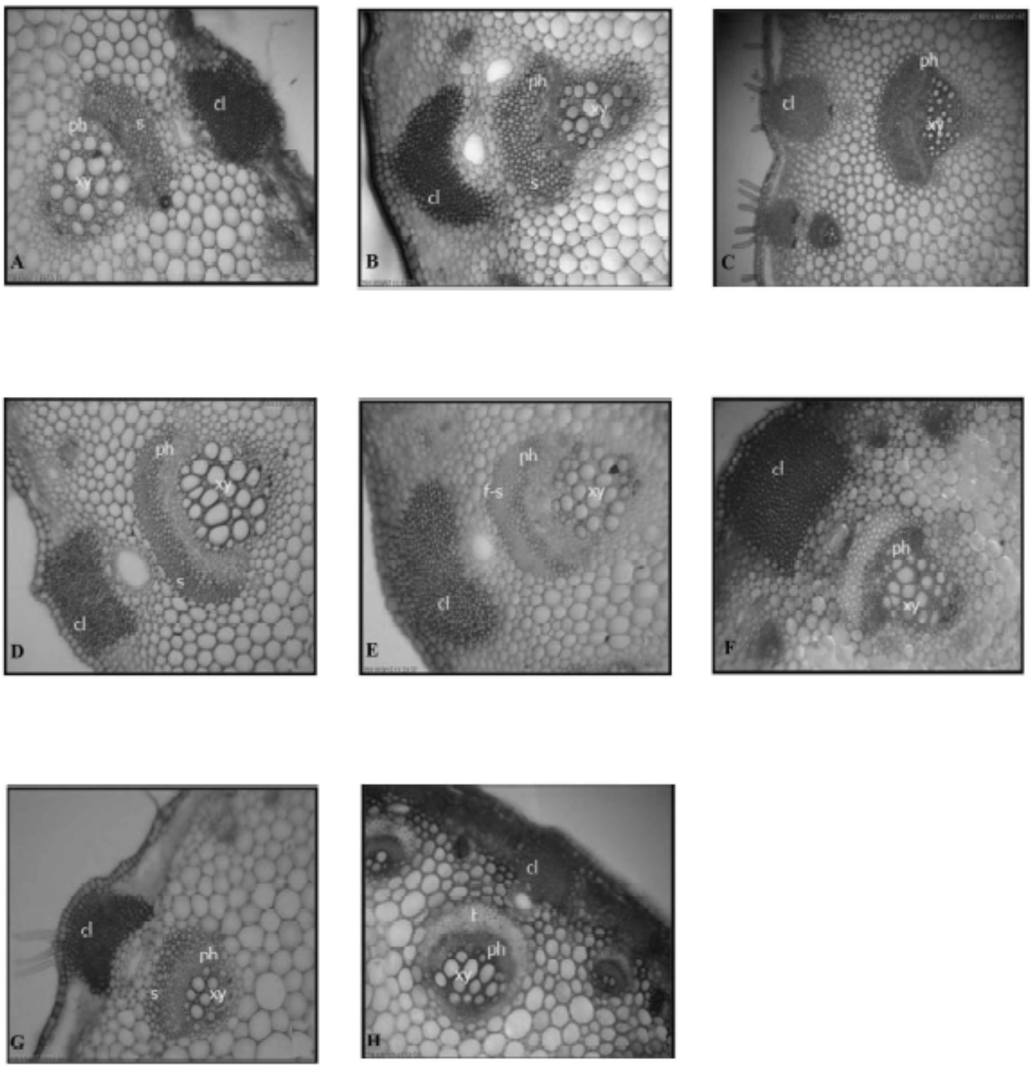


Figure 1



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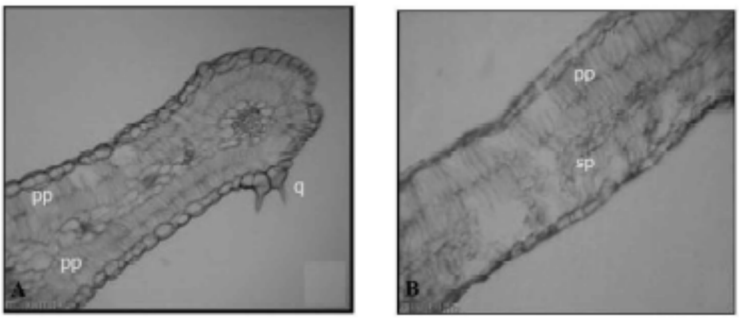

Figure 3

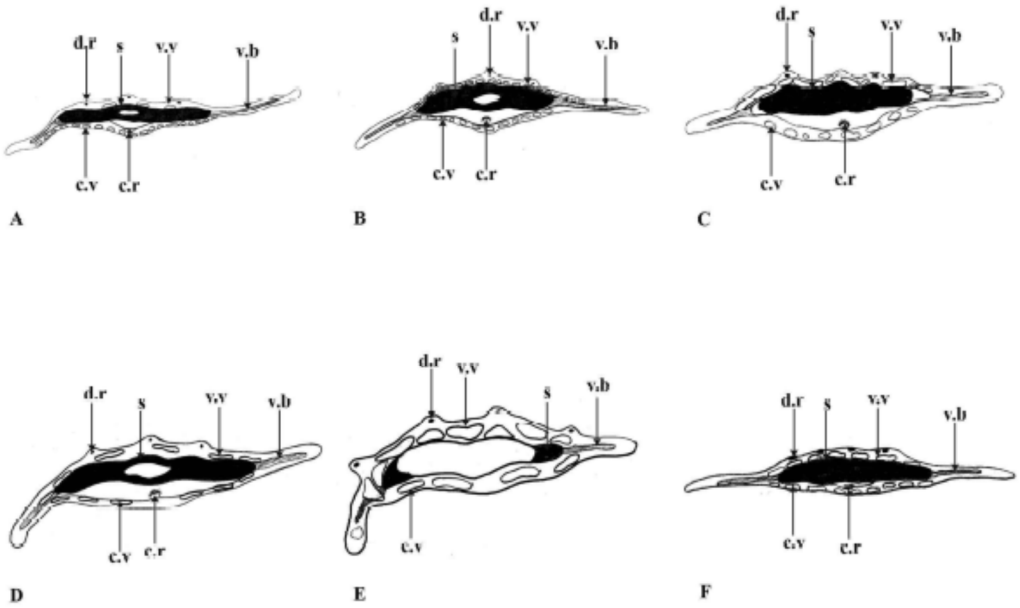

Figure 4

Figure 1: Cross section of stem: A) *Ferula gummosa*. B) *F. foetida*. C) *F. szowitziana*. D) *F. ovina.* E) *F. diversivittata*. F) *F. latisecta*. G) *F. flabelliloba*. H) *F. xylorhachis*.
f) fiber. S) sclerid. Xy) xylem. Ph) phloem. Ch) collenchyma. Cl) cluster vessels.

Figure 2: Cross section of petiole: A) *Ferula gummosa*. B) *F. foetida*. C) *F. szowitziana*. D) *F. ovina.* E) *F. diversivittata*. F) *F. latisecta*. G) *F. flabelliloba*. H) *F. xylorhachis*.
f) fiber. S) sclerid. Xy) xylem. Ph) phloem. Ch) collenchyma. Cl) cluster vessels.

Figure 3: Cross section of leaf. A) isolateral mesophyll in *F. ovina*. B) dorsi- ventral mesophyll in *F. diversivittata*
Pp) palisadic parenchyma. Sp) spongy parenchyma.

Figure 4: Cross section of mericarp. A) *F. foetida*. B) *F. szowitziana*. C) *F. gummosa*. D) *F. diversivittata.* E) *F. ovina*. F). *F. latisecta*.
s) endosperm. d.r) dorsal rib. c.r) commissural rib. c.v) commissural vitae. v.v) vallecular vitae. v.b) vascular bundle