Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2017, 9(1):104-109. DOI: 10.15835/nsb919950
Notulae Scientia Biologicae
Original Article

Anatomy and Micromorphology of *Inula helenium* subsp. *orgyalis* and *I. ensifolia* (Asteraceae) from Turkey

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Abstract

Inula helenium L. subsp. *orgyalis* (Boiss.) Grierson and *Inula ensifolia* L. were investigated anatomically and micromorphologically. The secretory cavities in the leaves and stem of both investigated taxa were located in the neighbourhood of the vascular bundles and in the rhizomes in the secondary cortex. The leaf mesophylls of investigated Inula taxa were homogeneous. Stomata were anomocytic in two species. The distribution and density of the eglandular and glandular trichomes provide information of taxonomical significance. Moreover, the cypselas of *I. helenium* L. subsp. *orgyalis* were homomorphic, whereas in *I. ensifolia* cypselas were heteromorphic. Additionally, the number of ribs, the shape of carpopodium and stylopodium were diagnostic taxonomic characters between the two taxa.

Keywords: anatomy, Asteraceae, *Inula,* micromorphology, SEM

Introduction

The genus *Inula* L. belongs to the family Asteraceae and comprises about 120 species, distributed mainly in Europe, Africa and Asia (Lack, 2007). In Turkey, there are about 28 species, 33 subspecies and varieties respectively of *Inula* and 8 of them are endemic. The rate of endemism of *Inula* species is 28.5% in Turkey (Güner *et al.,* 2012). *I. helenium* subsp. *orgyalis* is an endemic taxon to Turkey (Ekim *et al*., 2000).

Many of *Inula* species are antibacterial, antiseptic, antiinflammatory, antiulcerogenic, antipyretic, diuretic, antidiabetic, antirheumatic, antispasmodic, antihaemorrhoidal or anthelmintic (Nickavar and Mojab, 2003). Elecampane (*Inula helenium* L.) is considered a medicinal herb (Barnes *et al*., 2007). It is related to the presence of biologically active compounds, such as eudesmanolides, germacronolides, triterpenes, sterols and inulin, in different parts of plants (Sulborska, 2007). Perrot and Paris (1971) reported that *I. helenium* contains helenin in root. For these reasons, the species is of major interest for many pharmaceutical industries (Nikolakaki and Christodoulakis, 2004).

The use of anatomical and micromorphological characters has also played an important role in the systematic of this genus (Abid and Qaiser, 2004). Deryng (1961) was reported that secretory cavities were found in rays, xylem and phloem of the underground organs of this genus (Bukowiecki and Furmanowa, 1972). However, the information about anatomy of vegetative organs of *Inula* species is very scarce.

The micromorphological characters such as orientation, shape, size, colour of cypselas and nature of pappus bristles are very important in the seperation of *Inula* taxa (Jana and Mukherjee, 2012; Jana *et al*., 2013). Abid and Qaiser (2002) investigated cypsela morphology of *Dittrichia, Duhaldea, Inula, Iphiona* and *Pentanema* species from Pakistan and Kashmir and concluded that the morphology and anatomy of cypsela can be useful in identification of distinct taxa.

According to the available literature data, there are no detailed reports on the anatomy and micromorphological characters of Turkish *Inula* taxa. In this work, it was investigated the anatomy of the rhizome, stem and leaves and micromorphology and distribution of the trichomes on the stem, leaf and cypsela of *I. helenium* subsp. *orgyalis* which is an endemic taxon to Turkey and *I. ensifolia*, by using a light microscope (LM) and scanning electron microscope (SEM).

Materials and Methods

The plant specimens were collected at the flowering stage from the natural populations of North Anatolia, Turkey. Voucher specimens were deposited in the Herbarium of the Department of Botany, University of Ondokuz Mayıs, Faculty

Received: 21 Oct 2016. Received in revised form: 01 Mar 2017. Accepted: 24 Mar 2017. Published online: 30 Mar 2017.

of Art and Sciences, Samsun, Turkey (OMUB). Detailed localities of the collected specimens are given in Table 1.

Samples for anatomical studies were fixed in 70% alcohol. Cross-sections of rhizome, stem and leaves and surface sections of leaves were made by hand. For each structure, at least 30 preparations were observed and their photographs were taken with an Nikon-Coolpix P5100 digital camera. For scanning electron microscopic observations, dried stem, leaves and cypsela samples were mounted on stubs using double-sided adhesive tape and coated with 12.5-15.0 nm of gold. Coated samples were examined and photographed with JEOL-Neoscope JCM-5000 Scanning Electron Microscope (SEM).

The following formula was used to calculate the stomatal index (Meidner and Mansfield, 1968):

SI=S/E+S

where SI: Stomatal index; S: guard cells; E: epidermal cells.

The trichome terminology follows Metcalfe and Chalk (1950), Abid and Qaiser (2004) and Jana and Mukherjee (2013).

All anatomical measurements were made using a Image J program on the figures. A total of 19 characters were measured. These characters are given in Table 2. A student's t-test was used to analyse anatomical data and a p-value less than 0.05 was considered as statistically significant.

Results and Discussion

Anatomical characters

Comparative anatomical characters of rhizome, stem and leaf of *Inula* species are presented in Table 2.

Rhizome anatomy

In transverse section, the outer surface of the rhizome was covered by 4-6 layered periderm of which cells were dark coloured and squashed. In the external layer of cortex, secretory canals were present (Fig. 1). However, the secretory cavities were seen deeper in the cortex of *Inula ensifolia L.* (Fig. 1). Their shapes were close to rounded or oval. Outside of the phloem ring were located many sclerenchymatic elements. Sclerenchymatic cells were located on the cortical parenchyma. In *I. helenium* subsp. *orgyalis*, the pith was occupied with completely metaxylem elements, whereas in *I. ensifolia* it was parenchymatic (Fig. 1).

Stem anatomy

Epidermis was consisted of oval or rectangular cells with thick cuticle. The surface was covered with long one or multicellular protective hairs and glandular hairs. Several layers of cortex parenchyma and collenchyma were located beneath the epidermis (Fig. 2). The sclerenchyma fibres covered collateral bundles. The secretory cavities were located in the region of the phloem and xylem bundles (Fig. 2). The pith was formed by the parenchymatic cells that were thin-walled (Fig. 2).

Leaf anatomy

There was a thin cuticle on the upper and lower epidermis (Fig. 3). Surface of both epidermis was covered with long, multicellular eglandular and numerous glandular hairs. These trichomes were especially located at the lower epidermis. The

Table 1. Detailed localities of the collected *Inula* L. specimens

Taxa	Locality
Inula helenium L. subsp. *orgyalis* (Boiss.) Grierson	A5 Amasya, Güllüce village, 1,260 m, 28.07.2010, A. Akcin, OMUB 1552.
Inula ensifolia L.	A5 Amasya: Ovabaşı village, 1,100 m, 25.07.2010, A. Akcin, OMUB 1558.

Table 2. Anatomical measurements of examined *Inula* L. taxa. (data are given as mean ± sd, P < 0.05 denote a statistically difference between two species)

	Width (µm)			Length (µm)		
Anatomical characters	*I. helenium* subsp. *orgyalis*	*I. ensifolia*	p-value	*I. helenium* subsp. *orgyalis*	*I. ensifolia*	p-value
Root						
Thickness of periderm	66.63±18.667	111.65±35.466	0.000	-	-	-
Secretory canals in cortex	135.40±48.985	24.53±12.331	0.001	181.53±55.721	137.13±59.316	0.000
Diameter of trachea	38.68±7.952	21.03±6.073	0.000	-	-	-
Stem						
Epidermis cells	22.08±4.317	15.16±1.808	0.000	22.21±4.517	14.63±3.257	0.000
Collenchyma cells	65.82±19.031	82.04±6.831	0.001	-	-	-
Parenchyma cells	311.41±57.394	34.40±12.971	0.000	-	-	-
Sclerenchyma cells	197.82±55.414	63.55±17.309	0.000	-	-	-
Secretory canals	46.09±13.505	32.56±11.628	0.000	105.43± 21.723	64.94±10.724	0.000
Thickness of xylem	180.14±41.436	218.85±37.557	0.000	-	-	-
Thickness of phloem	97.90±16.107	60.92±11.295	0.000	-	-	-
Diameter of trachea	21.79±4.786	30.17±11.254	0.000	-	-	-
Diameter of pith cells	56.36±15.904	136.51±54.555	0.000	-	-	-
Leaf						
Epidermis cells	25.51±4.780	11.65±1.478	0.000	31.55±6.391	15.84±2.761	0.000
Thickness of mesophyll	74.14±19.141	203.14±18.147	0.000	-	-	-
Diameter of trachea	33.16±11.253	7.99±1.855	0.000	-	-	-
Thickness of sclerenchymatous sheath	121.34±32.350	59.38±16.349	0.000	-	-	-
Thickness of xylem	212.77±30.418	26.55±4.663	0.000	-	-	-
Thickness of phloem	80.18±8.726	26.55±4.663	0.000	-	-	-
Stomata	22.20±2.410	20.82±2.281	0.067	32.23±2.720	31.61±4.099	0.492

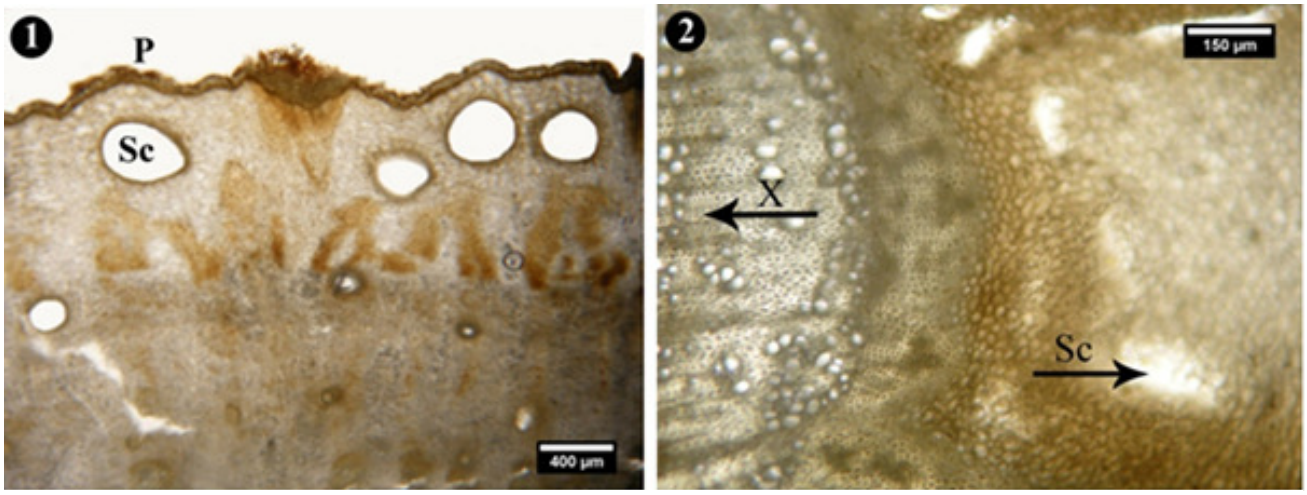

Fig.1.Cross-section of rhizome of *Inula* species. 1. *I. helenium* subsp. *orgyalis*, 2. *I. ensifolia*. P: periderm, Sc: secretory canals, X: xylem

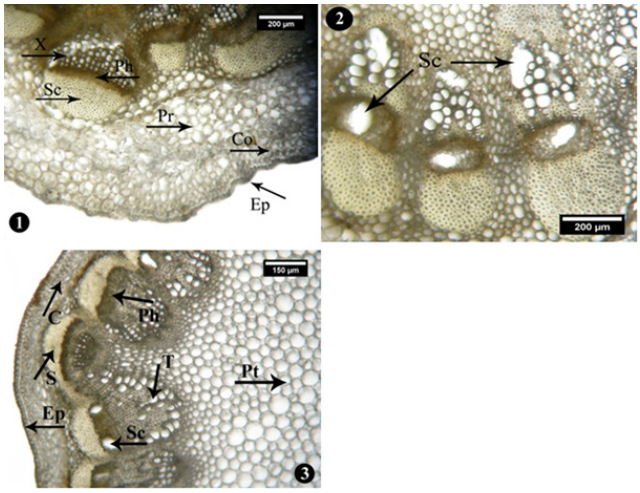

Fig. 2. Cross-section of stem of *Inula* species. 1-2. *I. helenium* subsp. *orgyalis*, 3. *I. ensifolia*. C: cortex, Ep: epidermis, Ph: phloem, Pt: pith, S: sclerenchyma, Sc: secretory canals, T:trachea, X: xylem

mesophyll was homogeneous. The anomocytic stomata were located at both epidermis (Fig. 3). However, stomata were especially abundant in the lower epidermis in both taxa. Stomatal index for abaxial epidermis is 20.46 ± 4.369 in *I. helenium* subsp. *orgyalis* and 19.91 ± 3.212 in *I. ensifolia*. Vascular bundles were surrounded by a sheath of sclerenchyma cells (Fig. 3). The secretory cavities were located by the side of the vascular bundles (Fig. 3).

Micromorphological characters

Trichome micromorphology

Two different trichome types on the stem and leaves of the investigated taxa were observed: glandular and nonglandular trichomes. Nonglandular trichomes were acicular or curved and made up from one or more cells (Fig. 4). Glandular trichomes were capitate or peltate with a very short stalk (Fig. 4). Subsessile or sessile glandular trichomes were mostly observed in *I. ensifolia*. They were common on the abaxial than the adaxial surface of leaves (Fig. 4).

Cypsela micromorphology

Cypselas were homomorphic in *I. helenium* subsp. *orgyalis*, whereas cypselas were heteromorphic in *I. ensifolia*. In *I. ensifolia*, disc cypsela was 8.03 ± 0.14 x 2.0 ± 0.21 mm including pappus, obovate in shape and black. Ray cypsela was 7.04 ± 0.12 x 1.0 ± 0.18 mm including pappus and black (Table 3). The surface of both cypsela was glabrous (Fig. 5). The number of ribs on cypsela varied: 14 in *I. helenium* subsp. *orgyalis*, 11 in *I. ensifolia* (Table 3). These ribs were present alternating with furrow in both taxa (Fig. 5). The carpopodium was irregular ring like in *I. helenium* subsp. *orgyalis*, whereas it was a complete ring-like in *I. ensifolia*. The stylopodium was inconspicous in *I. helenium* subsp. *orgyalis*, but the other taxa was enlarged. In the both cypsela, pappus

Table 3. Cypsela characters of investigated *Inula* L. taxa

Characters	*I. helenium* subsp. *orgyalis*	*I. ensifolia*
Type	Homomorphic	Heteromorphic
Shape	Oblong-oblongoid	Obovate in disc cypsela Oblanceolate in ray cypsela
Size (mm)	7.56±0.11x1.50±0.18	8.03±0.14x2.0±0.21 in disc cypsela 7.04±0.12x1.0±0.18 in ray cypsela
Colour	Yellow-brown	Black
Surface	Rough and glabrous	Glabrous
No of ribs	14	11
Carpopodium	Irregular ring like	Complete ring like
Stylopodium	Inconspicuous	Enlarged
Colour of pappus	White-yellow	White in disc cypsela Yellowish in ray cypsela

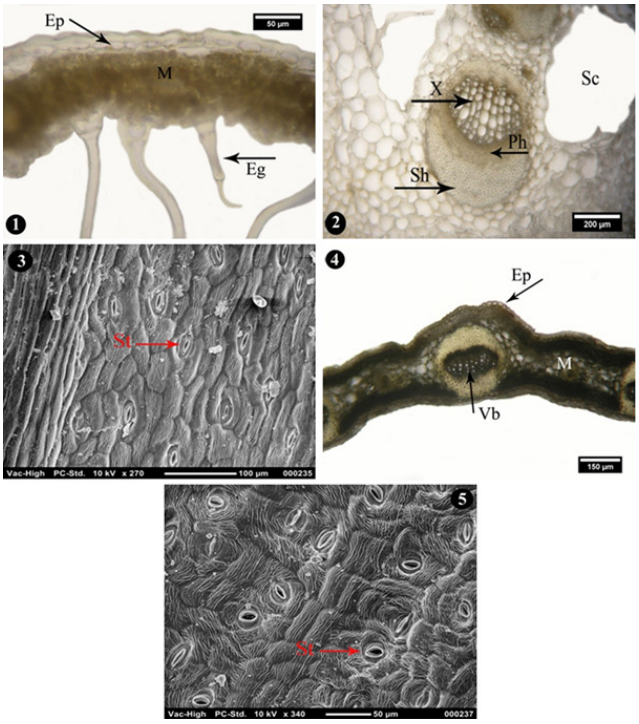

Fig. 3. Leaf anatomical structures of *Inula* species. 1-2. Cross-section of leaf of *I. helenium* subsp. *orgyalis*, 3. The lower surface of leaf of *I. helenium* subsp. *orgyalis* in SEM, 4. Cross-section of leaf of *I. ensifolia*, 5. The lower surface of leaf of *I. ensifolia* in SEM. Ep: epidermis, Eg: eglandular trichome, M: mesophyll, Ph: phloem, Sc: secretory canals, Sh: sclerenchymatous sheath, St: stomata, Vb: vascular bundle

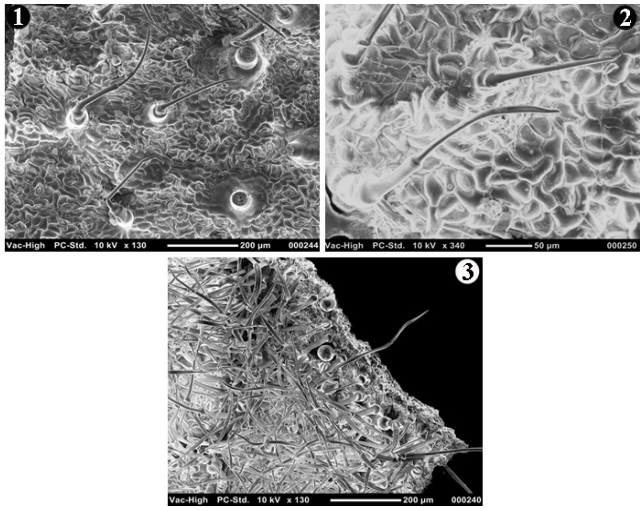

Fig. 4. Scanning electron micrographs (SEM) of trichomes of *Inula* species. 1. Non-glandular trichomes on stem of *I. helenium* subsp. *orgyalis*, 2. Eglandular trichomes on stem of *I. ensifolia*, 3. Glandular and eglandular trichomes on leaf of *I. ensifolia*

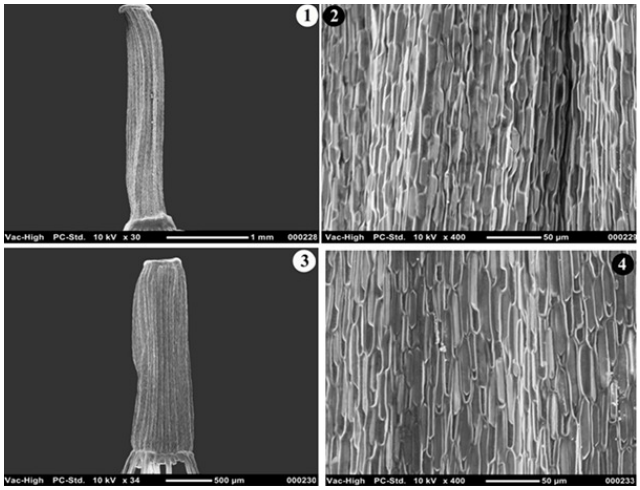

Fig. 5. Scanning electron micrographs (SEM) of cypsela of *Inula* species.1. General view of cypsela of *I. helenium* subsp. *orgyalis*, 2. Surface of cypsela of *I. helenium* subsp. *orgyalis*, 3. General view of cypsela of *I. ensifolia*, 4. Surface of cypsela of *I. ensifolia*

were consisted of serrulate-setose type of pappus bristles. The colour of pappus varied from yellowish to white (Table 3).

In the current study, the anatomical and micromorphological characters of *I. helenium* subsp. *orgyalis* and *I. ensifolia* were examined. It has reported that secretory structures and cavities are spread out in the Asteraceae family (Metcalfe and Chalk, 1950; Lersten and Curtis, 1988). As a result of the hereby study, one can conclude that the secretory cavities in the stems and leaves of both taxa were located in the neighbourhood of the vascular bundles and in the rhizomes in the secondary cortex. It was reported that stem and leaf secretory cavities were often located in the vicinity of phloem. This location may be used as an effective protection against phloem-feeding herbivores (Sulborska, 2007). Furthermore, it was shown that the secretory cavities may also contain substances toxic to some herbivores and insects (Maksymowych and Ledbetter, 1987).

On both the analysed species, the rhizome, stem and leaf anatomical structures were similar. However, statistically significant differences were found in mean of the measured all anatomical characters except for stomata length and width (Table 2, $P < 0.05$). In *I. ensifolia,* the secretory cavities in the rhizome were smaller and located deeper in the cortex compared to the other species (Table 2). A remarkable difference between taxa in rhizome anatomical structure was seen in pith region. In *I. helenium* subsp. *orgyalis*, the pith was occupied with completely metaxylem elements, whereas in *I. ensifolia* it was parenchymatic (Fig. 1).

The stem cortexes were more or less collenchymatized in the both investigated taxa. Some studies on *Inula* species such as *I. britannica* and *I. germanica* reported the existence of an collenchymatic layer in the stem (Toma *et al*., 2010; Afemei *et al*., 2011).

The present data are consistent with the results of Toma *et al*. (2010) and Afemei *et al.* (2011). There were significant differences in mean thickness of collenchyma, parenchyma and sclerenchyma layer, as well as in the lenght and width of secretory cavities in stem (Table 2, $P < 0.05$). The dimensions of secretory canals in *I. helenium* subsp. *orgyalis* were larger than the other *Inula* taxa (Table 2).

Metcalfe and Chalk (1950) reported that there were generally bifasial mesophylls in the family Asteraceae. In this study, it was seen that mesophyll was homogeneous in

investigated both *Inula* taxa (Fig. 3). Toma *et al*. (2010) observed that some *Inula* species such as *I. oculus-christi*, had homogeneous mesophyll, whereas *I. germanica* had dorsiventral mesophyll. The current results basically agree with the findings of Metcalfe and Chalk (1950). However, from Table 2, it is evident that the mesophyll in the leaf was thicker in *I. ensifolia*.

Furthermore, in *I. ensifolia* the sclerenchymatous sheat around the phloem was significantly wider (thicker) compared to that in midribs of *I. helenium* subsp. *orgyalis* leaves (Table 2). Diameter of vascular bundles in *I. helenium* subsp. *orgyalis* was much wider than that of those found in *I. ensifolia* (Table 2, $p<0.05$). This difference in thickness of sclerenchymatous sheath may be probably correlated with protection of the vascular bundles.

According to the results from the present study, the investigated two taxa have anomocytic stomata on both leaf surface (Fig. 3). It was reported that there were both anomocytic and anisocytic stomata in the family Asteraceae (Metcalfe and Chalke, 1950). Toma *et al.* (2010) determined that some Romanian *Inula* species have anomocytic stomata. The present findings are consistent with the results of Toma *et al*. (2010).

Studies of trichomes in Asteraceae have been found to be diagnostic by many researchers (Adedeji, 2004; Adedeji *et al*., 2007). In the present study, it was found that stem and leaf epidermis of both taxa have eglandular and glandular hairs (Fig. 4). Similar types of trichomes were observed in most of *Inula* species (Adedeji, 2004; Adedeji *et al*., 2007). However, distribution and density of the eglandular and glandular trichomes of *I. helenium* subsp. *orgyalis* were more abundant than *I. ensifolia*. Similarly, eglandular trichomes were shorter in *I. ensifolia* than the other investigated taxa. Also, glandular trichomes in *I. ensifolia* were sessile or subsessile, while in *I. helenium* subsp. *orgyalis* were mostly capitate or peltate (Fig. 4). The distribution of these trichomes may provide additional evidence to delimination of *Inula* species.

Investigated *Inula* species were distinguishable on the basis of cypsela micromorphology. Cypselas were homomorphic in *I. helenium* subsp. *orgyalis*, whereas in *I. ensifolia*, cypselas were heteromorphic (Fig. 5). In addition, the number of ribs, the shape of carpopodium and stylopodium were diagnostic taxonomic characters between studied *Inula* taxa. In *I. ensifolia*, stylpodium was enlarged, while in other studied species it was inconspicuous. In the present study, it was determined that carpopodium is an important taxonomical character. In *I. helenium* subsp. *orgyalis*, carpopodium was irregular ring-like. However, symmetric carpopodium observed in *I. ensifolia*. Jana *et al.* (2013) and Shekhar *et al.* (2011) reported that the characters of carpopodium and stylopodium of cypselas of the tribe Inuleae have taxonomic significance. The current results confirmed the existent data from the literature.

Finally, based on the results of the present study, it may be concluded that some of the micromorphological and anatomical characters are useful to distinguish within the species of the genus *Inula.* Further researchs on anatomical and micromorphological structure of the other *Inula* species could also provide insight into classification of *Inula* taxa.

Conclusions

The present paper demonstrated significant anatomical and micromorphological differences among two *Inula* L. taxa from Turkey. In *I. ensifolia*, the secretory cavities in the cortex were smaller and deeper in the rhizome. In addition, in *I. helenium* subsp. *orgyalis*, the pith was occuped with metaxylem elements, whereas in *I. ensifolia* it was parenchymatic. There were significant diffrences in mean thickness of collenchyma, parenchyma, sclerenchyma layer, the length and width of secretory cavities in stem. The leaf mesophyll of *I. ensifolia* was thicker than the other species. Furthermore, in *I. ensifolia* the sclerenchymatous sheath was significantly thicker compared to *I. helenium* subsp. *orgyalis* leaves. From all the studied micromorphological characters, the distribution and density of the eglandular and glandular trichomes was found to be most useful to deliminate the species.

References

Abid RD, Qaiser M (2002). Cypsela morphology of *Inula* L. (s.str.) and its allied genera (Inuleae-Compositae) from Pakistan and Kashmir. Pakistan Journal of Botany 34(3):207-223.

Abid RD, Qaiser M (2004). A micromorphological study for the generic delimitation of *Inula* L. (s. str) and its allied genera (Inuleae-Compositae) from Pakistan and Kashmir. Pakistan Journal of Botany 36(4):719-724.

Adedeji O (2004). Leaf epidermal studies of the species of *Emilia* Cass. (Senecioneae, Asteraceae) in Nigeria. Botanica Lithuanica 10(2):121-133.

Adedeji O, Ajuwon OY, Babawale OO (2007). Foliar epidermal studies, organographic distribution and taxonomic importance of trichomes in the family Solanaceae. International Journal of Botany 3(3):276-282.

Afemei M, Boz I, Toma C (2011). Histo-anatomical aspects refering to the vegetative organs of two species of *Inula* L. from Romanian Flora. Ştiintele Naturii 27(1):23-28.

Barnes J, Anderson LA, Phillipson JD (2007). Herbal medicines (3rd ed), Pharmaceutical Press, London pp 85-101.

Bhar I, Mukherjee SK (2004). Macromorphological and micromorphological study of cypselas in seven species of the tribe Anthemideae (Asteraceae). Journal of Economic and Taxonomic Botany 28(3):788-794.

Bukowiecki H, Furmanowa M (1972). Botanika Farmaceutyczna. PZWL, Warszawa pp 443-450.

Deryng J (1961). Atlas sproszkowanych roślinnych surowców leczniczych. PZWL, Warszawa.

Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N (2000). Türkiye Bitkileri Kırmızı Kitap [Red Data Book of Turkish Plants], Ankara: Turkish Association for the Conservation of Nature, No 18 pp 245.

Güner A, Aslan S, Ekim T, Vural M, Babaç MT (2012). Türkiye Bitkileri Listesi (Damarlı Bitkiler), [A Checklist of the Flora of Turkey, Vascular Plants]. İstanbul: Flora Araştırmaları Derneği ve Nezahat Gökyiğit Botanik Bahçesi Yayını.

Jana BK, Mukherjee SK (2012). Cypselar morphology of some species of the family Compositae and their taxonomic significance. International Journal of Pharma Sciences and Research 1(5):463-484.

Jana KR, Bar R, Mukherjee SK (2013). Cypselar morphology and anatomy of five species of the tribe Inulea-Asteraceae. International Journal of Pharma Sciences and Research 4(1):911-919.

Lack HW (2007). Tribe Cichorieae Lam. & DC. In: Kubitzki K (Ed). The families and genera of vascular plants, Vol 8, Flowering plants: Eudicots, Asterales. Berlin, Springer pp 180-189.

Lersten NR, Curtis JD (1988). Secretory reservoirs (ducts) of two kinds in giant ragweed (*Ambrosia trifida*; Asteraceae). American Journal of Botany 75 (9):1313-1323.

Maksymowych R, Ledbetter MC (1987). Fine structure of epithelial canal cells in petioles of *Xanthium pensylvanicum*. American Journal of Botany 74 (1):65-73.

Metcalfe CR, Chalk L (1950). Compositae. In: Anatomy of the dicotyledons. Leaves, stem and wood in relation to taxonomy with notes on economic uses. Clarendon Press, Oxford pp 782-804.

Nickavar B, Mojab F (2003). Antibacterial activity of *Pulicaria dysenterica* extracts. Fitoterapia 74:390-393.

Nikolakaki A, Christodoulakis S (2004). Leaf structure and cytochemical investigation of secretory tissues in *Inula viscosa*. Botanical Journal of Linnean Society 144:437-448.

Perrot E, Paris R (1971). Les plantes médicinales (I). Presses Universitaires de France, Paris.

Shekhar S, Pandey AK, Anderberg AA (2011). Cypsela morphology and anatomy in some genera formerly placed in *Inula* (Asteraceae: Inuleae-Inulinae). Rheeda 21(1):13-22.

Sulborska A (2007). Distribution and structure of internal secretory reservoirs on the vegetative organs of *Inula helenium* L. (Asteraceae). Acta Agrobotany 60(1):1-12.

Toma C, Grigore MN, Afemei M, Stanescu IE (2010). Histo-anatomical considerations on some romanian *Inula* L. species, with pharmacological action. Analele Ştiinţifice ale Universităţii Al I Cuza, Iaşi, Secţiunea a IIa. Biologie pp 5-13.