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COMPARATIVE ANATOMICAL STUDY OF THREE *CENTAUREA* SPECIES (ASTERACEAE) FROM IRAQ

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Abstract

In this study, anatomical features of three *Centaurea* species *viz. C. behen , C. cardunculus* and *C. rigida* (Asteraceae) were investigated for the first time from Iraq. Characteristics of epidermal strips and cross-sections of stem and leaf of the studied taxa were evaluated. Stem differences arise mainly in the development and arrangement of the collenchyma tissue and in the number and arrangement of the vascular bundles. The leaf exhibits more differences than similarities especially in structure of the midrib and the type of stomata and trichomes. Due to the absence of Kranz anatomy, all studied plant species are considered to be C3 plants. Anatomical data reported here represent a significant tools in separating between species studied.

Key words: Centaurea, Asteraceae, kranz anatomy, Iraq.

Introduction

Asteraceae (or Compositae) is the largest family of flowering plants with 20,000-35,000 species and 1500-1,911 genera occurred on all continents and in all environments except antarctica (Stevens, 2001; Funk *et al.,* 2005; Jeffrey, 2007; Rahman *et al.,* 2008; Bagal and Deokule, 2015; Katinas *et a.,* 2016; Royal Botanical Gardens Kew, 2016; Mandel *et al.,* 2019) and also one of the largest family in several countries like Turkey (Uysal *et al.,* 2010), India (Bagal and Deokule, 2015), Peru (Dillon and Alva, 2002), Chile (Moreira-Munoz and Munoz-Schick, 2007) as well as in Iraq (represented by nearly 400 species/according (Alkatib, 2000; Al-Rawi, 2014). *Centaurea* is one of the largest genera in Asteraceae with 250-700 species of herbaceous annual, biennial and perennial plants that distributed all around the world, particularly in America, Mediterranean region, and west Asia (Susanna and Garciai-Jacas, 2007; Shabestari *et al.,* 2013; Bancheva *et al.,* 2014; Tasar *et al.,* 2018).

In Iraq, the genus *Centaurea* contains more than 30 species occurred in various parts of the country (Al-Rawi, 2014). Systematics of this genus is problematic and its taxonomy mainly relies on the morphological characters (Garcia-Jacas *et al.,* 2001, Tasar *et al.*, 2018). Recently, the complexity of *Centaurea* has stirred much interest (Duran and Duman, 2002, Yuzbasioglu *et al.,* 2015) and the status of its taxa has been revised by several authors (Rahiminejad *et al.,* 2010, Ranjbar and Negaresh, 2013, Negaresh and Rahimibejad, 2014). Anatomical characters are an important tools in plant systematics (Lu *et al.,* 2008) and have been widely used in taxonomic treatments and systematic studies (Noman *et al.,* 2014). However, anatomical data on Asteraceae taxa, particularly on *Centaurea* species from Iraq are still very limited. Therefore, the present study was conducted to present detailed anatomical characteristics of three *Centaurea* species (*C. behen*, *C. cardunculus* and *C. rigida*) occurring in Iraq.

Materials and Methods

Plant samples were collected from Suliamaniya province (northern Iraq) during April-June 2018. Plant samples were identified by Iraqi National Herbarium and the identified samples were kept in the Department of Biology, College of Science, Tikrit University, Iraq. Stripped off epidermis and handmade cross sections of fresh and FAA (formaldehyde-acetic acid–alcohol) fixed

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stem and leaf of three *Centaurea* species (*C. behen, C. cardunculus* and *C. rigida*) were prepared (Al-Khesraji and Aziz, 1990). Iodine in potassium iodide (IKI) and safranin were mainly used to stain primary and secondary walls respectively. For each plant species studied, several measurements from three individual plants were used to check each anatomical character examined. Prepared slides were examined under Olympus light microscope and photographed with Sony digital camera.

Results and Discussion

Anatomical features of the studied species given below are based on both epidermal strips and cross sections of the stem and leaf of the three *Centaurea* species studied.

The stem (Tables 1, 2 and Fig. 1, 2)

Epidermal tissue system (ETS) : The epidermis of all studied species is a uniseriate complex tissue covered by cuticle and composed of polygonal ordinary cells , multicellular eglandular hairs (rarely mixed with multicellular glandular hairs, particularly in *C. cardunculus*) and tetracytic stomata type (rarely mixed with anisocytic type, particularly in *C. rigida*). The investigated taxa are nearly similar in dimensions of the ordinary cells and stomata. The highest stomatal density (number of stomata per unit area) was observed in *C. cardunculus* (58 per mm^2) followed by *C. behen* (32 per mm^2) and *C. rigida* (16 per mm^2). In all studied taxa, the stomata are at the same level of the epidermis. Hair type, particularly glandular hairs and the type and density of stomata are good characters in separating between species studied.

Ground tissue system (GTS): This system in the studied species consists of narrow cortex and wide pith. Cortex includes lamellar collenchyma mixed with angular one in the ridges and chlorenchyma and ordinary parenchyma in furrow regions (*i.e.* collenchyma in one side and chlorenchyma and ordinary parenchyma in other side occurred in alternating manner below epidermis). Thickness of collenchyma tissue and the number of its locations varied between species studied, few and narrow collenchyma locations in *C.behen* are a good character distinguishing this species from the other species studied. The parenchymatous pith in all examined species occupies

Table 1: Characteristics of the stem epidermis of *Centaurea* species (micrometer).

Type	Stomata		Ordinary cells		Species
	Density	L × W	Shape	L × W	
Tetracytic	32	30-35×20-25	polygonal	65-210 × 20-25*	*C. behen*
Tetracytic	58	20-30× 15-20	polygonal	75-125 × 15-20	*C. cardunculus*
Tetracytic& anisocytic	16	20-25×25-30	polygonal	60-180 × 15-20	*C. rigida*

L=length , W=width , (*) numbers in the table based on 10 replicates.

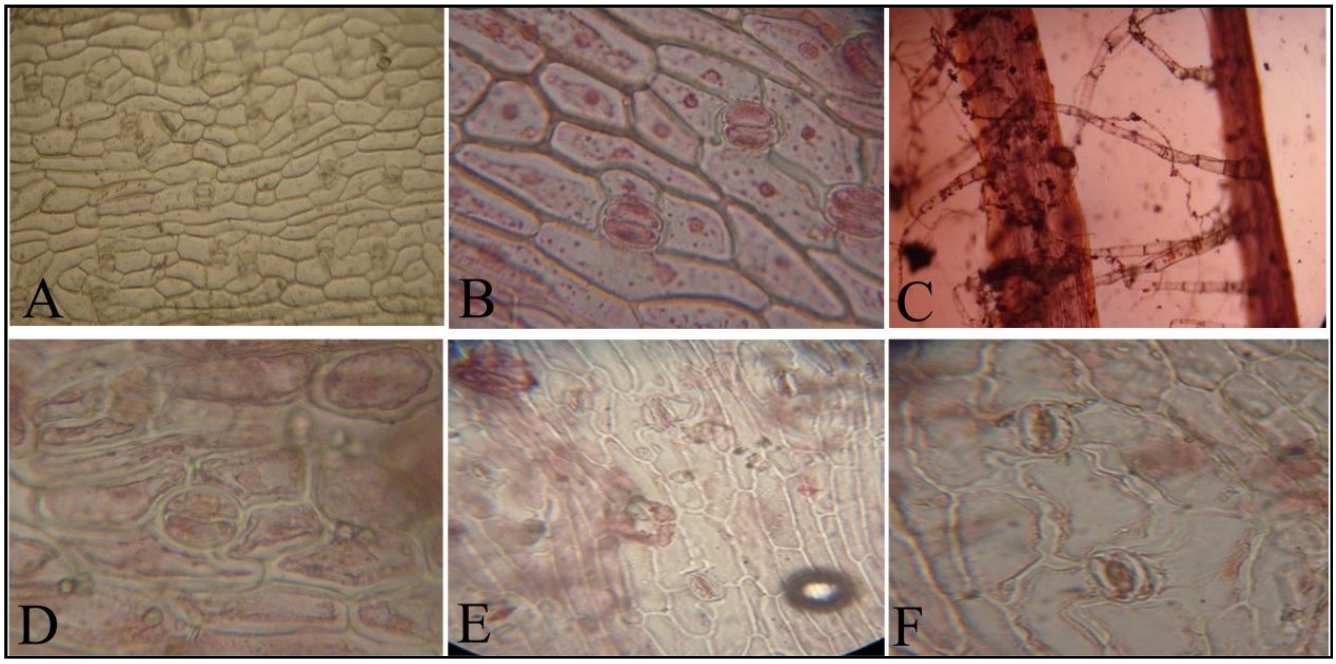

Fig.1: Stem epidermis of the studied taxa. A,B: *C. behen* ; C,D: *C. cardunculus* ; E,F: *C. rigida*. In C, note multicellular eglandular hairs abundant in all species studied.

most of the stem cross section.

Vascular tissue system (VTS) : This system in the studied species composed of two rings of collateral vascular bundles, outer ring with large vascular bundles facing the epidermis and inner ring with smaller vascular bundles facing the pith. In outer ring, thick sclerenchymatous caps occur in below and above the vascular bundles while they present in above the phloem in the inner ring . Size, number and arrangement of the vascular bundles varied in each and between species examined. The largest vascular bundles in all studied species are in location facing the collenchyma while the small bundles facing either the chlorenchyma (as in the outer ring) or the pith (as in inner ring). *C. cardunculus* showed the highest number of vascular bundles compared with the other species studied. Similar stem characters was previously reported in other *Centaurea* species and this may reflect a natural relationship between the studied species of this genus . In general, the present anatomical features of the stem were in agreement with the previous studies on the stem anatomy of the other *centaurea* species , particularly the occurrence of collenchyma in alternating manner with chlorenchyma and the size and arrangement of the vascular bundles in the two rings (Bercu and Bavara, 2012; Ozcan *et al.,* 2014; Tasar *et al.,* 2018).

Table 2: Characteristics of the stem cross section of *Centaurea* species (micrometer).

Pith	No. of vascular bundles	No. of vascular rings	Cortex thickness	Species
Solid	25–30	2	75 – 100*	*C.behen*
Solid	124–160	2	260 - 300	*C.cardunculus*
Solid	34–45	2	150 - 200	*C.rigida*

The leaf (Tables 3-5; Fig. 3-5):

ETS: Adaxial (upper) epidermis composed of ordinary cells, irregular in shape with undulate cell walls in *C. behen* and polygonal with straight walls in *C. cardunculus* and *C. rigida*. Ordinary cells exhibited largest dimentions in *C. behen* compared with other species studied. Both *C.*

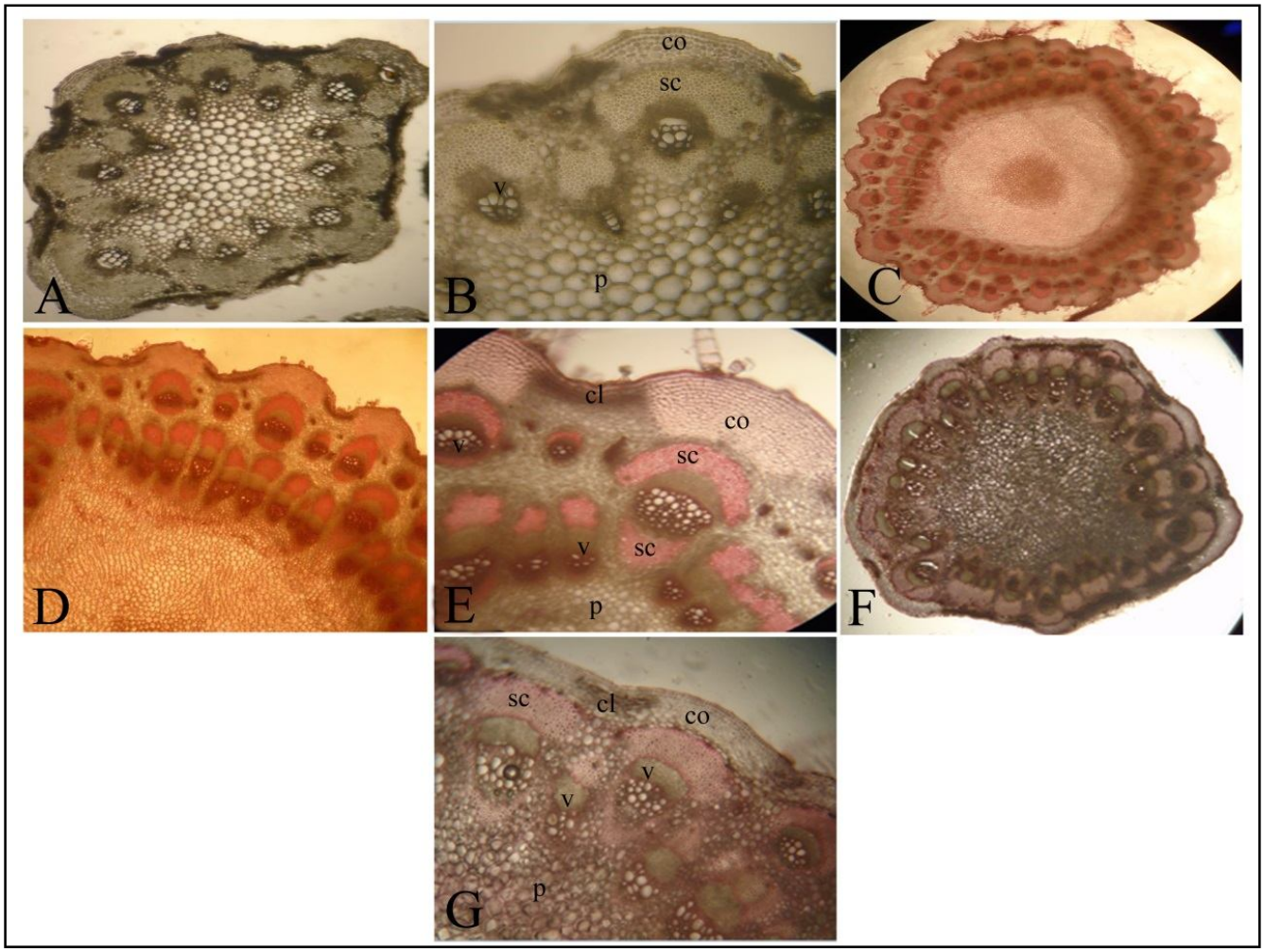

Fig. 2 : Stem cross section . A, *C. behen* ; B, *C. cardunculus* ; C. *rigida* .co, collenchyma; cl, chlorenchyma; sc, sclerenchyma; v, vascular bundle, p, pith

behen and *C. cardunculus* showed stomata of tetracytic type while *C. rigida* showed anisocytic type. Stomata are more abundant in *C.behen* (253 per mm^2) and *C.cardunculus* (221 per mm^2) than in *C.rigida* (163 per mm^2). In abaxial epidermis. The ordinary cells are polygonal in all species studied and present largest dimensions in *C. behen* and *C.cardunculus* compared with those in *C. rigida*. All studied species showed abaxial epidermis with tetracytic type. Mixed stomatal type are rarely observed in *C. behen*. Stomata are more abundant in *C. carunculus* (326 per mm^2) than in other species studied. Due to the presence of stomata on both adaxial and abaxial epidermis, the leaf in the studied taxa are described as amphistomatic Glandular hairs are only present in *C. rigida* (including the midrib region) while multicellular eglandular hairs occur in all taxa investigated. Eglandular hairs occur on the adaxial epidermis in *C. behen* and on both adaxial and abaxial epidermis in *C. cardunculus* and *C, rigida*. The shape and dimension of ordinary cells, type and density of stomata and trichomes (hairs) of the leaf are important taxonomic tools in separating between the species studied.

Table 3: Characteristics of the adaxial epidermis of *Centaurea* species (micrometer).

Stomata			Ordinary cells		Species
Type	**Density**	**L x W**	**shape**	**L x W**	
Tetracytic ,rarely mixed	253	25-35×20-35	Irregular with	40 - 74 × 25 - 400*	*C.behen*
with anisocytic			undulate walls		
Tetracytic	221	20-30×20 - 25	Polygonal with	25 - 80 × 15 – 30	*C.cardunculus*
			straight walls		
Anisocytic	163	×20-25 25 - 30	Polygonal with	30 - 75 × 20 – 30	*C.rigida*
			straight walls		

L=length , W=width , (*) numbers in the table based on 10 replicates

Table 4: Characteristics of the abaxial epidermis of *Centaurea* species (micrometer).

Stomata			Ordinary cells		Species
Type	**Density**	**L x W**	**shape**	**L x W**	
Tetracytic, rarely mixed	189	25-35×20-30	polygonal	40 - 125 × 25 – 60*	*C.behen*
with anisocytic					
tetracytic	326	20-30×20 - 25	Polygonal	30- 125 × 20- 25	*C.cardunculus*
tetracytic	184	×20-25 25 - 30	Polygonal	30 - 60 × 20 - 30	*C.rigida*

L=length , W=width , (*) numbers in the table based on 10 replicates

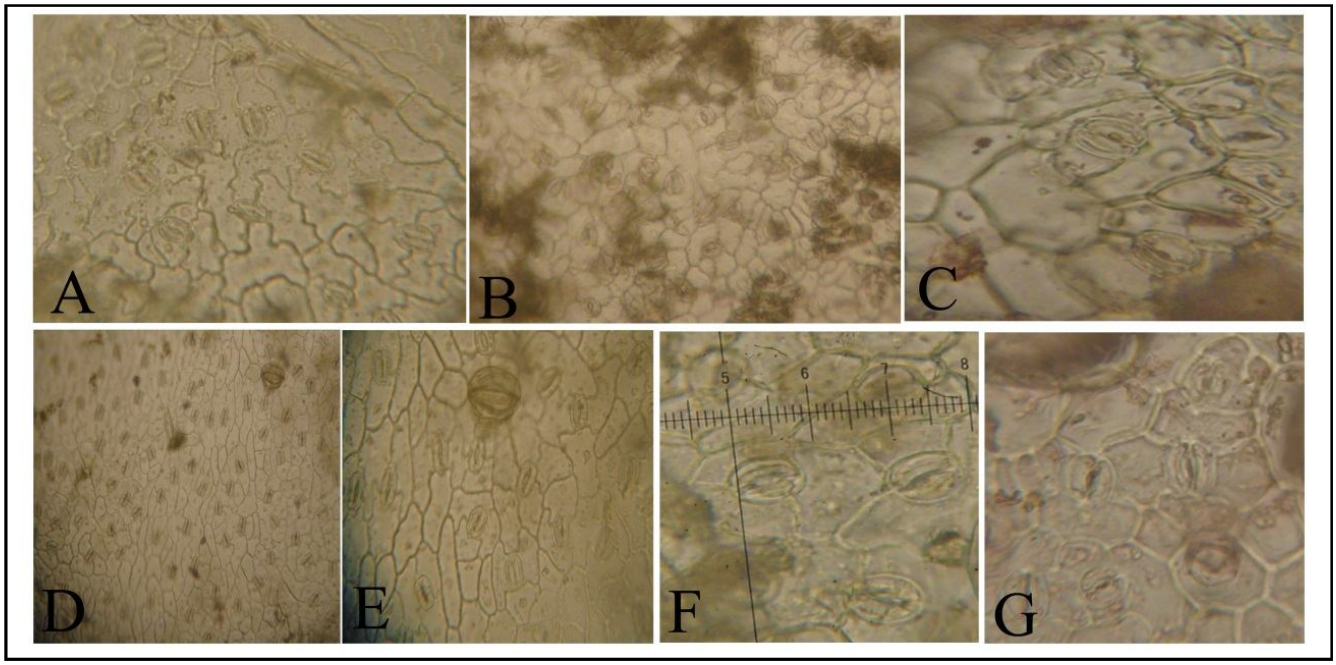

Fig. 3: Stomata in epidermal strip . A-C:adaxial epidermis;D-G:abaxial epidermis . A, D,E : *C. behen* ; B,F : *C. cardunculus* ; C,G: *C. rigida.*

GTS: The mesophyll in the studied Centuarea species is hetergenous isolateral (*i.e.* showing adaxial (under adaxial epidermis) and abaxial (adjacent to abaxial epidermis) palisade parenchyma between them spongy tissue. The adaxial palisade parenchyma in all studied species is thicker than the abaxial one. The GTS in midrib region of all taxa examined consists of lamellar collenchyma contineous below adaxial epidermis and scattered under abaxial epidermis, chlorenchyma alternating with collenchyma and ordinary parenchyma occupying most of the midrib region.

VTS : Leaves of all studied species showed closed collateral vascular bundles. These bundles are without living bundle sheath (or Kranz anatomy, the first step in C4 evolution) (Gowik and Westhoff, 2011), and this suggests that the studied plant species are of C3 photosynthetic pathway (*i.e.* C3 plants) (Al-Khesraji and Mohammed, 2013). Not all *Centaurea* species are C3 plants, some species of this genus have been previously reported as C4 plants (Krookston and Moss, 1970). Number of vascular bundles in the midrib region is an important character to separate between species where the highest number of

Table 5: Characteristics of the leaf cross section of *Centaurea* species (micrometer).

Midrib		Mesophyll		Species
No. vascular bundle	Thickness	Thickness	Type	
5 – 6	1850 – 2000	250 - 330*	isolateral	*C.behen*
8-10	2300 – 2450	130 – 160	isolateral	*C.cardunculus*
3-5	1100 – 1250	240 – 290	isolateral	*C.rigida*

* numbers in the table based on 10 replicates

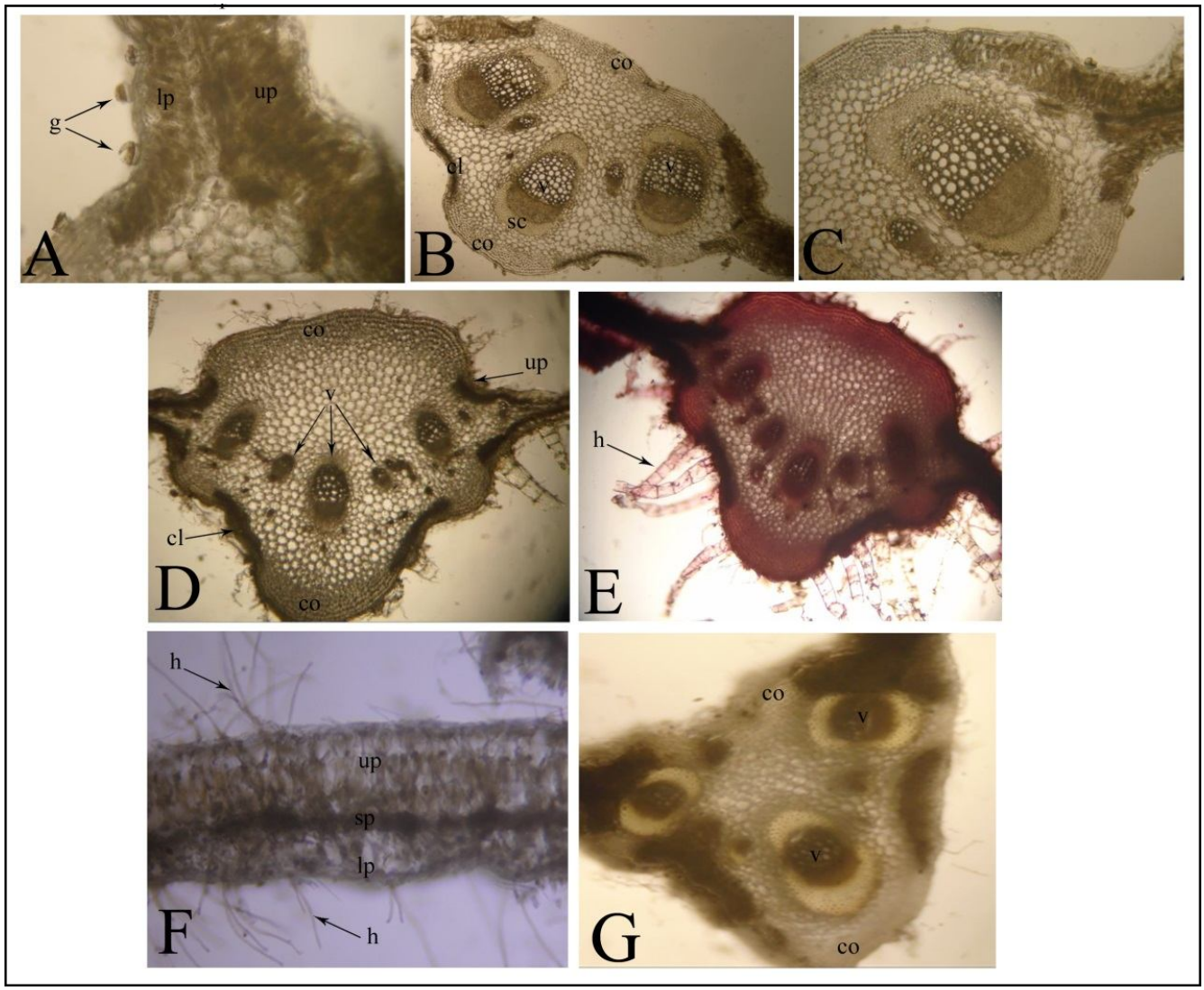

Fig. 4: Leaf cross section. A-C: *C. behen*; D(IKI stained), E (safranin stained): *C. cardunculus*; F, G:*C. rigida*. co, collenchyma; cl, chlorenchyma; sc, sclerenchyma; v, vascular bundle; pl, palisade parenchyma; up, upper palisade; lp, lower palisade; sp, spongy tissue; g, glandular hair; h, eglandular hair.

vascular bundles is in *C.cardunculus* (8-10 bundles) followed by *C.behen* (5-6 bundles) and *C.rigida* (3-5 bundle). Several studies were mentioned the importance of leaf structure in distinguishing *Centaurea* species (Lu *et al.,* 2008, Tasar *et al.,* 2018). However, the above leaf and stem characteristics exhibited by the studied species indicate the importance of the internal structure of these two organs in the identification of the plant species and in separating between species as well.

Conclusions

This study showed that the studied *Centaurea* species are similar in their basic internal structure of both the stem and leaf, but they also present differences. In both stem and leaf (including midrib region), these differences mainly include the type and density of stomata, type of hairs, number and arrangement of vascular bundles. In this study, anatomical characters were found as important tools in the taxonomy of the *Centaurea* species studied.

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