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A COMPARATIVE ANATOMICAL STUDY ON GENUS *PULICARIA* GAERTN. (COMPOSITAE) FROM TURKEY AND ITS TAXONOMIC IMPLICATION

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Abstract

The genus *Pulicaria* Gaertn. belongs to the tribe *Inuleae* Cass. and it is represented by six species *P. arabica* (L.) Cass., *P. armena* Boiss. & Kotschy ex Boiss., *P. dysenterica* (L.) Gaertn., *P. odora* (L.) Rchb., *P. sicula* (L.) Moris, and *P. vulgaris* Gaertn. in Turkey. *P. armena* is endemic to Turkey. A comparative anatomical study on *Puliaria* species growing in Turkey is presented. Cross-sections of stem, leaves, and surface sections of leaves of all species were taken. Anatomical description of *Pulicaria* species was given and species were compared. Noteworthy anatomical features were glandular and non-glandular trichome type, mesophyll cells, stomatal index, and stomata structure. Thirteen anatomical characters were quantified, organized in a data matrix, and analyzed with UPGMA methods. It is shown that leaf and stem anatomy data provide characters that are taxonomically useful.

Key words: Anatomy, Asteraceae, *Pulicaria*, Turkey.

Introduction

The genus *Pulicaria* Gaertn. comprising of about 149 taxa is distributed in Europe, Africa, and Asia (Kadereit & Jeffrey, 2007; The Plant List, 2013). *Pulicaria* is represented by shrubs, shrublets, perennial or annual herbs (Kadereit & Jeffrey, 2007). The genus belongs to the tribe *Inuleae* Cass. and it is represented by 6 species of annual or perennial herbs in the flora of Turkey and one of them is endemic (Davis, 1975; Güner *et al*., 2012). The family of Compositae is very large and widely distributed all over the world. The anatomical structure of the family shows considerable diversity. According to Metcalfe & Chalk (1950), some particular anatomical characteristics are of diagnostic value and have taxonomic importance within the family, e.g., types of glandular and non-glandular trichomes, secretory and laticiferous canals, the occurrence of medullar and cortical vascular bundles. Janaćković *et al*., (2019) also emphasized that morphological, micromorphological, and anatomical data are important in the systematics of Compositae. Drury & Watson (1966) examined phloem fibers, resin canals in the stem, and the presence of crystals in the achene wall of thirty-seven species in the tribe Inuloideae. Two *Pulicaria* species (*P. vulgaris* Gaertn. and *P. dysenterica* Gaertn.) were included in the study and crystals were observed in their achene wall. Resin canals were not found in studied *Pulicaria* species. They classified non-glandular trichomes into two types (Type A and type B). The cells are relatively large and thick-walled, often striated or slightly granular in surface view in Type A. In type B, one to three short, thin-walled basal cells are attached to a greatly elongated and often thick-walled, tapering terminal cell. Type A was observed in the studied *Pulicaria* species. Coutinho *et al*., (2011) studied on pollen morphology of Iberian *Pulicaria* species (*P. dysenterica* (L.) Gaertn., *P. microcephala* Lange, *P. odora* (L.) Rchb., *P. paludosa* Link, *P. sicula* (L.) Moris, *P. vulgaris* Gaertn.). According to their results, qualitative characters of the exine sculpture, especially the spines, were the most useful to define *Pulicaria* pollen types and separate the species. Nevertheless, separation of all species was not complete, only separation of *P. vulgaris* and *P. microcephala* was accomplished.

Some *Pulicaria* species are used in traditional folk medicine. For instance, species (*P. arabica* (L.) Cass., *P. dysenterica* (L.) Gaertn., *P. jaubertii* E. Gamal-Eldin, *P. odora* (L.) Reichenb., *P. undulate* (L.) C.A. Mey., *P. vulgaris* (L.) Gaertn., *P. vulgaris* Gaertn.) are used for abdominal pains, ankle sprain, ankle swelling bronchitis, burns, colds, contusions, cough, diabetes, flatulent colic, headache¸ influenza, leg pain, malaise, stomach troubles, whiteness on hands (Camejo-Rodrigues *et al*., 2003; Novais *et al*., 2004; Kızılarslan & Özhatay, 2012; Tuzlacı, 2016; Al-Fatimi, 2019) Different types of phytochemical from *Pulicaria* have been reported, including essential oils, flavonoids, phenolic derivatives, monoterpene derivatives, sesquiterpenes, diterpenes, triterpenes, steroids, and some others (Liu *et al*., 2010). Essential oil and different extracts of some *Pulicaria* species have shown antibacterial, antifungal, and antileishmanial activity (Ezoubeiri *et al*., 2005; Ali *et al*., 2012; Asghari *et al*., 2014). According to Sharifi-Rad *et al*., (2014), the essential oil of *P. vulgaris* showed that all assayed concentrations substantially inhibited the growth of *B. cereus, S. aureus, E. coli, P. aeruginosa, A. niger*, and C. *albicans*; and the findings of the cytotoxicity assay showed that there was the substantial cytotoxic activity of the essential oil against the MCF-7 and Hep-G2 cell lines. In Hanbali *et al*., (2005)'s study, the essential oil of *Pulicaria odora* showed inhibition zones against *Streptococcus C, Bacillus cereus, Enterococcus faecalis*, and *Pseudomonas vulgaris*. In each case, the activity of *P. odora* essential oil was higher than that of the standard antibiotic. The essential oil composition of *Pulicaria* species of Turkey was studied by Yüce-Babacan & Paksoy (2016). They found differences in major compounds of essential oils. The number of components in *Pulicaria* species was also different, and eighteen, forty-three, fifty-seven, sixty-two, sixty-five, and seventy-one components were determined respectively, in *P. sicula*, *P. vulgaris*, *P. arabica*, *P. armena*, *P. odora*, and *P. dysenterica*.

According to the literature, only a few anatomical studies have been reported in *Pulicaria* species; moreover, these are not comprehensive studies (Zareh, 2005; Ciccarelli *et al*., 2007; Perveen *et al*., 2016). No detailed studies on the anatomy of *Pulicaria* species were found. There are some anatomical studies on *Inula*, *Dittrichia* species, which are in the same tribe as *Pulicaria* (Werker & Fahn, 1981; Zareh, 2005; Karanović *et al*., 2016; Perveen *et al*., 2016; Aytas Akcın & Akcın, 2017). In this study, it is aimed to investigate detailed anatomical features of Turkish *Pulicaria* species for the first time and compare. The study also aimed to identify the systematic significance of anatomical features in *Pulicaria*.

Materials and Methods

Plant materials were collected between 2018-2019 by authors. Details of voucher specimens are given in (Table 1). Only *P. armena* Boiss. & Kotschy ex Boiss. was studied from herbarium material (ISTE 60993). The collected plant specimens were identified using the "Flora of Turkey and the East Aegean Islands" (Davis, 1975) and deposited in ISTE (the Herbarium of the Faculty of Pharmacy of Istanbul University).

The material was fixed in 70% alcohol - used for anatomical studies. Cross-sections of stem, leaves, and surface sections of leaves were taken by free hand and then stained with Sartur solution (Çelebioğlu & Baytop, 1949). Photographs of the well-stained sections were taken using an Olympus BH-2 light microscope equipped with Canon A 640 digital camera.

Thirteen anatomical features were used in a data matrix (Table 2), subsequently analyzed through UPGMA methods of PAST 1.81 (Hammer *et al*., 2001). The stomatal index was calculated according to the method of Meidner & Mansfield (1968). Anatomical measurements were made by using the image analysis systems KAMERAM (ARGENİT Microsystems).

Results

Anatomical measurements of stem and leaves of *Pulicaria* species are shown in (Tables 3 and 4). Photos of lamina epidermal surface, cross-section of leaves, and stem are shown in Figs. 1–3. Trichomes density of *Pulicaria* species is compared in (Table 5). Photos of non-glandular and glandular trichomes are given in Figs. 4 and 5.

Leaves anatomy: *Pulicaria arabica*: The leaf was bifacial. The upper epidermis cells were covered with a thick cuticle. The upper and lower epidermis cells single-layered and cell margins were undulate. Under the epidermis, there was 1–2 layers of cylindrical palisade parenchyma cells containing chloroplast. The spongy parenchyma has 3–4 layers and the cells were isodiametric. The lower epidermis cells were covered with a cuticle. The leaves were amphystomatic. The stomata index of the upper epidermis was 28.67 and the lower epidermis is 18.18. The upper and lower epidermis both had anomocytic stomata. There were glandular and non-glandular trichomes on both the upper and lower epidermis. There are three types of glandular trichomes. One of them was biseriate glandular trichomes with single-tier short stalk cells and 6–7 cell tiers, the second one was biseriate glandular trichomes had single-layer long stalk cells and following with 5 cell tiers. The third one was biseriate sessile glandular trichomes with 6 cell tiers. The non-glandular trichomes had 3 cells and a single web-like terminal cell.

Table 1. Collected species information.

Species	Locality	Herbarium number
P. arabica (L.) Cass.	Muğla: Bodrum, Göltürkbükü, 19.vii.2019, B. Gürdal, E. Özdemir Nath	ISTE 116891
P. dysenterica (L.) Gaertn.	İstanbul: Çatalca, Subaşı, 14.viii.2018, B. Gürdal, E. Özdemir Nath	ISTE 116730
P. odora (L.) Rchb.	Yalova: Armutlu, 06.vi.2019, B. Gürdal, E. Abamor	ISTE 116747
P. sicula (L.) Moris	Bursa: Nilüfer, Gölyazı, 19.x.2019, B. Gürdal, E. Özdemir Nath	ISTE 116910
P. vulgaris Gaertn.	Edirne: Enez, Sultaniçe, 20.viii.2019, B. Gürdal, E. Özdemir Nath	ISTE 116895

Table 2. Data Matrix. Characters and their coding.

Leaves	1.	**abaxial stomatal index: 0: < 20%, 1: > 20%**
	2.	adaxial stomatal index: 0: < 20%, 1: > 20%
	3.	Anamocytic stomata: 0: absent, 1: present
	4.	Leaves type: 0: equifacial, 1: bifacial
	5.	Biseriate glandular trichome with short stalk: 0: absent, 1: present
	6.	Biseriate glandular trichome with long stalk: 0: absent, 1: present
	7.	Biseriate sessile glandular trichome: 0: absent, 1: present
	8.	Uniseriate sessile glandular trichome: 0: absent, 1: present
Stem	9.	Biseriate sessile glandular trichome: 0: absent, 1: present
	10.	Biseriate glandular trichome with short stalk: 0: absent, 1: present
	11.	Biseriate glandular trichome with long stalk: 0: absent, 1: present
	12.	Uniseriate glandular trichome with short stalk: 0: absent, 1: present
	13.	Uniseriate sessile glandular trichome: 0: absent, 1: present

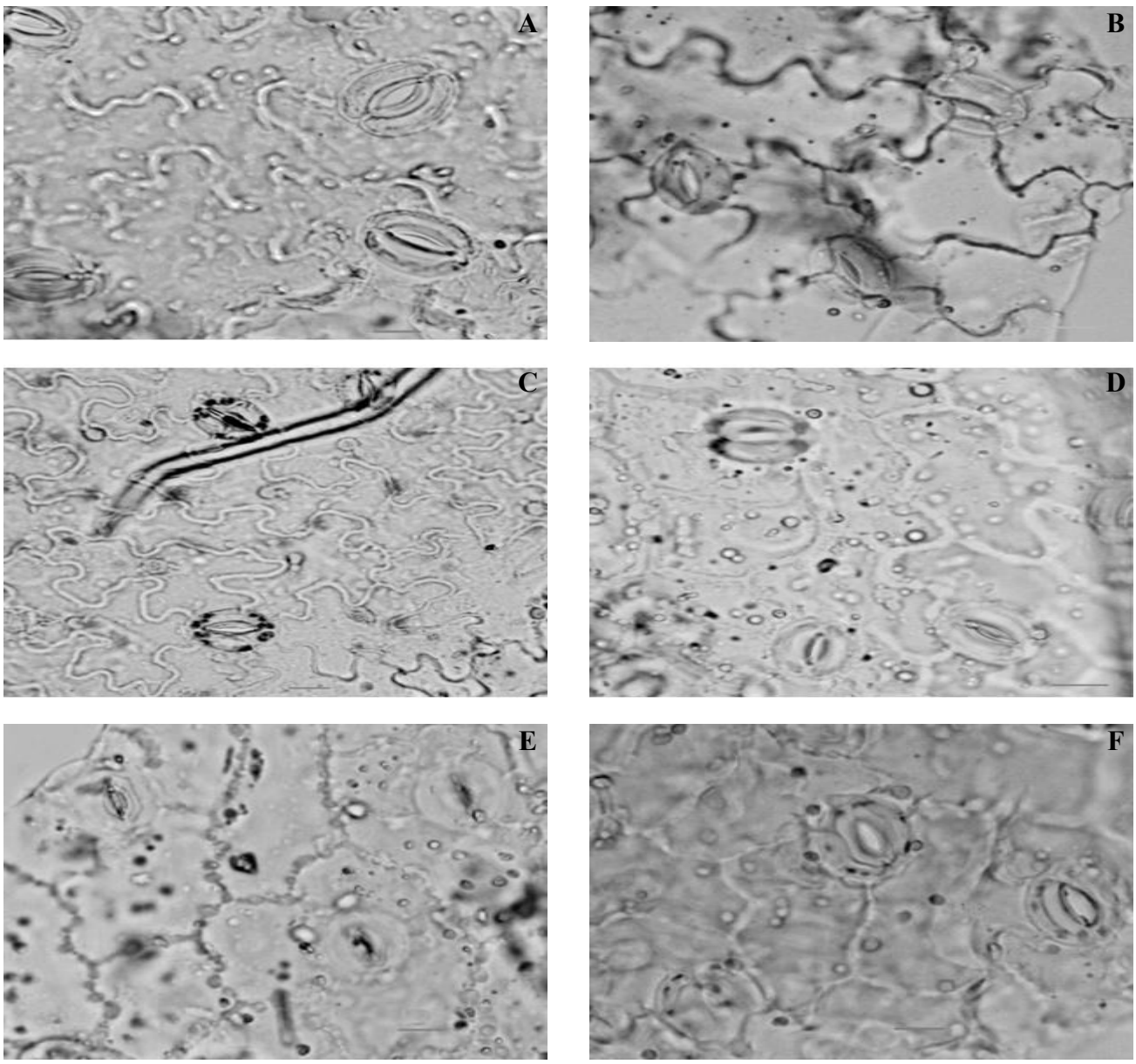

Fig. 1. Lamina epidermal surface of *Pulicaria* species. A. *P. arabica*, adaxial surface; B. *P. armena*, adaxial surface; C. *P. dysenterica*, abaxial surface; D. *P. odora*, abaxial surface; E. *P. sicula*, adaxial surface; F. *P. vulgaris*, abaxial surface (Scale bars: 10 μm).

Table 3. Anatomical measurements of leaf [Avr: average, Sd: standard deviation].

	P. arabica	***P. armena***	***P. dysenterica***	***P. odora***	***P. sicula***	***P. vulgaris***
			Upper epidermis			
Stomata type	anomocytic	anomocytic	anomocytic	anomocytic	anomocytic	anomocytic
Stomata index	28.67	20.40	5.13	13.68	15.47	18.70
Stomata length (μm) (Avr. ± Sd)	26.29±1.50	23.07±0.99	19.74±0.90	19.56±1.94	18.88±0.88	23.52±0.69
Stomata width (μm) (Avr. ± Sd)	19.15±1.71	16.19±1.06	16.74±0.42	18.10±1.12	19.16±1.11	17.63±0.39
Cuticle thickness (μm) (Avr. ± Sd)	5.74±0.91	5.35±0.42	9.99±1.31	6.18±0.81	4.83±0.79	6.06±0.41
Epidermis cell length (μm) (Avr. ± Sd)	14.06±0.97	11.56±1.5	9.48±1.24	18.64±2.43	15.43±1.95	15.08±2.44
Epidermis cell width (μm) (Avr. ± Sd)	24.59±3.33	22.90±3.10	18.01±1.99	16.57±3.30	25.92±4.17	22.80±2.46
			Lower epidermis			
Stomata type	anomocytic	anomocytic	anomocytic	anomocytic	anomocytic	anomocytic
Stomata index	18.18	18.31	19.52	17.61	13.38	20.31
Stomata length (μm) (Avr. ± Sd)	22.06±0.54	22.03±2.19	18.84±1.03	18.50±1.08	19.74±2.41	23.30±1.98
Stomata width (μm) (Avr. ± Sd)	17.03±1.21	17.16±0.56	16.32±1.37	16.68±1.19	18.20±1.48	19.36±1.55
Cuticle thickness (μm) (Avr. ± Sd)	6.86±0.54	4.78±1.29	2.79±0.26	5.07±0.69	5.47±1.04	6.92±0.44
Epidermis cell length (μm) (Avr. ± Sd)	15.43±1.73	15.01±1.00	13.95±1.57	12.56±1.72	12.47±1.41	15.71±3.48
Epidermis cell width (μm) (Avr. ± Sd)	20.16±2.72	25.48±1.43	16.83±2.76	13.28±1.86	14.41±1.64	25.40±5.39

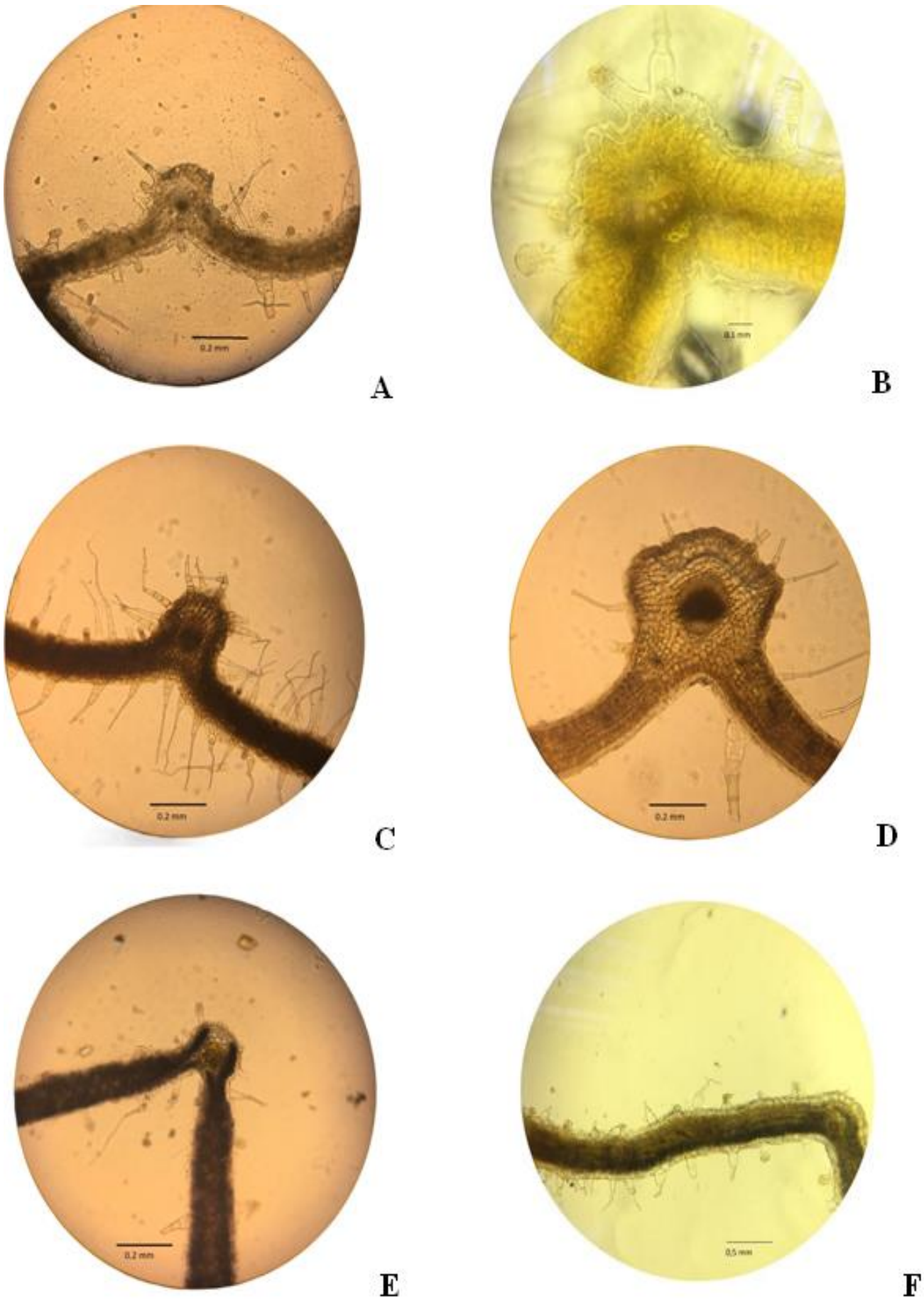

Fig. 2. Cross section of leaves in *Pulicaria* species. A. *P. arabica*; B. *P. armena*; C. *P. dysenterica*; D. *P. odora*; E. *P. sicula*; F. *P. vulgaris.*

Table 4. Anatomical measurements of stem [Avr: average, Sd: standard deviation].

	P. arabica	*P. armena*	*P. dysenterica*	*P. odora*	*P. sicula*	*P. vulgaris*
Epidermis cell length (µm) (Avr. ± Sd)	14.84±2.10	11.07±0.96	8.42±1.45	5.46±0.57	10.51±1.27	8.89±0.73
Epidermis cell width (µm) (Avr. ± Sd)	19.72±7.48	13.38±1.34	12.03±0.70	9.97±1.77	15.95±2.17	13.63±2.34
Pith ray cell	37.66±6.47	24.26±0.59	28.77±6.77	48.49±11.04	42.02±5.69	25.12±5.96
Trachea (µm) (Avr. ± Sd)	20.85±2.77	7.58±0.63	17.02±2.67	12.86±2.60	8.44±1.01	6.58±0.67
Endodermis cell length (µm) (Avr. ± Sd)	14.40±2.74	6.95±0.85	15.02±2.10	16.29±2.75	8.06±1.03	12.14±1.59
Endodermis cell width (µm) (Avr. ± Sd)	29.43±8.45	18.01±3.12	26.43±3.66	27.93±5.35	15.93±1.80	17.36±2.91

Table 5. Trichomes density of *Pulicaria* species.

	P. arabica	*P. armena*	*P. dysenterica*	*P. odora*	*P. sicula*	*P. vulgaris*
Stem						
Non-glandular trichomes	+	++	+++	+++	+	+
Glandular trichomes	+	+	+	++	+++	+
Leaves						
Non-glandular trichomes	+++	+++	++	++	+	++
Glandular trichomes	++	++	+	+	+++	+

+: few ++: moderate, +++: dense.

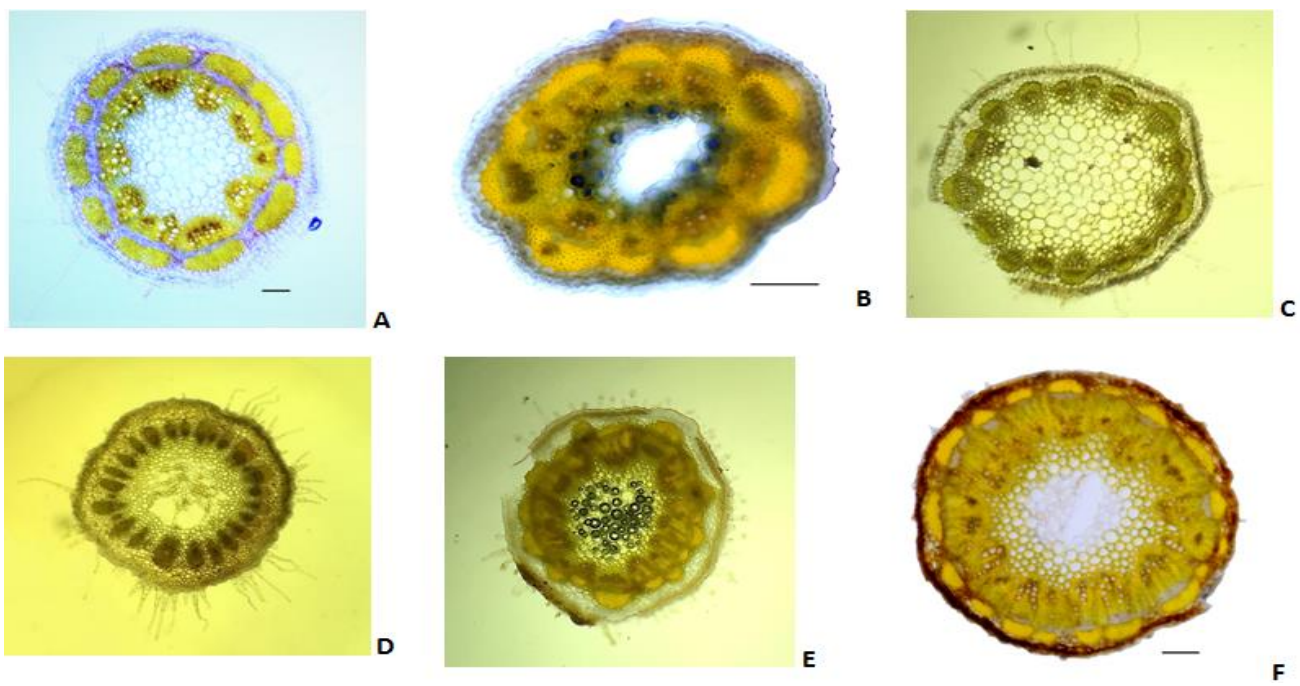

Fig. 3. Stem of *Pulicaria* species. A. *P. arabica*; B. *P. armena*; C. *P. dysenterica*; D. *P. odora*; E. *P. sicula*; F. *P. vulgaris* (Scale bars: 0.1 mm).

Pulicaria armena: The leaf was bifacial. The upper epidermis cells were covered with a thick cuticle. The upper and lower epidermis cells were single-layered with undulate cell margins. Under the upper epidermis, there was a single layer of cylindrical palisade parenchyma cells. The starch grains were observed in the parenchyma. The spongy parenchyma had 2–3 layers of isodiametric cells. The lower epidermis cells were covered with a thinner cuticle according to the upper one. The leaves were amphystomatic. The stomata index of the upper epidermis was 20.40 and the lower epidermis was 18.31. The stomata in the upper and lower epidermis were anomocytic. There were glandular and non-glandular trichomes on both the upper and lower epidermis. Three types of glandular trichomes were present (i) biseriate glandular trichomes with single-tier long stalk cell followed by 6 cell tiers (ii) biseriate glandular trichomes with single-tier short stalked cell and 5 cell tiers, (iii) biseriate sessile glandular trichomes with 4 cell tiers. The non-glandular trichomes consisted of 3–4 cells and a web-like terminal cell.

Pulicaria dysenterica: The leaf was bifacial. The upper epidermis cells were covered with a thick cuticle. The upper epidermis was single-layered and cell margins were undulate. Under the upper epidermis, there was a single layer of cylindrical palisade parenchymatous cells having chloroplast. The spongy parenchyma had 4 layers and the cells were isodiametric. The lower epidermis cells were covered with a comparatively thinner cuticle than the upper one. The leaves were amphystomatic. The lower epidermis contained more stomata than the upper epidermis. The stomata index of the upper epidermis was

5.13 and the lower epidermis was 19.52. The upper and lower epidermis both had anomocytic stomata. There were glandular and non-glandular trichomes on both the upper and lower epidermis. There were two types of glandular trichomes (i) biseriate glandular trichomes with one tier long stalked cell followed by 5 celled tiers (ii) biseriate sessile glandular trichomes with 5 celled tiers. The non-glandular trichome consisted of 2–4 cells and web-like terminal cell.

Pulicaria odora: The leaf was bifacial. The upper epidermis cells were covered with a thick cuticle. The upper epidermis cells were rectangular and single-layered. Under the upper epidermis, there was 1–2 layer of cylindrical palisade parenchyma cells having chloroplast. The spongy parenchyma had 3–4 layers and the cells were isodiametric. The cuticle of the lower epidermis was thinner than the upper one. Stomata occured on both sides of the leaves, but the lower epidermis contained more stomata than the upper epidermis. The stomata index of the upper epidermis was 13.68 and the lower epidermis was 17.61. The upper and lower epidermis both had anomocytic stomata. Two types of glandular trichomes were observed viz. biseriate sessile glandular trichomes with 6–7 cell tiers and uniseriate sessile glandular trichomes with 8 cell tiers. Non-glandular trichomes had 3–4 cells and web-like terminal cell on both the upper and lower epidermis.

Pulicaria sicula: The leaf was equifacial. The upper and lower epidermis cells were covered with a cuticle. The upper epidermis was single layered. Under the lower and upper epidermis, there were 1–2 layered cylindrical palisade parenchymatous cells which contained chloroplast. In the middle part, there was spongy parenchyma which had 3 layers and the cells were isodiametric. Stomata occured on both sides of the leaves. The stomata index of the upper epidermis is 15.47 and the lower epidermis was 13.38. The upper and lower epidermis both had anomocytic stomata. There were glandular and non-glandular trichomes on upper and lower epidermis. There were three types of glandular trichomes: (i) biseriate glandular trichomes with 1–2 tier short by stalked cell and 5–6 celled tiers, (ii) biseriate glandular trichomes had 1–2 layer long stalk cells and followed with 6–7 cell tiers. The third one was biseriate sessile glandular trichomes with 6 celled tiers. The non-glandular trichomes had 3–4 cells and a single web-like terminal cell.

Pulicaria vulgaris: The leaf was bifacial. The upper epidermis cells were covered with a cuticle. The upper epidermis cells were single-layered. Under the upper epidermis, there were 1–2 layers of cylindrical palisade parenchymatous cells. The starch grains were also observed in the parenchyma cells. The spongy parenchyma had 3–4 layers and the cells were isodiametric. The leaves were amphystomatic. There were more stomata in the lower epidermis than the upper epidermis. The stomata index of the upper epidermis was 18.70 and the lower epidermis is 20.31. The upper and lower epidermis both had anomocytic stomata. There were glandular and non-glandular trichomes on both the upper and lower epidermis. There were two types of glandular trichomes. One of them was biseriate glandular trichomes with single-tier shortly stalked cell and 5 celled tiers, the second one was biseriate sessile glandular trichomes with 5 celled tiers. The non-glandular trichome consisted of 3–4 cells and web-like terminal cell.

Stem anatomy: *Pulicaria arabica*: In the transverse section, the stem was more or less rounded. One layered epidermis, made up of oval to rectangular cells, covered with a cuticle was present and there were non-glandular and glandular trichomes. There were biseriate glandular trichomes with 6–7 cell tiers and the non-glandular trichomes had 3 cells and 1 web-like terminal cell. The cortex was composed of 3–4 layered parenchymatic cells. The single-layered endodermis consisted of rectangular cells. The phloem was surrounded by 5–7 layers of sclerenchymatous cell. The xylem comprised of trachea and tracheids. It had collateral vascular bundles. A large pith parenchymatous cell was present in the central region.

Pulicaria armena: In the transverse section, the stem was more or less round. The epidermis consisted of oval-rectangular cells with thin cuticles and was covered with non-glandular and glandular trichomes. Biseriate sessile glandular trichomes with 5–6 cell tiers were present. The non-glandular trichomes had 2–3 cells and 1 web-like terminal cell. The cortex region was very narrow and composed of 2–3 layered parenchymatic cells. The endodermis was single-layered. The sclerenchymatous was cells 3–5 layered, covering collateral vascular bundles. Orbicular parenchymatous cells were present in the central region.

Pulicaria dysenterica: In the transverse section, the stem was more or less round. The epidermis was composed of oval-rectangular cells and covered by a thick cuticle. It contained non-glandular and glandular trichomes. Biseriate sessile glandular trichomes with 3–4 cell tiers. Another type was biseriate glandular trichomes with 2 tiers long stalked cell and followed by 3 cell tiers. The non-glandular trichomes had 2–3 cells and 1 web-like terminal cell. The cortex consisted of 3–5 layered parenchymatic cells. The endodermis was 1–2 layered. The phloem was surrounded by 7–8 layers of sclerenchyma cells. The xylem comprised of trachea and tracheids. It had collateral vascular bundles. The pith comprised of hexagonal or orbicular parenchymatous cells.

Pulicaria odora: In the transverse section, the stem was more or less round or polygonal. The epidermis was covered by a cuticle with non-glandular and glandular trichomes, biseriate sessile glandular trichomes with 6 cell tiers. Uniseriate sessile glandular trichomes with 6–7 cell tiers were also present. The non-glandular trichomes had 3 cells and 1 web-like terminal cell. The epidermis was composed of a single layer of isodiametric cells. The collenchyma tissue was located under the epidermis which was 1–2 layered. The cortex, consisting of 4–6 layered parenchymatic cells was located under the collenchyma. The endodermis was distinct and composed of one layer. The phloem was partly surrounded by 5–9 layers of sclerenchyma cells. The xylem comprised of trachea and tracheids. It had collateral vascular bundles. The pith was present at the middle of the stem and was filled up with large parenchymatous cells.

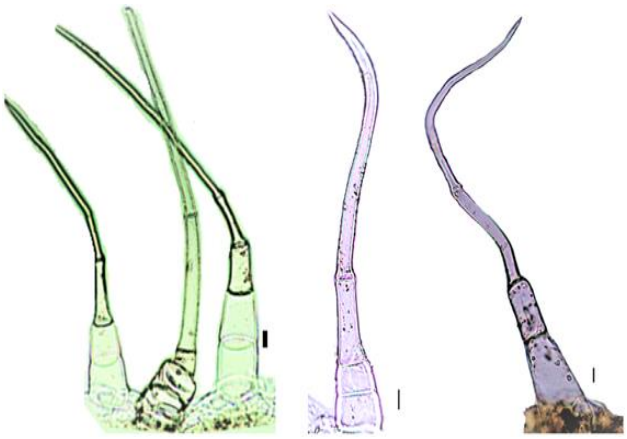

Fig. 4. Non-glandular trichomes observed on studied *Pulicaria* species. (Scale bars: 10 µm).

Pulicaria sicula: In the transverse section, the stem was more or less round. The epidermis was composed of oval-rectangular cells with thick cuticles and covered with non-glandular and glandular trichomes. There were 4 types of glandular trichomes; biseriate sessile glandular trichomes with 6 cell tiers, biseriate glandular trichomes with 3–4 tiers long stalked cell and following with 4–5 cell tiers, biseriate glandular trichomes with 1–2 tiers shortly stalked cell and following with 4–6 cell tiers, and uniseriate glandular trichomes with 1 tier shortly stalked cell and following with 3–4 cell tiers. The non-glandular trichomes had 3–4 cells and 1 web-like terminal cell. The collenchyma tissue was 3–4 layered. Under the collenchyma, parenchymatic cells were 2–3 layered. The endodermis was composed of a single-layer. The phloem was partly surrounded by 3–6 layers of sclerenchymatous cells. The xylem comprised of trachea and tracheids. It had collateral vascular bundles. The pith was made up of hexagonal parenchymatous cells.

Pulicaria vulgaris: In the transverse section, the stem was more or less round. The epidermis consisted of rectangular cells with thick cuticles. There were non-glandular and glandular trichomes. Biseriate sessile glandular trichomes with 5–6 cell tiers and non-glandular trichomes with 2–3 cells and 1 web-like terminal cell were present. The cortex consisted of 3–5 layered parenchymatic cells. The endodermis was single-layered. The phloem was partly surrounded by 4–5 layers of sclerenchyma cell. The xylem comprised of the trachea and tracheids. It had collateral vascular bundles. The pith was filled up with large parenchymatous cells.

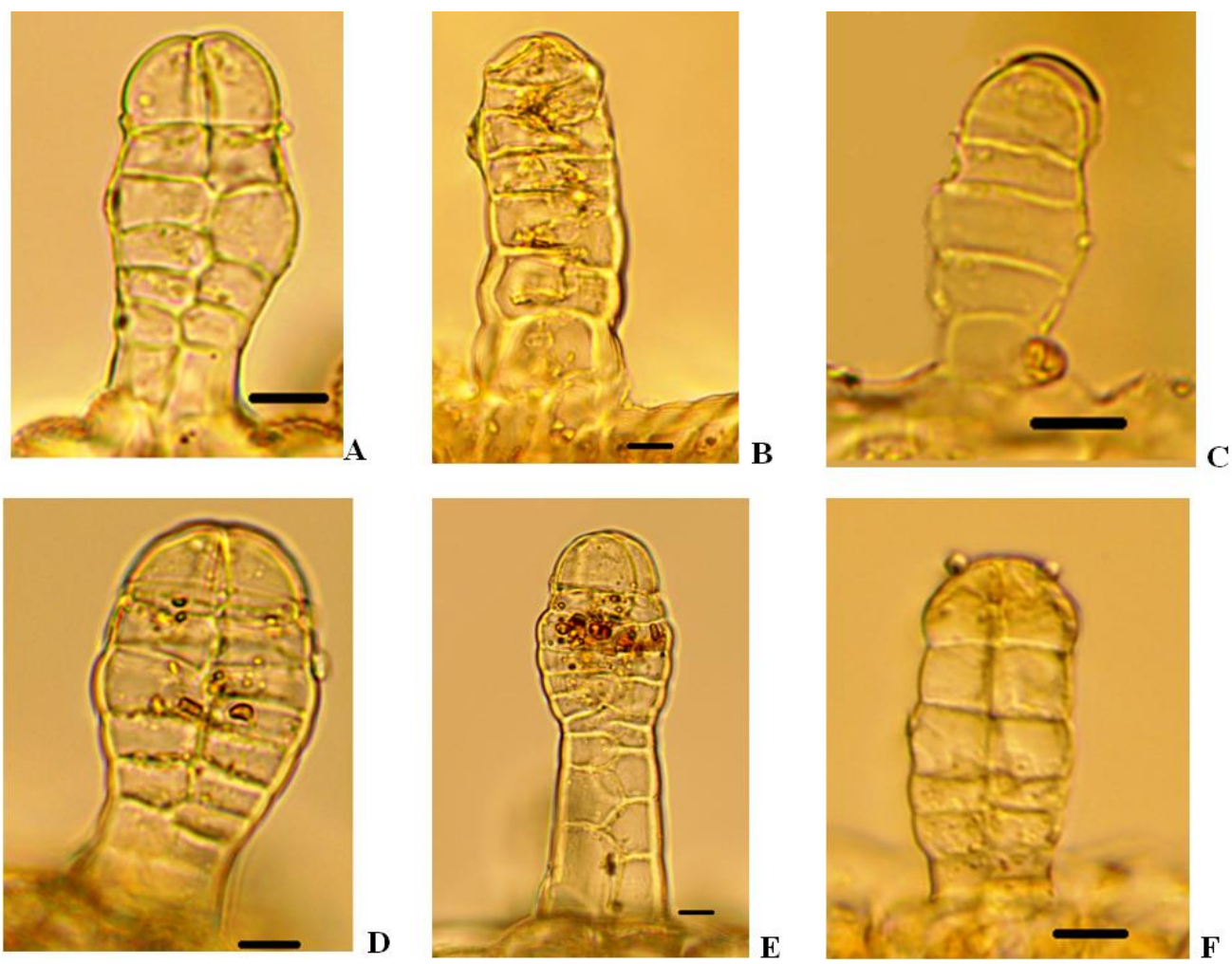

Fig. 5. Glandular trichomes types observed on studied *Pulicaria* species. A. Biseriate glandular trichome with one tier short stalk cell and following with 5 cell tiers. B. Biseriate sessile glandular trichome with 6 cell tiers. C. Uniseriate glandular trichome with 1 tier short stalk cell and following with 3–4 cell tiers D. Biseriate glandular trichome with 2 tiers short stalk cell and following with 6 cell tiers. E. Biseriate glandular trichome with 4 tiers long stalk cell and following with 5 cell tiers. F. Biseriate glandular trichome with 5 cell tiers (Scale bars: 10 µm).

Table 6. Morpho-anatomical data matrix for *Pulicaria* species based on scoring of character-states from Table 2.

	1)	2)	3)	4)	5)	6)	7)	8)	9)	10)	11)	12)	13)
P. arabica	0	1	1	1	1	1	1	0	1	0	0	0	0
P. armena	0	1	1	1	1	1	1	0	1	0	0	0	0
P. dysenterica	0	0	1	1	0	1	1	0	1	0	1	0	0
P. odora	0	0	1	1	0	0	1	1	1	0	0	0	1
P. sicula	0	0	1	0	1	1	1	0	1	1	1	1	0
P. vulgaris	1	0	1	1	1	0	1	0	1	0	0	0	0

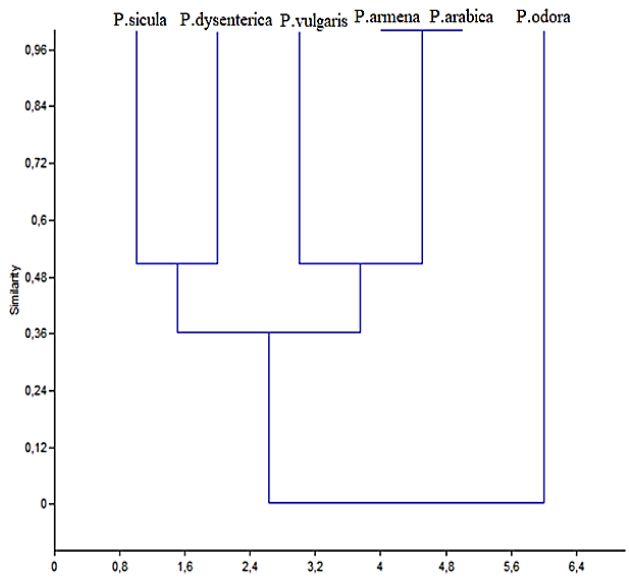

Fig. 6. UPGMA cluster of *Pulicaria* species, based on the morpho-anatomical data matrix presented in Table 6.

Discussion

In Flora of Turkey (Davis, 1975), *Pulicaria* species were first divided into two groups as perennials (*P. dysenterica* and *P. odora*) and annuals (*P. arabica, P. armena, P. sicula*, and *P. vulgaris*). Other distinctive characters are leaf shape, involucre feature, and ligule size. In the morphological description of species, some characters overlapped with each other. In this study, we compared anatomical characters of the species. Thus, with the help of anatomical characters, the distinctions between *Pulicaria* species have become clearer. The anatomical character matrix is presented in (Table 6 and UPGMA trees are shown in Fig. 6.

Glandular trichomes of variable morphology and density had been noticed in *Pulicaria* species. Comparison of leaf trichome density of *Pulicaria* species, the densest non-glandular trichome was observed in *P. arabica, P. armena*; more glandular trichomes were seen in *P. sicula*. When stem trichome density was compared, it was observed that the highest non-glandular trichome was in *P. dysenterica* and *P. odora*; the most glandular trichomes were also observed in *P. sicula*. Glandular trichomes types and density of *P. sicula* were significantly different among the studied *Pulicaria* species. Non-glandular trichomes in leaves and stem were found similar, only cell number was different among species. They all had a single web-like terminal cell. Metchalf & Chalk (1950) had already mentioned the importance of trichomes in the Compositae family. Non-glandular trichome type, as described in Metchalf & Chalk (1950), was web-like in examined *Pulicaria* species. Perveen *et al*., (2016) studied the trichome morphology of some Compositae species. Among them *Pulicaria angustifolia* and *P. carnosa* were examined. Their glandular trichomes were reported as multicellular, having a globular or rounded tips and rounded base. Werker & Fahn (1981) examined secretory hairs structure and secretions of *Inula viscosa* (L.) Aiton (it's a synonym of *Dittrichia viscosa* (L.) Greuter). It had very long, multicellular, and uniseriate non-glandular trichomes and also multicellular and biseriate glandular trichomes. Werker & Fahn (1981) also classified trichomes as sessile and stalked. According to Ciccarelli *et al*., (2007), biseriate glandular trichomes made up of 12–18 cells were found on the ovary of *Pulicaria dysenterica*.

Five main types of glandular trichomes were encountered in *Pulicaria* species. These were uniseriate sessile, uniseriate short-stalked, biseriate sessile, biseriate short-stalked, and biseriate long-stalked glandular trichomes.

In a study on essential oils of taxa belonging to the *Pulicaria* genus, Yüce-Babacan & Paksoy (2016) reported a difference between the essential oil yields of these taxa and the amount of essential oil 100 g. Essential oil yield of *P. sicula*, *P. arabica*, *P. odora*, *P. vulgaris*, *P. armena*, and *P. dysenterica* was found to be 0.2% (v/w), 0.4% (v/w), 0.5% (v/w), 0.5% (v/w), 0.5% (v/w), and 0.6% (v / w), respectively. According to this study, it was determined that the essential oil yield and components of *P. sicula* species were less than other species belonging to the genus. This is thought to be due to edaphic factors (because the habitat of this species is salty marsh areas along the sea and these areas are also poor in organic and inorganic content due to excessive salt and leaching). But in our study, it was observed that *P. sicula* has 4 different glandular hairs and they are very dense. So it was expected that the essential oil yield could be higher than the other species.

Stoma in the examined species were surrounded by 3–5 cells and stomata type was anomocytic. The stomatal index is 5.13–28.67 (upper surface) and 13.38–20.31 (lower surface). The lowest stomatal index of the upper epidermis belonged to *P. dysenterica* and the highest one was *P. arabica*. The lowest and highest stomatal index of the lower epidermis were in *P. sicula* and *P. vulgaris*, respectively. All leaves were also amphystomatic. Detailed anatomical measurements of the leaves are given in Table 2.

Zareh (2005) studied leaf anatomy of 22 species (including *Pulicaria incisa*, *P. vulgaris*, *P. inuloides*, *P. arabica*) in Egypt. Stomata were anomocytic as same in our studied *Pulicaria* species. Only non-glandular trichomes were reported. Among the *Pulicaria* species, trichomes were absent in *P. inuloides*. The presence of parenchymatous bundle sheath was observed in *P. incisa*.

The anomocytic stomata were also present in *Inula helenium* subsp. *orgyalis* and *I. ensifolia* (Aytas Akcın & Akcın, 2017).

Mesophyll of *Pulicaria* species was equifacial and bifacial types. Only the leaf of *P. sicula* was equifacial. This character was an important anatomical feature to differentiate *P. sicula* from other species. Drury & Watson (1966) mentioned the taxonomic importance of anatomical comparisons at the tribe level and compared foliar trichomes, resin canals in the stem and crystals of Inulodeae. *Pulicaria and Inula* are in the same tribe *Inuleae*. The anatomy of *Inula oculus-christi* was studied, and the mesophyll was a bifacial type (Karanović *et al*., 2016). It has anomocytic stomata. Two types of trichomes were observed as glandular, multicellular, biseriate trichomes and non-glandular, multicellular, uniseriate trichomes.

General characters of the stem were similar in all *Pulicaria* species. Measurements of epidermis cell, pith ray cell, trachea, endodermis cell are given in Table 3. One of the differences between species was the number of layers of sclerenchyma cells surrounding the phloem. In five *Pulicaria* species (*P. arabica, P. armena, P. odora, P. sicula*, and *P. vulgaris*) the endodermis was single-layerrd while the endodermis of *P. dysenterica* was 1–2 layered. Karanović *et al*. (2016) examined the anatomy of the *Inula oculus-christi* stem, which was round and the epidermis was covered with cuticle and trichomes. Its cortex parenchyma cells were composed of 6–10 layers and the phloem was surrounded by sclerenchyma cells. Stem anatomy of *Inula helenium* subsp. *orgyalis* and *I. ensifolia* was also studied by Aytas Akcın & Akcın (2017). Several layers of parenchyma and collenchyma were found beneath the epidermis. The secretory cavities were observed in the region of vascular bundles.

Conclusions

In this study, anatomical characteristics of *Pulicaria* species are compared and given in detail. Anatomical studies are important to differentiate *Pulicaria* species more clearly. *P. dysenterica* and *P. odora* are perennials and close to each other (Davis, 1975). Stomata index and glandular trichome types on leaf and stem can be used for distinguishing these species. *P. arabica, P. armena, P. sicula,* and *P. vulgaris* are annuals species. Morphological characters used to identify species are leaf, phyllary, and ligule length. Anatomically *P. sicula* can easily discriminate from others by its glandular trichome types and equifacial leaves. The other three species can be recognized by stomata index and glandular trichome types on leaf and stem. These anatomical findings have been shown as a good tool that can be used in combination with morphological characters.

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