

**ANATOMY AND TRICHOME CHARACTERISTICS OF ENDEMIC
TAXON *PHLOMIS RUSSELLIANA* (SIMS.) BENTHAM
AND THEIR SYSTEMATIC IMPLICATIONS**

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Abstract

The anatomy and trichome properties of *Phlomis russeliana* (Sims) Benthham were carried out in order to determine the useful characters for systematic purposes. *P. russeliana* is an Euxine element which grows in coniferous and deciduous woods and clearings at an altitude of 300 - 1700 m. This species is endemic among the Turkish Flora and it is characterized by yellow bilabiate. Some anatomical characteristics such as the number of pith rays in the root, the number of palisade and spongy parenchyma layer of the leaf, the types of leaf and stomata, petiole anatomy give information of taxonomical significance. Three main glandular types (peltate, capitate and stellate - with galandular and nonglandular arms coexistence were observed on the several organs of the species. In addition, three subtypes of nonglandular trichomes (simple, shield and stellate - with nonglandular arms only) were found in this study.

Introduction

Lamiaceae (the mint family) is a large group of angiosperms and can be identified easily by morphological characters. This family shows natural distribution having approximately 45 genera and about 546 species in Turkey (Davis 1982). According to many researchers, Anatolia is accepted as a gene center for this family (Başer 1993). Many species of Lamiaceae are used as herbal teas, spices, cosmetic materials in cosmetic industries and ornamental plants in Turkey. Some species of this family are traditionally used as medicinal plants in the Turkish folk medicines (Baytop 1984).

Phlomis L. is a large genus of Lamiaceae and unlike other genus, it is easily recognised. *Phlomis* sensu lato is morphologically unique as a lamioid group, but this morphology is not variable enough within the genus to make an infrageneric classification simple (Benthham 1832-1836). Moench (1974) determined morphological differences within *Phlomis* and he splited the taxon into two separate genera: *Phlomis* and *Phlomoides*. These features have also been recognised by many other authors (Benthham 1832-1836, Boissier 1879). The division of *Phlomis* species into two groups has been based on several traits, including habit (the *Phlomis* group are shrubs or sub-shrubs, while members of the group *Phlomoides* are herbaceous), certain leaf characters, the shape and colour of the corolla and strong cytological data.

The genus *Phlomis* has more than 100 species distributed in Euro-Asia and North Africa (Azizian and Moore 1982). The 52 taxa including 6 varieties, 12 natural hybrids and 34 endemic taxa are growing in Turkey (Demirci *et al.* 2006). Baytop (1999) reported that Turkish *Phlomis* species are used as herbal teas (Dağçayı), as tonic, carminative, appetizer and stimulants in the folk medicines and recognized by local names as “Balıkotu, Calba, Çapla or Şalba”. *Phlomis russeliana* (Sims.) Benthham used in this study is Euxine element which grows in coniferous and deciduous woods and clearings at an altitude of 300 - 1700 m (Fig. 1). It is a rare perennial herb to 100 cm and has yellow corolla. This species is endemic among the Turkish Flora and is

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characterized by yellow bilabiate (Davis 1982, Dadandi 2002). The local name of this species is “Akbaşı Çalba” in Turkey (Güner 2012).

Anatomical characters and trichome morphology of *P. russeliana* have not been studied before and there are very less investigations on *Phlomis* species. Therefore, the purpose of this study is to investigate anatomic characters of *P. russeliana* to determine the types of trichomes and their distribution on the various parts. In addition to this, the present study aims evaluating the usefulness of systematic characters.

Materials and Methods

The plant materials of *P. russeliana* were collected during the flowering period from natural populations in Amasya (Gölbeyi village, in the vicinity of Borabay Lake, North slopes, 1600 m, near the beech forest, June 2012, Cansaran 372) which is a city in the Black Sea region of Turkey. Plant samples for anatomical and trichome properties investigations were kept in 70% alcohol. For anatomical investigation, transverse sections of root, stem, leaf and petiole were made by hand using commercial razor blades and stained with safranin (Çelebioğlu and Baytop 1949). Measurements (to determine the min. and max. values of width - height measurements in various tissues) in the sections were performed under a Leica ICC50 HD binocular light microscope by using a Leica Digital Camera and the objectives used were, $\times 10$ and $\times 40$. Photographs were taken with a Leica ICC50 HD binocular light microscope and a Leica Digital Camera. Trichomes were obtained from stem, leaf, petiole, calyx and corolla by transverse and surface sections following the methods as described before. The types and distributions of trichomes are described. The general trichome terminology follows Metcalfe and Chalk (1972), Payne (1978) and Navarro and El Oualidi (2000).

Results and Discussion

The present paper provided useful data on the anatomy and trichome morphology of endemic *P. russeliana*. This is the first information on the characteristics of the species.

In cross-sections of root, stem, leaf, petiole of *P. russeliana*, the following characteristics were observed.

In the transverse section of the root, there was 9 to 10 layered peridermis on the outer surface of the root of *P. russeliana* (Fig. 2a). The dimensions of periderm cells were $25.38 - 70.69 \times 15.00 - 46.59 \mu\text{m}$ (Table 1). Beneath the periderm, there was multi-layered cortex, composed of ovoidal parenchymatic cells. There were xylem tissue composed of regular trachea and tracheid cells. The pith cells were also present in the center. The observations about the root showed a parallelism with the results of Metcalfe and Chalk (1957). Metcalfe and Chalk (1972) also determined some important anatomical information about the root anatomy of Lamiaceae family. They stated that the pith rays of roots of the family are 2 - 12 or more row cells. The pith rays found in the root of *P. russeliana* were composed 3 - 4 row cells. These findings were consistent with those of Metcalfe and Chalk (1972) and those of some studied species of Lamiaceae (Baran and Özdemir 2006, Özkan and Soy 2007).

P. russeliana is a typical characteristic species in Lamiaceae. The species had an angular stem. Its epidermis usually had one layer composed of ovoidal cells ($12.5 - 35.29 \times 14.31 - 23.76 \mu\text{m}$) (Table 1). The upper surface was covered with a cuticle. Transverse section of the stem revealed 9 - 10 layered collenchyma placed just beneath the epidermis (Fig. 2b). There were small groups of sclerenchymatous cells on the outer side of phloem. The xylem tissue existing beneath the cambium was composed of regular trachea and tracheid cells. The vascular bundles were collateral. The pith was wide and consists of flat cells with intercellular spaces. According to

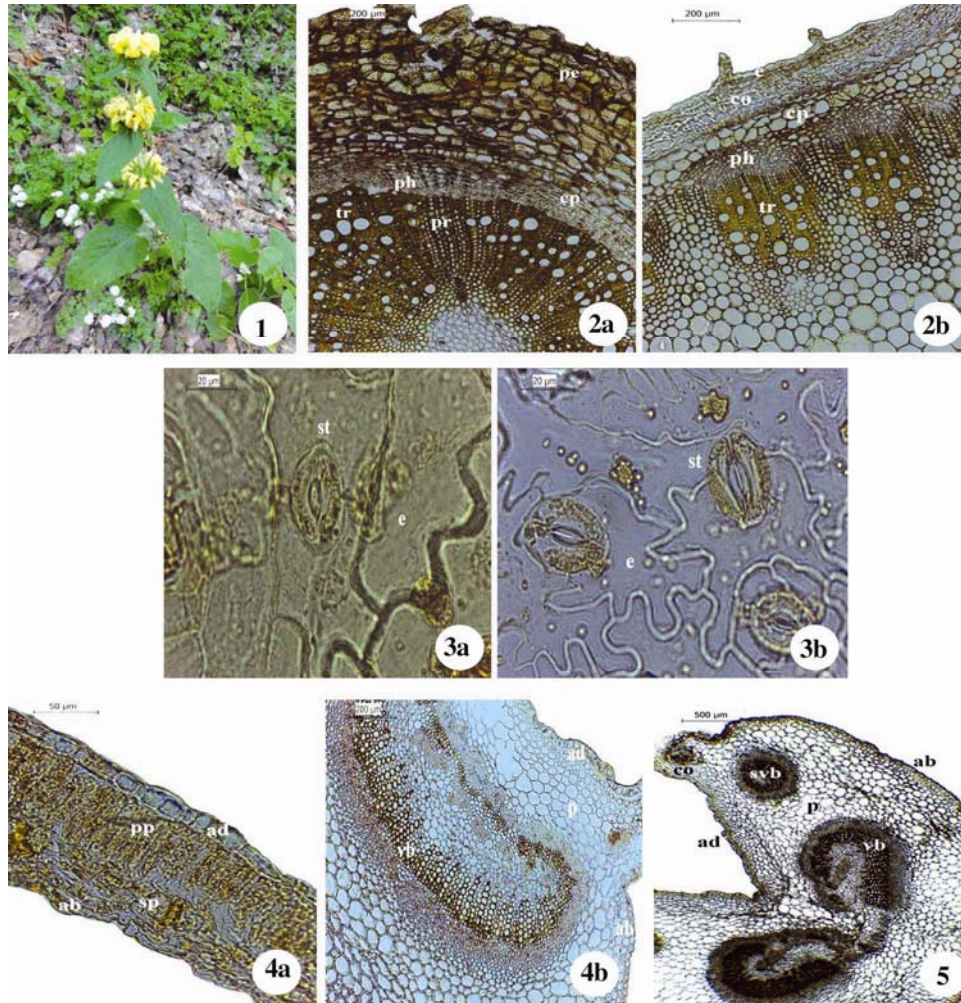
Metcalfe and Chalk (1972), the members of Lamiaceae have quadrangular stem and sclerenchymatous tissue surrounds the phloem groups of vascular bundles. In the transverse section of *P. russeliana*, the stem of this species was quadrangular and there were small groups of sclerenchymatous cells in the outer side of phloem in this study. Quadrangular stem and sclerenchymatous tissue surrounds the phloem groups of vascular bundles were observed in other members of Lamiaceae (Baran and Özdemir 2006, Kahraman *et al.* 2010).

Table 1. Anatomical measurements of various tissues of *P. russeliana*.

Parameter	Width (µm) min.-max.	Height (µm) min.-max.
Root		
Peridermis cell	25.38 - 70.69	15.00 - 46.59
Cortex cell	16.69 - 33.97	12.61 - 24.50
Trachea cell	16.64 - 66.16	16.20 - 53.87
Pith cell	13.44 - 33.39	11.80 - 28.28
Stem		
Epidermis cell	12.5 - 35.29	14.31 - 23.76
Cortex cell	16.00 - 62.72	13.58 - 49.99
Trachea cell	14.08 - 39.68	9.60 - 48.91
Pith cell	32.00 - 125.47	25.60 - 92.80
Leaf		
Adaxial epidermis cell	4.55 - 37.85	8.61 - 19.43
Abaxial epidermis cell	6.24 - 14.40	6.35 - 13.47
Palisade cell	6.62 - 14.25	53.29 - 76.60
Spongy cell	8.30 - 21.67	7.01 - 12.83
Mesophyll region	95.18 - 122.22	
Palisade region	53.29 - 76.60	
Spongy region	38.13 - 51.06	
Midrib trachea	11.90 - 29.83	10.02 - 20.10
Midrib parenchyma	18.74 - 69.29	12.36 - 69.94
Petiole		
Adaxial epidermis cell	7.40 - 14.10	8.30 - 18.10
Abaxial epidermis cell	18.81 - 42.29	8.05 - 19.83
Parenchyma cell	47.73 - 91.07	49.78 - 88.36
Trachea cell	12.80 - 16.00	20.86 - 29.50

In the leaves, the epidermis was single layered on upper and lower surface. There were glandular and non-glandular trichomes on epidermis. Just beneath the upper epidermis cells, there were one row of palisade parenchyma cells (Fig. 4a). The dimensions of the palisade parenchyma cells were 6.62 - 14.25 × 53.29 - 76.60 µm (Table 1). The 3 - 4 rows of spongy parenchyma existed beneath the palisade. The collateral vascular bundle was located in the midrib region (Fig. 4b). There were collenchyma cells on the outer side of phloem in the midrib. The stoma was diasitic and the leaf was bifacial (Fig. 3a,b and 4a). The stoma presented on upper and lower surfaces of the leaf (Fig. 3a,b). The diasitic stoma type was most common in Lamiaceae (Metcalfe and Chalk 1972). Diasitic stoma and bifacial mesophyll type were also observed on the leaf of

another members of Lamiaceae (Akçin *et al.* 2006, Öztürk Çalı 2014). A transverse section of the petiole show that there were glandular and non-glandular trichomes on epidermal cells which were ovoidal-rectangular shapes. Parenchymatic cortex cells were 9-10 layered and their dimensions were $47.73 - 91.07 \times 49.78 - 88.36 \mu\text{m}$ (Table 1). Metcalfe and Chalk (1972) reported that the structure of the vascular bundles in the petiole of the species in the Lamiaceae could be used as a diagnostic character. The structure of petiole shows differences between genera and species.



Figs 1-5: 1. General appearance of *Phlomis russeliana*. 2. The cross section of root (a) and stem (b) of *P. russeliana* pe: periderm, e: epidermis, cp: cortex parenchyma, co: collenchyma, pr: pith rays, ph: phloem, tr: trachea. 3. The surface sections of leaf of *P. russeliana*, (c) adaxial surface, (b) abaxial surface e: epidermis st: stoma. 4. The cross sections of leaf of *P. russeliana*. ad: adaxial epidermis cell, ab: abaxial epidermis cell, p: parenchyma, pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundle of midrib. 5. The cross section of petiole of *P. russeliana*. ad: adaxial epidermis cell, ab: abaxial epidermis cell, p: parenchyma, co: collenchyma, vb: vascular bundle, svb: subsidiary vascular bundle.

Besides, the useful petiole anatomical characters are determined in designated taxonomical structures of some species (Shaheen 2007, Eric *et al.* 2007). There were two-lobed collateral vascular bundles in the center and two small subsidiary bundles in the petiolar wings of *P. russeliana* (Fig. 5). Three or four layered collenchyma could be found under the adaxial and abaxial epidermis, which were 7 - 8 layered in the petiolar wings.

Glandular trichomes were peltate and capitate types. The peltate trichomes had a basal cell supporting a monocellular stalk cell which bears an head composed of four glandular cells. The anticlinal wall of the stalk cell was cutinized (Fig. 6a, b, c). Materials are secreted from the head cells through their outer wall into a space elevation of their common cuticle together with an outer layer of the cell wall. Peltate trichomes on the organs of Lamiaceae, frequently have multicellular secretory head cells with up to 16 head cells, a monocellular stalk and a basal epidermal cell (Hallahan 2000, Kamatou *et al.* 2006). It was found that *P. russeliana* has peltate trichomes consisting of a four celled head in a single circle (Fig. 6a). The present findings are consistent with previous reports (Corsi and Bottega 1999, Hallahan 2000, Kamatou *et al.* 2006).

It was also observed that peltate trichomes were present abundantly on the corolla, leaf surfaces, calyx and petiole whereas the same trichomes were rare on the stem of the plant (Table 2). These findings are in agreement with previous studies (Corsi and Bottega 1999, Serrato-Valenti *et al.* 1997).

Table 2. Data of the trichome types observed and their localization.

Plant organ	Glandular trichome types			Non-glandular trichome types		
	Peltate	Capitate glandular stalk 1-2 cells	Stellate type (with glandular and non-glandular arms coexisting)	Simple type (Unicellular and unbranched type)	Stellate type (with non-glandular arms only)	Shield type
		1	2			
Stem	+	-	-	-	+	-
Adaxial surface of leaf	+	-	-	-	+	-
Abaxial surface of leaf	++	+	-	-	+	-
Petiole	++	+	-	-	+	-
Calyx	+++	-	-	-	+	+
Corolla	+++	+++	++	++	+	-

(-), (+), (++) and (+++) denotes absence, a few and a lot of trichomes, respectively.

Another glandular trichome type is capitate glandular trichome. This type is composed of a basal epidermal cell, unicellular to multicellular stalk cell, a neck cell and a large, cutinized, unicellular secretory head (Fig. 6d, e). The capitate hairs, vary greatly in structure, size, proportions, occurrence on plant organs and manner of secretion. Capitate glandular trichomes are significant taxonomic character of the Lamiaceae (Navarro and El Oualidi 2000). The capitate trichomes were frequently observed in *P. russeliana* especially on the corolla of this plant (Table 2). It was reported that the capitate trichome types were found widely in the many species of Lamiaceae (Serrato-Valenti *et al.* 1997, Bisio *et al.* 1999). However, they greatly vary in structure and size. The findings of the present study are consistent with those of the previous reports. In this species, besides the peltate and capitate glandular trichomes, there were other kind of glandular

trichome: stellate trichomes with 3 - 5 arms, similar in their measures and general structure to the non-glandular ones previously described, but their arms support glandular cells at the end, or these coexist with non-glandular end cells (Fig. 6f). In the present study this glandular trichome type is only observed on the corolla of *P. russeliana*.

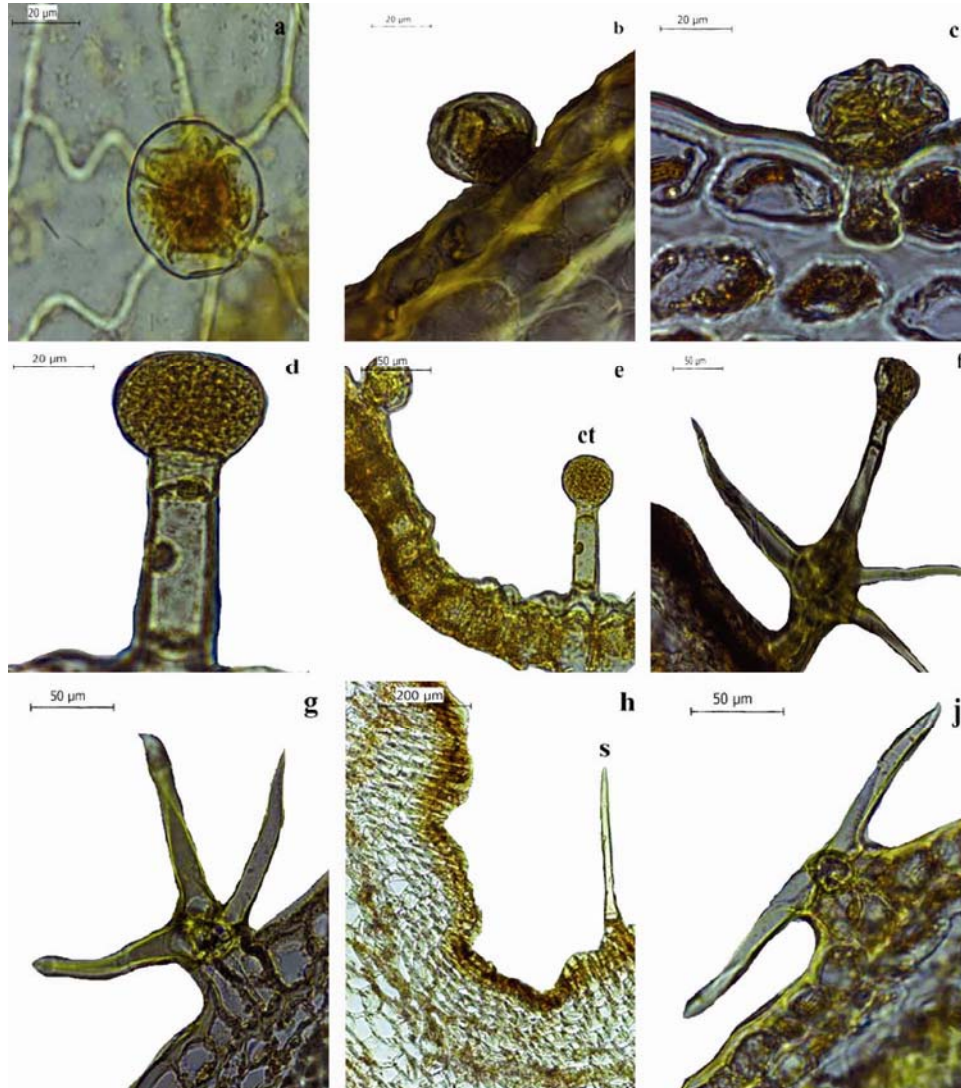


Fig. 6. Peltate glandular trichomes of *P. russeliana* on the leaf (a,c), petiole (b), glandular capitate trichomes on the corolla (d,e), stellate with glandular and non-glandular arms coexisting on the corolla (f), stellate type with non-glandular arms only on the calyx (g), simple non-glandular trichome on the petiole (h) and shield non-glandular trichome on the calyx (j).

Three types of non-glandular trichome were also noticed: stellate type with non-glandular arms only, simple and shield types (Fig. 6g, h, j). Stellate type with non-glandular arms only were determined abundantly on the abaxial surface of leaf, calyx and corolla. Davis (1982) reported that

stellate non-glandular trichomes were more abundantly on the abaxial surface of leaves than adaxial surface of *P. russeliana*. Another non-glandular trichome type was simple type. This type was unicellular, unbranched and acicular shape (Fig. 6h). Like simple non-glandular trichome type, shield type trichome was observed in another member of Lamiaceae (Özdemir 2011).

The taxonomic significance of the structure of trichomes is well known in the Lamiaceae (Metcalf and Chalk 1972, Kahraman *et al.* 2010). The morphology, distribution and frequency of glandular trichomes are used as discriminative characters at subfamilial level in the Lamiaceae (Ascensao *et al.* 1995). In the present study, three types of glandular trichomes were determined in *P. russeliana* species. These were peltate glandular, capitate glandular and stellate trichomes with 3 - 5 arms, which support glandular cells at the end, or these coexist with non-glandular end cells.

In conclusion, anatomical features and trichome morphology of *P. russeliana* were presented for the first time in this study. According to these results, anatomical features and trichome properties provide useful characters for distinguishing species in the *Phlomis* genus.

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