



Research Article

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Glandular and E glandular Trichomes of Endemic *Ballota glandulosissima* (Lamiaceae)

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Abstract

The aim of this study is to determine the taxonomic importance of the glandular and e glandular trichomes on the vegetative and reproductive organs of *Ballota glandulosissima*. This species is endemic to Türkiye and the Eastern Mediterranean element. It is among the medicinal plants of Türkiye. On the vegetative and reproductive organs of the studied species, both glandular and e glandular trichomes were seen. These trichomes were examined using light and stereo microscopes. The glandular trichomes were divided into peltate and capitate. The e glandular trichomes were grouped as branched (stellate, bicellular, and multicellular) and unbranched (simple unbranched e glandular trichomes). The stalk cell number of capitate glandular trichomes shows changes. According to the number of stalk cells, capitate glandular trichomes were grouped into uni, bi, tri, and multi-cellular trichomes. In the taxon, dendroid trichomes were not observed on the vegetative and reproductive organs. The stellate trichomes were dense on the upper and lower surfaces of the leaf, and calyx teeth, while capitate glandular trichomes were dense on the petiole, stem, leaf, and outside part of the calyx, and calyx teeth. Peltate glandular trichomes are dense on the calyx and corolla. Especially, the capitate glandular and stellate trichomes are found to have significant taxonomic characteristics in distinguishing this species.

Keywords: *Ballota glandulosissima*, endemic, trichome morphology

Endemik *Ballota glandulosissima* (Lamiaceae)'nın Salgı ve Örtü Tüyleri

Özet

Bu çalışmanın amacı, *Ballota glandulosissima*'nın vejetatif ve üreme organları üzerindeki örtü ve salgı tüylerinin taksonomik önemini belirlemektir. Bu tür Türkiye'de endemiktir ve Doğu Akdeniz elementidir. Türkiye'nin tıbbi bitkileri arasında yer almaktadır. İncelenen türün vejetatif ve üreme organları üzerinde hem örtü hemde salgı tüyleri görüldü. Bu tüyler ışık ve stereo mikroskoplar kullanılarak incelendi. Salgı tüyleri peltat ve kapitat olarak ayrıldı. Örtü tüyleri dallanmış (yıldızsı, iki hücreli ve çok hücreli) ve dallanmamış (basit dallanmamış örtü tüyleri) olarak gruplandırıldı. Kapitat salgı tüylerinin sap hücre sayıları değişiklik gösterir. Sap hücre sayılarına göre kapitat salgı tüyleri bir, iki, üç ve çok hücreli tüyler olarak gruplandırıldı. Taksonda, dendroid tüyler vejetatif ve üreme organları üzerinde gözlenemedi. Kapitat salgı tüyleri gövde, petiyol, yaprak ve kaliksin dış kısmında ve kaliks dişlerinde yoğunken, yıldızsı tüyler ise yaprağın alt ve üst yüzeyinde ve kaliks dişleri üzerinde yoğundur. Peltat salgı tüyleri kaliks ve korolla üzerinde yoğundur. Özellikle yıldızsı ve kapitat salgı tüylerinin bu türün ayırt edilmesinde önemli taksonomik karakterlere sahip olduğu tespit edilmiştir.

Anahtar kelimeler: *Ballota glandulosissima*, endemik, tüy morfolojisi

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INTRODUCTION

Ballota L. genus (Lamiaceae) is represented by 35 species in temperate and subtropical regions of the world and 12 species and 8 subspecies in Türkiye. Nine of these species are endemic to Türkiye, and the rate of endemism is quite high. This genus has high diversity in the Mediterranean Basin (Davis & Doroszenko 1982; Güner 2012; Morteza-Semnani & Ghanbarimasir 2019).

The fresh leaves, fruits, and seeds of *Ballota* species are very well known by the public. These species are frequently used for the treatment of wounds, burns, cough, insomnia, upper respiratory inflammation, diuretic, headache, nausea, eye infections, and hemorrhoids among people in Turkish folk medicine (Yeşilada et al. 1995; Baytop 1999; Dülger & Dülger 2012; 2017). Since *Ballota* species contain terpenoids, flavonoids, essential oils, saponins, tannins, and phenylpropanoids, these species have antiulcer, anticancer, antispasmodic, cholerectic, antihemorrhoidal, antioxidant, antibacterial, antiviral, antilisterial, diuretic, anti-inflammatory, anti-diabetic, sedative and astringent properties (Baytop 1999; Özbek et al. 2004; Saltan Çitoğlu et al. 2004; 2005; Sever Yılmaz et al. 2015; Riccobono et al. 2016; Rigano et al. 2017; Doğan & Duman 2019; Ağca et al. 2021). It has been reported that *B. glandulosissima* Hub.-Mor. & Patzak has strong antioxidant activity due to its high flavonoid content (Saltan Çitoğlu et al. 2004; Sever Yılmaz et al. 2015).

In many species of the Lamiaceae family, both glandular and eglandular trichomes were observed. Trichomes are used as significant taxonomic characters in distinguishing species and genera in the Lamiaceae family (Kandemir 2011; El-Deen Osman 2012; Seyedi & Salmaki 2015; Giuliani et al. 2021; Koçak & Kandemir 2023a; 2023b). Glandular trichomes are specialized secretory structures that vary in size, form, location and function in different plant species. These trichomes are generally separated into capitate and peltate (Werker et al. 1985; Navarro & El Oualidi 2000). The capitate trichomes have great importance in the systematic of the Lamiaceae family since these trichomes play an important role in the pollinization of family species (Kandemir 2011; Navarro & El Oualidi 2000). Generally, this type of trichomes has a longer stalk and a smaller head. However, peltate glandular trichomes have a short stalk or no stalk and a large secretory head which consists of 4–18 cells arranged in one or two concentric circles (Werker et al. 1985). Eglandular trichomes are also more common in the Lamiaceae family, and these trichomes were divided into four types: simple unicellular, simple multicellular, branched unicellular, and branched multicellular (Cantino 1990). Stellate trichomes are characteristic of some genera in the Lamiaceae family, such as *Marrubium* and *Ballota*.

It was aimed to determine taxonomic significance of eglandular and glandular trichomes on the vegetative and reproductive organs of *Ballota glandulosissima*. The other aim of this study is to separate the taxa *B. glandulosissima* and *B. saxatilis* Sieber ex C.Presl, which are very similar to each other according to their trichome micromorphology.

MATERIAL AND METHOD

The plant samples were collected from their habitat during the flowering period. The plant samples were described according to Davis and Doroszenko (1982). For the light microscopy observations (LM), some fresh plant samples were fixed in 70% alcohol solution for trichome investigations. The cross and surface sections of stem, leaf, petiole, calyx, and corolla were taken by hand using razor blades. Then preparations were made using Sartur reagent (Çelebioğlu & Baytop 1949). For the stereo microscopy observations, surface sections were taken from fresh plant and alcohol materials, and preparations were made. Preparations were photographed with a binocular light microscope,

with an equipped camera and 40X objectives (Leica ICC50 HD). The general classification of trichomes was done according to Cantino (1990) and Navarro & El-Qualidi (2000). The localities where the taxon was collected were listed below:

C3 Antalya: Finike Turuçoova-Elmalı road, machine areas, 175 m., 12.7.2021, N.Kandemir

C3 Antalya: Finike-Elmalı road, Arif Village, rocky areas, 700 m., 13.7.2021, N.Kandemir

C3 Antalya: Finike inner city, road to Finike Castle, 15 m., 13. 7.2021, N.Kandemir

RESULTS AND DISCUSSION

Morphology and localization of eglandular trichomes

Eglandular trichomes are common in the Lamiaceae family, where they may be unbranched and branched. On the vegetative and reproductive organs of this taxon, the two main different types of eglandular trichomes were seen: unbranched (simple unbranched eglandular trichomes) and branched (stellate, bicellular, and multicellular trichomes). In this study, simple unbranched trichomes were divided into four forms based on the shape and cell number: unicellular, bicellular, tricellular, and multicellular. While unbranched multicellular trichomes were rarely obtained on the stem, petiole, leaf, fruit, outside part of the calyx, calyx teeth, and corolla of this species, unbranched uni, bi and tricellular trichomes were densely obtained on the stem, petiole, calyx and corolla of *B. glandulosissima* (Figure 1A-D, Figure 2A-B and D, Figure 3A and 3E). There are unbranched unicellular trichomes between the vessels of the calyx (Figure 3A-E). These kinds of eglandular trichomes were reported in *B. kaiseri* Tachk., *B. undulata* (Sieber ex Fresen.) Benth., *B. saxatilis* and *B. pseudodictamnus* (L.) Benth (El-Deen Osman 2012) and on the surfaces of both vegetative and generative organs of *B. acetabulosa* (L.) Benth. (Giuliani et al. 2021).

In this species, three types of branched eglandular trichomes were recognized. The branched eglandular trichomes are bicellular, multicellular, and stellate trichomes. On the upper and lower leaves and the calyx teeth, multicellular and bicellular branched eglandular trichomes were obtained. These trichomes were reported in *B. kaiseri*, *B. undulata* and *B. saxatilis* (El-Deen Osman 2012).

Stellate trichomes are star-shaped and multicellular (Xiang et al., 2010). *Ballota* taxa were characterized by stellate trichomes on the upper and lower surfaces of leaves. El-Deen Osman (2012) reported the presence of stellate trichomes in *Ballota undulata*, *B. kaiseri* and *B. saxatilis*. These trichomes are densely seen on upper and lower surfaces, between the veins of the leaves, outside part of the calyx, and calyx teeth of this taxon (Figure 2C, Figure 3F and 3H). Especially, this type of trichomes is highly dense on the calyx and between the veins of the leaves of the studied taxon. The short stellate trichomes are densely seen only in the ridge parts of corolla's upper lip. However, long stellate trichomes were rare and seen only in the upper lip of the corolla. On the petiole and stem of the studied taxon, this type of trichome is not observed (Table 2). Stellate trichomes were reported in other *Ballota* taxa (*B. kaiseri*, *B. undulata* and *B. saxatilis*) (El-Deen Osman 2012). Generally, eglandular ones are mainly situated on the leaf and side of the calyx of the *B. glandulosissima*. In the studied taxon, stellate type of eglandular trichomes is abundance observed on the adaxial and abaxial surfaces of the leaf (Figure 2C).

In the studied taxon, dendroid trichomes were not seen on vegetative and reproductive organs. At the same time, there were no dendroid trichomes on vegetative and generative organs of *Ballota* taxa (excluding *B. acetabulosa*, *B. pseudodictamnus* (L.) Benth. ssp. *lycia* Hub-Mor and *B. cristata* P.H.Davis) distributed in Türkiye (Tezcan 2001). Also, dendroid trichomes were not observed in

some *Ballota* species (*B. kaiseri*, *B. saxatilis*, *B. undulata*) distributed in Egypt. But these trichomes were observed in *B. pseudodictamnus* and *B. damascens* growing in Egypt (El-Deen Osman 2012) and on the calyx and the corolla abaxial side of *B. acetabulosa* (Giuliani et al. 2021). The presence of both dendroid and stellate trichomes is of great importance in the separation of *Ballota* taxa (El-Deen Osman 2012). El-Deen Osman (2012) distinguished some *Ballota* taxa (*B. kaiseri*, *B. saxatilis*, *B. undulata* and *B. pseudodictamnus*) distributed in Egypt according to the presence or absence of dendroid and stellate trichomes.

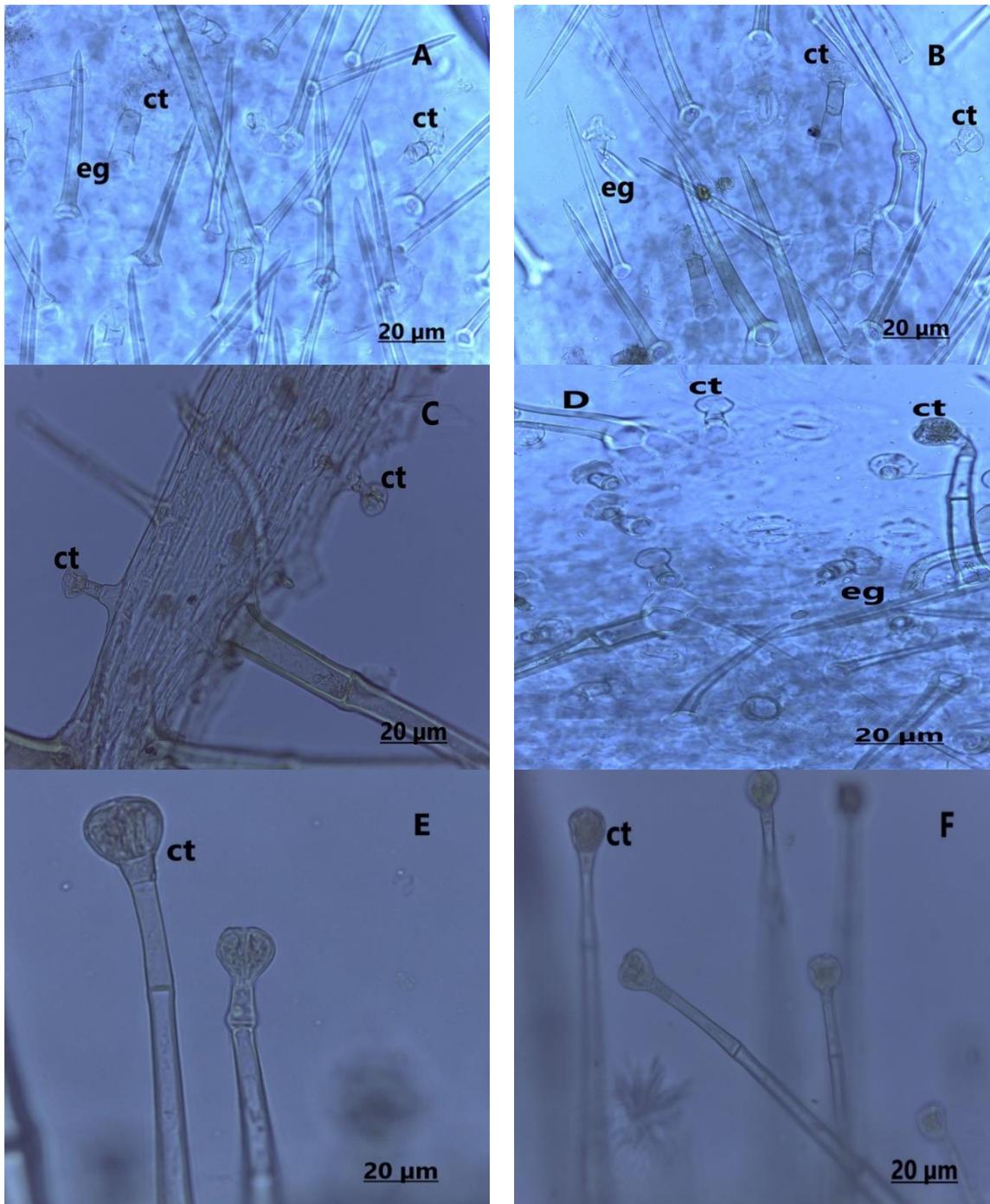


Figure 1. Continues.

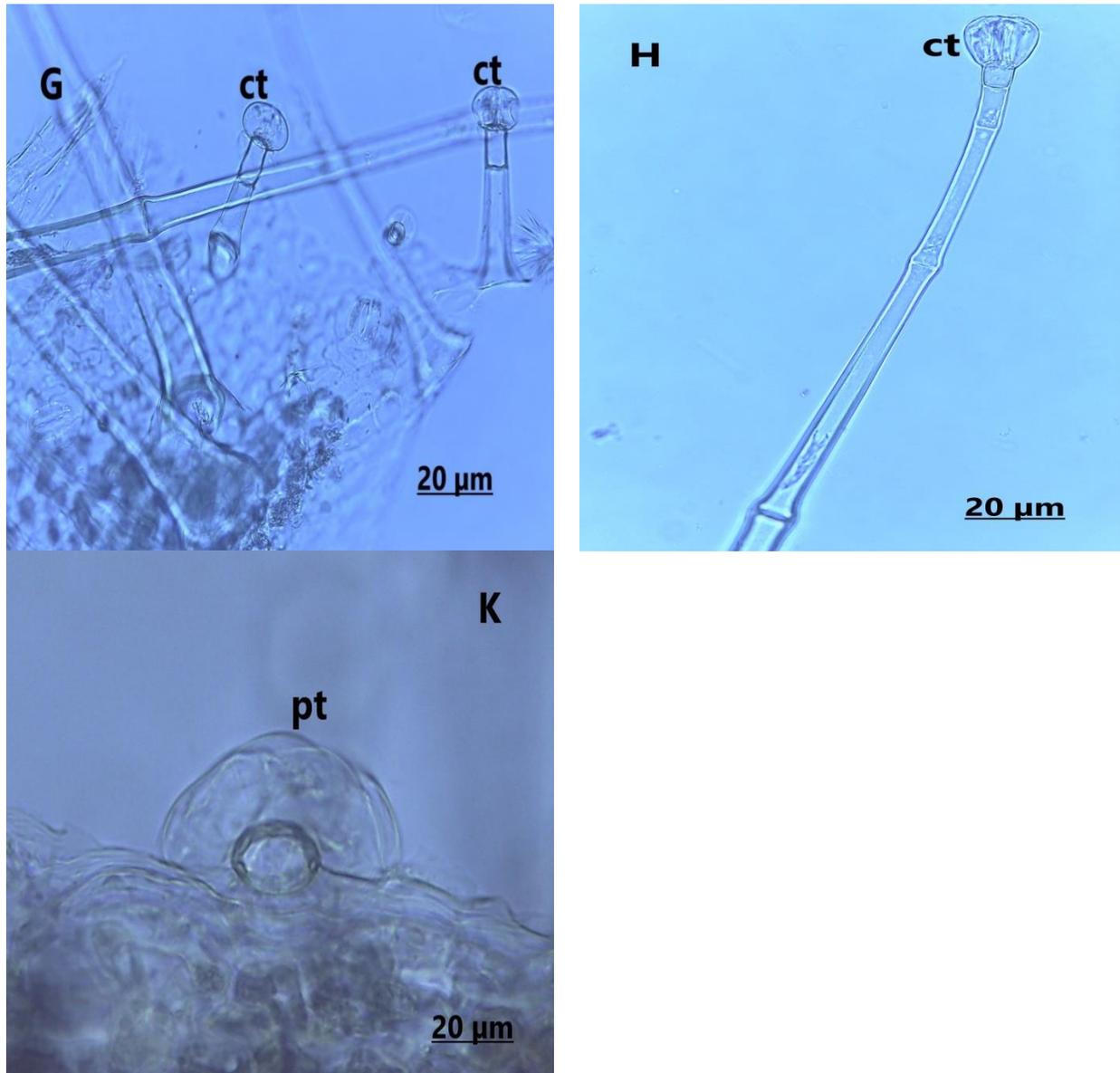


Figure 1. Glandular and eglandular trichome types on the stem. **A:** Unicellular eglandular and unicellular stalked with unicellular head capitate trichomes, **B:** Unicellular and bicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes, **C:** Multicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes, **D:** Bicellular eglandular and unicellular, tricellular stalked with unicellular, bicellular head capitate trichomes, **E:** Bicellular, tricellular stalked with unicellular, bicellular head capitate trichomes, **F:** Multicellular stalked with unicellular, bicellular head capitate trichomes, **G:** Bicellular stalked with bicellular head capitate trichomes, **H:** Multicellular stalked with bicellular head capitate trichomes, **K:** Peltate trichomes. **eg.** eglandular trichomes, **ct.** capitate trichomes, **pt.** peltate trichomes.

Morphology and localization of capitate glandular trichomes

Capitate glandular trichomes contain significant taxonomic characters since these trichomes play a significant role in the pollination of the Lamiaceae species (Navarro & El Oualidi 2000). These trichomes often vary in size, structure, abundance, and distribution among taxa. The capitate trichomes were detected abundantly on the vegetative and reproductive organs of *B.*

glandulosissima (Figure 1A-H, Figure 2A-B and 2D-H, Figure 3A-E and 3G-L). Specially, these trichomes were abundant on the stem, petiole, leaf, and calyx. In this study, capitate glandular trichomes were divided into four subtypes, which are unicellular stalked (subtype I), bicellular stalked (subtype II), tricellular stalked (subtype III), and multicellular stalked (subtype IV) stalked (Table 1). The number of head cells in the capitate trichomes can vary.

Unicellular stalked capitate glandular trichomes (Subtype I): The unicellular stalked capitate trichomes have a stalk cell and uni, bi, tri, and multicellular head. In this study, unicellular stalked capitate trichomes are uni and bicellular headed. The first form has a unicellular head and is present on the stem, petiol, leaf, calyx, and corolla of *B. glandulosissima* (Figures 1A-D, Figure 2A-B and 2D, Figure 3B, 3D-E). These trichomes are dense and present on the petiole and stem of this species. The second form has a bicellular head and is abundantly observed on the stem, petiole, and calyx of *B. glandulosissima* (Figure 1A-D; Figure 2A-B, Figure 3B and 3E). This type is rather rare on the leaf of *B. glandulosissima* (Figure 3D). The third form presents tricellular head and is seen on the stem, and petiole of *B. glandulosissima* (Figure 1B). The fourth form possesses a multicellular head, which is rarely observed on the stem of this species. The unicellular stalked capitate trichomes were found in *B. kaiseri*, *B. undulata*, *B. damascens* Boiss., *B. saxatilis*, *B. pseudodictamnus* (El-Deen Osman 2012). Moreover, these trichomes were detected on the vein system of the whole plant, especially on the leaf and corolla abaxial sides of *B. acetabulosa* (Giuliani et al. 2021). The unicellular stalked capitate trichomes were quite widespread in all Lamiaceae taxa (Giuliani & Maleci Bini 2008).

Bicellular stalked capitate trichomes (Subtype II): In this type of trichomes, stalk is two celled and the head has one, two and three cells. Unicellular, bicellular headed capitate trichomes are dense and observed on the stems, petiole, leaf, calyx, and calyx teeth of this species (Figure 1C, 1F-G, Figure 2A-B, 2D and 2H, Figure 3A-D and 3K). Tricellular headed capitate trichomes were obtained on the petiole of *B. glandulosissima*. Bicellular stalked capitate trichomes were observed in some *Ballota* taxa (*B. kaiseri*, *B. undulata*, *B. damascens*, *B. saxatilis*, and *B. pseudodictamnus*) (El-Deen Osman 2012) and on leaves and the calyx abaxial side of *B. acetabulosa* (Giuliani et al. 2021). These kinds of capitate trichomes were found in some members of the genera *Salvia*, *Stachys*, *Sideritis*, and *Scutellaria* (Giuliani & Maleci Bini 2008; Giuliani et al. 2020a; 2020b).

Tricellular stalked capitate trichomes (Subtype III): Subtype III capitate trichomes, which have three celled stalk and unicellular or bicellular head, are observed on the petiole, stem, calyx surface, calyx teeth, and leaf of *B. glandulosissima* (Figure 1D-F, Figure 2E-G, Figure 3A, 3D, 3H and 3L). In particular, these capitate trichomes are densely obtained on the calyx teeth, leaf margins, and leaf veins (Figure 3D and 3L). The kind of trichomes was reported in *B. kaiseri*, *B. undulata*, *B. damascens*, *B. saxatilis*, and *B. pseudodictamnus* (El-Deen Osman 2012).

Multicellular stalked capitate trichomes (Subtype IV): This type of trichomes has stalk multicellular celled (four, five, and six celled). The head part of this trichome is one or two celled. Multicellular stalked (subtype IV) capitate trichomes are densely seen on the stem, petiole, calyx teeth, and margin and veins of the leaf of *B. glandulosissima* (Figures 1F and 1H, Figure 2E-H, Figure 3D and 3G). But these trichomes were rarely found on the leaf lamina and corolla. This kind

of trichomes is found with stellate trichomes on the leaves of *B. glandulosissima*. Multicellular stalked capitate trichomes were recorded in only *B. saxatilis* and *B. undulata* (El-Deen Osman 2012). Also, these trichomes were obtained on the leaf adaxial side, on the sepal abaxial side and on floral peduncle *B. acetabulosa* (Giuliani et al. 2021) and on the reproductive organs of other Lamiaceae taxa (Giuliani & Maleci Bini 2008).

Morphology and localization peltate glandular trichomes

Peltate trichomes are also known as labiate type trichomes. In this study, peltate trichomes were scarcely seen on the stem, petiole and leaf while peltate trichomes were densely seen on the calyx and corolla (Figure 1K, Figure 2H and 2K, Figure 3M). These trichomes in the lower surface of the leaf of *B. glandulosissima* have 8 cells while these trichomes in the stem of *B. glandulosissima* have 4 celled. In the Lamiaceae family, the presence of peltate glandular trichomes was noted by some researchers (Metcalf & Chalk 1950; Cantino 1990; Werker et al. 1985; Salmaki et al. 2009; Kandemir 2011; Koçak & Kandemir 2023a; 2023b). Peltate trichomes were detected in *B. undulata*, *B. saxatilis*, and *B. kaiseri* (El-Deen Osman 2012). Eight-celled peltate trichomes were scarce on the leaves and abundant on calyx and corolla abaxial surfaces of *Scutellaria brevibracteata* subsp. *subvelutina* (Rech. f.) Greuter & Burdet (Giuliani et al. 2020a) and *B. acetabulosa* (L.) Benth (Giuliani et al. 2021).

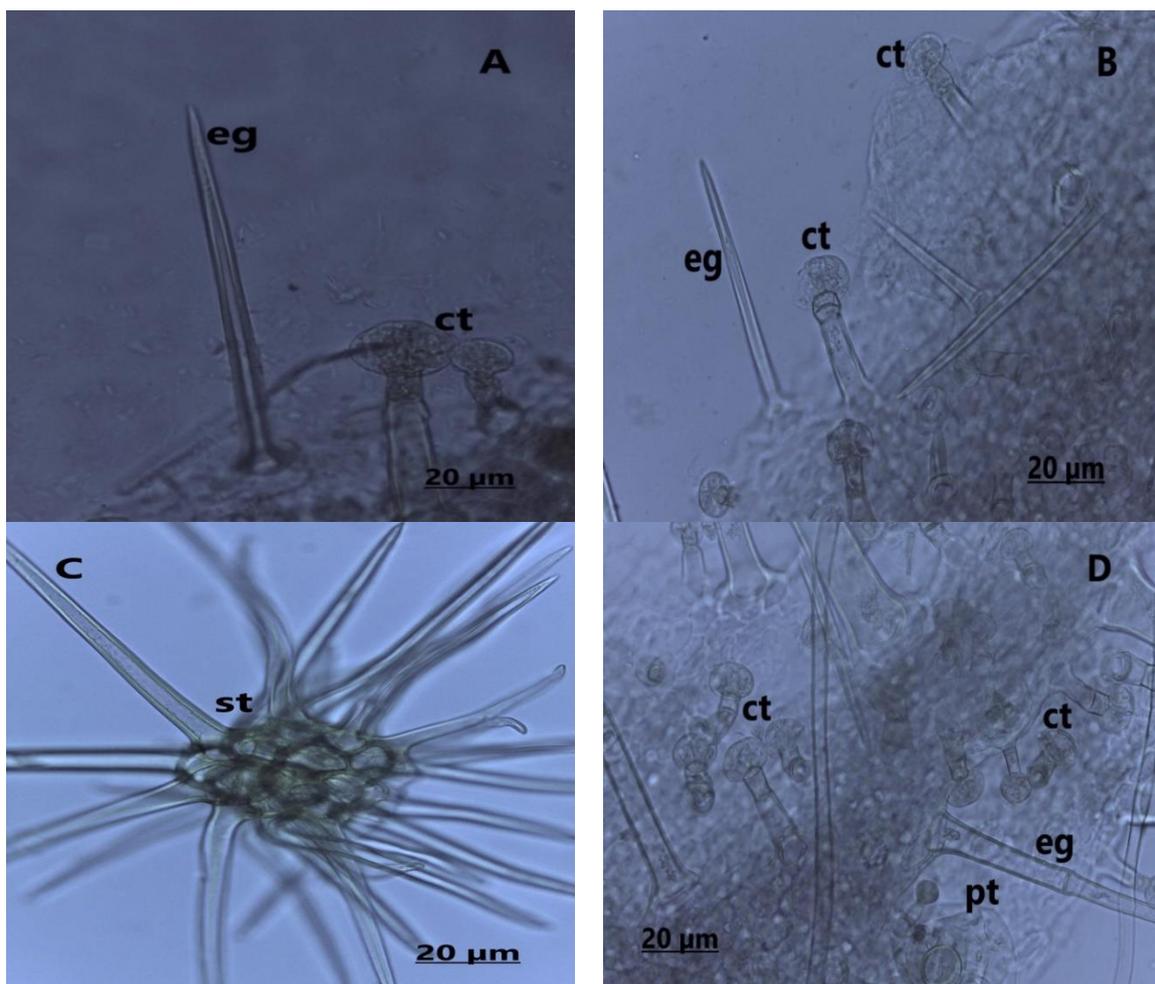


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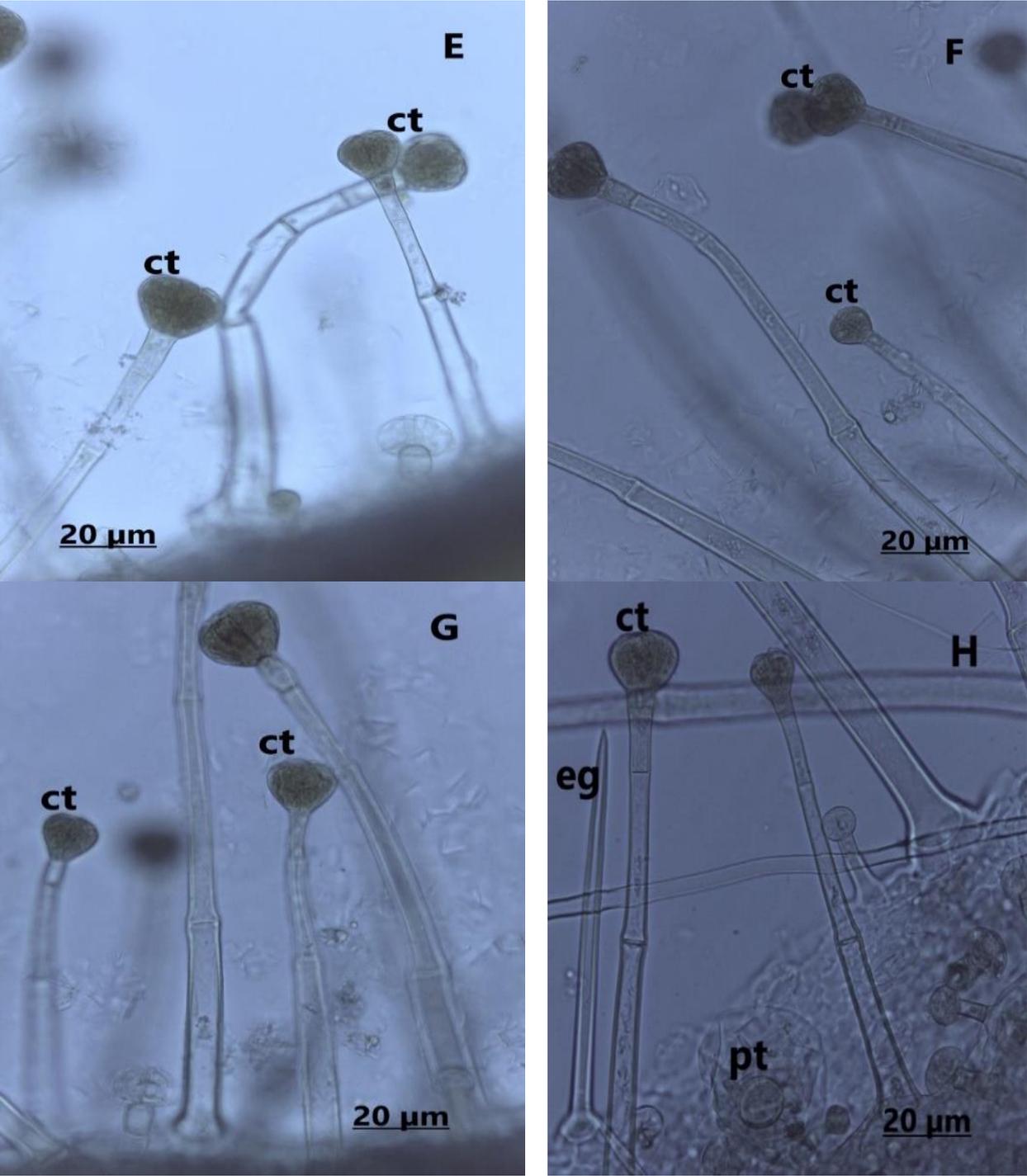


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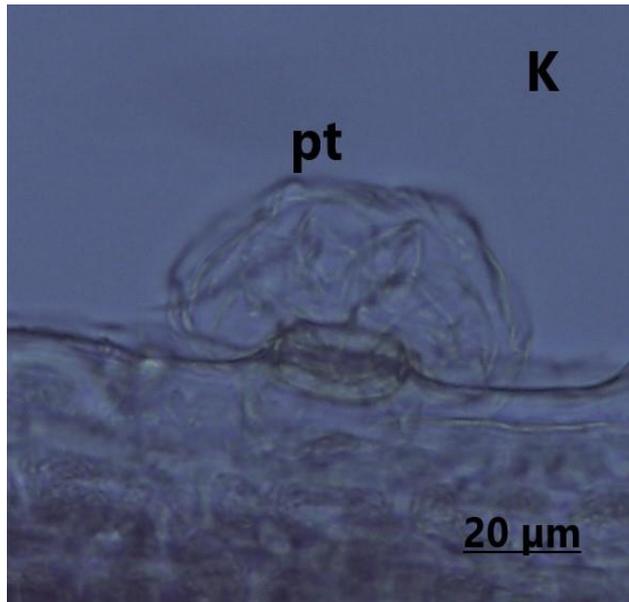


Figure 2. Glandular and eglandular trichome types on the petiole and leaves. **A:** Unicellular eglandular and unicellular long stalked with bicellular head capitate trichomes on the petiole, **B:** Unicellular eglandular and unicellular, bicellular stalked with unicellular head capitate trichomes on the petiole, **C:** Stellate trichomes on the leaf, **D:** Bicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes on the leaf, **E:** Multicellular stalked with bicellular head capitate trichomes on the petiole, **F:** Multicellular stalked with bicellular head capitate trichomes on the leaf, **G:** Tricellular stalked with bicellular head capitate trichomes on the leaf, **H:** Bicellular, tricellular, multicellular stalked with bicellular head capitate, peltate and unicellular trichomes on the leaf, **K:** Peltate trichomes on the petiole. **eg.** eglandular trichomes, **st.** stellate trichomes, **ct.** capitate trichomes, **pt.** peltate trichomes.

Trichome types on the vegetative and reproductive organs of the studied taxon are given in Tables 1 and 2.

Table 1. Glandular trichomes types and distribution on vegetative and reproductive organs of *Ballota glandulosissima*.

Organs of <i>Ballota glandulosissima</i>	Subtype I capitate	Subtype II capitate	Subtype III capitate	Subtype IV capitate	Peltate trichomes
Stem	+++	–	+	++	+
Petiole	++++	+	+	++	+
Margin and veins of leaf	++	++	++	+++	+
Lamina of leaf	++	++	+	+	+
Calyx teeth	++	–	++	+++	+
Outer surface of calyx	++	–	+	++	++
Corolla	++	–	–	+	++

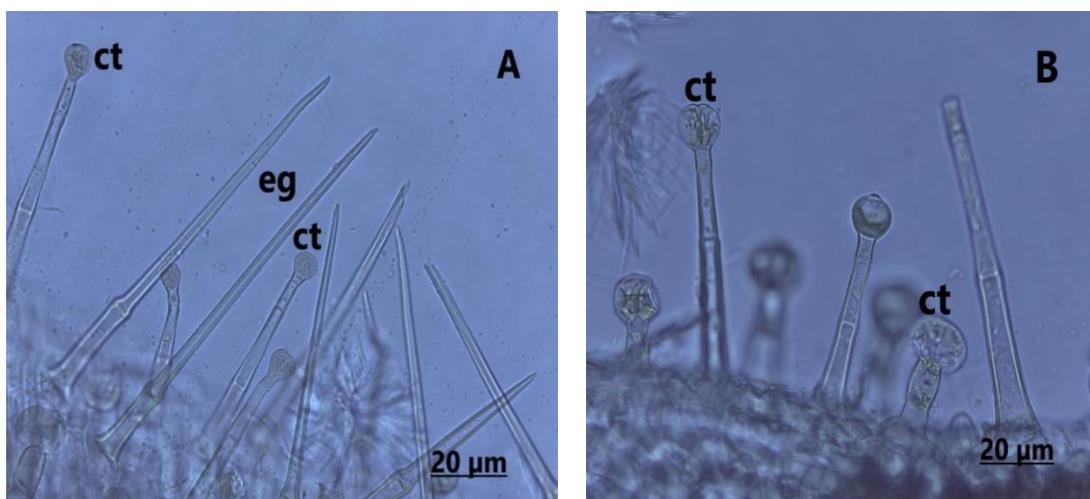
–: absent; +: scarce; ++: dense; +++: abundant

Table 2. Eglanular trichomes types and distribution on vegetative and reproductive organs of *Ballota glandulosissima*.

Organs of <i>Ballota glandulosissima</i>	Unbranched unicellular	Unbranched bicellular	Unbranched tricellular	Unbranched multicellular	Branched bicellular	Branched multi cellular	Stellate trichomes	Dendroid trichomes
Stem	++	++	++	+	-	-	-	-
Petiole	++	++	++	+	-	-	-	-
Margin and veins of leaf	-	-	-	-	+	+	+++	-
Lamina of leaf	-	-	-	+	+	+	+++	-
Calyx teeth	++	++	++	+	+	+	+++	-
Outer surface of calyx	++	++	++	+	-	-	+++	-
Corolla	++	++	++	+	-	-	++	-

In conclusion, on the vegetative and reproductive organs of the studied taxon, four main trichome types (peltate glandular, capitate glandular, unbranched and branched eglandular) were seen. Peltate glandular trichomes were rare on the vegetative and reproductive organs of the studied taxon, while capitate glandular trichomes (especially on the stem, petiole, leaf, and calyx surface, calyx teeth) and stellate trichomes (especially on the lower and upper surfaces of leaves, calyx and corolla) were dense on the vegetative and reproductive organs of studied taxon.

Trichome micromorphology provides significant data distinguishing sections and species. Our findings revealed that the density, type and distribution of trichomes provide important characters in distinguishing *B. glandulosissima* and *B. saxatilis*, which are morphologically very similar to each other. Glandular trichomes are similar in almost all *Ballota* species. However, glandular trichomes were denser in *B. glandulosissima* than in other *Ballota* species. The stellate trichomes are denser in *B. saxatilis* than in *B. glandulosissima*. On the other hand, stellate trichomes were not found in the petiole of *B. glandulosissima*, while stellate trichomes were found in the petiole of *B. saxatilis* (El-Deen Osman 2012). *B. glandulosissima* can be distinguished from *B. saxatilis* by the trichomes features mentioned above.

**Figure 3.** continues.

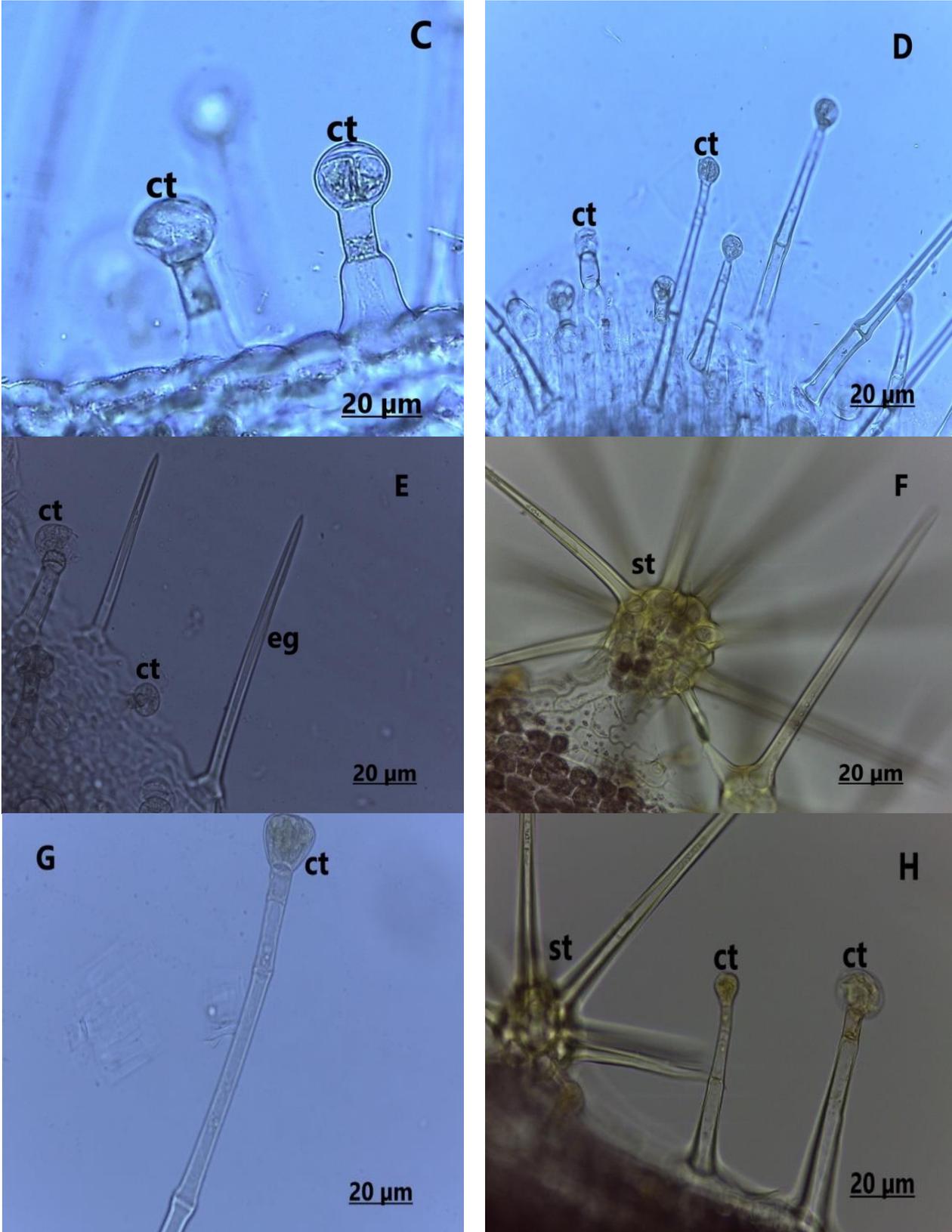


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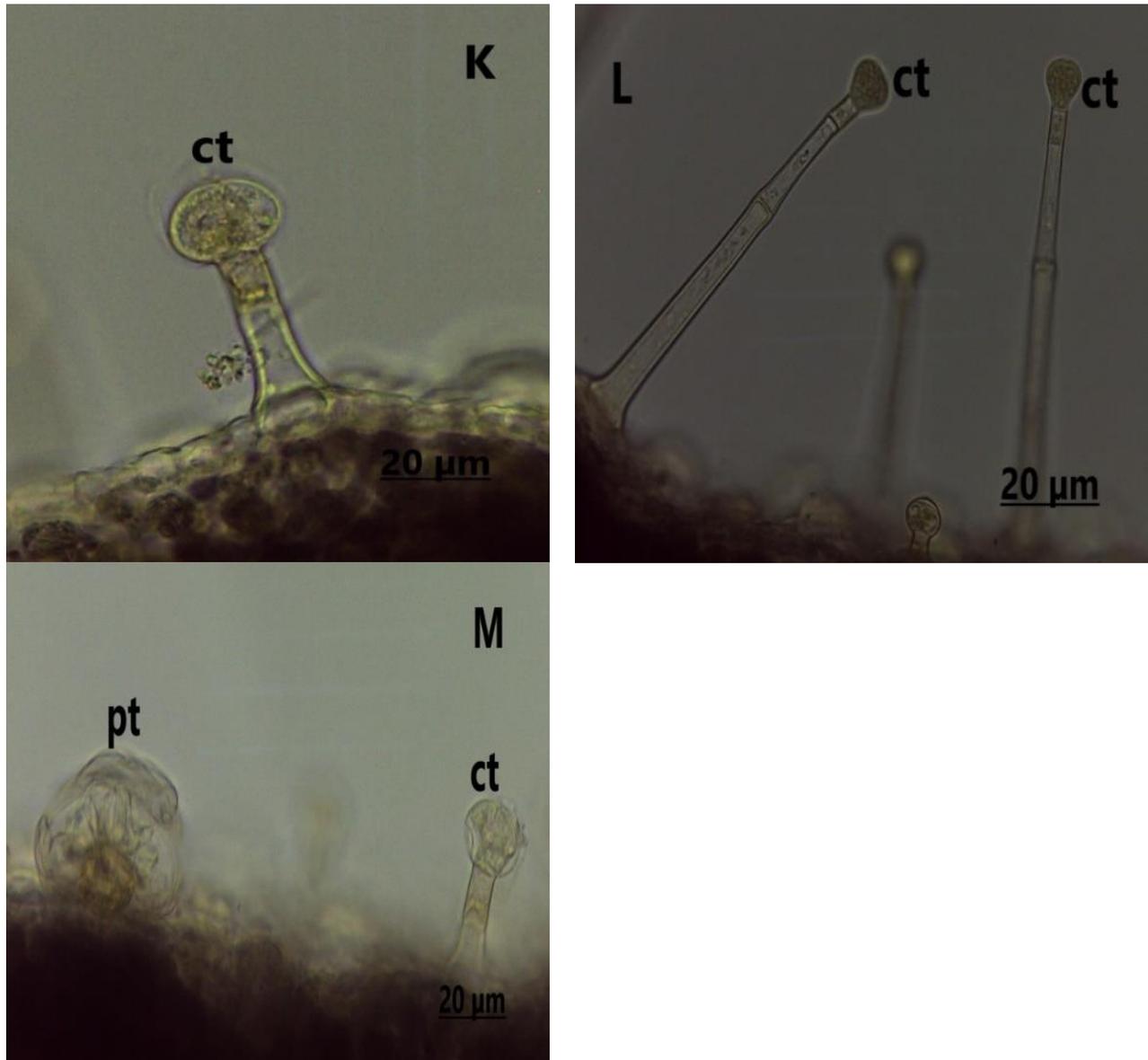


Figure 3. Glandular and eglandular trichome types on the calyx. **A:** Unicellular eglandular and unicellular, bicellular stalked with unicellular, bicellular head capitate trichomes, **B:** Unicellular and bicellular stalked with unicellular, bicellular head capitate trichomes, **C:** Unicellular, bicellular stalked with uni,bicellular head capitate trichomes, **D:** Unicellular, bicellular, tricellular stalked with uni, bicellular head capitate trichomes, **E:** Unicellular eglandular and unicellular, bicellular stalked with unicellular head capitate trichomes, **F:** Stellate trichomes, **G:** Multicellular stalked with bicellular head capitate trichomes, **H:** Bicellular, tricellular stalked with unicellular head capitate, stellate trichomes, **K:** Bicellular stalked with bicellular head capitate, **L:** Unicellular, tricellular stalked with unicellular, bicellular head capitate trichomes, **M:** Peltate and unicellular stalked with unicellular head capitate trichomes. **eg.** eglandular trichomes, **st.** stellate trichomes, **ct.** capitate trichomes, **pt.** peltate trichomes.

AUTHOR CONTRIBUTION STATEMENT

In this study; the study idea and design, data collection, analysis and interpretation of the results, and drafting of the article were made by Nezahat Kandemir.

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