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*Original Article*

# Morphological and Anatomical Study of *Phlomis bruguieri* (Lamiaceae), in the Iraqi Kurdistan Region

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## ABSTRACT

*Phlomis bruguieri* belongs to the Lamiaceae family. Plant specimens were collected from different locations in Iraqi Kurdistan, Sulaymaniyah-Garmian Province. A comprehensive biosystematics evaluation of *P. bruguieri* has never been conducted. Therefore, the objective of this study is to analyze the morphological and anatomical characteristics of *P. bruguieri*. This study states a significant role of the studied plant parts and whether they play a role in the classification of plants. The morphological characteristics of the vegetative parts were determined using fresh plant samples. For anatomical investigations, specimens were preserved in alcohol 70%. Transverse sections obtained from the species' roots, stems, leaves, and petioles were observed under a light microscope. The morphological findings were compared to the species descriptions in the Iranica and Turkey Floras. The anatomical findings were compared with earlier studies on the genus *Phlomis* and other Lamiaceae family members. The results of the study revealed that the significant taxonomic information is provided by the anatomical characters, which include the number of pith ray layers in the root, the thickness of the xylem and phloem tissues, the quantity of collenchyma and parenchyma layers, the thickness of the cortex tissue in the stem, the shape and number of vascular bundles within the midrib and petiole, the leaf and stomata kind, the shape of the crystal, and the trichome.

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*Keywords: Plant, Lamiaceae, Anatomy, Taxonomy, Iraq.*

## 1 Introduction

The Lamiaceae (Labiatae) family, which belongs to the Lamiales order, comprises a broad, cosmopolitan family with more than 230 genera and 7000 species [1-4]. The family's importance derives from its decorative and therapeutic qualities[5-7]. The majority of members of the Lamiaceae family are annual or perennial herbs with rectangular stems. Leaves are aromatic and composed of volatile oils; they are typically simple and arranged oppositely to one another. In addition to having a verticillastrate spiral pattern, the flowers are open-mouthed in shape, comprise 2 or 4 stamens and 5-lobed calyx. The aerial parts of the species in the Lamiaceae family are covered with numerous glandular trichomes. The fruit typically appears as a dry nutlet [8-10].

According to taxonomy, the genus *Phlomis* is a member of the tribe Phlomideae, which also comprises the genera (*Phlomis* and *Phlomoides*), the subfamily Lamioideae, the family Lamiaceae, and the order Lamiales [11, 12]. There are approximately one hundred species within the genus *Phlomis* [13], most of which are found in the northern areas of warm climates, particularly in Europe and Asia. In Iraq, this genus is estimated to have at least 6 to 9 species, the majority of which are found in the country's northern regions[14]. Small shrubs or perennial plants with stellate to dendroid hairs that might be pilose or tomentose, glandular or not. Leaves entire, crenate-dentate, or undivided. In the axil of flowering leaves, verticillasters are few to many-flowered, crowded or distant, and lack bracteoles, which can be few or numerous, subulate to ovate. The calyx is tubular or narrowly campanulate, with five teeth, five to ten veins, and equal or unequal teeth. Corolla is 2-lipped, purple, pink, or yellow (sometimes with a brownish upper lip); the tube is shorter than calyx; glabrous or annulate within. The fourth stamen can be included or extended; the anthers are in pairs and diverge; the style branches are uneven. trigonous, pubescent, or glabrous nutlets [15].

Some *Phlomis* species are used in traditional medicine because of their analgesic and antidiarrheal properties, as well as their capacity to heal wounds and haemorrhoids. There are few studies on *Phlomis's* pharmacological and biological impacts. Certain studies have shown a number of advantages, such as anti-

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inflammatory, immunosuppressive, and antioxidant properties that scavenge free radicals, as well as anti-bacterial, and anti-malarial properties [16]. Numerous glycoside groups were identified, including flavonoids, phenylpropanoids, iridoids, diterpenoids, and phenylethanoids. Some of these phenylpropanoids were determined in *Phlomis* species and showed significant biological activity, including anti-bacterial, cytotoxic, cytostatic, anti-inflammatory, and immunosuppressive properties [17]. The *Phlomis* species' essential oils exhibit antibacterial properties against microorganisms [12, 18, 19]. It has been demonstrated that oils from several *Phlomis* species possess antifungal activity against certain pathogenic strains of *Candida* sp [19, 20].

Since anatomy is one of the greatest fields of plant science and serves as a vital link between several other crucial parts, it has amassed a vast body of scientific information [21]. In this regard, anatomical investigation is essential. Several investigations have shown that specific anatomical properties, like the morphological properties of hair coverage, the shape of the stem, petiole, and midrib, the arrangement and structure of the vascular bundles, the sclerenchyma, and the collenchyma, offer important attributes that can be utilized as practical taxonomic tools in identifying Lamiaceae genera and species [11, 22, 23]. To differentiate between closely related *Phlomis* species based on morphological and anatomical characteristics, many taxonomic studies have been carried out [24]. *Phlomis monocephala's* primary distinguishing characteristics, according to Yetişen (2014), are the trichome structure, phloem fibers, and the occurrence of raphide crystals in the leaf [25]. Oran (2015) used the morphology of pollen grains to distinguish between six species of *Phlomis* found in Jordan [26]. *P. russeliana* is an endemic plant in Turkey. Çali (2016) analyzed this plant and looked at some anatomical features, including the frequency of pith rays in the root, the frequency of palisade and spongy parenchyma layers in the leaf, the types of stomata, and the anatomy of the petiole [27]. Yousefi et al. (2014) study of the anatomy, palynology, and trichome types of *P. olivieri*, an Iranian plant, revealed various traits of its aerial plant parts and identified five major trichome types. Additionally, it was the first time that dendritic trichomes in this species were reported [28].

In general, there are very few scientific studies on the anatomy and morphology of the species of the genus *Phlomis*, especially anatomy. To our knowledge, before this study, no comprehensive morphological and anatomical research had been conducted on *P. breugieri*, possibly with the exception of general observations reported in some Floras, such as Flora of Iranica [29] and Flora of Turkey [15]. The study aims are to determine the morphological and anatomical properties of different plant parts of *P. breugieri* from the family Lamiaceae.

## 2 Materials and methods

The study was conducted on freshly obtained *P. breugieri* materials that were taken on field trips from May to June 2024 from the Iraqi Kurdistan area, Sulaymaniyah, the Garmian Province, Kalar – Bawanur– Darbandikhan road (34.84241 N, 45.52656 E, 312 metres above sea level), and Darbandikhan – Bamo road (35.05070 N, 45.70510 E, 520 metres above sea level). A US-made Garmin 72 GPS device was used for determining the location, height above sea level, latitude, and longitude of the specimens that had been collected. Turkey and Iranica Flora [15, 29] were used to identify the collected plant sample. Figure 1 shows a map of *P. breugieri's* distribution. Fresh materials were used to determine measurements and morphological traits of the flower, stem, petiole, and leaf. Table 1 provides the biometric measures of the plant components whose morphological structures were analysed.

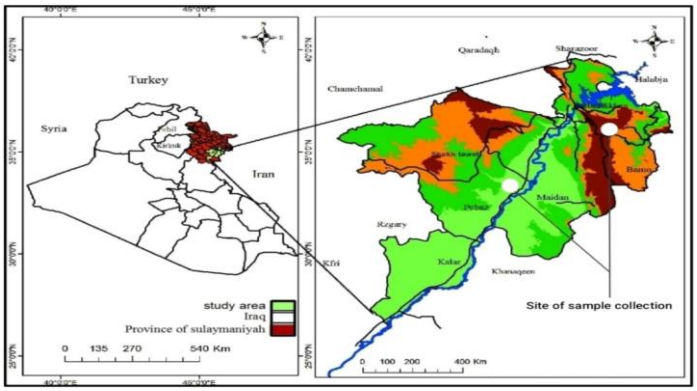


**Figure 1:** Distribution map of *P. bruguieri*.

After that, the sample was preserved in 70% alcohol for use in anatomical analysis. After recording the collection's date, location, and scientific name, the plant specimen was transported to the University of Garmian Research Center. A light microscope was used to examine the anatomical characteristics of the root, stem, leaf, and petiole in the University of Garmian Research Center. Safranin solutions were used to stain cross-sections of the root, stem, leaf, and petiole. The sections were measured under ×40 objectives, and five cells of different sizes (five replicates) were calculated to determine the dimension for each section. Table [2, 3, and 4] presents the biometric measures of the plant components whose anatomical structures were analyzed. The sample sections were manually cut using a razor blade [19, 30]. The sections have been photographed on camera using a Canon camera (DS126311, NO. 091025004604, JAPAN) attached to a Japanese-made Meiji TECHNO Compound Microscope MT4300L, model number 139635.

## 3 Results and Discussions

### 3.1. Morphological study

*P. bruguieri* is a perennial herb along the border line between Iran and Iraq and flowers in May and June. *P. bruguieri* grows in a variety of environments. This plant grows between 312 and 520 metres above sea level in mountainous areas next to rocky slopes and steppe vegetation. The specimen collection site could have unusually hot and dry summers, as well as possibly dry and cold winters. Even though *P. bruguieri* shares broad morphological traits with the Lamiaceae family. As part of its adaptation mechanism to drought stress, *P. bruguieri* uses thick-walled cells

in the epidermis and a relatively thick cuticle on its vegetative components, such as the stem and leaves. These constructions comprised several layers of obstacles that served as hydraulic controllers to control the water flow between the plant and the surrounding area [11].

As shown in Table 1 and Figures 2 and 3, the results in this study regarding the plant species generally concur with the descriptions given in the Flora of Turkey [15] and the Flora of Iranica [29]; nevertheless, some discrepancies were discovered, therefore these descriptions have been modified and broadened. This study found that different aerial plant parts were entirely covered in whitish stellate-tomentose hairs. The stem measured 30–40 cm in length. The leaf dimensions ranged from 4.5–10.1 cm in length and 1.5–2.3 cm in width. They were placed oppositely, oblong to lanceolate and linear, sharp at the tip and tapering towards the base, with entire margins. Its petiole measured 1.5–2.3 cm in length. The inflorescence resembles a verticillate cyme. Verticillasters have fallen leaves. In each verticillaster, 2–5 clusters of 8–9 flowers are grouped. Most floral leaves are sessile or shortly petiolate, ranging from linear to lanceolate. The dimensions of the bracts were 31–43 mm in length and 7–13 mm in width. The calyx was 30-38 mm long, tubular in shape, and very hairy. The calyx teeth were very long and varied in length, measuring 13–21 mm, while the calyx width measured 5–6 mm. The corolla was yellow, bi-labiate, mostly hairy, and measured 15–20 mm in length and 9–11 mm in width. Four stamens were present.

**Figure 2:** General appearance of *P. bruguieri.*

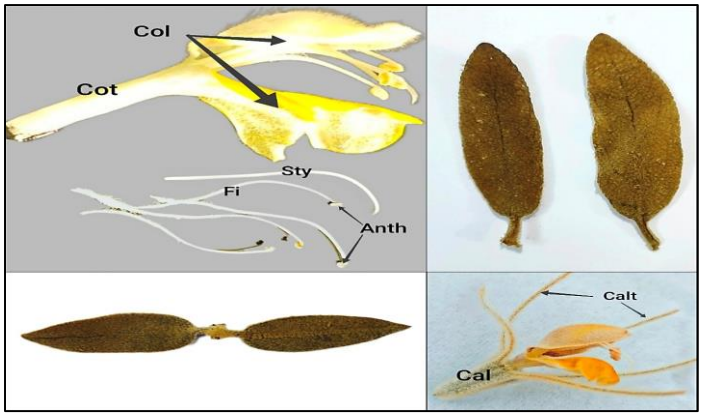


**Figure 3:** Morphological features of main parts of P. bruguieri: A, Col: Corolla lips; Cot: Corolla tube; Syl: Style; Fi: Filament; Anth; Anther; B: Leaf; C: Bract; D, Cal: Calyx; Calt: Calyx teeth.

**Table 1:** Morphological characteristics of P. bruguieri derived from current study and the Flora of Iranica [25] and also the Flora of Turkey [11].

| Parameters | Current study | Flora of Iranica | Flora of Turkey |
|---|---|---|---|
| **Stem** | Herb, 30-40 cm | 20-30 cm | Herb, 80 cm |
| **Leaf length** | 4.5-10.1 cm | Up to 10 cm | 4-12 cm |
| **Leaf width** | 1.5-2.3 cm | 3 cm | 0.8-2 cm |
| **Petiole** | 1-3.5 cm long | - | 2 cm long |
| **No. of flower in each bracelet** | 8-9 flowers | - | 6 flowers |
| **Verticillasters** | 2-5 | 3-7 | 2-5 |
| **Floral leaf** | Sessile or shortly petiolate | - | Sessile or shortly petiolate |
| **Bract length** | 31-43 mm | - | 10-25 mm |
| **Bract width** | 7-13 mm | - | - |
| **Calyx length with teeth** | 30-38 mm | 25-35 mm | 25-35 mm |
| **Calyx teeth length** | 13-21 mm, Unequal in length | Up to 15 mm, Unequal in length | 15-25 mm, Unequal in length |
| **Calyx width** | 5-6 mm | - | - |
| **Corolla length** | 15-20 mm | Up to 20 mm | 20-25 mm |
| **Corolla width** | 9-11 mm | 8-10 | - |
| **Corolla colour** | Yellow | Yellow | Yellow |
| **Corolla upper lips length** | 10-12 mm | 10 mm | - |
| **Corolla lower lips length** | 9-10 mm | 10 mm | - |

| | | | |
|---|---|---|---|
| **Corolla tube length** | 6-13 mm | - | - |
| **Stamen** | 21.5-24 mm | | |
| **Filament length** | 20-23 mm | - | - |
| **Anther length** | 1-1.5 mm | - | - |
| **Style length** | 24-26 mm | - | - |
| **Stigma length** | 1 mm | - | - |
| **Nutlets length** | 2.5-5.5 mm | - | - |
| **Nutlets width** | 1-2.5 mm | - | - |

Compared to the studied plant in this study, the Turkish flora has longer stems, leaves, calyx teeth, and corollas [15]. The study discovered that the criteria were higher than those observed in the Turkish flora, including leaf width, petiole, bract and calyx length, and number of flowers in each flower bracelet. Additional, never-before-published data about the width of the bract, calyx, and corolla, the length of the corolla tube (6–13 mm), the length of the upper and lower lips (10–12 mm), the length of stamen (21.5-24 mm) (filament length was 20–23 mm, and the length of the fertile anther was 1-2 mm), the length of the style (24–26 mm), the stigma (1 mm long), and the dimension of the nutlets (1-2.5 mm in width, and 2.5-5.5 in length) are also presented in this study. Additionally, in the current research, in comparison to the Flora of Iranica [29], bract length and width, calyx width, number of flowers in each bracelet, petiole, corolla tube, stamen (filament and anther), style, stigma length, and nutlet dimension are reported for the first time.

## 3.2 Anatomical study

### 3.2.1 Root

The cross sections of the roots of *P. bruguieri* have a thick layer of periderm on the outermost surface, with cells that looked to be breaking up or squished, which serve as a border. Periderm cells had dimensions ranging from 16.5–50.4 × 17–65.5 µm (Table 2, 3, and 4, Figure 4). There is a multilayered, 7-8-layer parenchymatic cortex located beneath the periderm. Above the phloem layers, present sclerenchyma groups consist of five to eight cells or more. Irregularly distributed trachea cells are in the xylem tissue beneath the phloem. The trachea and vessels make up the 150–187.5 µm xylem tissue structure. Cambium was not clear. 1–10 rows of rectangular cells make up the pith ray. The root's pith area, which is composed of parenchymatous cells that are polygonal or orbicular, is readily visible.

Pith rays on roots are a defining characteristic of the Lamiaceae family's root structure, according to Metcalfe and Chalk's (1972) analysis of some crucial anatomical data. According to their statement, the family's pith rays consist of two to twelve rows of cells or more [31]. Çalı (2016) found that the pith rays in the *P. russeliana* root consisted of three to four rows of cells [27]. In contrast, Yetişen (2014) reported that *P. monocephala* has 1-2 rows of pith rays in addition to 6-7 layers of cortex tissue [25]. The results of the present study aligned with the research conducted by Metcalfe and Chalk (1972) [31] as well as certain Lamiaceae species [32-35]. The comparison of the mean values of length and width of the results obtained in the current study from measured plant parts is explained in Table 3, which is compared with the results of the other studies on the species of this genus, as well as with *Salvia* as the largest genus and *Scutellaria* as the second largest genus of the family Lamiaceae.

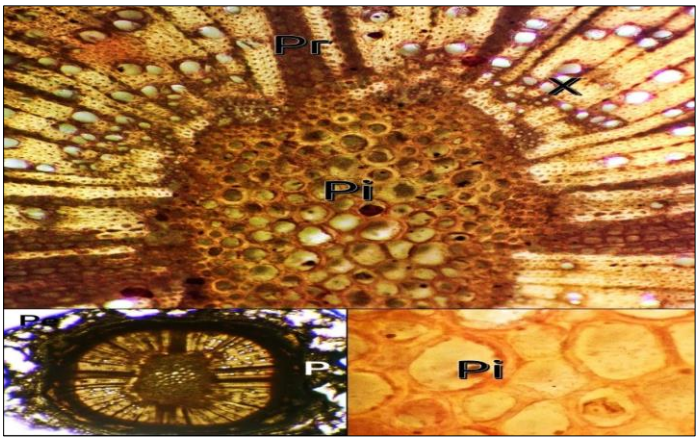


**Figure 4:** Transverse section of root of P. bruguieri: A (X=10), B (X=4), and C (X=40); Pr: Pith ray, X: Xylem; Pi: Pith region; Pe:Periderm; P: Parenchyma cell.

**Table 2:** Anatomical measurement of different tissues of *P. bruguieri.*

| Parameters | Width (µm) | Length (µm) | | |
|---|---|---|---|---|
| | Min | Max | Min | Max |
| **Root** | | | | |
| Periderm cell | 16.5 | 50.4 | 17 | 65.5 |
| Parenchyma cell | 12.5 | 30 | 22.5 | 50 |
| Trachea | 12.5 | 37.5 | 17.5 | 50 |
| Phloem | 10 | 15 | 10 | 25 |
| Pith cell | 15 | 67.5 | 22.5 | 75 |
| **Stem** | | | | |
| Cuticle | 2.75 | 5 | - | - |
| Epidermal cell | 10 | 15 | 15 | 27.5 |
| Parenchyma cell | 12.5 | 25 | 37.5 | 50 |
| Collenchyma cell | 7.5 | 15 | 12.5 | 50 |
| Trachea | 10 | 25 | 15 | 45 |
| Phloem | 5 | 10 | 10 | 17.5 |
| Pith cell | 27.5 | 42.5 | 30 | 50 |
| **Petiole** | | | | |
| Cuticle | 2.75 | 5 | - | - |
| Adaxial Epedirm | 12.5 | 20 | 15 | 30 |
| Parenchyma cell | 25 | 50 | 30 | 67.5 |

| | | | | |
|---|---|---|---|---|
| Trachea | 7.5 | 15 | 10 | 20 |
| Phloem | 5 | 7.5 | 5 | 12.5 |
| Abaxial Epedirm | 12.5 | 20 | 15 | 27.5 |
| **Leaf** | | | | |
| Cuticle | 3.25 | 6.75 | | |
| Upper Epedirm | 15 | 20 | 17.5 | 30 |
| Palisade Cell | 12.5 | 20 | 50 | 62.5 |
| Spongy Cell | 10 | 17.5 | 15 | 22.5 |
| Mesophyll region | 92.5 | 112.5 | - | - |
| Lower Epedirm | 15 | 22.5 | 20 | 30 |
| **Midrib** | | | | |
| Cuticle | 3 | 5 | - | - |
| Adaxial Epedirm | 10 | 18.75 | 13 | 24.1 |
| Parenchyma | 10 | 25 | 25 | 55 |
| Trachea | 12.5 | 25 | 17.5 | 24.75 |
| Phloem | 7.5 | 10 | 6.25 | 9.2 |
| Pith cell | 20 | 50 | 25 | 52.5 |
| Abaxial Epedirm | 10.5 | 17.5 | 12.5 | 19.25 |

Always members of the Lamiaceae family have scleranchymatous tissue around the phloem tissue of vascular bundles. *P. bruguieri* has broader root vessels than stem vessels [Tables 2 and 3], which is comparable with the findings of studies performed on the different species [27, 36-39]. The narrow vessels of the stem wood protect against cavitation, which can influence the distribution region of *P. bruguieri* due to the conduit width being positively correlated with drought-induced cavitations[40]. Studies have indicated that a species' susceptibility to cavitations can limit its range by influencing its ability to adapt to various conditions [41]. Psaras and Sofroniou (2004) reported the root vessels of *P. fruticosa* are wider than the stem vessels. The root vessels are nearly twice as wide as the stem vessels (52 vs. 27 µm). The conducting capacity of a tube is proportional to the 4th power of its radius; hence, a 52 µm root vessel has a conducting capacity that is around 14 times greater than a 27 µm stem vessel [42].

### 3.2.2 Stem

Within the Lamiaceae, *P. bruguieri* is a particularly characteristic species. As can be seen in Figure 5 and Tables 2, 3, and 4, its cross-section is quadrangular. The epidermis is made up of a single layer of approximately rectangular-oval cells with a thin coating of cuticle covering the cell walls. The thickness of the cuticle layer comprised 2.75–5 µm, and the dimensions of the epidermal cells comprised 10–15 x 15–27.5 µm. Single or multicellular glandular and non-glandular hairs are commonly seen in the epidermis. Cortex tissue (251-375 µm) is comprises 9–12 cellular layers. In the corners of the stem, four bundles of collenchyma were seen. The arrangement of collenchyma cells is five to seven rows in the corners and two to three rows between each corner. The collenchyma measures 7.5–15 x 12.5–50 µm and has an oval, angular form. Parenchyma consisted of oval, cubic, and parenchymatous cells arranged in 5-7 rows at the edges and 4-6 rows at the corners. The parenchyma cortical cells measured 12.5–25 x 37.5–50 µm in dimensions. Between the layers of parenchyma, there are crushed parenchymal cells.

The rows of pericycle fibers seven to nine were found outside the vascular tissue. Under the pericycle fiber, there are multiple layers of phloem. The thickness of the phloem tissue was 27.5–32.5 µm, and the phloem cell measured 5–10 x 10–17.5 µm. The xylem tissue has thick and heavily lignified cells and ranges in thickness from 137.5 to 162.5 µm. Compared to the phloem cell, the trachea cell was larger, measuring 10–25 × 15–45 µm. Collateral vascular bundles are positioned adjacent to one another and have their bundles separated at the stem's center by parenchymatous cells. The parenchyma cells in the pith area are either orbicular or polygonal, located in the center of the stem, and the calcium oxalate crystals were visible (Figure 8). The parenchymatous pith cells had dimensions of 27.5–42.5 x 30–50 µm and tiny intercellular spaces.

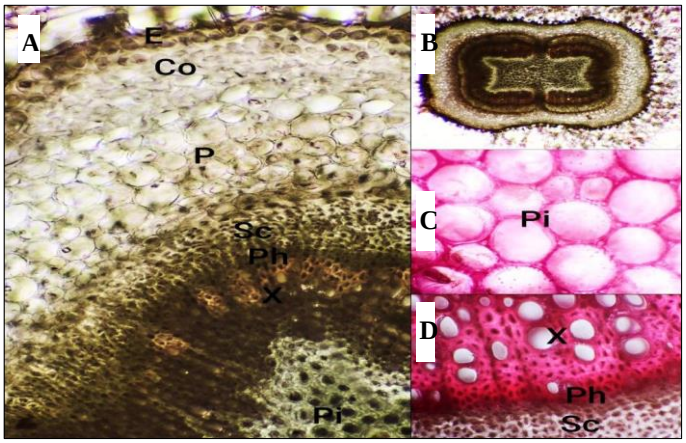


**Figure 5:** Transverse section of stem of P. bruguieri: A (X=10), B (X=4), C, and D (X=40); E: Epidermis; Co:Collenchyma; P: Parenchyma; Sc: Sclerenchyma; Ph: Phloem; X: Xylem;)Pi: Pith region.

Members of the Lamiaceae family have quadrangular stems, scleranchymatous tissue around the phloem tissue, and broad patches of collenchyma presenting at the corners [23, 31, 36, 37]. That is similar to the findings of the present study. The study conducted by Yetişen (2014) found that the cortex tissue thickness and the layers of the stem were greater than those found in this study, ranging from 15–58 µm and 4-6, respectively [25]. According to a study by Kahraman (2011), the parenchyma has between one and fifteen layers. In contrast, the collenchyma has between one and nine layers at the corners and four to five layers at the sides in *Salvia* species [38]. After analyzing the stem structure of *Salvia macrochlamys* species, Kılıç and Kılıç (2022) found that there are 1-3 rows of collenchyma cells located between the corners and 6–9 rows of these cells at the corners. The parenchyma had 4–8 rows at the corners and 6–10 rows at the borders [23]. The study found that the general anatomical structure of *P. bruguieri* is consistent with the Lamiaceae pattern described in the scientific literature. Additionally, the pith area of *P. bruguieri* is solid; this is a feature of *Phlomis* and other

members of the Lamiaceae, and it is in agreement with the results of several investigations [21, 23].

### 3.2.3 Petiole

As shown in Figure 6, the petiole of *P. bruguieri* in the transverse section reveals that the abaxial surface is convex and the adaxial surface is nearly concave. The adaxial surface cells measured 12.5–20 x 15–30 µm, almost the same as the abaxial surface cells, which were 12.5–20 x 15–27.5 µm. Both sides consist of just one layer of cells, with numerous glandular and non-glandular hairs and round, square, and rectangular epidermal cells covered in a cuticle layer ranging in thickness from 2.75 to 5 µm. Beneath the epidermis, the collenchyma tissue comprises three to five layers of cells. The dimension of parenchyma cells is 25–50 x 30–67.5 µm, and they are almost circular or hexagonal in shape. The dimensions of parenchyma cells range from 25–50 x 30–67.5 µm, with a hexagonal or nearly circular form. The cells contain intercellular spaces that contain crystals of calcium oxalate. Encircling the arc-shaped median vascular bundle that covers the inside of the petiole are parenchyma cells that are almost circular in shape and polygonal in shape. The petiolar vascular bundle was collateral kind. A well-developed sclerenchyma tissue is seen outside of the phloem and xylem. The phloem comprises irregular cells that measure 5–7.5 x 5–12.5 µm and consist of 2-4 layers. The vessel and trachea make up the xylem. The trachea has four to seven layers of normal cells, each measuring between 7.5-15 × 10-20 µm (Tables 2, 3, and 4).

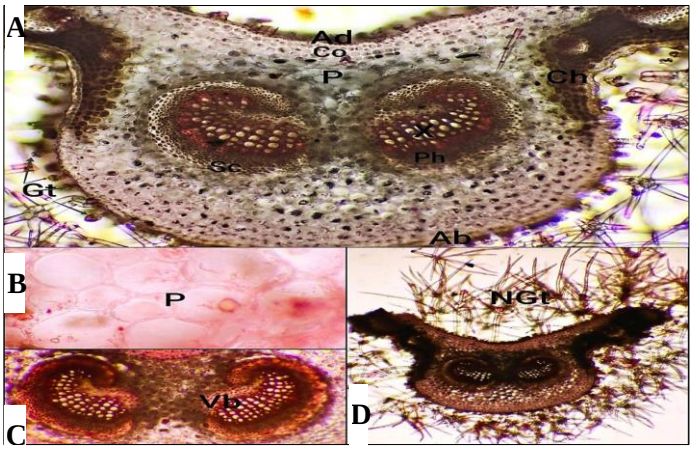


**Figure 6:** Transverse section of petiole of *P. bruguieri*: A (X=10), B, and C (X=40), D (X=4); Ad: Adaxial epidermis; Co:Collenchyma; Ch: Chlorenchyma; X: Xylem; Ph: Phloem; Sc: Sclerenchyma; Ab: Abaxial epidermis; Gt:Glandular trichome; P: Parenchyma; Vb: Vascular bundle; NGt: Glandular trichome.

The vascular bundle shape of the petiole in the Lamiaceae family serves as a diagnostic feature, allowing for species and genera differentiation [31]. El-Banhawy & Al-Juhani (2019) noted that *P. aurea* contains three unequal vascular bundles in the center and three auxiliary vascular bundles in the wings, as well as a distinctive round petiole shape [11]. While the petiole of *P. bruguieri* contains four vascular bundles, two of them are large, located in the center, and two are small-subsidiary, located in each wing. Underneath the vascular bundles and the petiolar wings are three to eight layers of chlorenchyma cells. As a result, the anatomical characteristics of the petiole could be help differentiate and separate this species from species belonging to the same genus and other genera. As shown in Table 4, the petiole of *P. russeliana* had a vascular bundle with two large sections in the center and one small section in each wing [27], *P. olivieri* (one large section in the center and one small section of vascular bundle in each wing) [28], *Salvia halophile* (six in the center and three small sections in each wing) [34], *S. limbata* (four large sections in the center and four small sections in each wing)[33], and *S. willeana* (one in the center and two small sections in each wing) [35].

### 3.2.4 Leaf and Midrib

In *P. bruguieri*, the cells that make up the epidermis are square, oval, and nearly rectangular. It is composed of a single layer. A thick, wavy cuticle covers the epidermis (Figure 7). Cuticle thickness varied between 3.25 and 6.75 µm. Numerous unicellular or multicellular glandular and non-glandular hairs covering the lower and upper epidermis. The upper and lower epidermis cells' dimensions, which were 15-20 x 17.5–30 µm and 15–22.5 × 20–30 µm, respectively, are almost comparable. Palisade (chlorenchyma) parenchyma cells, which are 1-2 rows and tubular, with dimensions ranging from 12.5-20 x 50-62.5 µm, and spongy parenchyma cells, which are 2-3 rows and irregular in shape, both constitute the mesophyll region thickness (92.5 to 112.5 µm). The equifacial leaf and stomata were present on both leaf surfaces (amphistomatic type), and the stomata were diacytic type. One projecting portion is the midrib area. The abaxial surface pointing inside (concave) and the abaxial surface pointing outward are slightly convex. The uniseriate, oval, and squarish cells that makeup both the adaxial and abaxial epidermis have dimensions of 10–18.75 x 13-24.1 µm and 10.5–17.5 x 12.5–19.25 µm, respectively. Additionally, a cuticle layer with a thickness of 3.25–6.75 µm covers them. Multiple layers of collenchymatous cells lie beneath the epidermis and act as support. Collenchyma cells are composed of 2–3 rows. Arc-shaped vascular bundles are surrounded by parenchymatic cells that are hexagonal or polygonal in shape. The dimensions of the cells are 10–25 x 25–55 µm, and they include intercellular spaces with an abundance of calcium oxalate crystal types. The midrib center is contained one main vascular bundle. The collateral vascular bundles are present. The xylem lies beneath the parenchyma. The trachea cell measured 12.5–25 x 17.5–24.75 µm, which was bigger. The phloem cells measured 7.5–10 × 6.25–9.2 µm the parenchyma. The trachea cell measured 12.5–25 x 17.5–24.75 µm, which was bigger. The phloem cells measured 7.5–10 × 6.25–9.2 µm, and they are situated beneath the xylem, as exhibited in Tables 2, 3, and 4. There are two to three layers of chlorenchyma cells beneath the midrib wings.

Leaves of Lamiaceae often show taxonomically useful traits in mesophyll structure and stomatal types. Lamiaceae is the family with the most prevalent diasitic stomas [31]. The leaves of various

Lamiaceae members showed the equifacial mesophyll type and diasitic stoma, which is consistent with the results of the current study [33]. For example, certain species, such as *S. limbata*, exhibit equifacial leaves [33]. Diasitic stoma was found in several *Phlomis* plants, such as *P. rigida*, *P. olivieri*, and *P. kurdica*, in a previous investigation [43]. On the leaves of certain Lamiaceae members, however, diasitic stoma and bifacial mesophyll types have been identified [28, 35]. In *Salvia halophile*, Kaya et al. (2007) identified the monofacial mesophyll type and the diasitic stoma[34]. In *Salvia suffruticosa*, Kılıç and Kılıç (2022) detected 1-2 rowed spongy parenchyma cells and 2-3 rowed palisade parenchyma cells. The midrib vascular bundles were collateral with an arcuate vascular bundle, and the mesophyll type was bifacial [23]. In agreement with the findings of the current study about spongy layers, Yousefi et al. (2014) found that *P. olivieri* possesses three to four layers of spongy parenchyma cells and one layer of palisade parenchyma [28]. Comparable to our results, El-Banhawy and Al-Juhani (2019) found that *P. aurea* had a similar number of palisade cell layers [11].

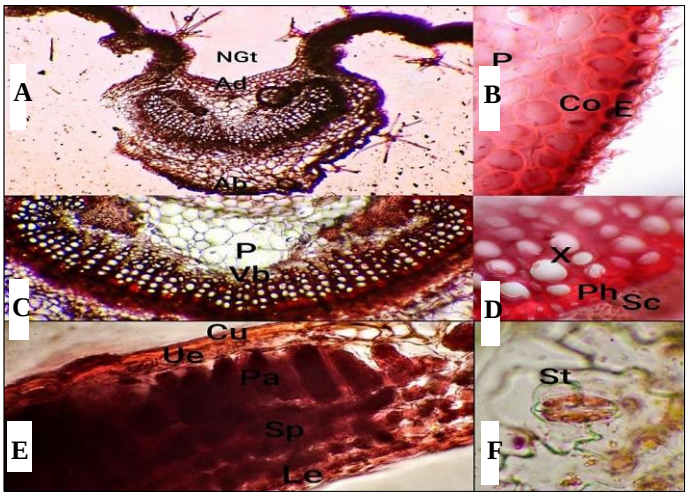


**Figure 7:** The transverse section of leafand midrib of *P. bruguieri*. A (X=4), B, D, E, and F (X=40), C (X=10); A, B, C, and D is Midrib, E, and F is Leaf, NGt: Non-Glandular Trichome; Ad: Adaxial surface; Ab: Abaxial surface; E: Epedermis; Co: Chollenchyma; P: Parenchyma; Vb: Vascular bundle; Sc: Sclerenchyma; Ph: Phloem; X: Xylem; Cu: Cuticle; Ue: Upper epidermis; Le: Lower Epidermis; Pa: Palisade parenchyma; Sp: Spongy parenchyma; St: Stomata.

**Table 3:** Comparative anatomy of the mean cell size of *P. bruguieri* derived from current study and other members of the Lamiaceae family.

| Parameters | | **Current study** | | ***Phlomis olivieri*** [28] | | ***Scutellaria* albida subsp. Velenovsky** [32] | | ***Scutellaria*. albida subsp. Colchica** [32] | | ***Salvia macrochlamys*** [23] | | ***Salvia suffruticosa*** [23] | | ***Salvia willeana*** [35] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Width | Length | Width | Length | Width | Length | Width | Length | Width | Length | Width | Length | Width | Length |
| **Root** | Periderm cell | 38.28 | 42.9 | - | - | 34.05 | 18.99 | 43.33 | 19.91 | 26.69 | 24.84 | 20.58 | 20.1 | 19.3 | 39.825 |
| | Parenchyma cell | 21 | 40.25 | - | - | 47.33 | 26.00 | 37.87 | 21.25 | 26.98 | 20.03 | 20.13 | 14.11 | 12.75 | 28.22 |
| | Trachea | 25 | 32.75 | - | - | - | - | - | - | 38.6 | 38.88 | 32.29 | 37.49 | 20.05 | 28.87 |
| | Phloem | 12.5 | 18.25 | - | - | - | - | - | - | - | - | - | - | - | - |
| | Pith cell | 41 | 47.75 | - | - | - | - | - | - | 14.45 | 21.81 | 9.64 | 10.31 | 11.05 | 21.6 |
| **Stem** | Cuticle | 3.8 | - | - | - | - | - | - | - | - | 5.6 | - | 5.8 | 4.92 | - |
| | Epedirm | 12.25 | 19 | 0.54 | 0.78 | 12.40 | 17.50 | 20.08 | 15.46 | 17.59 | 11.52 | 18.37 | 16.24 | 13.50 | 18.37 |
| | Parenchyma | 19 | 42.75 | 0.8 | 2.48 | - | - | - | - | 52.54 | 37.9 | 39.52 | 32.9 | 22.52 | 34.92 |
| | Cholenchyma | 11.5 | 33.75 | 0.88 | 1.54 | - | - | - | - | 24.73 | 21.71 | 24.61 | 23.03 | - | - |
| | Trachea | 18 | 30 | - | - | - | - | - | - | 32,28 | 35.23 | 19.29 | 19.42 | 18.30 | 23.35 |
| | Phloem | 6.75 | 14.35 | - | - | - | - | - | - | 11.33 | 6.27 | 7.47 | 5.43 | - | - |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pith | 35.5 | 40.25 | 2.16 | 2.48 | - | - | - | - | 117.77 | 121.91 | 51.9 | 49.89 | 44.42 | 52.42 |
| **Petiole** | Cuticle | 4.05 | | - | - | - | - | - | - | - | 3.33 | - | 4.39 | - | - |
| | Adaxial E. | 16.9 | 22.75 | 0.54 | 0.86 | - | - | - | - | 16.8 | 11.72 | 14.46 | 10.28 | 17.77 | 20.8 |
| | Parenchyma cell | 37.5 | 50.25 | 0.66 | 0.84 | - | - | - | - | 15.24 | 10.81 | 22.29 | 11.15 | 17.225 | 21.02 |
| | Trachea | 10.4 | 15.25 | 1.72 | 2.14 | - | - | - | | 48.05 | 44.3 | 41.72 | 44.33 | 31.95 | 39.78 |
| | Phloem | 6.55 | 9.6 | - | - | - | - | - | | 15.7 | 22.07 | 11.7 | 12.54 | 14.62 | 18.37 |
| | Abaxial E. | 17.5 | 22 | - | - | - | - | - | | 9.37 | 8.12 | 9.91 | 6.89 | - | - |
| **Leaf** | Cuticle | 5 | | - | - | 3.4-3.7 | | 3.65-2.1 | | - | 7.44 | - | 3.22 | 4.625 | - |
| | Upper E. | 17.5 | 24.25 | 0.68 | 1.26 | 30.13 | 24.39 | 29.86 | 28.62 | 24.77 | 17.93 | 20.62 | 14.24 | 13.600 | 21.675 |
| | Palisade Cell | 17.35 | 56.25 | 0.44 | 2.70 | 13.05 | 29.07 | 18.11 | 36.35 | 13.74 | 28.22 | 14.02 | 34.5 | 115.400 | 37.1 |
| | Spongy Cell | 14.65 | 19.95 | 0.88 | - | 17.90 | 13.51 | 17.98 | 16.22 | 12.10 | 17.72 | 14.02 | 18.4 | 13.725 | - |
| | Mesophyll region | 104.25 | - | - | - | - | - | - | - | - | 180,79 | - | 211.54 | 152.850 | - |
| | Lower E. | 18.9 | 26.2 | 0.52 | 0.72 | 16 | 14.23 | 11.08 | 11.58 | 21,14 | 16.13 | 19.05 | 12.43 | 29.050 | 36.3 |

**Table 4:** Comparison of anatomical characters of *P. bruguieri* derived from current study and other members of the Lamiaceae family.

| **Parameters** | **Current study** | ***P. russeliana*** [27] | ***P. monocephala*** [25] | ***P. olivieri***[28] | ***Scutellaria. albida* subsp. Velenovsky** [32] | ***Scutellaria. albida* subsp. colchica**[32] | ***Salvia. Halophila*** [34] | ***Salvia. limbata***[33] | ***Salvia. Willeana*** [35] |
|---|---|---|---|---|---|---|---|---|---|
| **Root** Xylem tissue size in (µm) | 150-187.5 | - | - | - | - | - | - | 800–1300 | - |
| No. of pith ray | 1–10 layered | 3-4 | 1-2 | - | 2-9 | 2-4 | - | 2–6 | 1-2 |
| **Stem** Cortex layers | 7-8 | - | 6-7 | - | - | - | - | - | - |
| Phloem tissue size (µm) | 27.5-32.5 | - | - | - | 24.20 | 27.41 | - | 80–120 | - |
| Xylem tissue size (µm) | 137.5-162.5 | - | - | - | 223.74 | 100.9 | - | 500–1150 | - |
| Cortex tissue size (µm) | 251-375 | - | 15-58 | - | 52.35-257.63 | 118.33 - 182 | - | 250–400 | - |
| Cortex layer | 9-12 | - | 4–6 | - | 3-8 | 5-8 | 4-7 | 7–10 | - |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Leaf** Mesophylll structure | Equifacial | Bifacial | - | | Bifacial | Bifacial | Monofacial | Equifacial | Bifacial |
| Palisade parenchyma layer | 1-2 | 1 | 1-2 | 1 | 1-2 | 1-5 | 4-6 | 2–3 | 2-3 |
| Spongy parenchyma layer | 2-3 | 3-4 | - | 3-4 | - | - | - | 1-2 | 3-4 |
| Upper epidermis versus lower epidermis | Nearly Equal | Larger | Larger | Nearly Equal | Larger | Larger | Larger | Large | Smaller |
| Type of stomata | Diacytic | Diacytic | - | Diacytic | - | - | Diacytic | Diacytic | Diacytic |
| No. of vascular bundles in the midrib | 1 large in the center | 1 in the center | 1 in the center | 1 in the center | 2 in the center | 1 in the center | 2 in the center | 1 in the center | 1 in the center |
| **Petiole** Number of vascular bundles | 2 large in the center and 2 small in each wing | 2 large in the center and 1 small in each wing | - | 2 large in the center and 1 small in each wing | - | - | 6 in the center and 3 small in each wing | 4 large in the center and 4 small in each wing | 1 in the center and 2 small in each wing |

### 3.2.5 Crystal Description

Crystals can provide highly diagnostic information on the anatomical structure of plants. The study's findings showed that the studied plant's ability to form calcium oxalate crystals is one of its distinguishing characteristics. Figure 8 illustrates the two different shapes of prismatic and styloid calcium oxalate crystal types that were discovered in the root, stem, petiole, leaf, and midrib of *P. bruguieri* in the current investigation.

Crystal forms can have diverse structural arrangements that vary throughout different taxa. The presence of unique crystal structures in Lamiaceae and associated taxa provides further diagnostic information for subfamily classification. The study by Tyagi et al. (2021) states that species from the Lamioideae and Nepetoideae sub-families were examined. Compared to taxa analyzed in other subfamilies, the most frequently detected crystal types in these subfamilies are styloids and prismatics, and their forms are distinctive [44]. However, in the other investigation, the raphide-type crystals were determined in the mesophyll tissue of *P. aurea* [11]. Different kinds of calcium oxalate crystals were found in the *Phlomis* species in the previous study. *P. kurdica*, for example, showed elongated prismatic crystals in flower and stem fragments, along with sand crystals in the flower sections. Furthermore, acicular crystals, which are long and tapered on both sides (needle-like), were seen in the flower portions of *P. rigida* [43].

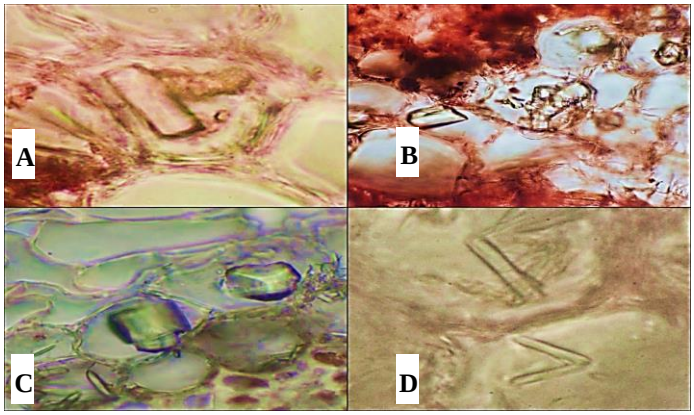


**Figure 8:** Different types of crystals in of *P. bruguieri.* A (X=100), B, C, and D (X=40); A, B, and D: Styloid type; C: Prismatic type.

### 3.2.6 Hair Description

Cells, or clusters of cells known as trichomes, appear as tiny hairs on the surface of the epidermis. As found, *P. bruguieri* has glandular and non-glandular hairs covering the stem, petiole, leaf, and midribs.

All plant sections in this species under examination exhibit fragments of capitate glandular trichomes. The capitated glandular trichome in *P. bruguieri* has been identified in the current study; these include 1-celled stalk and 2-celled stalk, which have different sizes and forms (Table 5 and Figure 9). According to Baran and Özdemir (2010), the capitate type comprises a basal epidermal cell, one or more stalk cells (varying in length), a neck cell, and a unicellular secretory head. It also depends on the plant organs it affects and how it secretes. It was discovered at varying frequencies in all of the investigated plant parts, but in higher amounts in the stem than the other plant parts[45].

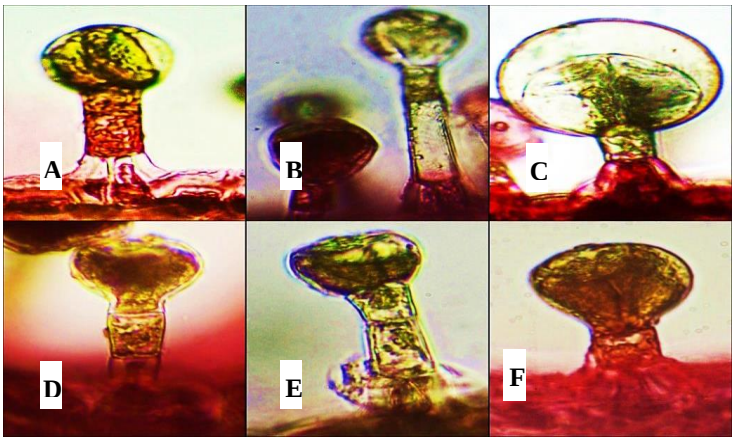


**Figure 9:** Different types of capitated glandular trichomes in *P. bruguieri*. A-F (X=40); A, B, C, D, and F: 1 celled stalk; D, and E: 2 celled stalk.

**Table 5:** Density and types of glandular and non-glandular trichomes in *P. bruguieri*.

| Plant organs | Capitated glandular trichome | | Non-glandular trichome | | |
|---|---|---|---|---|---|
| | 1 celled stalk | 2 celled stalk | Simple (Unicellular and unbranched) | Stellate type | Dendroid type |
| **Stem** | +++ | + | - | +++ | - |
| **Petiole** | ++ | - | - | +++ | + |
| **Leaf** | ++ | - | + | ++ | - |
| **Midrib** | ++ | - | - | + | + |

The Lamiaceae are known for their capitate glandular trichomes, an important taxonomic feature[46, 47], and for their morphology, distribution, and frequency, which are used as discriminative elements at the subfamilies level[48]. Capitate trichome types were observed to be broadly distributed among the several Lamiaceae species[49]. They were frequently found in *P.russeliana*[25] and *P. monocephala*[27]. Also, Gostin and Blidar (2024) reported it in several members of the *Phlomis* genus. The glandular trichomes' primary function is to defend plants from herbivores[47]. According to studies, plants with trichomes are less likely to be eaten by herbivores or attacked by parasitic organisms, and this effect has been seen in individuals of the same species with polymorphisms for trichome production[46]. Furthermore, *Phlomis* species are known to be attacked by phytophagous insects or infrequently eaten by herbivores[51]. Second, by eliminating volatile elements, the trichomes on sepals, petals, or even ovaries can aid in attracting pollinators[52]. Among the constituents found within the essential oil of *Phlomis* species, 1,8-cineole, linalool, and (E, E)-α-farnesene have been demonstrated to be attractive to a variety of pollinating insect species (Hymenoptera), and bicyclogermacrene for certain Diptera species[53]. Peltate trichomes are rare in the *Phlomis* genus. Therefore, peltate types of trichomes were not found in this study or in El-Banhawy and Al-Juhani's 2019 studies on *P. aurea*[11]. *P. russeliana*, an endemic species of Turkey, has been found to have peltate trichomes[23].

In the achieved results in the current study, different plant parts have varying numbers and sizes of non-glandular trichomes. Three kinds of non-glandular trichomes were also identified: simple (unicellular and unbranched), stellate (bifurcate or trifurcate, uniseriate or multiseriate with unicellular branch), and dendroid (with non-glandular branches). There were many branched stellate non-glandular trichomes visible on the stem, leaf petiole surface, and midrib. But compared to the branched stellated kind, the simple non-glandular trichome kind was relatively only detected on the leaf surfaces, and also dendroid non-glandular trichomes found in petiole and midrib (Table 5 and Figure 10).

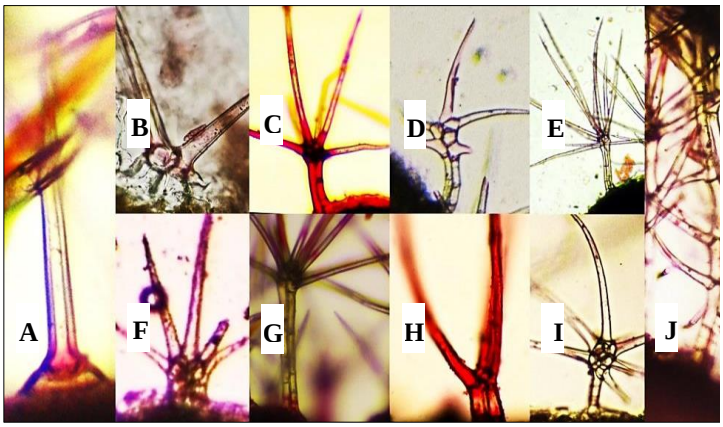


**Figure 10:** Types of non-glandular trichomes in P. bruguieri. A-J (X=10) A: Simple unicellular; B: Stellate (Bifurcate); C, and G: Stellate with multiseriate, unicellular branch; D, I and J: Dendroid; E: Stellate with uniseriate, unicellular branch; F: Stellate with unicellular branch; H: Stellate (Trifurcate)

According to the Cali et al. (2016) study, a simple non-glandular trichome type was found on the surfaces of leaves as well as in the stem, petiole, calyx, and corolla of plants. On the surface of the aforementioned plant organs, they discovered a form of trichomes that is non-glandular trichomes and branching[27]. In several plant parts, Saeidnia et al. (2015) identified the stellated non-glandular trichom type in *P. kurdica*, *P. olivieri*, and *P. rigida*[43]. Non-glandular trichomes of the Lamiaceae family produce a variety of substances (lipids, proteins, terpenes, alkaloids, phenolic compounds, and polysaccharides) that modulate interactions between plants and other species within the ecosystem rather than serving as inert structures with only a protective role against physical factors. They supplement the glandular trichomes' active defensive function for the plant[54]. A dendroid trichome with six branches is a distinguishing feature of *P. monocephala*[25]. Dendroid trichomes with mixed glandular and non-glandular branches are a distinguishing feature of *Salvia lanigera* var. grandiflora[55]. Within the Lamiaceae, the taxonomic importance of trichome structure has been widely recognized[31, 37].

## Conclusion

In conclusion, the transverse section of the anatomical structure of *P. bruguieri* exhibits features common within the Lamiaceae family. This study was the first to provide a detailed description of the morphological and anatomical characteristics of *P. bruguieri*. The results show that certain morphological and anatomical features of the stem, petiole, leaf, midrib, crystals, and trichome provide significant information for plant anatomy and taxonomy.

## Authors contribution

All researchers who participated provided an equal contribution to the preparation, writing, and submission of this study.

## Conflict of interests

No.

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