



## Comparative anatomy of flowering and sterile shoot leaf of *Hedera helix* L. (Araliaceae)

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### Abstract

*Hedera helix* L. is a medicinal plant and belongs to the Araliaceae family. Flowering shoots of the plant have elliptic-cordate and sterile shoots have palmately lobed leaves. Plant material was collected from Hacıkadın valley (Ankara-Turkey). The anatomical features of leaf and petiole were examined comparatively in this study. The results showed that both leaf types were dorsiventral. Single layer epidermis is covered by thick cuticle. The palisade parenchyma cell is oval-shaped in the flowering shoot mesophyll, also is elongated in the sterile shoots. In both leaf types, stomata are located in the lower epidermis. Flowering shoot leaf is covered with tufted hairs, sterile shoot leaf stellate hairs. The midrib of the leaves have a similar structure. Collenchyma tissue is located underside the upper and lower epidermal layers in the midrib. The vascular bundles are arc-form and tends to a ring-form. The sclerenchymatous tissue surrounds the vascular bundles and secretory canals are located in the adaxial side of the bundles. The leaves contain abundant druse crystals and starch grains. Petiole anatomy is similar in both leaf types. Unlike leaves, petioles are covered with stellate hairs, do not contain tufted-type. The collenchymatous tissue is located bottom the epidermal layer. The vascular bundles are embedded in the parenchymatous tissue and secretory canals are located on the adaxial side of petiole. Both petioles contain numerous druse crystals and starch grains.

**Keywords:** *Hedera helix*, Araliaceae, flowering shoot, sterile shoot, plant anatomy, Turkey

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## *Hedera helix* L. (Araliaceae)'in çiçekli ve çiçeksiz sürgün yapraklarının anatomik olarak karşılaştırılması

### Özet

*Hedera helix* L. Araliaceae familyasına ait tıbbi bir bitkidir. Bitkinin çiçekli sürgünleri eliptik-kordat ve çiçeksiz sürgünleri ise palmat yapraklara sahiptir. Bitki materyali Hacıkadın vadisinden (Ankara-Türkiye) toplanmıştır. Bu çalışmada yaprak ve yaprak sapının anatomik özellikleri karşılaştırmalı olarak incelenmiştir. İncelemelere göre, her iki yaprak tipinin de dorsiventral olduğu tespit edilmiştir. Tek tabakalı epiderma kalın kütikula ile kaplıdır. Çiçekli sürgünde palizat parankima hücreleri oval yapıda iken, çiçeksiz sürgünlerde uzamıştır. Her iki yaprak tipinde de stoma alt epidermada yer alır. Çiçekli sürgün yaprağı demet tüylerle kaplıdır, çiçeksiz sürgün yaprakları ise yıldız tüyler taşır. Yaprakların orta damarları benzer yapıya sahiptir. Kollenkima dokusu orta damarda alt ve üst epidermanın alt tarafında bulunmaktadır. İletim demetleri yay formunda ve halkalı yapıya yatkın bir şekildedir. Sklerenkima dokusu iletim demetini çevrelemektedir ve salgı kanalları iletim demetinin eksene bakan tarafındadır. Yapraklar bolca druz kristali ve nişasta taneleri bulundurmaktadır. Yaprak sapı anatomisi her iki yaprakta da benzerdir. Yaprakların aksine yaprak sapları yıldız tüylerle kaplıdır ve demet tüy bulundurmaz. Kollenkima dokusu epidermanın alt kısmındadır. İletim demetleri parankima dokusuna gömülüdür ve salgı kanalları yaprak sapının eksene bakan tarafındadır. Her iki yaprak sapı tipi de bolca druz kristali ve nişasta tanesi bulundurmaktadır.

**Anahtar kelimeler:** *Hedera helix*, Araliaceae, çiçekli sürgün, çiçeksiz sürgün, bitki anatomisi, Türkiye

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## 1. Introduction

*Hedera* L. genus is a member of Araliaceae family. The leaves of Araliaceae are alternate, simple and evergreen. The flowers of this family are usually small and globose umbels and also the fruit of the family is a berry. *Hedera* genus is represented with three species in Turkey namely *H. caucasigena* Pojarck., *H. colchica* (K. Koch) K. Koch and *H. helix* L. [1-4]. *H. helix* is known as English “ivy” or “common ivy” and also called as “duvar sarmaşığı” in Turkey [2, 3]. *H. helix* is woody perennial climbing over trees and creeping on the ground in woods. Leaves are on flowering shoots elliptic-cordate, on sterile shoots are palmately lobed [1].

In folkloric medicine, ivy leaves are used for the treatment of catarrhs of the respiratory tract and symptoms of chronic inflammatory bronchial conditions and this usage has been approved by the German Commission E [5]. The main components of *H. helix* are especially alkaloids, saponins, flavonol glycosides, hydroxycinnamic acid derivatives [6-8]. In addition to this effect, the spasmolytic, antiinflammatory, antimicrobial, anthelmintic, antitumor, antimutagenic, antioxidant and hepatoprotective activities have been reported with previous studies [9-12].

The correctly describe and determine of plants is very important, which are herbal medicine candidates and continue to be used in traditional folk medicine. Microscopic analysis of the anatomical structures of plants could provide useful information for the taxonomic classification. Light microscopy analysis is a common and effective method for the identification of medicinal plants [13].

In this study, comparative anatomical features of flowering and sterile shoots leaf and petiole of *H. helix* were investigated. Transverse and surface sections were examined with a light microscope and detailed photos were taken.

## 2. Materials and methods

The plant material was collected from Hacıkadın Valley (Ankara/Turkey) in 2020. A voucher specimen was deposited in the Ankara University Faculty of Pharmacy Herbarium (AEF 30690) in Turkey. The samples for anatomical studies were preserved in 70% alcohol.

The transverse and surface sections were cut by hand with razor blade in microscopic preparat form. The Sartur solution [14] was used in microscopic examinations. The anatomical analysis and the microphotographs were taken using the Leica DM 4000B.

## 3. Results

### 3.1. Leaf anatomy of flowering shoots

Transverse section of flowering shoot leaf was observed as Figure 1. The leaf is dorsiventral. Upper and lower epidermis were covered with a thick cuticle. The epidermal layer consists of square-rectangular cells and they are similar in terms of their sizes on both faces. In the midrib, both the upper and lower epidermal layers are supported by 1-4 rows of collenchyma. Parenchymatous cells arranged 5-7 layers, congested between the collenchyma and the main vein, contain druse crystals and secretory canals. The number of canals varies between 3-7 depending on leaf development. Mature leaf has 3 canals on the adaxial face and 4 canals on the abaxial face of main vein. The main vein is surrounded by sclerenchymatous tissue. In the main vein, xylem and phloem are arc-shaped and tend to ring-shaped. The mesophyll layer contains 4-5 rows of palisade parenchyma cells and 5-7 rows of spongy parenchyma cells. Palisade and spongy tissue cells contain abundant starch and druse crystals. Tufted hairs are observed in the whole epidermis, more dense in the lower epidermis. Stomata are found only in the lower epidermis.

Surface section of flowering shoot leaf was observed as Figure 2. Upper and lower epidermal cells are with sinuous anticlinal walls. Stomata are located only in the lower epidermis. The guard cells are accompanied by 3-4 subsidiary cells. In addition, wrinkles were observed in the lower epidermis cuticle layer. Tufted hairs are observed on both surfaces, but very dense on the lower epidermis.

### 3.2. Petiole anatomy of flowering shoots

Transverse section of flowering shoot petiole was observed as Figure 3. The transverse section of petiole is disk-like. The epidermis is single-layer, covered with a thick cuticle and has stellate hairs. The epidermal cells are square-rectangular. The underside of the epidermis layer is arranged by collenchyma tissue consisting of 3-5 rows. Interior part of the petiole is filled by large, oval-shape parenchymatous cells in lax form arranged. Parenchymatous cells include numerous druse crystals and starch grains. Individual vascular bundles were arranged in a ring-form in the parenchymatous tissue. The number of individual vascular bundles varies between 5-8 depending on leaf development. The adaxial side of each vascular bundle has numerous secretory canals.

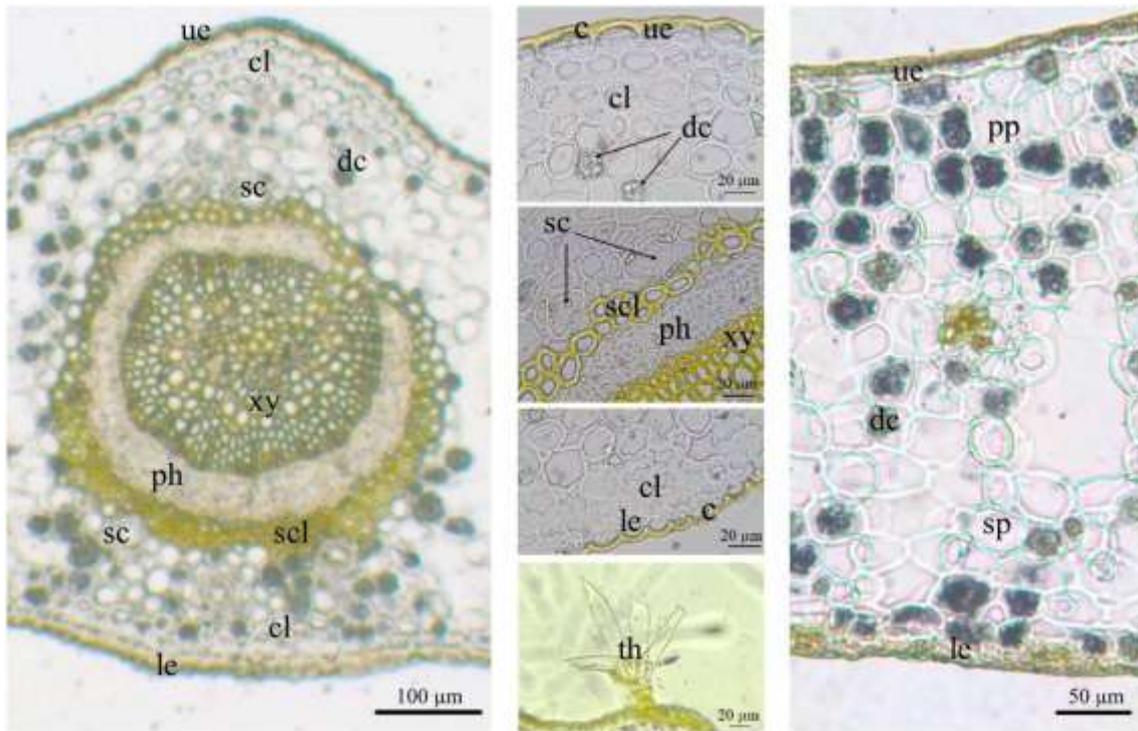


Figure 1. Transverse section of flowering shoots leaf; c: cuticle, cl: collenchyma, dc: druse crystal, le: lower epidermis, ph: phloem, pp: palisade parenchyma, sc: secretory canal, scl: sclerenchyma, sp: spongy parenchyma, th: tufted hair, ue: upper epidermis, xy: xylem



Figure 2. Surface sections of flowering shoots leaf; e: epidermis cell, le: lower epidermis, st: stomata, th: tufted hair, ue: upper epidermis

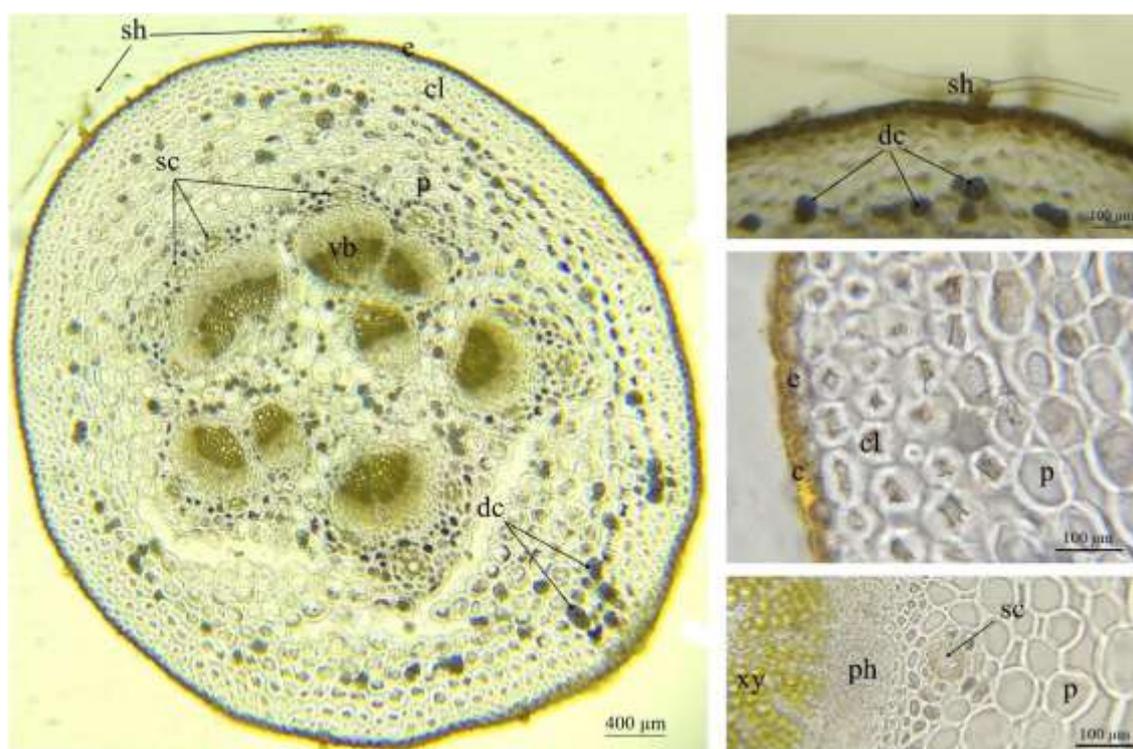


Figure 3. Transverse section of flowering shoots petiole; c: cuticle, cl: collenchyma, dc: druse crystal, e: epidermis, p: parenchyma, ph: phloem, sc: secretory canal, sh: stellate hair, xy: xylem, vb: vascular bundle

### 3.3. Leaf anatomy of sterile shoots

Transverse section of sterile shoot leaf was observed as Figure 4. The leaf is dorsiventral. Upper and lower epidermis were covered with a thick cuticle. The epidermal layer consists of square-rectangular cells and they are similar in terms of their sizes on both faces. In the midrib, both the upper and lower epidermal layers are supported by 1-4 rows of collenchyma. Parenchymatous cells arranged 3-5 layers, congested between the collenchyma and the main vein, contain druse crystals and secretory canals. Secretory canals are arranged in abaxial and adaxial face of the main vein. The adaxial face has 3 and abaxial face 4 channels, in the mature leaf midrib. The main vein has an arc-shaped phloem surrounding the xylem. Phloem often tends to surround the xylem. The sclerenchymatous tissue surrounding the main vein usually enters the vein, separating the xylem from the middle part into two. The mesophyll layer contains 2-3 rows of composed of columnar cells oriented perpendicular to the leaf surface palisade parenchyma cells and 5-10 rows of spongy parenchyma cells. The mesophyll layer contains 2-3 rows of composed of columnar cells oriented perpendicular to the leaf surface palisade parenchyma cells and 5-10 rows of spongy parenchyma cells. The mesophyll layer includes abundant starch and druse crystals. Stomata and stellate hairs are observed on the lower epidermis only.

Surface section of sterile shoot leaf was observed as Figure 5. Upper and lower epidermal cells are with sinuous anticlinal walls. Stomata are located only in the lower epidermis. The guard cells are accompanied by 3-5 subsidiary cells. Stellate hairs are observed on the lower epidermis.

### 3.4. Petiole anatomy of sterile shoots

Transverse section of sterile shoot petiole was observed as Figure 6. In the petiole transverse section, which appears as a disc, there is an epidermal layer consisting of square rectangular cells located in a single row under a thick cuticle layer. The epidermal layer is covered by a lot of stellate hairs. The underside of the epidermis is composed with 3-5 rows of collenchyma. Inside of petiole consists of numerous large, oval-shape parenchymatous cells. These cells contain druse crystals and starch grains. Each vascular bundle is embedded in the parenchymatous tissue in a ring-form. Numerous secretory canals are observed on the adaxial side of bundles.

## 4. Conclusions and discussion

It is very important to correctly define herbal-based pharmaceutical raw materials, which have traditional use and are considered as officinal drugs. In this case, besides morphological definitions, microscopic analysis is a practical and effective method. *Hedera helix* L. is a medicinal herb that is included in many pharmacopoeias. The leaves used

medicinally show different properties morphologically. Leaves are elliptic-cordate in flowering shoots, and palmately lobed in sterile shoots. In addition to the difference in leaf shape, the flowering and sterile shoots differ in several other phenotypic characteristics, for example habit of growth, light saturated photosynthetic rate and anthocyanin biosynthetic competence. Previous studies show that, in *H. helix*, the absence of anthocyanin in adult leaves is correlated with the lack of transcription of dihydroflavonol reductase, an enzyme in the anthocyanin biosynthetic pathway. The stable expression of such differences in phenotypic characteristics may be differential gene expression for these characteristics [15-19]. Therefore, it is important to show the microscopic features of that herbal drug.

The transverse section of the flowering and sterile shoots leaves indicates that, the leaf is a dorsiventral leaf. In both leaf types, the upper and lower epidermis layers consist of square-rectangular cells and are covered by a thick cuticle layer. The midrib of the leaves is generally covered with collenchyma cells in the ad- and abaxial direction just below the epidermis. The main vein is embedded in the parenchymal cells and surrounded by arc-shaped sclerenchymatous tissue. Phloem encloses the xylem by the abaxial in the shape of a crescent and often tends to cover the xylem completely. Savulescu and Luchian (2009) [20] reported in the midrib the vascular bundle in the form of arc, with outer xylem and inner phloem, covered by a sclerenchymatous tissue.

In the adaxial side of the main vein are located 3 and in the abaxial side 4 secretory channels. In generally, although previous studies have indicated that there are secretory canals, information on the number and location of these secretory canals is insufficient [20-23]. Our study provided sufficient information about the secretory canals in the leaf.

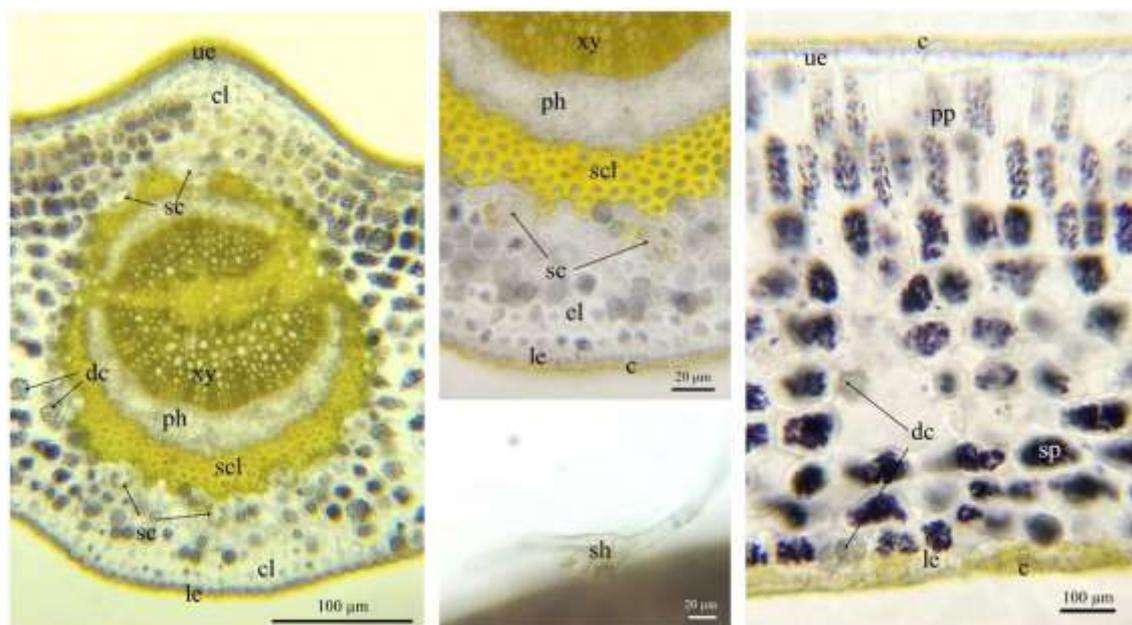


Figure 4. Transverse section of sterile shoots leaf; c: cuticle, cl: collenchyma, dc: druse crystal, le: lower epidermis, ph: phloem, pp: palisade parenchyma, sc: secretory canal, scl: sclerenchyma, sh: stellate hair, sp: spongy parenchyma, ue: upper epidermis, xy: xylem

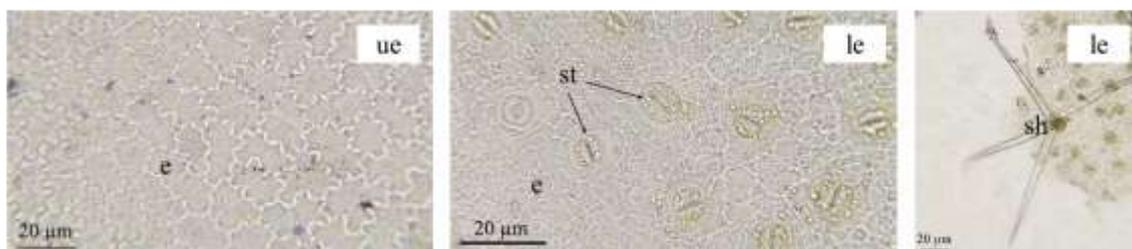


Figure 5. Surface sections of sterile shoots leaf; e: epidermis cell, le: lower epidermis, sh: stellate hair, st: stomata, ue: upper epidermis

In both leaf types, the midrib cross section shows a similar structure, while the mesophyll layer is different. In flowering shoot leaf mesophyll layer consists of 4-5 rows of palisade parenchyma and 5-7 rows of spongy parenchyma. In sterile shoot, leaf mesophyll consists of 2-3 rows of palisade parenchyma and 5-10 rows of spongy parenchyma. However, while the cells of the palisade parenchyma are elongated longitudinally in the sterile shoot leaf, it is almost the same as the spongy parenchyma cells in the flowering shoot leaf. In a previous study, it was noted that the leaf is bifacial and that the 2-3 rows of palisade parenchyma and 6-8 rows of spongy parenchyma are characteristic.

However, this study did not report the morphological shape of the leaves [23]. According to Petra et al. (2020) [22], in the two different *H. helix* samples (roof garden plant and typical garden plant), roof garden plant sample has longitudinally elongated palisade parenchyma cells in the mesophyll layer, while typical garden plant sample has oval palisade cells resembling spongy parenchyma. However, it was not reported to which shoot leaves examined in this study.

Large white hairs are characteristic of the Araliaceae family. These hairs can be used for taxonomic classification. The number and angles of the hair arms are the features used in classifying these hairs [21,24]. Metcalfe and Chalk (1965) [21] defined the hairs seen in the Araliaceae family as "shaggy, 2-armed, tufted, stellate, and peltate types". In our study, flowering shoot leaf carry on both upper and lower epidermal layers numerous tufted hairs. There are cover hairs only on the lower surface of the sterile shoot leaf and it is stellate type. In addition, all leaves both the mesophyll layer and the midrib contain abundant druse crystals. Stomata are located only in the lower epidermis and have 3-5 subsidiary cells. According to the report of Metcalfe and Chalk (1965) [21] lower epidermis with papillae is a diagnostic value. However, no papillae were observed in the lower epidermis in both leaf types in our study. Furthermore, previous studies have reported that the stomata are located only on the lower epidermis surface, the leaves include numerous druse crystals and the presence of stellate hair [20,22,23,25,26].



Figure 6. Transverse section of sterile shoots petiole; c: cuticle, cl: collenchyma, dc: druse crystal, e: epidermis, p: parenchyma, ph: phloem, sc: secretory canal, sh: stellate hair, xy: xylem, vb: vascular bundle

Identification of petiolar bundles in taxonomic classification provides an important diagnostic character [21,27,28]. Araliaceae family has rich leaf diversity. There are few studies on simple, lobed, and compound leaf petiole anatomy [20,27]. In this study, difference was not observed in both leaf type petiole. In transverse section, both petioles are disc-shaped, thick cuticle, and consist of single layer epidermis. Epidermal cells are square-rectangular with stellate hair. Sterile shoot petiole has more hairs. There are 3-5 rows of collenchyma tissue under the epidermal layer. The vascular bundles, in a ring form, are embedded in large parenchymatous cells. There are many secretory canals on the adaxial side of the vascular bundles. Additionally, druse crystals and starch grains are concentrated in the transverse sections both petioles. Previous studies report that *H. helix* petiolar bundles are embedded in a crescent-form or a ring-form parenchymatous tissue [21,23,27]. It appears in the form of a crescent in the transverse sections taken from the proximal and distal parts, while the middle part is in the form of a ring in transverse sections [27]. According to Amini et al., (2019) [23], that the petioles of sterile shoots consist 4-5 rows collenchymatous cells and 9-12 secretory canals, and epidermal layer carry on numerous stellate hairs.

In this study, the flowering and sterile shoots leaf and petiole anatomy of the *H. helix* were investigated comparatively. Leaves are on flowering shoots elliptic-cordate, on sterile shoots are palmately lobed. Due to this difference in phenotype, microscopic analysis of parts of a plant used as an herbal drug was considered a necessity. According to our microscopic analysis, both leaves have a thick cuticle layer, the epidermal cells are square-rectangular in shape. The midrib has collenchyma under the epidermis layer, the vascular bundles are in the arc form and

surrounded by a sclerenchymatous tissue. Mesophyll and midrib contain many druse crystals and starch grains. The stomata are located on the lower surface and have 3-5 subsidiary cells. The anticlinal walls of the epidermal cells are sinuous. The flowering shoot leaves have tufted hairs unlike the sterile shoot leaf and contain in palisade tissue oval, spongy-like parenchyma cells. The sterile shoot leaf differs with stellate hairs and palisade cells elongated. Both petioles have an epidermis tissue with a single layer of square-rectangular cells under the thick cuticle layer. The bottom of the epidermis layer is covered with collenchyma tissue. Vascular bundles are embedded in parenchymatous tissue. Petioles have only stellate hairs. Both leaves and petioles contain multiple secretory canals in the adaxial side of vascular bundles.

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