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Anatomical Investigation of the Leaves of Two Morphologically Similar Species, *Platanus orientalis* (Platanaceae) and *Liquidambar orientalis* (Altingiaceae)

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Abstract: In this study, anatomical characteristics of two species, *Liquidambar orientalis* and *Platanus orientalis* were investigated by examining the samples taken from dried drugs, under the light microscope. The anatomical structures of these two species, naturally occurring in Turkey have similar morphological features. They were examined in detail and the systematic characteristics were revealed. The mesophyll type of the leaves is dorsi-ventral and similar in both species. However, it is noted that the palisade parenchyma sequence was 2-3 layers at the *L. orientalis* samples while it was only monolayer in the *P. orientalis* ones. The stoma type was seen as anomocytic type (Ranunculous type) in the *Platanus* samples while it was a paracytic type (Rubiaceous type) in *Liquidambar* specimens. The mean tracheal diameter of *P. orientalis* in leaf lamina was 35.6 µm while that of *L. orientalis* was 17.6 µm. This value was estimated to be 29 and 10.2 µm in the petioles of *Platanus* and *Liquidambar*, respectively. In conclusion, the trachea diameter was measured wider in *P. orientalis* than that in *L. orientalis*. The other detected anatomic characters between the two species are presented in tables while the biometric measurements of cells and tissues were conducted and supported by the obtained images. This work is the first comparative microscopic research that illustrates the leaf epidermal micro-morphology and micro-anatomy of the species *L. orientalis* and *P. orie*ntalis.

INTRODUCTION

Altingiaceae is a small subfamily of the Hamamelidacea family which includes three genera (*Altingia*, *Liquidambar* and *Semiliquidambar*) and eighteen species belonging to them[1-3]. The two genera, *Liquidambar* and *Altingia* are naturally distributed mainly in Southeast Asia and America[4]. *Liquidambar*

was distributed in the same latitude in groups after the ice age and especially more pervasive in the tertiary period[3]. Later, it was wiped off the face of the Earth by cause of climate changes and massive glaciations. Today, only five species exemplify the genus[1,2]. While *L. styraciflua* L. is distributed in South America and *L. formosana* and *L. altingia* are found in South Asia, including South China, Vietnam and Taiwan. *L. orientalis* Mill. only grows in Turkey and has limited dissemination in the Southwestern shoreline such as Köyceğiz, Fethiye, Marmaris and Milas[5, 6]. It is one of the tertiary relict endemic tree species and has high importance in terms of plant biodiversity with local distribution in the southwestern part of Anatolia[7, 8]. As the remains of a warm-humid climate, its population is much destructed in the valleys of Pınargözü and Çürükdiri and faces the danger of extinction[9-11].

The early morphological foliar type and pollen grains of the *Liquidambar* and the Altingiaceae wood type date back to Paleocene[12-18]. *L. orientalis* exhibits different features from all other taxa in the genus by displaying deeply incised leaves with a subcordate base and 5-8 cm long petiole with varying leaf morphology from juvenile to mature ages[7]. *L. orientalis* var. *orientalis* and *L. orie*ntalis var. *integriloba* are two varieties of this species growing in Turkey[6]. Only a limited number of reports have been issued on the morphology and anatomy of these valuable taxa[19, 20, 7]. They are large trees, 25-40 m in height, deciduous with palmately lobed leaves arranged spirally on the stems. The flowers are small and formed by male inflorescence knobs and female inflorescence[21, 2, 3]. The species, growing as trees with 80-100 cm diameter in many places, show distribution together with the eastern plane (*Planatus orientalis*), east alder (*Alnus orientalis*), red pine (*Pinusbrutia*) and mostly with maquis elements[9].

L. orientalis (Fig. 1a) is generally called as ‘‘oriental sweetgum’’ and ‘‘stirace’’ in English, ‘‘styrax’’ in Spanish and Italian, ‘‘orientalischer Amberbaum’’ in German and ‘‘feng’’ in Chinese. In Turkish, it is known as “amber ağacı”, “günlükağacı”, “buhurağacı”, “Mia pelesengi”, “Miai sail” and “revganisuğla” because of the wood and gum-like structures which are traditionally used for wound healing by local people of the Aegean and Mediterranean regions[7, 6, 22, 23]. Also, those that grow in lower places are called “ova (lowland) günlüğü” and those that show the distribution at higher altitudes are called “dağ (mountain) günlüğü”[26]. The natural distribution of the sweetgum tree covers a restricted region in southwest Turkey. It is generally located within the borders of the province of Muğla and also scattered in Aydın-Denizli-Antalya-Burdur and İsparta[21, 26].

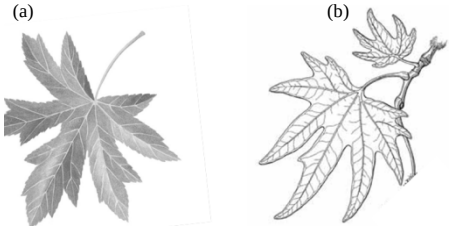

Fig. 1(a, b): General leaf illustration: (a) *Liquidambar orientalis*[24] and (b) *Platanus orientalis* (Drawing[25])

Today’s Platanaceae comprises only a single genus *Platanus*, represented by seven species and is distributed across the northern hemisphere. Five species are distributed in North America, one in Eurasia, *P. orientalis* and one in Vietnam, *P. kerrii*.[27, 28]. In all family members, the petiole has a circular concave covering the bud at the base and the large leafy stipules surround the branches. *Platanus*, like *Acer* and *Liquidamdar*, carries three to seven lobes on its leaf. *P. orientalis* has the maximum number of lobes on the leaves while *P. rasemose* exhibits mostly three lobes[29]. All species also have the same type of trichome bases. In particular, the presence of compound forms consisting of a ring-shaped surface trace associated with more than one underlying epidermal cell is characteristic of this family. These structures are also common in the extinct Platanaceae members and more clearly observed on the abaxial surface of *P. orientalis*, in the form of a compact structure that appears along with a rounded scar situated over the junction of underlying four epidermal cells[30, 28]. On the abaxial surfaces of leaves of all *Platanus* species, the trichome bases are often more difficult to distinguish because the cuticles are much thinner. Therefore, large-diameter bases associated with different basal epidermal cells can be clearly observed only in *P. orientalis*[28].

P. orientalis (Fig. 1b) is grown wild in Anatolia, as well as in parks, gardens and on the roadsides. These trees are ancient and some of them date back to very ancient times in history living up to 4,000 years and are preserved as monumental trees[31]. While it is called as “plane/plane tree or oriental plane” in English, it is known with the names of “Çınar, akkavak, beladan, biladan, buladan, çaymığ, çaynuğ, çilbirtir, çınarağacı, delikavak, doğuçınarı, gavlağan, gavlağanağacı, gavlan, kavak, kavlağan, kavlağın, kavlan” in different regions within the borders of Turkey[32-34]. A decoction, traditionally prepared from its dried leaves or bark, is used orally as antipyretic, diaphoretic, headache, local pains in addition to diuretic and laxative purposes in the Turkish folk medicine[35, 32, 34, 36].

In this study, the leaves of *Platanus orientalis* and *Liquidambar orientalis* species naturally occurring in Turkey are addressed concerning anatomical aspects. Leaf anatomical structures of these two species which are morphologically similar to each other were examined in detail and systematic characteristics belonging to these species were documented.

MATERIALS AND METHODS

The plant samples required for anatomical studies were collected from the following localities: B1 Balıkesir: İvrindi, Madra Mountain, Gebeçınar Village, creek edges, 350 m, 05.12.2014. SS 1507. C2 Muğla: Köyceğiz, Ortaca Region, 150 m, 04.15.2014. SAS 1200.

The systematical identification of the collected samples was conducted in accordance with the Flora of Turkey[5, 37-39]. The branches, leaves and fruits of the identified plants were labelled by preparing in the form of herbarium specimens and taken under protection at the Alanya Alaaddin Keykubat University Botanic Laboratory in Antalya. Five samples were taken from the mature leaves of the plants for each species. The collected samples were dried in an NÜVE EN 500 brand oven at 45°C for 12 h, thereby converting the samples to drugs. Anatomical studies were carried out on leaf samples that became drugs. In anatomical studies; transverse and superficial samples of sectioning were taken from the leaves and after staining with Floroglusin-HCL Reagent, they were fixed into permanent pre-parates by using Glycerin-Gelatin method[40]. The obtained pre-parates were examined under the Olympus BX 53 binocular light microscope and their microphotographs were taken.

RESULTS AND DISCUSSION

Throughout the centuries, both *L. orientalis* and *P. orientalis* are originated from the tertiary period and presenting distribution as relicts and endemics in Turkey. Regrettably, little research has been conducted on the morphology and anatomy of these species which are of great scientific, economic and pharmacological importance. Here, a study was performed on micro-anatomical comparative leaf analysis of two morphologically similar species (Fig. 1), *Platanus orietalis* and *Liquidambar orientalis* which are defined by Ekim *et al*.[41], Ozhatay *et al*.[42] and Servet and Aktag[43] as endangered species under the International Union for Conservation of Nature (IUCN) Red List categories.

Microscope assisted distinguishing studies of the foliar epidermal anatomy play an essential role in the correct identification and discrimination of closely related species like *L. orientalis and P. orientalis*. For this purpose, the following analyzes were performed:

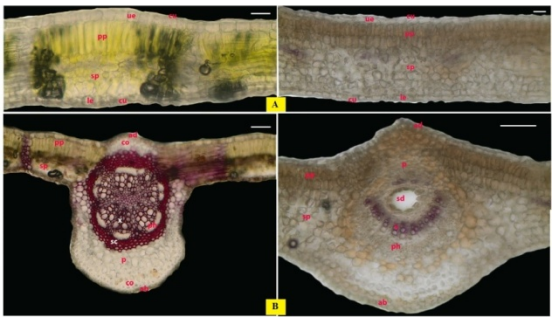

Fig. 2: Leaf anatomy of *P. orientalis* (left column) and *L. orientalis* (right column) species. A. mesophyll structures, B. leaf medium veins. CU: Cuticle, Ue: upper epidermis, pp: palisade parenchyma, sp: spongy parenchyma, le: lower epidermis, ad: adaxial epidermis, ab: abaxial epidermis, co: collenchyma, x: xylem, ph: phloem, sc: sclerenchyma, sd : secretion duct (Scale bar: 50 µm)

Leaf anatomy of the *Platanus orientalis*: When lamina was examined; on the outermost, there was a layer of epidermis with single-row round or cubic cells. The cells of the upper epidermis were observed to be larger, compared to the lower epidermis cells. The epidermis was covered with a thin cuticle (Fig. 2). Covering hairs were seen on both sides and there was no gland hair. Covering hairs were multicellular and had forklike shape. The mesophyll was dorsiventral and consisted of single-row palisade parenchyma and 3-5-row spongy parenchyma. Stomas were at the same level as the epidermis cells (amphistomatic); it usually showed an anomocytic sequence as seen in Rubiaceae. In the mid-vein of the leaf, vascular bundles were located just below the 1-3 rows of collenchyma cells, embedded in the parenchymatic cells. The bundles, whose numbers were between 6-8, formed a ring and were placed in a sclerenchymatous envelope surrounding them. In the transmission bundles, the xylem was directed inward; the phloem layer lied on the xylem, in the form of a hat. When examined petiole; on the outermost was a single-row epidermis layer covered with a thin cuticle (Fig. 3). The epidermis had dense, multicellular and branched cover hairs and a small number of capitated glandular hairs. The capitate hairs consisted of a 1-2 cell stem and a capitate type single-celled head. Just below the epidermis, there was a 2-3 rows annular collenchyma

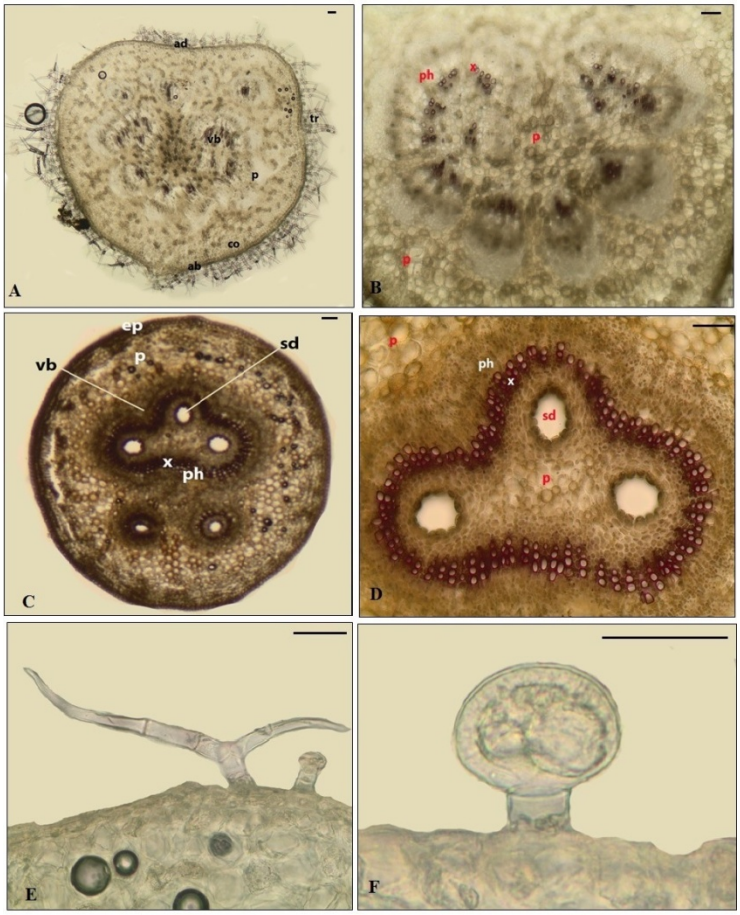

Fig. 3: Petiole anatomy of *P. orientalis* (A, B, E, F) and *L. orientalis* (C, D) species. Petiole general view (A-C). Petiole middle bundles (B-D). Multicellular branched trichome (E). Capitatetrichome (F). Ep: epidermis, p: parenchyma, sd: spongy parenchyma, ad: adaxial epidermis, ab: abaxial epidermis, co: collenchyma, x: xylem, ph: phloem, sc: sclerenchyma, sd: secretion duct. (Scale bar: 50 µm)

layer. The inside of the petioles were filled with parenchyma cells. In the centers, large crescent-shaped bundles were located while on the adaxial side, there were 3 small transmission bundles. The xylem was inward and the phloem was positioned outwards.

Leaf anatomy of *Liquidambar orientalis*: When the lamina was examined; on the outermost part, there were epidermis cells in a single row, round and cubic shapes, covered with a thin cuticle (Fig. 2). The upper epidermis cells were the same size as the lower epidermis cells. Cover hairs were very sparse and not branched and no glandular hairs were observed. Mesophyll type was dorsiventral and composed of 2-3 rows of palisade and 4-7 rows of spongy parenchyma. The stomas were at the same level as the epidermis cells (amphistomatic) and mostly show a paracytic (Rubiaceae type) sequencing. In the central leaf vein, just below the 1-2 rows of collagen, vascular bundles were embedded in the parenchymatous cells. In the vascular bundles, the xylem was directed inwards while the phloem layer was located on the hat-shaped xylem. At the very center of the vascular bundle, the secretion channel was seen.

The petiole was seen in round shapes in cross-sections (Fig. 3). The outermost layer was a single-row epidermis, covered by a thin cuticle. There were no covering and glandular hairs on the epidermis. Immediately below the epidermis was the collenchyma layer which was annular 2-3 in a row. The petiole was filled with parenchyma cells. In the centre there was a large crescent-shaped vascular bundle and on the adaxial side, there were three small transmission bundles. The

Table 1: Leaf anatomical characteristics of the species of *P. orientalisand L. orientalis*

Anatomical characters	Platanus orientalis	Liquidambar orientalis
Cover feather	+ (infrequently)	+ (infrequently)
Glandular hairs	+	-
Mesophyll type	Dorsiventral	Dorsiventral
Abaxial surface of the middle vessel	U-shaped	V-shaped
Palisade layer	one	2-3 layered
Spongy layer	3-5	4-7
Stoma type	Anomocytic	Paracytic
Xylem and phloem		Annular
Crystal	+ (single or clustered druse)	+ (single or clustered druse)
Secretion channels	-	+

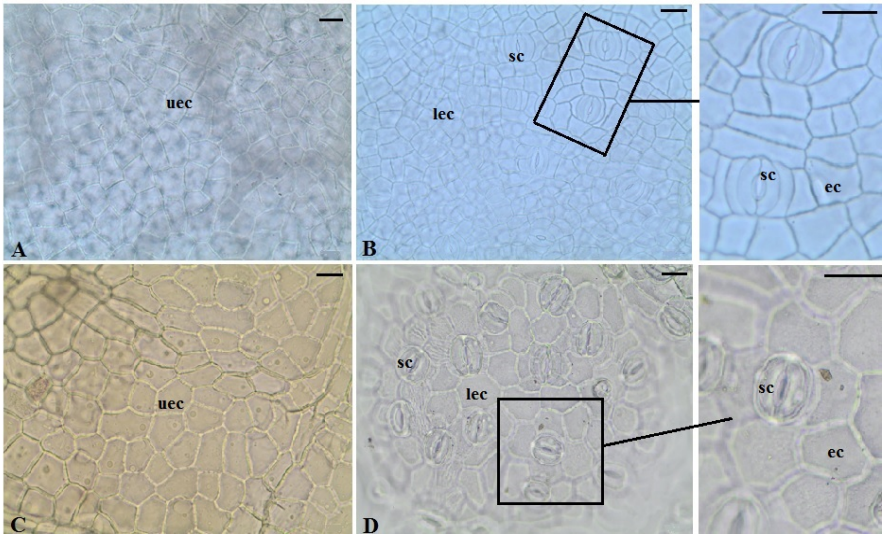

Fig. 4: Leaf epidermal surfaces of *L. orientalis* (A, B) and *P. orientalis* (C, D) species. Upper leaf surface (A-C). Lower leaf surface (B-D). Uec: Upper epidermis cell, lez: lower epidermis cell, sc: stoma cell, ec: epidermis cell (Scale bar: 20 µm)

xylem was inward and the phloem was outward. At the centre, there was a large and annular vascular bundle and just below, two small bundles. In the centre of the transmission bundles, secretion channels were seen.

The two species were chosen as the subjects of this study since their leaves display a close similarity in terms of morphology, although, they belong to different families. Through the detailed analysis of the data obtained as a result of the anatomical studies, anatomical characters between the two species have been documented (Table 1).

In the leaves examined as drugs, one of the most important characters used to separate the two species anatomically was the presence of trichomes. As it is well known, they are considered to be one of the most important anatomical characters used to distinguish species, systematically[44-48]. While the leaves were viewed to be covered with dense trichomes in *P. orientalis* specimens, though the cover hairs in the other parts were very rare (scattered or sporadic), they have never been observed in *L. orientalis* leaf samples. In addition, a rachis consisting of one, two or multicellular and capitate hairs in the form of a head consisting of one cell were also distinguished (Fig. 3). The mesophyll type of the leaves is dorsiventral and similar in both species. However, it is noted that the palisade parenchyma sequence was 2-3 layers in the *Liquidambar* samples while it was monolayer in the *Platanus* samples. Stoma type is an important character that can be used to differentiate taxa on family and genus level. This type was seen as anomocytic type (Ranunculous type) in the Platanus, whereas it was a paracytic type (Rubiaceous type) in the *Liquidambar* (Fig. 4). The secretion channels were found only in the leaves of Liquidambar''s vascular bundles (Table 1).

The diameter of the trachea is also another significative feature used to separate taxa based on the anatomical point of view[49, 50]. The mean tracheal diameter of *P. orientalis*in leaf laminate was 35.6 µm while that of *L. orientalis* was 17.6 µm. In the petiole, this value was measured as 29 µm and 10.2 µm in the specimens of *Platanus* and *Luquidambar*, respectively. It

was concluded that the trachea diameter of *Platanus* samples was wider than those of *Liquidambar* ones (Table 1).

CONCLUSION

This work is the first comparative microscopic research that illustrates the leaf epidermal micro-morphology and micro-anatomy of the species *L. orientalis* and *P. orientalis*. The results of comparative anatomy and morphology can be considered as important data for pharmaceutical industry researchers using the leaves of both species as drugs. In particular, by using the data obtained from this study, microscopic differences in dried and powdered leaf samples brought to the quality control laboratory through the commercial orders can be easily identified which will help minimize adulteration problems and produce higher quality and more effective drugs.

Inspired by this study, further research can be done to answer the following questions: What are the anatomical and morphological similarities and differences between flowers, pollens and fruits of these two species whose leaves are very similar although they belong to different families? To what extent do the factors such as altitude, wind, UV levels and growth under light/shade effect similarity and difference of the parameters? If there is a change affected by these factors are they also caused by genetic (hereditary) elements or are they just examples of plasticity (plant's inherited responses based on environmental conditions)? Do these factors cause any differences in reproductive biology (of both taxa), including flower production, pollination, fertilization and fruit content?

In conclusion, this study will be a useful source of information for future research to provide a contribution to a better quality of herbal medicine production and to ensure an opportunity to investigate the effects of changes in the anatomical and morphological structures of plant parts under various physical and chemical conditions.

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