

Studies on the anatomical structure of stems of willow (*Salix* L.) species (Salicaceae) growing in Ankara province, Turkey

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Abstract: This study was conducted on the anatomical structure of the stems of 9 *Salix* L. (willow) species (9 taxa) growing in Ankara, Turkey. These taxa are *S. triandra* L. subsp. *triandra*, *S. alba* L., *S. excelsa* J.F.Gmel., *S. fragilis* L., *S. babylonica* L., *S. caprea* L., *S. cinerea* L., *S. pseudomedemii* E.Wolf, and *S. amplexicaulis* Bory & Chaub. Illustrations and photographs were obtained of microscopic views of cross sections from the stems of each taxon. The differing anatomical structures of stems of the *Salix* species are suitable for use as an additional tool in identification. Our aim was to contribute information on anatomy for the taxonomy of this highly variable genus. The anatomical study of these 9 species does not indicate significant separations in morphological observations but instead yields data that can be used in taxonomy.

Key words: Willow, *Salix*, Salicaceae, stem anatomy

Ankara ilinde yetişen söğüt (*Salix* L.) türlerinin (Salicaceae) gövde anatomilerinin özellikleri

Özet: Bu çalışma, Ankara ili sınırları içinde yetişen 9 *Salix* L. (söğüt) türünün (9 takson) gövde anatomileri üzerinde gerçekleştirilmiştir. Bu taksonlar *S. triandra* L. subsp. *triandra*, *S. alba* L., *S. excelsa* J.F.Gmel., *S. fragilis* L., *S. babylonica* L., *S. caprea* L., *S. cinerea* L., *S. pseudomedemii* E.Wolf ve *S. amplexicaulis* Bory & Chaub'dur. Her bir taksonun gövde kesitlerinin mikroskopik görünümünün çizimleri ve fotoğrafları verilmiştir. *Salix* türlerinde farklı anatomik özellikler bu türlerin teşhisinde ek veri olarak kullanılabilir niteliktedir. Bu çalışmanın, yüksek derecede varyasyon gösterebilen bu cinsin taksonomisine anatomik olarak katkıda bulunması hedeflenmiştir. Dokuz takson üzerindeki çalışmanın anatomik bulguları morfolojik verilerde gözlenen belirgin ayrımlara neden olmamakla birlikte taksonomik açıdan kullanılabilir sonuçlar sunmuştur.

Anahtar sözcükler: Söğüt, *Salix*, Salicaceae, gövde anatomisi

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Introduction

The species of willow (*Salix* L.), belonging to the family *Salicaceae*, comprise deciduous and dioecious trees or shrubs. There are about 450 species of *Salix* known from all around the world (Argus, 1997); due to the complexity of the genus, however, classification remains difficult and there is some disagreement among authors regarding the exact number of species (Fang et al., 1999; Skvortsov, 1999; Ohashi, 2001; Heywood et al., 2006; Mabberly, 2008). Members of this group are found naturally or are planted at wetlands, marshes, river banks, and the sides of streams. Under suitable conditions, they are able to grow quickly. Turkey is rich in a number of willows species; including those that were introduced, 28 species grow in the country and 2 of them are endemic to Turkey (Skvortsov & Edmondson, 1970; Donner, 1990; Güner & Zielinski, 1993; Güner, 2000). Willows are an important group of plants because of their ecological, economical, and medicinal properties. In many countries of the world, including Turkey (Baytop, 1999), willow bark (Cortex *Salicis*) was commonly used as an antiinflammatory, analgesic, and antipyretic agent before the discovery of aspirin (acetylsalicylic acid), and it is still used in herbal remedies (Wichtl & Bisset, 1994; Commission, 1998). Willow is a species that also has many traditional uses because of its role in ethnobotanical practices, such as basketry production.

In addition to its phenotypical plasticity, the easy hybridisation of willows creates problems for the accurate identification and classification of specimens. The taxa of *Salix* are dioecious and many species have different times of development for flowers and leaves, making it difficult to observe all of the relevant characters on a single plant or specimen. The flower of *Salix* is furthermore too simple to afford adequate characters for taxonomy. Therefore, in addition to morphological characters, additional information, such as anatomical data, may provide help. Anatomic studies are very important for the identification of medicinal plants (Güvenç & Duman, 2010; Güvenç et al., 2011). The anatomy of willow stems and leaves has been investigated by numerous authors (Esau, 1965; Metcalfe & Chalk, 1965; Yazgan et al., 1986) and more recent studies aimed to combine the anatomy and physiology of the willows (Maroder et al., 2000; Cooper & Cass, 2001; Dong & Zhang, 2001). In this study, we investigated the stem anatomy of willow

taxa growing in the province of Ankara: *S. triandra* L. subsp. *triandra*, *S. alba* L., *S. excelsa* J.F.Gmel., *S. fragilis* L., *S. babylonica* L., *S. caprea* L., *S. cinerea* L., *S. pseudomedemii* E.Wolf, and *S. amplexicaulis* Bory & Chaub. Examinations were done on the cross sections of young stems, and possible characters that may aid in the taxonomy of the species are discussed. In the cortex parenchyma, sclerenchymatic elements were observed. In addition to druses, solitary calcium oxalate crystals were investigated. The results are supplemented by photographs and illustrations.

Materials and methods

The willow taxa used for this study were collected from different localities within Ankara Province. After comparing different specimens from different sites, the best representatives of the species were chosen for investigation. The names of the species and information about the collection sites are given in Table 1. Voucher specimens were deposited in the herbarium of Ankara University's Faculty of Pharmacy (AEF).

Anatomical research materials were preserved in 70% ethanol or used fresh. Cross sections of stems were hand-prepared from young (second year) stems from preserved or fresh material and boiled in Sartur reagent. Sartur reagent contains KI-I, aniline, Sudan III, lactic acid, alcohol, and water (Çelebioğlu & Baytop, 1949). Illustrations were made using a Leitz drawing prism attached to a Leitz-Wetzlar (45°) microscope. The cross sections were photographed with an automatic camera attached to an Olympus BX-50 microscope.

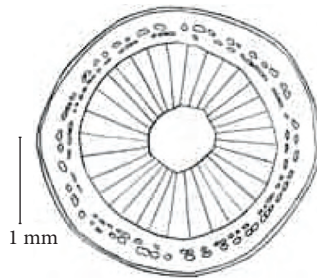
Results

Salix triandra subsp. *triandra* L.

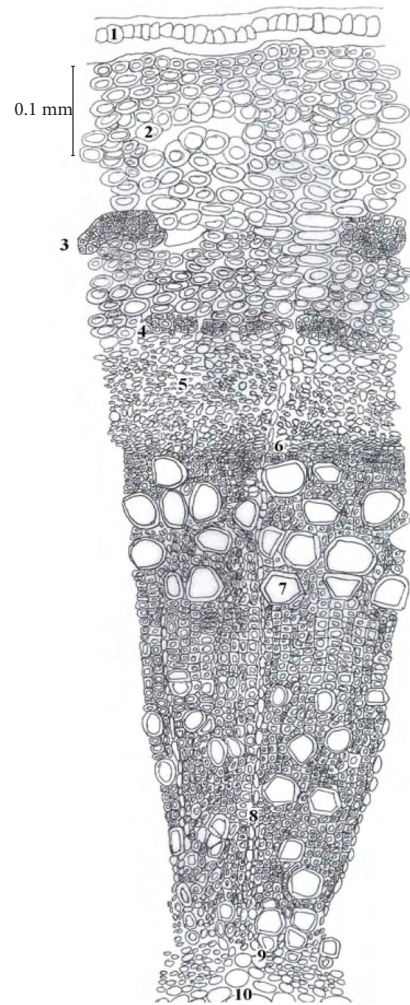
Young stems are circular in cross section (Figure 1). The pith appears circular. Epidermal cells are isodiametric, single-layered; outer and inner walls are rather thickened. These cells are covered with thick suberin (Figure 2). Collenchyma is not seen beneath epidermal layer. Cortex parenchyma, which is situated under the epidermis, is formed by thick-walled, oval, and starch-containing cells with a little space between them and the walls of the adjacent cells (Figure 3). No druse or other kind of calcium

Table 1. Locations of the studied *Salix* species.

Species	Locality	Herbarium number
<i>S. triandra</i> subsp. <i>triandra</i>	A4 Ankara: Sincan, along Ankara stream, 800 m, 22.3.2002, <i>O.Arihan</i>	(AEF 22548)
<i>S. alba</i>	B4 Ankara: Beynam, Beynam Atatürk Forest, Fındıcak Fountain, 1446 m, 29.4.2001, <i>O.Arihan</i>	(AEF 22668)
<i>S. excelsa</i>	B4 Ankara: Lake Eymir, 900 m, 28.8.2001, <i>O.Arihan</i>	(AEF 22707)
<i>S. fragilis</i>	B4 Ankara: Beynam, entrance point from the Ankara-Bala road to Beynam Atatürk Forest, 1250 m, 29.4.2001, <i>O.Arihan</i>	(AEF 22536)
<i>S. babylonica</i>	B4 Ankara: Lake Eymir, 900 m, 12.4.2001, <i>O.Arihan</i>	(AEF 22716)
<i>S. caprea</i>	A4 Ankara: Kızılcahamam, Soğuksu National Park, 1150 m, 31.3.2002, <i>O.Arihan</i>	(AEF 22556)
<i>S. cinerea</i>	A4 Ankara: Çubuk-Karagöl, around the lake, 1500 m, 21.4.2002, <i>A.Güvenç & O.Arihan</i>	(AEF 22482)
<i>S. pseudomedemii</i>	B4 Ankara: Beynam, Beynam Atatürk Forest, Fındıcak Fountain, 1446 m, 4.4.2001, <i>O.Arihan</i>	(AEF 22725)
<i>S. amplexicaulis</i>	A4 Ankara: Kızılcahamam, Soğuksu National Park, 1550 m, 6.11.2001, <i>O.Arihan</i>	(AEF 22616)

Figure 1. General view of the cross section of the stem of *Salix triandra* subsp. *triandra*.

crystal can be seen within the parenchymatic cells. Inside the cortex parenchyma there are some intracellular spaces. Sclerenchyma are seen within the cortex region as clustered packs and do not form large belts. The cells of the cortex parenchyma, which lies beneath the sclerenchyma, are smaller, more flattened, and contain much less starch, so this area is lighter in appearance. Below this layer, the phloem sclerenchyma is visible and is not as large as the sclerenchyma above. Phloem is composed of small, irregularly shaped, thin-walled cells. The cells that form the cambium layer are slender, flattened, and distinctly formed by 3 cells. Xylem is large and all elements of it are easily visible. Xylem is divided by single-celled horizontal layers of pith ray cells. These cells are seen easily within the xylem but are not dark. Rounded, dense parenchymatic cells of the xylem are seen in abundance when we approach the pith. Pith is formed by rounded parenchymatic cells. Both calcium crystals and starch are observed in this region (Figure 4). All layers of the anatomical structure of the stem can be seen in Figures 2 and 5.

Figure 2. Cross section of the stem of *Salix triandra* subsp. *triandra*, including anatomic properties: 1) epidermis, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays, 9) xylem parenchyma, 10) pith.

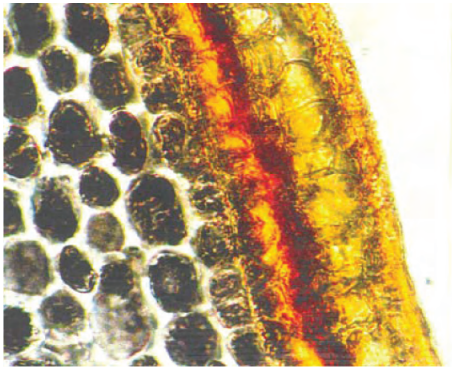


Figure 3. Epidermis and parenchyma of *Salix triandra* subsp. *triandra* (10×).

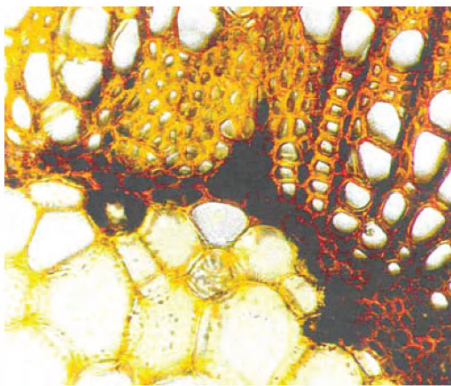


Figure 4. Pith and xylem cells of *Salix triandra* subsp. *triandra* (10×).

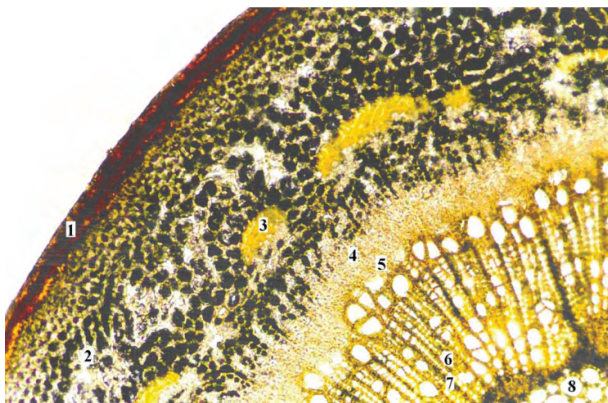


Figure 5. General view of the cross section of the stem of *Salix triandra* subsp. *triandra*, including: 1) epidermis, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem, 5) cambium, 6) xylem, 7) pith rays, 8) pith (4×).

Salix alba L.

Young stems are circular in cross section (Figure 6). The pith appears almost stellate. Suberin layer is thin, penetrating toward the border between the epidermis

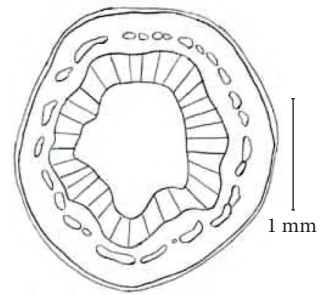


Figure 6. General view of the cross section of the stem of *Salix alba*.

cells (Figure 7). Epidermis, which is composed of a single layer of cells, has thickened walls that form convex projections outside. Cortex parenchyma is formed by thick-walled, spherical or oval-shaped, sometimes rounded, and starch-containing cells with a little intercellular space between the walls of the adjacent cells. Druses, sparsely distributed, can be seen within the parenchymatic cells. Sclerenchyma is seen within the cortex region. These bundles form

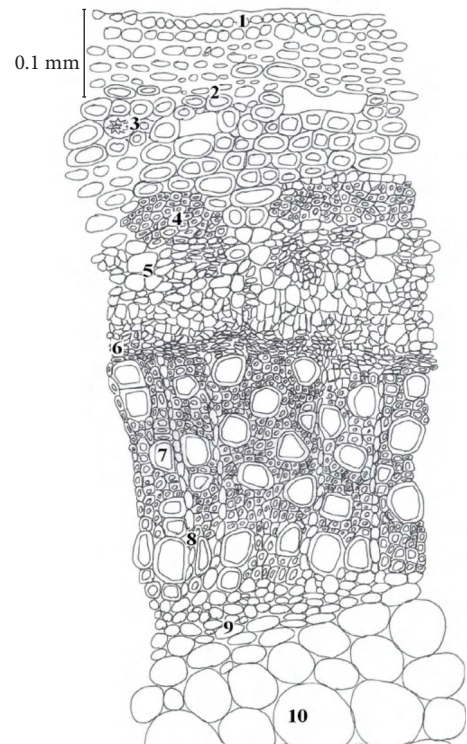


Figure 7. Cross section of the stem of *Salix alba*, including anatomic properties: 1) epidermis, 2) cortex parenchyma, 3) druse at cortex, 4) sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays, 9) xylem parenchyma, 10) pith.

belts. The cells of the cortex parenchyma, which lies beneath the sclerenchyma, are much smaller and contain less starch. Phloem sclerenchyma is visible. Phloem is composed of small to medium, irregularly shaped, thin-walled cells. The cells that form the cambium layer are slender, flattened, and formed by 3 cells. Xylem is divided by single-celled horizontal layers of pith ray cells. These rays are easily seen within the xylem (Figure 8). Xylem is not very large and all elements of it are easily visible (Figure 9). Rounded, sometimes oval, and dense parenchymatic cells of xylem are seen in abundance when we approach the pith. Pith is formed by rounded parenchymatic cells. No calcium crystal or starch is observed in this region. All layers of the anatomical structure of the stem can be seen in Figures 7 and 10.

Salix excelsa J.F.Gmel.

Young stems are circular in cross section (Figure 11). Pith appears stellate. Suberin layer is rather thickened, penetrating toward the border between

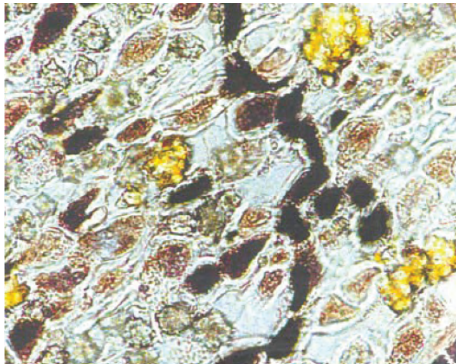


Figure 8. Pith rays and cortex parenchyma cells of *Salix alba* (10×).

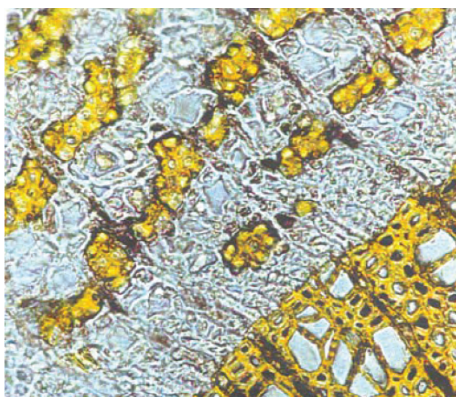


Figure 9. Phloem, cambium, and xylem cells of *Salix alba* (10×).

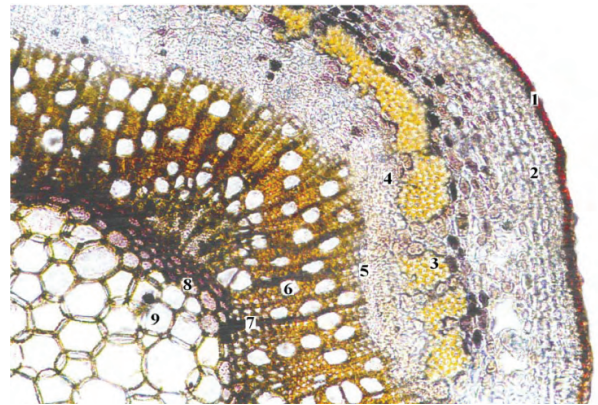


Figure 10. General cross section of the stem of *Salix alba*, including: 1) epidermis, 2) cortex parenchyma, 3) phloem sclerenchyma, 4) phloem, 5) cambium, 6) xylem, 7) pith rays, 8) xylem parenchyma, 9) pith (4×).

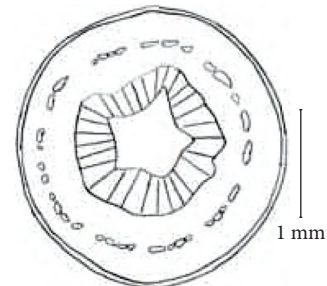


Figure 11. General view of the cross section of the stem of *Salix excelsa*.

the epidermis cells. Epidermis, which is composed of a single layer of cells, has thickened walls that form convex projections outside. Collenchyma is one-layered. Cortex parenchyma, situated beneath the collenchyma, is formed by thick-walled, spherical or oval-shaped, sometimes rounded, starch-containing cells with a little intercellular space between the walls of adjacent cells. Druses are dense and can be seen within the parenchymatic cells (Figure 12). Sclerenchyma is seen within the cortex region (Figure 13). These bundles are scattered and do not form belts. In the vicinity of these bundles are numerous druses. Cells of the cortex parenchyma, which lies beneath the sclerenchyma, are much smaller and contain less starch. Below this layer, phloem sclerenchyma is visible and is also scattered, like the sclerenchyma above. Solitary calcium oxalate crystals exist in some of the cells. Phloem is composed of small, irregularly shaped, thin-walled cells. Cells that form the cambium layer are slender, flattened, and distinctly

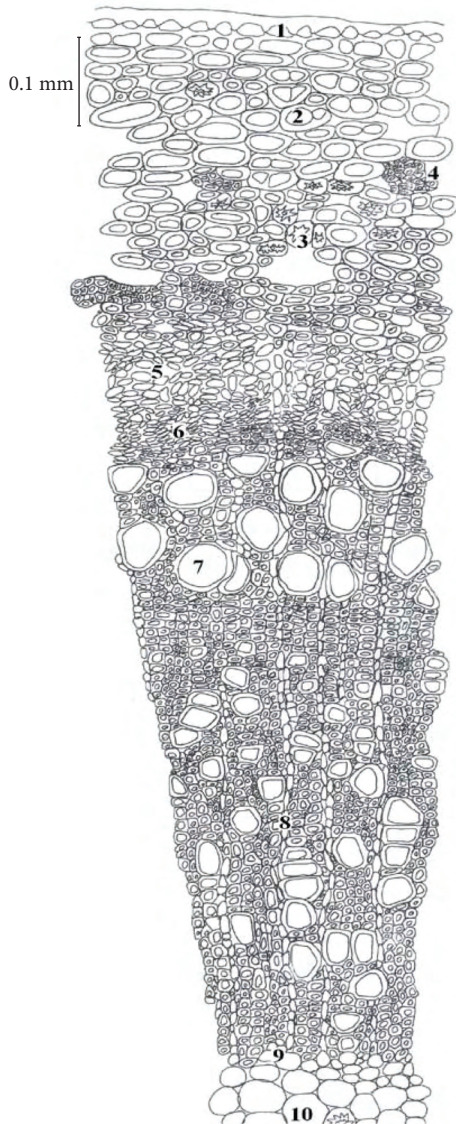


Figure 12. Cross section of the stem of *Salix excelsa*, including anatomic properties: 1) epidermis, 2) cortex parenchyma, 3) druse at cortex, 4) sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays, 9) xylem parenchyma, 10) pith.

formed by 3 cells. Xylem is not very large and all elements of it are easily visible. Xylem is divided by single-celled horizontal layers of pith ray cells. These rays are easily seen within the xylem. Rounded and dense parenchymatic cells of the xylem are seen in abundance when we approach the pith (Figure 14). Pith is formed by rounded parenchymatic cells. Druses can be seen in the pith region. All layers of the anatomical structure of the stem can be seen in Figures 12 and 15.

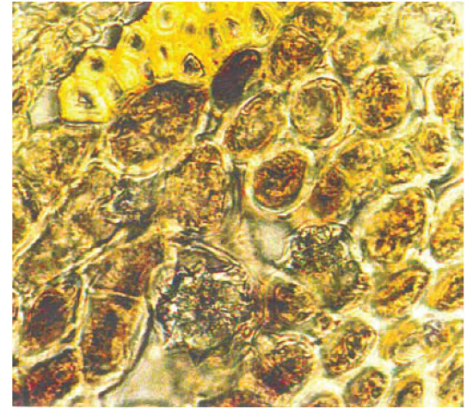


Figure 13. Cortex parenchyma and sclerenchyma of *Salix excelsa* (10×).

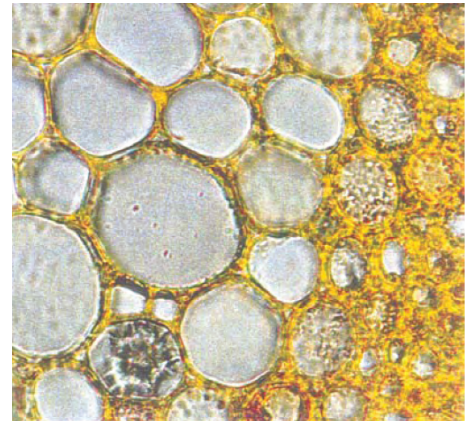


Figure 14. Pith, xylem cells, and druse of *Salix excelsa* (10×).

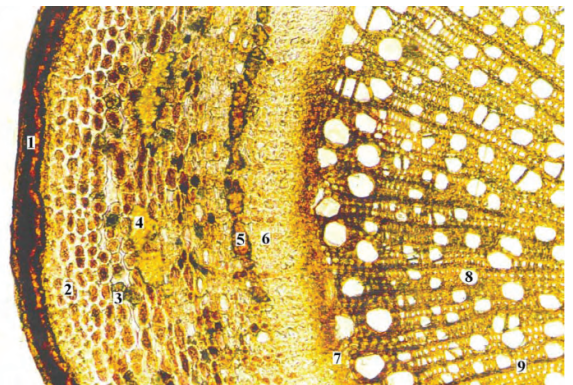


Figure 15. Cross section of the stem of *Salix excelsa*, including: 1) epidermis, 2) cortex parenchyma, 3) druse, 4) sclerenchyma, 5) phloem sclerenchyma, 6) phloem, 7) cambium, 8) xylem, 9) pith rays (4×).

Salix fragilis L.

Cross sections from young stems are rounded (Figure 16). Pith is almost stellate. Epidermis cells partially covered in suberin. Epidermis is composed

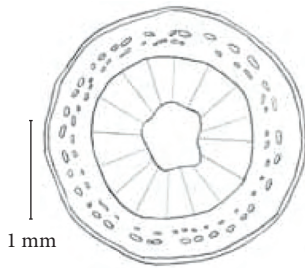


Figure 16. General view of the cross section of the stem of *Salix fragilis*.

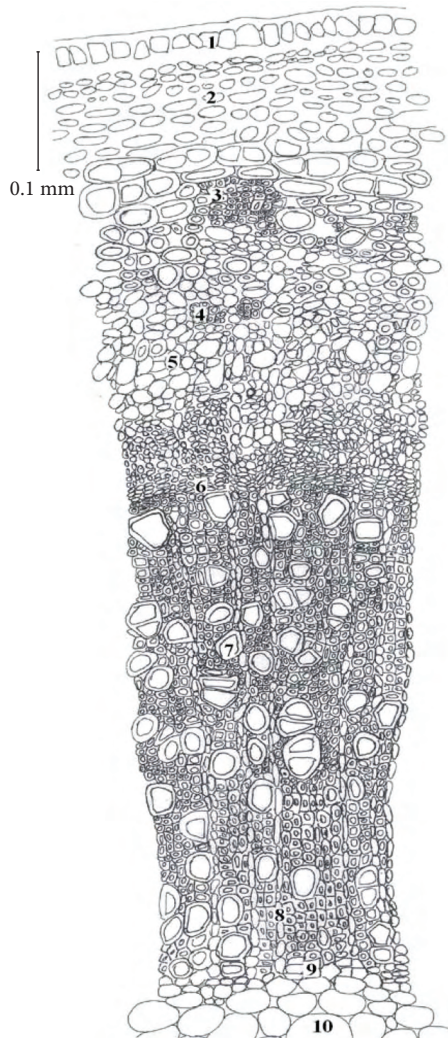


Figure 17. Cross section of the stem of *Salix fragilis*, including anatomical properties: 1) epidermis, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays, 9) xylem parenchyma, 10) pith.

of a single layer of cells. Collenchyma is single-layered. Cortex parenchyma, situated beneath this layer, has thick-walled, generally oval-shaped (Figure 17), starch-containing cells with intercellular space (Figure 18). Sclerenchyma is situated in cortex region. These bundles are dispersed and cover the stem cortex. Druses are found around those bundles. Small and less starch-containing phloem and phloem sclerenchyma are situated beneath the sclerenchyma. This sclerenchymatic layer is dispersed, like the one above. Simple crystals and druses are observed in some of the cells. Cells forming the multicellular cambium layer are thin and flattened. Xylem is not large and all elements are easily seen. Xylem is formed by a single layer of cells and divided by starch-containing pith rays. These rays are easily observed within xylem. Pith rays do not contain starch in cambium but their starch content becomes visible again when they are located within phloem. Pith is formed by rounded parenchymatic cells (Figure 19). Druses are visible in pith. All layers of the anatomical structure of the stem can be seen in Figures 17 and 20.

Salix babylonica L.

Young stems are circular in cross section (Figure 21). Pith appears as almost stellate. Epidermal cells are isodiametric to rectangular, single-layered; outer and inner walls are rather thickened. These cells are covered with thick suberin. Collenchyma is one-layered. Cortex parenchyma, which is situated beneath the collenchyma, is formed by thick-walled, spherical or oval-shaped, sometimes rounded, starch-containing cells with a little intercellular

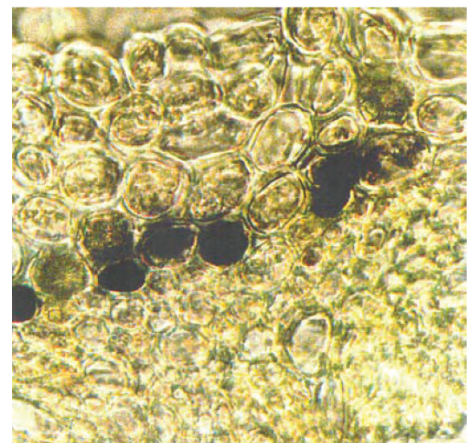


Figure 18. Cortex parenchyma cells of *Salix fragilis* (10 \times).

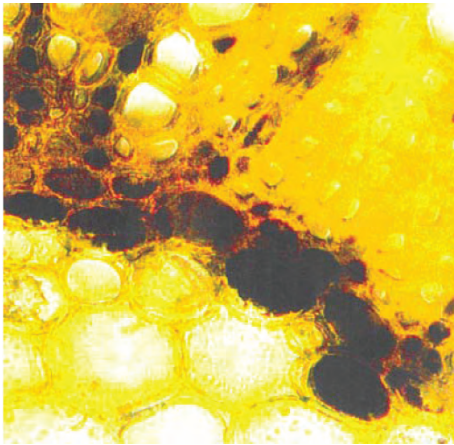


Figure 19. Pith and xylem cells of *Salix fragilis* (10×).

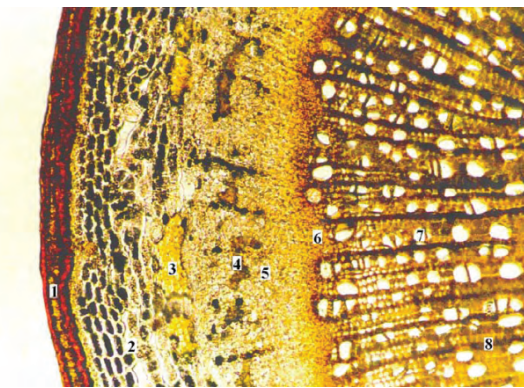


Figure 20. Cross section of the stem of *Salix fragilis*, including: 1) epidermis, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays (4×).



Figure 21. General view of the stem of *Salix babylonica*.

space between the walls of the adjacent cells (Figure 22). Druses, which are sparsely distributed, can be seen within the parenchymatic cells. Sclerenchyma is seen within the cortex region. These bundles are scattered and do not form belts. Cells of the cortex parenchyma, which lies beneath the sclerenchyma, are much smaller and contain less starch. Below this

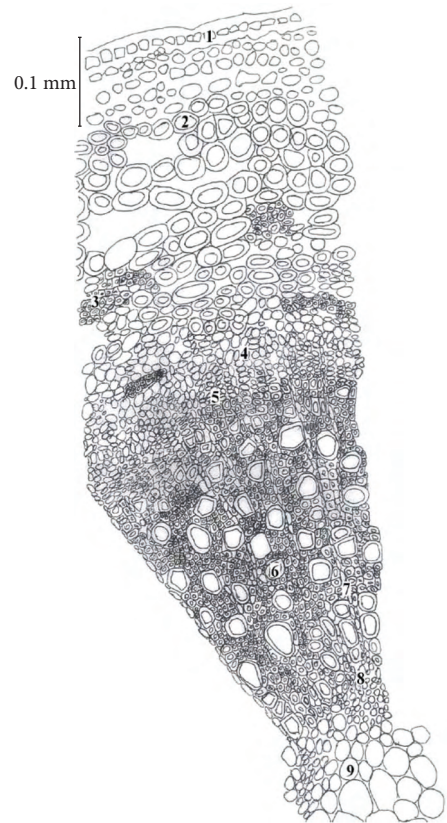


Figure 22. Cross section of the stem of *Salix babylonica*, including anatomic properties: 1) epidermis, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem, 5) cambium, 6) xylem, 7) pith rays, 8) xylem parenchyma, 9) pith.

layer, phloem sclerenchyma is visible and is both small in appearance and sparsely distributed. Phloem is composed of small, irregularly shaped, thin-walled cells. Cells that form the cambium layer are slender, multilayered, and flattened. Xylem is large and all elements of it are easily visible (Figure 23). Xylem

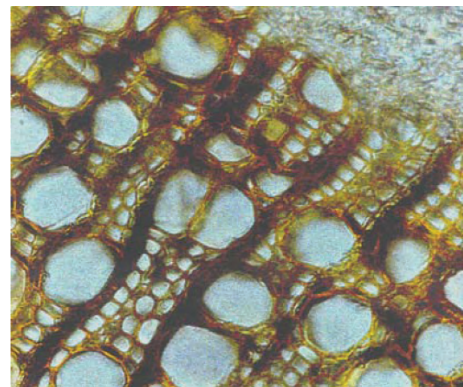


Figure 23. Xylem and cambium of *Salix babylonica* (10×).

is divided by single-celled horizontal layers of pith ray cells. These rays are easily seen within the xylem. Rounded and dense parenchymatic cells of xylem are seen in abundance when we approach the pith. Pith is formed by rounded parenchymatic cells. Druses can be seen in the pith region (Figure 24). All layers of the anatomical structure of the stem can be seen in Figures 22 and 25.

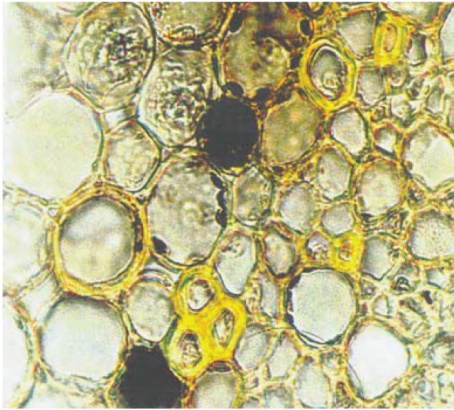


Figure 24. Starch and druses in the pith of *Salix babylonica* (10×).

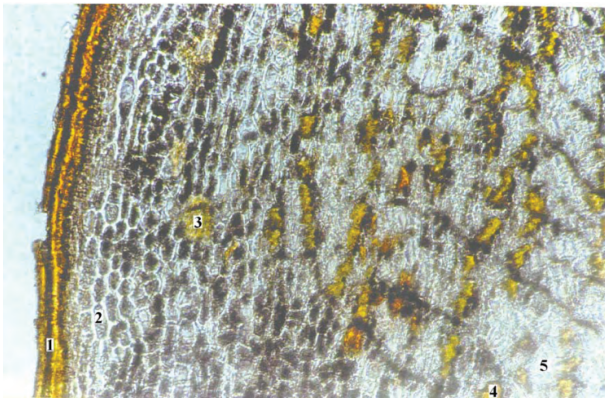


Figure 25. Cross section of the stem of *Salix babylonica*, including: 1) epidermis, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem sclerenchyma, 5) phloem (4×).

Salix caprea L.

Cross sections of young stems are rounded (Figure 26). Pith is rounded. Epidermis is formed of single-layered cells and the walls of those cells make convex projections to the outside. Collenchyma is single-layered. Cortex parenchyma beneath this layer has thick-walled, rounded, starch-containing cells with a little intercellular space. Druses are visible in parenchyma cells (Figure 27). Sclerenchyma exists in

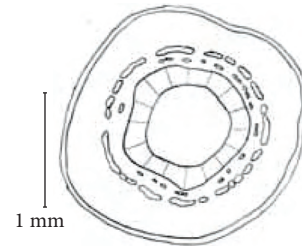


Figure 26. General view of the cross section of the stem of *Salix caprea*.

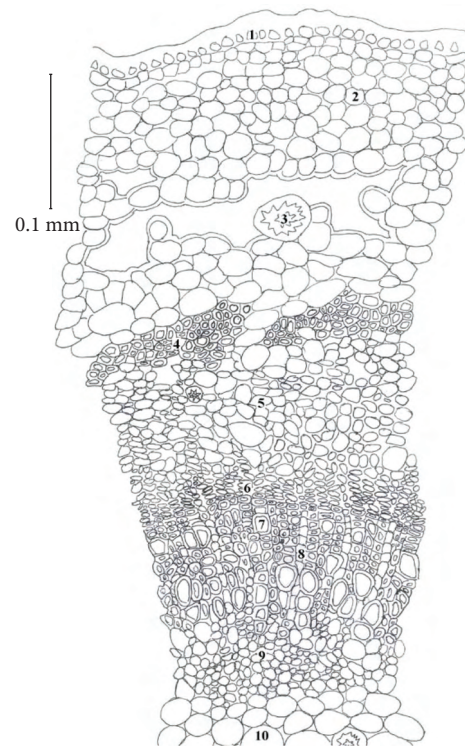


Figure 27. Cross section of the stem of *Salix caprea*, including anatomic properties: 1) epidermis, 2) cortex parenchyma, 3) druse, 4) phloem sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays, 9) xylem parenchyma, 10) pith.

the cortex region. Those bundles form a partial belt. Cortex parenchyma cells beneath the sclerenchyma are small and carry less starch. Phloem sclerenchyma may exist beneath this layer and appear dispersed, like the sclerenchyma above. Phloem is formed by small, irregularly shaped, thin-walled cells. Druses are observed in some cells (Figure 28). Cells forming the cambium layer are thin, flattened, and significantly multilayered (more than 4 layers of cells). All elements of xylem are easily observed.

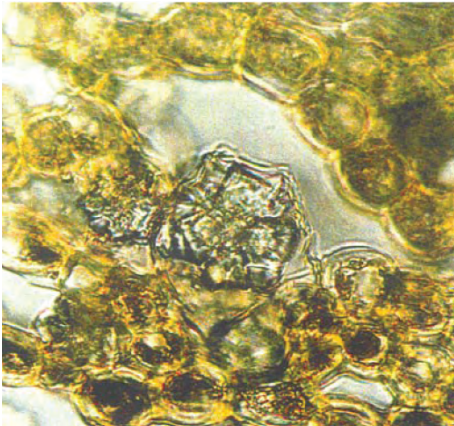


Figure 28. Druses in the cortex cells of *Salix caprea* (10×).

Xylem is divided by pith rays in a single row of cells. Those rays are easily observed within the xylem. Pith is rounded, formed by parenchymatic cells, and carries druse; starch is not observed (Figure 29). All layers of the anatomical structure of the stem can be seen in Figures 27 and 30.

Salix cinerea L.

Cross sections of young stems are rounded (Figure 31) and have a rough outer structure. Pith is large and not perfectly rounded. Epidermis cells are single-layered. Inner and outer walls of cells are covered by a thick suberin layer. This is covered by eglandular trichomes. Collenchyma can be seen and has 1 or 2 layers. Cortex parenchyma, beneath the collenchyma, has thick-walled, rounded, starch-containing cells with intercellular space (Figure 32). Druses are rarely seen in the cortex. Sclerenchyma is seen in the cortex

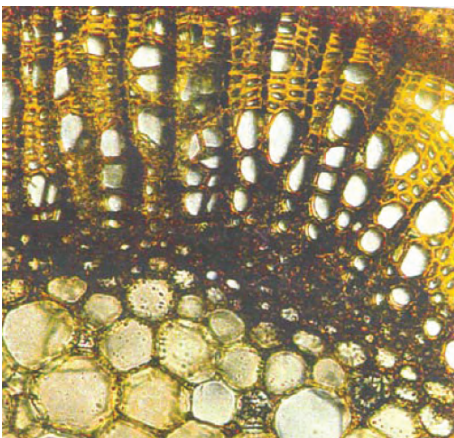


Figure 29. Pith and xylem of *Salix caprea* (10×).

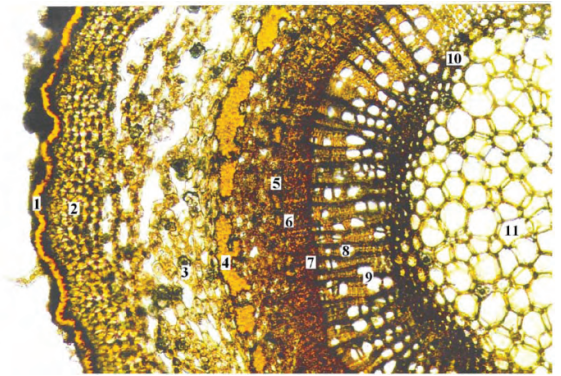


Figure 30. Cross section of the stem of *Salix caprea*, including: 1) epidermis, 2) cortex parenchyma, 3) druse, 4) sclerenchyma, 5) phloem sclerenchyma, 6) phloem, 7) cambium, 8) xylem, 9) pith rays, 10) xylem parenchyma, 11) pith (4×).

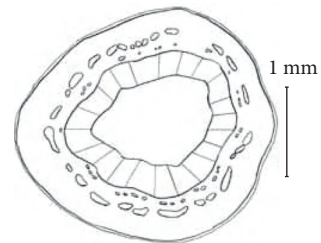


Figure 31. General view of the cross section of the stem of *Salix cinerea*.

as large clusters. Phloem sclerenchyma, formed of small bundles, is in a dispersed form. Phloem is formed by small and thin-walled cells. Druses are seen in some cells. Cambium is thin, flattened, and multilayered with 10 or 11 layers (Figure 33). Xylem is not very large and is frequently divided by pith rays, which are formed by a single row of cells. Those rays are easily observed in the xylem. Pith is formed by rounded parenchymatic cells and the intercellular space is very small (Figure 34). Druse and starch are found in pith. All layers of the anatomical structure of the stem can be seen in Figures 32 and 35.

Salix pseudomedemii E.Wolf

Young stems are circular in cross section (Figure 36). Pith appears almost stellate. Epidermis is mostly composed of a single- or sometimes 2-layered cells that are oval. Outer and inner walls of epidermis are covered with a thick suberin layer. Eglandular trichomes are seen in a large percentage (Figure 37). They are simple and unicellular. Trichomes are

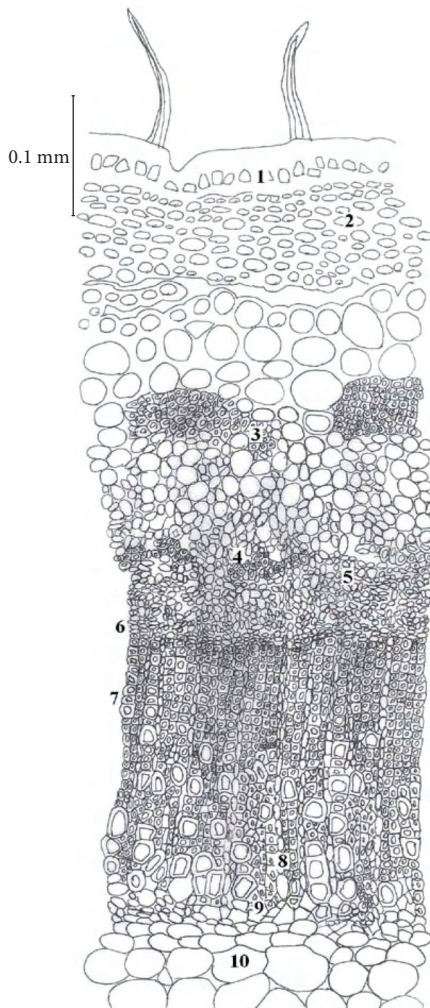


Figure 32. Cross section of the stem of *Salix cinerea*, including anatomic properties: 1) eglandular trichome, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays, 9) xylem parenchyma, 10) pith.

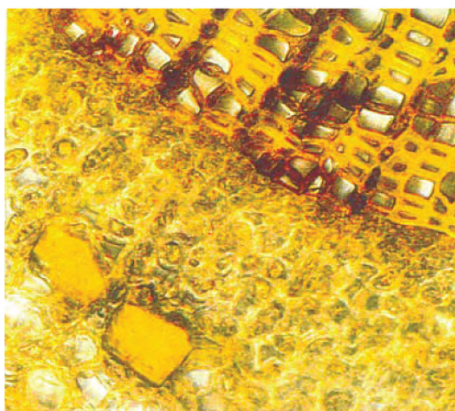


Figure 33. Xylem, cambium, and phloem of *Salix cinerea* (10×).

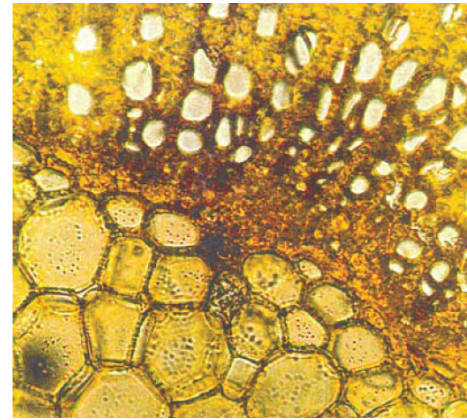


Figure 34. Pith, xylem cells, and druse of *Salix cinerea* (10×).

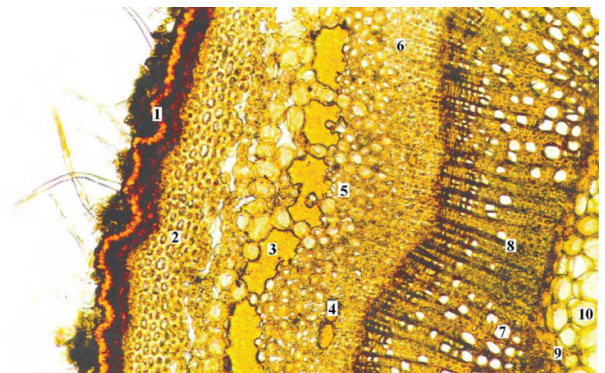


Figure 35. Cross section of the stem of *Salix cinerea*, including: 1) epidermis, 2) cortex parenchyma, 3) sclerenchyma, 4) phloem sclerenchyma, 5) phloem, 6) cambium, 7) xylem, 8) pith rays, 9) xylem parenchyma, 10) pith (4×).

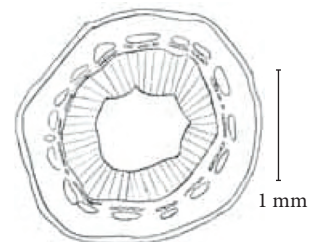


Figure 36. General view of the cross section of the stem of *Salix pseudomedemii*.

dense and can reach up to 0.5 mm in length (Figure 38). Collenchyma is generally one-layered. Cortex parenchyma, situated under the collenchyma, is formed by thick-walled, elliptic, starch-containing cells with a little space between the walls of the adjacent cells. Druses can be seen within some of the parenchymatic cells. Inside the cortex parenchyma

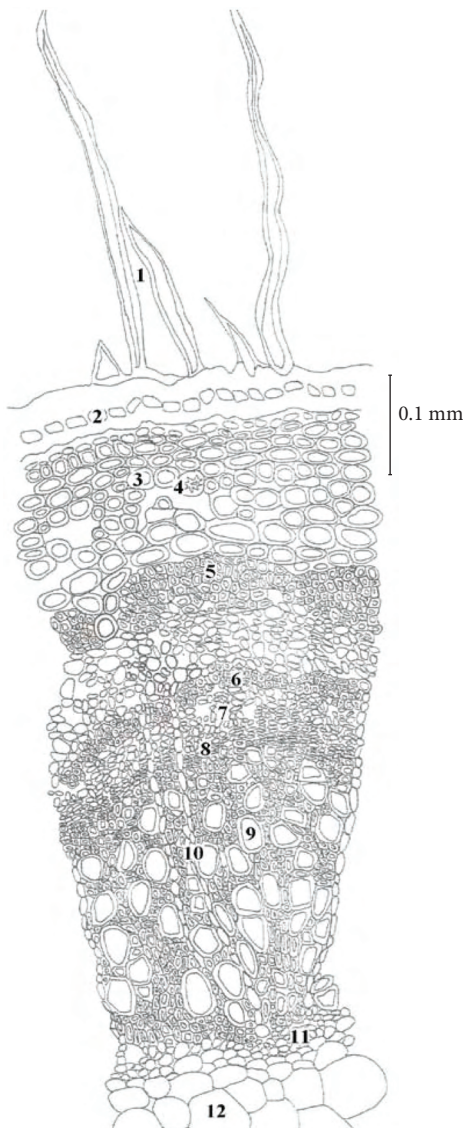


Figure 37. Cross section of the stem of *Salix pseudomedemii*, including anatomic properties: 1) eglandular trichome, 2) epidermis, 3) cortex parenchyma, 4) druse, 5) sclerenchyma, 6) phloem sclerenchyma, 7) phloem, 8) cambium, 9) xylem, 10) pith rays, 11) xylem parenchyma, 12) pith.

are some intracellular spaces. These areas are irregular in form and occurrence. Sclerenchyma is seen within the cortex region as big clusters. The cells of the cortex parenchyma, which lies beneath the sclerenchyma, are much smaller and contain less starch. Solitary calcium oxalate crystals exist in some cells. Below this layer, phloem sclerenchyma is visible, although it is not as large as the sclerenchyma above. Phloem is composed of small, irregularly

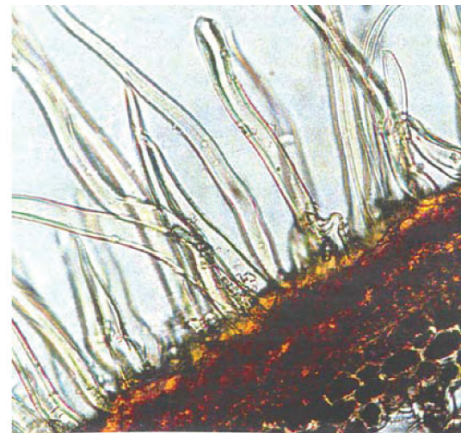


Figure 38. Eglandular trichomes of *Salix pseudomedemii* (10×).

shaped, thin-walled cells. Cambium cells are slender-shaped, flattened, and multilayered. Xylem is not very large and all of its elements are easily visible. Xylem is divided by single-celled horizontal layers of pith ray cells. These cells are seen as dark rays within the xylem because of their starch content. Rounded, dense, and starch-containing parenchymatic cells of xylem are seen in abundance when we approach the pith. Pith is formed by rounded parenchymatic cells. No calcium crystal or starch is observed in the pith (Figure 39). All layers of the anatomical structure of the stem can be seen in Figures 37 and 40.

***Salix amplexicaulis* Bory & Chaub.**

Cross sections of young stems are rounded (Figure 41). Pith appears rounded and does not constitute a large area. Epidermis is formed of a single layer of generally square-shaped cells. Cells are thick above

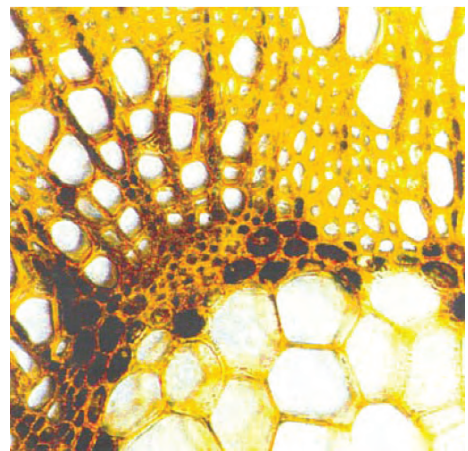


Figure 39. Pith and xylem cells of *Salix pseudomedemii* (10×).

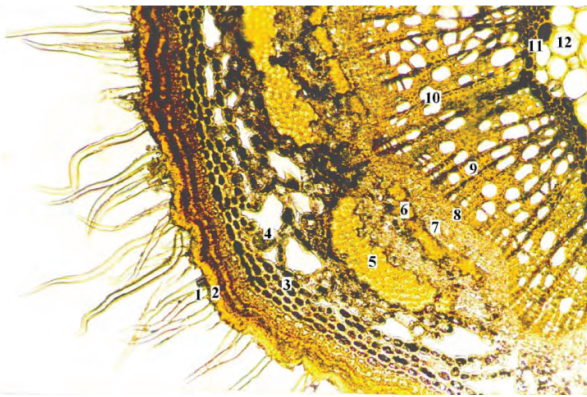


Figure 40. Cross section of the stem of *Salix pseudomedemii*, including: 1) eglandular trichome, 2) epidermis, 3) cortex parenchyma, 4) druse, 5) sclerenchyma, 6) phloem sclerenchyma, 7) phloem, 8) cambium, 9) xylem, 10) pith rays, 11) xylem parenchyma, 12) pith (4×).

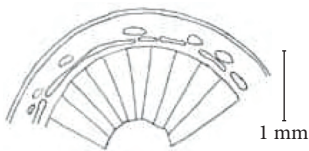


Figure 41. General view of the cross section of the stem of *Salix amplexicaulis*.

and below but thin-walled in the sides. No glandular trichomes are visible. Cortex parenchyma, beneath the epidermis, is formed by thick-walled, generally oval-shaped, rich starch-containing cells with a small intercellular space (Figure 42). Simple crystals are found in some parenchymatic cells (Figure 43). In addition, large or small clusters of sclerenchyma are seen in this region. Cortex parenchyma beneath these bundles has 1 or 2 layers and smaller cells that contain less starch. Beneath this layer, phloem sclerenchyma exists as a thick belt all over the stem. Phloem has small, generally cornered, irregularly shaped, thin-walled cells. Simple crystals are found in phloem parenchyma. Cells forming the cambium are pressed and remarkably 4-5 layered. Xylem constitutes a large area in the stem and all elements of the xylem are easily seen in this area. Xylem is divided by pith rays that are mostly single-layered cells. Pith rays are viewed as dark-coloured rays because of their starch content within the xylem. Pith is centred; xylem cells close to pith are small but larger through the centre. Pith is formed by rounded parenchymatic cells. Starch



Figure 42. Cross section of the stem of *Salix amplexicaulis*, including anatomic properties: 1) epidermis, 2) cortex parenchyma, 3) crystal, 4) sclerenchyma, 5) phloem sclerenchyma, 6) phloem, 7) cambium, 8) xylem, 9) pith rays, 10) xylem parenchyma, 11) pith.

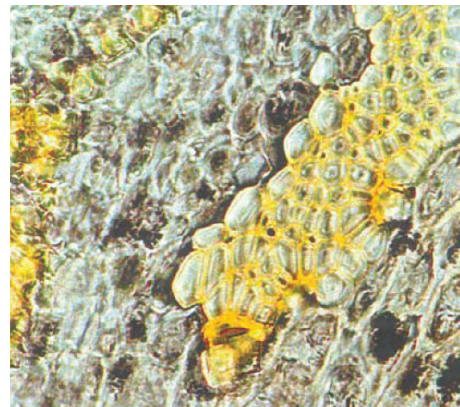


Figure 43. A crystal in the sclerenchyma of *Salix amplexicaulis* (10×).

and calcium oxalate crystals are not found in the pith (Figure 44). All layers of the anatomical structure of the stem can be seen in Figures 42 and 45.

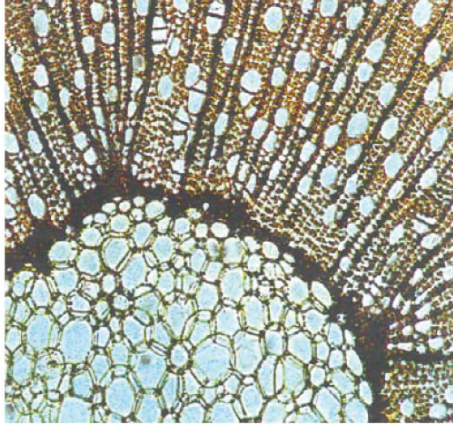


Figure 44. Pith and xylem cells of *Salix amplexicaulis* (10×).

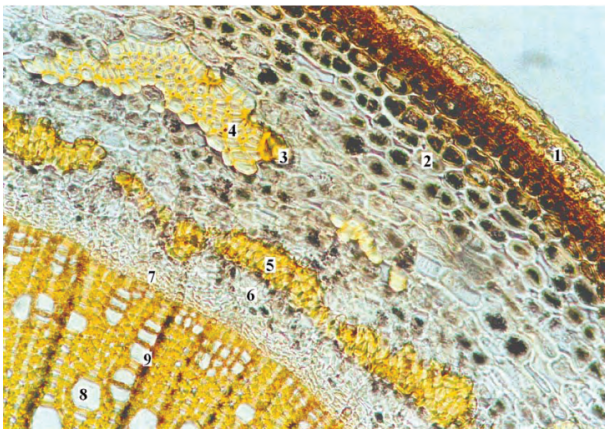


Figure 45. Cross section of the stem of *Salix amplexicaulis*, including: 1) epidermis, 2) cortex parenchyma, 3) crystal, 4) sclerenchyma, 5) phloem sclerenchyma, 6) phloem, 7) cambium, 8) xylem, 9) pith rays (4×).

Discussion

We have investigated the anatomical structure of young stems of some of the willow species growing in the province of Ankara. These species include *S. triandra* subsp. *triandra*, *S. alba*, *S. excelsa*, *S. fragilis*, *S. babylonica*, *S. caprea*, *S. cinerea*, *S. pseudomedemii*, and *S. amplexicaulis*. According to the records in the literature, there have not been any studies on the anatomy of willows in Turkey in this context, and such studies are similarly limited in other countries of the world. The most detailed information is

given in Metcalfe and Chalk's (1965) *Anatomy of Dicotyledones*. Our literature review indicates that only one previous study, "*S. alba* in Turkey" (Yazgan et al., 1986), provided a cross section of *S. alba* with schematic and anatomical illustrations. It has been observed that the anatomical structure of the stems of these selected willow species show general similarities. Suberin is found prominently in the outer layer in *S. triandra* subsp. *triandra* and *S. pseudomedemii*. E glandular trichomes are observed in *S. pseudomedemii* and *S. cinerea*, and this anatomical feature is apparently visible in the morphology as well (Arihan & Güvenç, 2009). The epidermis is generally formed by a single layer of cells. In *S. pseudomedemii*, there can be up to 2 layers of oval cells. The cortex parenchyma under the epidermis is formed by rounded, oval, thick-walled cells and is darkly viewed because of starch accumulation. They contain druses (except *S. triandra* subsp. *triandra* and *S. amplexicaulis*). Simple crystals can be seen in some of the cells (*S. excelsa*, *S. fragilis*, *S. pseudomedemii*, and *S. amplexicaulis*). The existence of druses and crystals in nonlignified tissues are in parallel with the information provided by Metcalfe and Chalk (1965). Another common feature shared by all of the species is the existence of intercellular space. From the literature, it is known that there exist some gaps within the periderm and cortex following the secondary growth of wood tissue (Esau, 1965). Such a partition was also observed in our study. Phloem sclerenchyma was found in all of the species except *S. alba* and *S. babylonica*. Sclerenchyma, which forms groups within the bark tissue, was observed in all of the species under investigation, and, furthermore, it was seen that in some of the species it is ordered so as to form belts within the stem. Single-celled parenchymatic pith ray cells are visible in all of the species and form a line between the bark and pith. Trachea and tracheids, which form the xylem, are fairly thin-walled. These 2 findings are also parallel to those described by Yazgan et al. (1986). As noted in that study, the cambium is mostly 3- or 4-celled. Druses are found in the pith of *S. excelsa*, *S. fragilis*, *S. babylonica*, *S. caprea*, and *S. pseudomedemii*; starch is found in *S. pseudomedemii*. In the *Salix* species, pith can be rounded (as in *S. triandra* subsp. *triandra*, *S. caprea*, *S. pseudomedemii*, and *S. amplexicaulis*), almost stellate (*S. alba*, *S. babylonica*, *S. cinerea*),

Table 2. A comparison of cross sections from the stems of 9 willow (*Salix*) species growing in Ankara, Turkey.

Stem	<i>S. triandra</i>	<i>S. alba</i>	<i>S. excelsa</i>	<i>S. fragilis</i>	<i>S. babylonica</i>	<i>S. caprea</i>	<i>S. cinerea</i>	<i>S. pseudomedemii</i>	<i>S. amplexicaulis</i>
Epidermal cells	1 layered rectangular, thick suberin layer	1 layered rectangular, 3 walls are thin, outer walls thick	1 layered	1 layered rectangular, thin	1 layered	1 layered, rectangular, thin walled, outer is covered with cuticle	1 layered, convex	1 sometimes 2 layered, oval, outer walls thick suberin covered	1 layered, rectangular shaped, below and upper walls thick, sides are thin walled
Cortex parenchyma cell	rounded, oval, starch containing	rounded, oval, thick walled starch, druse	spherical, oval, rounded, thick walled, starch, druse, crystal	oval, thick walled, starch, druse, crystal	rounded, thick walled, starch, druse	rounded, thick walled, starch, druse	rounded, thick walled, starch, druse	rounded, oval, thick walled, starch, druse, crystal	oval, thick walled, starch, crystal
Intercellular spaces	+	+	+	+	+	+	+	+	few
Phloem sclerenchyma	+	+	=	+	-	+	+	+	+
Xylem parenchyma	pale, starch granules (*)	starch granules+	starch granules(*)	dark, starch granules+	dark, starch granules+	starch granules+	starch granules+	dark, starch granules+	dark, starch granules+
Pith content			druse	druse	druse	druse	druse, starch		
Pith shape	rounded	close to stellate	stellate	stellate	close to stellate	rounded	close to rounded	close to stellate	rounded

+ : present; -: absent; *: rare

or stellate (*S. excelsa*, *S. fragilis*), as mentioned by Metcalfe and Chalk (1965). This property does not fit into a taxonomic order.

According to the characters mentioned in Table 2, *S. pseudomedemii* shows the greatest difference among these species. This finding is in parallel with the taxonomic status of the species (Arihan & Güvenç, 2009). A synopsis of the infrageneric taxa of the *Salix* species in Turkey has been made by Skvortsov and Edmondson in *Flora of Turkey* (Skvortsov & Edmondson, 1970). Species *S. caprea*, *S. cinerea*, *S. pseudomedemii*, and *S. amplexicaulis* are placed under the subgenus *Vetrix* Dumort.

The other 4 species investigated in this study belong to the subgenus *Salix*. None of them contain solitary calcium oxalate crystals except *S. pseudomedemii* and *S. excelsa*. The only species that has no calcium oxalate crystal is *S. triandra* subsp. *triandra*. According to molecular evidence (Azuma et al., 2000; Trybush et al., 2008; Chen et al., 2010), *S. triandra* subsp. *triandra* differs from other species of subgenus *Salix* and forms a distinct clade. Here the molecular evidence supports our own anatomical findings. The existence of solitary and clustered crystals in the unligified tissues is in accordance with Metcalfe and Chalk (1965). Phloem sclerenchyma is absent in *S. alba* and *S. babylonica*. Densely starch-containing (dark-coloured) xylem parenchymatic cells are found in *S. alba* and *S. pseudomedemii*. Druse in the pith can be seen in *S. excelsa* and *S. babylonica*. The pith of *S. alba* is stellate, as described by Metcalfe and Chalk, whereas the pith of *S. triandra* subsp. *triandra* is round; the other species are variously intermediate. Among these species, *S. alba* can be confused with *S. excelsa* and *S. babylonica* because of their general

similarity in terms of morphological appearance. The species *S. alba* and *S. babylonica* may be especially likely to be confused morphologically. According to our study, most of the characters are shared between these 2 species. Only 2 characters serve to distinguish them: the dark xylem parenchymatic cells in *S. alba* and the existence of druse in the pith of *S. babylonica*. Further morphological confusion may occur between *S. alba* and *S. excelsa*. Anatomically, *S. alba* can be distinguished from *S. excelsa* by the dark xylem parenchyma in *S. alba* and by the existence of phloem sclerenchyma, druses, and solitary calcium oxalate crystals in *S. excelsa*.

The bark anatomy of the 9 species that we investigated does not indicate a strong division, as is possible to determine from morphological and leaf anatomical characters. Further studies on this subject, however, may yield additional taxonomic data. Therefore, it is possible to use anatomical characters to differentiate between closely related species, although anatomical characters are not always as useful as morphological characters for plant identification. Some of the anatomical features used in this study, such as druses, crystals, or starch content, should be further tested in seasons other than summer, as a shift in the physiological state of the plant can change the accumulation of different materials. In further studies, the stability and reliability of these characters should be investigated since some of these characters may change according to environmental conditions and through hybridisation, a common occurrence in the genus *Salix*. It is hoped that the present study will provide a basis for future research on willow species in Turkey in addition to contributing to knowledge about those being examined in the greater Mediterranean region.

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