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Comparative leaf anatomy of three food plants that are used medically; *Mespilus germanica* L., *Malus sylvestris* (L.) Mill. subsp. *orientalis* and *Cydonia oblonga* Mill. (Rosaceae)

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Abstract

Anatomy of leaf cross sections were carried out on the three food plants that are used medically in the family Rosaceae; *Mespilus germanica* L. (Muşmula), *Malus sylvestris* (L.) Mill.(Elma) and *Cydonia oblonga* Mill. (Ayva) Anatomical features of the leaves were good taxonomic markers, especially concerning their palisade cells, cuticle, vascular bundles, crystals, stomatal index, and stomata structure. The bifacial blade has a heterogeneous and hipostomatic mesophyll and possesses a fascicular vascular system. Non-glandular trichomes are present in the lower epidermis. Paradermal sections of the lower epidermis disclose epidermal cells with undulate walls and anomocytic stomata type for *C. oblonga* and *M.sylvestris*, but actinocytic stomata type for *M. germanica*. It was calculated that the stomatal index ranges 16.08 µm-22.54 µm. This is the first study of leaf anatomy on the species.

Key words: Leaf Anatomy, *Rosaceae*, *Mespilus germanica, Malus sylvestris, Cydonia oblonga*

Introduction

The valuable fruits of *Rosaceae* family have formed an integral part of the human diet. Rosaceae is the third most economically important plant family in temperate regions. Fr uits of this family are extremely rich in compounds with strong antioxidant activities, such as L-ascorbic acid, phenolics including tannins, flavonoids and other phytochemicals beneficial for health. Biochemical profiles of several fruits have been estimated to provide new opportunities for both dietary and therapeutic purposes, such as

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the process of drug development (Rop et al. 2011). *Rosaceae* are considered valuable remedies and used frequently for various purposes.

M. germanica fruits are used in folk medicines, primarily for treatment of constipation, as a diuretic, or to rid the kidney and bladder of stones (Baytop, 1999). Its pulp or syrup is a popular remedy against enteritis. Stem bark decoction is used as an antihelmintic, leaves decoction is used in diabetes. In addition, *M. germanica* is used in animal (cattle) treatment (Tuzlacı & Tolon, 2000). For abdominal pain; *M. germanica* bark of the branches is boiled and administered orally, to treat tuberculosis; *Corylus maxima* Mill. leaves are mixed with the leaves of *M. germanica*. The concentrations of phenolic compounds and the antioxidative capacity are significantly altered by the degree of maturation of the medlar fruit, especially during the ripe to overripe periods (Gruz et al. 2011, Bibalani & Mosazadeh-Sayadmahaleh 2012).

C. oblonga Mill. fruits are eaten to stop diarrhoea. Leaves are used for common colds, flu, diarrhoea, cough, stomach ache, urinary disorders as tea (Yesilada et al. 1999). Also leaves decoction is used for cough, eczema and diabetes (Tuzlacı & Tolon, 2000).

M. sylvestris Mill. subsp. *orientalis* fruits decoction is used for cold and diabetes (Kargıoğlu et al. 2008; Ozturk & Altundag, 2011). Fresh and dried fruits are made compote (especially in winter) (Şenkardeş & Tuzlacı, 2014). Also leaf decoction is drunk with honey for bed wetting children and fruit infusion is used to cure asthma, bronchitis, cough (Kızılarslan & Özhatay, 2012).

The aim of the present study was to comparatively investigate the anatomical alterations taking place in the leaves of three food plants that are used medically. This is the first study of leaf anatomy on the species.

Material and Methods

All samples were collected from Istanbul, Usküdar in 2015 by the first author. Fresh leaves were cut from specimens and put into a 70% ethyl alcohol. Cross-sections were obtained by sectioning the leaves from the tip to 15–20 mm, and then stained with SARTUR solution (Çelebioğlu & Baytop 1949). Several slides were made and photographed for each

species with the aid of a light microscope (Olympus BH-2 and Canon A 640 digital camera). Stomatal index (SI) was calculated according to the formula of Salisbury (1927): SI = S/ (E+S) × 100, where S is the number of stomata per unit leaf area and E is the number of epidermal cells per unit leaf area.

Results

The leaf anatomy of the collected specimens was assessed in terms of their overall aspect including examination of leaf cross section, palisade cells, cuticle, vascular bundles, crystals, stomatal index, and stomata structure (Figures 1, 2, 3). Additionally, a number of parenchyma cells are oxaliferous – druses- raphide- prismatic - calcium oxalate crystals. Stomata are present in between the lower epidermal cells only – hipostomatic blade, these characteristics are similar all species. Paradermal sections of the lower epidermis disclose epidermal cells with undulate walls and anomocytic stomata type for *C. oblonga* and *M.sylvestris*, but actinocytic stomata type for *M. germanica*. It was calculated that the stomatal index ranges 16.08 µm-22.54 µm with SD ± 2.67 µm. The results were given in Table 1.

***Cydonia oblonga* Mill.**

Morphology: Leaves are ovate to oblong or occasionally suborbicular, up to 10 × 7 cm, entire, bilaterally white-tomentose at first, becoming glabrous above and densely villous beneath; petioles are 1-2 cm.

Leaf blade anatomy: Cross section of the bifacial blades reveals the upper epidermis, the mesophyll and the lower epidermis. The one-layered lower epidermis and upper epidermis are covered by an obvious outward cuticle. Trichomes were found longer and denser in the mid rib area than in the rest of the blades. The anomocytic stomata occurred more frequently on the lower epidermis and were usually surrounded by 3-4 ordinary epidermal cells. The palisade parenchyma was composed by three layers of cells, which were smaller and more elongated at the inner layer. In some sectors it was difficult to distinct. The spongy parenchyma had seven layers of cells with various shapes loosely arranged.

***Malus sylvestris* (L.) Mill. subsp. *orientalis* (A. Uglitzkich) Browicz**

Morphology: Leaves are elliptic to obovate or suborbicular, 3-8(-10) × 2-4(-5) cm, crenate or serrate, pilose when young, especially so above, on maturity glabrous or slightly tomentose above, tomentose or occasionally glabrescent beneath; petioles are tomentose, 2-5 cm.

Leaf blade anatomy: Cross section of the bifacial blades reveals the upper epidermis, the mesophyll and the lower epidermis. The one-layered lower epidermis and upper epidermis are covered by cuticle. Trichomes were found longer and denser in the mid rib area than in the rest of the blades. The anomocytic stomata occurred more frequently on the lower epidermis and were usually surrounded by 3-4 ordinary epidermal cells. The palisade parenchyma was composed by two layers of cells with numerous chloroplasts and 7-8 layers of spongy tissue with well aligned cells and few chloroplasts.

***Mespilus germanica* L.**

Morphology: Leaves are lanceolate to obovate, 5-12 × 2-6 cm, entire or serrulate, bilaterally adpressed-pubescent or subglabrous above and adpressed-pubescent beneath, especially along the midrib, petioles are 1-2 cm.

Leaf blade anatomy: Cross section of the bifacial blades reveals the upper epidermis, the mesophyll and the lower epidermis. The one-layered lower epidermis and upper epidermis are covered by cuticle. The genus *Mespilus* may have a multilayered epidermis (Aldasoro et al. 1988), but epidermis is a layer in this study. It has seven-eight cells at the bottom of the hair. The mid rib is very prominent on the lower surface of the blade. Loosely arranged layers of parenchyma cells without chloroplasts surround the vascular bundle. The palisade parenchyma was composed by two layers of cells with numerous chloroplasts and 7-8 layers of spongy tissue with well aligned cells and few chloroplasts.

Table 1: Comparative anatomical characteristics of *Cydonia oblonga, Malus sylvestris* and *Mespilus germanica*

Examined characteristics	*C. oblonga*	*M. sylvestris*	*M. germanica*
Length of midrib (mm)	0.76	0.83	0.99
Width of midrib (mm)	0.74	0.68	0.87
Collencyma thickness (mm)	0.43	0.32	0.21
Sklerencyma thickness (mm)	0.026	0.016	0.077
Epiderma thickness (µm)	2.6	2.4	1.7
Diameter of vascular bundle (mm)	0.82	0.75	0.92
Palisade thickness (µm)	44.73 2-layer, elongate	89.71 3-layer, prismatic	79.92 2-layer, prismatic
Spongy thickness (µm)	63.91 irregular	49.23 isodiametric	75.72 isodiametric
Stomatal index	22.54	21.13	16.08
Calcium oxalate crystals	druse	druse and prismatic	prismatic
Stomata type	anomocytic	anomocytic	actinocytic
Cells of the upper epidermis	almost undulate	pentagon or hexagon	distinctly undulate
Cells of the lower epidermis	almost undulate	pentagon or hexagon	distinctly undulate

Discussions

Past morphological studies included general vegetative habit and branching, bark, dormant buds, foliage, inflorescences, flower, pome features, some anatomical characteristics of pomes and seeds (Sterling, 1965a, b; Phipps et al., 1991; Rohrer et al., 1991, 1994; Aldasoro et al. 1988). This generated a large set of characters from which Phipps et al. (1991) used some for their cladistic analysis. But this is the first study of leaf anatomy on the species.

Unfortunately, anatomical and morphological data studied are not sufficient to explain the current distribution of genera, but the data can be used as reference for identification of herbal drugs.

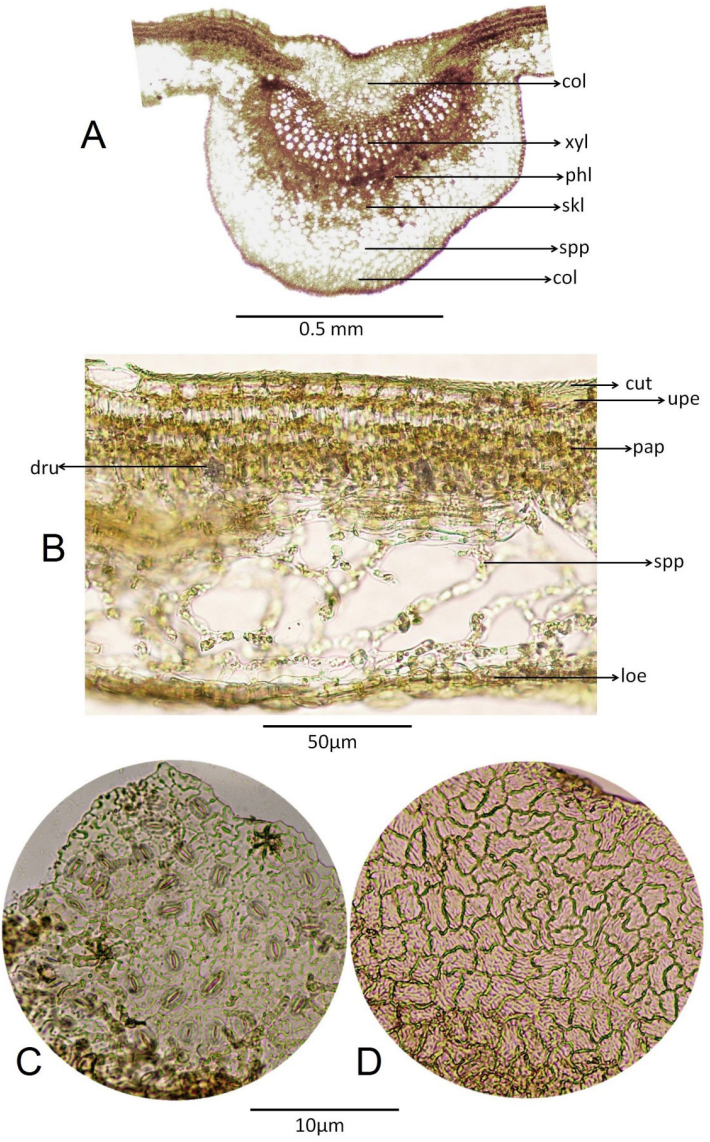

Figure 1: *C. oblonga:* A- Cross section of the mid rib; col- collenchyma, scl – sclerenchyma, xyl – xylem, phl – phloem. B- Cross section of the blade; dru- druse, cut – cuticle, upe - upper epidermis, pap - palisade parenchyma, spp - spongy parenchyma, loe - lower epidermis. C- Paradermal section of the lower epidermis. D - Paradermal section of the upper epidermis.

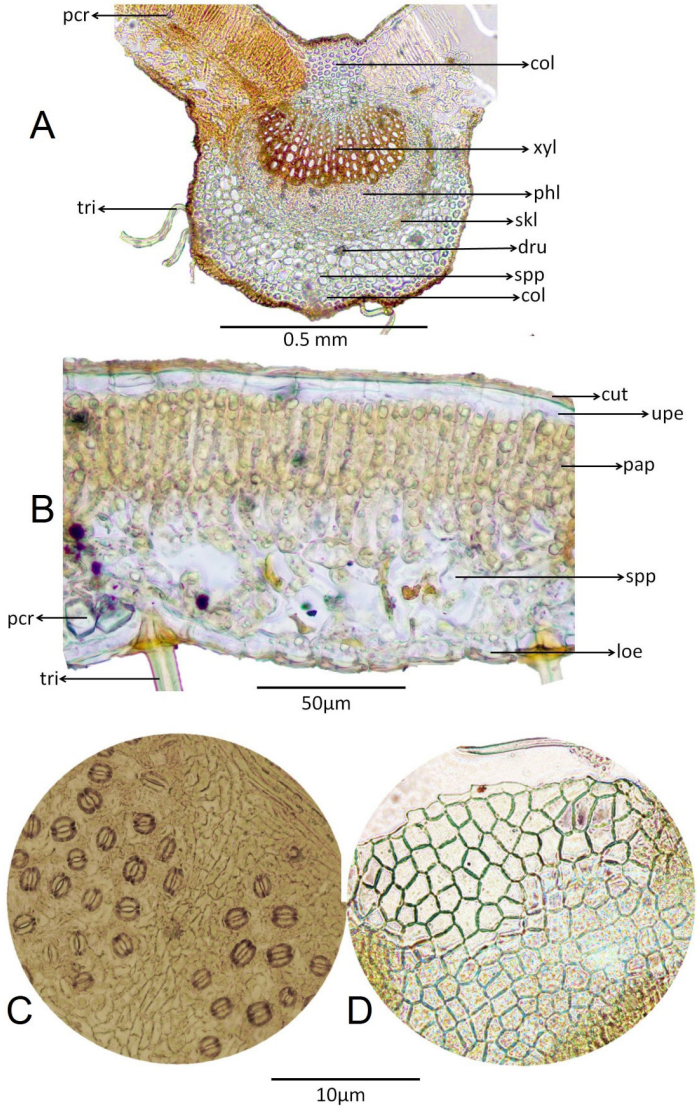

Figure 2: *M. sylvestris:* A- Cross section of the mid rib; col- collenchyma, scl – sclerenchyma, xyl – xylem, phl – phloem, dru- druse, pcr- prismatic crystal, tri - trichomes. B- Cross section of the blade; cut – cuticle, upe - upper epidermis, pap - palisade parenchyma, spp - spongy parenchyma, loe - lower epidermis. C- Paradermal section of the lower epidermis. D - Paradermal section of the upper epidermis.

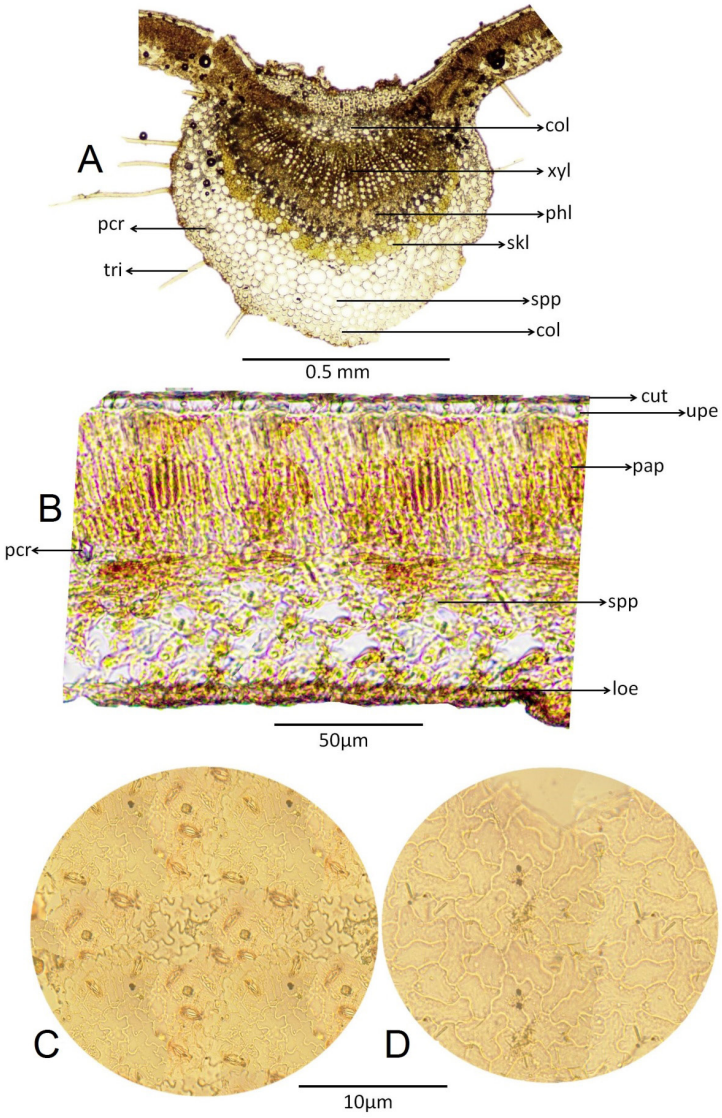

Figure 3: *M. germanica:* A- Cross section of the mid rib; col- collenchyma, scl – sclerenchyma, xyl – xylem, phl – phloem, dru- druse, pcr- prismatic crystal, tri - trichomes. B- Cross section of the blade; cut – cuticle, upe - upper epidermis, pap - palisade parenchyma, spp - spongy parenchyma, loe - lower epidermis. C- Paradermal section of the lower epidermis. D - Paradermal section of the upper epidermis.

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