Anatomical features of *Fraxinus ornus* L. growing in Iraq

Muazaz Azeez H. AL-Hadeethi  
Department of biology, College of Education for Pure Sciences  
Ibn Al-Haitham, University of Baghdad, Iraq  
Lubni_a75@yahoo.com

Lanja hewa Khal  
College of Agricultural Sciences Engineering, University of Sulaimani

Jwad Kadhem Ali  
College of Agriculture, University of AL-Qasimi Green, Iraq.

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Abstract

*Fraxinus ornus* L. is considered as a special species that is frequently planted as a decorative tree in most of the country. The cross-sections of the root and stem are circular in shape and in the secondary growth stage, the vascular tissue in the root and stem consists of secondary xylem in radial rows and the type of vessels in the xylem are ring pours wood. Epidermal cells of leaves undulate on the upper and lower side, hairs are uniseriate and unicellular and the stomata appeared in the abaxial surface only is anomocytic type. The vertical-section of blade leaf includes upper epidermis and lower epidermis followed by the palisade layer and spongy layers. The cross-section of petiole horseshoe shape and the vascular bundles are covered by sclerenchyma cells and distributed as groups.

Keywords: Anatomy, *Fraxinus ornus* L., Oleaceae family, Iraq.

1. Introduction

*Fraxinus ornus* L. is a deciduous tree from Oleaceae family, native in Europe and introduced to Iraq, the ancient name in Iraq Manna ash tree. Ibn AL-Baitar (c. 1240) mentioned the name Dardar and Lisan At Tair (Bird’s Tongue) used to description the Elm of tree, after him Ibn Wafid used the other name Lisan AL-Asfur (sparrow tongue) referring to the fruit of Ash not the Elm [1].

The trees of *Fraxinus ornus* are medium in size. The plant with male flowers is isolated from the plant that carries the female flowers. The fruit are known as samara and the seeds are known keys or helicopters [2]. In Greek mythology, they thought that the Meliae nymphs came from of the ash tree, especially the *Fraxinus ornus*, in Norse mythology, the Ash tree symbolized the first man or yew tree and they were considered as trees where ancient religious rituals were held, also namely needle ash or Eddas, or Vetgrønster Vida in ancient sources [3].

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Elsewhere in Europe mythology, it was written in it that the ash leaves were ejected the snakes by using the branches of the tree [4]. Also, in the Irish folklore mention that the shadows of ash tree would harm the crops and in Cheshire a county in western central England, and can be used to treat the warts and disease of babies caused by vitamin D lack [5].

[6]. Refer that the wood of *Fraxinus ornus* tree is considered a hardwood, thickness, tough, elastic and very strong, usually used in the artificial goods like as arches, handles and other uses exacting that demanding high force, flexibility, the solid structure, elasticity and flexibility. In addition, the good looks make the ash tree a perfect wood for making the curved parts of the furniture like as staircases or some art objects that include torsions, arches or certain curvatures like as parts of an electric guitar, drum shells, office furniture, making baseball bats. The leaves of the ash tree are used for animal grazing, also cut off the branches in the autumn season can be supplied the domestic animals in winter [7]. The aim of this research is to distinguish the tissues that give this plant a force by the anatomical way to be used in the industrial materials in various fields of life. This anatomy study is also the first for this species in Iraq.

2. Material and Methods

2.1. Collection and authentication of plant material

*Fraxinus ornus* plant was gain from the herbal garden of AL-Gherai'at north of Baghdad (Latitude of AL-Gherai'at: 33.4051920, 44.3473190) at April 2018. The plant was specified by using the Flora of Iraq as a source [1]. Figure 1. The wet parts of samples of the plants were preserved in formalin acetic acid (FAA) [8]. For 24 hours and then moved in 70% alcohol. The epidermis was done by putting the leaves in 0.5% Hypochlorite AL-Sodium for 10-20 min to remove the chlorophyll, then washed off with distilled water and stained by Safranin 1% for 30-minute [9]. Finally, the epidermal samples were examined by Olympus KRÜSS light microscope then photographed using MC500 camera.

The stomatal index was extracted by [10].

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\text{Stomatal index} = \frac{\text{number of stomata}}{\text{number of stomata} + \text{number of ordinary epidermal cells}} \times 100
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For doing sectioning parts of root by hand section, the procedure was performed according to [11]. We was took the parts of plants that was put in 70% ethanol, then sectioned by a razor blade into thin and small pieces (4-6 cm) then put in 0.5% Hypochlorite AL-Sodium for 5 mint to clear the tissue and finally put on the slide with cover slides and DPX then, examined it by Olympus KRÜSS light microscope then photographed using MC500 camera. The Permanent slides of cross sections for the stem, leaves and petiole followed by [12]. As follows:

Leaves and petioles were cut into small pieces with a length of (0.5-2) cm. Pieces put in vials with (20) ml Formalin acetic acid (F.A.A.) and left for (20-24) hours at room temperature. The samples were currently washed with distilled water and put in 70% alcohol, then dehydrated in Tertiary Butyl Alcohol (TBA) series as in Table 1. Samples were put in paraffin four times; each time remained for three days, and then embedded in paraffin which was poured in boxes made of cardboard satin for sectioning with sliding microtome. The sectioning were performed using sliding microtome (leica SM 200R sliding) with a thickness of (8-12 µm), after sectioning the ribbons and put on a water bath (40-45 C°) with the aid of 0.5% gelatin to fix the ribbons on the slide and then put on hot plate (40 C°) for drying. The wax was removed from ribbon by passing the slides containing plants pieces in different solutions as following: (Xylene for (5mins.), Xylene + 100% alcohol (1:1) for (5 mins.), Ether + 100% alcohol (1:1) for (5 mins.), 100%
alcohol for (5 mins.), 95% alcohol for (5 mins.), 70% alcohol for (5 mins.), Stained in 1% safranin in alcohol for (12-24) hours, washed by distilled water 3-4 times for (1-2) sec., 95% alcohol for (10 sec.), Fast green for (1-3) sec., Clove oil + 100% alcohol + xylene (2:1:1) 2-3 times, Xylene + 100% alcohol (1:1) for (5 mins.), Xylene for (10 mins.).

Table 1. Tertiary butyl alcohol (TBA) dehydration series.

| TBA series | Distilled Water | Absolute Ethanol | 95% Ethanol | TBA | Time |
|------------|---------------|------------------|-------------|-----|------|
| TPA1       | 50%           | 0                | 40%         | 10% | 3-6 h|
| TPA2       | 30%           | 0                | 50%         | 20% | 3-6 h|
| TPA3       | 15%           | 0                | 50%         | 35% | 24 h |
| TPA4       | 0             | 0                | 45%         | 55% | 24 h |
| TPA5       | 0             | 25%              | 0           | 75% | 24 h |

3. Results and Desiccation

3.1. Description of species:

*Fraxinus ornus* L. is a genus of flowering plants medium to large trees, around 7-10.5 m and can reach up to 22 m, the stem cylindrical and the leaves are opposite in whorls of three, shiny green color, petiolet and pinnately Figure 1. Most *Fraxinus* having male and female flowers on separate plants are used as an ornamental, the seeds are known as keys or helicopter and the type of fruit are known as a samara. The result agrees with [13,14,15].

3.2. Cross section of roots:

The cross section of root circular shape and in the secondary growth stage, the average thickness of epidermis 5.5 μm and the cortex are very thin were the average reached to 27.5 μm, the vascular bundle consist from xylem and phloem and the average of vascular bundle thickness are 115.6 μm, the type of vessels in the xylem are ring pours wood. The pith very wide Table 2. & Figure 2. The results agree with [16].

3.3. Cross section of stems:

The cross section of stem is circular shape, also they are in secondary growth stage like root, the average thickness of periderm is 4.5 μm, the cortex includes 4-6 layered from collenchyma tissue beneath the periderm and 7-10 layers of parenchyma tissue. The vascular tissue includes from the secondary xylem in radial rows and the type of vessels in the xylem are ring pours wood, this is considering an important taxonomic characteristic for distinguishing between species within one genus and among other genus within same family; the secondary phloem is outside the xylem Table 2. & Figure 3. The results are confirmed with [17].

3.4. Leaves section:

Epidermal cells of blade are undulate on the upper and lower side. Hairs are uniseriate and unicellular have conical shape. Stomata is appeared in the abaxial surface only and the adaxial surface free from stomata, the type of stomata is anomocytic type, the stomatal frequency 80/mm² on the abaxial side, and the stomatal index 3.11, also the stomata length reached to 23-29 (26.5) μm and the width is 22-25 (23.5) Figure 4. The results agrees with [18]. The measurements of leaf blade and midrib appear in the Table 1.The vertical section of blade leaf covering by cuticle, the average of the thickness 2.5 μm, upper epidermis is single layer contain from large cells oval in shape the average thickness 6.5 μm, follow the epidermis one palisade layers, 29.1 μm, under it five spongy layers the average thickness 52.1 μm and the lower epidermis like the upper (9.5 μm), Figure 5A.
The edge shape of leaf like a snake curved to the down Figure 5B. This feature is considered as special characters to discriminate between species in the same genus [19].

Vascular bundle, open and collateral consists of xylem and phloem.

3.5. Cross section of petioles:

The cross-section of petiole horseshoe shape, the epidermis is single-layered, the cortex includes 2-3 layered of collenchyma cells under the epidermis and 3-4 layered of parenchyma cells. The vascular bundle is a continue and includes from xylem and phloem covered from outside by sclerenchyma tissue and distributed as groups. The pith located in the center includes parenchyma cells isodiometric shape throw of it the ordinary schizogenous intercellular space Figure 6.

Table 2. The measurements characters of root and stem in the Fraxinus ornus L.

| Characters | Cuticle Thickness | Epidermis Thickness | Cortex Thickness o | Vascular Bundle Thickness |
|------------|-------------------|---------------------|--------------------|--------------------------|
| Root       | nil               | 4.5-6 (5.5)         | 20.5-28.3 (27.5)   | 110-120.5 (115.6)        |
| Stem       | 2.3-2.6 (2.6)     | 3.5-5.5 (4.5)       | 20.5-28.3 (27.5)   | 110-120.5 (115.6)        |

Table 3. The measurements characters of leaves in the Fraxinus ornus L.

| Characters | Cuticle Thickness | Upper Epidermis Thickness | Lower Epidermis Thickness o | Palisade Layers Thickness | Spongy Layers Thickness |
|------------|-------------------|---------------------------|-----------------------------|---------------------------|-------------------------|
| Leaf       | 1.5-2.7 (2.5)     | 6.7-7.2 (6.5)             | 8.3-11.1 (9.5)              | 27.2-31.5 (29.1)          | 48.4-56.5 (52.1)        |

Figure 1. Tree and leaf in the Fraxinus ornus L.
Figure 2. Cross-section of root in the *Fraxinus ornus* L (40X).

Figure 3. Cross-section of stem in the *Fraxinus ornus* L. (40X).
Figure 4. Upper and Lower of epidermis of leaf in the *Fraxinus ornus* L. (40X).

Figure 5. Longitudinal-section of blade of leaf in the *Fraxinus ornus* L. (A: 40X) and (B: 10X).
4. Conclusion

Based on the results, *Fraxinus ornus* L. is considered as a special species which is frequently grown as a decorative tree in most of the countries. Also, the hardwood of this tree is used in artificial goods and because most of the anatomical features are unclear for many researchers. This work clarifies the anatomical features of this species to help many researchers and agriculturist to know how can care and breed this plant.

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