Anatomical study of the *Carissa macrocarpa* (Apocynaceae family) in Iraq

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**Abstract.** This study was conducted to examine the anatomical aspects of *Carissa macrocarpa*, of stem, leaf and leaf venation. The results obtained showed that the tissue of the studied parts have important anatomical characteristics in terms of the shape of the cross-sectional of stem and vertical-sectional of leaves. The stem section appeared in circular and the midrib was crescent shape and the stomata appeared on the upper surface of the leaf only, Tetracytic in type. Druses crystals and Aleurone granules appeared on both surfaces of leaves. The venation type was Brochidodromous in which the secondary veins do not end at the edge of the leaf, and each secondary race is connected with the higher race and linked together with a series of prominent arches.

**Keywords.** *Carissa macrocarpa*, Anatomical study, Apocynaceae, Iraq.

1. **Introduction**

The Apocynaceae family consists of 300 genera and more than 1300 species spread all over the world and in Iraq have 4 wild species and 7 cultivated species [1]. The species is considered domestic in South Africa and is grown in Egypt [2]. Most of the plants in this family were herbs or shrubs or trees with milky juice, the leaves were simple, bracelet or alternate with smooth edge, the flowers radial symmetry and the seeds are often covered with hairs [3]. Most of the plants are grown for decoration, but many species are poisonous to humans and animals if they eat the fruit and their vegetative compositions, extracting a number of drugs and tanning materials [4]. We did not find published data provides clarify anatomical information to describe the species under study in Iraq, so the aim of this study provide and description anatomical features and enhanced measurements and forms which can be adopted the morphological studies of this species and applications of phylogenic relationships [3, 5, 6, 7].

2. **Materials and Methods**

The present study relied on the fresh samples of the species Carissa macrocarpa (Figure 1) collected at the flowering stage from the Botanical Garden of the College of Education for pure sciences-Ibn Al-Haitham, parks, public parks and some office located within the areas of AL-Adhamiya, Alkraiat, and...
Al-Ameriya in the Baghdad at the period from (20/12/2017 - 20/3/2018). The diagnosed of the species by taxonomic keys and fluorescent Arabic and international as well as Internet sources. The fresh samples of the stem and leaf were cut up to 2-3 cm from the middle of each part [8]. The samples were fixing by formalin acetic acid alcohol (F.A.A.) for 24 hours at room temperature according to [9] and then washed with alcohol concentration 70% to remove traces of the fixative solution and then kept in the same concentration of alcohol in the refrigerator until used. Several samples of preserved samples were selected to prepare the epidermal of leaves to study the stomata and the other Accessories of the epidermis. The epidermis of the leaf was peeling by a razor blade also the samples of stems and leaves have cut off by using the hand sectioning method follows by [10] were the parts of stem sectioned into thin pieces by a razor blade too. All the samples of the stem, leaf, and epidermis of leaf were put in the 0.5% sodium hypochlorite for 5 mints to remove the chlorophyll pigments, then the samples were transported to Petry Dish with Safranin pigment to gives color to the tissue of stems. Finally, the samples of the stem were put on the slides and mounted by cover slides with D.P.X. and fixed by Olympus light microscope then photographed using the Omax camera. The measurements of tissue were made using the Ocular micrometer and the characters were studied that are, the epidermis features, the cross-section of the stem and the cross-section of leaves also the dimensions of the stomata were measured and the stomatal index was calculated according to [11].

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\text{Stomatal index} = \frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Number of ordinary epidermal cells}} \times 100
\]

Also doing clearing of vegetative leaves to identify the system of venation as followed [12]. The study also relied on the terms contained in [3, 5, 6, 13].

3. Results and Discussion

3.1. Cross section of stem

The cross-section of the stem circular shape (Figure 2). The epidermis of stem consists of small cells, simple, uniseriate and cubical shape with straight walls, the thickness of it reached 13.4 µm. the epidermis covered by cuticle except for the position of stomata. The cortex consists from two layers the first layer it’s the collenchyma tissue under the epidermis consists from 5 rows free of intercellular
spaces and the thickness of it reached to 75 µm, the second layer it’s chlorenchyma tissue consist from parenchyma cell riches of chlorophyll, the cell were very thin circular to polygonal shape and throw it the intercellular spaces, the thickness of it reached to 133 µm. the average thickness to all cortex reached 210 µm. The vascular bundles open and collateral ensue as a wavy circular ring around the pith, the thickness of it 20 µm consist of phloem and xylem. the phloem consists of sieve tubes and companion cells have a transparent area being responsible for the milky white secretions that come out when the stem is broken and this is agreed with [2]. The thickness of phloem reached to 8 µm and the xylem thickness reached to 10 µm, also the number of vessels rows in xylem reached to 8-10 rows (Table 1). The pith consists of many layers of parenchyma cells storage, circular to polygonal shape with thin walls throw it intercellular spaces, the pith occupied the central part with solid tissue, the Druses crystals diffuse in the cortex and pith, and this is agreed with [2].

Table 1. characters cross section of stem in the species Carissa macrocarpa (µm).

| Epidermis thickness | Collenchyma thickness | Chlorenchyma thickness | Cortex thickness | Phloem thickness | Xylem thickness | Number of xylem rows | Vascular bundles thickness |
|---------------------|-----------------------|-----------------------|-----------------|-----------------|----------------|----------------------|--------------------------|
| 7.5-15.2 (13.4)     | 70.4-78 (75)          | 125.9-135.8 (133)     | 200-215 (210)   | 6-10 (8)        | 8-12 (10)      | 8-10 (8)              | 15-26 (20)               |

Figure 2. Cross section of stem in the species Carissa macrocarpa X40.
3.2. Leaves

3.2.1. Service view of leaves

The ordinary epidermal cells on both sides of leaf consist from oblong to-coordinate cells like bee cells with straight walls to semi-wavy on the side (Figure 3) this is due to changes in the shape of the outer tangential walls and the inner tangential walls of the cells [14]. The average of cell long on the upper epidermis of leaf reached to 46.2 µm and the average of width 43.2 µm also the average of cell long on the lower epidermis reached to 32 µm and the average of width 29 µm.

3.2.2. The Complex of stomata of leaves

The stomata appeared on the lower surface only and these results agree with [1]. Their diffused on that surface varied as they appeared in high density, were circular in shape, and the stomatal model was Tetracytic type, which is surrounded by four normal cells on that surface (Figure 3). It is possible to rely on the variation in the dimensions of stomata and stomatal evidence as an important anatomical characteristic that helps in the diagnosis and isolation of the species, it is also a demonstration of plant efficiency in photosynthesis [2].

3.2.3. Longitudinal section of leaves

The epidermis of blade was characterized by upper epidermis and lower epidermis, but they were almost identical, were consist from simple and uniseriate epidermal cells, with square shapes to polygonal ridges, stacked together, and straight walls have low wavy, and the lower epidermis appeared thinner and more stomata than the upper epidermis, the average thickness of upper epidermis reached to 22 µm, while the average thickness of the lower epidermis was 10 µm. The epidermal cells covered a layer of the thick-toothed cuticle (Figure 4). The mesophyll tissue, which follows the upper Palisade parenchyma and the lower spongy parenchyma, the type of this arrangement of mesophyll known bifacial and this type is a common form in the most leaves of plants. The palisade layer consist from 1-2 rows of oblong cells with narrow intercellular space between cells, its longitudinal axis is perpendicular to the leaf surface and contains chloroplasts, the average thickness of palisade layer reached to 62 µm, so the spongy layer of mesophyll consists from loose tissue have air lacunae between the cells and the cells irregular shape.
consist from druses crystals and ailerons grains, the rows of it reached to 2-3 rows and the average thickness of spongy layer reached to 94 µm, so the average of thickness of blade reached to 120.8 µm (Table 2). The result of the study of appeared that the midrib has almost straight from the upper to the zigzag from the lower (Figure 4) the tissue of midrib consist from two types of cells, the Angular collenchyma, occupied the corners of the middle race against the vascular bundle and exchanged with chlorenchyma cells, spherical to polygonal shape, containing an abundance of chloroplasts. The Crystals were also observed in the tissue and around the vascular bundles. The study showed that the vascular tissue in the midrib is only an extension of the vascular strand from the stem to the blade. Therefore, the vascular tissue retained its components of xylem and phloem elements. The vascular tissue appeared in the midrib in the form of veins, consisting of vascular bundles branching into several intertwined branches and thus the main vascular bundle showed the largest bundles in the middle ovate broad shape, and the average thickness of the vascular bundle was 33 µm. In addition to the emergence of sub-vascular bundles spread in the leaf blade less in size as we move away from the midrib, 1-2 rows of parenchyma cells surrounded by the vascular bundle compose the bundle sheath. The results of the present study confirmed that there are differences in the anatomical characteristics shown by the leaves of the species under study and the leaf consider is the most plant-specific part of the anatomical characteristics and the most consistent, this agrees with [8] that appeared the leaf has been used extensively to solve the most difficult taxonomic problems between different genus and species.

Table 2. Characters of longitudinal section of blade and midrib of leaves in the species Carissa macrocarpa (µm).

| Upper Epidermis thickness | Lower Epidermis thickness | Palisade layer thickness | Spongy layer thickness | Blade thickness | Vascular bundles thickness |
|---------------------------|---------------------------|--------------------------|------------------------|-----------------|---------------------------|
| 18-24 (22)                | 9.2-13.1 (10)             | 220-305 (62)             | 260-300 (94)           | 115-130 (120.8) | 20-38 (33)               |

*The number between parentheses refer to average

3.3. Crystals

The results of the present study showed crystals and their forms, and that one plant species may contain two or more types of crystals of different shapes and sizes and this is due to the form and nature of the isolated cell Idoblast, which acts as a template responsible for the shape of the crystal, the
crystals consist from calcium oxalate is a toxic acid which is produced by the plant's biological activity. Oxalic acid is a toxic acid and is therefore transformed by cells into insoluble compounds in the form of crystals that minimize their toxic effect [1]. The druses crystals appeared as a diffuse on the upper and lower leaves surfaces and appeared within the spongy layer and parenchyma tissue of their blade, as well as in the tissue of the cortex and pith of stem (Figure 5).

3.4. Starch grains

The starch is one of the most important materials stored in plant cells and the composition of these granules depends on the physiological conditions associated with the plant [15]. The phenotypic characteristics of these plants vary depending on the location and shape of the starch grains like the center of the grain formation (hilum), and the presence or absence of layers as well as the nature of those granules layers of being simple or complex or semi-complex [16]. The starch grains appeared in the parenchyma tissue as complex types have two hilum and the arrangement of the layers around each of them, the hilum circular shape un-centric location (Figure 6) also showed the Aleurone grains in the epidermis of leaves (Figure 7). Starch grains are an important anatomical characteristic in isolating plant species [17].

Figure 5. Druses crystals of the leaves in the species Carissa macrocarpa X100.

Figure 6. Starch grains of the leaves in the species Carissa macrocarpa X100.

Figure 7. Aleurone grains of the leaves in the species Carissa macrocarpa X100.
3.5. **Venation system of leaves**

The type of venation in the leaves of this species has a reticulate system, a characteristic of Dicotyldone [18], which was a type of Camptodromous, in this type the midrib penetrates the blade longitudinally, characterized by being larger than the rest of the veins, thicker and longer than the rest veins, the secondary veins. Branching from the midrib don’t reach to the margin of leaf and this veins branching and bifurcate and come back together to form a brochidodromous network type, in this type the veins do not end at the edge of the leaf and each secondary veins is connected with the higher vein and linked to each other composes prominent arches (Figure 8).

![Figure 8. Venation pattern of the leaves in the species Carissa macrocarpa (cm).](image)

4. **References**

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