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Cladode and fruit anatomy of *Opuntia ficus-indica* (L.) Miller in Türkiye

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ABSTRACT

Opuntia ficus-indica (L.) Mill. belong to the Cactaceae family, since can grow in conditions where other plants cannot survive, it is distributed in many parts of the world due to its socioeconomic, nutraceutical, and ecological properties. Especially, its fruits and cladodes are used as herbal medicine for various health problems in different countries. Pharmacological effects such as antioxidant, antimicrobial, anticancer, antiulcer, hepatoprotective, wound healing, hypocholesterolaemia, anti-diabetic, and anti-obesity were demonstrated with conducted studies. In this study, the anatomy of cladode and fruit of *O. ficus-indica* grown in Türkiye has been studied in detail. Cyclocytic stomata were detected on both fruit and cladode surfaces. Abundant calcium oxalate crystals and mucilage cells were detected in both fruit and cladode. The importance of anatomical study was emphasized for plants to be used for food and medicinal purposes.

Keywords: *Opuntia ficus-indica*, cladode, fruit, anatomy

INTRODUCTION

Opuntia ficus-indica (L.) Mill. is a perennial drought-resistant plant in the Cactaceae family, adapted to arid and semi-arid areas. The plant, which is generally known as "prickly pear" in the world, is known by names such as "Frenk

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inciri, Hint inciri, Kaynana dili" in Türkiye. Although its origin is Mexico, it is grown in many parts of the world today such as Mediterranean countries, South Africa, and North and South America[1-3].

Shrub or small tree with the succulent trunk, which is 2-4 m high, are spread. As the trunk ages, woody branches form in the lower part. The trunk has undergone metamorphosis by taking the shape of a leaf and is called a cladode. Its trunk has an articulated structure, and the joints are 10-40 × 7-20 cm in size, ranging from oblong-elliptical to obovate and flattened. Caducous leaves are about 3 mm long and subulate. As with other plants, they also develop spines rather than leaves, these spines are white in colour. They may develop spiny structures called glochids are weak, thin, small brush-like structures, alongside or in place of these spines and yellow colour. Glochids are found in small meristematic eyes called areoles. Glochids are found in the areole space in clusters of 7-12. Areoles do not have spines and their number varies between 1-6. The surfaces of spines are rough, however, glochids have smooth ones. The flowers are hermaphrodites and bright yellow. The berry is oval, 5-10 cm long, red, yellow, orange, or purplish. There are areoles on the berry, the berry usually does not bear spines. The seed has bony testa. It is whitish, and numerous[3-5].

Food products made from its fruit and cladodes are plentiful. Its fruits are utilized to produce a variety of items such as jams, juices, alcoholic beverages, natural sweeteners, body lotions, shampoos, and creams. Its cladodes are consumed as vegetables. Their principal usage is flour, which can be used in place of maize or wheat flour in baked goods including bread, cookies, and cakes[6]. In Italy, cladodes are used directly for skin diseases, viral infections (Herpes), and joint pain, while in South Africa it is recommended for haemorrhoids and toothache[7,8]. In Ethiopia, the cladodes are set on fire for anthrax in livestock and applied to the affected area when hot, and crushed and rubbed into their skin for lice or flea infestations[9]. In the Philippines, the decoction of cladodes is recommended for diarrhoea[10]. In Bolivia, it is recommended as moxibustion for angina, headache, and oedema, while its sap is consumed for cough. The sap obtained by crushing it is used for burns in the form of direct application, for hair such as shampoo, for kidney pain directly or by heating, and its decoction for body cleansing[11,12]. While the jam of the fruit is consumed for cough and cold in Italy, the fruit is eaten directly for gastritis in Ethiopia[7,13].

The plant grows naturally in the Mediterranean and Aegean regions of Turkey and also is cultured in these regions[14]. The decoction of cladodes is used for bronchitis by drinking a glass three times a day, and its mash is used by making compresses for rheumatism in Turkey[15]. Its cladodes and fruits are applied

with salt once a day for 1-2 weeks for dislocation and tonsillitis by heating, boiling, or in the form of cataplasm[16]. The fruit is eaten as fruit after peeling the prickly skin in the Mediterranean Region of Turkey (Fig. 1). This fruit is also eaten fresh for stomach-ache, 10 times a day on an empty stomach[17]. After peeling, it is consumed for diabetes, and anaemia, as well as a laxative and aphrodisiac. Peeled fruits are also used for moistening in the form of mash[18, 19].

Figure 1. *Opuntia ficus-indica* fruits and peeling off the prickly skin of the fruit (Photo. A. Köroğlu)

It is reported that especially, flavonoids, phenolic acids, betalains, carotenoids, and sterols in the fruit[20-30]. The fruit also is rich in Vitamin C and E [20, 21, 25, 31]. Its seeds are regarded as a good source of unsaturated fatty acids[25,32]. It has been indicated that the cladodes contain especially flavonoids, phenolic acids, tannins, and carotenoids[33-39].

Antioxidant, antimicrobial, antileishmanial, and anticancer activities of the fruit have been demonstrated by *in vitro* studies[20,24,25,28,30,39-42]. Its neuroprotective, sedative, hypocholesterolemic, antiulcer, and hepatoprotective effects have been approved by *in vivo* studies[20,29,43,44]. Especially, antioxidant and antimicrobial activities studies have been carried out on the cladodes *in vitro*

[34,35,37,45,46]. The pharmacological effects of cladodes such as antiulcer, wound healing, anti-inflammatory, hepatoprotective, antidiabetic, antispasmodic, hypocholesterolemic, and anti-obesity have also been demonstrated by *in vivo* studies[34,35,47-52]. The number of anatomy studies on the cladode is limited[53-55]. In addition, no clear study was found about the anatomy of the fruit, except for the structure of the pericarp of the seed[56].

This study aims to determine the anatomical features of the fruit and cladode of *Opuntia ficus-indica*, which is of great importance in terms of both medicinal and food use.

METHODOLOGY

The fruits and cladodes samples were collected in Antalya-Olympos (Türkiye) on August 22, 2017 (Figure 1-2) and preserved in 70% alcohol. The specimens were identified by Professor Ayşegül Köroğlu. At least 5 different samples were examined, and cross and surface sections were taken. The cross and surface sections taken from these samples were examined under the light microscope (Leica CME) with Sartur reagent [57] and their images were obtained with the Leica DFC280 camera.

Figure 2. *Opuntia ficus-indica* (Photo. A. Köroğlu)

RESULTS and DISCUSSION

Anatomy of the cladode blade

The morphological view of the cross-section of the cladode blade is shown in Figure 3. In the cross-section, it is surrounded on the outside by a thick waxy cuticle layer. The epidermis cells are rectangular and single-row. The hypodermis is usually 4-rowed and the walls of its cells are thickened. Big druses are observed in this part. The chlorenchyma consists of multi-row, thin-walled, and long rectangular-shaped cells with plastids. It contains a large number of druses and starches. Mucilage cells are observed in the chlorenchyma. Below the chlorenchyma is the part where the vascular bundles are located. The vascular bundles are of the closed collateral bundle type, with a xylem on the inside and phloem on the exterior. The core is usually composed of round and colourless cells. It carries abundant druses and starches (Figure 4).

Figure 3. The morphological view of the cross-section of the cladode blade

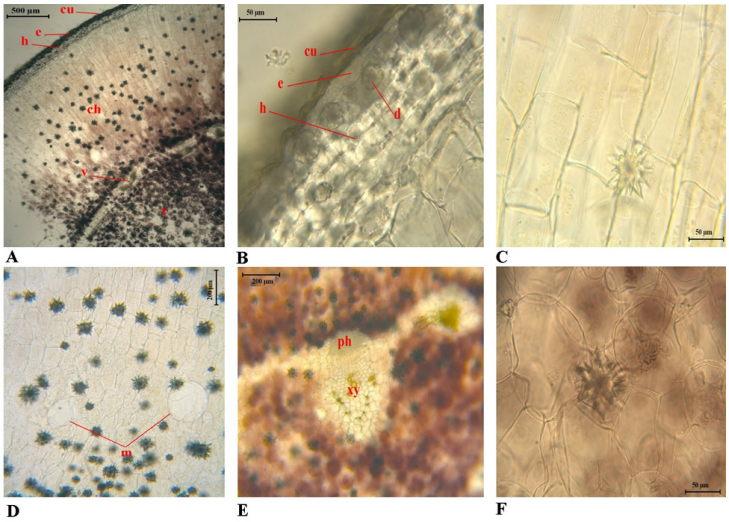

Figure 4. The microscopic image of the cross-section of the cladode blade; **A., B.:** General view, **C.** Druse in chlorenchyma, **D.** Mucilage cells in the chlorenchyma, **E.** Vascular bundle, **F.** Druse in the core. c: core, ch: chlorenchyma, cu: cuticula, d: druse, e: epidermis, h: hypodermis, m: mucilage cells, ph: phloem, v: vascular bundle, xy: xylem.

In the surface section, the epidermis cells are polygonal and slightly wavy-walled. Cyclocytic stomata were observed, the subsidiary cells of which were not very clear. The presence of druses and mucilage cells was observed (Figure 5).

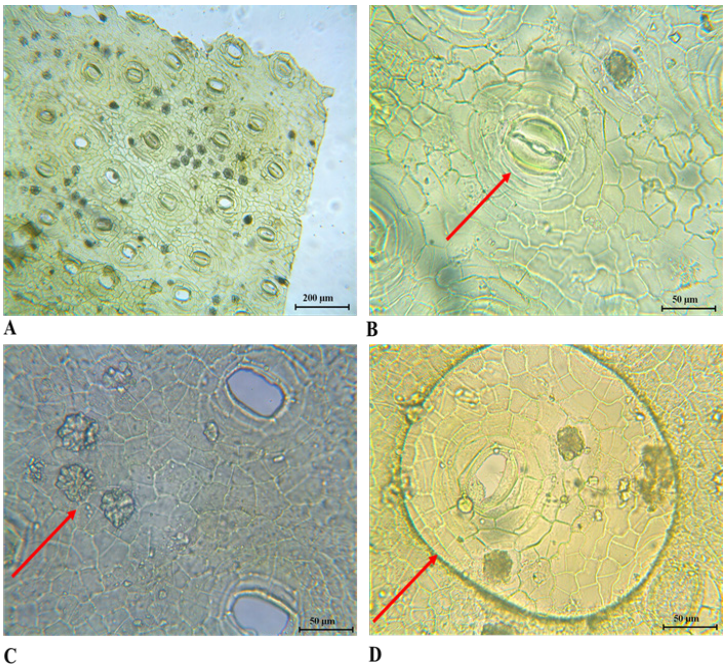

Figure 5. The microscopic image of the surface section of the cladode; **A.** General view, **B.** Stomata, **C.** Druses, **D.** Mucilage cell.

Anatomy of the fruit

The morphological view of the fruit and morphological view of the cross-section of the fruit are shown in Figures 6 and 7, respectively. In the exocarp, the thick waxy cuticular layer is located. Usually, rectangular and single-row cells make up the epidermis. The cells of the hypodermis usually have four rows and it has thicker cell walls. In the hypodermis, abundantly big druses are seen. Multi-row colourless long cylindrical parenchyma cells are located under the hypodermis. The mesocarp consists of irregularly shaped paranchymatic colourless cells with plastids. The presence of mucilage cells was observed in the mesocarp. Druses are observed more intensely in the mesocarp than in the exocarp. The vascular bundles are of the closed collateral bundle type and scattered in the mesocarp (Figure 8).

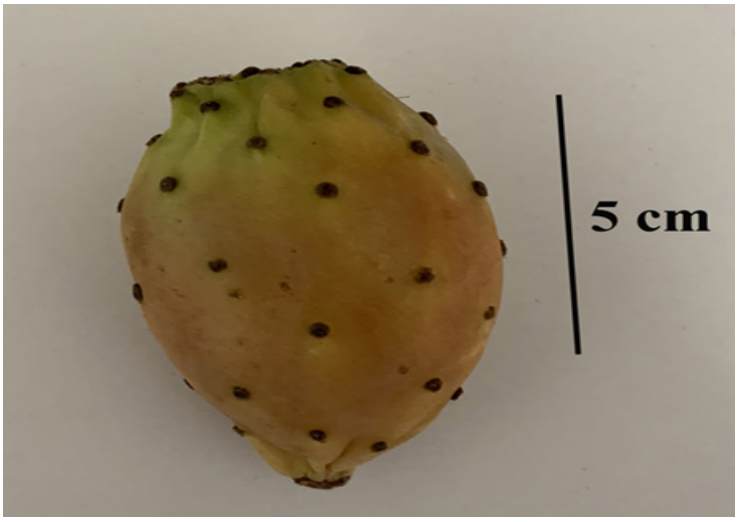

Figure 6. The morphological view of the fruit of *Opuntia ficus-indica*

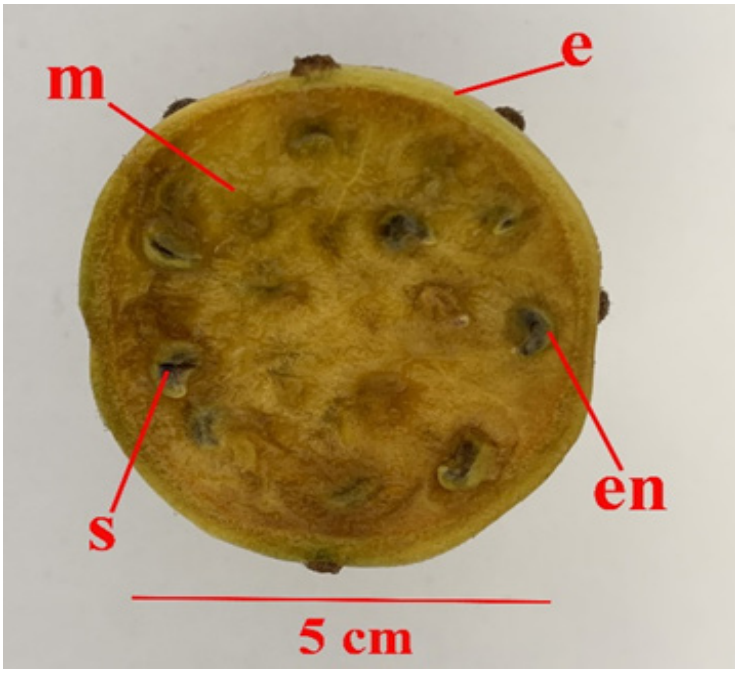

Figure 7. The morphological view of the cross-section of the *Opuntia ficus-indica* fruit; e: exocarp, m: mesocarp, en: endocarp, s: seed

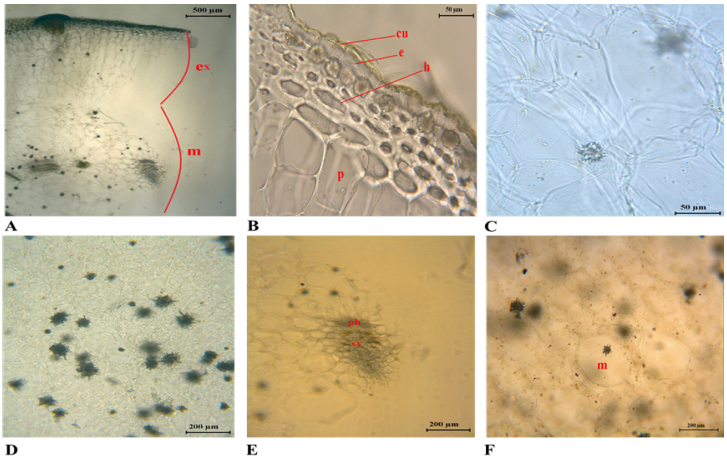

Figure 8. The microscopic image of the cross-section of the *Opuntia ficus-indica* fruit; A., B.: General view, C. Druse in exocarp parenchyma, D. Druses in the mesocarp, E. Vascular bundle, F. Mucilage cells in the mesocarp. e: epidermis, ex: exocarp, cu: cuticula, h: hypodermis, m: mesocarp, m: mucilage cell,.p: parenchyma, ph: phloem, xy: xylem.

In the surface section of the fruit of *Opuntia ficus-indica*, the epidermis cells are irregularly shaped and have slightly wavy walls. Cyclocytic stomata are present and the cells adjacent to the stoma are not clear. Abundant large druses are observed on the surface of the fruit (Figure 9).

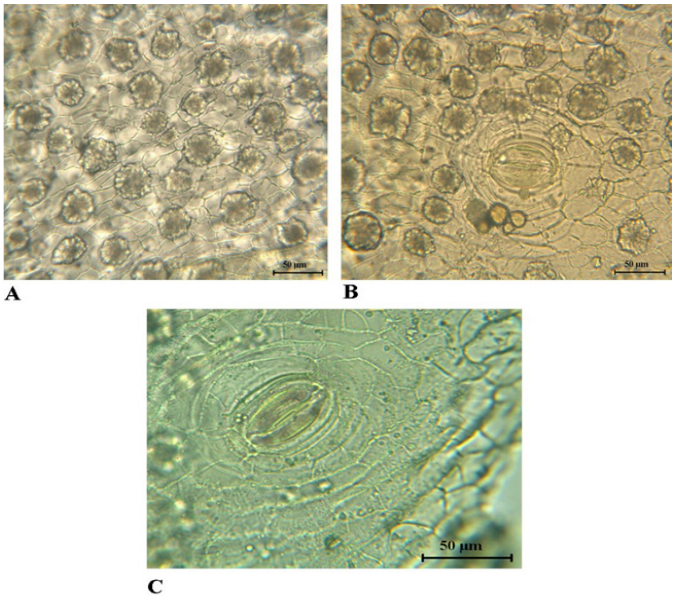

Figure 9. The microscopic image of the surface-section of the fruit; A. General view, B. Stomata and druses, C. Stomata

Different parts of *O. ficus-indica* are used in various fields such as health, nutrition, and cosmetics. Fruits and cladodes contain high amounts of important nutrients. The fruits and cladodes have been used in traditional folk medicine for many years in different parts of the world for different purposes. There are many studies about the chemical properties and biological activities of fruits and cladodes. However, the number of anatomy studies on the fruit and cladode is limited. Anatomical studies are very important for the identification of microcharacters of medicinal plants part (drugs).

As a result of the anatomical examinations; cyclocytic stomata with unclear adjacent cells in both cladode and fruit surface sections; on the other hand, abundant calcium oxalate crystals were detected in the cross and surface sections of two different parts of *Opuntia ficus-indica* (Figures 4, 5, 8, 9). It was observed that the hypodermis layer was collenchymatic in the cross-section of the cladode and fruit (Figures 4A, 4B, 8B and 9B). Mucilage cells were characteristically determined on the cladode surface and among the parenchyma cells in the cross-section (Figures 4D and 5D). In addition, mucilage cells were determined in the mesocarp part of the fruit (Figure 8F). The anatomical findings we obtained are generally combined with the source data found compatible[53-56]. The presence of pseudohypoderm in the cladode and that this structure has a collenchymatic and mucilage structure were stated by Metcalfe and Chalk (1965) [58], but this structure was not observed in our study and the presence of mucilage was found in the chlorenchyma part.

The main purposes of calcium oxalate crystal production in plants are high-capacity calcium regulation and herbivore defence. The crystals consist of calcium that the plant takes from the outside and oxalate that it biologically synthesizes. Studies have shown that ascorbic acid is a precursor in the biosynthesis of oxalate. Since ascorbic acid is also an antioxidant substance, there may be a relationship between antioxidant activity and the presence of calcium oxalate crystals. Because it increases the amount of oxalate excreted in urine and leads to the development of kidney stones, oxalate content may be harmful to human health[59].

In this study, the anatomical structures of *Opuntia ficus-indica* fruit and cladode grown in Türkiye were examined using light microscopy for the first time. The anatomical structure of the fruit has been revealed in detail. Cladodes and fruits, which are consumed for their medicinal and nutritive properties, contain plenty of druses. For this reason, clinical studies should be conducted on whether excessive or long-term consumption carries risks for human health.

STATEMENT OF ETHICS

No needed.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

GK: Designed and performed the study, and wrote the first draft. AK: Collected the plant, and reviewed the manuscript.

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