

MICROMORPHOLOGY AND PHYTOCHEMICAL SCREENING OF *OPUNTIA LITTORALIS* ENGLEM. CLADODES

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The present study was conducted to investigate the micromorphological structure and phytochemical constituents of *Opuntia littoralis* stem (cladodes) growing at the western Mediterranean coast of Egypt. The cladodes were collected during the wet season (February) and dry season (September) of 2015. The size of the cladode cells differ from wet to dry season, where both composed of thin cuticle covering the epidermis, hypodermis layer, parenchyma with storage cells and scattered bicollateral vascular bundles. The stem cells facing the sun contain pheloderm layer to protect the plant. The presence of high amounts of calcium oxalate crystals was also demonstrated. Phytochemical screening of *Opuntia littoralis* cladodes showed that it contains many of active phytochemical constituents such as; flavonoids, tannins, carbohydrates, glycosides, terpens and coumarines.

Keywords: *Opuntia litorallis*, micromorphology, epidermis, hypodermis, phytochemical constituents, flavonoids

Opuntia cactus is a group of xerophytic plants including about 200 to 300 species growing, mainly in arid and semi-arid regions, as small ground-hugging plants to quite massive trees, with the majority as erect or trailing shrubs. These plants show high ecological adaptive characteristics, where they can be encountered in virtually all climatic conditions (Stintzing and Carle, 2005). Whereas *Opuntia* cacti originate from Mexico, are cultivated in both hemispheres and on all continents, except Antarctica (Inglese et al., 2002). They bear fruits and stems, which are traditionally utilized for their medicinal and cosmetic purposes, forage building material, and source for natural colors (Stintzing and Carle, 2005). However, their economic importance is mainly restricted to fresh fruit consumption in their

countries of origin (Sáenz-Hernández, 1995 and Sáenz-Hernández and Sepúlveda, 2001). Moreover; they are also exported to the European fresh fruit market (Mizrahi et al., 1997 and Sáenz-Hernández et al., 2002). Cladodes of *Opuntia* are determinate organs that attain their mature length and width during one season, but remain photosynthetically active for many years (North et al., 1995). In the future, declining water resources and global desertification may even increase the importance of *Opuntia* spp. as an effective food production system including both fruits and vegetative parts (Stintzing and Carle, 2005).

Micromorphological aspects, such as size and structure of the vessels, thickness and shape of cells and the inclusion of crystals, are used as parameters in plant identification based on histological analysis (da Silva et al., 2010). They are also considered useful in the determination of fodder quality (Brito et al., 1997) and plant resistance to disease (Santos et al., 2005). As these variables are interrelated and constitute an important complementary tool for micromorphological studies, morphometrics provides information that allows for quantitative analysis of different tissues. Micromorphological and morphometric descriptions of the anatomical structure of cactus epidermis may clarify important mechanisms for the selection of material that is more resistant to pests (da Silva et al., 2010).

Medicinal plants (fruits, vegetables, herbs, etc.) are a source for a wide variety of natural products, such as the phenolic acids and flavonoids, which are very interesting for their antioxidant properties (Wong et al., 2006). In addition to their ability to act as an efficient free radical scavenger (Katalinic et al., 2006) their natural origin represents an advantage over synthetic antioxidants, which their use is being restricted due to their carcinogenicity (Farhan et al., 2012). Moreover, phenolic compounds are a unique category of phytochemicals especially in terms of their vast potential health-benefiting properties. They have multiple biological effects and also act as antioxidants by preventing the oxidation of low density lipoproteins, platelets, aggregation and damage of red blood cells (Cheynier, 2005). These secondary metabolites present in plants vary according to their age and maturity (Pandey et al., 2011).

According to Mauseth (2005), the micromorphology of the Opuntioideae is not known in detail. In addition, the fruits of the prickly pear exhibit both intra-site and inter-site variability in the shape, color, weight, sugar, acids, antioxidants, etc. These parameters vary from one cultivar to another and are strongly influenced by the environment (Bouzoubaâ et al., 2014). In this context, the present study aims to find out the phytochemical constituents and micromorphological characteristics of *Opuntia littoralis* growing at the western Mediterranean coast of Egypt.

MATERIALS AND METHODS

The present study was carried on the developing modified stem (cladodes) of *Opuntia littoralis*, which were collected during the wet season (February) and dry season (September) of 2015 from Wadi Majed, Matrouh (25 km from Matrouh) at the Northern Mediterranean Coast of Egypt (Fig. 1).

1. Micromorphology

Fresh *Opuntia littoralis* cladodes were collected and preserved at FAA 70%; then were dehydrated by chloroform and alcohol and transferred to wax. Thin sections (8-10 microns in thickness) were cut from the cladodes using the microtome. Samples were double stained with saffranin and fast green (Johansen, 1940). Epidermal layer was separated using acetic acid: hydrogen peroxide mixture (1:1).



Fig. (1). *Opuntia littoralis* Englem. Growing at Wadi Majed, Matrouh (N.B: The photograph was taken by the author).

2. Phytochemical Screening

Freshly collected plant cladodes were dried and then coarsely powdered. One hundred gram of the coarse powder were extracted using 70% ethanol till clearness. The extract was filtered and subjected to qualitative tests for the identification of various phytochemical constituents (Brinda et al., 1981; Anonymous, 1990 and Lala, 1993). Presence of alkaloids was assessed using Dragendorff's test (Adegoke et al., 2010), while carbohydrates and proteins using Molisch and Biuret tests, respectively (Boxi et al., 2010). In addition, cardiacglycosides were assessed using concentrated H₂SO₄ test (Obianime and Uche, 2008), coumarin using alcoholic sodium hydroxide, flavonoids by Pew's tests (Peach and Tracey, 1956), saponin using foam test (Adegoke et al., 2010), tannins by ferric chloride test and terpenes using Salkowski's test (Obianime and Uche,

2008). Moreover, volatile oils were assessed using oil distillation method. All these procedures are underlined by Allen (1989).

RESULTS AND DISCUSSION

1. Micromorphology

The present study shows that most of the micromorphological features of *Opuntia littoralis* cladodes support the anatomical patterns found in Cactaceae. Mostly, the epidermis with thin walled cells covered by a thick hydrophobic cuticle; the chlorophyllous hypodermis with many crystals; the parenchymatous tissue specialized in photosynthesis and water storage; and the presence of vascular bundles are the main features previously described for Cactaceae (Terrazas and Mauseth, 2002). This plant has thin walled cuticle outside the epidermal cells, however the new cladode had a special tissue composed of a layer of meristematic cells similar to the phelloderm that produces two layers of cells similar to that of the cork, but non-lignified and they were transparent (Fig. 2). In addition, this tissue disappeared, as the plant became older, and may be replaced with the hypodermal cells. These results are similar to Mauseth (2005), where a multiseriate hypodermis with extremely thick walls and crystals is almost universally present in Opuntioideae and Cactoideae.

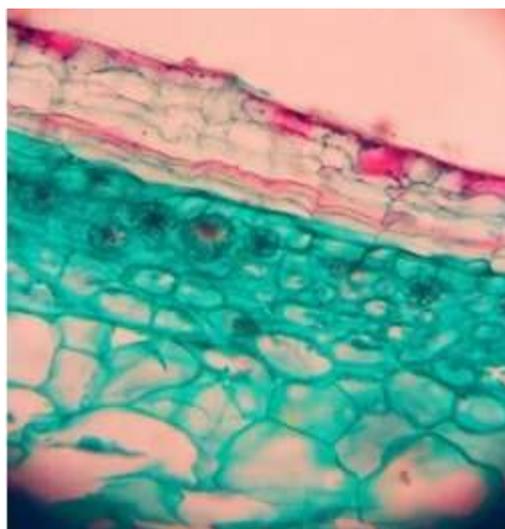


Fig. (2). T.S. of *Opuntia littoralis* cladode shows the hypodermal layer (X25).

Giovanna et al. (2009) showed in the transverse section, that the cladode skin consists of a thin walled cuticle covering a hypodermis of three or four layers of cells with heavily thickened walls. The epidermis is Egyptian J. Desert Res., **67**, No. 1, 155-170 (2017)

composed of a single layer of cells tabular in shape and filled with chloroplasts, while the hypodermal cells contain numerous calcium oxalate druses. In many Opuntioideae and Cactoideae, hypodermis cells have such thick hard walls that microtoming them is almost impossible and many contain druses in one or several layers of hypodermis. Similar results were recorded by many researchers (Conde, 1975; Mauseth and Ross, 1988; Mauseth, 1996, 1999; Mauseth and Kiesling, 1997; Mauseth and Plemons-Rodriguez, 1998 and Loza-Cornejo and Terrazas, 2003). According to Martin and Juniper (1970), the hypodermis helps to protect the inner tissues from the outer extreme conditions. Similarly, water stress and high temperature have been reported to cause an increase in the amount of plant cuticles (Lee and Priestly, 1924 and Skoss, 1955). The cells of the ground tissue remained meristematic for a long time and by dividing contribute largely to increase in girth of axis.

By studying the surface view of epidermis, it was found that the epidermal cells were composed mainly of several stomata (Fig. 3). The stomata, composed of two guard cells, were raised above the epidermal cells and appear bigger than the other tissues, although they are sunken. These results coincide with Giovanna et al. (2009). In accordance with a previous report of Rodríguez et al. (2007), the cladodes of *Opuntia ficus indica* contain significant amounts of calcium oxalate crystals as druses and these are present in all the tissues. The formation of these crystals is generally associated with calcium regulation mechanisms in tissues and organs (Hudgins et al., 2003), calcium storage when soil levels increase (Franceschi and Horner, 1980) or defense from herbivores (Ruiz et al., 2002). According to a number of studies, it is likely that the function of these crystals in cacti is to promote protection from herbivores and/or reflection of excessive sunlight, thereby avoiding damage to the chlorophyll parenchyma (Gibson and Horak, 1978). Calcium has an important role in water retention of succulent tissues to regulate the osmotic pressure in the cells. It has been shown that the size of these crystals increased as a function of maturation (Rectamal, 1987). The existence of calcium oxalate could limit the calcium bioavailability, which could also have maturation dependence.

Sharp spines were found on both sides of the stem mixed with glochides (a cluster of hairs). The thorns and hairs were developed from the so called "areoles". In this areole one or two unicellular thorns, which are bigger than hairs, were found (Fig. 4).

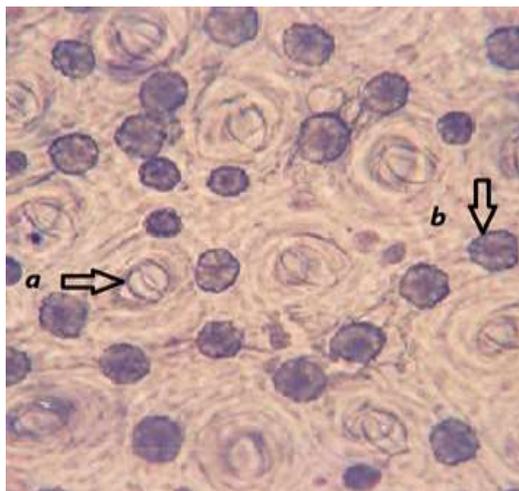


Fig. (3). Surface view of the epidermis of *Opuntia littoralis* shows a: the stomata, b: Numerous druses of calcium oxalate (X25).

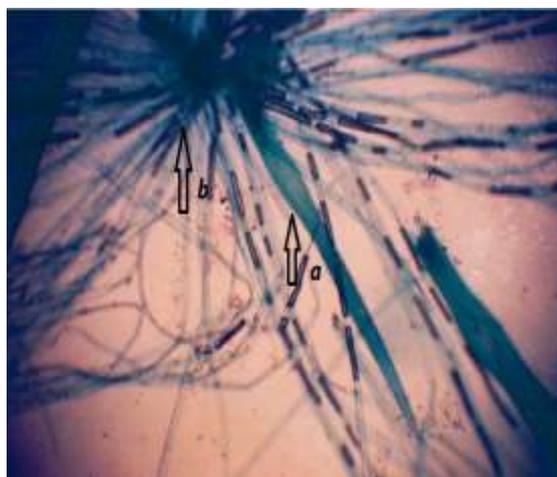


Fig. (4). (a) The sharp spines, (b) glochides found on the surface of *Opuntia littoralis* cladodes (X4.5).

In the present study, it was found that during the plant maturation, several cells rapped and act as cells with the mucilage that contains a high level of absorbed water (Fig. 5). They are found mainly on the tissues that faces the outer atmosphere. The remaining cells of the stem were parenchyma, which are smaller and denser near the surface and larger inside. These results were described previously by Mauseth (2005), who showed that the mucilage cells with intracellular secretion may be common in this family. They are present in *Pereskia*, *Maihuenia* and all samples of Egyptian J. Desert Res., **67**, No. 1, 155-170 (2017)

Opuntioideae. Canals filled with mucilage cells are very unusual, but appear to have originated three or more times in cacti in Opuntioideae; such as the species with flattened cladodes (Mauseth, 1980), and in Cactoideae; such as *Uebelmannia gummifera* (Nyffeler, 1997 and 1998).

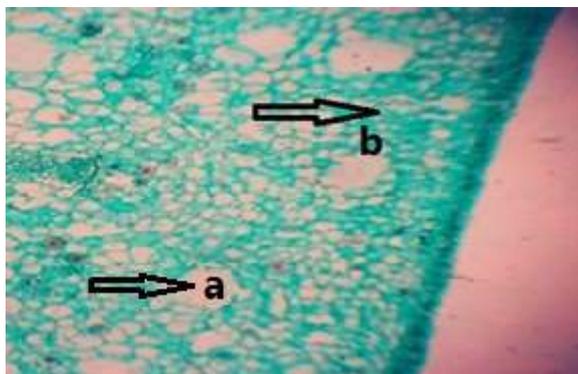


Fig. (5). T.S in *Opuntia littoralis* cladodes shows, (a). the storage cells (b). parenchyma cells (X25).

The main vascular tissue, which composed of xylem vessels with spiral lignifications mixed with few tracheids and fibers are shown in Fig. (6). In addition, the vascular system spread through this tissue consists of vascular bundles with meta-xylem and proto-xylem (Fig. 7). Each bundle has an external and internal phloem (baicollateral bundle) with no secondary structure. Similar results indicated that the vascular tissue lies in a single layer at the junction of the green chlorenchyma and the colorless central core tissue Giovanna et al. (2009). Moreover, a single mass of barrel tracheids may sometimes appear to be associated with more than one bundle. These cells have shape midway between that of barrel and that of a spindle; they are provided with locular or spiral thickening ridges, which are inserted on the thin wall by their narrow edge, and project far into the cell lumen. Generally, these tracheids were also distinguished from the actual vessels by lack of perforation (Metcalf and Chalk, 1950).

The lack of an extensive system of cortical bundles in Opuntioideae may be an important evolutionary limitation. Even with a thick cuticle, an epidermis will lose water to a dry atmosphere (Nobel, 1988), and this will require movement of water from the stele to the outer cortex, hypodermis and epidermis; if these tissues are remaining alive. In narrow stems with a thin cortex, diffusion is adequate, but with the progressive evolution of a broad cortex, diffusion gradually becomes too slow. Virtually, all Cactoideae have a set of cortical bundles vascularizing the cortex and keeping the hypodermis and epidermis hydrated, so the cortex is free to evolve to almost unlimited width (Mauseth and Sajeve, 1992). The evolution of cortical bundles must be much more difficult than the evolution of the ubiquitous

persistent epidermis and palisade cortex, because cortical bundles are absent from all non-cactus stem-photosynthetic, stem succulent plants and from most Opuntioideae.

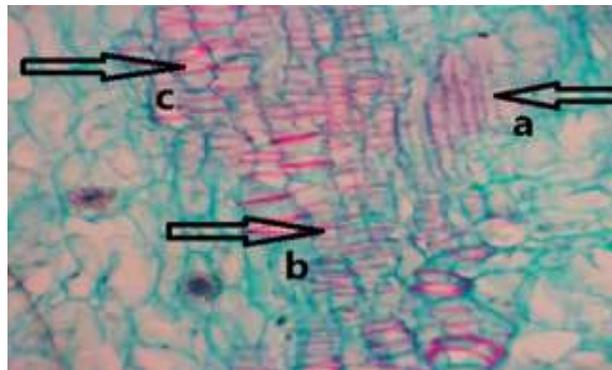


Fig. (6). T.S in *Opuntia littoralis* cladodes shows (a). fibers, (b). xylem vessels with spiral lignifications, (c). tracheids (X10).

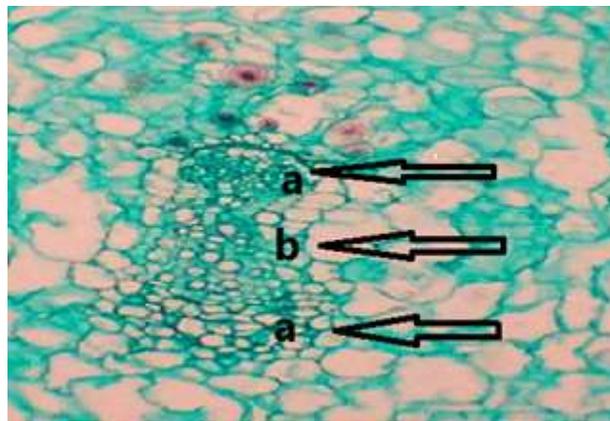


Fig. (7). T.S in *Opuntia littoralis* cladodes shows the bicollateral vascular bundle with internal and external phloem. (a). internal and external phloem, (b). xylem (X10).

Cells of the inner cortex often have thin plicate walls and thus can lose water easily and shrink; and then transferring their water reserves to the outermost chlorophyllous cells, which have thicker, non-plicate walls and cannot shrink so easily. All members of Opuntioideae, like all the non-cactus stem-photosynthetic, stem-succulent plants, have such a thin (just a few millimeters) photosynthetic cortex (Mausth, 2005). At the end of dryness season, the cladode become smaller and losses a lot of moisture and became wrinkled, the cells became smaller and the cytoplasm gathered at the side of the cell, the mucilaginous material coagulated around the cytoplasm

and the rest of the cell became nearly empty. Also many of the ground tissue were erupted and lose their liquid, the crystals of calcium oxalate became larger and some insoluble materials were observed in the epidermis cells (Fig. 8).

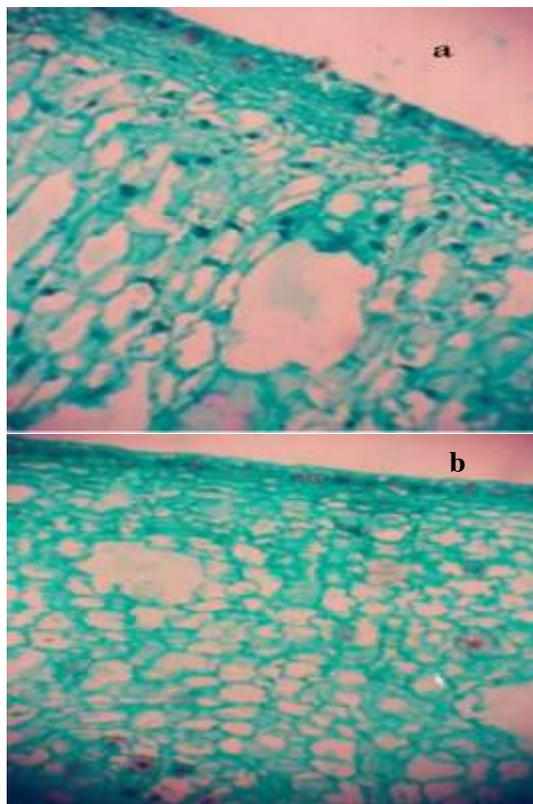


Fig. (8). T.S in *Opuntia littoralis* cladodes at (a). the dry season, (b). the wet season (X25).

2. The Phytochemical Screening

The results for preliminary phytochemical screening carried out on *Opuntia littoralis* cladods summarized in table (1), showed that flavonoids, carbohydrates, glycosides, tannins, coumarines, proteins, fatty acids, phenolic compounds and terpenes are present while absence of alkaloids, saponnins and volatile oils.

The *Opuntia* cladodes serve as a source of varied number of phytoconstituents (Chauhan et al., 2010). The composition varies depending on the edaphic factors at the cultivation site, climate and the age of the plant (Batista et al., 2003). These results were in accordance with Dib et al. (2013), who demonstrated the presence of flavonoids, tannins, terpenes and absence of alkaloids, saponnins and volatile oils at the cladodes of *Opuntia*

ficus indica. Also, Suryawanshi and Vidyasagar (2016) revealed the presence of phenolic compounds, flavonoids, alkaloids, glycoside and steroids, while, absence of terpinoids and saponins in alcoholic extract of cladodes of *Opuntia dillenii* Haw. The residue of a methanolic extract from *Opuntia dillenii* cladodes fed to male rats (250 mg/kg body weight) for 60 days reveals a significant reduction in weight and changes in the structure of the genital organs with a total lost in fertility. The effects could be attributable to the detected flavonoids (Gupta et al., 2002). Halmi *et al.* (2016) stated that flavonoids, steroids, tannins, and reducing sugars were detected and devoid of alkaloids on *Opuntia ficus indica* cladodes. The analgesic and anti-inflammatory activity of the extract would be due to the presence of sterols (campesterol, B sitosterol, lupeol), phenolic compound or alkaloids.

Table (1). The preliminary screening of the stem ethanolic extract of *Opuntia littoralis*.

| Phytochemical compound | Results |
|------------------------|---------|
| Alkaloids | - |
| Carbohydrates | + |
| Glycosides | + |
| Flavonoids | + |
| Tannins | + |
| Cumarines | + |
| Protein | + |
| Saponins | - |
| Terpenes | + |
| Fatty acids | + |
| Volatile oil | - |

As reported above several of the secondary metabolites occur in *Opuntia littoralis* and they may contribute a great deal to the antioxidant activity of a different solvent extract obtained from cladodes. Abdul Aziz et al. (2017) showed that the plant is used as antispasmodic, to treat muscle pain and anti-inflammation.

CONCLUSION

Investigating the micromorphological characters of *Opuntia litorallis* clarifies how the plant can adapt to unfavorable environmental conditions as drought, sunlight and water loss. As reported above, the phytochemical analysis conducted on the plant extract revealed the presence of several of the secondary metabolites that may contribute a great deal to the antioxidant activity of extract obtained from cladodes, which are known to exhibit medicinal activities.

The presence of tannins, which have antibacterial activity in the plant, explains the traditional use that is actually to treat diarrhea. Presence of flavonoids on alcohol extract of the cladode of *Opuntia litorallis* revealed the traditional use of the plant as anti-inflammatory, anti-diabetic and antioxidant.

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التركيب التشريحي والحصر الكيميائي لسيقان نبات التين الشوكي الأحمر

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تهدف تلك الدراسة إلى إلقاء الضوء على التركيب التشريحي والمركبات الكيميائية لسيقان نبات التين الشوكي الأحمر. حيث تم تجميع عينات من سيقان النبات المتحورة بداية من شهر فبراير حتى شهر الجفاف سبتمبر ٢٠١٥. وقد إتضح أن هناك إختلاف في حجم خلايا السيقان بين موسم المطر وموسم الجفاف، حيث تكونت السيقان في خلال الموسمين من خلايا البشرة مغطاة بطبقة رقيقة من الأدمة ثم طبقة من الخلايا البارنشيمية بها خلايا تجميع المياه والسوائل والحزم الوعائية. وقد تميزت خلايا السيقان المواجهة للشمس بوجود طبقة فلينية. كما لوحظ تراكم الكثير من بلورات أوكسالات الكالسيوم بخلايا السيقان. كما أوضحت نتائج الحصر الكيميائي للساق على إحتوائه على العديد من المركبات الكيميائية الفعالة من الفلافونيدات، السكريات، التربينات والمركبات الفينولية والبروتين والأحماض الدهنية.