

Morphological and leaf anatomical structure of *Pimpinella cypria* Boiss.

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Abstract

Pimpinella cypria Boiss (Apiaceae) is an endemic species of Turkish Republic of North Cyprus. It is locally known as 'Cyprus-rocky anise'. Three species of the genus *Pimpinella* that are *P. cretica* Poir., *P. peregrina* L., and an endemic *P. cypria* grow in Cyprus. Specimens of *P. cypria* were collected from their nature habitats; St. Hilarion castle, and specimens were dried according to the standard procedures, as herbarium specimens and kept in the Eastern Mediterranean University, Faculty of Pharmacy Herbarium (EMUH). Anatomical structure of leaves were examined. Leaves were bifacial, multicellular and long hairs were observed especially on the midrib. Leaves are amphistomatic where stomata are less on upper epidermis than those of lower epidermis. Stomata guard cells have a characteristic shaped resembling kidney.

Keywords

Apiaceae, anatomy, leaf, *Pimpinella cypria*, North Cyprus

Article History

Submitted: 28 August 2020

Accepted: 30 October 2020

Published Online: December 2020

Article Info

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Research Article:

Volume: 3

Issue: 3

December 2020

Pages: 169-181

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INTRODUCTION

Cyprus is the third largest island in the Eastern Mediterranean region after the Sicilia and Sardinia islands that is a crossing point of Asia, Africa and Europe. It measures 240 kilometres long and 100 kilometres wide. Cyprus is 75 kilometres away from Turkey. Other neighbouring regions include Syria (105 kilometres away) and Lebanon (108 kilometres to the east), Israel (200 kilometres to the southeast) and Egypt (380 kilometres to the south). The land of North Cyprus is divided into three areas; Besparmak Mountains – lying along the cape of Korucam to cape of Zafer, Mesaria plain- extending from Guzelyurt district to the eastern coastline and the third plain along the shore in the North (Yildirim, 2010). The general view and location of North Cyprus is shown in Figure 1. Apart from limited number of floristic studies (Meikle, 1977; Viney, 1994; Viney, 1996) carried out in North Cyprus, the data related with the anatomical structure of the leaves of *P. cypria* are scarce. Palynological studies belonging to the endemic taxa were published by Yıldız

et al., (2009) and Gücel *et al.*, (2008). The aim of the present study was to investigate the anatomical structures of the leaves of *Pimpinella cypria*.

According to the Flora of North Cyprus is one of the richest floras in the Mediterranean region (Meikle, 1977). The flora comprises 1649 indigenous taxa (species and subspecies), 254 introduced taxa occurring in the wild, 43 hybrids and 81 species with unclear status (as at March 2019). For the Flora of North Cyprus; it has 1610 species and 1738 taxa (Viney, 1994; Viney, 1996) 19 of species endemic for the Northern Cyprus (Yildiz and Gücel 2008). Many species of North Cyprus especially endemics are guaranteed because of they are smaller and few populations. Many of these species become endangered by the human influence; increase of the danger depends on the changes in agriculture, increasing the tourism activity and urbanization of natural places. The location and distribution of the species shown in Table 1 (Yildiz and Gücel 2008; Gücel *et al.*, 2009).



Figure 1: Location of South and North Cyprus.

Table 1: Location of some endemic species of Northern Cyprus.

Taxa , family	Locality
<i>Delphinium fissum</i> subsp. <i>caseyi</i> (Burt) C.Blanché & Molero (Ranunculaceae)	Kyrenia, St. Hilarion castle, Southwest limestone hill, north slope.
<i>Brassica hilarionis</i> Post (Brassicaceae)	Kyrenia, Girnekaya, scrubs and limestone cliffs.
<i>Arabis cypria</i> Holmboe (Brassicaceae)	Kyrenia, St. Hilarion castle limestone cliffs and rocks.
<i>Dianthus cyprius</i> A.K.Jacks. & Turrill (Caryophyllaceae)	Nicosia, Halevga, rocks and near road, southeast slopes.
<i>Silene fraduatric</i> Meikle (Caryophyllaceae)	Nicosia, Halevga, under forest.
<i>Sedum lampusae</i> Boiss. (Crassulaceae)	Kyrenia between Halevga- Girnekaya north slope Kyrenia from Halevga to Girnekaya, north slope.
<i>Pimpinella cypria</i> Boiss. (Apiaceae)	Kyrenia, St. Hilarion castle, rocky places. Kyrenia, from to Nicosia, under St. Hilarion castle.
<i>Ferulago cypria</i> Post (Apiaceae)	Famagusta between Geçitköy-Geçitkale, near road
<i>Limonium albidum</i> (Guss.) Pignatti subsp. <i>cyprium</i> Meikle (Plumbaginaceae)	Kyrenia, Tatlısu Village, sea level.
<i>Onosma caespitosum</i> Kotschy (Boraginaceae)	Nicosia, Halevga-Kalavaç road. Nicosia, Buffavento castle, south slopes.
<i>Salvia veneris</i> Hedge (Lamiaceae)	Nicosia above Değirmenlik lake, sandstone hills.

Chemical composition and medicinal usage of the family Apiaceae and genus *Pimpinella*

This family plants accumulate in flavonoids, mainly in the form of flavones and flavanols; significant antioxidant, antispasmodic and anti-inflammatory effects, due to the effects they are often used for muscle pain, stomach cramps, irritable bowel syndrome and nausea (Gebhardt *et al.*, 2005). Common flavonoids are; apigenin, quercetin, lutein, bisabolene, rutin. Vegetables in the family Apiaceae e.g fennel, carrot, parsley, celery are high in polyacetylene especially falcarinol, some research shows to be cytotoxic to five different cancer cell line to

against acute lymphoblastic leukemia (Zidorn *et al.*, 2005). Another important constituent is coumarin. It is common in all plants and expansively distributed, but for the carrot families (Apiaceae or Umbelliferae) are high in coumarin. Coumarins has vascular effect, hepatoprotective effect, anticancer effect, antispasmodic effect, hormonal effect and immune enhancing effect (Stansburg 2016). Common coumarins are osthol, umbelliferon, scopoletin, bergapten, angelicin, imperatorin, avicennin, avicenol, xanthotoxin etc., in the terms of plants which are rich in coumarin especially 'osthol'; shows anti-aggregating effects, antiproliferative effects on vascular smooth

muscle, protective effect on liver and antihypertension effects (Ramesh and Pugalendi 2005; Lee *et al.*, 2003; Guh *et al.*, 1996; Chiou *et al.*, 2001). ‘Umbelliferone’ constituent shows an anti-hyperlipidaemic, improve the glycaemic action in diabetic patient and most important activity is anticancer effect include the inhibition of cell proliferation and the induction of apoptosis. This constituent also shows as an antiviral effect as well as direct effect on skin disorders, it’s photosensitizing (Ramesh and Pugalendi 2005; Okamoto *et al.*, 2005). Approximately 70-80% of world population treated with the traditional medicine practices by using the plants. People uses plant extract for medicinal purposes by making the syrup, tablets and oral spray to treated the anxiety, central nervous system disorder, some spasmodic disorders and decreases some pain about headache, teeth or throat (Arceusz *et al.*, 2010). Also, the essential oils are obtained from the plants; they have special odour and aroma therapeutic properties. They have antimicrobial, antioxidant and anticancer effects (Hammer *et al.*, 1999; Jayaprakasha *et al.*, 2002; Lee and Shibamoto 2002; Vardar-Unlu *et al.*, 2003). For the Apiaceae family especially Anise and Caraway seeds are very important effects on traditional medicine. Anise (*Pimpinella anisum*) is an important plant and spice; used in pharmacy, perfume and

food industry. It has antispasmodic, antioxidant, antimicrobial, insecticidal and antifungal activity (Tunc and Sahinkaya 1998; Tirapelli *et al.*, 2007; Tepe *et al.*, 2006; Ozcan and Chalchat 2006; Gulcin *et al.*, 2003). Also, it can be used in throat pain, flu and expectorant (Blumenthal 1998). Some hormonal effect has been noted in this family especially in Fennel (*Foeniculum vulgare*) and Anise (*Pimpinella anisum*) seeds tea promote lactation in nursing women and some gonadotropin activity in central nervous system (Stansburg 2016; Tabanca *et al.*, 2004). Lastly this family plants can be used in allergy treatment; *Angelica* species has been found to have an antihistaminic and antiserotonin effect, so used in asthma or bronchitis (Matsuda *et al.*, 2002). It has cognitive function to improve the enhance memory especially the essential oil forms (Stansburg 2016). For this family plants mostly, contraindications are limited to hypersensitivity to active substance. Many of the oils in this family especially green anise (*Pimpinella anisum*) are neurotoxic because of the presence of particular ketones or phenonic ethers (Price and Price 2011). Some species produce phototoxic substances mainly furanocoumarin constituent species may cause photodermatitis also some members (carrot, celery, fennel, parsley, parsnip) exhibit a cytotoxic effects

(Stansburg 2016). Apart from some chemical analysis; *P. cypria* contains essential oils which is 81.7% of the total composition. The essential oils are; oxygenated sesquiterpenes (33.9%), sesquiterpenes (22.0%), monoterpenes (11.4%), oxygenated monoterpenes (2.6%) and phenylpropanoids (7.5%).

The main components of the oil were (*Z*)- β -farnesene (6.0%), spathulenol (5.9%), *ar*-curcumene (4.3%), and 1,5-epoxy-salvial(4)14-ene (3.8%).

P. cypria essential oil demonstrated moderate antimicrobial activity against Gram-negative and Gram-positive bacteria except for *Candida albicans*. Additionally, it has insecticidal activity against yellow fever mosquito's (*Aedes aegypti*) and it shows a cytotoxic effect (Tabanca *et al.*, 2016).

The genus *Pimpinella* in North Cyprus

According to the Flora of North Cyprus published by Viney, 2000, the genus is represented by 4 species; *Pimpinella anisum* L., *P. cretica* Poir., *P. peregrina* L. and *P. cypria* Boiss. *P. cypria* (North Cyprus burnet) locally known as Cyprus-rocky anise (kibris tas anasonu) (Anon 2011). *P. cretica*, slender erect annual plant with 40 cm height, zigzag-branched above, basal leaves are simple, roundish with scalloped edge, the oval segments often 3-lobed uppermost with narrow segments; umbels opposite with leaves, flowers are

white or pinkish and frequent in north-central area. *P. peregrina* hairy biennial plant to 1m with a carrot-shaped taproot, basal leaves are simple or ternate but normally withered by flowering time, upper leaves pinnate with 5-7 oval, toothed segments, umbels dropping in bud and flowers are white. Very local in Kyrenia range; shady banks in Lapta (Viney 1994; Viney 1996).

Morphological characteristics of *Pimpinella cypria* Boiss.

Pimpinella cypria is a herbaceous perennial (Apiaceae/ Umbelliferae) which is an endemic species to North Cyprus. Erect with a stout, woody rootstock clothed in the upper part with the remains of withered and indurated leaf-sheaths; stems conspicuously sulcate, rather densely (rarely sparingly) clothed with soft spreading hairs, not much branched except in the inflorescence; basal leaves oblong, pinnate with 5 (less commonly 3) segments, or rarely undivided and flabelliform, up to 12 cm long and 7 cm wide; segments broadly ovate-cuneate, up to 4 cm long 3 cm wide, thinly puberulous above, more densely pubescent below with prominent reticulate nervation, apex obtuse, margins bluntly toothed and irregularly lobed, the terminal segment often deeply 3-lobed; petioles up to 12 cm long, densely pubescent, canaliculate above, basal sheaths conspicuous, overlapping, thick, distinctly nerved; stem

leaves very sparse, similar to basal or sometimes divided into two narrow, linear or oblanceolate, pinnatisect or lacinate segments, shortly petiolate or sessile; umbels terminal, in lax spreading panicles, 6-14-rayed; peduncles 2-9 cm long; bracts and bracteoles wanting; rays shortly hispidulous, spreading, subequal up to 3 cm long at anthesis; flowers mostly hermaphrodite and fertile, numerous and rather dense in each umbellule; pedicels hispidulous, up to 5 mm long at anthesis; sepals obsolete; petals dirty white, broadly oblong-ovate, unequally 2-lobulate, flattish, about 1 mm long 0.9 mm wide, conspicuously unequally-2-lobulate, flattish, about 1mm long, 0.9 mm wide, conspicuously pilose dorsally; filaments up to 1.5 mm long, glabrous, inflexed; anthers yellow, oblong, about 0.5mm long, 0.4 mm

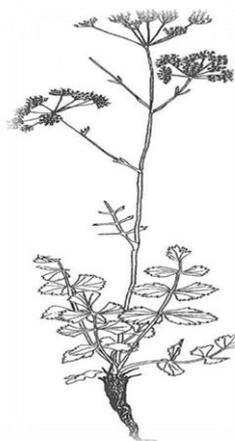


Figure 2: General habit of *P. cypria* by D.A.Viney.

wide; stylopodium convex or shortly conical, about 0.6 mm diam. with a crenulate margin; styles filiform, spreading, about 2mm long; stigmas. Fruit narrowly ovoid-ellipsoid, about 4 mm long, 2.5 mm wide, laterally compressed, conspicuously white-pilose; carpophore deeply bipartite; mericarps dark brown when ripe, convex dorsally with 5 distinct, pallid, filiform ridges, attenuate towards apex; endosperm flattish along its commissural face.

Distribution of *Pimpinella cypria*

P. cypria is common quite on North facing slopes beside the steps leading up to the St. Hilarion castle, rocky places; 800 m, Girne (Kyrenia) from Alevkayasi – North slope; 820 m, Girne (Kyrenia) near Girnekaya, north slope, rocky places; 750-800 m. *P. cypria* grown in a sunny, sheltered and rocky places.



Figure 3: *P. cypria* in its natural habitat by G Konstantinou.



Figure 4: Inflorescences of *P. cypria*.

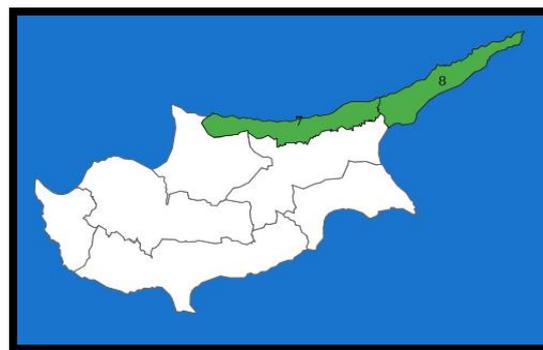


Figure 5: Distribution of *P. cypria* (green parts) (dynamic checklist- Flora of N. Cyprus).

MATERIALS

Field surveys about research material was conducted on April- 14.04.19, and May 24.05.19. Young and mature individual of *P. cypria* are shown in Figure 6.

The plant specimens that was collected on 14.04.19 (young individuals) and 24.05.19 (mature individuals) then dried according to the standard procedures and transformed into the Herbarium of Eastern Mediterranean University, Faculty of Pharmacy (EMUH), the number of the specimen SNM 002.

Study area

The survey area located in Northern Cyprus, at the west of the Besparmak

Mountains; St. Hilarion Castle. It has rocky places and above 700 m sea level. The picture and map of the study area are shown in Figure 7.

In literature review, it was seen that *P. cypria* is found in North Slope on Kyrenia mountains; Kyrenia from Halevga to Girnekaya and St. Hilarion castle. This species grows in rocky places, Halevga and St. Hilarion castle rocky places have similar topography (Gucel and Yildiz 2008).



Figure 6: Basal leaves of young *P. cypria* in April (left) and flowering stage of mature *P. cypria* in May (right) (taken by author).

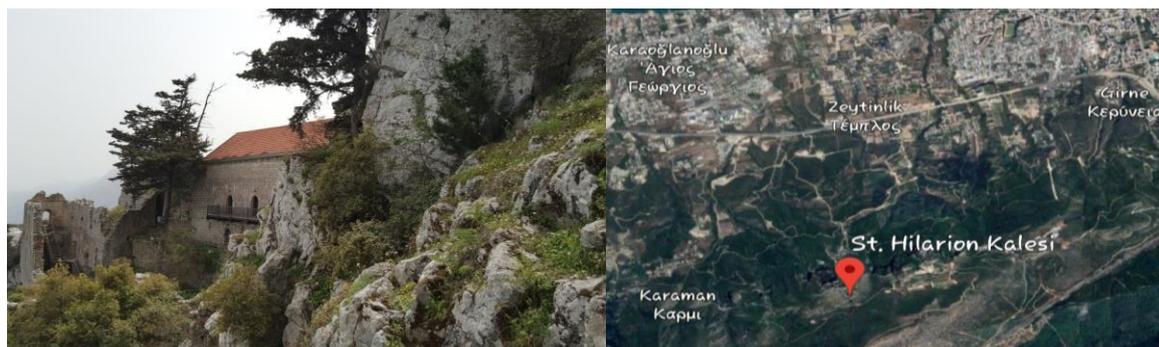


Figure 7: Study area (left; picture taken by author) and map.

METHODS

In this study; the anatomical and morphological characteristics of leaves are examined. Morphological examinations were carried out with the help of literature reviews. The general view as well as the shapes of the leaves drawn by hand due to their significance in the identification of the specimens also taken photographs by mobile phone. For anatomical examination were taken cross and superficial section of leaves that use by hand with the aid of razor blade. In cross sections were taken perpendicular to the long axis (90°) with the help of razor blade. In superficial sections were taken both upper and lower layer,

peeled the layers (upper& lower) separately and carefully pulled out the transparent layer from foliole, after placed the samples into the microscope slides then dropped Sartur Reagent which is ‘developed in 1949 by two Turkish scientists, Sarim Celebioglu and Turhan Baytop. It is prepared to identify many elements in one preparation’ (Celebioglu and Baytop 1949), to the sample and close with coverslide, heated on hot plate because Sartur reagents must be activated by using heat. Finally slides examined under microscope and photographed by mobile phone.

RESULTS

Morphological examination

The plant is perennial with 60-70 cm height. Leaves are compound and imparipinnate with 7 leaflets, basal leaves

are hairy and the stem is erect with soft hairs (Figure 8). Flowers are dirty white showing a characteristic features of an umbel.



Figure 8: Compound basal leaves and leaflet (taken by author).

Anatomical examination

Anatomical investigation based on the superficial and cross section of leaves.

Cross-section

Leaves exhibit dorsiventral (bifacial) structure. Epidermis is distinctly, has long and multicellular hairs for both upper and lower epidermis; upper epidermis hairs are thin while lower epidermis hairs are thickly and compactly but on midrib hairs distinctly seen and more than hairs has been observed than upper and lower epidermis. Interior of leaf between upper and lower epidermis contain veins and parenchymatous cells, differentiated into two region; palisade parenchyma below the upper epidermis which is vertically elongated, parallel and cylindrical cells and rich in chloroplast therefore they are the main set of photosynthesis and spongy parenchyma between the lower epidermis

and the palisade parenchyma which is oval, rounded and branched also they have chloroplast and cell walls are thin. Both palisade and spongy parenchyma has crystals while on palisade parenchyma has more than the spongy parenchyma. Vascular bundles not seen distinctly so some literature review shows; It is made up of a number of vascular bundles of varying size, found at the boundary between the palisade and spongy parenchyma, they are almost rounded, possess both phloem and xylem which lie on the same radius. Xylem lies towards the upper side of the leaf while phloem is found towards the lower surface, xylem consists of vessels, tracheids, xylem parenchyma and a few xylem fibres, xylem parenchyma stores food and allows lateral movement of water and mineral salts, phloem parenchyma cells store food and help in the lateral conduction of food'

(Fritsch and Salisbury 1943). However, on result photographs have not been viewable because of the sartur reagent; when drop

sartur reagent on to leaves; parenchymatous cells has been become dark purple because of the starch content of plant (Figure 9).

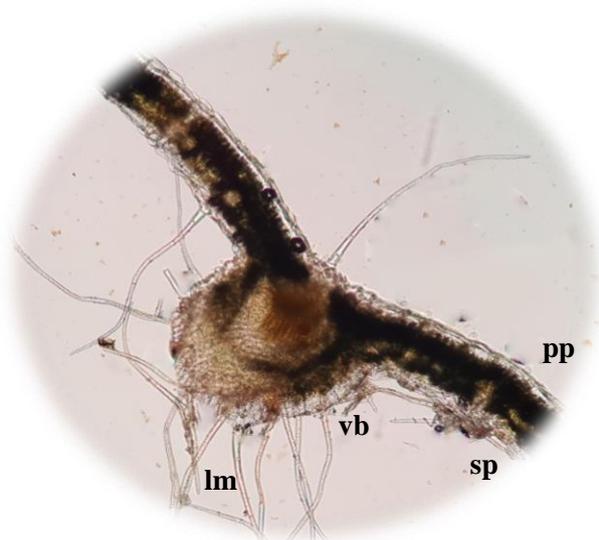


Figure 9: Cross section of the leaves (midvein).

pp: palisade parenchyma, sp: spongy parenchyma, vb: vascular bundles (xylem & pholem), lm: long and multicellular hairs .

Superficial sections- abaxial and adaxial surface

Shown as an amphistomatic characteristic feature which means both upper (adaxial) and lower(abaxial) sides have stomata on their surfaces; tiny pores in tissue to allow

gas exchange and each stomata has a narrow pore bounded and controlled by small specialised kidney-shaped epidermal cells called guard cells. However *P. cypria* stomata on upper (adaxial) side lesser than lower (abaxial) side (Figure 10 and 11).

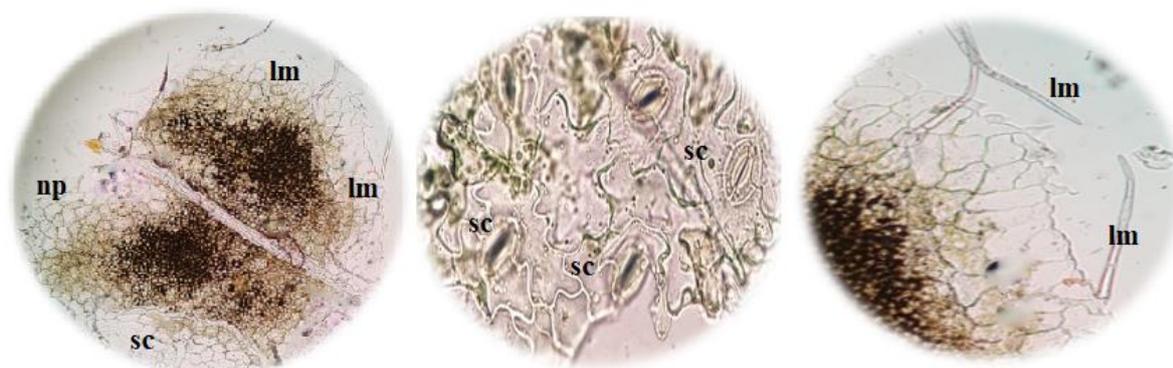


Figure 10: Upper epidermis (adaxial epidermis).

lm: long and multicellular hairs, np: normal polyhedral cells, sc: stomata cell.

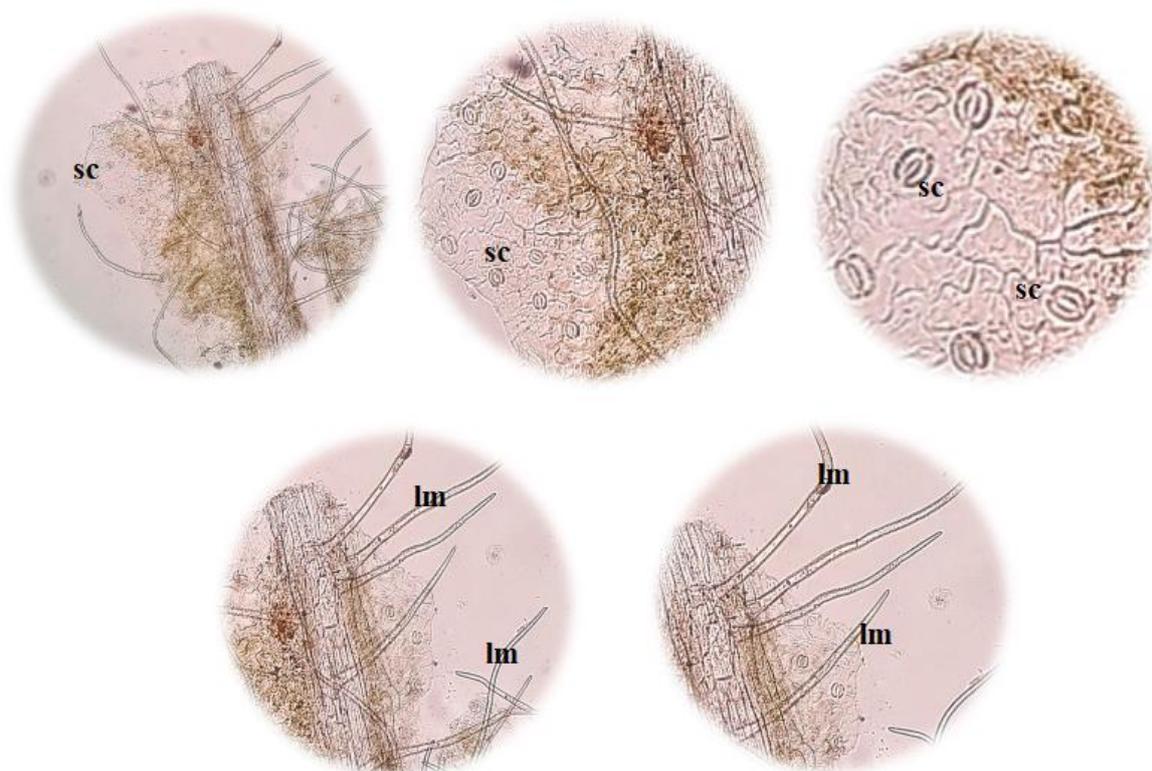


Figure 11: Lower epidermis (abaxial epidermis).
lm: long and multicellular hairs, sc: stomata cell.

CONCLUSION

In conclusion, the main objective of this study is to investigate the anatomical and morphological characteristics of the leaves which are presented for the first time. Young and mature individuals collected from nature habitat with the different times after that cross-section and superficial section were taken from the leaves and examined under microscope. During the observations; leaves are exhibit

dorsiventral structure, upper epidermis rich in palisade parenchyma and lower epidermis rich in spongy parenchyma, long and multicellular hairs are observed for both sides, on the upper epidermis hairs were thinly while on the lower epidermis hairs were thick and compactly. This plant shows amphistomatic structure however on the upper epidermis has lower stomata than lower epidermis.

ACKNOWLEDGEMENTS

This study is based on graduation thesis of the first author carried out in limited time in the student laboratory of EMU Faculty of Pharmacy. We would like to thank Prof. Dr. Salih Gücel for his help in the Herbarium of NEU for examining the herbarium specimens and growing area information. We also thank Sultan Ogmen and Ertugrul Ozbil for their contributions.

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