Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

EMU Journal of Pharmaceutical Sciences

EMUJPharmSci

Anatomical studies of *Dracocephalum moldavica* L., *Ocimum basilicum* L. and *Agastache rugose* (Fisch. & C.A.MEY) Kuntze (Lamiaceae) used for cardiovascular diseases in traditional Uyghur medicine

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Abstract

Traditional Uyghur Medicine (TUM) is widely used in Xinjiang Uyghur Autonomous Region in the east-west of China. TUM divided cardiovascular diseases according to causes and symptoms into several different types with different treatments to gain effective results. In this study, it was attempted to research anatomical characteristics of three frequently used herbs in cardiovascular disease treatments in TUM: *Dracocephalum moldavica, Ocimum basilicum,* and *Agastache rugosa,* all from Lamiaceae family. Leaf and stem anatomies of these three plants were examined in detail.

Keywords

Agastache rugose, Dracocephalum moldavica, Ocimum basilicum, TUM.

Article History

Submitted: 17 December 2019 Accepted: 23 March 2020 Published Online: March 2020

Article Info

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Research Article:

Volume: 3 Issue: 1 March 2020 Pages: 16-28

INTRODUCTION

The history of Traditional Uyghur medicine (TUM) can be traced back to 2500 years ago (Zhao *et al.,* 2017). TUM interacted with other medical theories, such as traditional Chinese Medicine, ancient Greece medicine, Egyptian Medicine, Arabian Medicine, and Indian Medicine throughout history. Currently, it has formulated a sophisticated and systematic theoretical system (Umar *et al.*, 2015). It is composed of different fundamental theories such as Erkan theory (fire, air, water, earth), four temperaments (cold, heat, moist, dry) and the four body fluids, also called Humor (blood, phlegm, yellow bile, black bile) (Mattohti, 2015; Aibai, 2007).

TUM divided cardiovascular diseases into two types: Humoral and non-humoral. Depending on the cause of the disease, TUM grouped them into cold, hot, dry and moisture-induced types. Treatments are applied according to the groupings, mentioned above, and the symptoms such as, heart palpitations, heart pain, heart pressure, heart 'chi' progression, angina, high blood pressure (Aibai, 2007). Mixed herbal preparations are preferred to be used instead of single herb usages and three Lamiaceae herbs, *Dracocephalum moldavica* L., *Ocimum basilicum* L., *Agastache rugosa* (Fisch. & C.A.Mey.) Kuntze are frequently chosen.

D. moldavica is an annual herb, which is distributed in China, Russia, Siberia, Eastern Europe, Central Europe and South to Kashmir (Flora of Xinjiangensis, 2004). The root or the whole plant of *D. moldavica* has medicinal value with clinical applications (Chinese Materia Medica, 2005). The main components of the aromatic oil (oil content is 0.01-0.17 %) obtained from the whole grass are citral (25-68 %), geraniol (80 %), nerol (7 %) and thymol (Feng and Li, 2015). It has extensive usage both in Uyghur Medicine and Tibet Medicine for cold, headache, sore throat, bronchitis, asthma, jaundice, vomiting, blood stasis, dysentery, heart disease, neurasthenia and also it is used as an anticancer agent (Miernisha *et al.*, 2015).

O. basilicum is an annual herb. The herb sweet basil is native to tropical Asia, Africa and America. It was being cultivated in Eygpt 300 years ago, reached England in 16th century, and North America in 17th century, and currently it is cultivated all over the world. The entire plant contains volatile oil and coumarins. Flowers contain ursolic acid, oleanolic acid, and β-sitosterol. Seeds contain oil over 16.8 % (Yi *et al.,* 2004). Moreover, it

is used for the treatment of cardiac insufficiency, heart fibrillation, cold cough and diarrhea in TUM (Umar *et al.*, 2014).

A. rugosa is commonly known as the Korean mint, and its native range is from Russian Far East to East Asia. Major components are essential oil (0.28 %) which mainly consists of more than 80 % is methylchavicol (Chinese Materia Medica, 2005). It is used as a supplement for spleen, stomach and liver disorders. Also, sometimes it is used as an anti-depressant (Gong *et al.*, 2017).

The present study aims to investigate the anatomical and morphological alterations taking place in the stem and leaves of three Lamiaceae plants that are used for cardiovascular diseases in TUM in order to assist in the identification of the plant materials.

MATERIALS AND METHODS

All samples were collected from Karakash, Xinjiang, China, in 2015. The voucher specimen was identified by the authors and deposited at the Herbarium of Faculty of Pharmacy, Istanbul University (ISTE). The Voucher numbers are ISTE 115639 for *D. moldavica*, NU 37 for *A. rugose*, ISTE 115640 for *O. basilicum*. Fresh stems and leaves were cut from specimens and put into a 70 % ethyl alcohol solution. Transverse and longitudinal sections of the samples were prepared freehand using razor blades. The sections were placed on glass slides and then stained with SARTUR solution (Celebioglu and Baytop, 1949). Several slides were made and photographed for each species with the aid of a light microscope (Olympus BH-2 and Canon A 640 digital camera).

RESULTS

D. moldavica L.

Stem anatomy

In transection, the stem presented a square shape. The epidermis appears in a single layer with thick, papillae cuticle. Intense glandular and non-glandular trichomes can be observed. The top of most of the non-glandular trichomes is multicellular. Most of the glandular trichomes were composed of a single row cell, the head of which are multiple, large-scale cells. The cortex is formed by 4-5 layers angular collenchyma cells with thickened at the corner and sclerenchyma among them. The vascular cylinder presents a thin phloem outward and thick xylem inward. The pith is

composed of broad parenchymatous cells with thin walls. Sclerenchyma is also located in the perimedullary region (Figure 1).

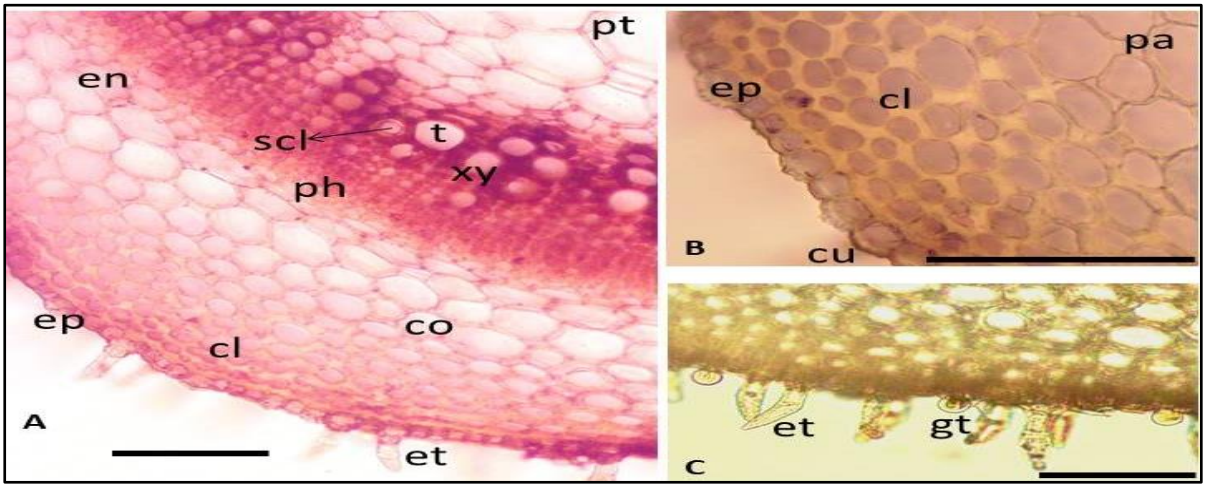

Figure 1: A) Stem anatomy of *D. moldavica*, B) Collenchyma, C) Trichomes:
ep: epidermis, gt: glandular trichome, cu: cuticle, pa: parenchyma, cl: collenchyma, et: eglandular trichome, en: endodermis, ph: phloem, xy: xylem, t: trachea, scl: sclerenchyma, pt: pith region, co: cortex (scale 0.1 mm).

Leaf anatomy

Cross-section of the bifacial blades reveals the upper epidermis, the mesophyll, and the lower epidermis. The upper and lower epidermis, one layer and isodiametric, were both composed of a row of thick radical walls and cuticles, and lower epidermis has thicker radical walls. They are amphistomatic leaves. The epidermal glandular and non-glandular trichomes and stomata are distributed on both epidermises. There are several types of glandular trichomes, one of which is the characteristic Lamiaceae family: single stalk cell and eight head cells. The non-glandular trichome observed in the leaf corresponds to the same type found in the stem (Figure 2). The leaf is dorsiventral and is formed by two layers of palisade and 4-5 layers of spongy parenchyma. The palisade parenchyma was composed of two layers of cells, which were smaller and more elongated at the inner layer. In some sectors, it was difficult to distinguish. The spongy parenchyma had 4-5 layers of cells with various shapes loosely arranged. Secondary vascular systems can be observed.

The leaf is amphistomatic. In both upper and lower epidermis, stomata are diacytic type with two subsidiary cells, and one is bigger than the other. The anticlinal walls are wavy. There are more glandular trichomes and non-glandular trichomes on the lower epidermis, and the non-glandular trichomes observed in the leaf corresponds to the same type found in the stem (Figure 3).

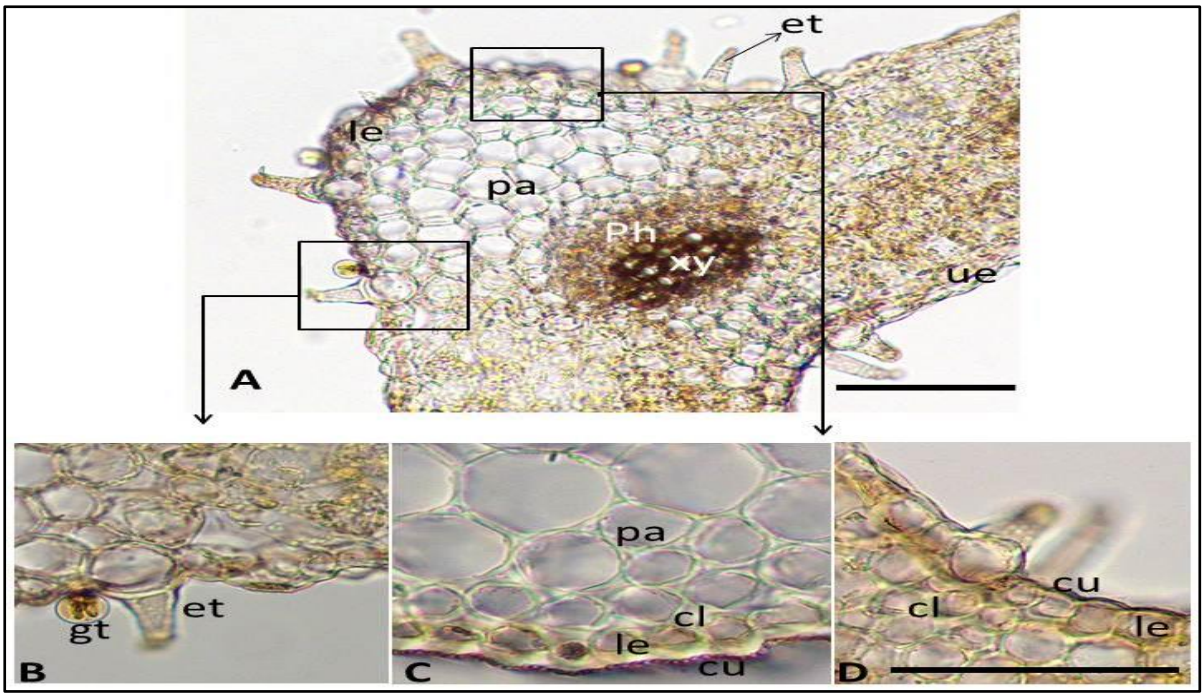

Figure 2: A) Midrib area in leaf of *D. moldavica,* B) Trichomes, C-D) Cuticle
ue: upper epidermis, le: lower epidermis; et: eglandular trichome, pa: parenchyma, ph: phloem, xy: xylem, gt: glandular, cu: cuticle, cl: collenchyma (scale 0.1 mm).

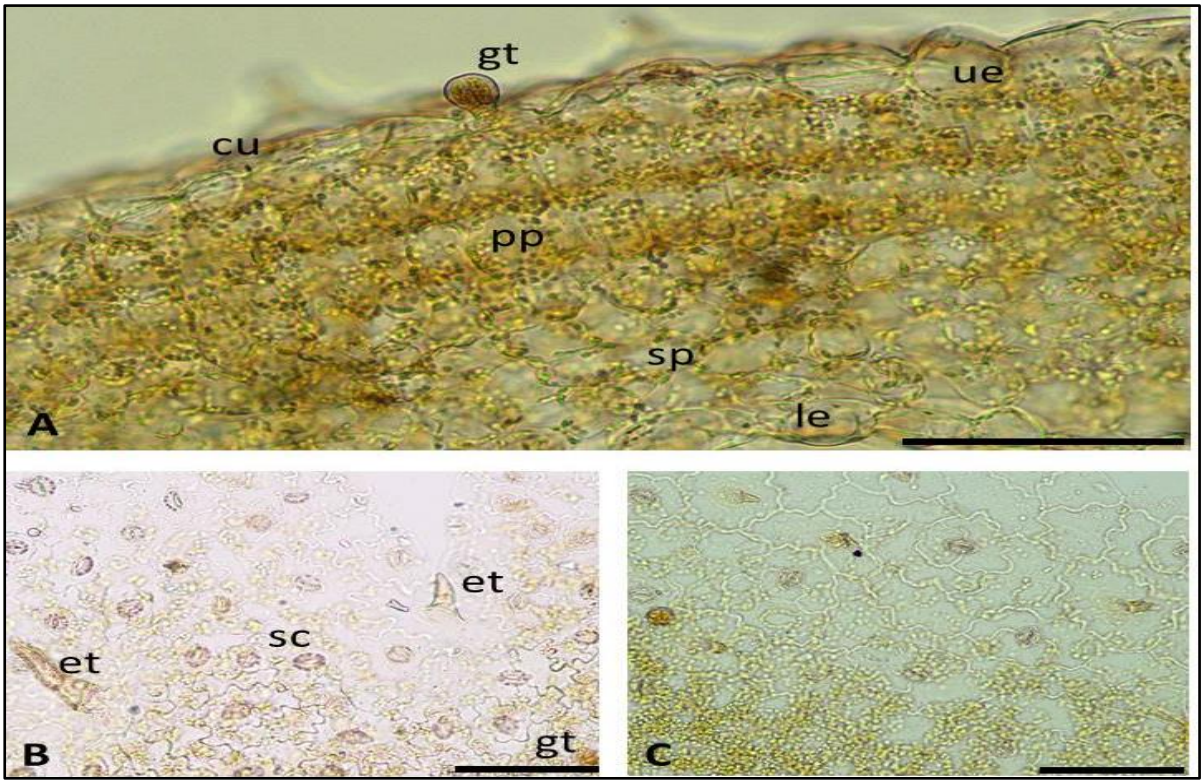

Figure 3: A) Leaf anatomy of *D. moldavica,* B) Upper epidermis, C) Lower epidermis.
ue: upper epidermis, gt: glandular trichome, le: lower epidermis, cu: cuticle, pp: palisade parenchyma, sp: spongy parenchyma, et: eglandular trichome, sc: stoma cell (scale 0.1 mm).

***O. basilicum* L.**

Stem anatomy

The overall appearance is rectangular, with a large pith area. It shows a typical character, consists of 3 parts; epidermis, cortex, central cylinder. Epidermis is a single-row, thickened outer wall with rectangular cells and is covered with cuticle and cuticle is flat. It is covered with glandular and non-glandular trichomes (Figure 4).

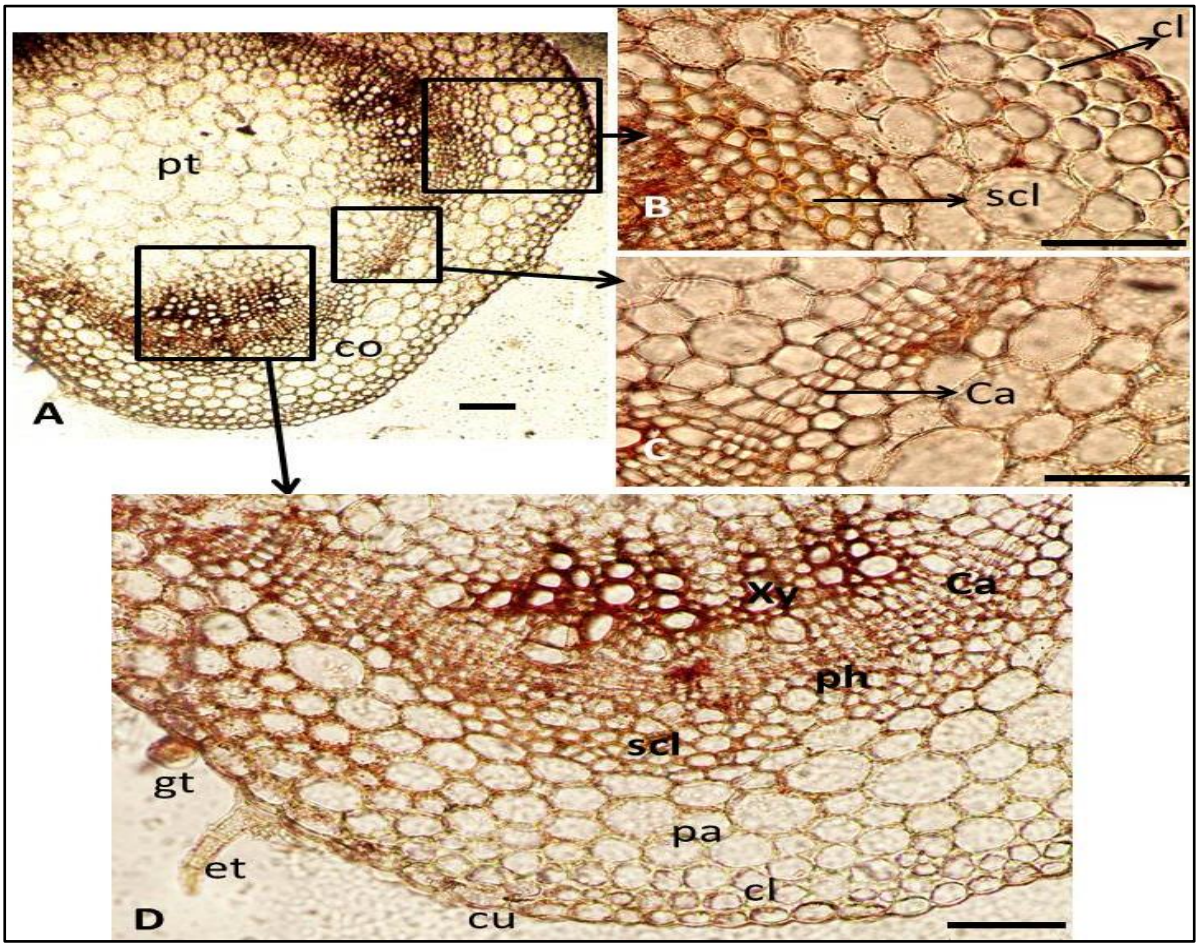

Figure 4: A) Stem anatomy of *O. basilicum*, B) Cortex, C) Cambium, D) Vascular tissues.
ep: epidermis, gt: glandular trichome, cu: cuticle, ca: cambium, pa: parenchyma, cl: collenchyma, et: eglandular trichome,en: endodermis, ph: phloem, xy: xylem, t: trachea, scl: sclerenchyma, pt: pith region, co: cortex (scale 0.1 mm).

Leaf anatomy

In the transverse section, the leaf is bifacial. The epidermis is uniseriate and covered by a thin cuticle with intense non-glandular trichomes, which has two or three bifurcated hooks and characteristic Lamiaceae family glandular trichomes. The collenchyma is a single layer. The collateral vascular system is surrounded by a thin uniseriate sclerenchyma cell (Figure 5).

In mesophyll, the upper epidermis is manifesting 1-2 rows of palisade parenchyma, then 3-4 rows of oval or round shaped sponge parenchyma with

irregular spaces between cells. The lower epidermis cells are smaller than the upper. Both epidermises are composed of ellipse-shaped cells without spaces among them. The epidermis on both sides are covered with a cuticle and some glandular and non-glandular hairs on them (Figure 6).

The leaf is amphistomatic. In both upper and lower epidermis, stomata are diacytictype with two subsidiary cells, and one is bigger than the other. The anticlinal walls are wavy. There are more glandular trichomes and non-glandular trichomes on the lower epidermis, and the non-glandular trichome observed in the leaf corresponds to the same type found in the stem. Cubic crystals can be observed (Figure 6).

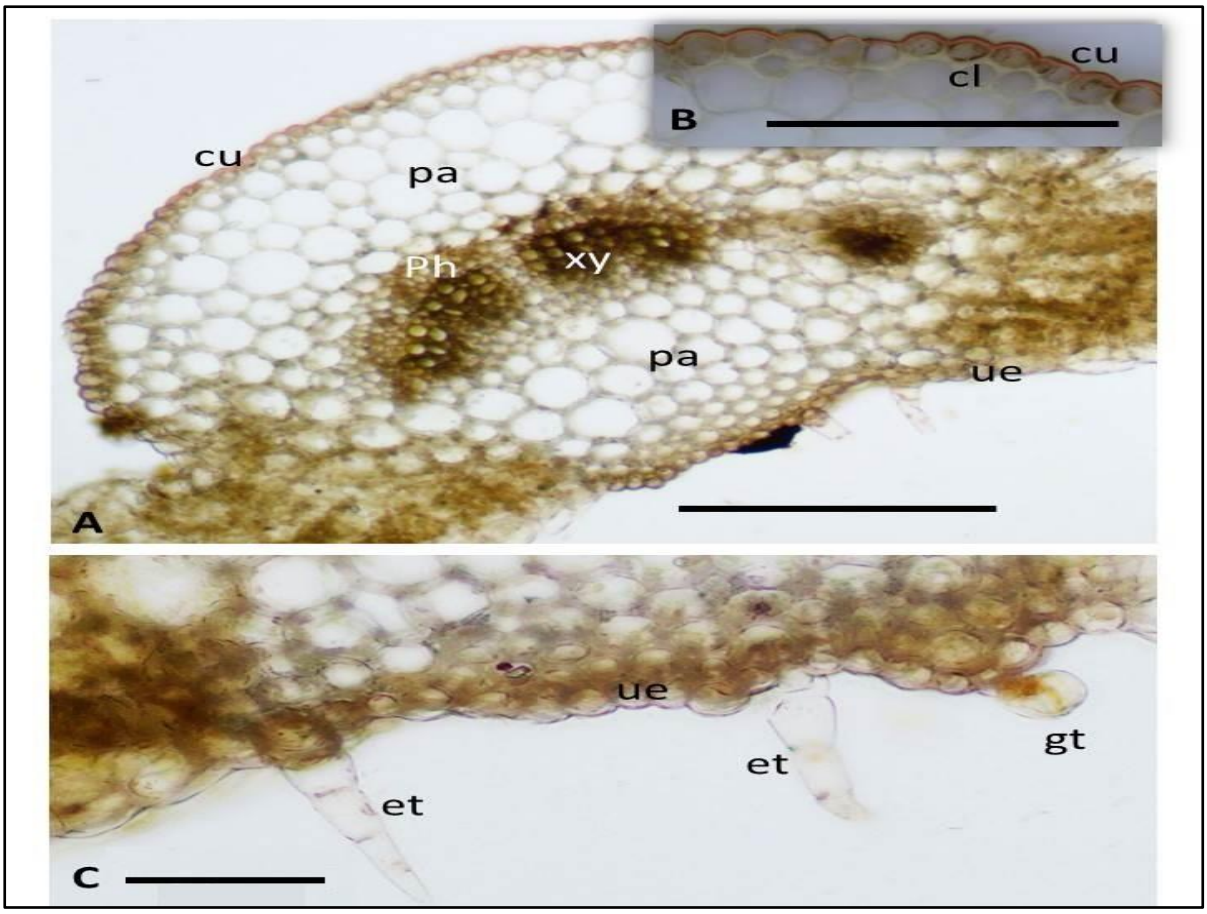

Figure 5: A) Midrib area in leaf of *O. basilicum* (scale 0.5 mm), B) Lower epidermis, C) Upper epidermis (scale 0.1 mm).
le: lower epidermis; et: eglandular trichome, pa: parenchyma, ph: phloem, xy: xylem, gt: glandular, cu: cuticle, cl: collenchyma.

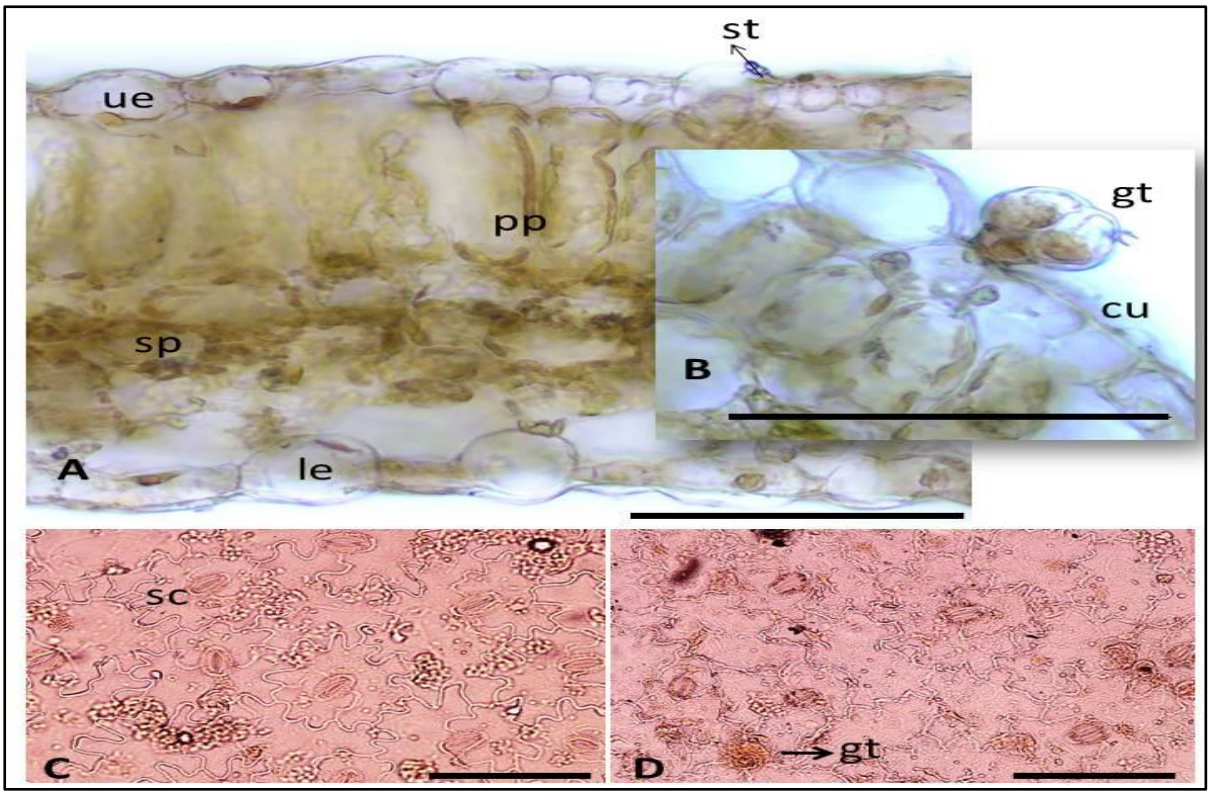

Figure 6: A) Leaf anatomy of *O. basilicum* B) Upper epidermais and glandular trichome, C) Lower epidermis, D) Upper epidermis.
ue: upper epidermis, gt: glandular trichome, le: lower epidermis, cu: cuticle, pp: palisade parenchyma,sp: spongy parenchyma, sc: stoma cell (scales: 0.1 mm).

***A. rugosa* (Fisch. & C.A.Mey.) Kuntze**

Stem anatomy

In transection, the stem presented a rectangular shape, and there is a broad pith area. The epidermis appeared in a single series with thickened and papillae cuticles. There are several layers of cells in the cortex. Beneath the epidermis, there were about 8-9 layers of angular collenchyma and 3-4 rows of oval or round shaped sponge parenchyma cells with space among them. The sclerenchyma was formed by thickened cells containing lignin, leading to a sclerenchymatous ring in the cortex. The endodermis was formed by a layer of cells around the cortex. The vascular system presented cambia, forming phloem outward and xylem inward. Druse crystals can be observed on phloem (Figure 7).

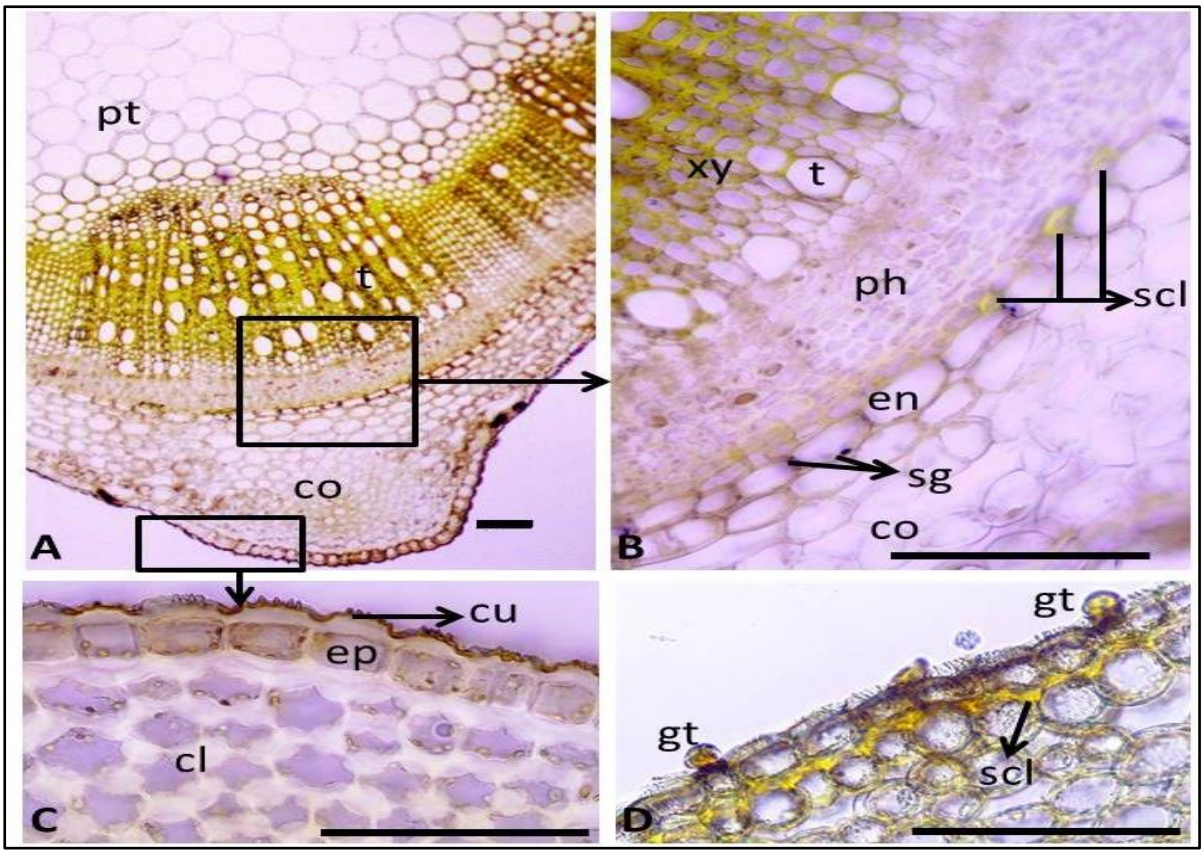

Figure 7: A) Stem anatomy of *A. rugosa*, B) Endodermis, C) Collenchyma, D) Sclerenchyma.
ep: epidermis, gt: glandular trichome, cu: cuticle, pa: parenchyma, cl: collenchyma, en: endodermis, ph: phloem, xy: xylem, t: trachea, scl: sclerenchyma, pt: pith region, co: cortex, sg: starch grains (scale 0.1 mm).

Leaf anatomy

The leaves of *A. rugosa*, in frontal view, showed epidermal cells with wavy anticlinal walls, which are relatively thin on both sides, and covered by thick and papillae cuticles. The leaves are hypostomatic. The epidermis is uniseriate. Beneath the epidermis, on both sides, there are 1-2 strata collenchyma, 2-3 layers of sclerenchyma with small round cells and 5-6 layers of parenchyma with thin walls are apparent. The vascular system was represented by an open arc, forming phloem outward and xylem inward (Figure 8). The leaves are bifacial, which contains 1-2 layers of palisade and 3-4 layers of sponge parenchyma, taking up the half mesophyll area, respectively. Druse crystals can be observed.

Only the lower epidermis of the leaves is hypostomatic and have stomata on it. The lower epidermis is composed of informal shaped cells with spaces among them, and epidermis cells of the lower surface are bigger than the upper. The anticlinal walls are wavy. Some glandular and non-glandular hairs on them. In lower epidermis stomata are diacytic type with two subsidiary cells, one is bigger than the other, and stomata are plentiful. Druse crystals can be observed on both epidermis (Figure 9).

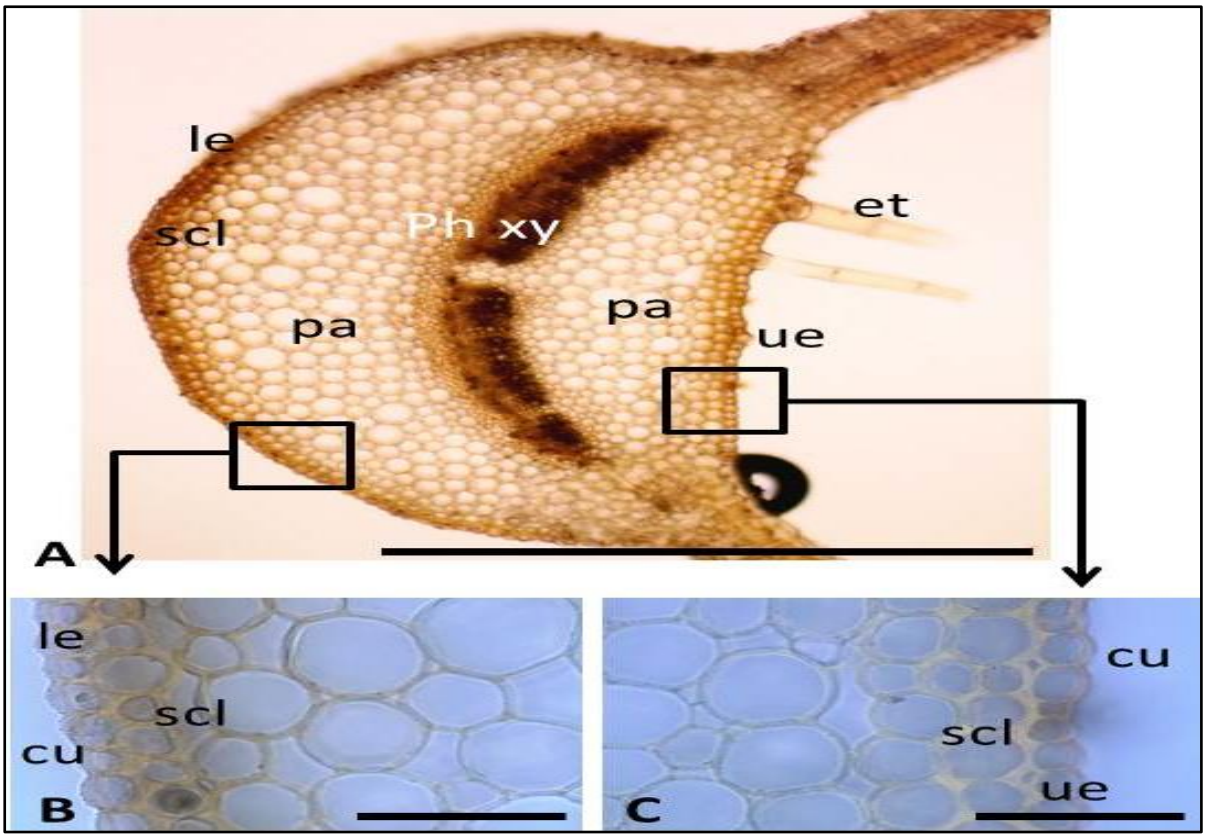

Figure 8: A) Midrib area in leaf of *A. rugosa* (scale 1 mm), B) Lower epidermis, C) Upper epidermis (scales 0.1 mm).
ue: upper epidermis, le: lower epidermis; et: eglandular trichome, pa: parenchyma, ph: phloem, xy: xylem, cu: cuticle, scl: sclerenchyma.

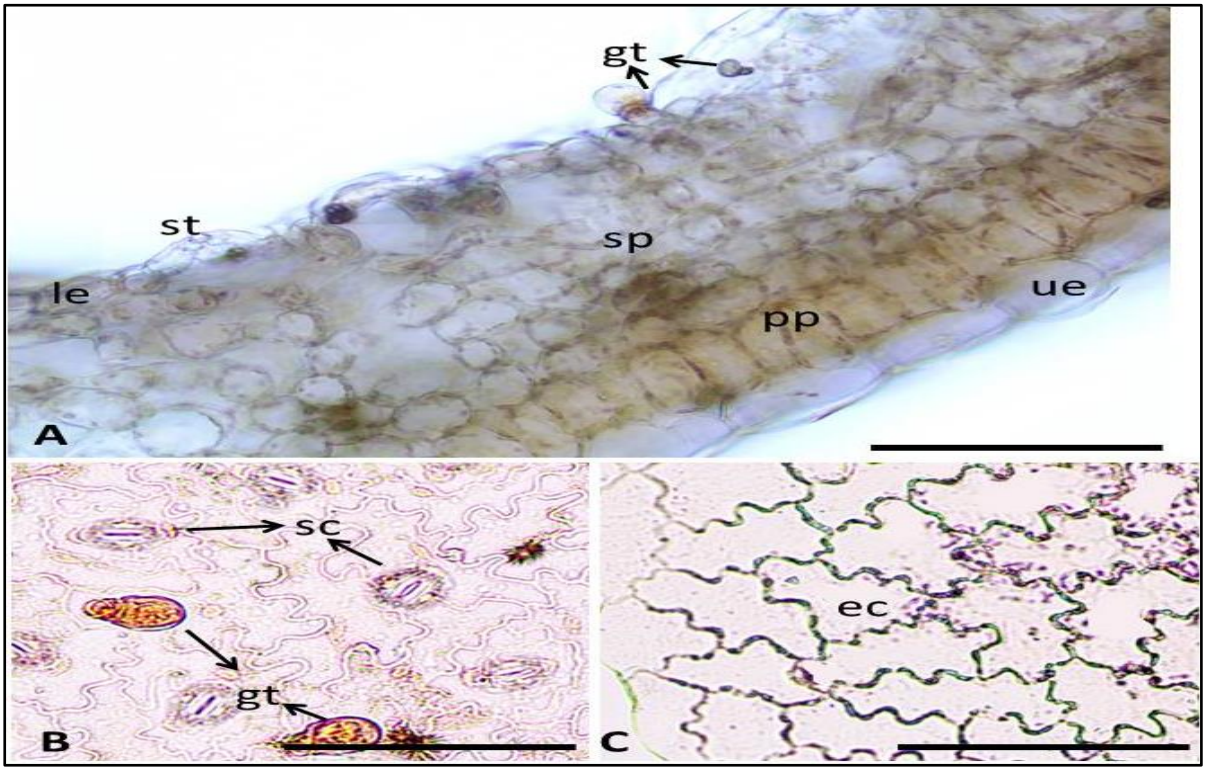

Figure 9: A) Leaf anatomy of *A. rugosa*, B) Lower epidermis, C) Upper epidermis.
ue: upper epidermis, gt: glandular trichome, le: lower epidermis, cu: cuticle, pp: palisade parenchyma, sp: spongy parenchyma, et: eglandular trichome, sc: stoma cell, ec: epidermis cell (scale 0.1 mm).

Table 1: Anatomical characteristics of *D. moldavica*, *O. basilicum* and *A. rugosa.*

	D. moldavica	***O. basilicum***	***A. rugosa***
Glandular trichome	+	+	+
Eglandular trichome	+	+	+
Crystals	-	+	+
Leaf type according to mesophyll	Bifacial	Bifacial	Bifacial
Leaf type according to stomata	Amphistomatic	Amphistomatic	Hypostomatic
Stomata types	Diacytic	Diacytic	Diacytic
Palisade layers	2	Single	Single

DISCUSSION

D. moldavica, O. basilicum and *A. rugosa* (Lamiaceae) have important medical use in cardiovascular diseases (Chinese Materia Medica, 2005; Maimaitiyiming *et al.*,2014; Huang and Liang, 1999) and always have the priority when making decoctions or preparations for the cardiovascular disorders like angina, heart palpitation, abnormal heart rhythms or coronary artery diseases (Miernisha *et al.*, 2015).

The anatomical findings of *A. rugosa* are presented for the first time in this study. Although there are no detailed anatomical studies on the other two species, anatomical studies have been carried out giving structural details such as feather structures, depot materials, growth hormones and secretory channels (Dmitruk and Weryszko-Chmielewska, 2010; Li and Ding, 2001). All anatomical findings are summarized in the table 1.

Cross sections taken from the stem of these plants have exhibited a monolayered epidermis which is composed of oval, cubic, or rectangular cells. The upper surface of epidermis is covered with a thin cuticle and contains glandular and eglandular trichomes. Eglandular trichomes are curved, simple cells made up from one to three cells and arranged in a single row containing acuticle with or without micropapillae. Capitate trichomes are small in size and either consists of a short unicellular stalk and a globose or pear-shaped head cell, or two-cellular stalks and a globose or pear-shaped head cell. Epidermis also includes diacytic stomata type. Underneath the epidermis, multilayered collenchyma cells are located at the corners and there are 1–3 rows of chlorenchyma cells between them. The parenchymatic cortex tissue consists of 7–10 layered of oval, ovate, or orbicular parenchymatous cells. The single-layered endodermis consists of generally oval cells.

In the cross section of the lamina, there is a thin cuticle on the upper and lower epidermis. Both epidermal cells are mono

layered, isodiametric, and rectangular, oval, or cubic in shape. Surface of epidermis are covered with eglandular (1–4 cells) and glandular trichomes. Stomata are present on both surfaces of the lamina (amphistomatic type) in *D. moldavica* and *O. basilicum.*

The identification of pharmaceutically used species in pharmacopeias traditionally relies, besides phytochemical characterization, on morphology and anatomy. Morphological and anatomical characteristics are thus mandatory for drugs' pharmaceutical identification and purity control. Through the comparative study of the anatomy of these three species, we hope to have furthered as well to have laid the foundation for an accurate evaluation of different sources of Lamiaceae as to ensure their safety and efficacy.

ACKNOWLEDGMENTS

We thank Scientific Research Project Unit of Istanbul University for financial support (project no. TYL-2017-25278).

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