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**Morphological and anatomical studies of *Ocimum basilicum* L.
cultivated in Al-Marj city, Libya**

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Abstract

Ocimum basilicum L. belonging to the *Lamiaceae* family known as the 'King of Herbs' has been used as traditional medicine for various ailments. The aim of the present study is to provide new properties for the identification and description of this species. The morphological and anatomical patterns were investigated by light microscope. The features of floral morphology such as verticillaster number, bract, calyx, corolla and stamens are important characters to distinguish this species. The stomata are present in both epidermises, being of diacytic and anisocytic types. Vascular supply of stem consists of four arcs while in leaves and petiole are continuous bundles. Mesophyll dorsiventral of one row of rectangular palisade tissue adaxially followed rows of spongy tissue. We observe morphologically different types of glandular and non-glandular trichomes in all the studied organs. The different morphological characters in the trichomes have very important taxonomic values.

Key words : *Ocimum basilicum*, morphology, anatomy, glandular and non-glandular trichomes, Al- Marj (Libya).

دراسة الشكل الظاهري والتركيب التشريحي لنبات *Ocimum basilicum* L. المزروع في مدينة المرج/ ليبيا

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الملخص :

الريحان *Ocimum basilicum* L. ينتمي إلى العائلة الشفوية ويعرف باسم ملك الأعشاب كما يستعمل كدواء تقليدي للعديد من الأمراض. الهدف من هذه الدراسة هو تزويد صفات جديدة لتعريف و وصف هذا النوع. تمت دراسة الصفات الظاهرية والتشريحية باستخدام المجهر الضوئي. تعتبر الصفات الظاهرية للزهرة كعدد النورات اللولبية، القنابة، الكأس، التويج و الاسدية من الصفات المهمة لتمييز هذا النوع. توجد الثغور على كل من البشرة العليا و البشرة السفلى وهي نوعين، نوع تكون فيه إحدى الخلايا المساعدة المحيطة بالخلايا الحارسة تختلف عن الباقي والنوع الآخر فيه خليتين مساعدتين تحيط بالخلايا الحارسة. الجهاز الوعائي بالساق يكون على هيئة أربعة أقواس بينما في الورقة والعنق يكون على هيئة حزم متصلة. النسيج المتوسط للورقة يتكون من صف واحد من النسيج العمادي ذو خلايا مستطيلة متبوعة بعدة صفوف من النسيج الإسفنجي. نلاحظ وجود أنواع

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مختلفة ظاهريا من الشعيرات الغدية وغير الغدية في جميع الأعضاء النباتية المدروسة. هذه الاختلافات تكون ذات قيم تصنيفية مهمة جدا.

الكلمات المفتاحية : *Ocimum basilicum* ، التشكل ، التشريح ، trichomes الغدية وغير الغدية ، المرج (ليبيا).



Introduction

Ocimum genus, which belongs to the tribe Ocimeae, subfamily Nepetoideae, the order Lamiales and family Lamiaceae. There are about 150 species in this genus broadly dispersed over the warm regions of the globe, represented by cultivated species in Libya [1]. *The genus Ocimum* is one of the economically important groups of aromatic herbal plants widely used in perfumery, flavouring and pharmaceutical products [2]. It can be applied in food preparations, such as meat, fish, butter, cheese, and beverages [3,4,5]. *Ocimum basilicum* L. (Sweet basil) is one of the most popular and healthy culinary herbs in the world. It is referred to as the 'King of Herbs' has been used tremendously as traditional medicine for many ailments [6]. Several species of the *Ocimum* genus possess multiple biological activities such as, in vitro antimicrobial, antiviral, antimalarial activities and in vivo analgesic, anti-inflammatory, antidiarrhoeal, antidiabetic, anticancer, radiation protective, anti-hyperlipidemic activities, etc. [7, 8, 9, 10, 11, 12,13]. Essential oils extracted from fresh parts of the plants can be used as aromatic additives in food, pharmaceuticals, cosmetics and to improve shelf life of food products [14].

Many publications pointed out on the importance of morphological features in delimitation and identification in some Lamiaceae species [15,16,17,18,19]. The anatomical studies of vegetative organs are important for characterization of Lamiaceae taxa [20, 21, 17, 22]. The glandular trichomes and their distribution [23], stomatal distribution and other anatomical characters provide significant taxonomic information [24, 18, 25].

In this concern , *Ocimum basilicum* L. (Basil or Sweet Basil) was chosen to be the subject of the current study because its economic importance as an ornamental, spice, culinary and medicinal herb; yet anatomical structures of Basil herb are poorly investigated. Thus, this study aimed to investigate the morphological and anatomical structures of vegetative organs of Basil plant.

Material & methods

The plant species investigated in this study is *Ocimum basilicum* L. Plant sample was collected from Alef area (32.48191° N,20.82316° E) in Al-Marj city (Libya), between October and November of 2022 and March and May of 2023 when the plants is in full bloom. The collected samples were identified by Flora of Libya [1,26].

1-1 Epidermal Study

In the epidermal analysis, the method of [27] was used. The adaxial and abaxial epidermal peels were obtained using sharply pointed forceps. Peels were stained with 1 % safranin, rinsed with distilled water to remove excessive stain and were then mounted in a drop of pure glycerol on clean glass slides.

1-2 Anatomical Study

Free hand section of stem, leaves and petiole were taken, stained with methylene blue and mounted in glycerol and observed under light microscope and photographed [28].

Results

2-1 Morphological characteristics (Fig.1a,b&c)

Perennial shrub, up to 126cm tall. Stem square and tomentose. Leaves (5.2 x 3.2 cm) opposite, petiolate and fragrant where scratched. Lamina simple, ovate, green and tomentose on both the surfaces, serrate margin, acute apex and asymmetrical base. Inflorescences verticillaster (verticals 3-6 flowered). Bract (0.9-x0.5cm) sessile, obanceolate, tomentose and green. Flower bisexual, pedicel (0.3cm). Calyx (0.5x0.4cm) campanulate and green, 5-toothed, the upper tooth flat and rounded. Corolla (0.6-0.8cm) whitish purple, bilabiate; the lower flat, the upper lip with 4 lobes. Stamens 4 and free, anthers yellow and disk 4 lobed. Stigma shortly bifid.



Figure1: Morphology of *O. basilicum*: a. Whole plant, b. Leaf, c. Inflorescence and flower, d. Lamina surface. Di .S.: Diacytic stomata, An.S.: Anisocytic stomata

2-2 Lamina eqidemal characteristics

Leaf amphistomatic. Two types of stomata, diacytic and anisocytic. Abaxial and adaxial epidermal cells are irregular with wavy walls (Fig. 1d).

2-3 Anatomical characteristics:

2-3-1 Stem anatomy:

Stem in cross section quadrangular, composed of four ridges alternating with the same number of furrows. Epidermis uniseriate, epidermal cells are radially with thin cuticle. Trichomes are various kinds. The non-glandular hairs are uniseriate consisting of one to six cells (hook, rod and flagellate shape). The glandular hairs consist of capitate and peltate hairs. Capitate hairs are composed of one base cell, one-three stalk cells and a one head cell. Cortex in the 4 ribs occupied by angular collenchymas (3-5 rows) followed by polyhedral parenchyma (3-5rows). Vascular supply represented by four arcs of large collateral bundles located opposite the ridges, one to three minor collateral bundles between arcs located and cambium zone is recognized as continuous ring between phloem and xylem. The pith occupies large portion in the center and consists of large polygonal parenchymatous cells (20 rows) (fig.2 a).

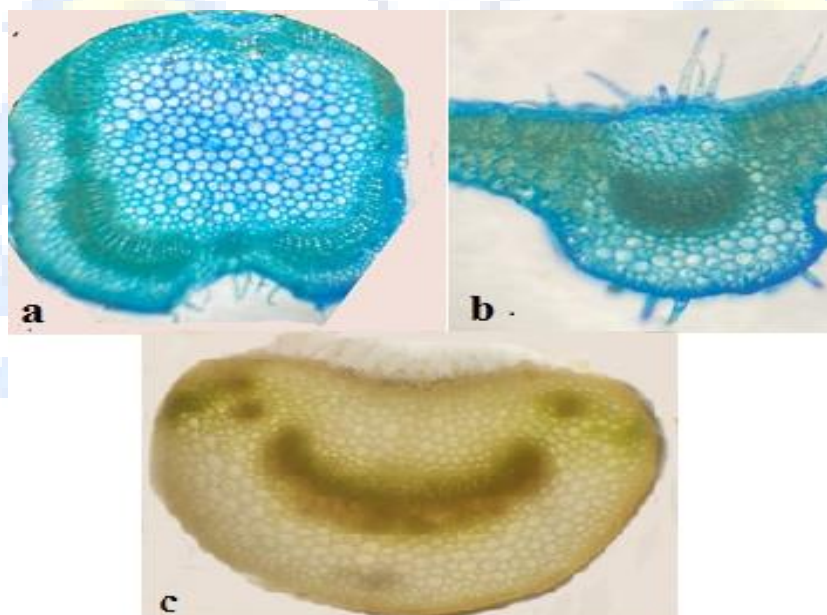


Figure2: Anatomy of *O. basilicum*. a. Stem, b. Leaf, c. Petiole

2-3-2 Leaf anatomy:

Epidermis, uniseriate and upper and lower cells are tangentially to papillose with thick cuticle. Trichomes are various kinds. The non-glandular hairs are uniseriate consisting of one to six cells (hook, rod and flagellate shape). The glandular hairs consist of capitate and peltate hairs. Capitate hairs are composed of one base cell, one-three stalk cells and a one head cell. Mesophyll dorsiventral of one row of rectangular palisade tissue adaxially followed by 3-5 rows of spongy tissue. Midrib region is flat adaxially and convex abaxially, occupied by angular collenchymas (1-2rows) followed by 4-5 rows of polyhedral parenchyma. Vascular supply collateral by the xylem toward the adaxial surface and the phloem toward the abaxial surface (fig.2 b).

2-3-3 Petiole anatomy:

Petiole in cross section reniform shape. Epidermis uniseriate, epidermal cells are tangentially with thin cuticle. Trichomes are various kinds. The non-glandular hairs are uniseriate consisting of one to 4 cells. The glandular hairs consist of capitate hairs which are composed of one base cell, one-three stalk cells and a one head cell broad. Cortex occupied by angular collenchymas (2-3 rows) followed by polyhedral parenchyma (7-8 rows). Vascular supply represented by continuous shallow arc vascular bundle in addition to one or two lateral bundle dispersed toward the wing (fig.2c).

Discussion:

In many studies, morphological, epidermal and anatomical characteristics were important in the classification of vascular plants [29, 30, 31, 32, 33, 34].

Most of the morphological features exhibited in our results were mentioned by [1], but some them not mentioned, such as leaf margin shape, apex shape, bract characteristics and the color of anthers. Morphological characters are very important in delimitation and identification in some Lamiaceae species [15, 16, 17, 18, 19]. In previous studies on Lamiaceae species; morphological characters such as stamen type, verticillaster number, calyx shape, corolla shape, corolla length and upper lip shape, are distinctive characters in the identification of species [35, 36].

Some of the published articles noted one types of stomata on leaves of basil, where observed [37] the anisocytic type, while [38] noted the presence of diacytic type. According

to our results, leaves of *O. basilicum* have two types of stomata, which are anisocytic and diacytic (Fig1d).

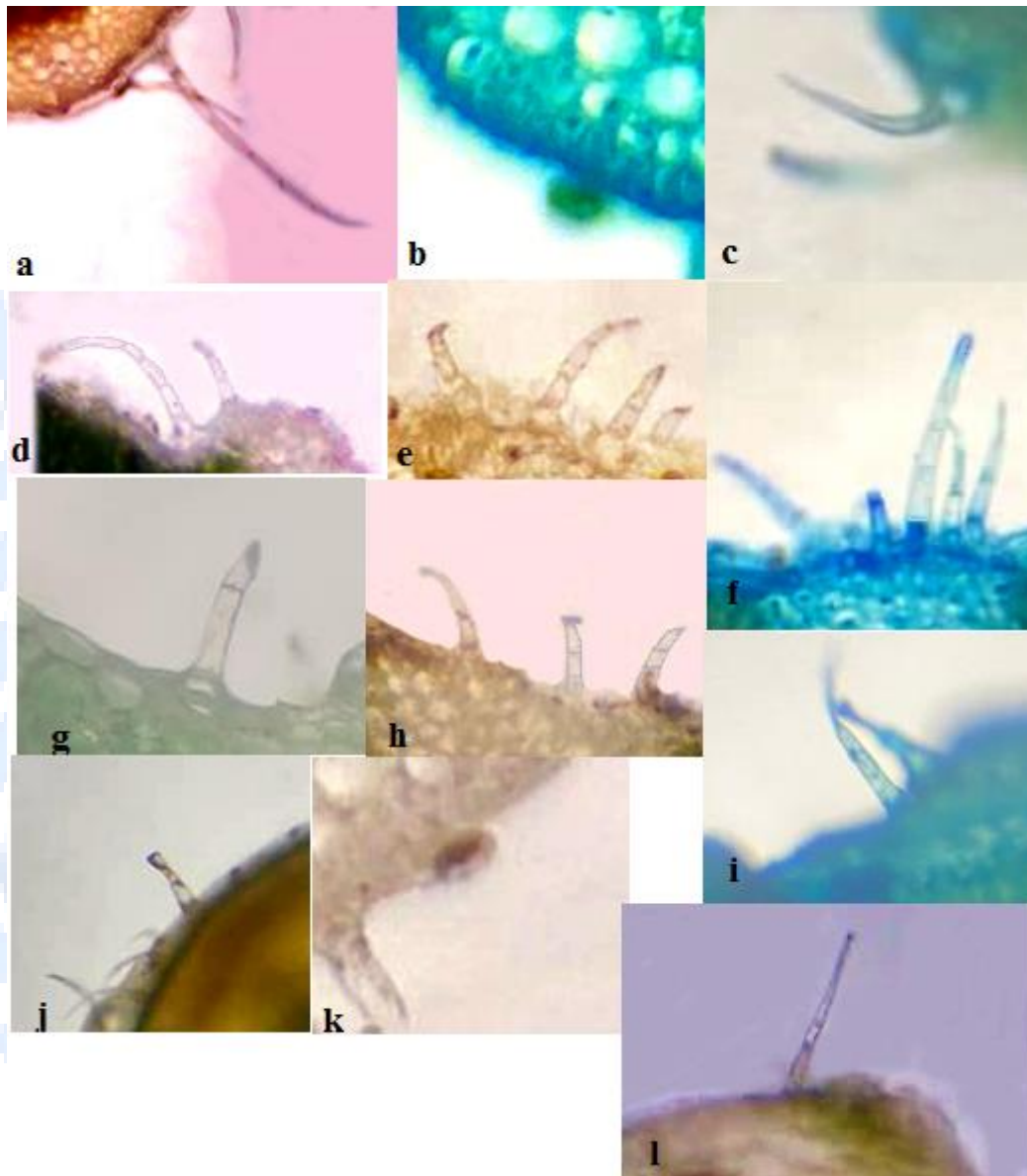


Figure3: Types of trichomes in *O. basilicum*:

Non-glandular trichomes: **a.** Flagellate shape; with 6 cell. **C.** Hook shapoe. **d.** four celled. **e.** one-three celled. **f.** two-four celled. **g.** Rod shape. Glandular trichomes: **h,I,j&l.** Capitate. **b&k.** Peltate trichomes.

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[39,40] reported that, vegetative parts of the aromatic plants belonging to the family *Lamiaceae* are covered with trichomes, including non-glandular and glandular or secretory trichomes. In the present study, the results recorded different types of non- glandular and glandular trichomes of the stem, leaves and petiole.(Fig.3). [41] mentioned that, for the *Lamiaceae*, there are different plants species can have different densities of non-glandular and glandular trichomes, and different morphological features which could be of very important taxonomic values. According to our study, the non-glandular trichomes of stem and leaves consist of 1-6 cells (Fig.3). This result in disagreement with [38], who found that the non-glandular trichomes of two parts consist of 3-5 cells.

[42] pointed out that stems in many genera and species of *Lamiaceae* are quadrangular in transverse section with well-defined groups of collenchyma in the four angles. This is in harmony with our results (Fig2a).

In current results, Mesophyll is dorsiventral of one layer of rectangular palisade tissue adaxially followed by 3-5 layers of spongy tissue, both epidermis are uniseriate, consisting of tangential- papilose cells, midrib represented by one layer of collenchymas and vascular system collateral type (Fig.2b). These results are in agreement with [38].

[43] described the internal structure of petiole of *O. americanum* L., who observed collateral type of vascular supply with additional accessory vascular bundles in wings located one on each side. Also in our study on petiole of *O. basilicum* showed collateral vascular supply with one-two additional accessory bundles (Fig.2c).

Conclusion:

O. basilicum L.(sweet basil) a perennial herb belongs to *Lamiaceae*. It has been used extensively in medicine. Sweet basil is also used as a kitchen herb and culinary herb. In our study, the leaf is amphistomatic with diacytic and anisocytic stomata. Anatomical studies of stem, leaves and petiole are important in identification of species. Trichomes of stem, leaf and petiole are various kinds. The morphologically different types of glandular and non-glandular trichomes have very important taxonomic values.

Our study suggests that in the future, the basil plant should be studied taxonically with a large groupe of members of the *Lamiaceae* family.

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