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MORPHOLOGICAL ANALYSIS DEPICTING DIFFERENT IDENTIFYING FEATURES OF *OCIMUM GRATISSIMUM* L.

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Ocimum gratissimum L. is immensely used in traditional medicine due to its medicinal properties and are used to treat painful and difficult diseases (Ganguly *et al.,* 2021; Ugbogu *et al.,* 2021). Standardization of crude drug and need of correct identification of medicinal drugs is essentially required in the herbal formulations (Ganguly and Kumar, 2022). Adulteration and substitution have been a common problem with medicinal plants of more than one species growing at the same geographical locations (Ahmed and Hasan, 2015). Every plant has different anatomical and morphological features in nature (Eko, *et al.,* 2020). Different species of same genus shows morphological as well as anatomical differences when matured. As the growth of the plants, varies holistically on species to species. These features also help to place a particular genus in respective families (Sarjani *et al.,* 2017). The morphological study not only limits to out looking features but also includes micro-morphological analysis of anatomy of the plant which becomes a part of identifying characters in a taxonomic description (Simpson, 2006). This is considered as an old method but becomes very essential basis for the researchers to identify the plant in the field during collections (Stuessy, 2009). The inner anatomical structures have been the characteristic features of particular plant and have served to plant systematics since several centuries (Judd, 2022). It also determines the phylogenetic relationships and helps in understanding the ancestral connections. The morphological features are observed by binocular microscopes and hand lens whereas, the photomicrograph of the anatomical features is taken by using light microscopes and electron microscopes. The anatomy of vegetative structures like stem, leaf, roots, petioles and taxonomic dissections of reproductive structures like flowers and ovaries are observed. More fine features are captured in scanning electron microscopy which is done on leaf, seeds and pollens. These features add on to genetic relationships and variations (Eko, *et al.,* 2020). The genetic variation can be noticed when same species grow at different geographical location but attributes to variation in morphology for different environmental conditions (Steenis, 2010). The present study deals with exploring the different morphological and anatomical features of different parts of *Ocimum gratissimum* which helps it to stand out from other species of *Ocimum* and concluding to correct identification by highlighting its features.

Collection: The plant was collected from local area of Baidyabati, Hooghly, India (22º47’48”N and 88º20’02”E) and was sent to Central National Herbarium, Botanical Survey of India, Howrah, India for authentication.

Equipments: Alcohol, safranin, light green, glyrecol, DPX, formalin, pith, binocular microscope, magnifying glass, compound microscope and Scanning Electron Microscope (SEM).

The morphological features of leaf, stem, petioles and roots are identified according to the described terminologies entitled in a book (Evans, 2009). The fresh samples were taken and transverse sections were made with a sharp blade by placing it between pith and double stained with safranin and light green followed by several washing with alcohol grades. The sections were

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mounted on a clean slide with glycerol and observed user the light microscope and photomicrographs were taken of different fields. The sections were later made permanent with DPX (Eko *et al.,* 2020).

The seeds and pollen were shade dried. Acetolysis was performed for pollen samples. Both seed and pollen samples were stored in Eppendorf and sent to S.N. Bose Innovation Centre, University of Kalyani, West Bengal, India. The dried pollen and seed samples were mounted on stubs and then coated with gold-palladium for 4 minutes and then photomicrographs were taken using EVO LS 10, Carl Zeiss Scanning Electron Microscope at 5000x magnification (Halbritter 1998).

Morphology

General morphology

It is a strong and stout shrubby and branched plant with sweet aroma, 1—3m tall; stem erect green at juvenile stage and light brown when fully matured, pubescent, quadrangular, woody and not easily breakable, much branched; leaves are in opposite decussate pattern of arrangement, of various sizes ranging from 2-15 cm × 1-7 cm, petiolate, 6-18 cm long, ovate-lanceolate, apex acute, base cuneate, decurrent and entire, margins are deeply serrated, pubescent, reticulate venation; inflorescence verticillaster, flowers arranged in terminal raceme with 90 to 150 flowers in each; flowers are white, petiolate, hermaphrodite, zygomorphic, 4-6 mm ; calyx 2-lipped, gamosepalous, campanulate, pubescent, persistent, 2-3 mm long, in fruit 5-6 mm, upper lip recurved and round, lower lip 4, narrow, much shorter than the upper lip; corolla five, gamopetalous, campanulate, bilabiate, white, 4-6 mm in length, pubescent outside the lower lip, upper lip truncate, lower lip flat and entire; stamens 2+2, didynamous, attached to corolla tube; filament simple, straight, 6 mm in length; anthers bilobed, sagittate, attachment at adaxial surface; pistil syncarpous, style simple, stigma bifurcated, ovary 4-chambered, with one ovule in each; fruits nutlet; seeds brown, spherical, with ornamented hard outer covering (Fig. 1). Fig. 2 shows different parts of *Ocimum gratissimum* with scale.

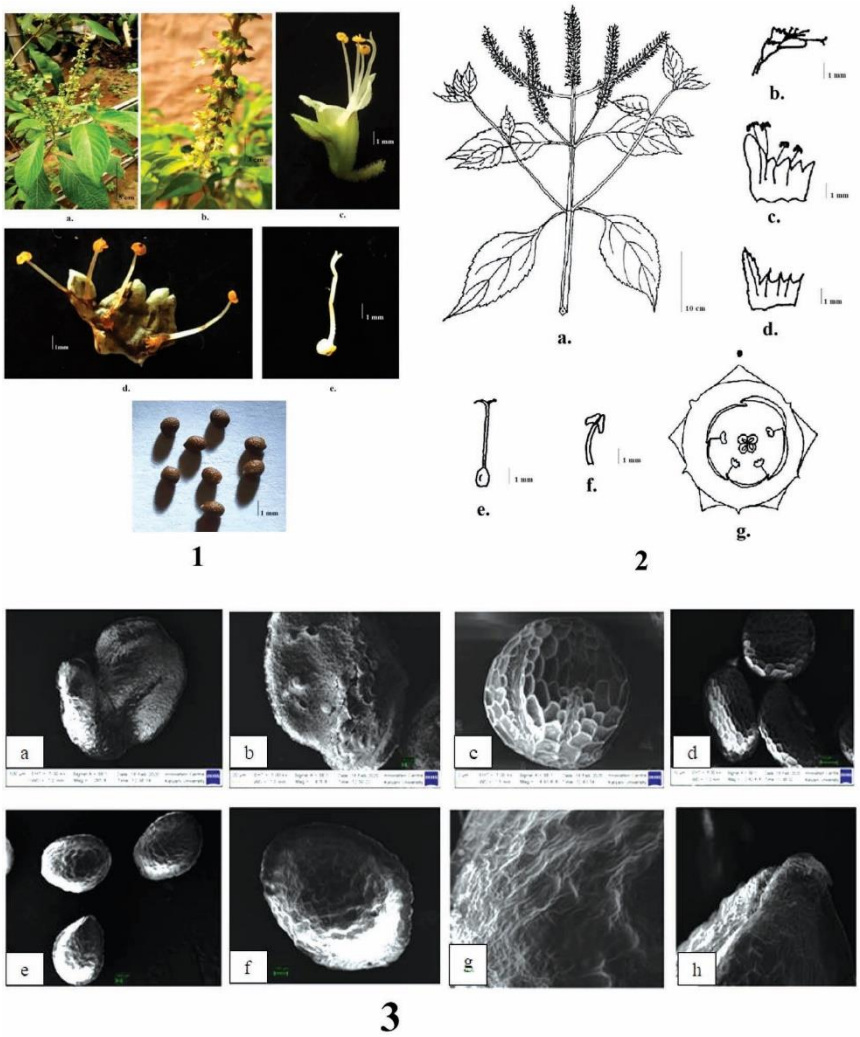
i. Flowering and fruiting time: June-July and November-February
ii. Habitat: Terresterial
iii. Mode of propagation: Seeds
iv. Scanning Electron Microscopic (SEM) studies:

The scanning electron microscopy of pollen sample shows yellow coloured pollen, free, elliptic shape, colpate suture and lophoreticulate ornamentation with prominent ridges and furrows, NPC 643 and the seed sample shows brown colour, with hard covering, free, sheprical shape, colpate suture and reticulate ornamentation with prominent ridges and furrows (Fig. 3).

Anatomy

Transverse Section of Leaf

The transverse section of midrib is a pot-shaped structure which shows cuticularized upper epidermal cell which contain covering, septate, tapering trichomes, glandular trichomes and bristles followed by hypodermis and collenchyma cells. The outer cortex is composed of thin walled parenchymatous cell which contains starch grains and prismatic crystals of Ca-oxalate in some. The vascular bundle is endarch type with protoxylem towards the center and metaxylem towards the periphery. The phloem cells are present just above the metaxylem. Pericyclic fibres are present in the region of pericycle which provides strength to the plant as it is a sclerenchymatous tissue. The pith is parenchymatous. Parenchymatous cortex is continued after



Figs 1-3: 1. External morphology of *Ocimum gratissimum* L. (a) Habit; (b) Inflorescence; (c) A single flower; (d) Gynoecium; (e) A flower dissected showing stamen attachment to corolla tube; (f) Seeds. 2. Illustrations of different parts of *Ocimum gratissimum* L. (a) Habit; (b) A single flower; (c) Corolla split open; (d) Calyx split open; (e) Gynoecium; (f) Androecium; (g) Floral diagram. 3. Photomicrograph showing Scanning electron microscopy (5000X) (a) An entire anther; (b) pollens are coming out bursting the anther wall; (c) an entire pollen grain with lophoreticulate ornamentation on the exine and a prominent colpate suture; (d) group of pollens having elliptical shape; (e) spherical shaped seeds; (f) single seed having reticulate ornamentation; (g) reticulate ornamentation having ridges and furrows on the surface of seed coat; (h) the colpate suture of seed

the vascular bundle followed by collenchymatous layer of cells, lower epidermis made up of elongated cells covered by cuticles and interrupted by the presence of diacytic stomata (Fig. 5).

The lamina shows upper epidermal cells covered by cuticles and trichomes followed by elongated rectangular palisade cells filled with starch grains and cell content. The spongy round shaped cells are found below the palisade cell which is followed by cuticularized lower epidermis interrupted by stomata at several places (Fig. 4).

Transverse Section of Petiole

The transverse section of petiole shows the upper epidermis comprising of elongated cells covered by cuticles. The covering, multicellular and glandular trichomes and bristles are present on the upper epidermis. The epidermal layer is followed by hypodermis and collenchyma cells with depositions. The outer cortex is composed of thin walled parenchyma cells which contains starch grains and prismatic crystals of Ca-oxalate. The vascular bundle is endarch type, ie, the protoxylem is present towards the pith and metaxylem towards the periphery. The pith is parenchymatous. The phloem is present on the outer side of the metaxylem. The pericyclic fibres are present on the pericyclic region. The parenchymatous cortex is continued after the vascular bundle followed by collenchymatous layer of cells, lower epidermis made up of elongated cells covered by cuticles (Fig. 6).

Transverse Section of Stem

Young stem of *Ocimum gratissimum* is almost square in outline and shows the following tissues in transverse section.

Epidermis: it is single-layered with tubular cells without intercellular spaces but externally covered by a layer of cuticle. Multicellular hairs and stomata are present in the epidermis.

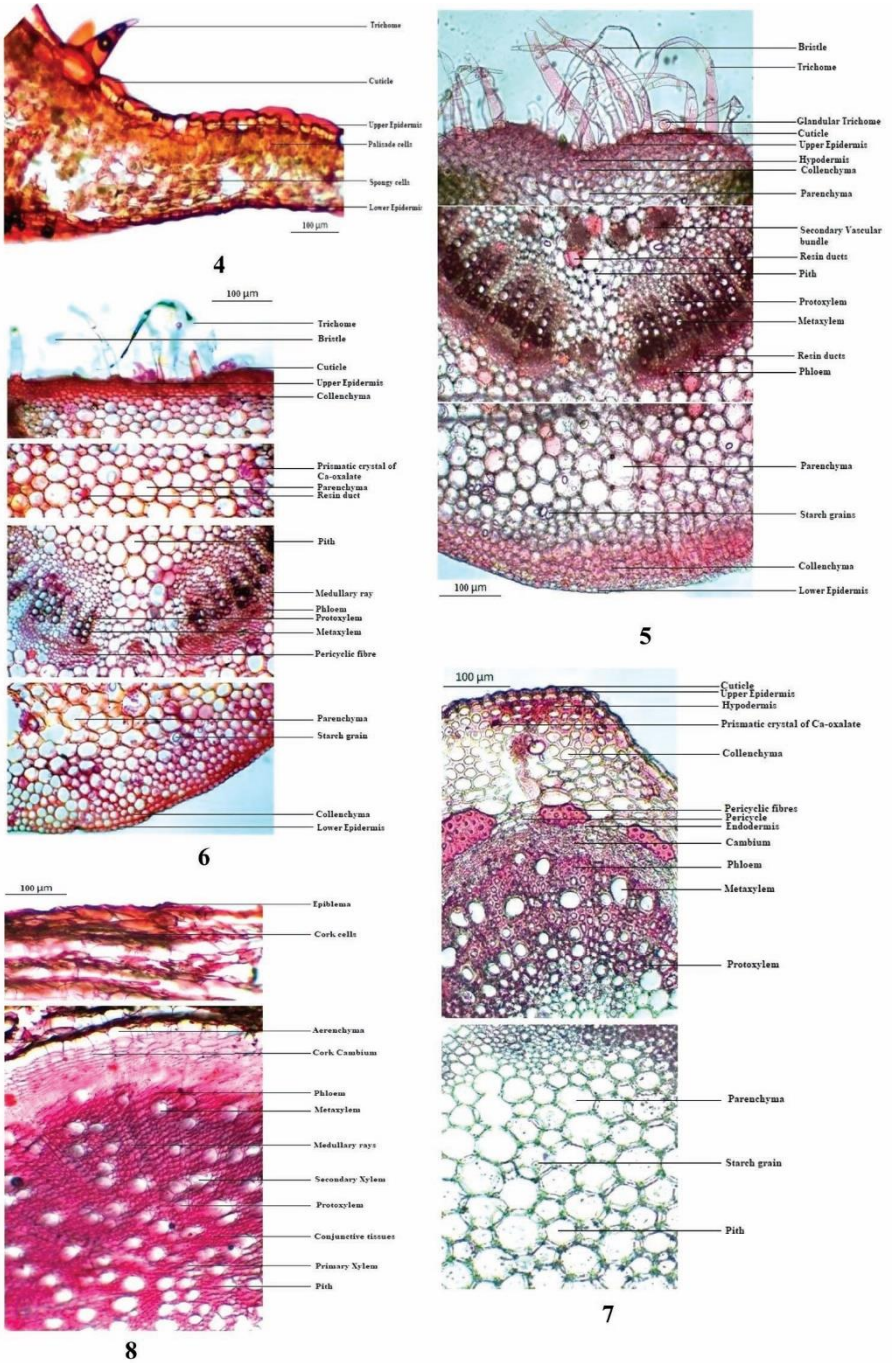
Cortex: The cortex in this stem is comparatively shallow but differentiated into three zones- the hypodermis, parenchymatous middle zone and the starch sheath. The collenchymatous hypodermis is densely aggregated at the corner of the stem. These diagonally placed collenchymatous patches form the I-girders to give mechanical support to the stem. These patches extend from the corners to some extent but do not form the continuous band of hypodermis. The innermost layer of the cortex is the starch sheath consisting of barrel-shaped compactly arranged cells with abundant starch grains. In between the hypodermis and the starch sheath a few layers of thin-walled parenchyma cells remain. The cells of this layer contain abundant chloroplasts and appear as a green belt under the microscope without stain.

Stele: The central core of tissues made of vascular strands and interstellar ground tissues encircled by the starch sheath in the stele. Internal of the starch sheath a few layers of sclerenchyma form a continuous band. It may be called pericycle or perivascular tissue. Large parenchymatous pith present at the center presses the vascular bundles towards the periphery. The primary medullary rays become indistinct due to secondary growth in thickness.

The vascular bundles are conjoint, collateral and open and remains arranged more or less in a ring. The phloem remains towards the periphery having sieve tubes, companion cells and phloem parenchyma. Internal to the phloem there is a strip of cambium, made of a few layers of fusiform cells appearing more or less rectangular in outline. Internal to the layer of the cambium xylem occurs with usual tracheary elements- tracheids and tracheae, parenchyman and fibres. Xylem is endarch, i.e., protoxylem remains towards the center and metaxylem towards the circumference (Fig. 7).

Transverse Section of Root

The transverse section of root shows the epiblema layer followed by cork cells which is composed of rectangular cells, followed by few layers of phelloderm and phellogen. The corticle cells are made up of sclerenchymatous tissue. The vascular bundle consists of scattered protoxylem and metaxylem which are embedded in the conjunctive tissue and divided by elongated cells forming the medullary rays. The phloem is present on the peripheral side which is encircled my sclerenchymatous pericyclic fibre. The secondary xylem and phloem are also present in a scattered manner (Fig. 8).



Figs 4-8: 4. Transverse section of leaf showing portion of lamina of *Ocimum gratissimum* L. 5. Transverse section of leaf showing portion of midrib of *Ocimum gratissimum* L. 6. Transverse section showing portion of petiole of *Ocimum gratissimum* L. 7. Transverse section showing portion of stem of *Ocimum gratissimum* L. 8. Transverse section showing portion of root of *Ocimum gratissimum* L.

The earlier study done on *Ocimum gratissimum* (Rawat *et al*., 2016) reveals that it has a height of 102.6 cm, a canopy average of 787.53cm^2, dark brown stem; ovate-lanceolate lamina with serrated margins, a length of 12.4cm; flowers creamy white, seeds globose, brown. According to literature (Rawat *et al*., 2016) 1000 seeds weigh about 0.90 g. It is also described as a tall, profusely branched perennial shrub, 1–2.3m high, distributed throughout the country and in Lakswadeep island and often cultivated, leaves are ovate, coarsely crenate-serrate, gland-dotted, pubescent on both the surfaces, flowers pale greenish yellow, in simple or branched racemes, moderately close whorled, nutlets sub-globose, rugose, brown, with glandular depression, not mucilaginous when wetted (Anonymous, 1966). The present morphological study reveals similarity with earlier studies. Compared to earlier data, the present study has elaborated the description, as briefed in the following, which is likely to help in correct identification of the plant up to species level. The scanning electron microscopic features of pollen grains were studied in *Ocimum basilicum* where the exine sculpturing of pollen structure was elaborated (Rashid *et al*., 2019). No such study was conducted on this *Ocimum gratissimum*. The present study shows similarity with the studies done on other species of *Ocimum*, but such studies on *Ocimum gratissimum* were yet to be undertaken. So, the present study thoroughly elaborated the characteristic features of both pollen and seeds, thus helping in correct identification of the plant. The anatomical study of the plant which was undertaken earlier (Rawat *et al*., 2016) gave the summary of cross section of stem only. Although there are reports of earlier morphological studies on *Ocimum gratissimum* L. (Prabhu *et al*., 2009 and Christian, 2012); the present study describes the plant thoroughly covering different parts to enable correct identification of the plant. The anatomical characters were not explored in the earlier studies in such an elaborative way and so the present study was needed in order to fill in the lacuna. Earlier work was done on stem and leaves, while the present study has revealed the internal organization of all parts of the plant covering the root and the shoot systems. The detailed anatomical description, as presented is likely to prove useful in identification of the species as well as preparation of a standard for prevention of adulteration and quality control of the drug.

In conformity with the contemporary global interest in herbal remedies, is a holistic contribution towards understanding of medicinal potential of leaves, stems, roots and flowers of *Ocimum gratissimum* L. The noteworthy contributions of this work to science are conclusively enumerated which includes thorough description of different plant parts in terms of morphology and anatomy which has been presented so as to enable their correct identification on which depends the life and safety of the patient.

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