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Taxonomic Comparison of Two *Ocimum* Species – *Ocimum gratissimum* L. and *Ocimum sanctum* L.

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Abstract*—Plant morphology offers insights into the external form and structural development of plants, providing a window into their evolutionary origins and progressions. Variations in morphology can be instrumental in discerning genotypic variations within species and individual populations. A comprehensive understanding of the morpho-physiological, anatomical, and genetic dimensions of commercial, industrial, and endangered plants is pivotal for devising quality standards and conservation blueprints. The Ocimum genus, including Basil, is renowned for its intricate diversity, comprising approximately 65 documented species with several synonymous classifications. Such taxonomic intricacies largely arise from genetic diversifications induced by cross-pollination and varied environmental factors. Historically, genotype identifications leaned heavily on morphological markers, with descriptors like leaf and flower color serving taxonomic classification. However, continuous cultivation and frequent hybridizations have birthed multiple species, subspecies, and varieties, many of which display morphological similarities despite genetic disparities. The Lamiaceae family, with its universal presence, holds significant economic merit, and members like Ocimum find recurrent mentions in traditional medicinal practices. Given the phytochemical variability across Ocimum species, a thorough characterization addressing their anatomical and morphological aspects becomes imperative.This research aims to delve into a comparative analysis of two specific Ocimum species: Ocimum gratissimum L. and Ocimum sanctum L., emphasizing their taxonomical distinctions. Our evaluation involved a rigorous examination of both micro and macromorphological characteristics including stomatal index. Preliminary results underscored pronounced morphological and anatomical variations between the two species. In essence, our findings suggest distinct morphological disparities between the studied Ocimum species, reinforcing the necessity of accurate species identification for pharmacognostical endeavors. To bolster these findings and foster a holistic comparison between Ocimum gratissimum and Ocimum sanctum, future research endeavors should encompass molecular-level exploration.*

***Keywords*—** *Ocimum, morphology, taxonomy, anatomy, stomatal index, Lamiaceae.*

I. INTRODUCTION

The mid-20th century saw a surge in scientific investigations into the traditional medicinal uses of Tulsi (Holy basil). While most evidence of its medicinal properties comes from experimental animal studies, a few human studies have also been conducted. These studies have attributed a range of notable properties to various parts of the Tulsi plant, including antimicrobial, adaptogenic, anti-diabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radio-protective, neuroprotective, cardio-protective, and larvicidal/mosquito repellent effects[1] .

Animal studies by Mediratta et al, [2] have demonstrated the immune modulatory properties of Tulsi leaf extract. The human immune system is highly complex, and a fine balance exists between health and disease. Any substance, be it synthetic or biological, that can enhance, suppress, or modulate the immune system is deemed an immune modulator. In India, it's commonly believed that consuming Tulsi leaves on an empty stomach boosts immunity. The *Ocimum* genus, including Basil, is diverse and complex with about 65 recognized species and numerous considered synonyms[3]. This taxonomic complexity is largely attributed to genetic diversity driven by cross-pollination and various environmental influences. The greatest species diversity is found in Africa's tropical rain forests, but several Basil species are also native to India[4]. To date, nine *Ocimum* species have been documented in India, three of which are exotic: *O. americanum* L., *O. minimum* L., and *O. africanum* Lour. However, identifying distinct *Ocimum* species can be challenging, requiring consideration of numerous traits.

Earlier identifications of genotypes were primarily based on morphological characteristics. For taxonomic classification, taxonomists have developed descriptors like leaf shape and color, flower color, etc. However, due to extensive cultivation and the occurrence of inter and intra-specific cross hybridization, these plants have evolved into numerous species, subspecies, and varieties that exhibit polyploidy and are not significantly different morphologically[5]. This has resulted in significant genetic variations within and between *Ocimum* species. They display considerable morphological differences and variations in growth characteristics, reproductive behavior, and chemical composition, which are also influenced by environmental factors. Consequently, these morphological descriptors can cause confusion in the taxonomy of *Ocimum* species.

In this context, the current study is focused on analyzing and comparing two species of *Ocimum*, specifically *Ocimum gratissimum* L. and *Ocimum sanctum* L., from a taxonomic perspective.

II. MATERIALS AND METHODS

Plant materials:

The present study focuses on two particular species: *Ocimum gratissimum* L. and *Ocimum sanctum* L. *Ocimum*

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sanctum, also known as *Ocimum tenuiflorum*, carries various common names including Krishna tulsi, Holy basil, tulasi, and tulsi. This species is typically an aromatic herb, under shrub, or shrub, characterized by its potent aroma emanating from oil glands. Cultivated for its medicinal properties and religious significance, this herb can reach about 4.5 feet in height. Revered in many parts of India, the herb is carefully nurtured.

Methodologies:

Macromorphological Observations:

The research undertook an in-depth macromorphological analysis, capturing detailed aspects of *O. gratissimum* and *O. sanctum.* This examination covered primary features like stem, leaf, inflorescence, flower, and fruit, using both visual observations from live specimens and photographic documentation. Key measurements, such as stem length, leaf dimensions, and sizes of floral parts like calyx, corolla, and stamen, were meticulously noted.

Micromorphological Analysis:

The micromorphological segment of the study assessed finer details. Using an ocular micrometer, dimensions like epidermal cell measurements, cortex and pith widths, and the sizes of palisade and spongy cells were precisely gauged.

Anatomical Investigations:

Preparation of Sections for Anatomical Study:

For the anatomical study, young fresh portions of the stems and leaves were selected. Cross-sections of the stem and leaf midrib were made and subsequently examined using a compound microscope, and photographs were taken using a digital camera. In a similar manner, the stomata of both *O. gratissimum* and *O. sanctum* were analyzed under a compound microscope.

Stomatal Study:

For stomatal examination, fresh leaves were submerged in water. This process not only ensured they remained hydrated but also facilitated easy extraction of epidermal cells. Razor blades were employed to carefully peel the abaxial leaf surfaces. The acquired peels were then set in Canada balsam, readying them for microscopic evaluation as described by Chaudhary & Imran[6]. This procedure allowed for an accurate determination of factors such as stomatal presence on epidermal surfaces, stomatal type, along with its density, frequency, and index.

Stomatal index:

Stomatal index (I) is the average number of stomata cells present per square millimeter of the epidermis of the leaf.

$$I = S / E + S,$$

where I = stomatal index, S = number of stomata cells per unit area, and E = number of epidermal cells per unit area.

III. Results and Discussion

Morphological characteristics of Ocimum gratissimum:

It is an aromatic, perennial herb, 1-3 m tall; stem erect, round-quadrangular, much branched, glabrous or pubescent, woody at the base, often with epidermis peeling in strips (Fig. 1a). Leaves are 5-12cm. long, ovate, acute base petiole as long as the lamina. Stem is green, woody, erect, hairs present. Inflorescence is thyrsus, arranged in a terminal, 5-15 cm long; rachis lax, softly pubescent; bracts sessile, ovate, 3- 12 mm x 1-7 mm, acuminate, caducous; pedicel 1-4 mm long, spreading or ascending, slightly curved and branching (Fig.2a). Flowers are **s**mall, hermaphrodite, calyx is bilipped, 2 ±0.5mm long, in fruit 5-6 mm, pubescent, upper lip rounded and recurved, reflexed in fruit, lower lip with 4, narrow, pointed teeth, central pair of teeth minute and much shorter than the upper lip; Lower calyx teeth minute, upper most tooth rounded, longer than the lowest ones, curved upwards in fruits (Fig. 3a). Corolla campanulate, 3.5-5 mm long, bilipped, greenish to creamy white, pubescent outside, upper lip truncate, four-lobed, lower lip longer, declinate, flat, entire (Fig. 3b). Stamens 4, declinate, didynamous, epipetalous, filaments distinctly exserted, upper pair with a bearded tooth at the base (Fig. 3c). Ovary superior, bicarpellary, each bicelled, style bifid. Stigma bifid, gynobasic style, length of gynoceum is 7±0.5 mm (Fig. 3d). Fruit is composed of four dry, one-seeded nutlets that are subglobose, 1.5 mm long, rugose, and brown; the outer pericarp does not become mucilaginous in water. The nutlets are wrapped in a persistent calyx, which is the bottom lip closing the mouth of the fruiting calyx.

Figure 1a&b: *O. gratssimum* and *O. sanctum*

Morphological characteristics of Ocimum sanctum:

A perennial aromatic plant with woody root-stock; branchlets purplish (Fig. 1b). Leaves 2-4 cm, long, simple opposite green or purple leaves that are strongly scented and hairy stems. Petioles are present on oval, up to 5 cm long leaves that are typically somewhat serrated. The young stem is quadrangular in outline. Inflorescence is raceme type (Thyrsus), 5-10 cm length (Fig. 2b). The bracts are many and have a caudiform shape. Small, hermaphrodite flowers, Calyx glabrous within, fruiting calyx spreading, 4-5.5 mm long (Fig. 3a). Corolla 3-4 mm long. upper calyx lobe abruptly acute, lower two teeth long, spine-tipped (Fig. 3b). There are 4 stamens. Filament length is 1 mm. Filament color is white (Fig. 3c). Bicarpellary, syncarpous, superior ovary, axile of placentation, tetralocular with a single ovule in each locule, disc located under the ovary, gynobasic style, and bifid stigma (Fig. 3d). Plants are excellent seed producers; the seeds are tiny and white in appearance.

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Figure 2a&b- Inflorescence of *O. gratssimum* and *O. sanctum*

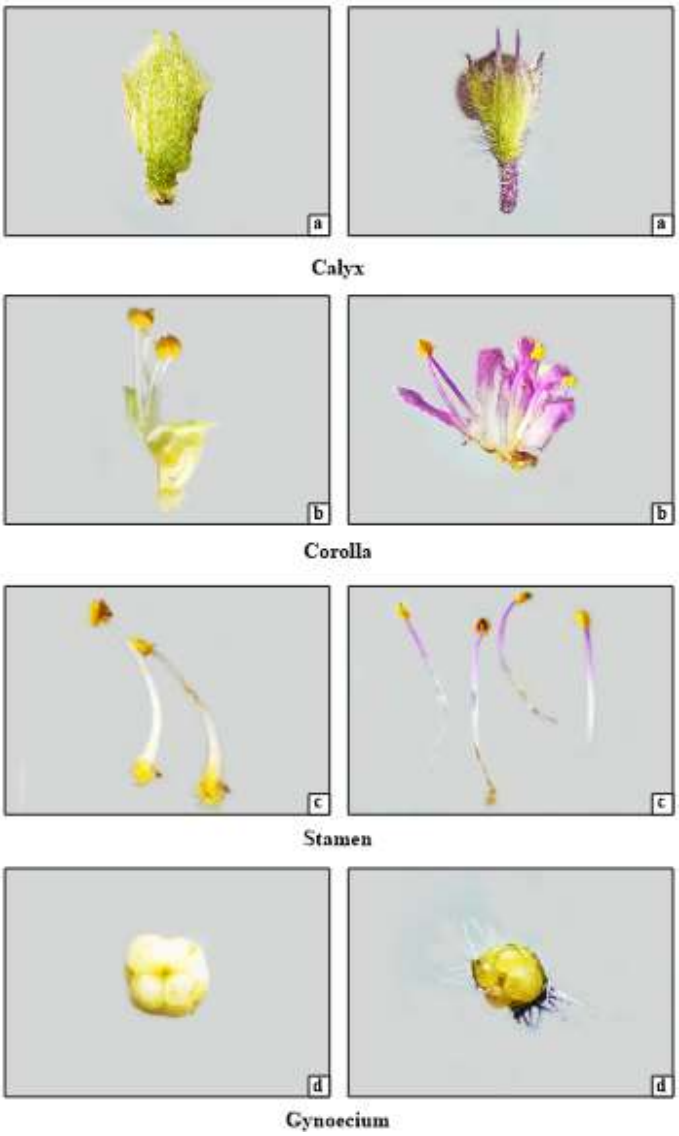

Figure 3a-d: Floral parts of *O. gratssimum* and *O. sanctum*

The two species of *Ocimum* showed significant variation of phenotypic characters like lamina length of *O. gratissimum* (12.4 cm), *O. sanctum* (4.3 cm), Lamina shape is sub-ovate in *O. sanctum*; while ovate in *O. gratissimum*. Lamina margin is sub-serrate in *O. sanctum* while serrate in *O. gratissimum*. The plant height varies from 51.46 cm to 102.6 cm. The smallest plant population of *O. santum* and the tallest plants of *O. gratssimum* were observed. Stem colour is, purple green in *O.sanctum*, dark brown in *O. gratissimum*. Flower colour sowed a great variations among these species of *O. sanctum* (purple white), *O. gratissimum* (creamy white). Seed shape is globose in two pecies of *Ocimum*, seed colour is brown in *O. gratissimum* and *O. sanctum*. 1000- seed weight ranges from 0.30 to 0.90 g, bold and high weight seed found in *O. gratissimum* (0.90 g), and *O. sanctum* (0.30 g).

Micro morphological and Anatomical Characteristics of Ocimum species:

Ocimum gratissimum

Stem: The central cylinder and the bark make up the two halves of the hexagonal stem's cross section (Fig. 4a). The three main tissues that make up the thin bark are the collenchyma, cortical parenchyma, and epidermis. The epidermis is made up of a thin, cellulose-based wall and a single base of tiny, adjacent rectangular cells. The cortical parenchyma is made up of thin-walled polygonal cells arranged in many layers. Compared to the bark, the center cylinder is more developed.

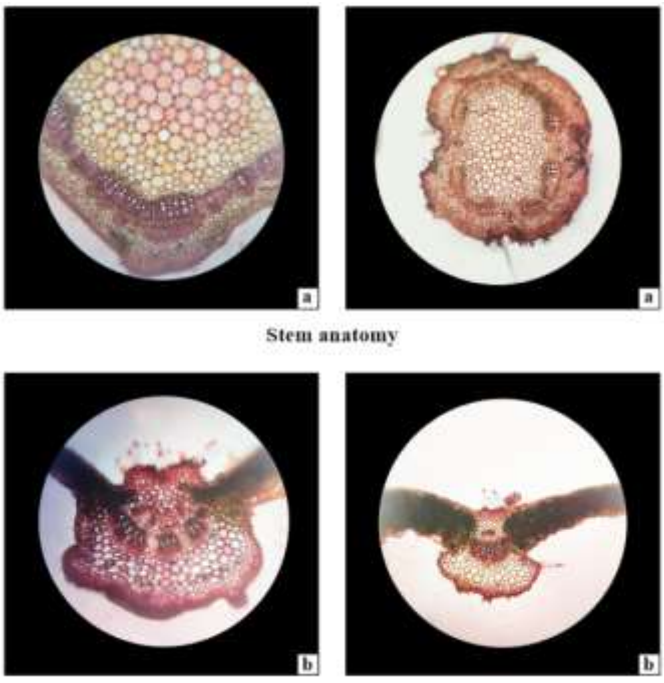

Figure 4a&b: Stem and midrib anatomy of *O. gratssimum* and *O. sanctum*

Leaf midrib: The cross section consist of spongy parenchyma, cutinized lower and upper epidermis, few layers of collenchyma, primary phloem and medullary parenchyma, bristles are also seen (Fig. 4b).

Stomata: Anomocytic stomata is seen on the abaxial surface of the leaves. This type stomata have guard cells that are surrounded by cells that have the same size, shape and

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arrangement as the rest of the epidermis cells (Fig. 5a). Stomatal index was 15 ±2.

Trichomes: In *O. gratissimum* non-glandular trichomes were observed more in the stem and leaves. The presence of capitate, peltate and non-glandular types was also observed on the stem. Non-glandular trichomes were observed on adaxial side of leaves. Trichomes with homogenous morphology were also observed.

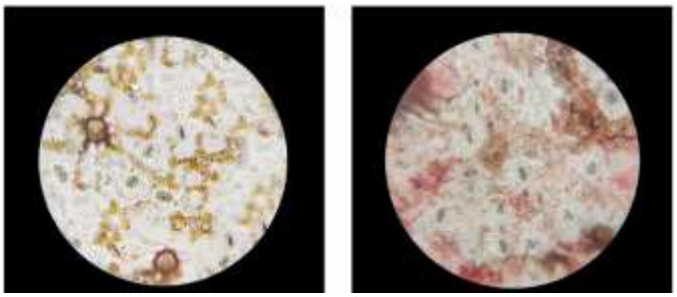

Figure 5a&b: Stomata of *O. gratssimum* and *O. sanctum*

Ocimum sanctum

Stem: The young stem is quadrangular in outline. Outermost layer is epidermis (EP) composed of tangentially elongated isodiametric cells and covered by their cuticle. Hypodermis is slightly collenchymatous (Coll). Cortex (Co) is parenchymatous with air spaces. Stele has four vascular bundles between them. Vascular bundles are collateral and open (Fig. 4a).

Leaf midrib: consisting of single layered epidermis composed of thin-walled, oval cells having a number of covering and glandular trichomes; covering trichomes multicellular 1-8 celled long,rarely slightly reflexed at tip; glandular trichomes short, sessile with 1-2 celled stalk and 2-8 celled balloon-shapedhead, measuring 22-27 in dia;three vascular bundles situated centrally, middle one larger than other two; xylem surrounded by phloem; cortical layers reduced towards apical region (Fig. 4b).

Stomata: Stomata seen were anomocytic & diacytic, and is on the abaxial surface of the leaves. They have guard cells that are surrounded by cells that have the same size, shape and arrangement as the rest of the epidermis cells (Fig. 5a). Stomatal index was 18 ±2.

Trichomes: A transverse section of the leaf shows an epidermis composed of one layer of cells, with thin cuticle and many glands, glandular hairs and a few stomata; lower epidermis possesses numerous stomata, some glands, glandular and non-glandular hairs; the glandular hairs are stalkless and with unicellular head. The non-glandular hairs are uniseriate, multicellular and often very long. Glands consist of a few cells, containing essential oils and are found in depressions of the upper and lower epidermis.

Both the species have a quadrangular (or potentially hexagonal) outline. Both have an outermost layer of epidermis composed of tangentially elongated isodiametric cells. The cells are covered by a cuticle. Hypodermis is slightly collenchymatous in both, the cortex is parenchymatous with air spaces and have four vascular bundles between them and are collateral and open. In cross section, the stem of *Ocimum gratissimum* is hexagonal, with a bark and a central cylinder. This specificity isn't provided for *Ocimum sanctum*. The bark of *Ocimum gratissimum* is thin and comprises three primary tissues: epidermis, cortical parenchyma, and collenchyma. Epidermal cells for *O. gratissimum*, is made up of a single base of small, contiguous, rectangular cells with a thin, cellulose wall, the cortical parenchyma has several layers of polygonal cells with thin walls. The central cylinder is more developed compared to the bark. Such specifics aren't provided for *Ocimum sanctum*. From the provided points, while there are many similarities in the anatomical features of the stems of *O. sanctum* and *O. gratissimum*, specific details about the hexagonal stem and the differentiation of bark from the central cylinder are highlighted for *O. gratissimum* but not for *O. sanctum*.

The genus *Ocimum* encapsulates a wide array of species, each with distinct morphological and anatomical features. As per study of Rawat et al.,[7] provided insights into four different species of *Ocimum*, each with its colloquial names. Their distinct structural attributes can play a pivotal role in ensuring the correct identification of these plants, which is paramount in pharmacognostical evaluations. In a subsequent investigation, a more extensive characterization of six *Ocimum* species was undertaken, inclusive of chlorophyll estimation. Notably, *O. gratissimum* L. emerged with the highest chlorophyll levels in both young and mature leaves, eclipsing its counterparts. Additionally, *O. gratissimum* L. showcased the highest carotenoid pigments in its young leaves, while mature leaves of *O. sanctum* L. reigned supreme. These pigmentations not only define the visual appeal but also point towards potential medicinal and nutritional properties[8]. Morphological and anatomical variations have far-reaching implications, especially in the drug manufacturing sector. Since the quality assurance of drugs is paramount, the ability to correctly identify and differentiate plant species is crucial. Many a time, due to vernacular names or untrained workers, there is a risk of adulteration. As indicated by Parida et al., [8], the findings from this study act as a cornerstone for the Ayurveda, Siddha, and Unani (ASU) drug preparations in the Indian System of Medicines, ensuring authentic raw material for the desired formulations.

Rocha et al., [9] in their study on *O. basilicum*, shed light on its unique anatomical structure. Below the epidermis of *O. basilicum*, annular collenchyma layers were discerned, juxtaposed with a cortical region exhibiting fundamental parenchyma. This structural alignment not only provides insight into the plant's physiology but also its potential adaptive strategies. Furthermore, the flower structure, identified as bilabial with five petals and sepals, accords with the characteristics of the Lamiaceae family, as highlighted by Basílio et al., [10].

IV. Conclusion

The detailed morphological and anatomical investigations into the two species, *Ocimum sanctum* and *Ocimum gratissimum*, have provided a comprehensive understanding of their structural nuances. Despite sharing the same genus and displaying numerous anatomical similarities, distinct

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differences were evident. Specifically, the hexagonal stem structure and the differentiation of bark and central cylinder in *O. gratissimum* set it apart from *O. sanctum.* Such variations play a critical role in taxonomic identification and may also influence the ecological adaptability and medicinal properties of these species. As we continue to explore and understand the vast botanical world, it's clear that even closely related species can harbor a treasure trove of differences. These findings not only reinforce the importance of detailed morphological and anatomical studies in botanical sciences but also emphasize the need for thorough documentation to preserve the rich biodiversity and facilitate future research endeavors.

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