

# Anatomical Study of Six *Ocimum* Species: The Valuable Method Used in Indian Systems of Medicines (ISM)

## Research Article

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### Abstract

This study aimed to characterize the morphological and anatomical traits of six *Ocimum* species namely *Ocimum tenuiflorum* L. Syn. *O. Sanctum* L. (green var. known as Radha Tulasi), *O. tenuiflorum* L. (purple var. known as Krishna Tulasi), *O. basilicum* L. (green var. known as Sugandhi Tulasi), *O. basilicum* var. *pupurascens* L. (purple var. known as Durlabha Tulasi), *O. canum* Sims. (Kanakan Tulasi) and *O. gratissimum* L. (Rama Tulasi). Chlorophyll estimation of young and matured leaves was also estimated. Chlorophyll a, Chl. b and total chlorophyll was maximum in both young and matured leaves of *O. gratissimum* L. compared to other species. Carotenoid pigments was maximum of 203.94 mg/ml in young leaves of *O. gratissimum* L. and 244.92 mg/ml in matured leaves of *O. sanctum* L. Comparative morphological characters and anatomical variation were studied for identification of the desired species in drug manufacturing industries before making formulations. This study will certainly help to ensure the quality of the crude drug as these are collected by untrained workers and supplied in dry condition to the drug manufacturing industries. Therefore, there is a chance of adulteration because of vested interest or ignorance as many plants are known by common names or in vernacular names. It was found that the six species can be identified and authenticated using these experimental findings before preparation of compound Ayurveda, Siddha and Unani (ASU) drugs in Indian System of Medicines.

**Key Words:** Adulteration, Anatomical identification, Chlorophyll estimation, *Ocimum* spp., Indian Systems of Medicine.

### Introduction

Medicinal plants play an important role in the health of individuals and human beings. The Indian System of Medicine is the culmination of Indian thought of medicine for healthy living. It is combined with other system of medicine like Greece (resulting in Unani Medicine) or Germany (Homeopathy) or our scriptures/sages which gave us the science of Ayurveda, Siddha and also Yoga and Naturopathy for successful management of various disease conditions. The medicinal properties of these plants are based on bioactive constituents that produce a particular action on human body. Therefore, plant remains to be the major source of medicine from the ancient time. Among the medicinal plants, *Ocimum* is the important genus of the subfamily Nepetoideae under the family Lamiaceae. The word is derived from the Greek word "Ozo" meaning "smell" and is called as king of herbs due to its use in traditional systems of medicine (1, 2). It has been reported that genus *Ocimum* has more than 160 species

and is the largest genera of which 65 species are native to *Ocimum* and the remaining should be considered as synonyms (3). In India, about nine species of *Ocimum* have been reported including three exotic species namely, *O. americanum* L., *O. minimum* L. and *O. X africanum* Lour. (4). Different *Ocimum* species exhibit variation in physical appearance, chemical composition and therefore all the species possess different pharmacological activities. Botanical, pharmacological, phytochemical, ethnomedicinal and toxicological informations were collected and reviewed on *O. sanctum* L. (5).

The present study was conducted with the aim of identifying six different species of *Ocimum* based on anatomical differences before using for making the formulations in drug industries. Besides this chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were also evaluated in these six species. *Ocimum* species show wide morphological variations, growth attributes, reproductive behavior including variations in chemical composition in relation with environmental factors (6) because of genetic variations in their inter and intra specific variability. Advancement in classification of *Ocimum* was introduced (7, 8) based on essential oil compositions from the aerial parts. Chemotaxonomy can be used to differentiate the genotypes of the same species. As it generates confusions because of variation in chemical composition due to environmental influence, therefore anatomical evaluation and

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authentication of desired species is the easiest and cost effective method for proper identification before making the formulations in drug industries. There is confusion in vernacular name, as the plant parts or the whole plant is collected by unskilled labour and from them the crude drug is procured by the drug industries, therefore this plant parts should be examined anatomically. In this study six *Ocimum* species are differentiated based on morpho-chemical-anatomical characterization.

## Materials and Methods

### Collection of Samples

The fresh plants were collected from medicinal plant garden of Centurion University of Technology and Management, Bhubaneswar Campus, Odisha which is located in 20.1756 degree N latitude and 85.7066 degree E longitude during the month of February and March. These plant species were identified with the literature data available in the department of Botany. In the present study six *Ocimum* species namely *Ocimum tenuiflorum* L. Syn. *O. Sanctum* L. (green var. known as Radha Tulasi), *O. tenuiflorum* L. (purple var. known as Krishna Tulasi), *O. basilicum* L. (green var. known as Sugandhi), *O. basilicum* var. *pupurascens* L. (purple var. known as Durlabha), *O. canum* Sims. (Kanaka Tulasi) and *O. gratissimum* L. (Rama Tulasi) were taken for morphological study (Fig.1- 6), anatomical study of stem and chlorophyll estimation of young and matured leaves. Among these two varieties from *Ocimum tenuiflorum* L., two varieties of *O. basilicum* L. and one variety from *O. canum* Sims. and one from *O. gratissimum* L. were taken for this study (Table 1).

**Table 1. *Ocimum* species with common and regional names**

Sl. No.	<i>Ocimum</i> species	Common Name	Odia Name
1	<i>Ocimum tenuiflorum</i> L. Syn. <i>O. sanctum</i> L. (green variety)	Sacred basil/holy basil	Radha Tulasi
2	<i>O. tenuiflorum</i> L. Syn. <i>O. sanctum</i> L. (purple variety)	Sacred basil/holy basil	Krishna Tulasi
3	<i>O. basilicum</i> L. (green variety)	Sweet basil	Sugandhi Tulasi/ Babu Tulasi / Dahana Tulasi
4	<i>O. basilicum</i> var. <i>pupurascens</i> L. (purple variety)	Purple basil/Red Rubin basil	Durlabha Tulasi/ Marua Tulasi

5	<i>O. canum</i> Sims.	Hoary basil/Wild basil	Kanaka Tulasi/ Landa baguli
6	<i>O. gratissimum</i> L.	Shrubby basil/ African basil	Rama Tulasi/ Bana Tulasi/ Ghoda Tulasi/ Gandha Tulasi

### Anatomical Study

The experiment was conducted in the experimental laboratory of Botany Department of Centurion University of technology and management, Bhubaneswar Campus, Odisha. Stems of these species were washed thoroughly with tap water. Uniformly dissected fine, thin transverse hand sections were selected for each species separately, stained with safranin-O and observed under compound microscope.

### Chlorophyll Estimation

Finely cut fresh leaves of 0.5mg of *Ocimum* spp. was taken separately and grinded by motor pestle with approximately 10 ml of 80% acetone. It was then centrifuged at 10,000rpm for 10 minutes at 4°C. The supernatant was collected and absorbance at 470nm, 646.8 nm, and at 663.2 nm. was recorded using UV-visible spectrophotometer for each species separately. The concentrations of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were calculated using the following equation (9).

- Chlorophyll a (mg/ml)=12.25 (A663.2nm)-2.79 (A646.8nm)
- Chlorophyll b (mg/ml) =21.50 (A646.8nm)-5.10 (A663.2nm)
- Total chlorophyll [(a+b) mg/ml] =7.15 (A663.2nm) +18.71(A646.8nm)
- Carotenoids = [100 (A470nm)-(1.82chl<sub>a</sub>+85.02chl<sub>b</sub>)]/198

## Results and Discussion

### Morphological Characteristics

Morphological studies of six *Ocimum* species showed a high level of variability (Fig. 1-6). For identification of six species of *Ocimum*, morphological attributes like leaf shape, colour, leaf apex, stem, inflorescence, flower and seed were taken in to consideration (Table 2) as they play the major role (10). These results coincided with the literature data carried on four species of *Ocimum* (11).

**Table 2. Morphological Characteristics of six *Ocimum* Taxa**

Sl. No.	<i>Ocimum</i> Taxa	Morphological characters
1	<i>O. canum</i> Sims.	Leaf ovate, obovate, hairy, inflorescence greenish in colour, flower white to whitish gray, calyx is green in colour and two upper calyx lobes are pubescent, seeds globose and dark brown in colour
2	<i>Ocimum tenuiflorum</i> L. Syn. <i>O. sanctum</i> L. (green variety)	Leaf ovate, obovate, elliptic- oblong and hairy, obtuse to acute at apex, inflorescence verticillaster, green in colour, flower white, calyx green, pubescent, seeds globose, brown in colour
3	<i>O. tenuiflorum</i> L. Syn. <i>O. sanctum</i> L. (purple variety)	Leaf ovate, obovate, elliptic- oblong and hairy, obtuse to acute at apex, inflorescence purple in colour, flower purple, calyx purple, pubescent, globose seeds, brown in colour
4	<i>O. gratissimum</i> L.	Leaf petiolate, ovate, obovate, elliptic-lanceolate, acute or slightly acuminate at apex, distinctly serrate along margins, glabrous except hairy mid rib, inflorescence greenish in colour, flower yellowish white, calyx green, pubescent, seeds subglobose, brown
5	<i>O. basilicum</i> var. <i>purpurascens</i> L. (purple variety)	Leaf elliptic-lanceolate, leaf apex, stems with sparse hairy, inflorescence greenish in colour, flower pinkish white, calyx greenish purple and some cases purple, ellipsoid seeds, black in colour
6	<i>O. basilicum</i> L. (green variety)	Leaf ovate-lanceolate to oblong- lanceolate, acute leaf apex, leaf lamina glabrous, vein-lets hairy, inflorescence greenish in colour, flower whitish pink, calyx light green, hairy, brownish black ellipsoid seeds



**Fig. 1. *Ocimum canum***  
(Kanaka Tulasi)



**Fig. 2. *O. sanctum***  
(Radha Tulasi)



**Fig. 3. *O. sanctum***  
(Krishna Tulasi)



**Fig. 4. *O. gratissimum* L.**  
(Rama Tulasi)



**Fig. 5. *O. basilicum* var. *purpurascens* L.**  
(Durlabha Tulasi)



**Fig. 6. *O. basilicum* L.**  
(Sungadhi Tulasi)

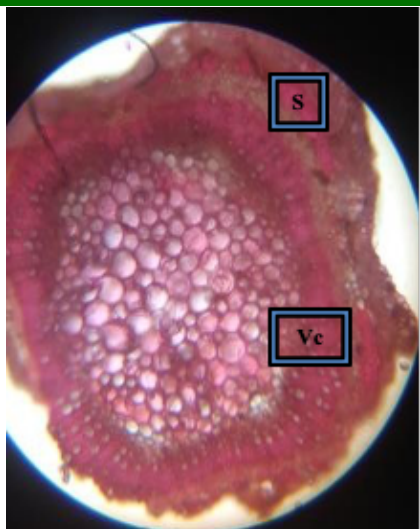


Fig. 7. *Ocimum canum* (Kanaka Tulasi)

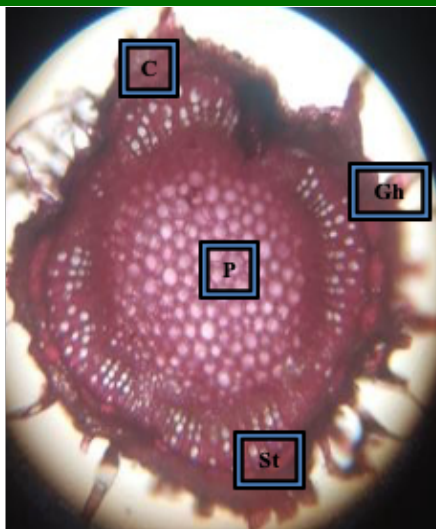


Fig. 8. *O. sanctum* (Radha Tulasi)

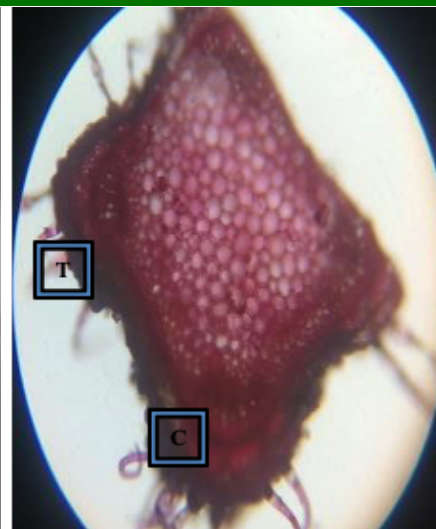


Fig. 9. *O. sanctum* (Krishna Tulasi)

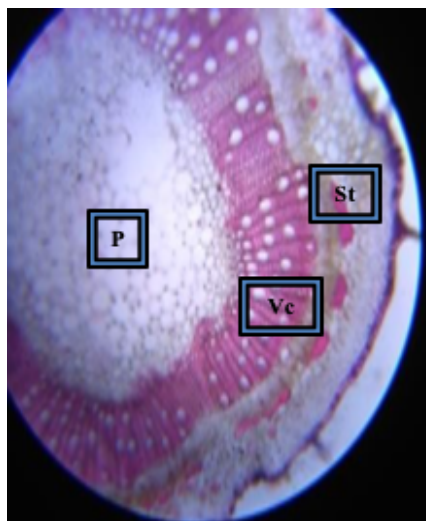


Fig. 10. *O. gratissimum* L. (Rama Tulasi)

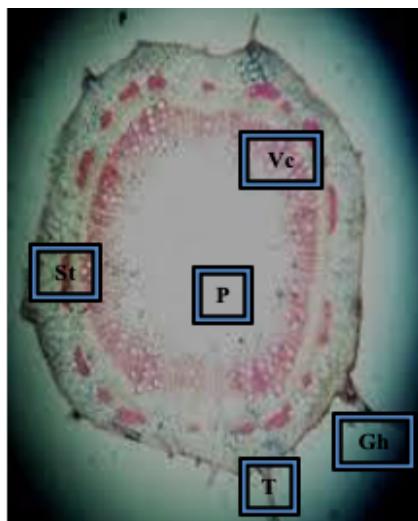


Fig. 11. *O. basilicum* var. *purpurascens* L. (Durlabha Tulasi)

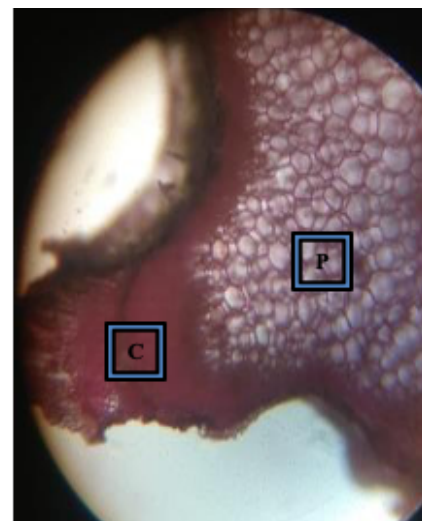


Fig. 12. *O. basilicum* L. (Sungadhi Tulasi)

(\*C: Collenchyma; Gh: Glandular hairs; P: Parenchyma; S: Sclerenchyma; St: Stone cells; T: Trichomes; Vc: Vascular cylinder)

**Anatomical Characteristics**

***Ocimum canum* L. (Kanaka Tulasi)**

The transverse section of stem showed rectangular in shape with four ridges and grooves. The epidermis layer is uni-seriate, and has been covered by a thin cuticle layer. The parenchymatous cortex is collenchymatic in a hypodermal position. The patches of stone cells were mostly found to confine to four ridges. Very few stone cells were noticed in the shallow portion of the stem. The large bundles have radial ranges of ligneous vessels separated by uni- or multi-seriate areas of parenchymatous cells. Four large vascular bundles were also observed at each corner. The number of glandular and tectorial trichomes were very few. The tectorial trichomes are uniseriate, consisting of three cells, with an acute apex and a bi- or multicellular basis (Fig. 7).

***O. tenuiflorum* L. (Radha Tulasi)**

Transverse section of *O. sanctum* (Radha Tulasi) is rectangular in outline with well developed four ridges in four corners of the stem. Number of trichomes and

glandular hairs are present covering the entire surface of the stem. The epidermis is composed of a thin cuticle covering externally. Collenchymatous tissue is located under the epidermis just below the ridges. The cortex consists of parenchymatous cells with air spaces. Small groups of stone cells were arranged ring like layer in the peripheral cortical region with more patches of stone cells in less developed ridges. Vascular bundles are conjoint, collateral and endarch type. Endodermis is conspicuous. Vascular bundles are more prominent in four corners. The cambium activity produces phloem to the exterior and xylem to the interior. Ray parenchyma located in between the vascular bundles. Pith is composed of polygonal, oval and spherical parenchymatous cells with small intercellular spaces (Fig. 8).

***O. tenuiflorum* L. (Krishna Tulasi)**

Transverse section of *O. sanctum* L. is rectangular in outline with four well developed ridges in four corners. Trichomes are mostly confined to the four corners and very few trichomes are present in the furrows. The epidermis is composed of a thin cuticle

covering externally. Collenchyma tissue is located under the epidermis, patches of collenchymatous cells were found to be confined to the corners. The cortex consists of parenchymatous cells with air spaces. Vascular bundles are conjoint, collateral and endarch type. Endodermis is conspicuous. Vascular bundles are more prominent in four corners. Ray parenchyma located in between the vascular bundles. Pith is composed of polygonal, oval and spherical parenchymatous cells with small intercellular spaces (Fig. 9.).

***O. gratissimum* L. (Rama Tulasi)**

The cross section of is nearly quadrangular in outline (Fig. 10) and divided in to two parts; the bark and the central cylinder. The bark is thin, has three primary tissues (epidermis, cortical parenchyma and collenchyma). The epidermis is consists of a single base of small contiguous rectangular cells, the wall is thin. The cortical parenchyma consists of several layers of polygonal cells with thin walls. Group of stone cells are present just below the collenchymatous mass of cells below the less developed ridges. Very few stone cells are also observed in furrows. Sclerenchymatous tissue surrounds the phloem at each corners of stem. Few trichomes especially glandular trichomes are present. The central cylinder is more developed than the bark. The primary wood, secondary phloem and secondary wood are arranged in radial alignment. The pith is occupied by parenchymatous cells and constitutes large polygonal cells (Fig. 10).

***O. basilicum* var. *purpurascens* L. (Durlabha Tulasi)**

The transverse section showed epidermis with very few trichomes. The cortex consists parenchymatous tissue with air spaces. Patches of stone cells are present in the cortical region surrounding the peripheral region of the central vascular cylinder (Fig.11). Vascular bundles are conjoint, collateral and endarch type. Endodermis is conspicuous. The cambium activity produces phloem to the exterior and xylem to the interior. Ray parenchyma located in between the vascular bundles. Pith is composed of polygonal, oval and spherical parenchymatous cells with small intercellular spaces.

***O. basilicum* L. (Sugandhi Tulasi)**

The epidermis is single layered without trichomes on the surface of stem. More pronounced collenchyma tissue is located in four ridges. The cortex consists of parenchymatous tissue with air spaces. Vascular bundles are conjoint, collateral and endarch

type. Endodermis is conspicuous. The cambium activity produces phloem to the exterior and xylem to the interior. Ray parenchyma located in between the vascular bundles. Pith is composed of polygonal, oval and spherical parenchymatous cells with small intercellular spaces (Fig. 12). Phylogenetic evolution study was conducted and correlation was made on a number of varieties of genus *Ocimum*. Different morphological variations were also studied (12).

**Chlorophyll Estimation**

Chlorophyll a, b, total chlorophyll and carotenoids of both young and matured leaves of six species of *Ocimum* were estimated and presented in Table 3. It was revealed that total chlorophyll content in matured leaf of *O. sanctum* L. (Radha Tulasi or Green Tulasi) was maximum of 36.79 mg/ml whereas least amount of 12.65 mg/ml of Chl-a was recorded in young leaf. Maximum of 126.1 mg/ml carotenoid was estimated in matured leaf.

Data depicted in Table 3, revealed that Chl.a was found to be maximum of 19.18 mg/ml in matured leaves of *O. basilicum* L. (Purple) followed by 19.04, 18.77, 18.18, 17.67 and 13.83 mg/ml in *O. gratissimum* L., *O. sanctum* L. (Purple), *O. canum* L., *O. basilicum* L. (Green) and *O. sanctum* L. (Green) respectively. Chl-b was estimated with 32.85 mg/ml in matured leaves of *O. gratissimum* L. followed by 28.15 mg/ml in *O. basilicum* L. (Purple), 23.28 mg/ml in *O. sanctum* L. (Purple), 22.95 mg/ml in *O. sanctum* L. (Green) and 20.72 mg/ml in *O. canum*L. Least *Chl.a* of 18.34 mg/ml chl b was reported in *O. basilicum* L. (Green). It was also observed that total chlorophyll content in matured leaves of *O. gratissimum* L.(Rama Tulasi) was maximum of 51.89mg/ml followed by 47.34mg/ml in *O. basilicum* L. (Purple), 42.05mg/ml young leaf with 44.05mg/ml whereas the amount of Chl.a of 17.01 mg/ml and 19.04 mg/ml in *O. sanctum* L. (Purple), *O. canum* L. with 38.91, *O. sanctum* L. (Green) with 36.79 mg/ml and *O. basilicum* L. (Green) with 36.02 mg/ml respectively. Maximum of 244.92 mg/ml carotenoid was estimated to be present in matured leaf of *O. sanctum* L. (Purple) followed by 237.45, 230.56, 129.71, 126.1 and 121.60 mg/ml in *O. gratissimum* L., *O. basilicum* L. (Purple), *O. canum* L., *O. sanctum* L. (Green), and *O. basilicum* L. (Green) respectively. It was also revealed that in all cases the young leaves contain less quantity of chlorophyll a, chlorophyll b, and total chlorophyll and carotenoid pigments in comparison to matured leaves that indicates the efficiency of matured leaves for increasing drug efficacy.

**Table 3. Pigment Estimation in *Ocimum* species**

<i>Ocimum</i> spp.	Pigments (mg/ml)							
	Chl. a		Chl. b		Total Chl		Carotenoid	
	Young leaves	Matured leaves	Young leaves	Matured leaves	Young leaves	Matured leaves	Young leaves	Matured leaves
<i>O. basilicum</i> L. (Green)	15.23	17.67	9.29	18.34	24.53	36.02	116.36	121.60
<i>O. basilicum</i> L. (Purple)	9.07	19.18	10.49	28.15	19.57	47.34	93.28	230.56
<i>O. canum</i> L.	16.73	18.18	17.76	20.72	34.49	38.91	113.57	129.71
<i>O. gratissimum</i> L.	17.01	19.04	27.04	32.85	44.05	51.89	203.94	237.45
<i>O. sanctum</i> L. (Green)	12.65	13.83	21.27	22.95	33.92	36.79	118.51	126.1
<i>O. sanctum</i> L. (Purple)	16.65	18.77	20.05	23.28	36.71	42.05	201.79	244.92

Literature data revealed the diversity of nine *Ocimum* genotypes based on morphological, chemical, RAPD identification and taxonomic classification (13). This indicated that for genotyping study RAPD technique is needed but for preparation of drug formulations, the anatomical study is a valuable and important easiest tool for identification of the raw drug before making the formulations. The morphological characteristics studies are the basic requirement for identification of plants but in drug manufacturing industry, anatomical study of dry collected raw materials should be checked as a quality control parameter. Data reported on four *Ocimum* species viz. *O. basilicum* var. *thysiflora*, *O. sanctum*, *O. gratissimum* and *O. viride* was reviewed and correlated with the present findings and proved to be useful in correct identification (14).

### Conclusion

In this article the diversity of six *Ocimum* species was described in terms of morphological, anatomical and also based on chlorophyll content analyses. Based on the anatomical features it would be easier to distinguish six different *Ocimum* species from each other. All the species possess different pharmacological activities because of the huge variation in the chemical composition. This experiment helps to give the easiest methods to identify the species in crude dry form before preparation of formulations and is a valuable tool for authentication of the desired species. Morphological and anatomical characters play an important role in plant based crude drug identification and authentication and also helps to differentiate the species.

### Conflict of Interest

None

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