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A critical study on genus *Ocimum* of Dibrugarh District of Assam

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Abstract

The genus *Ocimum* belonging to family Lamiaceae with more than 160 species and is distributed mainly in Africa, Asia and America. The present study aims to characterize the morphological and anatomical traits of five species of *Ocimum* namely *O. sanctum* L. (purple variety), *O. basilicum* L., *O. gratissimum* L., *O. americanum* L., and *O. sanctum* L. (green variety) collected from the district. Variation of stomatal index and density were seen among all the species. *O. sanctum* L. is believed to be sacred or holy plant and has been widely used to treat fever, common cold, flu, and other respiratory disease. The stomatal index ranged from 41.8% to 37.5% in *O. sanctum* L. (purple variety) and *O. gratissimum* L. on the abaxial surface while on the adaxial surface the highest stomatal index ranged from 40.5% in *O. sanctum* L. (purple variety) to 31.8% in *O. gratissimum* L. The highest stomatal density (96.7mm^2) was found in *O. sanctum* L. (green variety) and lowest stomatal density (71.2mm^2) was found in both *O. basilicum* L. and *O. americanum* L. on the abaxial surface while on the adaxial surface it ranges from 76.3mm^2 in *O. sanctum* L. (purple variety) to 31.8mm^2 in *O. gratissimum* L.. By taking 44 morphological traits and 5 OTU a dendrogram was constructed using an analysis software SPSS 26.0 version which shows *O. sanctum* L. (purple variety) and *O. basilicum* L. having more similarity and has the lowest morphological variation distance 23. *O. sanctum* L. (green variety) has significant differences from all the other species with a square Euclidean distance of 66.

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Introduction

With around 220 genera and nearly 4000 species worldwide, the family Labiatae (Lamiaceae) is one of the largest and distinctive flowering plant groups which are distributed over the globe. Various members of this family have yielded numerous physiologically active essential oil. This family is one of the world's most important suppliers of vegetable, culinary, and medicinal plants. *Thymus*, *Salvia*, *Mentha*, *Origanum*, *Coleus* and *Ocimum* species are utilised in cuisine and industry. In modern and traditional medicine, various species of the family are utilised (Naghibi *et al.,* 2022). *Ocimum* is a significant genus in the Lamiaceae family belonging to subfamily Nepetoideae. The name *Ocimum* was derived from a Greek word "Ozo" which means smell or to be fragrant and is known as 'king of herbs'. According to reports the genus *Ocimum* contains around 160 species and is the largest of the genera, with 65 species unique to *Ocimum* and others being synonyms. About nine *Ocimum* species have been reported in India, including three foreign species: *O. minimum*, *O. x africanum*, *O. americanum* (Parida *et al.,* 2020).

From sea level to 1800 meters the species is widely distributed in the warmer regions of both the hemisphere, having three centres of diversification (a) subtropical and tropical parts of Africa (b) tropical region of Asia and (c) tropical America. This species is found in India, Australia, Brazil, Nepal, Egypt, The Philippines, Pakistan, Western Asia, Arab countries and Malaysia (Zahran *et al.*, 2020; Chutia *et al.*, 2016; Chowdhury *et al.,* 2017). *O. basilicum* (Ban Tulsi), *O. camphora*, O. *canum* (Dulal Tulsi), *O. gratissimum* (Ram Tulsi), *O. kilimandscharicum* Guerke (Kapoor Tulsi), *O. micranthum*, *O. sanctum* L. (Tulsi) are some examples of well-known species of this genus that thrive in many regions of the world. Species such as *O. gratissimum, O. basilicum*, *O. canum, O. basilicum* var. *purpurascens*, and *O. sanctum* are grown in North East India (Kalita *et al.*, 2013). From extract of fresh leaf and stems of various phenolic compounds are yielded namely rosameric acid, cirsilineol, isothymusin, circimaritin, and apigenin as well as significant amount of eugenol, two flavonoids such as vicenin and orientin which have shown medicinal effect such as hepatoprotective, cardioprotective, neuroprotective, chemopreventive, antioxidant, immunomodulatory, anticancer, antimicrobial, antipyretic, antiulcer, anti-inflammatory and other medicinal importance (Deshmukh *et al.*, 2015). The many *Ocimum* species in the Lamiaceae family are revered as holy plant. They are present in almost every household and are revered as "God" (Rawat *et al.*, 2016).

The morphological and anatomical characteristics are crucial for the standardisation of crude drugs. Important taxonomic characteristics for quality control and certification of medicinal plants includes anatomical characterisation for identification and comparison. Differences in the climatic factors to which plants are subjected during growth may have a direct impact on the morpho- anatomical structures. Additionally, these studies on the morpho-physiological, anatomical, and genetic features of industrial and commercial crops as well as endangered species are crucial for creating effective quality parameter standards and conservation methods, respectively (Chowdhury *et al.,* 2017).

This study aims to present the numerical taxonomic analysis of the five different species of genus *Ocimum* collected and identified based on their morphological structure (Fig 1). Variations in their anatomical structure, stomatal index and density are recorded. Traditional and religious uses of each species have also been discussed.

Material and methods

Collection of samples

The present study was carried out on the five different species of *Ocimum* collected in the district of Dibrugarh during the period ranging from October 2021 to June 2022. Dibrugarh covers 3381 square kilometres in total. The district is located at an altitude of 99 to 474 meters between 27°5′38′′ N and 27°42′30′′ N latitude and 94°33′46′′ E to 95°29′8′′ E longitude with an average yearly temperature of 23.9°C and also receives 276 cm of rain on an annual basis (www.dibrugarhonline.in). The district's soil is predominantly fertile alluvial soil, with varied

proportions of sand and clay adjacent to the Brahmaputra River (www.dibrugarh.gov.in). Five species of *Ocimum* namely *O. americanum* L., *O. basilicum* L., *O. gratissimum* L., *O. sanctum* L. (green variety) and *O. sanctum* L. (purple variety) were taken for morphological study, anatomical study of stem, trichomes and their stomatal density and index. The scientific names along with their location and GPS are shown in Table 1.

Table 1. Collection details of *Ocimum* species from different sites of Dibrugarh District.

SL	Species	Location	GPS
1.	*O. sanctum* L. (purple variety)	Lakhi Nagar, Japara Gaon	27.449975° N; 94.915376° E
		West Milan Nagar	27.46094° N; 94.910308° E
		Namrup	27.188378° N; 95.354329° E
2.	*O. basilicum* L.	Chabua	27.482666° N; 95.172068° E
		Dibrugarh	27.462143° N; 94.870827° E
		Khania Gaon	27.464342° N; 94.916515° E
3.	*O. gratissimum* L.	Officer's Colony	27.465574° N; 94.913544° E
		West Milan Nagar	27.464663° N; 94.914604° E
		Taor Gaon	27.43662° N; 94.931209° E
4.	*O. americanum* L.	West Milan Nagar	27.460501° N; 94.910802° E
		Railway Colony	27.46382° N; 94.914855° E
		Chowkidingee	27.465533° N; 94.912323° E
5.	*O. sanctum L.* (green variety)	Amolapatty	27.465085° N; 94.917617° E
		Babulal Poddar Path	27.481074° N; 94.90518° E
		Mancotta Road	27.477844° N; 94.908356° E

Field equipments used for Taxonomic study

Tools such as diggers, trowel, blade, knife, secateurs, pair of forceps, newspaper, polythene bag, pocket lens, field notebook, cans containing preservatives, pen, camera and a standard questionnaire was used during the sample collection.

Collection of specimen

Plant specimens were collected from different locations. A specimen must contain all their parts such as roots, stem, leaf, flower, fruits etc.

A good quality of photograph is taken in the camera before cutting the important parts of the plant. Each sample was gathered separately in different polythene bags to prevent mixing and should be marked carefully. The collected samples were mounted into herbarium by following herbarium techniques and were preserved in formaldehyde solution for anatomical and stomatal study.

Drying and Pressing of the Specimen

Plant materials should be put between newspapers and pressed securely with plant press after collection. Plants should be dried as soon as possible after being prepared for a herbarium. The plants must be pressed before wilting. Newspaper should be changed every 2 to 4 days then several times more at longer intervals.

Poisoning of the specimens

The collected dried specimen was poisoned by dipping it into 0.1% mercuric chloride ($HgCl_2$) solution to minimise insect and fungal attack. Broad tipped forceps are used to remove well dried specimens from the solution after one or two minutes of soaking, and they are then left on a wire mesh to drip out the excess solution for a while.

Mounting of specimen

Different methods can be used to add the adhesive. The blank herbarium sheet of 41 x 29 cm is used to adhere all the plant pieces pressed gently and carefully. The glue is attached to the tag to mount the sheet.

Collection of data

Interactions were made with the locals for the essential data using questionnaires with collection number their habit, habitat, local name, flowering season, collection date and are taken down in a notebook. The collected plant parts were preserved in the formaldehyde solution.

Identification

A plant is identified by studying its characters, comparing them to flora of the area, its family, genus, and species keys, then compare them to full description and illustration. The plant specimen was identified in consultation with the taxonomists of Department of Botany, Assam down Town University, Guwahati.

Analysis of Data

44 morphological characters were taken such as colour of leaves, calyx, corolla, length of calyx, corolla, and stamens were noted for the 5 OTU under this study which are listed below in Table 2. Character states were established through examination of both fresh and herbarium specimens, and they were used as multistate characters. To clarify the hypothesised evolutionary relationships among the species, a dendrogram was constructed using SPSS 26.0 version as shown in Fig 2. With particular attention to taxonomic and morphological characteristics, morphometric study of the five species under the genus *Ocimum* of family Lamiaceae was conducted. Based on square Euclidean distance, morphological variance between the investigated species was conducted.

Table 2. Morphological characters used in numerical analysis.

SL	Characters	Taxa				
		O. sanctum L. (purple)	*O. basilicum* L.	*O. gratissimum* L.	*O.americanum* L.	*O. sanctum* L. (green)
1	Habit: Herb 1/ Shrub 2	1	1	2	1	1
2	Plant height: less than 1 m 1/ more than 1m 2	1	1	2	1	1
3	Stem surface: Glabrous 1/ Pubescent 2	2	2	2	2	2
4	Nature of Stem: Erect 1/ Weak 2	1	1	1	1	1
5	Type of Stem: Herbaceous 1/ Woody 2	2	1	2	2	2
6	Stem colour: Purplish to reddish brown 1/ Greenish to brown 2/ Greenish to light brown 3	1	2	3	2	2
7	Type of Phyllotaxy: Alternate 1/ Opposite decussate 2/ Opposite superposed 3/ Whorled 4	2	2	2	2	2
8	Leaves colour: Green - purple 1/ Green 2/ Light Green 3	1	2	2	2	3
9	Leaf outline : Linear 1/ Cordate 2/ Ovate 3	3	3	3	3	3
10	Leaf apex : Acute 1/ Acuminate 2	1	1	2	1	1
11	Leaf base: Obtuse 1/ Cuneate 2	1	1	2	2	1
12	Leaf margin: Serrate 1/ Entire 2	1	1	1	1	1
13	Leaf adaxial surface: Pubescent 1/ Glabrous 2	1	1	1	1	1
14	Leaf abaxial surface: Pubescent 1/ Glabrous 2	1	1	1	1	1
15	Leaf length (in cm): 1.93 cm 1/ 1.98 cm 2/ 2.23 cm 3/ 3.01 cm 4/ 6.5 cm 5	2	1	5	4	3
16	Leaf vein colour: Purple 1/ Green 2	1	2	2	2	2
17	Leaf Width(in cm): 0.65 cm 1/ 0.71 cm 2/ 1.07 cm 3/ 1.12 cm 4/ 3.96 cm 5	3	2	5	1	4
18	Petiole Length (in cm) : 0.5 cm 1/ 0.6 cm 2/ 0.8 cm 3/ 0.9 cm 4/ 1 cm 5	3	2	5	1	4
19	Petiole Surface: Pubescent 1/ Glaborous 2	1	1	1	1	1
20	Inflorescence : Verticillaster 1/ Cyathium 2/ Hypanthodium 3	1	1	1	1	1
21	Pedicel length (in cm) : 0.2 cm 1/ 0.3 cm 2	1	1	1	2	1
22	Pedicel surface: Pubescent 1/ Glaborous 2	1	1	1	1	1
23	Floral Symmetry : Actinomorphic 1/ Zygomorphic 2	2	2	2	2	2
24	Flower length (in cm): 0.6cm 1/ 0.8 cm 2/ 0.9 cm 3/ 1 cm 4	3	2	3	4	1
25	Corolla Colour: Purple 1/ White 2/ Yellowish white 3/ Whitish pink 4	1	2	3	2	4
26	Corolla length (in cm): 0.2 cm 1/ 0.4 cm 2/ 0.6cm 3	2	2	2	3	1
27	Corolla sinus: Shallow 1/ Deep 2	1	1	1	1	1
28	Corolla surface outside: Pubescent 1/ Glabrous 2	1	1	1	1	1
29	Calyx Colour: Green- purple 1/ Light Green 2/ Green 3	1	2	3	3	3
30	Calyx length (in cm): 0.2 cm 1/ 0.4 cm 2	2	1	2	2	1
31	Calyx Sinus: Shallow 1/ Deep 2	1	1	1	1	1
32	Calyx surface outside: Pubescent 1/ Glabrous 2	1	1	1	1	1
33	Inflorescence: Purple 1/ Green 2	1	2	2	2	2
34	Inflorescence length (in cm): (5-7) 1/ (7-9) 2/ (9-11) 3	1	3	2	2	1
35	No. of flower per inflorescence : (5-10) 1/ (10-15)2/ (15-20) 3	3	1	1	1	3
36	Androecium: Dialdelphous 1/ Didynamous 2	2	2	2	2	2
37	Stamen length long (in cm): 0.3cm 1/ 0.4 cm 2/ 0.5 cm 3/ 0.6 cm 4	3	2	3	4	1
38	Stamen Length short (in cm) : 0.2 cm 1/ 0.3 cm 2/ 0.4 cm 3/ 0.5 cm 4	2	2	3	4	1
39	Gynoecium Length (in cm): 0.4 cm 1/ 0.6 cm 2/ 0.8 cm 3	2	2	3	2	1
40	Style Length (in cm): 0.2 cm 1/ 0.5 cm 2/ 0.6 cm 3	2	2	3	2	1
41	Style Surface: Pubescent 1/ Glabrous 2	2	2	2	2	2
42	Seed Colour: Black 1/ Brown 2	2	1	2	1	2
43	Seed Shape: Ellipsoid 1/ Globose 2/ Sub- globose 3	2	1	3	2	2
44	Seed Length (in cm) : 0.1 cm 1/ 0.2 cm 2	1	1	1	1	1

Anatomical Study

This experiment was conducted in the laboratory of Assam down Town University. Preserved or fresh stems of these species were completely cleaned in tap water. The part of stem to be sectioned must be held firmly in between the thumb and index finger. By moving the blade back and forth, a section was cut and placed in watch glass, filled with water. Double staining procedure is followed using safranin –O and fast green. A thin section of stem was selected separated and placed in a clean glass slide and was stained with safranin- O for each species separately. A drop of glycerine was applied, and then the cover slip was placed on top which was now observed under microscope under various magnifications (Parida *et al.,* 2020; Rawat *et al.*, 2016).

Trichome study

This investigation includes both fresh and samples preserved in formaldehyde solution. When studying the thin hand cross sections of the preserved or living materials cut using a sharp blade were stained with Safranin and mounted with glycerine and observed under a microscope. The structural trichome variations between the species were captured on the camera (Sanoj *et al.,* 2021)

Stomatal Study

Stomata are seen on both the surfaces of leaf and can also be found on fruits, inflorescence, petioles, stems.

Materials required

Specimen, brush, pair of forceps, replica, glass slide, cover slip, ocular micrometer, stage micrometer, needle are required to conduct the stomatal study

Calibration of Ocular micrometer

A stage micrometre is a scale with 100 divisions of 1mm, so 1 division equals 0.01mm, 10 µm. The stage micrometer is placed on the microscope's stage and focused it at the desired magnification. The ocular micrometre is placed in one of the microscope's eyepiece. The ocular micrometer and stage micrometre is aligned while looking through the eyepiece. The number of divisions overlapping the ocular micrometer and stage micrometer is counted. The stage micrometer can be removed and a slide containing the sample material can be put for measurement and observations once the ocular scale has been calibrated for different magnifications of 10x, 45x, 100x etc.

Determination of stomatal density

The leaf sample is collected and washed under running tap water to eliminate dust or debris and let them dry. The epidermal layer is taken off by hand if possible; otherwise a layer of replica (commonly available adhesive) is applied to peel off the surface and is laid on the surface so that the imprinted surface is on the upper side. The number of stomata present in the microscopic view can be recorded in order to calculate the stomatal density which is given in terms of stomata/mm^2 as shown in Table 5. The total count of stomata is noted at a certain magnification. Using an ocular scale, the diameter is determined. The following formula can now be used to compute an area of a circle in a microscopic field by πr^2. Similarly, the stomatal density of upper and lower surface of leaves is done for all the species.

Determination of Stomatal Index

Stomatal index can be determined by using, stomatal index (%) =(S/S+E) ×100, where S and E are the number of stomata and epidermal cells. This can be measured on both the surfaces of leaves (Paul *et al.*, 2017).

Ethnobotanical Study

The ethnobotanical study was done by submitting a questionnaire. For each plants information regarding the plant's uses were recorded in the field notebook along with date and location of the interview or inquiry, the name of the informants, their tribe and community of origin. Data sheets carrying information such as the field number of the voucher of the plant specimen, the botanical name, the family, local name, the locality or village, their uses were taken from the ethnic community. A study regarding the religious belief, traditional and medicinal use in the daily life of the local people of different localities was also attempted (Table 7).

Result and discussion

A total of 5 species of *Ocimum* were explored from various places of Dibrugarh district of Assam. The vernacular names were identified in consultation with the locals with a standard set of questionnaires (Table 3). These were then identified in consultation with the taxonomists of Assam down town University.

Description of the plant

Ocimum has a thryse inflorescence with opposite 1-3 flowered cymes. Verticels are two opposite cymes that are normally developed on the axis. The calyx is usually funnelled shaped or tubular with a slightly curved or straight form. The calyx has 4-lobed lower lip and a single upper lip, two lateral and two median lobes comprise the anterior lip. Fruit usually causes the calyx to expand. The corolla is usually straight; however, it might be slightly bent downward. There are usually two pairs of stamens, one near the mouth of corolla and other at the base. In every species the ovary is divided into four sections. These portions mature into mericarps, which are single seeded round or spherical nutlets mostly glaborous and rarely pubescent (Nahak *et al.*, 2011).

Table 3. Common name, local name and synonym of the collected species.

SL	Scientific Name	Common name	Local Name	Synonym
1.	*O. sanctum* L. (purple variety)	Sacred Basil	Kola Tulsi	*O. tenuiflorum* L.
2.	*O. basilicum* L.	Sweet Basil	Ram Tulsi	*O. thyrsifolium* L.
3.	*O. gratissimum* L.	Shrubby Basil	Ram Tulsi	*O. viride* Willd.
4.	*O. americanum* L.	Haory Basil	Ban Tulsi	*O. canum* Sims.
5.	*O. sanctum* L. (green variety)	Sacred Basil	Boga Tulsi	*O. tenuiflorum* L.

Key to Species (Sanoj et al., 2021)

1a. Stem trichomes only glandular type 2

1b. Stem trichomes both glandular and non-glandular types 3

2a. Foliar glandular trichomes only capitate type ....*O. gratissimum*

2b. Foliar glandular trichomes only peltate type ..... .*O. basilicum*

3a. Stem or foliar trichomes having internodal thickening 4

3b. Stem or foliar trichomes lack internodal thickening ..... *O. americanum*

4. Foliar trichomes only capitate type ....*O. tenuiflorum*

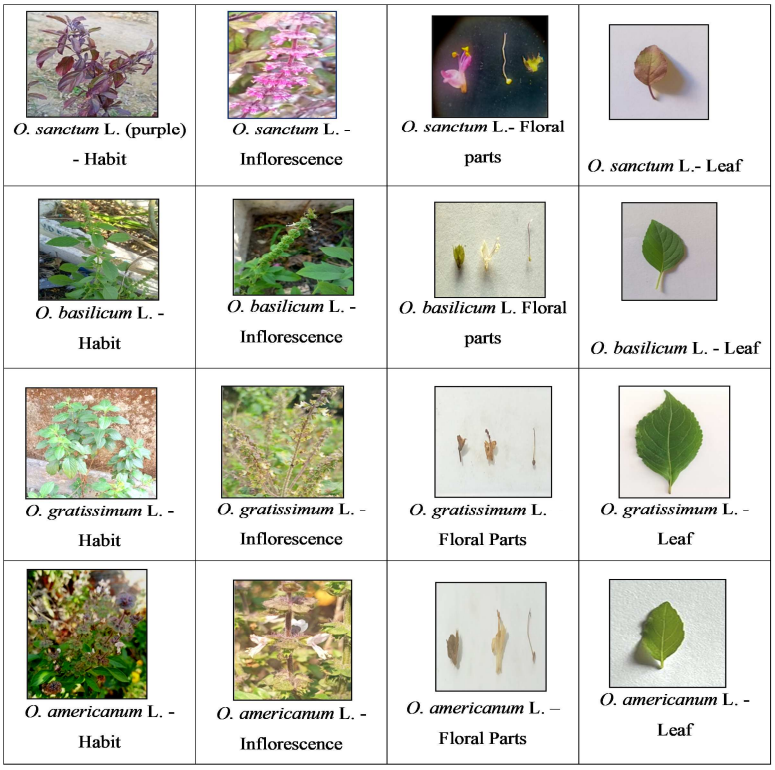


Fig. 1. *Ocimum* species collected from Dibrugarh district.

Data Analysis

In the present survey a total of 5 fresh species of genus *Ocimum* have been collected from the district of Dibrugarh. In general flowers of *Ocimum* are easily identified by their verticellaster inflorescence, bilabiate calyx and corolla with didynamous stamens.

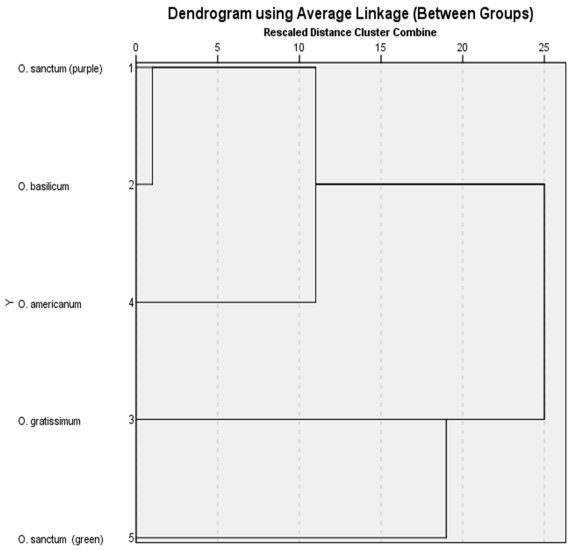


Fig. 2. Dendrogram showing the relationship between each species.

The taxonomy of *Ocimum* species relied mostly on morphological traits. Identification of the species is greatly aided by plant habit, stem structure, leaf shape, margin, apex and base, petiole pubescence, flower colour and structure, fruit and seed. In the cluster analysis 44 morphological characters are analysed and employed.

O. sanctum (purple variety) highly similar to *O. basilicum* as showed in the cluster analysis and has the lowest morphological variation distance of 23. *O. basilicum* characterized by obtuse leaf base and *O. sanctum* (purple variety) also having the same leaf base. *O. americanum* is highly dissimilar to *O. sanctum* (green variety) as showed in the cluster analysis and has the highest morphological variation distance of 66.000. *O. americanum* is characterized by cuneate leaf base, seed colour black while *O. sanctum* (green variety) is characterized by obtuse leaf base, and brown seed colour. *O. sanctum* (purple variety), *O. basilicum, O. americanum* have great similarity among themselves having acute leaf apex, having pubescent calyx, corolla. *O. sanctum* (green variety) has significant differences of all existing studied species with a square Euclidean distance of 66 as shown in Table 4.

Table 4. Morphological variation between the studied species based on Squared Euclidean Analysis.

Proximity Matrix					
	Squared Euclidean Distance				
Case	1:*O. sanctum* L. (purple)	2:*O. basilicum* L.	3:*O. gratissimum L.*	4:*O. americanum* L.	5:*O. sanctum* L. (green)
1:*O. sanctum* L. (purple)	0.000	23.000	45.000	36.000	36.000
2:*O. basilicum* L.	23.000	0.000	54.000	31.000	35.000
3:*O. gratissimum* L.	45.000	54.000	0.000	47.000	41.000
4:*O. americanum* L.	36.000	31.000	47.000	0.000	66.000
5:*O. sanctum* L. (green)	36.000	35.000	41.000	66.000	0.000

Anatomical Study

All the species of *Ocimum* have quadrangular stem with four ridges, the cuticle follows the epidermis. Trichomes were present on the surface of stem except *O. basilicum* L. The cortex is made up of parenchymatous cells with air gaps. The four corners showing vascular bundles are collateral, conjoint and endarch varieties. Between the vascular bundles is a ray parenchyma. Pith is made up of parenchymatous cells with small intercellular gaps that are polygonal, oval, and spherical. The epidermis of *O. gratissimum* L. is made up of single base of tiny, closely spaced rectangular cells with a thin cell wall. Just below the collenchymatous mass of cells beneath the less developed ridges, there is a group of stone cell. In furrows, very few stone cells have been found. Stems of *O. americanum* L. hardly have any stone cells. In both the varieties of *O. sanctum* L. parenchymatous, collenchymatous cells are present (Parida *et al.,* 2020)

O. sanctum L. (purple variety)

The stem is quadrangular in shape. Trichomes and glandular hairs were present on the stem. Cuticle is followed by epidermis. There are vascular bundles present between the ray parenchyma.

The vascular bundles consist of xylem, phloem and cambium. Pith is oval or spherical in shape (Fig 3).

O. basilicum L.

The stem is quadrangular in shape. Trichomes were seen on the surface of the stem. Ray parenchyma is present in between the vascular bundles. Pith is made up of oval or spherical shape (Fig 4).

O. gratissimum L.

The stem is quadrangular in shape. Epidermis is made up of rectangular cell covered with the cuticle. Vascular bundle comprises of xylem, phloem and cambium. The phloem is encircled by sclerenchymatous tissue at each corner of the stem. Pith is made up of polygonal cells (Fig 5).

O. americanum L.

The stem is quadrangular in shape with trichomes. Outermost layer is the epidermis covered by cuticle. Stone cells were found on the four ridges. Vascular bundles were observed. The pith is polygonal in shape (Fig 6).

O. sanctum L. *(green variety)*

The stem is quadrangular in shape. Trichomes and glandular hairs were present on the stem. Cuticle is followed by epidermis. There are vascular bundles between the ray parenchyma. The vascular bundles consist of xylem, phloem and cambium. Pith is oval or spherical in shape (Fig 7).

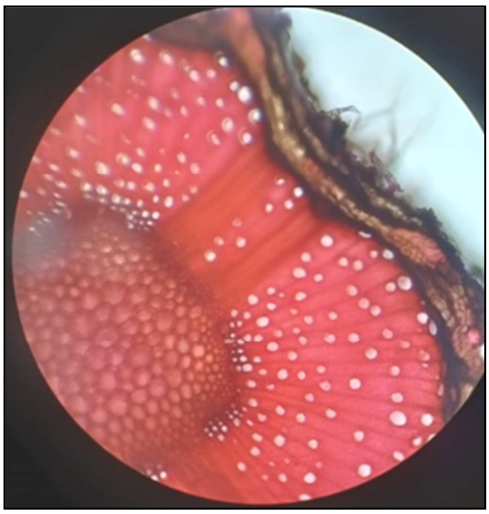


Fig. 3. *O.sanctum* L. (purple).

Fig. 4. *O. basilicum* L.

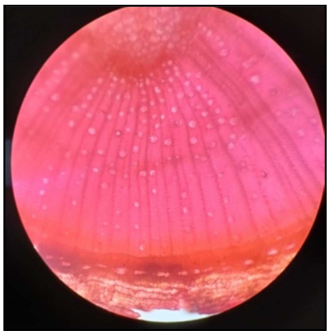


Fig. 5. *O. gratissimum* L.

Fig. 6. *O. americanum* L.

Fig. 7. *O. sanctum* L. (green).

Trichome study on leaves

In *O. americanum*, capitates trichomes had a single-celled base and head, whereas peltate trichome could have either a single celled head or multicellular head.

The non-glandular uniseriate trichomes have a multicellular and unicellular base and an acute apex. However, *O. basilicum* displayed both glandular and non-glandular foliar trichomes, the latter of which had a multicellular head. The segmented, uniseriate non glandular trichomes had a sharp apex. On the adaxial side of the leaves of *O. gratissimum*, capitates and non-glandular trichomes were examined. While the non-glandular trichomes are lengthy, multicellular or unicellular with an acute apex, the capitate form revealed a unicellular base, stalk and head.

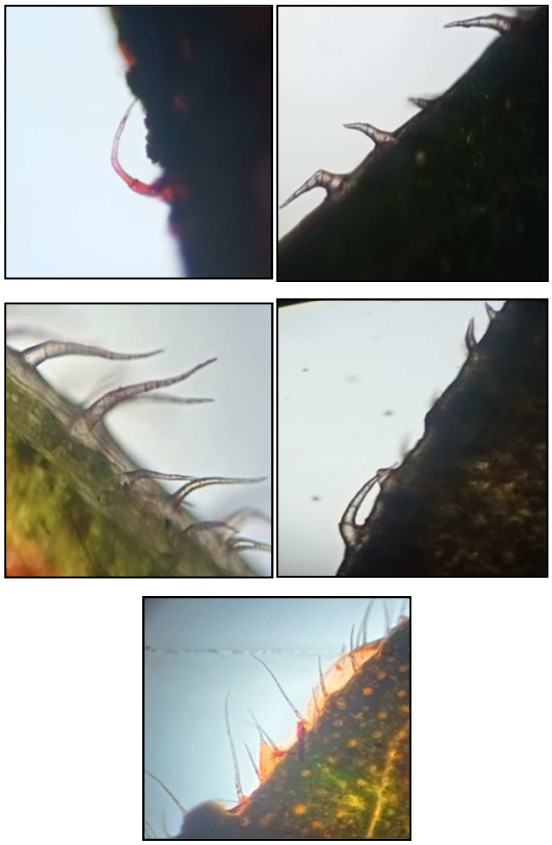
The characteristics features that set *O. sanctum* apart from other species are its capitates and non-glandular trichomes. In this instance, the capitate form had a single celled stalk and a secretarial mono or multicellular head. The long, segmented, uniseriate non glandular trichomes had a unicellular or multicellular base and broad or acute apex (Sanoj *et al.,* 2021).

Trichomes can be seen on the adaxial and abaxial of leaves, and they serve a specialized purpose, such as offering protection from environmental stress. Multi cellular, non-glandular trichomes are present in almost all the species of *Ocimum*. Both green and purple variety of *O. sanctum* shows multicellular or unicellular and non-glandular trichomes while both *O. gratissimum* and *O. americanum* have multicellular and non-glandular trichomes (Fig 8).

Stomatal density on leaf surfaces

On the abaxial surface of *O. basilicum* (65.79mm²) the highest stomata density is seen, while on the adaxial surface of *O. lamiifolium* (10.53mm²) the lowest is observed (Abdulrahaman *et al.,* 2005).

Highest stomatal density is observed on the abaxial surface of *O. sanctum* (green variety) having 96.7mm². The lowest stomatal density is seen on the adaxial surface of *O. gratissimum* with 31.8mm². Abaxial surface of both *O. basilicum* and *O. americanum* has the same stomatal density of 71.2mm². In *O. gratissimum* a huge difference of stomatal density in both the abaxial and adaxial surface of leaf is found. The lowest stomatal density is present in *O. gratissimum* (31.8mm²) in the adaxial surface and the highest stomatal density in *O. sanctum* (purple variety) (76.3mm²). On the abaxial surface of *O. sanctum* (green variety) (96.7mm²) the highest stomatal density is seen and the lowest is present on both *O. basilicum* and *O. americanum* (71.2mm²).



O. sanctum L. (purple) *O. basilicum* L. *O. gratissimum* L. *O. americanum* L. *O. sanctum* L. (green)

Fig. 8. Trichome structure of each species.

Table 5. Stomatal density of the studied species.

SL	Taxon	Leaf surface	Stomatal density (inmm²)
1.	*O. sanctum* L. (purple variety)	Adaxial	76.3
		Abaxial	91.6
2.	*O. basilicum* L.	Adaxial	56.01
		Abaxial	71.2
3.	*O. gratissimum* L.	Adaxial	31.8
		Abaxial	76.3
4.	*O. americanum L.*	Adaxial	45.8
		Abaxial	71.2
5.	*O. sanctum* L. (green variety)	Adaxial	66.1
		Abaxial	96.7

Stomatal index on leaf surfaces

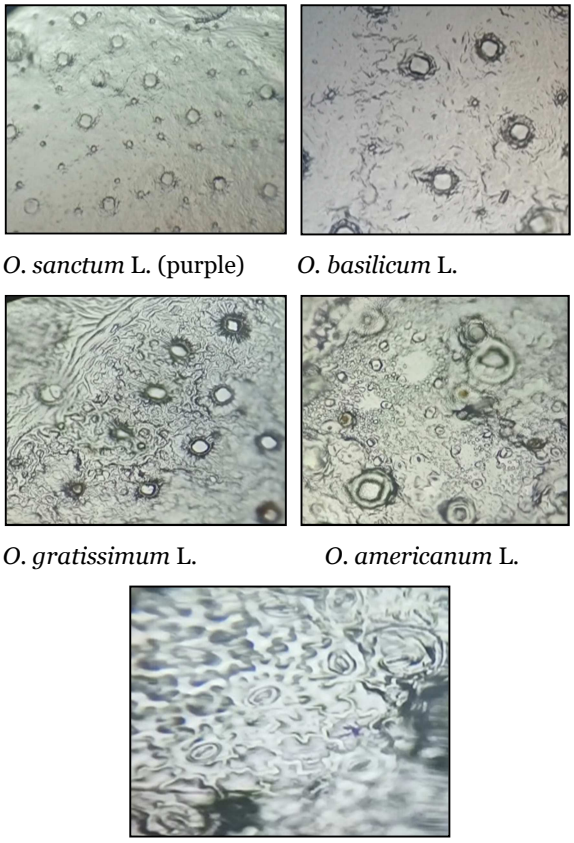
The leaves of stomata have amphistomatic leaves meaning having stomata on both the surfaces. The former displaying epidermal and stomatal cell, while

the latter one exhibiting trichome characteristics. In *O. americanum* the abaxial surface has complex types that are paracytic, diacytic and anisocytic stomata. Diacytic stomata are most common followed by anisocytic and paracytic stomata While in *O. basilicum* the adaxial surface have complex types that are diacytic, anisocytic, and paracytic stomata. Diacytic stomata are most common followed by anisocytic and paracytic stomata. The abaxial surface of *O. gratissimum* has anisocytic, diacytic, and paracytic stomata. Diacytic stomata are most common followed by anisocytic and paracytic stomata. The leaf surface of *O. basilicum* has more stomata, with a stomatal index of 18.87%, compared to *O. lamiifolium*, which has a lower index of 3.36% (Abdulrahaman *et al.,* 2005)

All the species have amphistomatic leaves meaning have stomata on both the abaxial and adaxial surface. Among all the five species of *Ocimum* found in Dibrugarh district *O. sanctum* (purple variety) having the highest stomatal index with 41.8% in the abaxial surface of the leaf. *O. gratissimum* has the lowest stomatal index on the adaxial surface 31.8%. It is seen that stomata are abundant on abaxial surface than on the adaxial surface. The abaxial surface of *O. gratissimum* and the adaxial surface of *O. americanum* have almost the same stomatal index (37.5%). Among all the species the adaxial surface of *O. gratissimum* has the lowest stomatal index of 31.8% while the highest stomatal index is present in *O. sanctum* (purple variety) having 40.5%. On the abaxial surface of *O. sanctum* (purple variety) highest stomatal index is seen with 41.8% whereas *O. gratissimum* has the lowest stomatal density of 37.5%.

Table 6. Stomatal index of the studied species.

SL	Taxon	Leaf surface	Number of stomata	Number of epidermal cell	Stomatal Index (%)
1.	*O. sanctum* L. (purple variety)	Adaxial	60	88	40.5
		Abaxial	72	100	41.8
2.	*O. basilicum* L.	Adaxial	44	76	36.6
		Abaxial	56	92	38.3
3.	*O. gratissimum* L.	Adaxial	28	60	31.8
		Abaxial	60	100	37.5
4.	*O. americanum* L.	Adaxial	36	60	37.5
		Abaxial	56	84	40
5.	*O. sanctum* L. (green variety)	Adaxial	52	88	37.1
		Abaxial	76	108	41.3



O. sanctum L. (purple) *O. basilicum* L.

O. gratissimum L. *O. americanum* L.

O. sanctum L. (green).

Fig. 9. Stomata of the five species of *Ocimum*.

Traditional and religious use

Ocimum genus is regarded as one of the most remarkable herbs. Ayurvedic medicines uses Tulsi extracts for cold, stomach problems, headaches, heart diseases, inflammation, poisoning and malaria (Pattanayak *et al.*, 2010). Tulsi extracts mixed with lukewarm water can be used internally and for exterior detoxification, cleansing, and purification. It is also used to treat skin conditions including ringworms and itching. Fresh leaves can be taken raw, pasted, powdered or as herbal supplements. Tulsi also known as Tulasi or Vaishnavi is an Indian sacred plant (*Ocimum tenuiflorum* L. syn *O. sanctum* L.). According to Hindu belief, plants have tremendous spiritual, medical, and therapeutic value. It is considered by Hindus to be an earthly embodiment of the goddess Tulsi, who is revered as a major Vishnu worshipper. Tulsi is grown in the heart of Hindu household's central courtyards in India. These plants are commonly found in front of or near Hindu homes, often in specific containers or brick (Upadhyay., 2017).

In Indian culture, down the ages it can be found that in most Indian homes it is worshipped and adored. In the month of Kartik, which follows Sharad Poornima, Hindus undertake a special Tulsi puja. Every evening during this month the tradition of lighting of lamp is performed that involves the worship of Tulsi, which is considered fortunate for the home. Women waters the plant and the soil, each part of the plant including stems, leaves as well as the seeds are regarded as sacred. Tulsi is revered in ancient literature as one who assists in connecting people to God (Deshmukh *et al.*, 2015). It is utilized in practically every religious ceremony and is frequently delivered to gods as garlands. Leaves are employed in a variety of events including births, weddings, religious rituals and funerals (Hanumanthaiah *et al.*, 2020)

The present study conducted in the district of Dibrugarh, Assam. According to the indigenous people of the area, the presence of this herb is to shield the house from all types of negative or evil influences, prevent from diseases. The herb is considered pure and is planted in front or outside their home. Leaves of Tulsi are used in Vishnu Puja and their seeds are used to make garland. People perform a special puja during the Kartika month and light diyas every evening during this month. The Assamese people celebrate this festival as Kati Bihu and plant a Tulsi plant on this auspicious day which usually falls on the 3rd week of month October.

Ethnobotanical use

Tulsi extracts and a heated combination cleanse, purify, and detoxify the body on the inside as well as the outside. Fine slurry of leaves is beneficial to the skin, leaf extracts can be consumed raw or added to drinks, and herbal supplements might be in powder, paste or other forms. It is typically employed for reducing symptoms of flu, cold, sore throat, headache and fever. Tulsi tea is powerful for respiratory disease (Upadhyay., 2017).

In the present study it is said that Tulsi leaf extracts are mixed with honey and their intake has been useful against cold, cough. Tulsi tea has also been used to against respiratory diseases. It is believed that Tulsi leaves added to food items before eclipse helps in maintaining the purity and safety of all food products. Tulsi leaves have a wide range of benefit and is also known to cure some skin disease by applying its paste directly on the affected area. It is also believed sprinkling Tulsi water to the house helps get rid of the evil spirit. Table 7 shows the ethnobotanical uses of *Ocimum* collected from the information given by the locals in the district.

Table 7. Ethnobotanical uses of each collected species.

SL	Taxa	Ethnobotanical Uses
1.	*O. sanctum* L. (purple variety)	Fever, Common Cold, Coughs, Sore Throat, Respiratory Disorder, Children's Ailments, Mouth Infections, Skin Disorders, Rashes. Worshipped to keep evil spirits away and also for prosperity and happiness of family.
2.	*O. basilicum* L.	Cough and fever
3.	*O. gratissimum L.*	Plant used to cure sickness, headache, sunburn, used to treat skin conditions, stomach ache, inflammation to ears, eyes and throat
4.	*O. americanum* L.	Cough, fever, mouth ulcer, dysentery
5.	*O. sanctum L.* (green variety)	Fever, Common Cold, Coughs, Sore Throat, Respiratory Disorder, Children's Ailments, Mouth Infections, Skin Disorders, Rashes. Worshipped to keep evil spirits away and also for prosperity and happiness of family.

Conclusion

Plant morphology refers to the study or investigation of a plants origin, structure and form. In order to identify these species and to control and evaluate their quality, anatomical characteristics are crucial [8]. In this study the diversity of five different *Ocimum* species using morphological, anatomical characters that can be used for taxonomic delimitation. It will provide numerical taxonomy which has been done in these species. This study helps to distinguish the closely related species. Due to extreme differences in chemical composition, every species has a unique set of pharmacological properties. The obtained information may be combined with information from other sources, such as molecular taxonomic methods, to improve the accuracy of taxonomic revision of species of *Ocimum*. The identification and verification of plant based crude drugs depends heavily on morphological and anatomical characteristics, which also serve to distinguish between various species (Parida *et al.,* 2020).

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