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Research Article

A COMPARATIVE STUDY OF MORPHOLOGICAL AND ANATOMICAL STRUCTURES OF FOUR *OCIMUM* SPECIES IN UTTARAKHAND, INDIA

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ABSTRACT

In traditional systems of medicine along with holistic approach, different parts of basil (Ocimum spp.) have been prescribed for the treatment of various ailments. Morphological and anatomical characters play a vital role in plant based crude drug identification and standardization. This study aimed to characterize the morphological and anatomical structure of four different species of Ocimum such as Ocimum basilicum var thyrsiflora L. commonly named as Thai basil; Ocimum tenuiflorum L. as Holy or sacred basil; Ocimum gratissimum L. as Shrubby basil or Clove basil and Ocimum viride Willd. as Temple basil or Fever plant of Sierra Leone. This study is useful to correct identification judging the authencity of the plant and to differentiate these species from each other's while undertaking pharmacognostical characterization and evaluation.

Key words: Basil, Anatomical, Morphological, Medicinal crude drugs.

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INTRODUCTION

Medicinal plants are of great importance to human health. That is why the world Health Organization estimates that 80% of the populations in developing countries rely on traditional medicine for their primary health care (Nathiya et al., 2012)1. Therefore, medicinal plants have played an important role in the socio-cultural and therapeutic need of people. Some of these plants are used as spices and food. Medicinal plants being as a key natural resource and potentially secure drugs are contributing a significant role in mollifying human health by contributing nil side effects herbal medicines. The high cost of allopathic medicine and their potential side effects, encouraged the people to use the traditional medicine.

The *Ocimum* (Basil) comprises some of the most popular herbs known for aromatic, cuisine, medicinal, ornamental, sacred and several other aesthetic properties in the world. It belongs to the family Lamiaceae, sub family ocimoideae and includes more than 65 different species and varieties distributed in the tropical regions of Asia, Africa, Central and South Africa considered as one of the largest genera of the Lamiaceae family. The name *Tulsi* is derived from 'Sanskrit' which means "matchless one" (Ghosh 19952 and Simpson and Conner 1986)3. In traditional systems of medicine, different parts (leaves, stems, flowers, roots, seeds and even whole plant) of *Ocimum* have been recommended for the treatment of various ailments i.e., bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, chronic fever and insect bite. The essential oil of *Ocimum* spp. were rich in camphor, citral, eugenol, geraniol, linalool, linalyl acetate and methyl chavicol etc., and being harnessed for successful utilization in industry. There is a variation in the production of these products among different species of *Ocimum*. Therefore, precise characterization such as anatomical and morphological features of promising species of *Ocimum* is felt necessary.

Standardization of herbal drugs is pre-requisite as per global market of herbal medicine. Morphological and anatomical characters play a vital role in crude drug standardization (Agarwal *et al*., 2013)4. Anatomical characterization is important taxonomic parameters for the certification and quality control of medicinal plants for comparison and correct identification. An investigation of anatomical structures of the species organs is necessary (Sinnott, 1960)5. The present study therefore provides an anatomical characterization of the stems of four species of *Ocimum* of industrial importance.

The morpho-anatomical structure may be directly affected by variations in the environmental conditions to which plants are exposed during development. The morphological variations help in understanding the genotypic difference in the species and population studied. In addition, such studies on morpho-physiological, anatomical and genetic aspects of commercial and industrial crop and endangered species are essential to develop successful standardization or quality parameter and conservation strategies respectively (Ojha, 2014)6. The morphological and anatomical account of a medicinal plant is the first step towards establishing the correct identity and the degree of purity of such materials.

MATERIAL AND METHODS:

The plant selected for the present study such as *Ocimum basilicum* var. *thyrsiflora* (L.) Benth. (EC 387839), *Ocimum tenuiflorum* L. (NRO 42.), *Ocimum gratissimum* L. (SKV/RRA/AK/1850) and *Ocimum virdi* Willd. (NRT 04) belongs to the family Lamiaceae. The field experiment was conducted in the experimental farm field of ICAR-NBPGR, Regional Station-Bhowali, Niglat, Distt- Nainital($29^0$20´N latitudes, $79^0$30´E longitudes) at an altitude of 1450 m in two consecutive year 2014-15, using ABD(Augmented Blocks Design) method in three rows (8 Plants in each row). The type of soil was grey-brown. Seeds were sown in the greenhouse in the first week of March and the seedlings were planted in the field beds of size 2.0 X 1.2 m; distance plant to plant 25 cm and row to row distance 60 cm in the last week of April. Plants at flowering stage were used for morphological characterization by randomly selected 5 plants of each accessions or variety. Minimum and maximum temperature from March to Sept 5.37^0C to 18.20^0C and 17.87^0C to 29.37^0C with average rainfall 228 mm in 2014 and 5.20^0C to 18.32^0C and 16.83^0C to 28.58^0C with 229 mm of rainfall in 2015. Irrigation was provided on daily basis up to 15 days after transplanting and establishment of plant population. From time to time irrigation and in cultural practices were coined out. The quantitative and qualitative characters were measured before harvesting. In order to document the morphological, agro- botanical and economic traits, the standard descriptors was used (Singh *et al*., 2013)7.

For morphological characterization colour of aerial parts, odour, taste, size and shape of lamina, lamina margin, plant height, stem colour, flower colour, seed shape, seed colour, 1000-seed weight and plant canopy were recorded. For anatomical investigation the fresh or preserved (in FAA) materials were taken for microscopic studies. Middle part of plants stem was taken for the anatomical studies. Section taken by moving the blade back and forth and placed in watch glass containing water. Thin section were selected and placed in chloral hydrates and cleared by boiling. Cleaned sections were stained by using Phloro-glucinol and concentrated hydrochloric acid. One thin section was taken out and mounted on a clean glass slide. A drop of glycerin was added and covered with the cover slip. This slide was observed under microscope and photomicrographs of with different magnifications were taken with Olympus CX 31 Lica ATC 2000 (digit 3) microscopic unit.

RESULTS

Morphology:

The four species of *Ocimum* were shown a great variation of phenotypic characters like lamina length of *O. gratissimum* (12.4 cm), *O. tenuiflorum* (4.3 cm), *O.b*. var *thyrsiflora* (5.7 cm) and *O. viride* (5.6 cm). Lamina shape is sub-ovate in *O.b*. var *thyrsiflora*, *O. tenuiflorum*; ovate in *O. gratissimum* and *O. viride*. Lamina margin is sub-serrate in *O.b.* var *thyrsiflora* and *O. tenuiflorum* while serrate in *O. gratissimum* and *O. viride*. The plant height varies from 51.46 cm to 102.6 cm (Fig:-1). The smallest plant population of *O. tenuiflorum* and the tallest plants of *O. gratssimum* were observed. The plant canopy was ranged from 692.24 cm^2 of *O. tenuiflorum* to 3843.94 cm^2 of *O. viride* (Fig:-1.1). Stem colour is light green in *O.b.* var. *thyrsiflora*, purple green in *O. tenuiflorum*, dark brown in *O. gratissimum* and dark green in *O. viride*. Flower colour sowed a great variations among these four species of *Ocimum* i.e., *O.b*. var. *thyrsiflora* (white), *O. tenuiflorum* (purple white), *O. gratissimum* (creamy white) and *O. viride* (pink purple). Seed shape of *O.b*. var *thyrsiflora* was ellipsoid and globose in rest three species of *Ocimum* was recorded. Seed colour is black in *O.b.* var. *thyrsiflora* and *O. viride* and brown in *O. gratissimum* and *O. tenuiflorum.* 1000-seed weight ranges from 0.30 to 0.90 g, bold and high weight seed found in *O. gratissimum* (0.90 g), *O.b*. var. *thyrsiflora* (0.80 g), *O. viride* (0.50 g) and *O. tenuiflorum* (0.30 g) (Table 1).

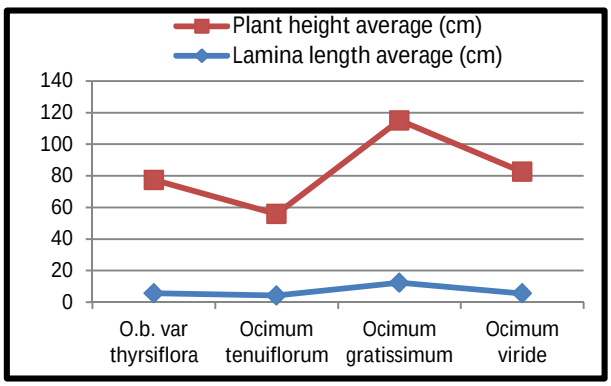

Figure 1: Quantitative characters on the basis of Average value of four species of *Ocimum*.

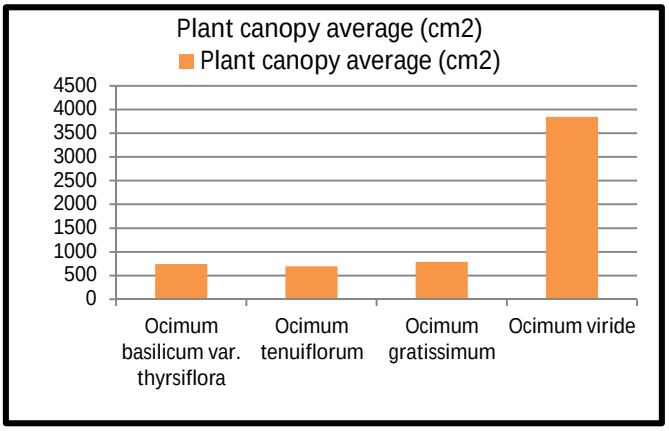

Figure 2: Plant canopy (cm^2) of four species of *Ocimum*.

Table 1: Morphological features of the four species of *Ocimum*

Parameter	Species of *Ocimum*			
	***Ocimum basilicum* var. *thyrsiflora* (L.) Benth. (Thai basil)**	***Ocimum tenuiflorum* L. green type (Holy or sacred basil)**	***Ocimum gratissimum* L. Clove basil**	***Ocimum viride* Willd. Fever plant of Sierre Leone**
Odour	Clove like scent	Sweet, green herbaceous top noted and appreciable note of cloves under tones.	Strongly scented than other varieties or species	Highly scented, district
Taste	No pungent, Soft	Light pungent, light bitter	Clove-mint type, strong pungent	Sweet, light pungent and betel like taste
Lamina length (cm)	5.7	4.3	12.4	5.6
shape of lamina	Sub-ovate	Sub-ovate	Ovate-lanceolate	Ovate
Lamina margin	Sub serrate	Sub serrate	Serrate	Serrate
Plant height (cm)	71.68	51.46	102.6	76.9
Stem colour	Light green	Purple green	Dark brown	Dark green
Flower colour	White	Purple white	Cream colour	Pink purple
Seed shape	Ellipsoid	Globose	Globose	Globose
Seed colour	Black	Brown	Brown	Black
1000-Seed weight average	0.80	0.30	0.90	0.50
Plant canopy average (cm^2)	741.20	692.24	787.53	3843.94

Anatomy: I. *Ocimum basilicum* var *thyrsiflora* L.: The stem has a primary structure only in the upper third part, and a secondary structure in the other two. The epidermis layer is uniseriate, and has been covered by a thin cuticle layer. The parenchymatous cortex is collenchymatic in a hypodermal position. The large bundles have radial ranges of ligneous vessels separated by uni- or multi-seriate areas of parenchymatous cells. The sclerenchyma and fibers, at the end of the large phloem vascular bundles, have less thickened but still cellulosic walls, which will be thicker and lignified from the base to the top of the stem. The number of glandular and tectorial trichomes per surface unit is decreased from the base to the top of the stem. The tectorial trichomes are uniseriate, consisting of three cells, with an acute apex and a bi-or multicellular basis {Fig 4 (a)}.

Figure 3: Plant population of Four species of *Ocimum*

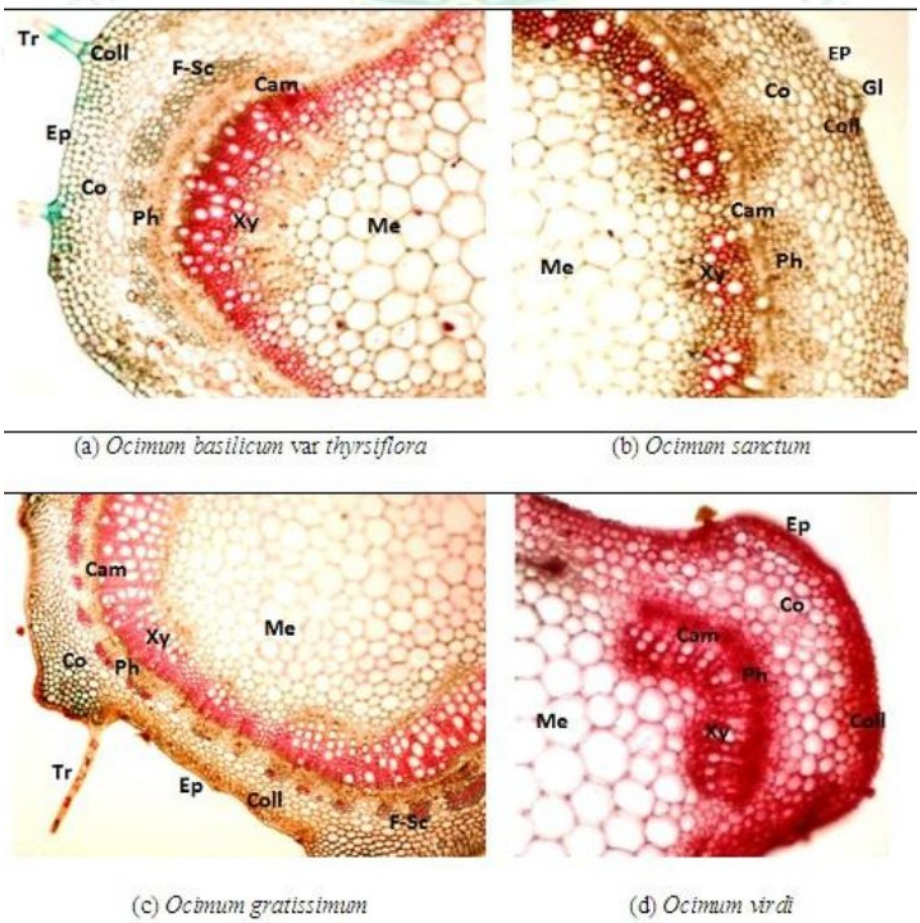

Figure 4: Stem anatomy of Four species of *Ocimum*

Abbreviations: *Ep: Epidermis, Coll: Collenchyma, Co: Cortex, F-Sc: Fiber- Sclerenchyma, Ph: Phloem, Xy: Xylem, Me: Medulla, Gl: Glandular trichome, Tr: Tectorial Trichome.*

II. *Ocimum tenuiflorum* L.: 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. Xylem (Xy) is without fiber tracheid with libriform fibers. Pith (M) in centre consists of lignified parenchymatous cells. sclerenchyma and fibers, at the end of the large phloem vascular bundles are present. Scleranchymatous tissue surrounds the phloem group of vascular bundles, as can be seen. Secondary thickening develops from cambial activity producing thin phloem ring towards the exterior and thicker xylem ring on the interior. Xylem is without fiber tracheid with libriform fibers {Fig 4 (b)}.

III. *Ocimum gratissimum* L.: The cross section of hexagonal stem {Fig 4 (c)} has 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 and cellulose. The cortical parenchyma consists of several layers of polygonal cells with thin walls. The central cylinder is more developed than the bark. A fundamental parenchyma is observed in which differs from primary tissues (wood, phloem parenchyma) and two secondary tissues (wood and secondary phloem). The primary wood is centrifuged and the secondary phloem and secondary wood are arranged in radial alignment. sclerenchyma and fibers, at the end of the large phloem vascular bundles are present. Scleranchymatous tissue surrounds the phloem group of vascular bundles, as can be seen. The narrow parenchyma includes meatus and is formed of large polygonal cells cellulose walls {Fig 4 (c)}.

IV. *O. virdi* Willd. : 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. Xylem (Xy) is without fibre tracheid with libriform fibres. Pith (M) in centre consists of lignified parenchymatous cells. Pith in the centre consists of lignified parenchymatous cells {Fig 4 (d)}.

DISCUSSION:

These four species of *Ocimum* showed a great variability in morphology but anatomically the variations is not very fine. Earlier studies confirmed great morphological variability available in *Ocimum basilicum* (Nurzynska-Wierdak, 20138; Negi *et al*, 20159; Rawat *et al*., 2016)[10]. Morphological study of *O. gratissimum* was done by Chirstian 2012[11], Prabhu *et al*., 2009[12]. Morphological variability in holy basil was earlier reported by Malav *et al*., 2015[13]. The quadrangular transaction is frequently described for Lamiaceae (Metcalf and Chalk, 1988[14]; Barroso, 1991)[15], as well as the evident collenchymas in the four angles (Cronquist, 1981)[16], which is considered of diagnostic value according to Metcalf and Chalk 1988[14]. Metcalf and Chalk (1972)[17] also determined some scleranchymatous tissue surrounds the phloem groups of vascular bundles, as can be seen the same in the two species of basil (*Ocimum basilicum* var. *thyrsiflora* and *Ocimum gratissimum*). The sclerenchyma is the predominant support tissue for species that grown in dry environments as recorded by Faria (2008)[18]. Pharmacognostical characterization of *Ocimum tenuiflorum* and *Ocimum gratissimum* is earlier done by (Agarwal *et al*., 20134; Fofie *et al*., 2014[19])

CONCLUSION:

Plant morphology is study of the physical form and external structure of plants. It represents the study of the development, form, and structure of plants and origin (Harold et al., 1987)[20]. The present study will help to maintain proper standards of assigned medicinal plants and may result to check other adulterated mixture of fake crude drugs. This will also assist to maintain their uniqueness of well known ancient system of medicines. Thus in recent years there has been an emphasis in pharmacognostical standardization of medicinal plants of therapeutic potential.

Anatomical characters of diagnostic value in four species of basil were recorded in the present study. This information is important, as it helps in the identification of these species and contributes to its quality control, and evaluation. This investigation helps to differentiate from the closely related other species and varieties of *Ocimum*.

REFERENCES:

1. Nathiya S, Santhi N and Kalaiselvi S, A comparative study on ontogenic expression of antioxidants and secondary metabolites in *Withania somnifera*: *Int. Res. J. Pharm*., 2012, 3(1), 2010-1015.
2. Ghosh GR Tulsi (N.O. Labiatate, Genus- *Ocimum*), New Approaches to Medicine and Health. NAMAH., 1995, 3, 23-29.
3. Simpson BB and Conner OM, Economic Botany Plants in Our World. McGraw Hill Book Company, Hamburg, 1986.
4. Agarwal S, Kumar VR and Kumar A, Pharmacognostical Characterization of *Ocimum* spp.: *Journal of* Pharmacognosy *and Phytochemsistry*, 2013, 2(1), 219-224.
5. Sinnot EW, Plant Morphogenesis, MCGraw-Hill Co, New York, USA, 1960.
6. Ojha SN, Studies on Ecological, Taxonomic, Ethnobotanical and Chemotypic Variation in 'Oregano' (*Origanum vulgare* L.) and Prospectus of its Cultivation. Ph.D. dissertation. Kumaun University, Nainital, 2014.
7. Singh BM, Mahajan, RK, Srivastva, U and Pareek, SK,. Minimal Descriptor of Agri- Horticultural Crops, Part-IV: Medicinal and Aromatic Plants, ICAR-NBPGR, Pusa, New Delhi, 2013.

8. Nurzynska-Wierdak, R, Analiza zawartoscii skladu chemicznego olejku dwoch form bazylii wonnej (*Ocimum basilicum* L.). Ann. UMCS, EEE, IX Suppl., 2001, 189-193.
9. Negi K.S, Ojha SN, Koranga SS, Rayal A and Mehta PS, Sweet basil ke Saundrya Udyog mein badti mang: *Kheti*, 2015, 66(10), 18-22.
10. Rawat Rajni, Negi, KS, Mehta, PS, Tiwari Vandana, Verma SK and Bisht IS, Study of six varieties of sweet basil (*Ocimum basilicum* L.) and their morphological variations: *J. Non- Timber For. Prodts*, 2016, 23(1), 17-22.
11. Christian OA, Agro-morphological variability of *Ocimum gratissimum* L. and three other accessions of basil in south-western Nigeria. *The African J. of Plant Sci. and Biotech.* 2012, 7(1), 89-92.
12. Prabhu KS, Lobo R, Shirwaikar AA and Shirwaikar A, *Ocimum gratissimum*: A Review of its Chemical, Pharmacological and Ethnomedicinal Properties: *The Open Complementary Medicine Journal*, 2009, 1, 1-15.
13. Malav P, Pandey A, Bhatt KC, Krishnan, S and Bisht IS, Morphological variability in holy basil (*Ocimum tenuiflorum* L.) from India. *Genet Resour Crop Evol.*, 2015, 62, 1245–1256.
14. Metcalfe CR and Chalk L, Anatomy of Dicotyledons. Vol II. Clarendon Press, Oxford, 1988.
15. Barroso GM, *Sistematica de angiospermas do Brasil*. Vicaso, Universidade Federal de Vicaso, 1991.
16. Cronquist A, An Integrated System of Classification of Flowering Plant. Columbia University Press, New York, USA; 1981.
17. Metcalfe CR and Chalk L, Anatomy of Dicotyledons. Vol I. Clarendon Press, Oxford; 1972.
18. Faria MT,.Morfologia,anatomia, histoquimica de especies Dogenero hypenia Mart. ex Benth. In: Lamiaceae Ocorrentes no Cerrodo do Goias. Dissertacao de Mestrado [Harley R (ed.)], programa de pos-graduacao em Botanica, Universidade Federal de Goias; 2008.
19. Fofie NBY, Coulibaly K and Kone BD, Phamacognostic study of *Ocimum gratissimum* Linn.: Pharmafooe plant. *J.Pharmacognosy and Phytochemistry*, 2014, 2(5), 74-79.
20. Harold CB, Alexopoulos CJ and Delevoryas T. Morphology of Plants and Fungi. 5th ed. Harper-Collins, New York; 1987.