





A taxonomic review of the genus Ocimum L. (Ocimeae, Lamiaceae)

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Abstract

The plants of the genus Ocimum L. are rejuvenating herbs due to their aesthetic and magnanimous healing nature. Ocimum L. is a pivot of modern research such as phytochemistry, molecular and medicinal biology. However, there needs to be more taxonomic interpretation and identification of the genus, summing up the need for our study. The morphological discrepancies and nomenclature of Ocimum L. are jumbled up, the same species has been named multiple times by multiple authors. It culminates in erroneous evaluation for taxonomy-based research. Our indignation highlights the taxonomic alterations and modifications that Ocimum L. has undergone since primeval period. The genus's classical to the modern taxonomic transition has not been studied hitherto. The aim is to provide precise information on the antecedent and contemporary stature of Ocimum L. The present study attempts to provide taxonomic clarity for a highly economic genus in order to enhance the commercial perspective and efficiency of selective breeding practices. The present study emphasizes identifying samples with their type, protologue, and herbarium specimen and extricate between ecotype, morphotype, and chemotypes. Molecular taxonomy can provide better insights and transparency. But for a better exposition, such studies must revolve around correctly identified species. The selection of representatives from different populations of the same species can help understand the consistency of vegetative and reproductive characteristics. In this review, few corrections have been provided for misidentified or mistaken species. The species with incorrect nomenclature has been identified and corrections have been suggested. For a better understanding, habit photographs of Indian Ocimum L. have been furnished and morphological features (vegetative and reproductive) for proper diagnosis and correlation has been provided. Specimen examination either through physical or digital herbaria is an obligatory criterion for an error-free taxonomic inquest. Our findings will help to achieve accuracy while interpreting the genus and its species. The retrospection will aid in floristic, biodiversity, taxonomic, phylogenetic, biochemistry and molecular systematic related works. The observations will apprehend the existing gap concerning the taxonomy of Ocimum L.

Keywords

Ocimum; identification; morphology; molecular; taxonomy

Introduction

The 'Empress of all herbs' Basil is known as Tulsi or Tulasi in Hindi, an incarnation of Hindu goddess Lakshmi. The term 'Basil' descended from the Greek word 'Basileus/Basilikos,' which means princely or Royal. The plants

can be given in Kings Hand or Royal House because of their astounding fragrance (1). Basil is a term that manifests all the aromatic members of Ocimum L. The genus 'Ocimum' signifies fragrant-lipped, a term procured from 'okimon' and 'ozein,' which purports the herbs having smell or odor. 'Ocimum' is a Latinized term derived from the ancient Greek word ' Ω κἰμον' (Okimon) owing to an ending 'um,' which implies neuter genera. The earliest reference to ' Ω κἰμον' was put forward by Theophrastus (2). The genus erstwhile named Basil (2), Okiminon / Okimon / Akinos / Okimoides / Okimone / Ocimastrum (3), Ocymum (1,4-7), Ocimum (4,5,6), Ozymum (7), Ocymi / Ocymo (1,8), Basilic (9,10), Ocimon / Okimon (11), Ozymum / Ozimum (12),

Ocimum L. is native to warm tropical and temperate regions. The primary centre of origin is Africa (most species are confined to this continent), Tropical Asia, Central and South America, while the secondary origin is India (13). Ocimum L. is chiefly an East Indian genus (6). It is an indigenous genus of India, and its species are disbursed throughout the country. Ocimum L. belongs to the tribe Ocimeae Dumort., subfamily Nepetoideae (Dumortier) Luersson, the largest group of the mint family Lamiaceae Martinov. The tribe Ocimeae Dumort. is the most speciose group incorporating nine genera distributed in the tropics and sub-tropics. Ocimum L. is the type genus of Ocimeae, specified with concave compressed corolla lip, declinate stamens, synthecous anthers, and explosively releasing pollen (14). Dumortier first traced Ocymeae (=Ocimeae); subsequently, Bentham described it as Ocimoideae (Ocymoideae). As the name doesn't accord with ICBN, it was rectified into Ocimeae. Briquet carved Ocimeae (Ocimoideae) into three subtribes Hyptidinae, Moschosminae (=Ociminae), and Plectranthinae (15). Ocimeae Dumort. was split into sub-tribe Hyptidinae, Hanceolinae, and Ociminae (including Basilicum, Orthosiphon, Ocimum, and Platostoma) (16). The sub-tribe Ociminae (Dumort.) Schmidt was first introduced by Ryding while studying the pericarp structure of Ocimeae Dumort. where sub-tribe Ociminae split up into three informal groups. The Ocimum group holds Ocimum L. with Becium Lindl., Erythrochlamys Gürke, Hemizygia (Benth.) Briq. and Syncolostemon E. Mey. ex Benth. (17).

Numerous nomenclature and classification disputes have muddled up the genus. Furthermore, we have perceived the same while examining the pieces of literature. The complexity and confusion are due to polyploidy and intra and inter-specific hybridization. It resulted in plants with vast morphological variations, leading the way for many subspecies, varieties, and form. Such forms have created uncertainty, accentuating the need to revise the taxonomy (18). Due to immense morphological variability, the genus is convoluted regarding phylogenetic relationships. The tribe Ocimeae, the largest group in Lamiaceae in South East (S.E) Asia, consists of nine genera and the tribe badly needs revision (16). The taxonomic complexity has been studied by Paton (19), Khosla (20), and Grayer et al. (21). The Indian literature furnishing the taxonomy of Ocimum L. based on cytology and phytochemistry is somewhat complicated (20). A taxonomy and phylogeny study reported seven species of *Ocimum* L. in India (22). Their description as a taxonomic revision of *Ocimum* L. must be more supportive. A revision needs rigorous and extensive analysis of a taxon, herbarium specimen analysis, interpretations, corrections, historical account, and developments. Few exemplary taxonomic works have been put through, a synopsis (19) and a revision of Ocimeae in S.E Asia (16). In India, no such taxonomic study has been accomplished for *Ocimum* L.

Ocimum L. constitutes annual herbaceous, fragrant members and a few perennial shrubs. The species of Ocimum L. are widely cultivated and naturalized in open or disturbed areas, roadside, and forest areas. Few species are cultivated commercially and kept as pot herbs in homes and gardens. The Basil is characterized by the presence of the upper lobe of the calyx, which is large and decurrent. The plants in the genus are sweet-scented herbs or shrubs. Stem – quadrangular; Leaves – spreading, green or purplish green; Inflorescence - dense or lax; Bracts ovate or obovate, sessile or subsessile; Flowers - verticillate spiked, terminal or axillary; Pedicel -slender, pubescent, same or longer than calyx; Calyx – two lipped, upper one wider, rounded and lower lip four toothed; Corolla tube short, two-lipped, superior lip equally four fids, inferior lip longer; Corolla-crimson, pinkish white or white, purple white, lobes pubescent on back, posterior lip with two obovate oblong median lobes slightly longer or equal than two oblong lateral lobes, anterior lip obovate oblong; Stamens - four, filaments declinate, out of which two are shorter; stamens posterior pair may have a small tuft of hairs at base; Gynoecium – style with two sub equal lobes; nutlets oblong, smooth or rough, may or may not be mucilaginous when wet. (Fig. 1).

The taxonomic study of *Ocimum* L. needs to be explored more. The present study is an attempt to surmount the drawback. The work enumerates the correct taxonomic knowledge and identification of the *Ocimum* L. species. Accurate identification will help in applied research to get more authenticated results while implementing *Ocimum* L. analysis. The notes obtained from the literature were framed for reviewing the taxonomic status of the genus with a more conclusive and precise outcome. Our work is an effort to streamline the taxonomical ramifications of *Ocimum* L.

Materials and Methods

The review has been done using web sources of BHL, Google Books, Internet Archive, JSTOR, NCBI, PubMed, Science Direct, Scopus, Smithsonian Library, Springer, Web of Science, etc. We have also gathered information from relevant literature (Floras, Journals, Records, Revisions) available at BSI, Shillong. The classical pieces of literature incorporated in the citation of homotypic and heterotypic synonyms of *Ocimum* L. were examined. The research articles primarily searched for review, taxonomy, revision, and floras executing *Ocimum* L. The inputs were retrieved using search terms/keywords such as Basil, Basil in India, Basil and its uses, diversity of tulsi, *Ocimum* species,

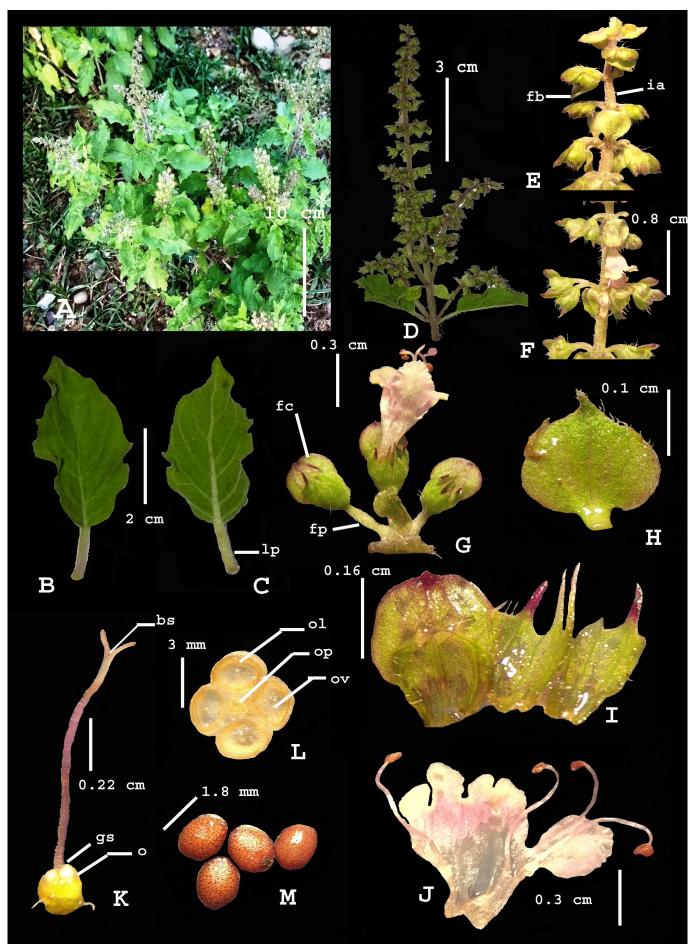


Fig. 1. Representative of Ocimum L. (O. tenuiflorum L.) (A) habit (B) adaxial view of leaf (C) abaxial view of leaf (D) inflorescence (E) inflorescence tip (ia-inflorescence axis; fb-flower bract) (F) closure view of inflorescence showing floral arrangement (G) complete flower along with fruiting calyx (fc) and flower pedicel (fp) (H) bract I calyx (J) corolla showing epipetalous stamen (K) gynoecium exhibiting gynobasic style (gs), bifid stigma (bs) and ovary (o) (L) ovary T.S revealing ovules (ov), ovary locule (ol) and placenta (op) (M) nutlets. ©Mamita Kalita

Ocimum research articles, marker-based study in Ocimum

Ocimum, molecular taxonomy of basil, taxonomic revision, morphology and taxonomy of Ocimum, etc. Only the significant, definitive, and peer-reviewed research articles on Ocimum L. were included in our analysis. The poor research findings and contradictory records were excluded, along with data obtained from dubious and predatory journals. All the relevant facts and particulars of Ocimum L. were acquired, analyzed, and reviewed under five sections: The initial system of classification includes the early position of the genus between the 16th to 19th centuries; the second section describes the classification of the phylogenetic system followed by the ongoing status of the genera. The fourth and fifth sections included the documentation on Indian Ocimum L. and current taxonomic inquests, respectively.

The photographs of six *Ocimum* L. species were captured using a digital camera (Fig. 2). The habit, vegetative and floral characteristics were studied by alluring a representative of the genus (*O. tenuiflorum* L.), collected during the flowering and fruiting phase (Fig. 1). The micromorphological features were investigated using a Labomed CZM4 stereo zoom binocular microscope, and photo plates were prepared using Adobe Photoshop 7.0. The *Ocimum* L. representatives were analyzed with their type specimens, consulting regional and national herbaria, such as ARUN, ASSAM, CAL (acronyms given by the Botani-

cal Survey of India) and GUBH. The microfilms of herbarium specimens from online databases BSI-IVH, JSTOR, KEW, G, MNHN, MO, and NY were also consulted for correct interpretations and conclusions.

Results

The initial system of classification

In the artificial (arbitrary) system of classification, Theophrastus tells of Basil (O. basilicum) as herbaceous plants with woody roots, flowering for a long duration (2). According to Pliny, the Elder Ocimum must be avoided as it is detrimental to human health, similar to the belief of Chrysippus. He mentioned around 35 remedies of Ocimum used by people in succeeding ages (23). Dioscorides sketched out Ocimum (Basil) in aromatics (Okiminon), living creatures (Okimon), roots (Akinos), and other herbs and roots in Okimoides (Ocimoideae). Four species exist in Okiminon and Okimone; one in Akinos; two in Okimoides. The Akinos and Okimoides are also familiar as Ocimastrum (a name given by Romans) (3). Casper Bauhin cited Ocimum as Okimon Dioscoridi, a more than hundred-year-old odoriferous genera with 11 species. The species differ in leaf size, aroma, and corolla color. The 11 species and their synonyms were named as per the binomial system of nomenclature (24). Andrea Caesalpino kept Ocymum inside Ocimoides, which, in turn, contained herbs and suffruticose



Fig. 2. Ocimum L. species distributed in India (A) O. africanum Lour. (B) O. kilimandscharicum Gürke (C) O. americanum L. (D) O. tenuiflorum L. (E) O. gratissimum L. (F) O. basilicum L. (G) O. filamentosum Forssk

Source: (A-F) @Mamita Kalita; (G) Reproduced with permission from @Swarochi Tathagath (iNaturalist photo 163564483)

(perennial herbs having a woody base) (4). John Ray tells of Ocimum/Ocymum (De Ocymo-Basil) as plants with spiked spiral flower arrangements (Verticillatis). While describing the transformation of species into plants, he brought up Ocimum into Serpyllum. He has also, mentioned five species, O. magnum, O vulgatius, O minimum, O. indicum, O. minus angustifolium foliis serratis (polynomial name) in Floriferae of Herbaea (Herbaceous plants) (1). Tournefort availed only corolla features, construe Ocimum (Basilic) as single petal flower (labiate) with upper corolla erect and lower curly. He set the genus in 4th class De herbis & suffruticibus, flore monopetalo, labiato (single petal, lipped flower, herbs and suffruticose in habit) of group Simplices Monopetali, Petalodes within Herbae (9). In his Biblotheca Botanica, Linnaeus appraises Dioscorides and Theophrastus as exceptional Phytologists. In 1742, Linnaeus classified Ocymum in the 14th class Didynamia (four stamens in pairs of unequal length), order Gymnospermia (5), besides other Labiatae Juss. genera. (=Lamiaceae, ICN 2017, Article 18.5). Later, he placed the genus Ocymum in Verticillatae. He considered the basal tooth region of upper stamina (stamens) as an essential and universal characteristic of Ocymum L. He depicted five species (O. frutescens, O. basilicum, O. minimum, O. tenuiflorum, O. menthoides) (25). Rumphius described three Ocimum (=Ozimum) species as Basilicum agreste, B. indicum, and Ozimum citratum (12). A Commentary by Linnaeus (8), comprehends B. agreste as Ocymum gratissimum, B. indicum as Ocymum, Ozymum citratum as O. tenuiflorum, and Majana rubra as O. frutescens. Majana rubra was erroneously reduced by Linnaeus to O. frutescens. While recognizing the error, Linnaeus made Rumphian description and figure as a fundamental basis of O. scutellarioides L., the base of Coleus scutellarioides Benth. He further described Ocimum as a genus having two-lipped calyx, i.e., Calyces bilabiati (26). B. agreste Rumph. (as incarnated in Herbarium Amboinense) reduced as Ocimum tenuiflorum, supplementing three additional species as O. gratissimum, O. americanum, and O. scutellarioides (27).

In light of the natural system of classification (based on natural affinities), Michael Adanson emphasized floral characteristics, raising Ocimum Tourn. (vertical spike flower, calyx five-toothed) as Ocimon Lat., Okimon Ippokr., Basilic Gall. (Lat-Latin; Ippokr-Hippokrate and his work; Gall-Barrelier and his work) (11). A.L De Jussieu placed Ocimum L. (Basilic) in the fourth category of 6th order Labiatae, characterized by bilabiate calyx, bilobed corolla, and four fertile stamens. Labiatae, in turn, placed in the 8th class (Corolla hypogyna) of Monopetalae amongst Dicotyledonae (28). A.P. de Candolle allocates Labiatae in Irregularies (unequal or dissimilar lobes) of Gamopetales with lobes arranged in valves or lips (29). Lamarck epitomizes 21 species of Ocymum as BASILIC (10) and, together with Candolle, gave an account of BASILIC (30). They put forth three species, Ocymum basilicum as Basilic commum, O. bullatum as B. crepu, and O. minimum as B. nain. Ocimum L. was placed in the subtribe *Euocimeae* of the tribe *Ocimoideae* (31,32). Ocimum L., held in tribe Ocimoideae (having declinate stamens) under 150th order Labiatae, was quartered into four sections based on filament features, Ocimodon Benth. (19 species), Hemizygia Benth. (15 species), Hierocimum Benth. (11 species), and Gymnocimum Benth. (08 species) along with 43 species placed in Species exclusae, a list of species excluded from *Ocimum* L. and identified as different species (33). Stephan Endlicher located Ocimum L. in sub-tribe Moschosmeae (corolla subequal, lowest one declinate, narrower and flat), tribe Ocimoideae Benth. (declinate stamens, lobes of two or four upper planes are subequal) in order Labiatae, cohort Gamopetalae of fourth section Acramphibrya (peripheral terminal vegetation) (34). Bentham and Hooker placed deflexed fruiting calyx Ocimum L. in the subtribe Euocimeae (four sub-equal corolla lobes, calyx lobe or posterior tooth is wider than rest) of tribe Ocimoideae (four perfect declinate stamens, rarely two). The tribe was set in Labiatae of series Bicarpellatae (hypogynous, stamens as many as corolla lobes), sub-class Gamopetalae (united petals), class Dicotyledones (two cotyledons, pentamerous or tetramerous flowers) of Phanerogams (31).

Bentham's first remarkable botanical contribution relates to 11 species in *Labiatae* genera in the *Ocymum* section. He considered the decurrent margins of the calyx's upper tooth and the corolla's flat lower lip as distinguishing characteristics of the genus. Moreover, he recognized nine *O. basilicum* L. varieties for the first time. Bentham suggested considering them as varieties originating in gardens, which might have arisen from two wild species, *O. basilicum* var. *pilosum* and *O. basilicum* var. *glabratum* (6). Later, he segregated the tribe *Ocymoideae* into three sections, *Ocymodon* Benth., *Hierocymum* Benth., and *Gymnocymum* Benth. The tribe *Ocimoideae* was first published in the article Labiatae and later carved into the sub-section *Euocimeae* and *Lavanduleae* (31).

Ocimum L. was erroneously considered Becium Lindl., Erythrochlamys Gürke., Hyperaspis Briq., and Nautochilus Bremek. Becium Lindl. set out by Lindley (35) contains 35 species. He appraises the dissimilarity of Becium Lindl. from other Ocymoideae genera. Engler brought Erythrochlamys Gürke as a new genus (36). Hyperaspis gen. nov. was first encountered by Briquet together with one species (37). He suggested that Hyperaspis Briq. differ from all other members of Ocimoideae, occupying the group of Ocimoideae-Moschosminae, a special place with marked affinities for Erythrochlamys Gürke and Ocimum L.

Ocimum L. in light of the phylogenetic system of classifi-

A.W. Eichler developed the first evolutionary or phylogenetic system. He cited *O. basilicum* (kitchen and oil perfumery usage) in series Labiatiflorae (didynamous stamens), situated in Sympetalae (fused petals), class Dicotyleae (dicots), division Angiospermae of sub kingdom Phanerogamae (seed plants). His classification scheme (38) became the foundation for the Engler system. Engler and Prantl relate *Ocimum* L. including *Becium* (einschl. Becium Lindl.) in Ocimoideae-Moschosminae, sub-class Metachlamydeae (Sympeatalae) segregating from genus *Hemizygia* Briq. and *Erythrochlamys* Gürke. They cleave

Ocimum L. into three sections Ocimodon Benth. (rear stamens with an appendage at base), Hierocimum Benth. (tufts of hair present and without appendage at the bottom), and Gymnocimum Benth. (bare without appendage). They further split Sect. Ocimodon Benth. into Basilica Briq., Gratissima Benth. and Hiantia Benth. based on calyx features (39).

Bessey's classification (40) was modern, revised, and developed on Benthamian classification. Lamiales were held in super-order Strobiloideae-Sympetalae-Polycarpellatae (carpels many, petals united, actinomorphic), class Oppositifolia (Dicotyledoneae) of phylum Anthophyta (flowering plants). He stated the origin of Oppositifolia from Cycadean (gymnosperm), which orderly evolved into progressive groups. The former has sequentially evolved into syncarps to polycarpellate to Dicarpellate, having Gentianales, Polemoniales, Scrophulariales, and Lamiales (characterized by strobiloid flower). The strobiloid attribute was also recognized by Hans Hallier. His classification of phylogeny was parallel to the Besseyan system, both adhering to the monophyletic origin of phyla (41). Hutchinson arranged families of flowering plants based on probable phylogeny placing Ocimum L. (odoriferous, herbaceous, and rarely woody) in the last family Labiatae of division Herbacea (annual, biennial, perennial herb or shrubs derived from herbs), sub-phylum Dicotyledons and phylum Angiospermae (42). Takhtajan carves Ocimum L. including Becium Lindl., Erythrochlamys Gürke. and Nautochilus Bremek. in subtribe Ocimeae, tribe Nepetoideae, super-order Lamianae (also called Lamiiflorae), sub-class Lamiidae together with Lamiales and Lamiaceae kept in Magnoliopsida (dicot flowering plants) (43). Arthur Cronquist propounds a similar scheme of the Takhtajan classification. He advocated parallel evolution in the gynoecium of Lamiales, an order allied to Solanales and Gentianales. Lamiales was retained in the sub-class Asteridae, which he communed as the most advance and recently evolved. Most of the Sympetalae members of the Engler system belong to the Asteridae. The evolution corresponds with the evolution of pollinating agents such as insects. Lamiaceae of Asteridae is an advanced family arranged along with three other families (Boraginaceae, Lennoaceae, and Verbenaceae) (44).

The genus is monophyletic in origin (45) if the restrained genera Becium Lindl. and Erythrochlamys Gürke are included within it. Ocimeae Dumort. is monophyletic, which embraces monophyletic subtribes Lavandulinae, Hyptidinae, Ociminae, and Plectranthinae (46). Takhtajan, Cronquist, and Hutchinson had complementary opinions regarding the monophyletic theory of evolution. However, Engler & Prantl's classification system trusted in polyphyletic origin, and Thorne's perception is paraphyletic. In the contemporary world, a more appropriate, stable, and advanced (molecular-based) phylogenetic classification exists, i.e., APG (Angiosperm phylogeny Group) classification, which supports the monophyletic origin of Angiosperms. In APG, Ocimum L. is positioned in sub tribe Ocimeae Dumortier along with eight other genera. The order Lamiales (inclusive of sub-family Nepetoideae, family Lamiaceae) was also treated as monophyletic and placed in Euasterids I of clade Asterids (47). Given APG III, Lamiales was allocated in the clade Lamiids (synonym of Euasterids) of Asterids. Also, it was accustomed to the Takhtajan classification having more admissibility as he favored small and natural groups where characteristics could be easily held up (48). In recent APG IV, Asterids placed in Superasterids with newly introduced and related orders of Lamiales (Icacinales, Metteniusales, Vahliales) (14).

Current status of Ocimum L.

ICN rules have frequently not been applied in naming the species of the genus; the same species has been named numerous times (45). A few taxonomic statements were appended (49) while describing six species of Ocimum L., so the genus is represented as Ocimum L. emend. (ICN Article 47.1). The nomenclature entanglement of the genus is such that 463 names are subjugated in Ocimum L., which includes 291 synonyms, 144 accepted, 18 unchecked, and 10 ambiguous (50). Following the records, there are 118 species of Ocimum L., including 97 accepted and 21 doubtful names (51). Over 2022, the same database counted 117 species of Ocimum L. inclusive of 105 accepted and 12 doubtful. Globally, Ocimum L. is represented by 150 species (52) and 64 species (45). There are 65 truly accepted species, 08 subspecies, and 09 varieties of Ocimum L. distributed pantropically (53). The only vulnerable species is O. fischeri Gürke, and the endangered ones are O. urundense Robyns & Lebrun, according to IUCN red list 2011 and 2016, respectively (54). Considering the tree definition given by IUCN's Global Tree Specialist Group (GTSG), O. ellenbeckii Gürke and O. grandiflorum Lam. are the two tree species of Ocimum L. (55). Ocimum × africanum Lour. is a nothospecies (ICN Article 3.2) resulting from a cross between O. americanum L. (hoary Basil) and O. basilicum L. (sweet Basil). There are three autonyms (ICN Article 26.1) in Ocimum L., O. gratissimum subsp. gratissimum, O. Basilicum var. basilicum, O. gratissimum var. gratissimum). The Ocimum section Hierocymum endure O. tenuiflorum L., and the Ocimum section Ocimum comprises O. americanum L., O. basilicum L., O. forskoelei Benth., and O. kilimandscharicum Gürke. The former section has been referred to as the Sanctum group (56) and the latter as the Basilicum group (20). Such infrageneric classification delineating Ocimum L. into Basilicum and Sanctum groups is frequently used in economic and industrial literature (52,57). Such a classificatory system should not be used as a standard as the classification does not comply with ICN (45).

The type specimen of *Ocimum* L. is Lectotype (LT): *Ocimum basilicum* L., designated by N. L. Britton et Millspaugh, Bahama Fl. 380. 26. 1920. The Indian subcontinent is epitomized by seven species, two sub-species, and four varieties. The species are: *Ocimum africanum* Lour. (specific epithet *africanum* refers to its wide distribution in the continent of Tropical Africa), *O. americanum* L. (*americanum* signifies the nativity or origin being America), *O. basilicum* L. (*basilicum* and *basilikos* term commend as royal), *O. filamentosum* Forssk. (filament is marked and osum-fully developed) *O. gratissimum* L. (*gratissimum* explains an exaggerated expression of pleasantness due to

the aroma), *O. kilimandscharicum* Gürke ('kili' suggests the species origin from the Kilimanjaro hills of Kenya, and 'scar' means steep rocky eminence or bare region on the sloppy side of the mountain), *O. tenuiflorum* L. (tenuiflorum means the small, slender flowers). The infraspecific taxa of *Ocimum* L. are: two sub-species (*O. gratissimum* L. subsp. *gratissimum*; *O. gratissimum* L. subsp. *iringense* Ayob. ex A.J. Paton) and four varieties (*O. basilicum* L. var. *basilicum*; *O. basilicum* var. *minimum* (L.) Alef.; *O. gratissimum* L. var. *gratissimum*; *O. gratissimum* L. var. *macrophyllum* Briq.)

Documentation of Indian Ocimum L.

Preliminary Documentation

The 18th century Flora on the Indian subcontinent reveals eight species of Ocymum L. (58). O. africanum Lour. was first traced by Loureiro depicted along with O. basilicum L., O. gratissimum, and O. minimum (non L.) Lour. He considered O. minimum L. as Ocymum citratum Rumph. Amb., a species found in India in various forms and habitats (59). Curtis furnished the first illustration of hoary Basil (O. canum), treated distinctly from O. album described by Linnaeus. Also, it is claimed as new and relative to O. sanctum L. and O. tenuiflorum L. from India (60). Wallich traced out East Indian plant species where Ocymum is placed in the tribe Ocymoideae and split into two groups (i) Caule herbacea (stem herbaceous) include O. basilicum, O. canum, and O. thyrsiflorum (ii) Caule fruticoso (stem bushy) covers O. tenuiflorum, O. adscendens, O. gratissimum, and O. diffusum (doubtful as an Orthosiphon). He divided O. basilicum into six races pilosum, majus, glabratum, album, difforme, purpurascens (61). Ocymum caryophyllatum Roxb. (=0. basilicum L.) is native to India. Initially, the seeds were donated by Dr. Rottler along with O. thyrsiflorum Willd. (=O. basilicum L.) (62). The Description of Indian plants recounts 13 Ocymum species inclusive of O. gratissimum L. having a more substantial degree of fragrance but O. thyrsiflorum Willd. was the most fragrant and prettiest among all the Ocymum species of India. In British India, Ocimum L. was delineated by Hooker in the subtribe Euocimeae of tribe Ocimoideae and described five species of Ocimum L. of India, namely O. canum Sims. (=O. americanum L.), O. basilicum L., O. gratissimum L. (having var. suavis), O. adscendens Willd., O. sanctum L., and O. minimum L. as a doubtful species which might be a cultivated form of O. basilicum L. In accord with Hooker, O. sanctum (=0. tenuiflorum L.), the Indian native is doubtfully indigenous (32). The confusion between O. canum Sims. and O. americanum L. emerged due to the type specimens. The lectotype LINN-749.9 is a young representative of O. americanum L. devoid of fruiting calyces and the type of O. canum Sims. is an illustration (60) that does not specify stem pubescence. Prain gave an account of four Ocimum L. species, including Ocimum sanctum, O. gratissimum, O. canum, and O. basilicum, with two varieties (purpurascens and thyrsiflora) based on spike features (63). Cooke identified four species O. sanctum L., O. canum Sims., O. gratissimum L. (doubtfully indigenous), and O. adscendens Willd. in Labiatae (64). He found a cultivated form of O. basilicum L. and var. thyrsiflora Benth. (most important variety for

Bombay presidency). The native plants of the province (India) portrayed four species of *Ocimum* L. as *O. gratissimum* L., *O. basilicum* L., *O. canum* Sims., and *O. sanctum* L. (65). Gamble depicted three varieties of *O. basilicum* L. (var. thyrsiflorum, purpurascens, pilosum Benth.) and four species as *O. gratissimum* L., *O. canum* Sims., *O. sanctum* L., and *O. adscendens* Willd. (66).

Contemporary documentation

The Indian origin, O. tenuiflorum L. (holy Basil), is recognized as African cultivated species (16). The species is incorrectly known as O. sanctum L. (currently synonym) which emulates the vernacular name holy Basil (20). It is considered an environmental weed invasive to India. The Sanskrit authors have distinguished two varieties of holy Basil based on the differences in leaf color. However, they are a cultivar of O. tenuiflorum L. (cv. Radha and cv. Krishna), originating through selective breeding. National Medicinal Plants Board (NMPB 2019) prioritized O. tenuiflorum L. among 32 medicinal plants that need conservation. A similar species in appearance, growth, and habitat is O. kilimandscharicum Gürke (camphor basil). The species is alien to Indian flora. The exotic camphor-yielding plant is found in both wild and cultivated states. The species was imported from India in 1953 and cultivated in Thailand (16). During the physical herbarium visit at CAL, we found a few write-ups in herbarium sheets and by correlating all of them we can conclude that Camphor basil seeds were acquired by BSI, Shillong from Forest Research Institute, Dehradun in 1949 and successfully grown in Batasipur, Rangapahar, Umsaw experimental garden of the North East province. We found that O. kilimandscharicum Gürke herbarium specimens present in ASSAM were prepared either from Nursery or Dehradun during 1949-50. Dr. Janaki Ammal (Director, BSA, during 1952) might have procured the nursery specimen (BSA 4993) from ASSAM. This might be why several documentations has stated its distribution in N.E India. We have discerned no herbarium specimens from CAL and ARUN. The species is not being reported from N.E. India in wild circumstances, as validated by POWO (Plants of the World Online). Another specimen was received by ASSAM herbarium from CDRL (CSIR- Drug Research Laboratory) Jammu & Kashmir on 07-07-1959 (exotic from Kenya); however, it is the deficit of accession number.

O. filamentosum Forssk. (long-stamen basil) was earlier known as O. adscendens Willd. On examining, O. adscendens Willd. at KEW, Harley found resemblances with Becium Lindl. He documented Becium Lindl. as a new distributional record to India because of Becium filamentosum Forssk. Chiov. and concluded O. adscendens Willd. as a synonym (67). However, the former species is a basionym and homotypic synonym of O. filamentosum Forssk. Another physical scrutiny of a herbarium specimen is the type specimen present in CAL herbarium. Ocimum exsul Collett et Hemsl. (CAL0000020414/415) is a member of Becium Lindl. and a synonym of Ocimum filamentosum Forssk. which in turn, is a basionym of Becium filamentosum (Forssk.) Chiov. Becium Lindl. (type: Becium bicolor Lindl.) was subjugated as separate genera of Labiatae (68).

However, Becium Lindl., Hyperaspis Briq., Erythrochlamys Gürke., and Nautochilus Bremek. now remain as synonyms of Ocimum L. The Neotype (LINN-749.2) of O. gratissimum L. (clove basil) is a cultivated species of Uppsala, originally from India (19). Suddee et al. recognized two varieties of O. gratissimum L. subsp. gratissimum (var. gratissimum and var. macrophyllum) and their distribution from India. Var. macrophyllum is distinct from var. gratissimum in having lax inflorescence and sparse indumentum. This incarceration is held up by referencing Indian material, where the discontinuity between the two varieties is also supported. Also, var. macrophyllum was introduced from or to India and later disseminated through African cultivation (16).

O. americanum sensu A.J Paton is a north Indian native species, more minor than O. basilicum L., sometimes called O. canum Sims. (69). The correct nomenclature for the former is O. americanum L. The type specimens were regarded as different (13), although they were concluded as the same based on the observations of Paton and Putievsky (69). They suggested the voucher specimen of O. canum Sims in the rise of such ambiguity. (13) referred to as interpretative Epitype for both O. americanum L. and O. canum Sims. (69). Paton considers two varieties of O. americanum sensu A.J Paton., var. americanum and var. pilosum (Willd.) based on stem indumentum. He considered O. basilicum var. pilosum as the type specimen of O. americanum var. pilosum (Willd) Paton (19), now recognized as a synonym of O. africanum Lour. (lemon basil). Although O. americanum var. americanum is incognito and var. pilosum (Willd.) is a species called O. africanum Lour. (=Ocimum × citriodorum Vis.). The O. americanum L. (hoary Basil) and O. basilicum L. (sweet Basil) cross resulted in O. × citriodorum Vis. (13), a correct name for O. americanum sensu Pushpangadan & Sobti non L. (69). The epithet citriodorum has been frequently used as a basionym for treating the entity at the infraspecific rank of O. basilicum L. (57). The Bush basil O. minimum L. was considered as a variety of O. basilicum L. (13, 57) but is often mentioned as a separate species (19,69). However, it is a varietal synonym for O. basilicum var. minimum (L.) Alef. (53).

Current status on the taxonomic research

In *Ocimum* L., inter and intra-specific hybridization results in cultivars, chemotypes, and plants with vast morphological variation. According to Paton, morphological traits alone are unreliable to substantiate results when the characters overlap. Molecular taxonomic and morphological studies provide authenticity regarding identification, characterization, phylogeny, and classification based on shared traits. RAPD (Random amplification of polymorphic DNA) studies have shown that *O. africanum* Lour. And *O. basilicum* L. are different species, and both are related to *O. americanum* L. (70). At CAL herbaria, we have come across the specimen of T. Chowdhury (*O. citriodorum*, DDTC-317, 22-10-2015, no accession number), we conclude it correct with rectification in nomenclature as *O. africanum* Lour.

In view of Kumar *et al.* (71), the phylogeny and taxonomy of *Ocimum* L. are doubtful. They performed ISSR

(Inter simple sequence repeats) and psbA-trnH (noncoding fragment between psbA and trnH of chloroplast genome) markers genotyping for Ocimum L. They found the former more effective. Based on phylogeny, the species were bunched into three groups, Basilicum, Gratissimum, and Sanctum. The genotyping revealed the closeness of Basilicum-Sanctum and Gratissimum-Basilicum groups predicated through ISSR and psbA-trnH, respectively (Fig. 3). The interpretation seems correct and analogous to the groupings of Engler and Prantl (39) and Paton (19). Based on morphological interpretations, the present study suggests both Basilicum (O. basilicum L., O. africanum Lour. O. americanum L., O kilimandscharicum Gürke) and Sanctum (O. tenuiflorum L., O. filamentosum Forssk.) as concomitant. O kilimandscharicum Gürke might act as a knot (link) between the two groups. They further claim O. viride Willd. and O. gratissimum L. as different species and used them in their phylogenetic analysis. On close examination of the images provided in their study (71) we found it as either chemotypes or ecotypes of O. gratissimum L. O. viride Willd. is incognito and exists only as a synonym for O. gratissimum L. Also, O. adscendens Willd. is an ordinal synonym of *O. filamentosum* Forssk. nevertheless, the image of the species is correctly identified.

Six Ocimum L. species and six varieties of O. basilicum L. were investigated through nuclear DNA content and chromosome number (72). The results suggested the prevalence of infra-generic groups within Ocimum L. They suggested O. basilicum clade (varieties, cultivars of O. basilicum and O. minimum) are tetraploids and O. americanum clade (O. basilicum var. purpurascens, O. africanum, O. americanum) as hexaploids. Our observation suggests that both clades, in a true sense, form the Basilicum subsection of Ocimum L. However, the differences in ploidy might be due to the use of cultivars and varieties (not true species) for their study and O. minimum L. var. purpurascens is no more valid, O. minimum L. (=O. basilicum var. minimum (L.) Alef.) is a variety, and var. purpurascens doesn't exists.

Four clusters of Ocimum L. supported by high bootstrap values of AFLP (Amplified fragment length polymorphism) are O. africanum/O. americanum, O. basilicum, O. gratissimum, and O. tenuiflorum. In the Neighbor-joining (NJ) analysis, six accessions of O. basilicum 'Erevanskii' came within O. basilicum (cluster) and hinge on O. africanum/O. americanum (cluster) due to parsimony analysis. O. kilimandscharicum Gürke was away from both the clusters; however, it has more affinity with O. africanum/O. americanum and O. tenuiflorum but has a low bootstrap percentage (73). ISSR and RAPD interpretation shows speciesspecific DNA fragments in O. sanctum L. and O. basilicum L. The amplicons, which are unique and don't show intraspecific polymorphism, were regarded as species-specific markers. No such amplicons were recovered in O. americanum L. and O. polystachyon L. (74). They found shuffling of O. polystachyon L. amidst O. basilicum L. genotypes; the reason might be the non-existence of the former. It can be a form of O. basilicum L. (presently a synonym).

The species of Ocimum L. were validated using three

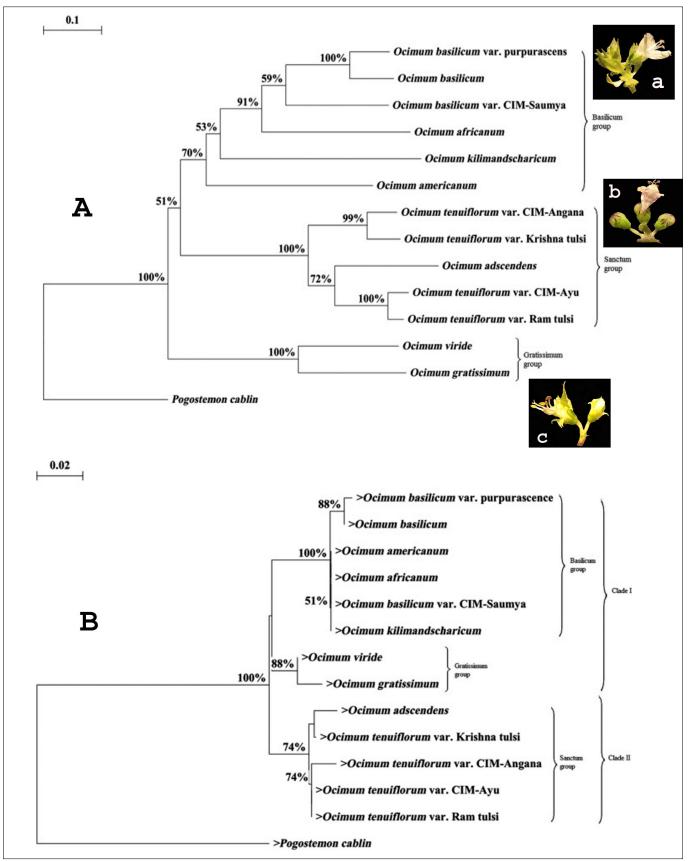


Fig. 3. Phylogram/dendrogram showing *Ocimum* L. species in three cluster Basilicum, Sanctum and Gratissimum using NJ (neighbour joining) method among seven species of *Ocimum* L. The phylogenetic trees are resulted from **A** ISSR, showing more efficiency in clustering than **B** psbA-trnH dataset; the groups are illustrated through floral representative, (a) *Ocimum basilicum* L. (b) *Ocimum tenuiflorum* L. (c) *Ocimum gratissimum* L.

Source: (A and B) reproduced with permission from Kumar et al. (2016); (a-c) ©Mamita Kalita

barcodes rbcL (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit), matK (Maturase K), and psbA-trnH of the chloroplast genome. The psbA-trnH spacer was more useful as a discrimination tool since it gives more variation at the inter-species level (75). They pointed to the

morphological similarity between *O. tenuiflorum* L. and *O. filamentosum* Forssk. The image of the latter has been misidentified since it is devoid of peculiar characteristics (serrated, ovate-oblong leaves, 3-5 cm raceme with few distant verticils) of *O. filamentosum* Forssk. Another mo-

lecular scrutiny related to ScoT (Start Codon Targeted) polymorphism of *Ocimum* L. has shown more polymorphic percentage than ISSR. The dendrogram obtained from ScoT analysis is well-founded, justifiable, and informative compared to ISSR. Two species (*O. viride* and *O. sanctum*) have been out-grouped (76), possibly due to poor diagnosis during species identification. *O viride* Willd. is currently regarded as a synonym of *O. gratissimum* L. subsp. *gratissimum*.

Plastid markers rbcL, matK, trn L-F, trnH-psbA are suitable in the formation of the exclusive monophyletic clade of O. tenuiflorum L. and another clade having O. africanum Lour., O. americanum L., O. basilicum L., and O. kilimandscharicum Gürke. Such differences in clade formation are due to high divergence showing a long history of evolution in comparison to low divergence that leads to clustering (77). The species O. tenuiflorum Vana 8258 is misidentified, the correction is given as O. americanum L. Also, rectifying image O. tenuiflorum 5751 as O. campechianum Mill. and O. campechianum 7564 as O. tenuiflorum L. The genus Ocimum L. is diversified with heterogeneity in forms, cultivars, varieties, hybrids, and ecotypes. The inter and intra-crossing tendency results in morphotypes and chemotypes. The genotype controls the chemotype; however, developmental and environment-related patterns regulate their expression. In keeping with Jurges et al. (2018), photon quality modulates the development and responses of oil glands (essential oil in Ocimum L.). Again, the magnitude of secondary metabolites revolves around the plant's age, light concentration, and other abiotic factors. So, the plants of Ocimum L. have morphologic diverseness together with varied bioactive compounds profile.

Conclusion

The present study has primarily focused on Ocimum L. (taxonomic background) and its species distributed in India. We have tracked down much antiquity literature for better understanding and inference. In our study, we have analyzed the taxonomic position of Ocimum L. in artificial, natural, and phylogenetic classification systems, demarcating the features and criteria used for the hierarchical arrangement. Also, photographic representation of the habit, micro-morphological features have given a clear understanding in anticipating Ocimum L. Further, we had extricated the documentation based on Indian Ocimum L. to know the taxonomic development route of the genus. The molecular taxonomy specifics of Ocimum L. were evaluated to correlate with classical taxonomy (morphology based). The phylogenetic clades obtained from marker genotyping were close to phenetic dendrograms. For molecular and biochemistry based research, we encourage proper validation and identification of specimens before experimentation. It has helped us in reaching clarity and a conclusion. The hybridization tendencies of specific and infra-specific taxa are high, and with 65 species, we can expect a magnificent divergence. The frequent misinterpretations occur between O. africanum Lour./ O. basilicum L. and O. americanum L.; confusion among the cultivars or morphotypes of O. basilicum L. and O. gratissimum L. The usage of synonyms such as O. sanctum (for O. tenuiflorum L.) and O. citriodrum Vis. (for O. africanum Lour.) should be avoided. Similarly, O. viride Willd. and O. suave Willd. are chemotypes of O. gratissimum L., and their stature as distinct species is not justifiable. The sweet Basil (O. basilicum L.) has a sufficient number of cultivars, ecotypes, and chemotypes. Using varietal names except for var. basilicum and var. minimum is discouraged. The ongoing records were examined interchangeably with past ones for suitable repercussions. We have provided a few corrections for research articles of utmost importance in the present scenario. Substantial analysis of nomenclature disputes, taxonomy, and classification has been attained. Meticulous observation and interpretations will help us to overcome the problems pertaining to Ocimum L.

Abbreviations

L./LINN-Linnaeus; ICBN-International code of Botanical Nomenclature; BHL-Biodiversity Heritage Library; JSTOR-Journal Storage; NCBI-National Center for Biotechnology Information; BSI-Botanical Survey of India; ARUN-Arunachal Pradesh Regional Centre, Itanagar, Arunachal Pradesh; ASSAM-Eastern Regional Centre, Shillong, Meghalaya; CAL-Central National Herbarium, Howrah, West Bengal; GUBH-Gauhati University Botanical Herbarium; IVH-Indian Virtual Herbarium; KEW-Royal Botanic Garden, Kew; G-Conservatoire et Jardin botaniques de la Ville de Genève; MNHN-Muséum national d'Histoire naturelle; MO -Missouri Botanical Garden's Herbarium; NY-New York Botanical Garden Herbarium; ICN-International Code of Nomenclature for Algae, Fungi, and Plants; IUCN-International Union for Conservation of Nature; BSA-Central Regional Centre, Allahabad, Uttar Pradesh; DNA-Deoxyribonucleic acid.

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Authors contributions

ND has conceived the topic for review work. MK has carried out the literature study and presented the information. MK drafted the manuscript; ND scrutinized the review data and provided rectifications. ND and MK have finalized the manuscript for correspondence. Both authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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