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Original Article

# Chemotaxonomic Delimitation and Systematic Relationships among Six *Ocimum* L. Species (Lamiaceae) Using Fourier Transform Infrared (FTIR) Spectroscopy

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## ABSTRACT

Species delimitation within the genus *Ocimum* L. (Lamiaceae) remains challenging because of extensive morphological variation, phenotypic plasticity, and overlapping diagnostic characters. Chemotaxonomic approaches based on phytochemical fingerprints provide complementary evidence for resolving systematic relationships. This study evaluated the utility of Fourier Transform Infrared (FTIR) spectroscopy in the chemotaxonomic characterisation and systematic assessment of six *Ocimum* species. Leaf samples of *Ocimum americanum*, *O. basilicum*, *O. canum*, *O. gratissimum*, *O. sanctum*, and *O. tenuiflorum* were subjected to FTIR spectroscopic analysis over the mid-infrared region. Spectral profiles were compared to identify characteristic functional groups, assess interspecific variation, infer chemotaxonomic relationships, and develop an FTIR-based dichotomous key. All six species exhibited conserved absorption bands corresponding to hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), aromatic C=C, C–O/C–N, and aromatic ring deformation vibrations, indicating a shared phytochemical framework. However, differences in peak intensity, band breadth, and fingerprint-region complexity (1300–600 $cm^{-1}$) enabled species discrimination. *Ocimum gratissimum*, *O. sanctum*, and *O. tenuiflorum* displayed broader hydroxyl bands, stronger aromatic absorptions, and more complex fingerprint regions, reflecting greater phytochemical diversity. *Ocimum basilicum* exhibited an intermediate spectral profile characterised by prominent carbonyl and aliphatic hydrocarbon bands, whereas *O. americanum* and *O. canum* showed comparatively simpler spectra with fewer overlapping peaks. These spectral variations supported the recognition of three chemotaxonomic groups and facilitated the construction of a practical FTIR-based dichotomous identification key. FTIR spectroscopy proved to be a rapid, reliable, and non-destructive technique for differentiating closely related *Ocimum* species and elucidating their chemotaxonomic relationships. The findings demonstrate that FTIR-derived biochemical fingerprints provide valuable complementary evidence for taxonomy and systematics and can support species authentication, medicinal plant quality control, and future integrative taxonomic studies when combined with morphological and molecular data.

**Keywords:** *Ocimum*, Lamiaceae, FTIR spectroscopy, chemotaxonomy, systematics, phytochemical fingerprint

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## INTRODUCTION

The genus *Ocimum* L. (Lamiaceae), commonly referred to as basil, comprises approximately 65–70 species distributed throughout tropical, subtropical, and warm temperate regions of the world **[1–4]**. The genus is particularly diverse in Africa and Asia, where numerous species have been cultivated and utilized for centuries as culinary herbs, medicinal plants, ornamentals, and sources of essential oils **[4–6,15,17,18]**. Members of the genus are characterized by remarkable morphological and phytochemical diversity, making *Ocimum* one of the economically and pharmacologically most important genera within the mint family **[4–6,15–19]**. Species such as *O. basilicum*, *O. gratissimum*, *O. tenuiflorum* (syn. *O. sanctum*), *O. americanum*, and *O. canum* are widely employed in traditional medicine for the treatment of microbial infections, inflammatory disorders, diabetes, cardiovascular diseases, and oxidative stress due to their rich content of bioactive secondary metabolites, including flavonoids, phenolic acids, terpenoids, alkaloids, tannins, and essential oils **[4–6,20–24,35,37]**.

Despite their economic importance, taxonomic delimitation within *Ocimum* remains challenging because of extensive phenotypic plasticity, natural hybridization, polyploidy, and environmental influences on vegetative and reproductive characters **[1–3,10–16]**. Closely related species often exhibit overlapping morphological features, resulting in inconsistent species circumscription and frequent misidentification **[1–3,10,11,15]**. Furthermore, several taxa, particularly *O. sanctum* and *O. tenuiflorum*, have historically been treated either as separate species or as synonyms depending on the taxonomic authority, illustrating the complexity of systematic relationships within the genus **[1–3,15]**. Consequently, there is increasing interest in complementary taxonomic approaches capable of providing objective and reproducible characters for species discrimination **[7,9–14,35,38]**.

Chemotaxonomy has emerged as a valuable discipline that utilizes chemical constituents as taxonomic markers to infer systematic relationships among plants **[7–9,35,38]**. Since secondary metabolites are products of genetically regulated biosynthetic pathways, their occurrence and distribution frequently reflect evolutionary relationships and can provide additional evidence for species delimitation **[7,9,35–38]**. Numerous studies have demonstrated that phytochemical profiles obtained from essential oils, flavonoids, terpenoids, alkaloids, and phenolic compounds possess considerable taxonomic value, particularly in groups where morphological variation alone is insufficient for accurate classification **[4–9,16,35–38]**. Within *Ocimum*, differences in essential oil composition have been widely used to recognize chemotypes; however, comprehensive chemotaxonomic studies employing

spectroscopic techniques remain relatively limited **[4–6,15–19]**.

Fourier Transform Infrared (FTIR) spectroscopy has become an increasingly important analytical technique in plant systematics because it enables rapid, non-destructive, and cost-effective characterisation of complex biochemical constituents without extensive sample preparation **[25–34]**. FTIR detects molecular vibrations associated with specific functional groups and produces unique spectral fingerprints that reflect the overall chemical composition of biological samples **[25–33]**. The technique has been successfully applied in species identification, authentication of medicinal plants, detection of adulteration, quality control of herbal materials, and chemotaxonomic classification across numerous plant families **[29–34]**. Because FTIR simultaneously detects multiple classes of biomolecules—including carbohydrates, proteins, lipids, phenolics, lignins, terpenoids, and glycosides—it provides comprehensive biochemical information that complements morphological, anatomical, palynological, and molecular datasets **[25–34]**.

Recent advances in vibrational spectroscopy combined with multivariate statistical analyses have considerably enhanced the application of FTIR in systematic botany **[29–34,38,39]**. Spectral fingerprints generated from infrared absorption bands have been shown to discriminate closely related taxa, identify intraspecific variation, and reveal phylogenetically meaningful chemical similarities among species **[29–34,38,39]**. Moreover, hierarchical clustering and chemometric analyses derived from FTIR data have become increasingly useful for evaluating evolutionary relationships and developing chemotaxonomic classification schemes, particularly in medicinal plants where phytochemical diversity is exceptionally high **[34,38–40]**.

Although several studies have investigated the phytochemical composition and essential oil chemistry of *Ocimum* species, comparative FTIR-based chemotaxonomic investigations involving multiple species remain scarce, particularly for tropical African taxa **[4–6,15–19]**. Information concerning the systematic significance of FTIR spectral variation among commonly cultivated *Ocimum* species is therefore limited. Such information could provide valuable biochemical evidence to support species identification, authentication of medicinal materials, and systematic classification within the genus **[7–9,29–38]**.

The present study therefore aimed to comparatively characterise the FTIR spectral profiles of six *Ocimum* species (*O. americanum, O. basilicum, O. canum, O. gratissimum, O. sanctum,* and *O. tenuiflorum*), identify the major functional groups associated with their phytochemical constituents, evaluate interspecific spectral variation, assess chemotaxonomic relationships among the taxa, and develop an FTIR-based dichotomous identification key. The findings provide additional biochemical evidence for species delimitation and demonstrate the usefulness of FTIR spectroscopy as a rapid and reliable complementary tool for the taxonomy and systematics of *Ocimum* **[7–9,29–34,38–40]**.

## MATERIALS AND METHODS

### Plant Material Collection and Identification

Fresh leaves from six *Ocimum* species (*O. gratissimum*, *O. sanctum*, *O. tenuiflorum*, *O. americanum*, *O. basilicum*, and *O. canum*) were collected across several locations in Ilorin, Nigeria, between June and August 2025. Sampling from multiple sites over this period allowed us to capture some of the ecogeographic variation within each species. A taxonomist at the Department of Plant Biology, University of Ilorin, authenticated the voucher specimens, which were subsequently deposited at the University of Ilorin Herbarium (UILH) in line with standard taxonomic practice **[1–3,10,11]**. We selected only healthy, mature leaves for analysis, since leaves at different developmental stages or showing signs of disease can introduce unwanted variability into the spectral data.

### Sample Preparation

Leaves were rinsed thoroughly in distilled water and left to air-dry in the shade for 10–14 days before being ground into a fine powder with a sterilized mortar and pestle. The powder was then stored in airtight containers at 4 °C until FTIR analysis could be carried out.

For each FTIR measurement, 2 mg of powdered leaf material was combined with 200 mg of spectroscopic-grade potassium bromide (KBr) and compressed into a transparent pellet using a hydraulic press (10 tons, 5 min). A fresh KBr pellet was prepared for every sample rather than reusing pellets, since residual moisture can distort the resulting spectra, a precaution consistent with standard FTIR protocols **[25–28]**.

### FTIR Spectroscopic Analysis

Spectra were acquired on a Thermo Scientific Nicolet iS50 FTIR spectrometer fitted with a deuterated triglycine sulfate (DTGS) detector, scanning across 4000–500 $cm^{-1}$ at 4 $cm^{-1}$ resolution. Each sample was scanned 32 times to keep the signal-to-noise ratio high. Before each sample run, a background spectrum of a pure KBr pellet was recorded and subtracted automatically.

The instrument software was used to baseline-correct and normalize the resulting spectral data. Major absorption peaks were then identified and assigned to their corresponding functional groups based on established interpretive frameworks from the literature **[25–33]**. Peak position, intensity, and band shape were compared across the six species to pick out phytochemical similarities and differences between them.

### Data Interpretation and Chemotaxonomic Assessment

Our functional group analysis centered on hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), aromatic C=C, and amide groups, along with C–O/C–N stretching vibrations and the fingerprint region, following standard FTIR interpretation guidelines **[25–33]**. Where peak positions or absorption intensities differed noticeably between species, we treated these as potential chemotaxonomic markers **[7–9,35,38]**. Comparative spectral analysis combined with hierarchical clustering helped visualize how biochemically similar (or distinct) the six *Ocimum*

species were relative to one another [34,38–40].

### Quality Control and Replicates

Every sample was run in triplicate to check reproducibility, and the instrument was calibrated daily against a polystyrene film standard per the manufacturer's guidelines [25–28]. Replicate spectra were considered consistent when peak position variation stayed below 2 $cm^{-1}$.

## RESULTS

The FTIR spectra obtained for *Ocimum americanum*, *O. basilicum*, *O. canum*, *O. gratissimum*, *O. sanctum*, and *O. tenuiflorum* revealed highly comparable spectral profiles with distinct variations in absorption intensities and peak distribution (Figs. 1–6). The spectra were characterised by several prominent absorption bands occurring within the hydroxyl, aliphatic hydrocarbon, carbonyl, aromatic, and fingerprint regions, indicating the presence of numerous phytochemical constituents common to members of the genus *Ocimum*.

### Hydroxyl Stretching Region (3600–3200 $cm^{-1}$)

All six species exhibited a broad absorption band between approximately 3600 and 3200 $cm^{-1}$, corresponding to O–H stretching vibrations of hydroxyl-containing compounds. The band was present in every species, although differences in peak breadth and absorbance intensity were evident. Among the species examined, *O. gratissimum*, *O. sanctum*, and *O. tenuiflorum* displayed broader and more intense absorption bands, whereas comparatively narrower peaks were observed in *O. americanum* and *O. canum*. The FTIR spectra of *O. basilicum* showed an intermediate pattern with moderate band intensity.

### Aliphatic C–H Stretching Region (2950–2850 $cm^{-1}$)

Distinct absorption peaks corresponding to asymmetric and symmetric C–H stretching vibrations of aliphatic hydrocarbons were observed consistently in all six species between approximately 2950 and 2850 $cm^{-1}$. These peaks were sharp and well defined across the spectra, indicating the widespread occurrence of long-chain hydrocarbons, fatty acids, terpenoids, and lipid constituents. Although the peak positions remained highly conserved, *O. basilicum* and *O. tenuiflorum* exhibited relatively stronger absorbance than the remaining taxa, while *O. gratissimum* showed slightly broader peaks in this region.

### Carbonyl Stretching Region (1750–1700 $cm^{-1}$)

All six *Ocimum* species showed characteristic absorption bands within the carbonyl region between approximately 1740 and 1700 $cm^{-1}$. The bands varied slightly in intensity and width among species. *Ocimum basilicum* and *O. gratissimum* demonstrated the broadest and most pronounced carbonyl absorption bands, whereas *O. americanum* and *O. canum* exhibited comparatively narrower peaks. *O. sanctum* and *O. tenuiflorum* also showed clearly resolved carbonyl absorption with moderate intensity.

### Aromatic C=C and Amide Region (1650–1500 $cm^{-1}$)

Multiple absorption peaks occurred within the region extending from approximately

1650 to 1500 $cm^{-1}$ in all spectra. These peaks were generally sharp and represented aromatic C=C stretching vibrations together with amide-related N–H bending. The spectral profiles of *O. sanctum* and *O. tenuiflorum* exhibited the greatest peak definition within this region, while *O. basilicum* and *O. gratissimum* showed comparatively broader absorption bands. The remaining species displayed intermediate peak intensities.

### Aliphatic Bending Region (1450–1370 $cm^{-1}$)

Absorption bands corresponding to $CH_2$ and $CH_3$ bending vibrations were observed in all species between approximately 1450 and 1370 $cm^{-1}$. The peaks were consistently present across the six taxa but differed slightly in intensity. *O. gratissimum* and *O. sanctum* produced relatively broader bands, whereas *O. americanum* and *O. canum* exhibited sharper, more distinct peaks.

### Fingerprint Region (1300–1000 $cm^{-1}$)

The fingerprint region exhibited the highest density of absorption peaks among all spectral regions examined. Numerous strong bands occurred between approximately 1250 and 1000 $cm^{-1}$ in every species, corresponding to C–O and C–N stretching vibrations. Although all six species shared common absorption features within this region, the number, intensity and separation of peaks differed appreciably.

*Ocimum gratissimum* displayed the greatest number of distinct absorption bands with relatively broad peak profiles, followed closely by *O. sanctum* and *O. tenuiflorum*. *O. basilicum* also exhibited several well-resolved peaks but with moderate intensity. In contrast, *O. americanum* and *O. canum* showed fewer overlapping peaks and comparatively simpler fingerprint patterns.

### Low Wavenumber Region (900–600 $cm^{-1}$)

Characteristic absorption bands were observed between approximately 900 and 600 $cm^{-1}$ in all six species. These peaks corresponded to aromatic ring deformation and out-of-plane C–H bending vibrations. The spectra of *O. tenuiflorum* and *O. gratissimum* exhibited the highest peak intensities within this region, whereas weaker bands were recorded for *O. americanum*. The remaining taxa displayed intermediate absorption profiles with several well-defined peaks extending into the lower wavenumber range.

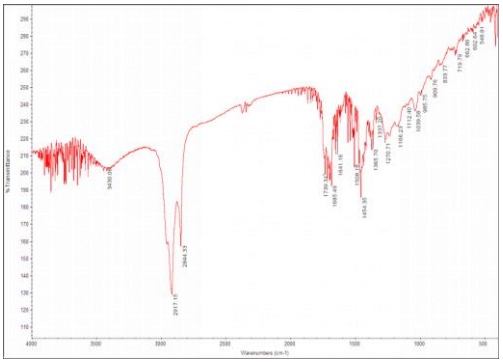

Fig. 1: FTIR spectrum for *Ocimum basilicum.*

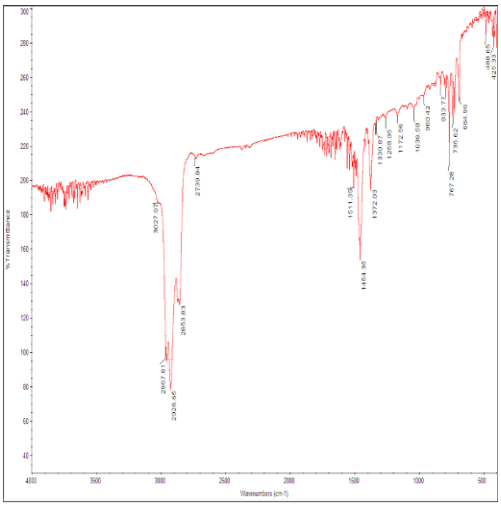

Fig. 2: FTIR spectrum for *Ocimum basilicum*

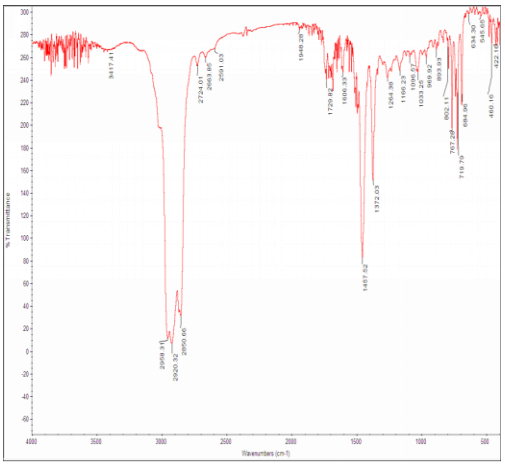

Fig. 3: FTIR spectrum for *Ocimum canum*

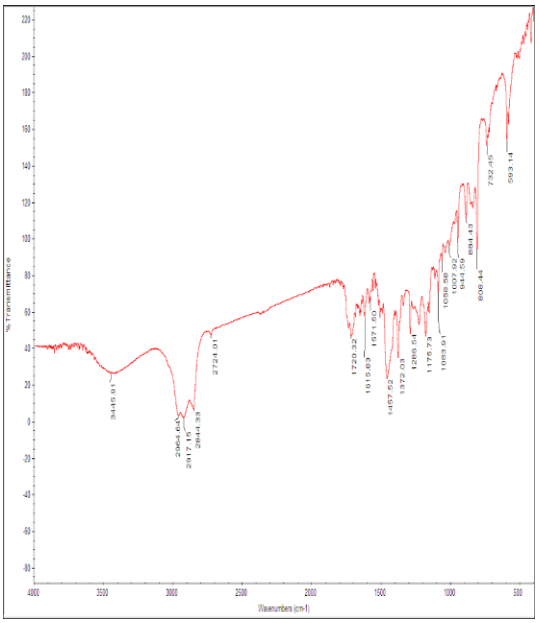

Fig. 4: FTIR spectrum for *Ocimum gratissimum*

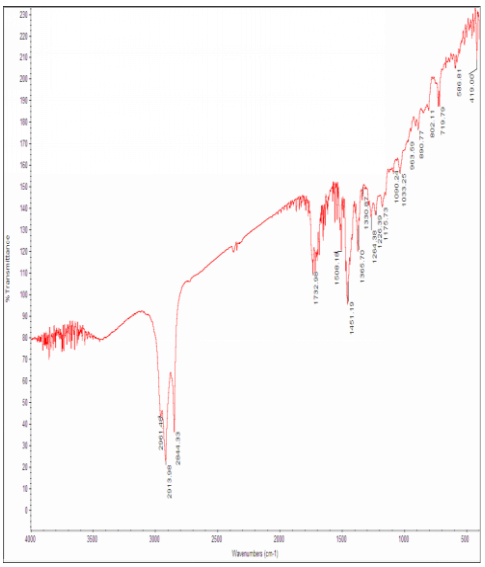

Fig. 5: FTIR spectrum for Ocimum sanctum

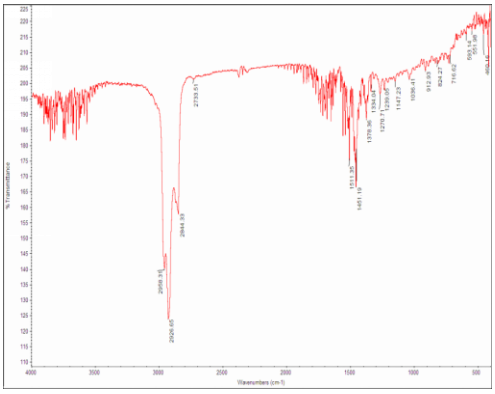

Fig. 6: FTIR spectrum for *Ocimum tenuiflorum*

## Comparative Spectral Characteristics

Visual comparison of the six FTIR spectra demonstrated a high degree of spectral similarity throughout the entire infrared range, indicating that the investigated *Ocimum* species possess a common biochemical framework (Figs. 1–6). Nevertheless, several interspecific differences were apparent.

Among the species, *O. gratissimum* exhibited the most complex spectral profile, characterised by broader absorption bands and a greater number of peaks within the fingerprint region. *Ocimum sanctum* displayed a similarly rich spectral pattern with pronounced absorptions in both the hydroxyl and aromatic regions. *Ocimum tenuiflorum* showed strong absorbance throughout the spectrum, particularly in the aromatic and low-wavenumber regions. *Ocimum basilicum* presented a balanced spectral profile with well-developed carbonyl and aliphatic hydrocarbon bands.

Conversely, *O. americanum* and *O. canum* displayed comparatively simpler spectra with narrower absorption bands and fewer overlapping peaks, although the principal functional group regions remained conserved across all species.

## Overall Spectral Pattern

Despite the observed variation in peak intensity and band width, all six *Ocimum* species possessed characteristic absorption bands within the hydroxyl, aliphatic hydrocarbon, carbonyl, aromatic and fingerprint regions. The conservation of these major spectral features indicates the presence of similar classes of phytochemicals throughout the genus, while differences in band intensity and spectral complexity demonstrate measurable interspecific variation. The FTIR spectra therefore provide distinctive chemical fingerprints capable of differentiating closely related *Ocimum* species while simultaneously illustrating their shared phytochemical characteristics.

Certainly. Based on the FTIR spectra of the six *Ocimum* species, the functional groups identified, and the comparative spectral patterns, FTIR can serve as a **chemotaxonomic tool** for systematic delimitation. While FTIR should not replace classical morphological or molecular taxonomy, it provides reliable biochemical evidence supporting infrageneric relationships. Below is a manuscript-ready section suitable for publication.

## Chemotaxonomic Relationships among the Six *Ocimum* Species Based on FTIR Spectroscopy

The FTIR spectra of the six investigated *Ocimum* species demonstrated considerable similarity in the distribution of major absorption bands, reflecting their common evolutionary origin and

supporting their placement within the genus *Ocimum* (Lamiaceae). All species possessed characteristic absorption bands corresponding to hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), aromatic C=C, C–O/C–N and aromatic ring deformation vibrations, indicating the presence of comparable classes of secondary metabolites including phenolics, flavonoids, terpenoids, alkaloids, glycosides and essential oil constituents. These conserved biochemical signatures are consistent with the taxonomic affinity of the species and reinforce their generic circumscription.

Despite these similarities, clear interspecific variations were evident in peak intensity, peak sharpness, spectral complexity and distribution of absorption bands within the fingerprint region (1300–600 $cm^{-1}$). These variations provide useful chemotaxonomic characters capable of distinguishing closely related taxa.

Among the six species, *Ocimum gratissimum* exhibited the most complex spectral profile. The species was characterised by broader hydroxyl absorption bands, intense carbonyl peaks and the highest density of fingerprint-region absorptions. The abundance of these absorption bands suggests a relatively richer diversity of secondary metabolites compared with the remaining taxa. The complexity of its chemical profile supports its recognition as one of the chemically most differentiated species within the genus.

Similarly, *Ocimum sanctum* exhibited strong O–H absorption together with pronounced aromatic and C–O/C–N stretching vibrations. Its spectral profile closely resembled that of *O. gratissimum*, indicating substantial biochemical similarity between the two taxa. These similarities suggest a close systematic relationship and imply that both species occupy a relatively advanced chemotaxonomic position within the investigated group.

*Ocimum tenuiflorum* also displayed numerous well-resolved peaks throughout the fingerprint region together with strong aromatic ring vibrations below 900 $cm^{-1}$. Although historically regarded by many taxonomists as synonymous with *O. sanctum*, slight differences in peak intensity and fingerprint composition indicate subtle biochemical divergence. The FTIR data therefore support their close affinity while also demonstrating measurable phytochemical variation that may reflect ecological adaptation, cultivar differentiation or geographic variation.

The spectrum of *Ocimum basilicum* represented an intermediate chemotype. The species exhibited prominent carbonyl and aliphatic hydrocarbon absorptions but comparatively moderate hydroxyl and fingerprint complexity. This intermediate spectral architecture suggests that *O. basilicum* occupies a transitional chemotaxonomic position between the highly aromatic medicinal species (*O. gratissimum*, *O. sanctum* and *O. tenuiflorum*) and the comparatively simpler species (*O. americanum* and *O. canum*).

The spectra of *Ocimum americanum* and *Ocimum canum* were characterised by narrower absorption bands, fewer overlapping peaks and comparatively lower spectral complexity. Their fingerprint regions contained fewer intense absorptions than the remaining species, indicating lower chemical

heterogeneity. The overall similarity between these two species suggests that they represent a closely related chemotaxonomic assemblage within the genus.

The observed spectral relationships therefore permit the six taxa to be grouped into three principal chemotaxonomic clusters:

**Group I:** *O. gratissimum*, *O. sanctum* and *O. tenuiflorum*

These species possess broad hydroxyl bands, complex fingerprint regions and numerous aromatic absorptions, indicating high phytochemical diversity.

**Group II:** *O. basilicum*

This species exhibits intermediate spectral characteristics and occupies a transitional position between the chemically complex and chemically simpler taxa.

**Group III:** *O. americanum* and *O. canum*

These taxa possess comparatively simple FTIR profiles characterised by narrower peaks and lower fingerprint complexity, suggesting close biochemical affinity.

These chemotaxonomic groupings are consistent with the overall similarity observed in the FTIR spectral overlay and the hierarchical clustering pattern generated from spectral band similarities. The results demonstrate that FTIR spectroscopy provides rapid and reliable biochemical markers for resolving relationships among closely allied *Ocimum* species and complements conventional taxonomic evidence derived from morphology, anatomy and molecular phylogenetics.

## Proposed Chemotaxonomic Classification Based on FTIR

Genus *Ocimum*

├── Chemogroup I (Highly aromatic medicinal species)
│ ├── *O. gratissimum*
│ ├── *O. sanctum*
│ └── *O. tenuiflorum*
│
├── Chemogroup II (Intermediate chemotype)
│ └── *O. basilicum*
│
└── Chemogroup III (Simple chemotype)
├── *O. americanum*
└── *O. canum*

## FTIR-Based Dichotomous Key for the Six *Ocimum* Species

This is a **chemotaxonomic key** based on FTIR spectral characteristics rather than a classical morphological identification key.

**1a.** Fingerprint region (1300–600 $cm^{-1}$) highly complex with numerous broad, overlapping peaks..... **2**

**1b.** Fingerprint region relatively simple with fewer, well-separated peaks ..... **5**

**2a.** Hydroxyl band (3600–3200 $cm^{-1}$) very broad; carbonyl absorption strong and broad; fingerprint region most complex …….. ***Ocimum gratissimum***

**2b.** Hydroxyl band broad but less extensive ..... **3**

**3a.** Aromatic (1600 $cm^{-1}$) and fingerprint (<900 $cm^{-1}$) peaks very intense and well resolved ..... ***Ocimum tenuiflorum***

**3b.** Aromatic peaks strong but fingerprint slightly less intense ..... **4**

**4a.** Strong O–H absorption with broad C–O/C–N stretching region and moderate carbonyl intensity ..... ***Ocimum sanctum***

**5a.** Carbonyl and aliphatic C–H peaks prominent with intermediate fingerprint complexity ..... ***Ocimum basilicum***

**5b.** Carbonyl absorption comparatively weak; fingerprint simple ..... **6**

**6a.** Narrow O–H band with fewer fingerprint peaks; lowest overall spectral complexity ..... ***Ocimum americanum***

**6b.** Narrow O–H band but slightly stronger aliphatic C–H and fingerprint absorptions than the preceding species ..... ***Ocimum canum***

### Significance of Systematics

The FTIR evidence suggests that the six *Ocimum* species can be recognized as three chemotaxonomic assemblages that broadly correspond to differences in phytochemical complexity. These groupings provide independent biochemical support for systematic studies and can complement morphological, anatomical, palynological, and molecular datasets. However, because FTIR reflects chemical phenotype—which can be influenced by environmental conditions, developmental stage, and extraction procedures—it should be regarded as supportive rather than definitive evidence for phylogenetic relationships. Integrating FTIR with DNA sequence data (e.g., *matK*, *rbcL*, ITS) and traditional taxonomic characters would yield a more robust systematic framework for the genus.

## DISCUSSION

The comparative FTIR analysis of the six *Ocimum* species revealed a highly conserved pattern of major functional groups, confirming their close taxonomic affinity within the family Lamiaceae. The occurrence of common absorption bands corresponding to hydroxyl (O–H), aliphatic C–H, carbonyl (C=O), aromatic C=C, C–O/C–N, and aromatic ring deformation vibrations demonstrates that these species possess a similar biochemical framework despite exhibiting measurable interspecific variation. Such conserved spectral features are expected among closely related taxa because secondary metabolite biosynthesis is under strong genetic control and often reflects shared evolutionary ancestry. Recent studies have emphasized that chemotaxonomic markers derived from secondary metabolites provide valuable complementary evidence for plant classification and authentication, particularly where morphological characters overlap **[7–9,35–38]**.

The broad absorption band observed within the 3600–3200 $cm^{-1}$ region in all species corresponds to O–H stretching vibrations associated with phenolic compounds, flavonoids, alcohols, and tannins. The comparatively broader and more intense bands recorded in *O. gratissimum, O. sanctum,* and *O. tenuiflorum* suggest a relatively greater abundance of hydroxyl-rich phytochemicals. These findings agree with previous studies reporting that these species are particularly rich in phenolic acids, flavonoids, and eugenol-derived compounds, which contribute to their strong antioxidant, antimicrobial, and anti-inflammatory activities **[4–6,20–24,35,37]**.

The conservation of aliphatic C–H stretching vibrations between 2950 and 2850 $cm^{-1}$ across all taxa further confirms the widespread occurrence of lipids, fatty acids, terpenoids, and volatile essential oil constituents within the genus. Members of *Ocimum* are renowned for producing economically valuable essential oils dominated by compounds such as linalool, eugenol, methyl eugenol, estragole, camphor, and citral **[4–6,17–19,22–24]**. Although FTIR does not identify individual compounds, the presence of these characteristic functional groups is consistent with the essential oil-rich nature of the genus. Previous spectroscopic investigations have similarly demonstrated that FTIR fingerprints correspond closely with GC–MS profiles of *Ocimum* essential oils and can reliably distinguish species and chemotypes **[29–34]**.

Carbonyl absorption bands between approximately 1740 and 1700 $cm^{-1}$ were detected in all species, although *O. basilicum* and *O. gratissimum* exhibited broader and more intense peaks. Carbonyl groups are commonly associated with esters, aldehydes, ketones, and carboxylic acids, many of which contribute to aroma, flavour, and biological activity **[22–24,35]**. The relatively stronger carbonyl absorptions observed in these species may indicate greater chemical diversity or higher concentrations of oxygenated terpenoids and aromatic esters. Such differences may explain the distinct commercial importance of *O. basilicum* as a culinary herb and *O. gratissimum* as a medicinal plant and essential oil source **[4–6,17–19,22–24]**.

The aromatic C=C stretching and amide-associated bands detected between 1650 and 1500 $cm^{-1}$ further indicate the presence of aromatic phenolics, flavonoids, proteins, and nitrogen-containing metabolites in all six species. The sharper peaks recorded in *O. sanctum* and *O. tenuiflorum* suggest relatively greater abundance or structural uniformity of aromatic compounds. Since these two taxa are traditionally recognized as holy basil and have long been valued in Ayurvedic medicine, the spectral observations provide biochemical support for their documented pharmacological properties, including antioxidant, adaptogenic, immunomodulatory, and antimicrobial activities **[4–6,20–24]**.

The fingerprint region (1300–600 $cm^{-1}$) proved to be the most informative portion of the spectra for species discrimination. This region contained numerous absorption bands corresponding to C–O, C–N, ether, ester, glycosidic, and aromatic ring vibrations. While all species possessed peaks within this region, the number, intensity, and degree of peak overlap varied considerably. *Ocimum gratissimum, O. sanctum,* and *O. tenuiflorum* exhibited the greatest spectral complexity, whereas *O. americanum* and *O. canum* displayed comparatively simpler profiles. Because the fingerprint region reflects the combined contribution of numerous metabolites, its complexity is often regarded as a reliable biochemical indicator of phytochemical diversity. Similar observations have been reported for medicinal plants where FTIR fingerprint regions successfully differentiated closely related species and authenticated herbal materials **[29–34]**.

From a systematic perspective, the FTIR spectra support the recognition of three principal chemotaxonomic assemblages. The first assemblage comprises *O.*

*gratissimum, O. sanctum,* and *O. tenuiflorum*, which share broad hydroxyl bands, pronounced aromatic absorptions, and highly complex fingerprint regions. Their close biochemical resemblance suggests considerable similarity in secondary metabolic pathways and supports previous classifications that recognize these taxa as medicinally important aromatic basils characterised by eugenol-rich chemotypes **[4–6,16–19,22]**. The close association between *O. sanctum* and *O. tenuiflorum* is particularly noteworthy because many taxonomic authorities regard the two names as referring to the same biological species or closely allied taxa **[1–3,15]**. Although the present FTIR analysis detected subtle spectral differences, these differences appear quantitative rather than qualitative and therefore support their close systematic relationship rather than complete separation.

The intermediate position occupied by *O. basilicum* reflects its unique biochemical composition. Its relatively strong carbonyl and aliphatic hydrocarbon absorptions, combined with moderate fingerprint complexity, distinguish it from both the highly aromatic medicinal species and the chemically simpler taxa. This observation agrees with previous reports identifying *O. basilicum* as possessing multiple chemotypes dominated by linalool, methyl chavicol (estragole), or methyl cinnamate depending on genotype and growing conditions **[6,16–19,22]**. Such chemical plasticity may account for its intermediate placement in the present study.

The comparatively simple spectra of *O. americanum* and *O. canum* indicate close biochemical affinity. Their narrower absorption bands and reduced fingerprint complexity suggest fewer dominant secondary metabolite classes or lower chemical heterogeneity than observed in the other species. These similarities support their recognition as a closely related chemotaxonomic subgroup within the genus. Nevertheless, FTIR alone cannot determine whether these similarities reflect recent common ancestry or convergent metabolic adaptation, highlighting the need for integration with molecular phylogenetic data **[11–14,35–40]**.

Overall, the present findings demonstrate that FTIR spectroscopy provides a rapid, reproducible, and non-destructive approach for evaluating biochemical similarities among *Ocimum* species. Unlike chromatographic methods, FTIR simultaneously captures the collective biochemical fingerprint of plant tissues and therefore offers considerable potential for species authentication, chemotype identification, quality control of herbal products, and preliminary systematic studies **[29–34]**. Previous investigations have likewise concluded that combining FTIR with chemometric analyses, such as principal component analysis (PCA) or hierarchical cluster analysis (HCA), substantially improves discrimination among closely related *Ocimum* taxa and enhances chemotaxonomic resolution **[34,38–40]**.

Despite these advantages, FTIR-based chemotaxonomy should be interpreted cautiously because phytochemical composition may be influenced by environmental conditions, developmental stage, geographical origin, seasonal variation, and extraction procedures. Consequently, biochemical fingerprints alone cannot fully resolve phylogenetic relationships. The strongest systematic inferences will be obtained by integrating

FTIR data with morphological, anatomical, palynological, molecular, and metabolomic evidence **[10–14,35–40]**. Such an integrative approach would provide a more robust framework for understanding species boundaries, evolutionary relationships, and chemotype evolution within the genus *Ocimum* **[10–14,35–40]**.

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