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# Untargeted metabolomics and targeted approach of essential oils to differentiate five *Ocimum* species using gas chromatography coupled with mass spectrometry

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## ABSTRACT

The chemical profile of essential oils (EOs) obtained from *Ocimum* species can be used to identify species and assess the potential applications for them. Some kinds of basil (*Ocimum* L.) are used as medicinal herbs because of the distinct biological activity of their essential oils. The goal of this study was to use chemometrics to distinguish between five closely related species of *Ocimum*, viz., *O. basilicum* Linn., *O. canum* Sims., *O. citriodorum*, *O. gratissimum* Linn. and *O. sanctum* Linn. to distinguish between different volatile organic compounds (VOCs) based on their EOs. Through the use of gas chromatography-mass spectrometry (GC-MS) operated under screening mode to determine the compositions of the EOs, total 119 metabolites were found. The VOC composition of the investigated species allowed for distinct differentiation, as demonstrated by untargeted metabolomics and multivariate analysis using Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA). Simultaneously using GC-MS selected ion monitoring (SIM) method was established for the simultaneous four major components like Linalool, Caryophyllene, Estragole and Eugenol of quantitative estimation in five different Ocimum species, as well as providing methodological reference for quality control. Based on chromatographic data, Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) heat map experimental design were able to successfully distinguish between the five related species. The content was detected in varying proportions between batches of the same species, according to quantitative evaluation i.e., (i) Three different of batches *Ocimum basilicum* Linn., Estragole was 52.9 %w/w as higher content, Linalool 18.19 %w/w, Caryophyllene 0.3 %w/w, and Eugenol were not detected, (ii) Three different of batches *Ocimum canum* Sims., Linalool was founds as > 70.82 %w/w as higher content, Caryophyllene 3.41 %w/w, Eugenol 0.8 %w/w and Estragole were not detected in all three different batches, (iii) Three different of batches *Ocimum citriodorum*., Caryophyllene was found around 34.36 %w/w, Linalool 6.29 %w/w; Eugenol 10.64 %w/w and Estragole were not detected in all three different batches, (iv) *Ocimum gratissimum* Linn., in three different of batches, Eugenol was found in high content up to 65.77 %w/w, Linalool 1.77 %w/w respectively, Caryophyllene 10.8 %w/w and Estragole not detected in all three different batches, (v) *Ocimum sanctum* Linn., Eugenol were in high content at 57.21 %w/w, Caryophyllene up to 19.85 %w/w, Linalool and Estragole both were not detected in all three different batches; this could be because of the species nature and the varying climatic circumstances. *Ocimum* essential oil is a potent antibacterial, antioxidant, repellent, insecticidal, larvicidal, nematocidal, and therapeutic agent (anti-inflammatory, antinociceptive, antipyretic, analgesic, immunomodulatory, etc.) with a vast range of biological action.

**Keywords:** *Ocimum* species, Linalool, Caryophyllene, Estragole, Eugenol, GC-MS, Untargeted metabolomics.

## INTRODUCTION

*Ocimum*, a genus belonging to the Lamiaceae family, is renowned for its fragrant and medicinal properties and is recognized as the foremost family of plants that produce essential oils. This genus encompasses over 150 species that are extensively cultivated and found in both tropical and temperate regions, commonly referred to as "basils," with Tulsi being one of the most prominent names. The contributions of certain plant species to the scientific domain have been significant since ancient times and continue to influence contemporary research, particularly due to their numerous medicinal applications. These applications include antimicrobial, antioxidant, repellent, insecticidal, larvicidal, nematocidal, and therapeutic properties, such as anti-inflammatory, antinociceptive, antipyretic, antiulcer, analgesic, anthelmintic, anticarcinogenic, skin permeation enhancement, immunomodulation, cardio-protection, and antilipidemic effects [1]. The species *Ocimum basilicum* (commonly referred to as sweet basil), *Ocimum gratissimum* (known as clove basil or wild basil), and *Ocimum sanctum* (often called holy basil) are widely cultivated across various regions, including East Asia, Europe, America,

and Australia, primarily for their essential oil production. Additionally, *Ocimum americanum*, previously classified as *Ocimum canum*, encompasses wild varieties found in India, with its essential oil being utilized for multiple commercial applications [2]. The diverse species within the *Ocimum* genus exhibit a range of medicinal properties attributed to their extensive biological activities, leading to their incorporation into various traditional and indigenous medicinal practices. Furthermore, these species are recognized for their unique aroma and flavour profiles, which are derived from high-quality essential oils and aroma compounds that are highly valued in the food, fragrance, cosmetic, and pharmaceutical sectors [3,4,5]. The essential oil composition of *Ocimum* taxa is notably complex, exhibiting significant variations influenced by factors such as genotype, morphotype, chemotype, environmental conditions, climate, and agronomic practices associated with different cultivars [6]. Chemical characterization techniques can facilitate the differentiation of accessions based on the concentration of specific compounds, thereby elucidating the intrinsic variability among accessions of the same species. Occasionally, various chemotypes such as eugenol-rich variants of *O. gratissimum* and *O. tenuiflorum* [7] have been documented, alongside an Estragole-rich chemotype (87%) of *O. tenuiflorum* originating from Australia [8]. The essential oil composition of plants within the *Ocimum* genus, particularly *O. basilicum*, has been extensively studied, as chemotaxonomy serves as a valuable tool for evaluating both inter and intra-species variability [9]. Analyses of the essential oils from *Ocimum* species have demonstrated significant diversity in their chemical constituents, with distinct chemotypes identified across different geographical regions. The predominant components found in *Ocimum* species include phenyl propanoids (such as eugenol, methyl eugenol, Estragole, and methyl cinnamate), monoterpenoids (including Linalool, 1,8-cineole, camphor, limonene, ocimenes, citral, and thymol), and sesquiterpenoids, primarily β-caryophyllene, β-selinene, α-selinene, β-bisabolene, elemol, and germacrene D. [10,11,12,13].

The primary aim of the current research was to chemically analyze and quantify the essential oil of the specified *Ocimum* species evaluated for the study of major molecules like Linalool, Caryophyllene, Estragole and Eugenol in the five *Ocimum* species *viz. O. basilicum Linn., O. canum Sims., O. citriodorum, O. gratissimum Linn., O. sanctum Linn.* using gas chromatography coupled with a single quadrupole mass spectrometry (GC-MS). In the present study, GC-MS analysis is applied for first time to evaluate essential oil of *Ocimum* species in order to quantify the major target molecule and simultaneously the characterization and their identification of untargeted molecules during the profiling of each *Ocimum* species. Identification of molecules showed the extensive variations in their compositions by using chemometrics to distinguish between five closely related species of *Ocimum.*

## MATERIALS AND METHODS

### Sampling and source of essential oils

All essential oil of *Ocimum* species were provided by Dabur Research and Development Centre (DRDC) inventory division and authenticated by Bio Resources Development (BRD) Division after extraction from aerial parts of *Ocimum* species *viz. Ocimum basilicum* Linn., *Ocimum canum Sims., Ocimum citriodorum, Ocimum gratissimum Linn., Ocimum sanctum Linn.* of fresh herb and were subjected to hydro-distillation in a Clevenger type apparatus for 3 hours. The extracted essential oils were measured directly in the extraction burette. The oil samples were dehydrated over anhydrous sodium sulfate ($Na_2SO_4$) and kept in a cool and dark place until further analysis [14].

### Chemical and reagents

All standards Linalool ($C_{10}H_{18}O$, Purity 97%); Caryophyllene ($C_{15}H_{24}$, Purity 80%), Estragole ($C_{10}H_{12}O$, Purity 98%) and Eugenol ($C_{10}H_{12}O_2$, Purity 98.5%) were purchased from Supelco (Bellefonte, PA, USA). Methanol was LC-MS CHROMASOLV, ≥ 99.9 % grade and purchased from Honeywell Research Chemicals (Germany). Analytical grade sodium sulphate anhydrous was obtained from Sigma Aldrich. Ultra-pure water was prepared by using a Milli-Q water purification system (Millipore, Molsheim, France).

### Standard solutions preparation

Stock standard solutions of each compound (4.202, 3.342, 4.402 & 4.085 mg/ml respectively corresponds to conc. of Linalool, Caryophyllene, Estragole and Eugenol) were prepared by weighing of powder or liquid and dissolved in 50 ml of Methanol. Standard solutions were stored in a refrigerator ($T \leq 5$ °C). Mix intermediate and working standard solution was prepared by adequate dilution of the corresponding stock solution with acetone and stored under refrigeration ($T \leq 5$ °C).

### GC-MS analysis

Before analysing essential oil of *Ocimum* species in order to quantify target molecule, each sample 0.2 g approx. was weighed into 20 mL amber colour volumetric flasks. 5 ml methanol was added and shake well to homogenize and made up to the desired volume. Filter through 0.45µ membrane filter & filtrate 0.5 mL was used for the quantification of targeted molecules in basil oil samples and in case of higher concentration of target analyte diluting samples as per appropriate manner which to become under desired linearity range. The samples for identification of untargeted molecules were prepared by diluting 1:5 ratio in methanol which was subjected to injection by GC-MS.

### Gas chromatography-mass spectrometry (GC-MS) Quantitative analysis

The GC–MS system consisted of a model Agilent 8890 Gas Chromatograph coupled with PAL RTC 120 (Series 2) autosampler and model 5977 C GC/MSD Single quadrupole mass detector was used for analysis followed by selected ion monitoring (SIM) mode (Agilent Technologies, Santa Clara,) equipped with Agilent CP9205; Agilent make VF-Wax MS capillary column (30 m × 250 µm ID x 0.25 µm) column. The carrier gas Helium was used (99.999% purity) with flow rate of 1.2 mL/min. The volume of the sample utilized was 1 µL, accompanied by a split ratio of 100:1. The temperature program for the oven commenced at 80 °C for a duration of 2 minutes, subsequently increasing at a rate of 5 °C per minute to reach 120 °C, where it was maintained for 1 minute. Following this, the temperature was elevated at a rate of 30 °C per minute to 220 °C, which was sustained for 11 minutes. A post-run phase of 3 minutes at 230 °C concluded the procedure. The overall duration of the run was approximately 28 minutes, inclusive of a solvent delay of 5 minutes. The temperatures of the injection port and the electron transfer line were set at 220 °C and 280 °C, respectively. Mass spectra were obtained using electron ionization mode (EI) at an energy of 70 eV, while selective ion monitoring (SIM) was employed for quantification, with a dwell time of 13 ms per ion. The typical retention times recorded were 9.84 minutes for Linalool, 11.05 minutes for Caryophyllene, 12.32 minutes for Estragole, and 16.10 minutes for Eugenol. The characteristic ions (m/z) and retention times for these four analytes are detailed in Table 1.

### Qualitative analysis and Statistical Evaluation of Analytical Data

The GC-MS analyses of samples were performed on Agilent 8890 Gas Chromatograph coupled with PAL RTC 120 (Series 2) autosampler and model 5977 C GC/MSD Single quadrupole mass detector, selected a HP-5MS UI capillary column (30 m × 250 µm ID x 0.25 µm film thickness) due to more detailed study of screening of compounds qualitatively i.e., identification, composition and mass confirmation for essential oil of *Ocimum* variety . Helium (1 ml/min) was used as carrier gas with split ratio of 1:100. The oven temperature was programmed from 60°C to 240°C, at 3°C/min, and further

programmed to 270°C at 5°C/min followed by post run was 4 min at 305 °C. The injector and mass transfer line temperature were 250°C. Other MS conditions were used electron impact ionization mode (EI) at 70 ev with mass scan range of 30–500 m/z; Mass Source and Quad temperature were 230°C and 150°C respectively.

Data processing for identification of volatile compounds of particular components of basil essential oil were achieved by comparing the retention indices (RI) determined by using C7 - C30 Saturated Alkanes Calibration Standard (Sigma-Aldrich, St. Louis, MO, USA) and mass spectra fragmentation patterns of each compound with RI and mass spectra present in the Wiley Registry 12th Edition / NIST 2020 Mass Spectral Library by MassHunter Workstation Qualitative Analysis Version 10.0.10305.0 (Agilent Technologies, Palo Alto, CA, USA) as well as with literature data [14]. Parameters of calibration lines of the selected components (Linalool, Caryophyllene, Estragole and Eugenol) present in the basil essential oils were calculated by using MassHunter Workstation Quantitative Analysis Version 12.0.893.1 (Agilent Technologies, Palo Alto, CA, USA) and Agilent MassHunter WorkStation Mass Profiler Professional (MPP) version 15.1 was used for the statistical evaluation of the results.

## RESULTS

### Quantification of four significant constituents in *Ocimum* oil using GC-MS

The essential oil constituents chosen for quantitative analysis were selected due to their scientific significance and pharmacological properties, identified as the predominant active volatile compounds in specific *Ocimum* species [1,14,15]. The five basil varieties examined revealed Linalool, Caryophyllene, Estragole, and Eugenol as key components, highlighting their relevance in the pharmaceutical, food, and cosmetic sectors. This study employed the GC-MS analytical technique to enhance the identification and quantification processes through selective ion monitoring (SIM) mode. The analytical method was developed in accordance with ICH guidelines, addressing calibration parameters such as linear range, correlation coefficient ($R^2$), slope, and interface, as well as specificity, sensitivity, and limits of detection and quantification (LOD and LOQ), determined via a signal-to-noise ratio approach. The mass selection criteria for identification and quantification using GC-MS were guided by the SANTE 11312/2021 [16] framework (Fig. 1). The performance and calibration parameters obtained are summarized in Table 2, indicating that the quantitation limits and linear ranges were suitable for the sensitive and reliable determination of the essential components in the basil oils extracted from the five varieties studied.

Based on our quantitative evaluation of GC-MS, individual concentrations of selected components were detected in the studied basil varieties: (i) Three different of batches *Ocimum basilicum* Linn., Linalool (18.96, 17.63, 17.99 %w/w respectively); Caryophyllene (0.3, 0.3, 0.29 %w/w respectively); Estragole (54.88, 52.80, 51.03 %w/w respectively) and Eugenol were not detected in all three batches, (ii) Three different of batches *Ocimum canum Sims.*, Linalool (70.05, 70.46, 71.97 %w/w respectively); Caryophyllene (3.43, 3.36 and 3.45 %w/w respectively); Eugenol (0.78, 0.77, 0.81 %w/w respectively) and Estragole were not detected in all three different batches, (iii) Three different of batches *Ocimum citriodorum.*, Linalool (6.11, 6.32, 6.44 %w/w respectively); Caryophyllene (33.32, 34.49, 35.28 %w/w respectively); Eugenol (10.31, 10.68, 10.94 %w/w respectively) and Estragole were not detected in all three different batches, (iv) *Ocimum gratissimum Linn.*, in three different of batches, Linalool (1.78, 1.73, 1.80 %w/w respectively); Caryophyllene (10.88, 10.54, 11.0 %w/w respectively); Eugenol (66.32, 63.92, 67.09 %w/w respectively) and Estragole not detected in all three different batches, (v) *Ocimum sanctum Linn.*, Caryophyllene (19.87, 19.48, 20.22 %w/w respectively); Eugenol (57.36, 56.13, 58.16 %w/w respectively), Linalool and Estragole both were not detected in all three different batches; concentration data is summarized in (Table 3). Box plots were used to depict the relative distribution of these marker compounds in the five species *Ocimum* varieties (Fig. 2).

### Identification of basil oil components in *Ocimum* species

Concurrently, an examination of the compounds identified through GC-MS profiling revealed significant variations in the chemical composition of specific species. These differences may be attributed to their presence in distinct eco-climatic zones and variations in edaphic factors. Our findings indicate that the majority of the identified compounds possess biological significance. The separation and identification of basil oil constituents across five different *Ocimum* varieties were conducted utilizing a GC-MS method specifically developed for this analysis. This method relied on the retention index (RI) established through a homologous series of n-alkanes (C7 - C30 Saturated Alkanes) Calibration Standard (Sigma-Aldrich, St. Louis, MO, USA) under consistent experimental conditions. Additionally, co-injection with standards or known essential oil constituents, along with mass spectra library searches (Wiley Registry 12th Edition / NIST 2020 Mass Spectral Library), facilitated the identification process. The relative quantities of individual components were assessed using the peak area normalization method and expressed as percentages (%), as illustrated in (Fig. 3).

A detailed comparative list of compounds across different species is illustrated in Table 4. The chemical profile of Ocimum oil indicates the presence of one hundred fifty volatile constituents, which are found in all five varieties of basil oil. Our investigations into the essential oils from *Ocimum* species demonstrate that the compounds present in significant quantities among the accessions of various species include α-Pinene, D-Limonene, (E)-Linalool oxide A, cis-Linalool oxide, Linalool, Estragole, Eugenol, Methyleugenol, α-Elemene, α-Neoclovene, Caryophyllene, Aromandendrene, α-Humulene, δ-Cadinene, α-Cubebene, α-Bisabolene, Caryophyllenyl alcohol, Caryophyllene oxide, and Acetyl eugenol.

The data presented in Table 4 reveal that *O. Citriodorum* essential oil is characterized by the presence of sixty-two volatile compounds, as illustrated in Figure 3. Caryophyllene emerged as the primary sesquiterpene, contributing 17.87-17.93% to the oil's composition. In addition, a variety of other compounds were found in notable concentrations. D-Limonene (0.64-2.64 %), Linalool (3.62-5.62 %), Eugenol (11.5-13.5 %), Copaene (1.59-1.67 %) , Cinnamaldehyde dimethyl acetal (2.38 -4.17 %), α-Elemene (2.55-2.75 %), α-Neoclovene (3.52-3.79 %), , γ-Cadinene (1.23-1.25 %), α-Longipinene (1.06-1.07 %), Aromandendrene (7.11-7.17 %), α-Humulene (6.02-6.1), δ-Cadinene (1.99-2.02 %), Caryophyllenyl alcohol (4.78-4.83 %), Fonenol (2.37-2.61 %), Ledol (1.31-1.36 %), Acetyl eugenol (1.06-1.08 %) and Rosifoliol (6.68-6.87 %). Major Caryophyllene content found in the range of 17.87-17.93 % in *O. Citriodorum* oil which was on the higher side as compared to the previous literature report on this species [14].

A comprehensive chemical characterization of *O. basilicum* oil identified thirty-four compounds that constitute the total oil profile (illustrated in Fig. 3). The analysis indicated that phenylpropanoids were the primary components, with Estragole accounting for 49.49–61.05% of the oil. Additionally, other notable compounds included (E)-Linalool oxide A (0.95-3.73%), Linalool (14.3-20.33%), α-Longipinene (1.26-1.83%), and 4-Methoxycinnamaldehyde (2.77-5.19%).

The oil analysis of *O.gratissimum* showed twenty one compounds constituting of about the oil (Fig. 3). The composition of basil oil was characterized by a predominance of phenylpropanoids, particularly Eugenol, which represented between 58.67% and 59.83% of the total oil content, followed by major sesquiterpene as Caryophyllene (17.69-18.61 %) was identified, monoterpene hydrocarbons as Linalool was (3.38–3.33 %). Other compounds present in substantial amounts were p-Cymene (1.42–1.57 %), 2-Bornanone (3.87–3.89 %), α-Terpineol

(1.18-1.19 %), α-Humulene (4.25-4.41 %) and Caryophyllene oxide (2.38–3.36 %).

Profiling of the essential oil from *O. canncum* identified a total of thirty-eight compounds that comprise the oil (Fig. 3). The analysis indicated that monoterpene hydrocarbons were the most abundant, with Linalool representing 50.81–51.26% of the composition. Caryophyllene followed at 7.66-7.98%, and α-Humulene was recognized as the major sesquiterpene, present at 3.71-3.8%. Other significant compounds included α-Pinene (1.59-1.61%), D-Limonene (3.05-3.3%), (E)-Linalool oxide A (1.94-2.06%), Eugenol (1.15-1.39%), Methyleugenol (2.92-2.94%), and α-Bisabolene (4.53-5.22%).

A total of twenty-six volatile compounds were identified in the essential oil of O. sanctum, as illustrated in Figure 3. The profiling of the essential oil revealed that phenylpropanoids were the predominant constituents, comprising 52.21-52.6% of the total oil. The major sesquiterpene identified was Caryophyllene, which accounted for 25.43-26.16%. Additionally, other compounds present in notable quantities included Eucalyptol (1.01-1.05%), Citronellol (0.88-0.97%), Copaene (0.89-0.93%), α-Humulene (6.91-6.97%), and δ-Cadinene (1.43-1.5%).

**Principle component analysis (PCA) and Hierarchical clustering analysis (HCA)**

PCA serves as an unsupervised statistical method aimed at reducing the dimensionality of extensive datasets, thereby highlighting distinctions among the volatile compositions of various Ocimum essential oil varieties. A total of one hundred fifty selected volatile compounds were analyzed using 3D PCA. The first three principal components explained approximately 80% of the total variance in the original dataset, with contributions of 36.4%, 27.57%, and 15.54%, respectively. The 3D score plot demonstrated a clear differentiation between O. Citriodorum essential oil and the other four Ocimum species, indicating that the selected compounds were significant for sample discrimination (Fig. 4). In a similar vein, one hundred nineteen selected volatile compounds were analyzed using Hierarchical Cluster Analysis (HCA). HCA is an effective technique for identifying subgroups within a dataset, allowing for the clustering of observations with similar abundance profiles. The results are illustrated in a dendrogram (Fig. 5), which classified Ocimum essential oils into five distinct clusters based on the compounds detected in five different basil oil varieties, with a cut-off value of 0.05.

**Table 1:** Characteristic ions (m/z) and retention times of the four analytes

| Compound | RT (min) | Quantifier m/z | Qualifier 1 | Qualifier 2 |
|---|---|---|---|---|
| Linalool | 9.84 | 93.1 | 69.1 | 81.1 |
| Caryophyllene | 11.054 | 91.1 | 105 | 133 |
| Estragole | 12.328 | 148 | 65.1 | 77.1 |
| Eugenol | 16.104 | 91 | 131 | 55.1 |

**Table 2:** Performance parameters and calibration data for determined basil essential oil components

| Compound | Linear range (mg/ml) | Correlation coefficient ($R^2$) | Calibration Equation | LOD (mg/ml) | LOQ (mg/ml) |
|---|---|---|---|---|---|
| Linalool | 0.00525 - 0.52523 | 0.999 | y = 51842.291257 * x - 41173.132684 | 0.00015 | 0.00045 |
| Caryophyllene | 0.00418 - 0.41772 | 0.999 | y = 68331.141440 * x - 47543.974359 | 0.00012 | 0.00035 |
| Estragole | 0.0055 - 0.5502 | 0.998 | y = 109388.052938 * x - 29053.613498 | 0.00015 | 0.00044 |
| Eugenol | 0.00511 - 0.51065 | 0.998 | y = 39126.230763 * x - 66145.018404 | 0.00012 | 0.00035 |

**Table 3:** Quantified basil oil components in five varieties of *Ocimum* Species

| Batch No. | Variety | Linalool | | Caryophyllene | | Estragole | | Eugenol | |
|---|---|---|---|---|---|---|---|---|---|
| | | (mg/ml) | (% w/w) | (mg/ml) | (% w/w) | (mg/ml) | (% w/w) | (mg/ml) | (% w/w) |
| **1** | | 189.6 | 18.96 | 2.98 | 0.3 | 548.80 | 54.88 | 0.00 | 0 |
| **2** | *O. Basilicum* | 176.3 | 17.63 | 3.02 | 0.3 | 527.99 | 52.8 | 0.00 | 0 |
| **3** | | 179.88 | 17.99 | 2.93 | 0.29 | 510.29 | 51.03 | 0.00 | 0 |
| **1** | | 700.46 | 70.05 | 34.25 | 3.43 | 0.00 | 0 | 7.76 | 0.78 |
| **2** | *O. Canum* | 704.6 | 70.46 | 33.65 | 3.36 | 0.00 | 0 | 7.72 | 0.77 |
| **3** | | 719.74 | 71.97 | 34.49 | 3.45 | 0.00 | 0 | 8.06 | 0.81 |
| **1** | | 61.07 | 6.11 | 333.15 | 33.32 | 0.00 | 0 | 103.09 | 10.31 |
| **2** | *O. Citriodorum* | 63.15 | 6.32 | 344.88 | 34.49 | 0.00 | 0 | 106.81 | 10.68 |
| **3** | | 64.42 | 6.44 | 352.83 | 35.28 | 0.00 | 0 | 109.38 | 10.94 |
| **1** | | 17.84 | 1.78 | 108.82 | 10.88 | 0.00 | 0 | 663.19 | 66.32 |
| **2** | *O. Gratissimum* | 17.34 | 1.73 | 105.41 | 10.54 | 0.00 | 0 | 639.23 | 63.92 |
| **3** | | 18.03 | 1.8 | 110.05 | 11 | 0.00 | 0 | 670.90 | 67.09 |
| **1** | | 0 | 0 | 198.67 | 19.87 | 0.00 | 0 | 573.58 | 57.36 |
| **2** | *O. Sanctum* | 0 | 0 | 194.79 | 19.48 | 0.00 | 0 | 561.27 | 56.13 |
| **3** | | 0 | 0 | 202.16 | 20.22 | 0.00 | 0 | 581.58 | 58.16 |

**Table 4:** Comparative basil oil composition (Content %) of five *Ocimum* Species- Identification and Characterization

| | | | *O. Basilicum* (3) | | | *O. Canum* (3) | | | *O. Citriodorum* (3) | | | *O. Sanctum* (3) | | | *O. Gratissimum* (3) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Name** | **RI (Exp.)** | **RI (Lit.)** | **1** | **2** | **3** | **1** | **2** | **3** | **1** | **2** | **3** | **1** | **2** | **3** | **1** | **2** | **3** |
| α-Pinene | 934 | 934 | - | - | - | 1.59 | 1.65 | 1.61 | - | - | - | 0.16 | 0.17 | 0.16 | - | - | - |
| Sulcatone | 986 | 984 | - | - | - | 0.39 | 0.39 | 0.39 | - | - | - | - | - | - | - | - | - |
| β-Pinene | 973 | 972 | - | - | - | 0.28 | 0.29 | 0.32 | 0 | 0.19 | 0.2 | - | - | - | - | - | - |
| p-Cymene | 1020 | 1018 | - | - | - | 0.19 | 0.19 | 0.2 | - | - | - | 0.36 | 0.36 | 0.31 | 1.42 | 1.45 | 1.57 |
| D-Limonene | 1029 | 1027 | - | - | - | 3.05 | 3.06 | 3.3 | 0.64 | 0.61 | 0.64 | 0.18 | 0.18 | 0.17 | - | - | - |
| Eucalyptol | 1031 | 1029 | 0.20 | 0.20 | 0.53 | 0.56 | 0.56 | 0.56 | - | - | - | 1.01 | 1.05 | 1.02 | - | - | - |
| β-Ocimene | 1033 | 1031 | - | - | - | - | - | - | 0.18 | 0.18 | 0.2 | - | - | - | - | - | - |
| (E)-Linalool oxide A | 1073 | 1078 | 0.95 | 3.79 | 3.73 | 2.06 | 2.02 | 1.94 | - | - | - | - | - | - | - | - | - |
| δ-2-Carene | 1019 | 1011 | - | - | - | - | - | - | 0.31 | 0.29 | 0.3 | - | - | - | - | - | - |
| cis-Linaloloxide | 1080 | 1078 | 0.95 | 3.66 | 3.60 | 2.73 | 2.7 | 2.62 | - | - | - | - | - | - | - | - | - |
| Linalool | 1101 | 1097 | 14.30 | 13.96 | 20.33 | 51.26 | 50.99 | 50.81 | 3.62 | 3.66 | 3.57 | - | - | - | 3.28 | 3.33 | 3.33 |
| E-myrtenol | 1114 | - | - | - | - | 0.27 | 0.26 | 0.25 | - | - | - | - | - | - | - | - | - |
| 2-Bornanone | 1146 | - | - | - | - | 2.10 | 2.11 | 2.08 | - | - | - | - | - | - | 3.87 | 3.88 | 3.89 |
| Borneol | 1157 | 1164 | - | - | - | - | - | - | - | - | - | 0.45 | 0.48 | 0.48 | - | - | - |
| α-Terpineol | 1191 | 1188 | - | - | - | - | - | - | 0.38 | 0.39 | 0.37 | - | - | - | 1.18 | 1.19 | 1.19 |
| cis-Ocimenol | 1192 | 1194 | - | - | - | 1.86 | 1.88 | 1.83 | - | - | - | - | - | - | - | - | - |
| Linalool-7-OH | 1195 | - | 1.03 | 1.06 | 0.34 | - | - | - | - | - | - | - | - | - | - | - | - |
| β-Terpinyl acetate | 1198 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.29 | 0.29 | 0.3 |
| Estragole | 1206 | 1202 | 49.49 | 48.55 | 61.05 | 0.75 | 0.79 | 0.78 | - | - | - | 0.43 | 0.46 | 0.46 | - | - | - |
| Anisaldehyde | 1254 | - | 0.65 | 0.62 | 0.00 | - | - | - | - | - | - | - | - | - | - | - | - |
| Geraniol | 1254 | 1252 | - | - | - | - | - | - | 0.24 | 0.25 | 0.24 | - | - | - | - | - | - |
| (E)-Cinnamaldehyde | 1270 | 1264 | - | - | - | - | - | - | 0.94 | 1.19 | 1.62 | - | - | - | - | - | - |
| D-neoisomenthol | 1273 | - | 0.46 | 0.46 | 0.00 | 0.22 | 0.18 | 0.2 | - | - | - | - | - | - | - | - | - |
| Decahydronaphthalen-2-ol | 1289 | - | - | - | - | 2.50 | 2.48 | 2.48 | - | - | - | - | - | - | - | - | - |
| Neryl acetal | 1358 | 1362 | 0.35 | 0.35 | 0.91 | - | - | - | - | - | - | - | - | - | - | - | - |
| Geranyl acetal | 1381 | 1380 | 0.73 | 0.75 | 1.73 | - | - | - | - | - | - | - | - | - | - | - | - |
| α-Cubebene | 1351 | 1353 | - | - | - | - | - | - | - | - | - | 0.23 | 0.22 | 0.22 | 0.18 | 0.18 | 0.2 |
| Nerolidol Z and E | 1359 | - | 0.30 | 0.33 | 0.00 | - | - | - | - | - | - | - | - | - | - | - | - |
| Eugenol | 1360 | 1361 | - | - | - | 1.15 | 1.29 | 1.39 | 11.5 | 11.49 | 11.61 | 52.6 | 52.14 | 52.21 | 58.67 | 59.29 | 59.83 |
| α-Guaiene | 1367 | 1365 | - | - | - | - | - | - | 0.54 | 0.56 | 0.52 | - | - | - | - | - | - |
| Anisaldehyde dimethyl acetal | 1368 | - | 0.32 | 2.85 | 2.91 | - | - | - | - | - | - | - | - | - | - | - | - |
| Longifolene-(V4) | 1372 | - | - | - | - | - | - | - | 0.52 | 0.52 | 0.5 | - | - | - | - | - | - |
| Ylangene | 1373 | 1375 | - | - | - | - | - | - | 0.26 | 0.23 | 0.21 | - | - | - | - | - | - |
| Copaene | 1377 | 1372 | - | - | - | 0.23 | 0.23 | 0.22 | 1.67 | 1.63 | 1.59 | 0.89 | 0.93 | 0.9 | 0.88 | 0.85 | 0.8 |
| Isomyrcenylacetate | 1387 | - | 0.20 | 0.22 | 0.00 | - | - | - | - | - | - | - | - | - | - | - | - |
| Cinnamaldehyde dimethyl acetal | 1401 | - | - | - | - | - | - | - | 2.38 | 3.09 | 4.17 | - | - | - | - | - | - |
| Methyleugenol | 1405 | 1396 | 0.16 | 0.18 | 0.00 | 2.94 | 2.92 | 2.92 | - | - | - | - | - | - | - | - | - |
| α-Elemene | 1409 | 1410 | - | - | - | - | - | - | 2.75 | 2.74 | 2.55 | 0.17 | 0.16 | 0,16 | - | - | - |
| α-Neoclovene | 1415 | 1416 | - | - | - | - | - | - | 3.79 | 3.52 | 3.77 | - | - | - | - | - | - |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Caryophyllene | 1421 | 1420 | 0.40 | 0.43 | 0.40 | 7.66 | 7.84 | 7.98 | 17.93 | 17.98 | 17.87 | 26.16 | 25.83 | 25.43 | 17.69 | 17.89 | 18.61 |
| β-Gurjunene | 1431 | 1429 | - | - | - | - | - | - | 0.53 | 0.53 | 0.52 | 0.18 | 0.19 | 0.21 | | | |
| α-Longipinene | 1437 | 1438 | 1.26 | 1.29 | 1.83 | | | | 1.06 | 1.07 | 1.07 | - | - | - | 0.75 | 0.74 | 0.74 |
| α-Cadinene | 1441 | 1442 | - | - | - | - | - | - | 0.21 | 0.21 | 0.21 | - | - | - | - | - | - |
| β-Panasinsene | 1443 | - | - | - | - | - | - | - | 0.28 | 0.3 | 0.29 | - | - | - | - | - | - |
| Cedrene-V6 | 1446 | - | - | - | - | 0.60 | 0.59 | 0.6 | - | - | - | - | - | - | - | - | - |
| Isocaryophyllene | 1451 | - | - | - | - | 0.24 | 0.24 | 0.24 | - | - | - | - | - | - | - | - | - |
| Aromandendrene | 1454 | 1454 | - | - | - | - | - | - | 7.11 | 7.22 | 7.17 | - | - | - | - | - | - |
| α-Humulene | 1455 | 1455 | 0.00 | 0.00 | 0.26 | 3.71 | 3.77 | 3.8 | 6.02 | 6.1 | 6.05 | 6.93 | 6.97 | 6.91 | 4.25 | 4.28 | 4.41 |
| Longifolene | 1462 | - | - | - | - | - | - | - | 0.21 | 0.2 | 0.2 | - | - | - | - | - | - |
| Valencene | 1475 | - | - | - | - | - | - | - | - | - | - | 0.17 | 27.781 | 27.786 | - | - | - |
| δ-Selinene | 1482 | - | - | - | - | - | - | - | 0.19 | 0.19 | 0.19 | - | - | - | - | - | - |
| α-Curcumene | 1484 | - | - | - | - | 0.16 | 0.16 | 0.16 | - | - | - | - | - | - | - | - | - |
| Germacrene D | 1486 | - | - | - | - | 0.20 | 0.21 | 0.28 | - | - | - | - | - | - | - | - | - |
| α-Selinene | 1497 | - | - | - | - | - | - | - | 0.28 | 0.28 | 0.28 | 0.21 | 0.21 | 0.22 | | | |
| Neoalloocimene | 1497 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| γ-Cadinene | 1499 | 1511 | | | | 0.82 | 0.82 | 0.82 | - | - | - | - | - | - | - | - | - |
| γ-Muurolene | 1500 | - | - | - | - | - | - | - | 0.45 | 0.46 | 0.47 | - | - | - | - | - | - |
| γ-Selinene | 1502 | 1502 | - | - | - | 0.18 | 0.19 | 0.19 | - | - | - | - | - | - | - | - | - |
| α-Muurolene | 1503 | 1504 | - | - | - | - | - | - | 0.36 | 0.37 | 0.36 | 0.17 | 0.17 | 0.18 | | | |
| α-Himachalene | 1510 | - | 0.16 | 0.17 | 0.26 | 0.96 | 0.96 | 0.96 | 0.33 | 0.35 | 0.35 | - | - | - | - | - | - |
| D-Germacrene | 1516 | - | - | - | - | 0.41 | 0.41 | 0.43 | - | - | - | - | - | - | - | - | - |
| β-Spathulenol | 1517 | - | - | - | - | - | - | - | 0.7 | 0.7 | 0.71 | - | - | - | - | - | - |
| δ-Cadinene | 1525 | 1521 | - | - | - | 0.68 | 0.71 | 0.71 | 2.01 | 1.99 | 2 | 1.43 | 1.48 | 1.5 | 0.95 | 0.95 | 0.96 |
| Elemol | 1544 | 1542 | 0.21 | 0.22 | 2.75 | 4.53 | 4.89 | 5.22 | 0.78 | 0.76 | 0.77 | - | - | - | 0.3 | 0.41 | 0.33 |
| Guaiol | 1551 | 1552 | - | - | - | - | - | - | - | - | - | 0.17 | 0.19 | 0.2 | | | |
| Betulenol | 1554 | - | - | - | - | - | - | - | - | - | - | 0.29 | 0.28 | 0.25 | 0.35 | 0.37 | 0.35 |
| Humulene epoxide I | 1555 | 1556 | - | - | - | - | - | - | 0.25 | 0.24 | 0.25 | - | - | - | - | - | - |
| Bicyclogermacrene | 1558 | - | - | - | - | 0.57 | 0.55 | 0.55 | | | | - | - | - | - | - | - |
| Longifolenaldehyde | 1561 | - | - | - | - | 0.2 | 0.19 | 0.2 | 0.19 | 0.19 | 0.19 | - | - | - | - | - | - |
| 4-Methoxycinnamaldehyde | 1568 | - | 2.77 | 5.20 | 5.19 | - | - | - | | | | - | - | - | - | - | - |
| Caryophyllenyl alcohol | 1573 | - | - | - | - | - | - | - | 4.78 | 4.83 | 4.82 | - | - | - | - | - | - |
| Isospathulenol | 1579 | - | 0.00 | 0.19 | 0.00 | - | - | - | | | | - | - | - | - | - | - |
| Fonenol | 1581 | - | - | - | - | - | - | - | 2.47 | 2.37 | 2.61 | - | - | - | - | - | - |
| Caryophyllene oxide | 1585 | 1592 | 1.14 | 1.01 | 1.27 | 1.75 | 1.48 | 1.31 | 0.39 | 32.075 | 32.075 | 4.16 | 4.22 | 4.58 | 2.38 | 3.57 | 3.36 |
| Globulol | 1585 | 1587 | - | - | - | - | - | - | | | | - | - | - | - | - | - |
| α-Elemene | 1587 | 1590 | 1.29 | 1.33 | 0.00 | - | - | - | 0.28 | 0.28 | 0.25 | - | - | - | - | - | - |
| 2-Methoxybenzyl acetate | 1587 | - | - | - | - | - | - | - | | | | - | - | - | - | - | - |
| 2-Methoxy-4-butylphenol | 1589 | - | 1.20 | 1.27 | 0.00 | - | - | - | | | | - | - | - | - | - | - |
| Neoalloocimene | 1595 | - | - | - | - | - | - | - | 0.18 | 0.19 | 0.18 | - | - | - | - | - | - |
| Ledol | 1603 | - | - | - | - | - | - | - | 1.33 | 1.31 | 1.36 | - | - | - | - | - | - |
| Diisobutyl | 1608 | - | - | - | - | - | - | - | 0 | 0 | 0.22 | - | - | - | - | - | - |
| Humulene oxide II | 1611 | 1606 | 0.60 | 0.51 | 0.65 | 0.45 | 0.37 | 0.34 | | | | 0.6 | 0.64 | 0.72 | 0.46 | 0.42 | 0.27 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Widdrol | 1619 | 1620 | - | - | - | - | - | - | 0.6 | 0.59 | 0.54 | - | - | - | - | - | - |
| p-Methoxy-α-methyl-cinnamic acid | 1621 | - | 0.59 | 0.59 | 0.00 | - | - | - | | | | - | - | - | - | - | - |
| Nepetalactone | 1632 | - | - | - | - | - | - | - | 1.12 | 1.12 | 1.15 | - | - | - | - | - | - |
| Isolongifolol | 1634 | - | - | - | - | - | - | - | 0.3 | 0.36 | 0.42 | - | - | - | - | - | - |
| γ-Eudesmol | 1638 | 1641 | - | - | - | - | - | - | | | | 0.18 | 0.21 | 0.23 | 0.19 | 0.11 | 0.15 |
| Duvatriendiol | 1647 | - | 0.26 | 0.27 | 0.00 | - | - | - | | | | - | - | - | - | - | - |
| Nerolidol-epoxyacetate | 1650 | - | 0.19 | 0.20 | 0.00 | - | - | - | 0.3 | 0.34 | 0.32 | 0.17 | 0.2 | 0.22 | 0.21 | 0.2 | 0.18 |
| Phenol, diethyl- | 1662 | - | 0.21 | 0.23 | 0.00 | - | - | - | | | | - | - | - | - | - | - |
| Solanesol | 1672 | - | - | - | - | - | - | - | 0.23 | 0.25 | 0.26 | - | - | - | - | - | - |
| α-Cyperone | 1674 | 1676 | - | - | - | 0.25 | 0.21 | 0.23 | | | | 0.76 | 0.92 | 0.69 | 0.34 | 0.64 | 0.56 |
| 3-Methyl-2-nitroso-phenol | 1695 | - | - | - | - | - | - | - | 0.51 | 0.49 | 0.49 | - | - | - | - | - | - |
| 3-Methoxycinnamaldehyde | 1702 | - | 0.35 | 0.81 | 0.84 | | | | | | | - | - | - | - | - | - |
| Diazoprogesterone | 1714 | - | - | - | - | - | - | - | 0.46 | 0.48 | 0.48 | - | - | - | - | - | - |
| 4-Hydroxy-2-methoxycinnamaldehyde | 1731 | - | - | - | - | - | - | - | - | - | - | | | | 0.2 | 0.19 | 0.18 |
| Coniferol | 1735 | - | - | - | - | - | - | - | - | - | - | 0.49 | 0.52 | 0.45 | 0.47 | 0.45 | 0.26 |
| Chrysanthenyl acetate | 1752 | - | 0.27 | 0.23 | 0.00 | 0.61 | 0.55 | 0.47 | - | - | - | - | - | - | - | - | - |
| Nerolidol-epoxyacetate | 1791 | - | 0.18 | 0.17 | 0.00 | - | - | - | - | - | - | - | - | - | - | - | - |
| β-cedrenoxide | 1871 | - | - | - | - | - | - | - | 0.22 | 0.21 | 0.22 | - | - | - | - | - | - |
| Neryl Acetate | 1912 | - | - | - | - | - | - | - | 0.43 | 0.44 | 0.44 | - | - | - | - | - | - |
| trans-D-dihydrocarveol | 1919 | - | - | - | - | - | - | - | 0.35 | 0.35 | 0.37 | - | - | - | - | - | - |
| m-Camphorene | 1954 | - | - | - | - | 0.41 | 0.4 | 0.38 | - | - | - | - | - | - | - | - | - |
| p-Camphorene | 1988 | - | - | - | - | 0.20 | 0.19 | 0.17 | - | - | - | - | - | - | - | - | - |
| Guaiacol, 6-allyl- | 2165 | - | - | - | - | - | - | - | 0.18 | 0.2 | 0.18 | - | - | - | - | - | - |
| Aceteugenol | 2188 | 2190 | - | - | - | - | - | - | 0.81 | 0.8 | 0.81 | - | - | - | - | - | - |
| Acetyl eugenol | 2205 | - | 0.15 | 0.15 | 0.00 | | | | 1.08 | 1.06 | 1.07 | - | - | - | - | - | - |
| Solanesol | 2339 | - | - | - | - | - | - | - | 0.26 | 0.24 | 0.24 | - | - | - | - | - | - |
| Geranyl linalool isomer B | 2394 | - | - | - | - | - | - | - | 0.51 | 0.5 | 0.5 | - | - | - | - | - | - |
| Rosifoliol | 2515 | 2517 | - | - | - | - | - | - | 6.68 | 6.8 | 6.87 | - | - | - | - | - | - |
| Geranyl linalool isomer | 2520 | 2521 | - | - | - | - | - | - | 0.24 | 0.2 | 0.33 | - | - | - | - | - | - |
| Tetramethrin | 2560 | - | - | - | - | - | - | - | 0.41 | 0.35 | 0.44 | - | - | - | - | - | - |
| trans-Isoeugenol | 2584 | - | - | - | - | - | - | - | 0.84 | 0.83 | 0.83 | - | - | - | - | - | - |
| Cyclomusk | 2597 | - | 0.31 | 0.32 | 0.00 | - | - | - | - | - | - | - | - | - | - | - | - |
| Spirojatamol | 2606 | - | - | - | - | - | - | - | 0.26 | 0.25 | 0.26 | - | - | - | - | - | - |
| Nitrosothymol | 2643 | - | - | - | - | - | - | - | 0.19 | 0.19 | 0.16 | - | - | - | - | - | - |
| Pogostol | 2699 | - | - | - | - | - | - | - | 0.22 | 0.18 | 0.19 | - | - | - | - | - | - |
| Guaiacol, 6-allyl- | 2742 | - | - | - | - | - | - | - | - | - | - | 0.33 | 0.4 | 0.17 | | | |
| Neoalloocimene | 2743 | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.18 | 0.00 | 0.00 |

Notes: Compounds were identified by comparison of their Retention indices (RI) and mass spectra with those of authentic compounds or with databases retention index, RI Exp. indicates retention indices determined on the HP-5MS column, RI Lit. indicates retention indices from literature (Raina et al., 2017) and NIST Library.

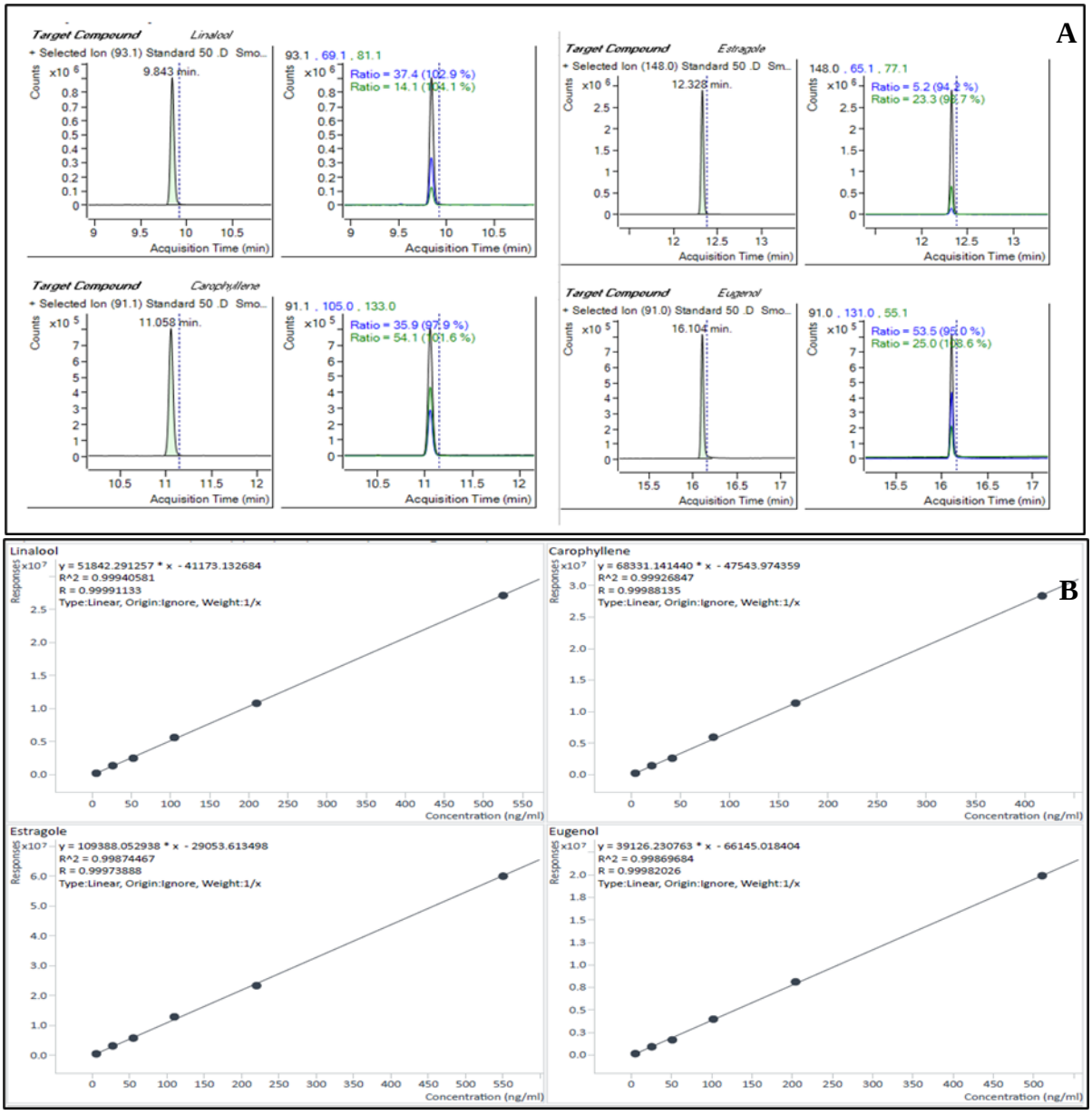



**Fig. 1:** GC-MS Quantitative Analysis - SIM chromatogram showing quantifier ion and qualifier ion of Linalool, Caryophyllene, Estragole and Eugenol in Standard (A); Linearity with six-point calibration (B)

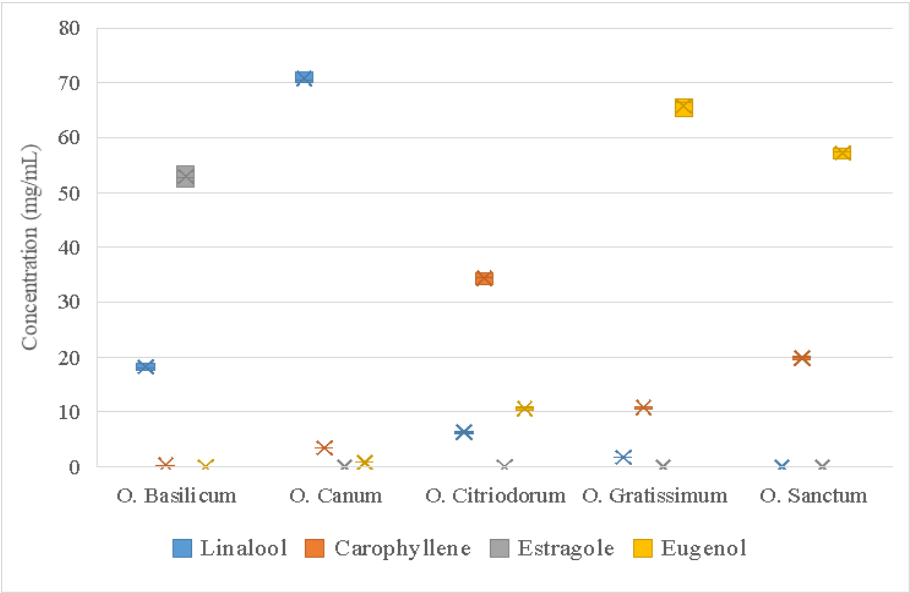
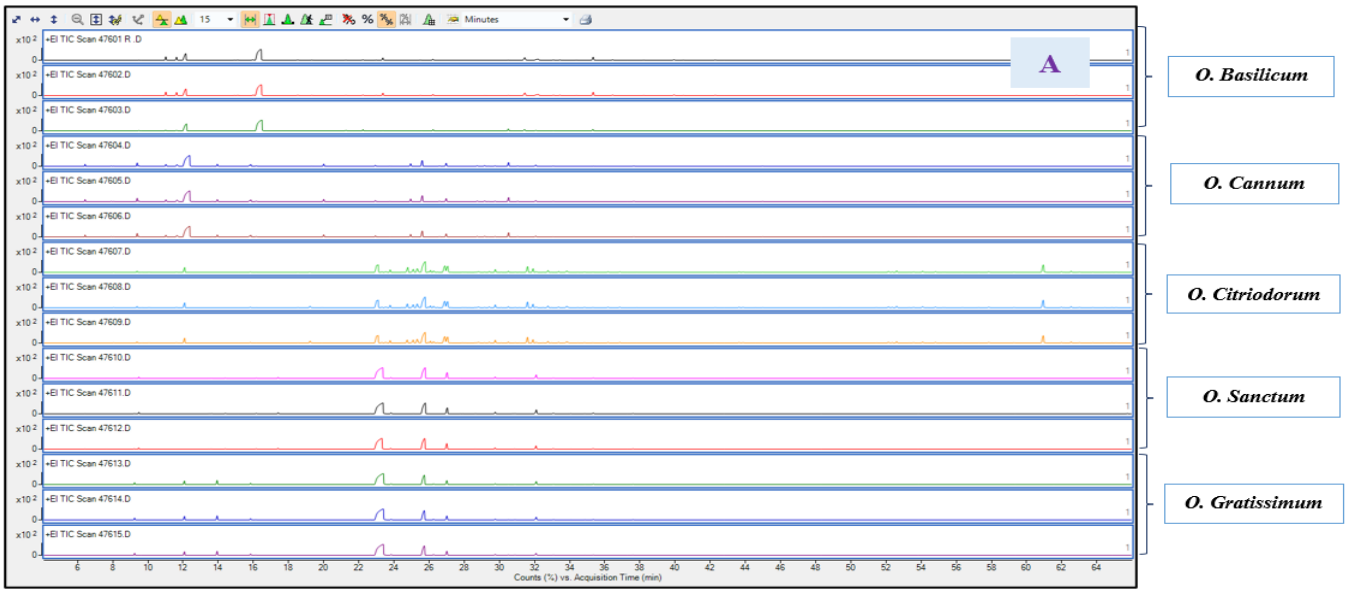
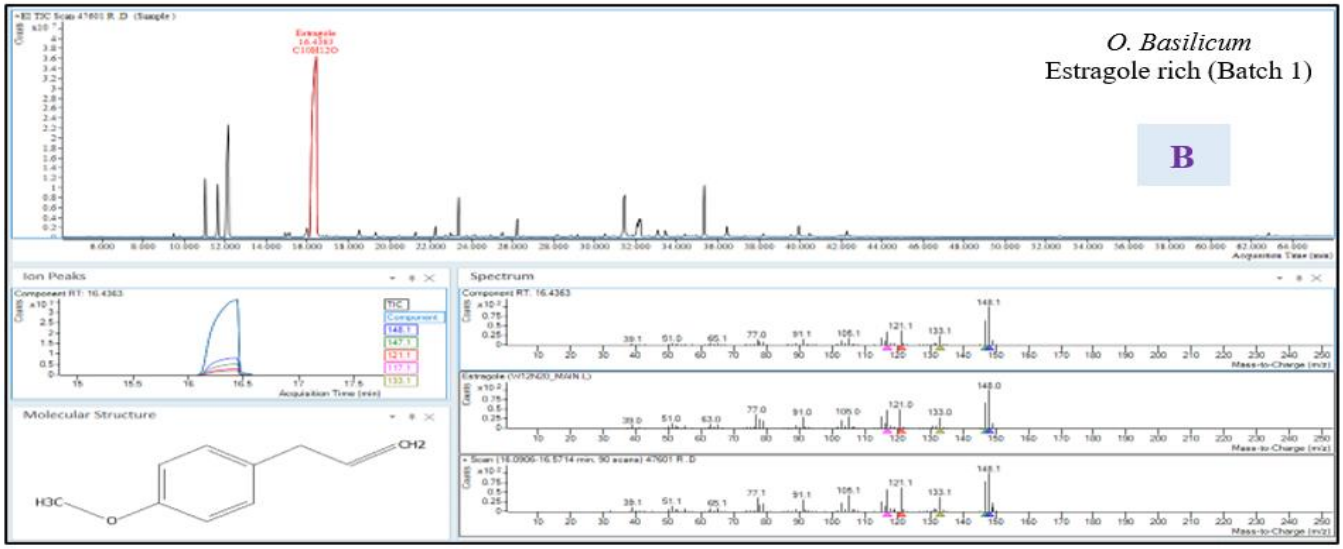
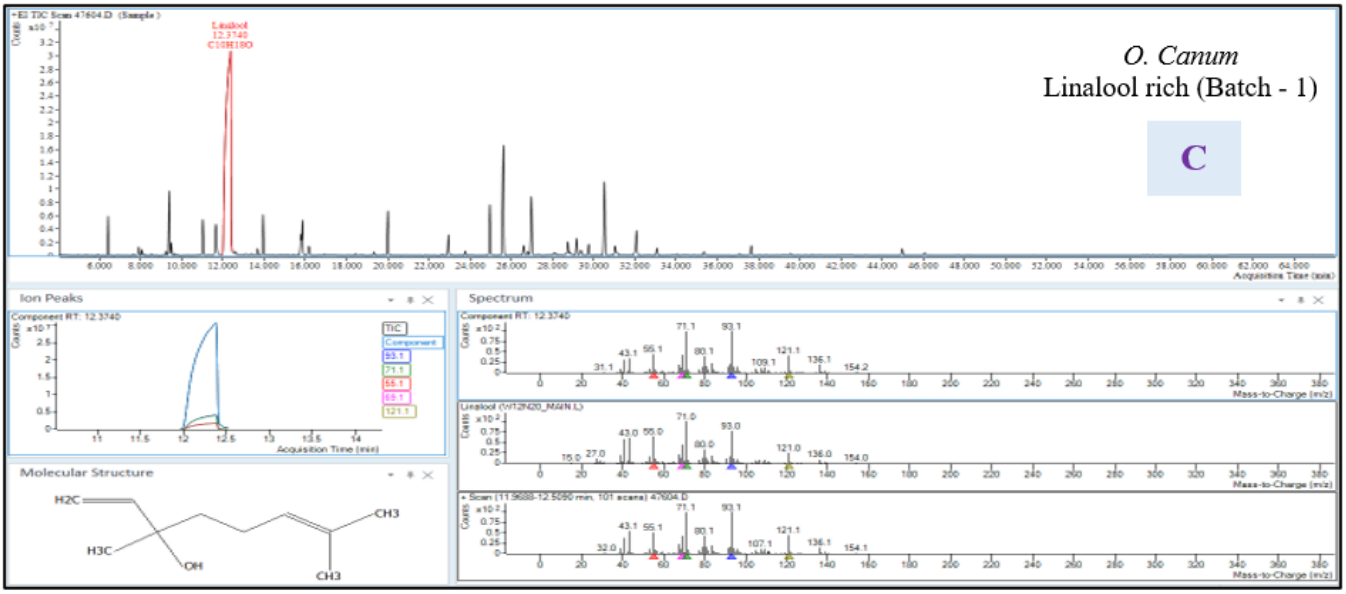



**Fig. 2:** Box plots showing normalized abundance and quantitative variation in the concentration (mg/ml) of Linalool, Caryophyllene, Estragole and Eugenol in basil varieties


A
O. Basilicum
O. Cannum
O. Citriodorum
O. Sanctum
O. Gratissimum


O. Basilicum
Estragole rich (Batch 1)
B
Ion Peaks
Spectrum
Molecular Structure


O. Canum
Linalool rich (Batch - 1)
C
Ion Peaks
Spectrum
Molecular Structure

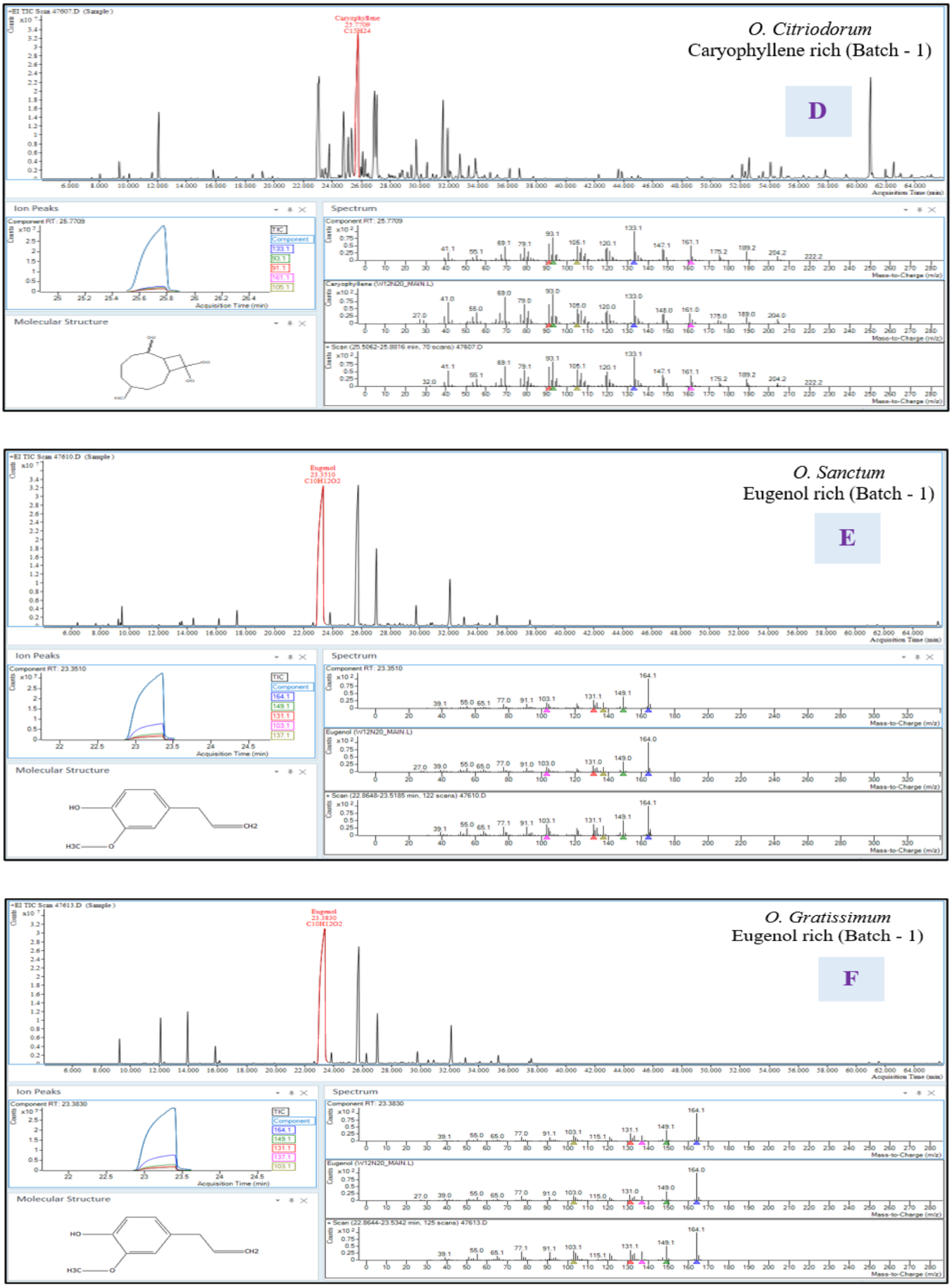



**Fig. 3:** GC-MS Chromatograms: (A) Comparative chromatographic profiles of all five basil varieties of essential oil; (B) *Ocimum basilicum* Linn; (C) *Ocimum canum Sims.* (D) *Ocimum citriodorum.;* (E) *Ocimum gratissimum Linn.;* (F) *Ocimum sanctum Linn.*

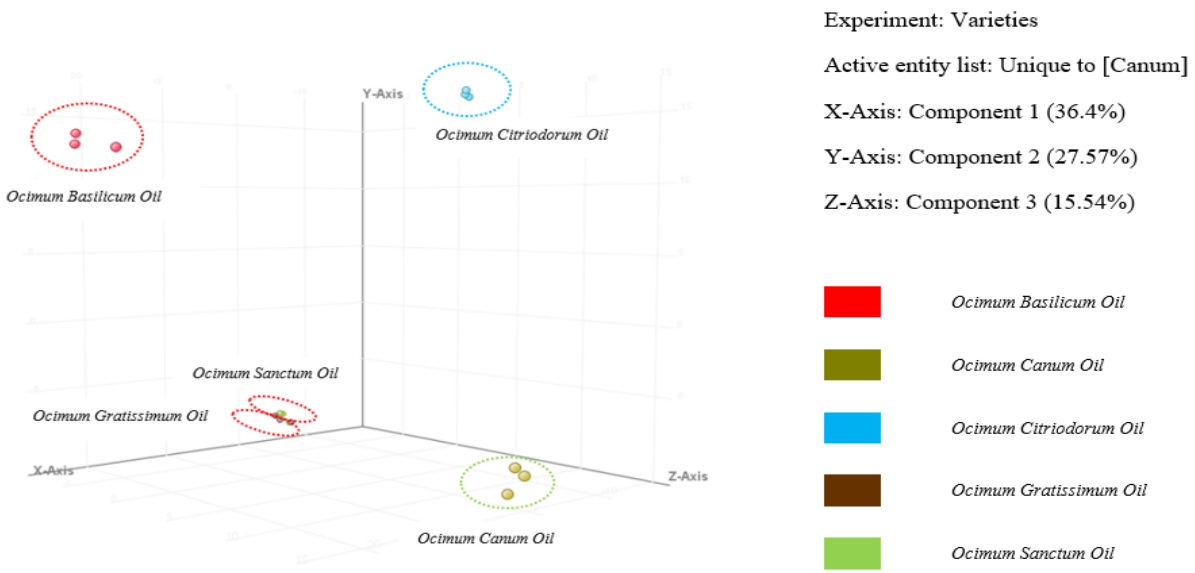



**Fig. 4:** 3D Principal component analysis (PCA) of five varieties of basil essential oil

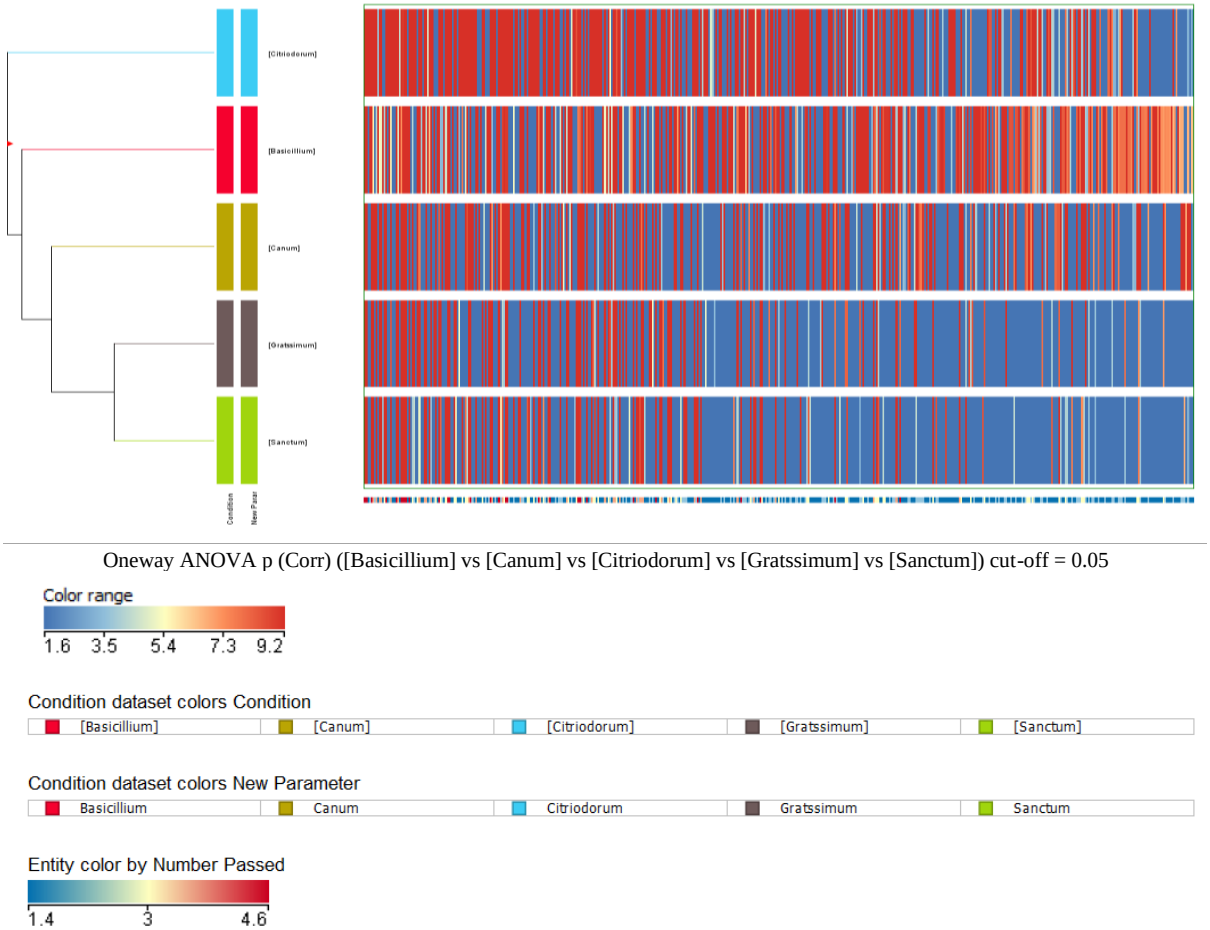



**Fig. 5:** Hierarchical clustering analysis (HCA) heat map for association of compounds detected in five different varieties of basil oil

## DISCUSSION

The research into the chemical composition of essential oils from 5 basil types constitute a vital clinical endeavour with far-accomplishing implications. These essential oils discover packages in diverse industries, consisting of pharmaceuticals, food, and cosmetics, making it vital to realise their difficult chemical profiles. Notably, this examines pioneers using a focused as nicely non-focused technique with GC-MS (Selective Ionization Mode for quantification and full screening mode for identification) to discover the presence of bioactive compounds in methanolic extracts of basil species [14,17,18]. This novel technique well-known shows a wealth of formerly uncharted chemical records that may have an effect on the nice and application of basil oils in various sectors. The GC-MS analysis, a cornerstone of this examines, exposed a terrific overall of 119 awesome peaks withinside the oils of those basil species. Each top represents a completely unique bioactive compound, and their identities have been showed through matching their retention instances and molecular formulation to recognised compounds as catalogued withinside the NIST library. Comparing the gas

chromatography (GC) records throughout 5 different *Ocimum* species, consisting of *O. basilicum*, *O. gratissimum*, *O. canum*, *O. sanctum* and *O. Citriodorum* offers a charming glimpse into the particular chemical profiles in their essential oils. *O. basilicum* distinguishes itself through its wealthy array of phenylpropanoids, drastically proposing the dominant compound Estragole, recognised for its candy and fragrant qualities, rendering it perfect for each culinary and medicinal packages [19,20,21]. *O. gratissimum* and *O. sanctum* evaluation showcases an awesome chemical signature in the main characterised through its excessive Eugenol content. Eugenol is widely known for its healing properties, consisting of antimicrobial and anti-inflammatory effects [22,23,24]. Meanwhile, *O. canum* is predominantly described through its monoterpene hydrocarbons, with Linalool taking the lead. Linalool, famed for its soothing and floral aroma, discover packages withinside the perfume and beauty industries [25,26]. *O. citriodorum* provides a chemical profile proposing phenylpropanoids, with Caryophyllene as a standout compound recognised for its anti-inflammatory properties, rendering *O. sanctum* treasured in medicinal contexts [19,27,28]. These discrepancies in chemical compositions a few of the *Ocimum* species underscore the various attributes and capability packages of each, underscoring the vital function of choosing the precise range for particular purposes, whether or not they be culinary, healing, or fragrant.

Principal Component Analysis (PCA) and Hierarchical Clustering Analysis (HCA) are valuable statistical techniques employed to derive insights from intricate datasets, particularly when managing a substantial number of variables, such as the volatile compounds present in *Ocimum* oils [29]. PCA enables the reduction of dimensionality in the dataset while preserving the most critical information. The initial three principal components account for roughly 80% of the variance in the data, and their depiction in a three-dimensional score plot illustrates significant separations, particularly between O. *Citriodorum* oil and the other four *Ocimum* species. This distinction suggests that the identified compounds are effective in differentiating among the various basil oil varieties. In contrast, HCA is a technique used to uncover subgroups or clusters within a dataset by aggregating observations specifically, the different basil oil samples based on similar compound abundance profiles. The outcomes are represented in a dendrogram, which classifies the basil oils into clusters according to the associations of the detected compounds. The determination of an appropriate cut-off value in the clustering process (in this case, 0.05) influences how closely compounds must be related to be included in the same cluster.

## CONCLUSION

The present study details the different composition of targeted molecules identified and quantified in five different varieties of basil oil. Based on the untargeted approach using the PCA model we were able to clearly differentiate the *Ocimum* species of basil oils and showed the percentage variation of the major molecules. The major chemical constituents like D-Limonene, Linalool, 2-Bornanone, Estragole, Eugenol, Methyleugenol, Caryophyllene, Aromandendrene, α-Humulene, Caryophyllenyl alcohol etc. were found in *Ocimum* species. Based on the scientific literature search no report was found for quantification study of basil oils in different *Ocimum* species following the mass criteria of GC-MS using SIM mode which clearly characterize the molecules in all the five varieties of *Ocimum* species. Simultaneously untargeted approach using PCA model also gives an additional information along with Hierarchical clustering analysis (HCA) heat map for association of compounds to show the resemblance between the *Ocimum* species of five different varieties. Consequently, this research is anticipated to aid in the chemical screening and quality assessment of raw materials for pharmaceutical and cosmetic applications. The variability in the chemical properties identified in this study underscores the necessity for precise botanical identification and an understanding of the plant's origin utilized in traditional medicine. This study can serve as a valuable source of enriched compounds that are significant for the production of various perfumery and healthcare products. Drawing from this experimental data, we can identify superior varieties of basil oil that demonstrate the diversity within Ocimum species.

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**Conflict of interest**

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