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Original article

Vibrational Spectroscopic Methods for the Identification and Distinction of Essential Oils in Genus *Ocimum* L.: A Chemometric Approach

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ABSTRACT

Essential oils are mainly analyzed by gas chromatography coupled with mass spectrometry (GC–MS). This is a time consuming technique which requires complex instrumentation assembly. Raman and FTIR spectroscopy are rapid and non-destructive techniques for qualitative characterization, differentiation of principal essential oil components, detection of adulteration in the oil, quality control of essential oil in industry as well as to distinguish different taxa. In the present study, five *Ocimum* spp. (*Ocimum tenuiflorum* L., *Ocimum gratissimum* L., *Ocimum* × *africanum* Lour., *Ocimum basilicum* L. and *Ocimum americanum* L) have been identified on the basis of essential oil by the Raman and FTIR spectroscopic techniques. It was found that methyl eugenol, eugenol, estragole, and camphor are major essential oils components present in *Ocimum* L. The results of PCA indicate that all five species of *Ocimum* L. form different clusters on the basis of principal essential oil components. The components of essential oil predicted by Raman spectroscopy correlate well with that of FTIR data. The present study demonstrates the use of Raman and FTIR spectroscopy techniques to distinguish taxa and characterization of essential oil components.

1. Introduction

The essential oils of *Ocimum* L. (Lamiaceae) are commercially valuable due to their huge applications in cosmetics, perfumes, medicines, aromatherapy and flavor for delicious food. Due to these usages the production and demands of these oils have been increased day by day (Lawrence, 1998; Padalia et al., 2013; Raina and Misra, 2017). There are different commercial applications of basil essential oil in different industrial products (Preedy, 2016; Sharmeen et al., 2021). According to a market research, the demand and production of essential oil will be increased during the years 2021–2027 (6Wresearch). The essential oils are complex secondary compounds of plant origin which are the mixture of different chemicals like terpene, phenylpropanoids, hydrocarbons and other sulphur or nitrogenous compounds. The characteristic aromatic smell of different basils is due to the presence of different major chemical components of essential oil in different ratio (Vina and Murillo, 2003). These oils components provide unique aroma to the basils. Some popular compounds like estragole or methyl chavicol, linalool and eugenol give specific taste and flavors to essential oils. The amount and composition of these characteristic

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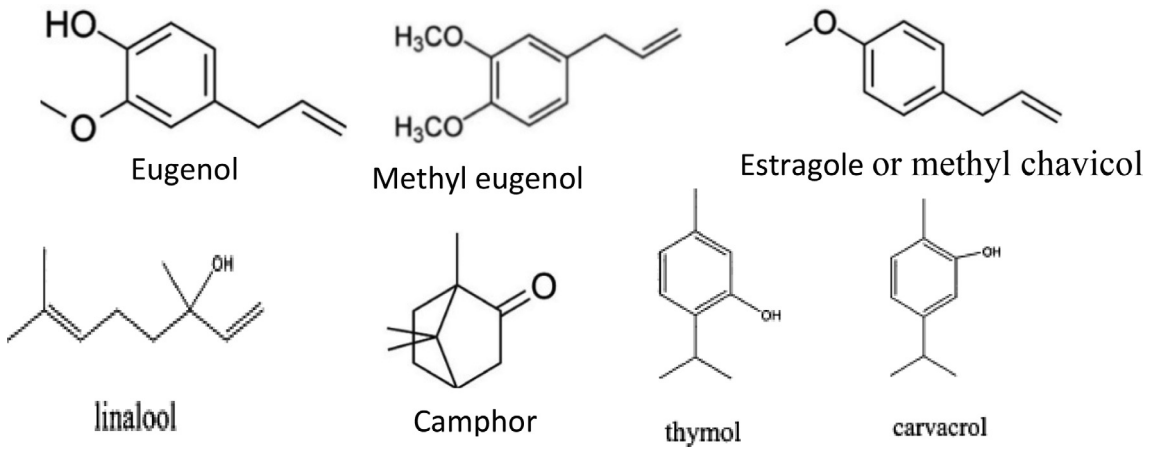

Fig. 1. Molecular structure of some essential oil components.

aromatic compounds depend on the chemodiversity (Satyal et al., 2016). The molecular structure of few essential oils components are given below (Fig. 1).

The distribution of plant essential oils is species specific. Basil oils are extracted mainly from foliar or aerial parts of the plants (Silva et al., 2016) in concentrated form through hydro-distillation or steam-distillation (Dhifi et al., 2016; El-Shemy, 2017). The characterization of essential oil not only depends on composition but also on growing season, area and environmental condition (Zouari et al., 2012). Besides, harvesting time is also important for recovery of maximum percentage of essential oil (Khalid et al., 2009; Yesil and Ozcan, 2021). Although essential oils are mixture of more than hundreds of chemical compounds but few main components or their definite ratios are responsible to distinguish the chemotypes in a species. The different species of *Ocimum* L. can be recognized on the basis of particular chemotypes (Table 1). The main components of *Ocimum* L. growing in India are estragole or methyl chavicol, eugenol, methyl eugenol, linalool, 1, 8-cineole. One can notice from the Table 1 that two main essential oil components viz. estragole or methyl chavicol (Simon et al., 1990; Lewinsohn et al., 2000; Özcan and Chalchat, 2011) and linalool (El-Soud et al., 2015) are most common chemotypes in the *Ocimum basilicum*. The essential oil of sacred basil *Ocimum tenuiflorum* is characterized by presence of high percentage of methyl eugenol and eugenol (Simon et al., 1990; Bhattacharya et al., 1996; Awasthi and Dixit, 2007; Sims et al., 2014), whereas *Ocimum americanum* is recognized by presence of camphor and limonene (Mondello et al., 2002; Chagonda et al., 2000). The essential oil of clove basil, *Ocimum gratissimum* is characterized by eugenol (Raina and Misra, 2017, Bhattacharya et al., 1996; Oyen and Dung, 1999). The major components of essential oil of *Ocimum* × *africanum* are citral and estragole or methyl chavicol (Tangpao et al., 2018; Gurav et al. 2021).

At present, essential oils are mainly analyzed by gas chromatography coupled with mass spectrometry (GC–MS) technique (Grayer et al., 1996; Joshi, 2017). This technique is time consuming and required complex instrumentation assembly, whereas Raman and FTIR based spectroscopic techniques are quick and non-destructive approach. These spectroscopic techniques work on the basis of interaction between light and molecules. Such interaction provide unique feature to molecules in form of spectrum that are utilized for recognition of these molecules in essential oils (Schulz et al., 2003; Agatonovic-Kustrin et al., 2020). Earlier, these technique has been utilized successfully for rapid determination of purity of essential oils used in industries. The closely related plant varieties can be differentiated with the help of Raman and FTIR spectroscopy (Wiwart et al., 2015; Chen, et al., 2019; Kolasinac, et al., 2022). These techniques need no pre-treatment of sample for the measurements. Here dried and fresh samples can be analyzed smoothly with very low interference of water content present in plant sample (Seidler-Lozykowska et al., 2010).

In this study, we have identified commercially important essential oils of 5 species of *Ocimum* viz. *Ocimum tenuiflorum, Ocimum gratissimum, Ocimum americanum, Ocimum* × *africanum and Ocimum basilicum* by Raman and FTIR spectroscopic technique. The spectra received from both spectroscopic techniques work as fingerprint to find its constituent molecules. Further, principle component analysis (PCA) and chemometric analysis have been employed to differentiate the closely related components of essential oils in these taxa.

2. Material and Methods

To get the essential oil, the plants of *Ocimum tenuiflorum*, *Ocimum gratissimum*, *Ocimum basilicum* and *Ocimum* × *africanum* were raised in the botanical garden of department of Botany, CCS

Table 1
Characteristic principal essential oil components in *Ocimum* L. species.

Essential oil source	Chemotype	Reference
Ocimum tenuiflorum L.	methyl eugenol, eugenol	Raina and Misra, 2017; Gurav et al., 2021; Anand et al., 2016.
Ocimum gratissimum L.	eugenol, thymol, geraniol	Bhattacharya et al., 1996; Oyen and Dung, 1999; Charles and Simon, 1992; Rawat et al., 2017; Olugbade et al., 2017; Vieria et al., 2001.
Ocimum basilicum L.	estragole or methyl chavicol, linalool, ctral, citral/linalool	Simon et al., 1990; Lewinsohn et al., 2000; Özcan and Chalchat, 2011; El-Soud et al., 2015.
Ocimum × *africanum* Lour.	estragole or methyl chavicol, linalool, citral (neral and geranial)	Raina and Misra, 2017; Gurav et al., 2021; Janesha et al., 2018; Pisutthanan and Pisutthanan, 2009.
Ocimum americanum L.	Camphor, Citral, limonene, linalool, methyl cinnamate	Mondello et al., 2002; Selvi et al., 2012.

University, Meerut (India). The naturally growing *Ocimum americanum* was collected from wild population. Aerial parts of flowering stage were collected during noon for maximum essential oil extraction. Collected plant material was cut in to small pieces and allowed to shade dry for removal of excess water.

Isolation of essential oil: The dried aerial parts of all five species were crushed separately into small sized pieces and it was followed by hydro-distillation in a Clevenger's apparatus for 3 hours. The oil was collected and dried over anhydrous sodium sulfate and keep in amber bottle at 4 °C for further analysis.

Pure standard compounds: Pure standards compounds of eugenol, citral, estragole or methyl chavicol, linalool, thymol, eucalyptol were purchased from Sigma-Aldrich and CDH India.

Raman Spectroscopy: Raman measurements were performed using Raman spectrometer (RIAFMR-785-C RI Instruments & Innovation India) equipped with a 785 nm diode laser and scattered light was detected by a TEC cooled CCD Linear Array detector. Approximately 5 µl of essential oil was used for spectral analysis. Each Raman spectrum was collected with a total integration time of 10 s by using maximum laser power of 458mw.

FTIRSpectroscopy: The FTIR spectra were recorded on Agilent ATR-FTIR Spectrometer (ATR module of Cary 630 FTIR, Agilent Technologies) using bounce diamond crystal from 600 cm^{-1} to 4000 cm^{-1} and equipped with Agilent Resolutions Pro Software, version 5.2 (G9222-64000). About 10 µl drop of essential oil was placed on a diamond ATR crystal for spectral analysis.

Chemometric Analysis: The hierarchical cluster analysis (HCA) was performed on Raman spectral data using Ward's algorithm to construct groups based on the spectral data ranging from 500 to 1800 cm^{-1}. PCA was performed by R-Studio software on twenty spectral data of each species for complete discrimination and to reduce complexity of data.

3. Results

The yield of essential oil was recorded on dry weight basis. It was recorded 2.2 %, 1.8 %, 2 %, 2.1 % and 1 % for *Ocimum basilicum*, *Ocimum americanum*, *Ocimum tenuiflorum*, *Ocimum* × *africanum* and *Ocimum gratissimum* respectively. The color of essential oil in *O. basilicum*, *O. americanum* and *O.* × *africanum* was whitish. In *O. tenuiflorum* and *O. gratissimum,* the color was yellowish.

Raman spectrum of essential oils for all the species and references used in this study are given in Fig. 2. Raman spectrum of *Ocimum tenuiflorum*, *Ocimum gratissimum*, *Ocimum basilicum*, *Ocimum* × *africanum* and *Ocimum americanum* are given in Fig. 2a-e respectively. Raman spectrum of references viz. eugenol, estragole or methyl chavicol, linalool, citral, eucalyptol, hexane and thymol are given in Fig. 2f-l respectively. The characteristics bands ranging between 1449 and 1450 cm^{-1} are most common bands that appear for all extracted essential oil samples. These bands can be attributed to CH_3/CH_2 vibrations (Baranska et al., 2013; Jentzsch et al., 2015). The major Raman peaks of essential oil samples along with corresponding vibrations are given in Table 2.

The observed different Raman peaks can be correlated with the characteristic feature of different compounds present in the essential oil. Raman peak intensities would be stronger for the compounds whose quantity is more in the essential oil. Fig. 2(a) shows the Raman spectra of *Ocimum tenuiflorum* essential oil where strong peaks appear at the wavenumber 1640, 1611, 1450, 1335, 1299, 1190, 1031, 907, 755 and 469 cm^{-1}. When these spectra of extracted oil are compared with reference sample spectra of eugenol, then it has been observed that most characteristics peaks match with the oil sample. The strongest band has been observed at frequency 1640 cm^{-1}. This band corresponds to exocyclic C═C of eugenol, whereas peak at 1611 cm^{-1} is attributed to the benzene ring of eugenol and methyl eugenol (Chowdhry et al., 2015; Wang and Sung, 2011). Next strong band at 1299 cm^{-1} is attributed to = CH bond.

The eugenol intensity of reference was found to be stronger than intensity of essential oil at 1450 and 755 cm^{-1}. The strong peak at 1281 cm^{-1} in the spectra of eugenol standard has been shifted to 1299 cm^{-1} along with shoulder peak of methyl eugenol at 1334 cm^{-1} (Chowdhry et al., 2015).

Fig. 2(b) shows Raman spectra in the sample of *Ocimum gratissimum*. The major Raman spectra of *Ocimum gratissimum* have been observed at 1641, 1449, 1298, 1211, 1191, 1034, 902, 799, 749, 649, 556 and 468 cm^{-1} wavenumber. The eugenol is main essential oil component of *Ocimum gratissimum*. This is also evident from similar Raman patterns of reference and extracted oil. The band at wavenumber 1641 cm^{-1} correspond to exocyclic C═C of eugenol, whereas 1298 cm^{-1} corresponds to = CH bond of eugenol. The strong peak of eugenol at 799 cm^{-1} has slightly been shifted to 801 cm^{-1} in spectra of *Ocimum gratissimum*.

Raman spectra of *Ocimum basilicum* essential oil where major peaks lie at 1645, 1619, 1449, 1302, 1185, 823 and 640 cm^{-1} wavenumber (Fig. 2c). The bands at 1645, 1619, 1449 cm^{-1} correspond to exocyclic C═C, ring quadrant stretch and CH3/CH2 respectively, whereas, band at 1300 cm^{-1} corresponds to ether Ar-O stretch. Two bands at 823 and 640 cm^{-1} of *Ocimum basilicum* essential oil match exactly with reference estragole or methyl chavicol reference that can be assigned to aromatic H, CH wag and Ring deformation (Jentzsch et al., 2015).

Fig. 2(d) represents Raman spectra in the sample of *Ocimum* × *africanum*. Major Raman peaks were found at 1639, 1611, 1449, 1294, 1180, 823 cm^{-1} wavenumbers. Here bands at 1639, 1611, 1294 cm^{-1} correspond to C═C stretch, ring quadrant stretch and to ether Ar-O- stretch, respectively (Schulz et al., 2003; Jentzsch et al., 2015). While bands at 1180, 823 and 643 cm^{-1} corresponds to plane ring C—H bond, aromatic H, CH wag and ring respectively (Jentzsch et al., 2015). The *Ocimum* × *africanum* is a natural hybrid of *Ocimum americanum* and *Ocimum basilicum* (Majdi, et al., 2020). Therefore, its major Raman peaks were found to be similar to *Ocimum basilicum* and reference extragole sample. It has also been observed that two peaks at 1639 and 1611 for *Ocimum* × *aftricanum* sample shift towards the higher wavenumber at 1645 and 1619 cm^{-1} in *Ocimum basilicum* sample. On the basis of previous studies, it has been found that citral, linalool and methyl chavicol are the three major essential oil components of *Ocimum* × africanum (Raina and Misra, 2017; Gurav et al., 2021; Janesha et al., 2018; Pisutthanan and Pisutthanan, 2009). When the extracted oil sample are compared with references then extragole reference matches with the sample, whereas linalool and citral peaks match partially. The presence of these components can also be sensed from its aroma, as these two taxa, *Ocimum* × *africanum* and *Ocimum basilicum* have quite similar smell. This indicate common aromatic substance in both the species.

According to Mondello et al., (2002) and Pandey et al., (2014) the two major components type of *Ocimum americanum* are citral and camphor type. The citral type is characterized by high amount of neral and geranial.

In *Ocimum americanum,* the major bands of Raman spectrum appear at wavenumber 476, 556, 652, 759, 861, 919, 951, 1019, 1094, 1305, 1449, 1645 and 1747 cm^{-1} (Fig. 2e). The two strongest bands appear at 652 and 1449 cm^{-1} wavenumber. These strong bands were associated with ring deformation of camphor (Jentzsch and Ciobota, 2014) and CH3/CH2 bending respectively. The weak band at 1747 appears a vibration mode of C═O stretch of camphor (Jentzsch and Ciobota, 2014). The thymol is also major essential oil component of *Ocimum gratissimum*. Similar Raman spectrum of thymol in hexane solution along with hexane

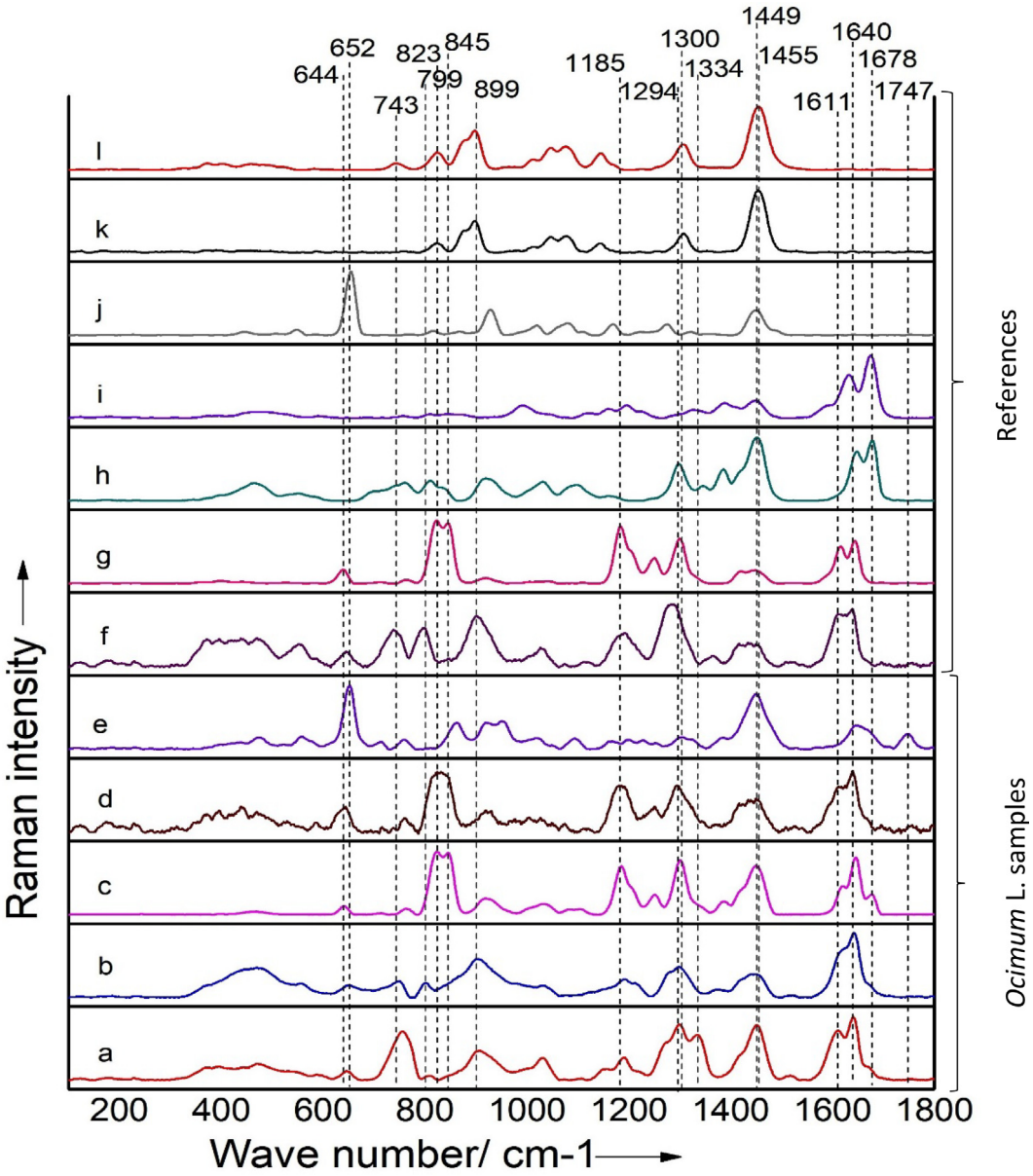

Fig. 2. Raman spectra of: (a) *Ocimum tenuiflorum*, (b) *Ocimum gratissimum*, (c) *Ocimum basilicum*, (d) *Ocimum* × *africanum*, (e) *Ocimum americanum*, (f) eugenol, (g) estragole or methyl chavicol, (h) linalool, (i), citral,(j) eucalyptol, (k) Hexane, (l) Thymol.

spectrum can be seen in Fig. 3. The Raman peak that appears at 743 for thymol sample is found to be the characteristic peak for thymol (Khoshroo et al., 2015). However, thymol is not observed in Raman spectrum of all essential oils sample of *Ocimum* L.

ATR-FTIR-Spectroscopy: The identity of all the above essential oil components were also confirmed with the help of FTIR Spectroscopy measurement.

The FTIR spectra of essential oil of five species of *Ocimum* L is given in Fig. 4a-e. The spectral data for *Ocimum tenuiflorum* and *Ocimum gratissimum* show clear identity and discrimination between methyl eugenol and eugenol types. Strong peaks at 2930 and 2933 cm^{-1} are due to CH and peak at 1638 corresponding to C═C aromatic moiety (Wang and Sung, 2011) were found similar in both the samples of *Ocimum tenuiflorum* and *Ocimum gratissimum*. The peaks at 1605 and 1591 cm^{-1} represent the vibrations of aromatic C═C (Nuchuchua et al., 2009). The pattern and position of peaks in *Ocimum gratissimum* oil were correlated well with previous work on eugenol by FTIR Spectroscopy (Pramod et al., 2015). The difference in pattern of peaks between both samples of *Ocimum tenuiflorum* and *Ocimum gratissimum* can be observed at 1418 and 1463 cm^{-1} for *Ocimum tenuiflorum* and 1431 and 1465 cm^{-1} for *Ocimum gratissimum* (Fig. 4 a, b). The peak at 1418 is weak and at 1463 is stronger in *Ocimum tenuiflorum* but in case of *Ocimum gratissimum* the peak at 1431 is stronger and 1465 is weaker for eugenol and methyl eugenol respectively.

The strongest bands for *Ocimum basilicum* and *Ocimum* × *africanum* have been observed at 1511 cm^{-1} for *Ocimum basilicum* and 1510 cm^{-1} for *Ocimum* × *africanum*. The second strongest peak was observed at 1244 cm^{-1}, whereas moderate bands at 1176 cm^{-1} and 807 cm^{-1} have been observed for both the species. The band at 913 cm^{-1} for *Ocimum basilicum* was shifted to 912 cm^{-1} for *Ocimum* × *africanum*.

In *Ocimum americanum*, the strongest band at 1743 cm^{-1} (Fig. 4, e) corresponds to carbonyl stretching (C═O) is of camphor (Michelina et al., 2018) and at 1375 cm^{-1} corresponds to methylene deformation (Gudi et al., 2015). The peaks at 2873,

Table 2
Assignment for the most intense characteristic Raman bands of essential oils extracted from *Ocimum tenuiflorum, Ocimum gratissimum, Ocimum americanum, Ocimum × africanum. and Ocimum basilicum*.

Essential oil	Wave numbers of bands (cm^{-1})	Proposed assignment
Ocimum tenuiflorum L. (Holly basil)	1640	Exocyclic C=C
	1611	Ring quadrant stretch
	1450	CH3/CH2 bend
	1299	=CH
	755	Ring deformation
Ocimum gratissimum L. (Clove basil)	1641	Exocyclic C=C
	1449	CH3/CH2 bend
	1298	=CH
	749	Ring deformation
	649	Ring deformation
Ocimum americanum L. (American basil)	1747	C=O stretch
	1645	C=C stretch
	1449	CH3/CH2 bend
	759	Ring deformation
	652	Ring deformation
Ocimum × africanum Lour. (African basil)	1639	C=C stretch
	1611	Ring quadrant stretch
	1449	CH3/CH2 bending
	1294	Rther Ar-O- stretch
	1180	C—H bond
	823	Aromatic H, CH wag
	643	Ring deformation
Ocimum basilicum L. (Sweet basil)	1645	C=C stretch
	1619	Ring quadrant stretch
	1449	CH3/CH2 bend
	1300	Ether Ar-O- stretch
	1185	C—H bond
	823	Aromatic H, CH wag
	640	Ring deformation

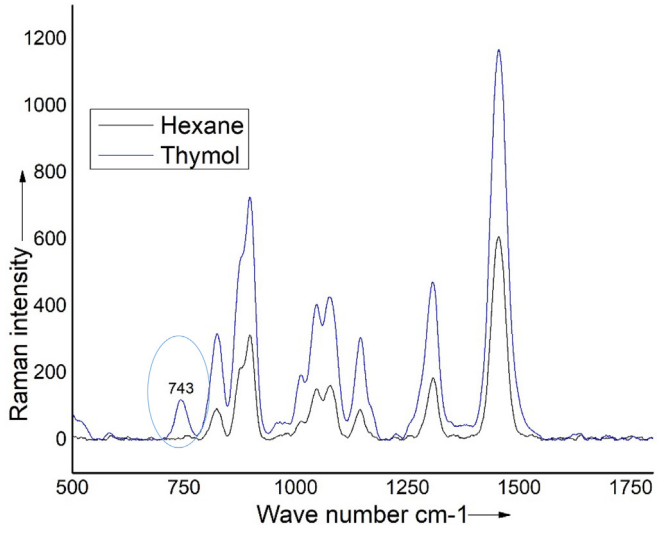

Fig. 3. Raman spectra of Hexane and Thymol. Peak at 743 in the raman spectra of Thymol differenciate the thymol from hexane.

2959, 1448, 1046 and 751 correspond to previous FTIR spectral data of camphor (Nunes et al., 2020). These results are in complete agreement with our FTIR Spectral data of camphor.

The fingerprinting zones of characteristic peaks for all samples are given in Table 3. Our spectral data agree completely with the results of Gas chromatography and vibrational spectroscopic work (Wang and Sung, 2011; Pramod et al., 2015) for characteristic components.

Principal Component Analysis (PCA): This analysis is based on dimensionality-reduction method in which dimension information

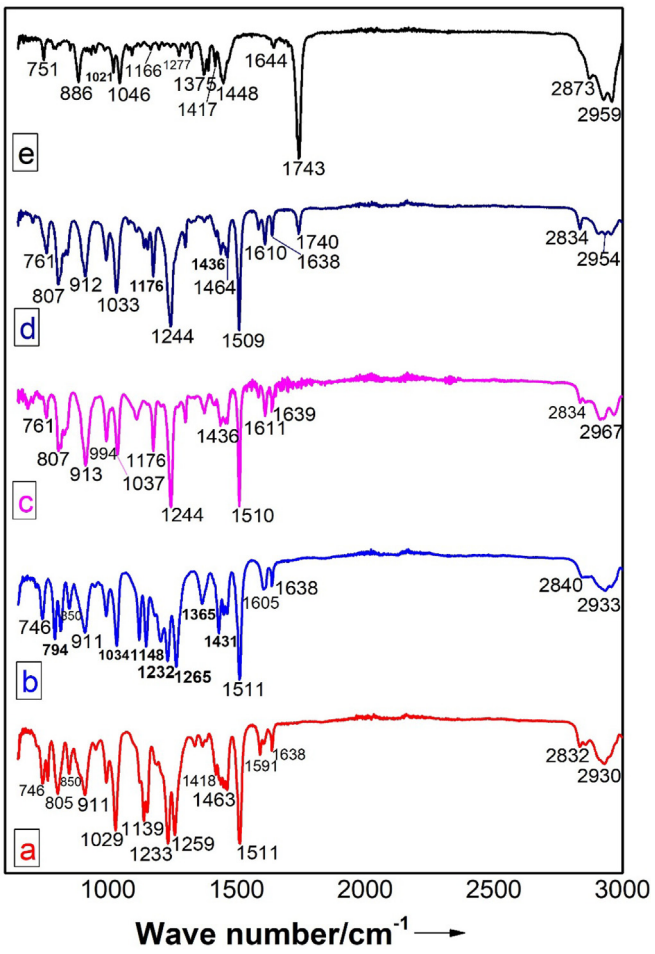

Fig. 4. FTIR-spectra of (a) *Ocimum tenuiflorum*, (b) *Ocimum gratissimum*, (c) *Ocimum basilicum*, (d) *Ocimum × africanum*, (e) *Ocimum americanum*.

of large data sets are transferred to less dimensional data sets. PCA analysis performed on the 20 data set points for each sample i.e. a total of 100 Raman essential oils data set (Fig. 5). First two PCA components (PC1, PC2) show major variance from others which is plotted in Fig. 5. Two principal component describes the 31 % of total variation and PC1 describes 23 % and PC2 describes 8 %. Here, essential oil PCs for five species are clustered into five distinct groups on the basis of Raman bands and each point represent a spectrum. Each species is clearly separated on the basis of their components. However, *Ocimum × africanum* and *Ocimum basilicum* are closely related species and contain estragole or methyl chavicol chemotype, yet PCA analysis clearly differentiate both types.

Hierarchical Cluster Analysis (HCA): The hierarchical cluster analysis was performed on spectral data of Raman spectroscopy from 500 cm^{-1} to 1800 cm^{-1}. This analysis clearly grouped samples according to their essential oil relationship among the investigated species (Fig. 6). The reference eugenol is clustered with *Ocimum gratissimum* (1 and 6 in Fig. 6), whereas estragole or methyl chavicol is clustered with *Ocimum × africanum* and *Ocimum basilicum* (4, 5 and 8 in Fig. 6). *Ocimum americanum* (2), *Ocimum tenuiflorum* (3), eucalyptol (7), linalool (9), citral (10), thymol (11) were segregated separately into individual cluster (Fig. 6).

4. Discussion

The essential oil components of five different species of *Ocimum* L. were investigated. In this study, Raman spectroscopy and FTIR

Table 3
Wavenumber of major peaks of FTIR-spectrum of *Ocimum tenuiflorum* (Ot), *Ocimum gratissimum* (Og), *Ocimum basilicum* (Ob), *Ocimum* × *africanum* (Oxa) and *Ocimum americanum* (Oa).

Frequency (cm-1)	Ot EO	Og EO	Ob EO	Oxa EO	Oa EO
3000–2700 cm^{-1}	2930	2933	2834, 2967	2834, 2954,	2873, 2959
2700–1800 cm^{-1}	–	–	–	–	–
1800–1550 cm^{-1}	1591, 1638	1608, 1638	1611, 1639	1610, 1638	1644, 1743
1550–1050 cm^{-1}	1233, 1259, 1511, 1418, 1463,	1148, 1232, 1266, 1431, 1465, 1511	1176, 1244, 1301, 1436, 1510	1176, 1244, 1301, 1436, 1509	1166, 1277, 1323, 1375, 1417, 1448
Below 1050 cm^{-1}	746, 805, 850, 911, 993, 1029	745, 794, 816, 850, 911, 993, 1034,	761, 807, 913, 994, 1037	761, 807, 912, 993, 1033	751, 886, 1021, 1046

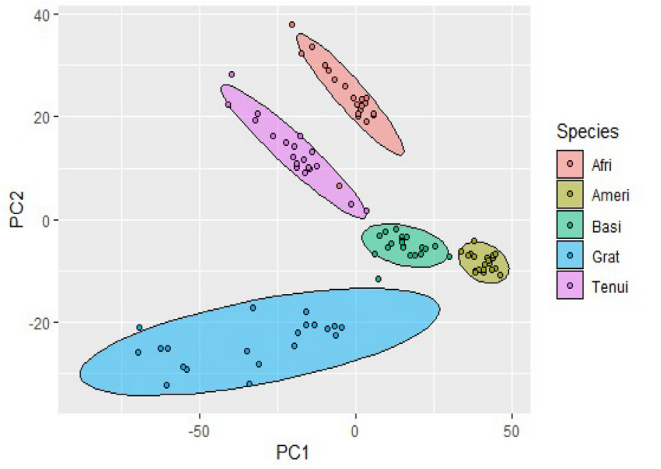

Fig. 5. Principal Component Analysis (PCA) of *Ocimum tenuiflorum* (Tenui), *Ocimum gratissimum* (Grat), *Ocimum* × *africanum* (Afri), *Ocimum basilicum* (Basi), *Ocimum americanum* (Ameri).

spectroscopy have been utilized successfully for qualitative characterization and differentiation of principal essential oil components and to distinguish different species of *Ocimum*. The investigations of principal essential oil components by these method are in complete agreement with the previous studies and published literature (Schulz et al., 2003; Wang and Sung, 2011; Baranska et al., 2013; Jentzsch and Ciobota, 2014; Chowdhry et al., 2015; Jentzsch et al., 2015). The spectra from both techniques were used as fingerprint. The hierarchical cluster analysis of essential oils were found into four groups according to their chemical similarity. The results of PCA also indicate that all five species of *Ocimum* form different clusters on the basis of principal essential oil components. The principal components of *Ocimum tenuiflorum*, *Ocimum gratissimum*, *Ocimum* × *africanum*, *Ocimum basilicum*, *Ocimum americanum* are methyl eugenol, eugenol, estragole, estragole and camphor respectively. The characteristic aroma of each species is due to these principal components. The species of *Ocimum* do not contain thymol, eucalyptol, citral and linalool.

In the Raman spectrum of *Ocimum tenuiflorum* strongest band has been observed at frequency 1640 cm^{-1} (Fig. 2a). This band corresponds to exocyclic C=C of eugenol, whereas peak at 1611 cm^{-1} attributed to benzene ring of eugenol and methyl eugenol (Chowdhry et al., 2015; Wang and Sung, 2011). Eugenol is main essential oil component of *Ocimum gratissimum* (Charles and Simon, 1992; Rawat et al., 2017). Raman spectrum band at wavenumber 1641 cm^{-1} corresponds to exocyclic C=C of eugenol (Fig. 2b), whereas 1298 cm^{-1} corresponds to = CH bond of eugenol (Chowdhry et al., 2015). Raman spectra of *Ocimum basilicum* (Fig. 2c) match exactly with reference of estragole or methyl chavicol and is in complete agreement with previous findings on estragole (Jentzsch et al., 2015).

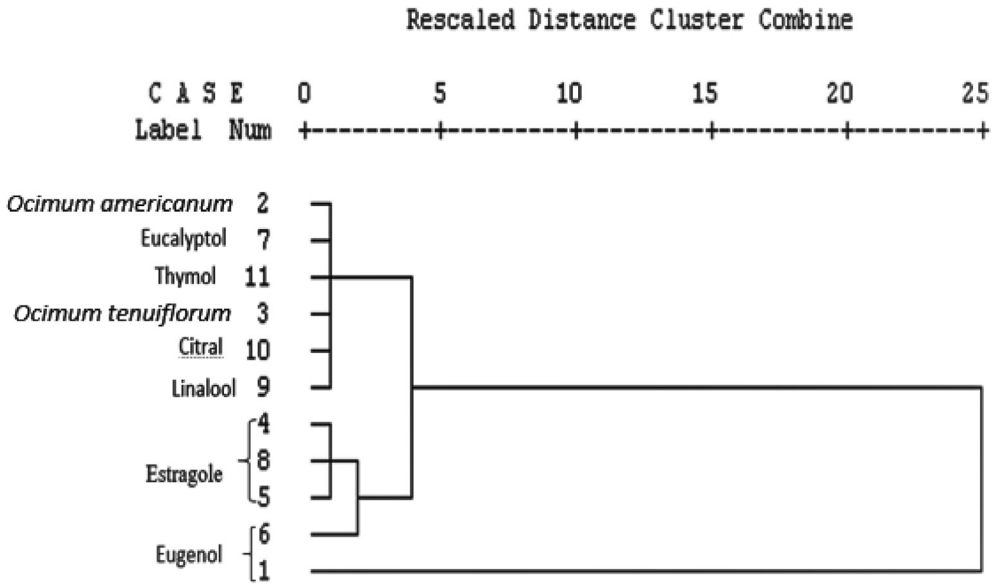

Fig. 6. Dendrogram (Ward's method) showing differentiation of essential oil based on the spectral data of Raman spectroscopy obtained from extracted oil and used standards. 1. Eugenol reference, 6. *Ocimum gratissimum*, 4. Estragole or methyl chavicol reference, 5. *Ocimum* × *africanum*, 8. *Ocimum basilicum*, 3. *Ocimum tenuiflorum*, 2. *Ocimum americanum* 7. Eucalyptol reference, 9. Linalool reference, 10. Citral reference, 11 Thymol reference.

Ocimum × *africanum* essential oil is characterized by estragole or methyl chavicol, linalool, citral (neral and geranial) rich chemotypes (Raina and Misra, 2017; Gurav et al., 2021; Janesha et al., 2018; Pisutthanan and Pisutthanan, 2009). Raman peaks (Fig. 2d) matched with spectra of estragole standard reference and previous work (Schulz et al., 2003; Jentzsch et al., 2015). *Ocimum* × *africanum* is a natural hybrid of *Ocimum americanum* and *Ocimum basilicum*, so the component of these species should be in the hybrid species (Majdi, et al., 2020). The presence of estragole in the hybrid indicates that *Ocimum* × *africanum* has acquired the estragole from *Ocimum basilicum*. It was observed that the hybrid *Ocimum* × *africanum* is chemically close to *Ocimum basilicum* in context of estragole parental component. Here, it can be concluded that the dominant gene of *Ocimum basilicum* for estragole has been acquired by the hybrid and this hybrid may be used for bioprospecting studies. This will help in reduction of exploitation of *Ocimum basilicum* utilized to extract this component. The smell of *Ocimum* × *africanum* and *Ocimum basilicum* are exactly similar. It could be due to similar aromatic constituent in both the taxa. The chemotypes in *Ocimum americanum* essential oil have been reported to be rich in Camphor, Citral, limonene, linalool, methyl cinnamate (Mondello et al., 2002; Selvi et al., 2012). In our study the bands at wavenumber 652 and 1449 cm^{-1} in *Ocimum americanum* essential oil associated with ring deformation of camphor (Jentzsch and Ciobota, 2014) and band at 1747 appears a vibration mode of C=O stretch of camphor (Jentzsch and Ciobota, 2014). Thus, these findings suggests that these techniques can be utilized for quick identification, discrimination and classification of different taxa (Kolasinac, et al., 2022).

5. Conclusion

The data of Raman spectroscopy and FTIR techniques provide clear identification and discrimination between principal components of investigated *Ocimum* species. The results of both techniques show complete reconciliation with earlier spectral data on individual principle components present in each of the essential oil samples. Our finding based on principal essential oil components and their aroma indicate that the different species of *Ocimum* can be encouraged for cultivation for different purposes. *Ocimum basilicum* and *Ocimum* × *africanum* have estragole. These have sweet smell and thus these species can be used for value addition and for perfumery. Similarly *Ocimum tenuiflorum* and *Ocimum gratissimum* have spicy smell. These species can be cultivated for value addition. *Ocimum americanum* is camphor rich and can be used for antiseptic and antimicrobial spray. Thus, both these techniques can be efficiently used for plant identification, chemical profiling and to distinguish taxa as well as characterization of essential oil component. Similar methodology and findings can be utilized to distinguish other closely related taxa at varietal and form ranks.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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