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Essential Oil Composition and Antimicrobial Activity of Methyl cinnamate-Linalool Chemovariant of *Ocimum basilicum* L. from India

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Abstract: The essential oils obtained from hydrodistillation of *Ocimum basilicum* L. harvested at four different growth stages during spring-summer and rain-autumn cropping seasons, were characterized using GC and GC-MS. The essential oil yield was found to vary from 0.28–0.32% and 0.40–0.52% during spring-summer and rain-autumn cropping season, respectively with its maximal at full bloom stage. Altogether, forty constituents, comprising 94.9–98.3% were identified represented by (*E*)-methyl cinnamate (36.6–66.4%), linalool (11.2–43.8%), and (*Z*)-methyl cinnamate (5.4-7.6%) as main constituents. Results showed that growth stages strongly influenced the chemical composition of the essential oil in two cropping seasons, particularly concerning to the content of (*E*)-methyl cinnamate and linalool. Seed setting stage was optimized for harvesting (*E*)-methyl cinnamate rich oil (66.4%) in rain-winter cropping season. The antimicrobial potential of the essential oil was tested against eight pathogenic bacteria and three fungal strains. Antimicrobial assay showed that the essential oil possessed good antibacterial activity against *Streptococcus mutans, Staphylococcus epidermidis, Escherichia coli*, and antifungal activity against *Candida kefyr* and *Candida albicans.*

Keywords: *Ocimum basilicum*; essential oil; (*E*)-methyl cinnamate; linalool; antibacterial activity; antifungal activity.

1. Introduction

Genus *Ocimum* (Lamiaceae) includes around 30 species from tropical and subtropical regions of Asia, Africa, Central and South America, with variable morphological and chemical features. Most members of this genus are annual or perennial, highly aromatic, branched herbs or shrubs [1,2]. These are accredited with various medicinal properties, and used in folk medicine for the treatment of abdominal pains, colds, coughs, measles, insomnia, rheumatism, sunstroke, gonorrhea, inflammation, snakebite/insect bites, stomach and kidney malfunctions etc [3,4]. Few member of the genus *Ocimum*, *viz*., *O. tenuiflorum* L., *O. gratissimum* L., *O. americanum* Sims, *O. basilicum* L., *O. kilimandscharicum* Guerke, and *O. micranthum* Willd are being commercially cultivated in different parts of the world for their dry herbs, essential oils and high-value aroma chemicals which are being used extensively in the food, perfumery, cosmetic and pharmaceutical industries [3-6]. The presence of essential oils with distinct compositions (aroma chemicals) determines the specific aroma and flavour

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of the *Ocimum* taxa. Moreover, the essential oil of *Ocimum* taxa showed huge inter and intraspecific compositional variability due to existence of numerous genotype/chemotypes/cultivars within taxa and because of endogenous variables, including variations in geographic range and climate, and agricultural conditions etc [4,7]. Monoterpenoids (linalool, 1,8-cineole, camphor, citral, thymol, ocimenes, geraniol), phenylpropanoids (methyl chavicol, eugenol, methyl eugenol, methyl cinnamate), sesquiterpenoids (β-elemene, β-caryophyllene, *trans*-α- bergamotene, (*E*)-α-bisabolene, β-bisabolene) are the prevalent components distributed in the essential oil of most of the studied *Ocimum* taxa [4-10]. Among the various species of basil, *O. basilicum* is considered most important for its sweet aromatic oil and cultivated in different regions. Based on essential oil composition of *O. basilicum*, four major chemotypes (methyl chavicol, linalool, methyl eugenol, and methyl cinnamate type) and numerous subtypes with mixed proportion of these constituents were identified in *O. basilicum* [6]. In addition to these, eugenol, *trans*-β-bergamotene/linalool, menthone/methyl chavicol, 1,8-cineole, camphor, citral, camphor/linalool, geraniol/linalool, (*E*)-anethole chemotypes, along with taxa marked by mixed composition of different proportion of these constituents, were also reported in *O. basilicum* from different geographic regions [7-19]. Earlier reports on the antimicrobial potential of *O. basilicum* revealed that basil oil and its specific constituents exhibited broad range of antagonistic activity against a wide range of microorganisms including various pathogenic Gram-positives, Gram-negative bacterial and fungal strains [15,17,20-24]. However, changes in chemical composition of basil essential oil, based on dominance of major constituents was found to have a direct effect on activity potential of basil essential oil [25-27]. Therefore, in continuation to our research work on Indian *Ocimum* taxa, in the present study we have investigated and compared the ontogenic variations in essential oil yield and composition of *O. basilicum* at different growth stages during two distinct cropping seasons (spring-summer and rain-autumn) using gas chromatographic retention index (RI) and mass spectral data. Moreover, the antibacterial and antifungal potential of the essential oil was tested against seven bacterial and three fungal strains.

2. Material and methods

2.1. Plant Materials

The experiment was conducted at the experimental field of CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Pantnagar, located between coordinates 29°N, 79.38°E at 243 m above mean sea level at foothills of Uttarakhand, India during spring-summer and rain-autumn seasons. The experimental site experiences subtropical and humid climate with hot summer and chilled winter. The maximum temperature ranges between 35–45°C, and minimum between 2–5°C. Monsoon usually breaks in mid June and continues up to September. The soil of the experimental site was sandy-loam in texture, with neutral pH. To study the variation in oil yield and compositional variability at different crop stage; the crop was raised in two different cropping seasons (spring-summer and rain–autumn). Seeds sown in the nursery on 15th January and 15th June for spring–summer and rain–autumn cropping seasons, respectively. Seedlings (45 days old) were transplanted in the field (plot size: 3.5 m × 3.5 m) and raised following normal agricultural practices. Three samples were harvested randomly at each stage of plant growth (vegetative, half bloom, full bloom and seed setting stage) for extraction of essential oils to study the yield and compositional variability during both cropping seasons.

2.2. Isolation of Essential Oils

Freshly harvested samples (100 g each) were hydrodistilled in a Clevenger apparatus for 3 h (until no more essential oil was recovered), in triplicate, for extraction of essential oil. The oils were collected, measured, dehydrated over anhydrous Na_2SO_4 and stored in amber vials in cool and dark place until further analysis. The extraction yield was calculated in mL of oil per 100 g of fresh plant material.

2.3. Analysis of Essential Oils

The composition of the essential oil was analysed by GC and GC-MS techniques. GC analysis of the essential oil samples was done on a DB-5 capillary column (30 m × 0.32 mm i.d., film thickness 0.25 µm) fixed inside the oven of NUCON Gas Chromatograph (model 5765). The column oven temperature was programmed from 60-230 °C, at the rate of 3 °C min^{-1}, using Hydrogen as carrier gas at constant flow rate of 1.0 mL min^{-1}. Injector and detector (FID) temperatures were 220 °C and 230 °C, respectively. Injection size was 0.02 µL neat (syringe: Hamilton 0.5 µL capacity, Alltech USA) with a split ratio was 1:40. GC-MS analysis was performed on PerkinElmer Turbomass Mass Spectrometer (Shelton, USA) fitted with Equity-5 fused silica capillary column (60 m × 0.32 mm; 0.25 µm film thickness; Supelco Bellefonte, PA, USA). The column temperature was programmed 70 °C, initial hold time of 2 min, to 250 °C at 3 °C min^{-1} with final hold time of 3 min, using helium as carrier gas at a flow rate of 1.0 mL min^{-1}. The injector, ion source and transfer line temperatures were 250 °C. The injection volume was 0.04 µL neat with split ratio 1:30, electron impact ionization mode (EI) with ionization energy 70 eV, and mass scan range of 40–400 amu.

2.4. Identification of Constituents

Characterization of constituents was achieved based on their retention time, retention index (RI) determined using a homologous series of *n*-alkanes (C_8-C_{24}) under the same temperature-programmed conditions, coinjection with standards (Aldrich and Fluka), MS Library search (NIST and WILEY), and by comparing with the mass spectral literature data [28]. Moreover, retention times of authentic compounds, standards/ marker constituents of known essential oils used to confirm the identities of the constituents. The content (%) of individual components of the oil is expressed as percent peak area relative to total peak area from the GC/FID analyses of the whole essential oil by electronic integration without response factor correction.

2.5. Antibacterial Assays

The antibacterial activity of the essential oil of *O. basilicum* from full bloom stage of the rain-autumn cropping season (main season) was determined using disc diffusion assay as per CLSI guidelines [29]. Inoculums of the test bacteria [Gram-positive: *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Streptococcus mutans* (MTCC 890); Gram-negative: *Escherichia coli* (MTCC 723), *Escherichia coli* (DH5α), *Klebsiella pneumoniae* (MTCC 109), *Salmonella typhimurium* (MTCC 98) and *Pseudomonas aeruginosa* (MTCC 741)] were prepared equivalent to McFarland Standard 0.5. Uniform bacterial lawns prepared using 100 µL inoculums on a Mueller Hinton agar plate. Filter paper discs (5.0 mm; Whatman) soaked with test essential oil was placed over seeded plates. The plates were incubated at 37 °C for 24 h. Activity was measured in terms of zone of growth inhibition (mm) determined by subtracting the disc diameter (*i.e.* 5.0 mm) from the total zone of inhibition shown by the test disc in terms of clear zone around the disc. The tests were performed in triplicate. The bacterial strains were procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology (IMT) Chandigarh, India. Antibacterial efficacy of the essential oil was also determined by Micro dilution broth assay using 96 'U' bottom micro-titer plates as per CLSI guidelines [30]. Samples were serially diluted two folds (in the range of 1000-1.95 µg/mL) in Mueller Hinton Broth (MHB). The broth was inoculated with 10.0 µL of diluted 24 h grown culture of test organisms with a titre equivalent to 0.5 McFarland standards. The inoculated plates were incubated at 37 ºC for 16–24 h and the growth was recorded spectrophotometrically at 600 nm using Spectramax 190-microplate reader (Molecular Devices, CA, and USA). The MIC value was determined from the turbidimetric data as the lowest concentration showing growth inhibition equal to or greater than 80% as compared to control. Norfloxacin and DMSO were used as a positive and negative control, respectively. Experimental observations were performed in triplicate to rule out any error during the antibacterial assay.

2.6. Antifungal Assays

The antifungal activity of the essential oil was tested againist *Candida albicans* (ATCC 14053), *Candida albicans* clinical isolates (AI) and *Candida kefyr* (ATCC 204093). Cultures of fungi were grown on Sabouraud Dextrose Broth (Hi Media Pvt, Ltd., India) for 24 hours at 37^0C and then turbidity was adjusted to 0.5 McFarland standards (approximately 1.2 x 10^6 CFU/mL). 100µL of inoculum (0.5 McFarland) of the fungal culture was withdrawn with caution and spread uniformly over the surface of Sabouraud Dextrose agar plate to get even lawn. 5µL of oil was impregnated on the sterile paper disc (5 mm diameter, Whatman No. 3 filter paper) and placed on the fungal lawns. The plates were then incubated for 24 h (37°C) following which the diameter of the inhibition zone was measured. The net zone of growth inhibition was determined by subtracting the disc diameter (i.e. 5 mm) from the total zone of growth inhibition shown by the test disc in terms of clear halo fungal lawn around the disc. MIC (minimum inhibitory concentration) was estimated using macro dilution broth assays. For this purpose, two-fold serial dilution series was employed to assess the MIC of given oils. In each assay, 10 µl of fungal culture (0.5 McFarland) prepared as before was added to the 1.0 ml medium and incubated at 37 ± 1°C and the killing or inhibition was examined by visible turbidity. MFC (minimal fungicidal concentration) was determined by plating 100 µl from each tube used for determining MIC and observed for any growth after 2 days of incubation. Ketoconazole was used as standard in antifungal activity evaluation [31].

2.7. Statistical Analysis

The content of linalool, (*E*)-methyl cinnamate, (*Z*)-methyl cinnamate representing 84.9%-90.7% of the oil compositions of different stages of *O. basilicum* in two cropping season were subjected to hierarchical cluster analysis for reflecting similarity or differences in their compositions. Moreover, the percentages of sixteen major constituents viz. menthyl chavicol, linalool, nerol, neral, geraniol, geranial, camphor, (*E*)-anethole, α-terpineol, (*Z*)-methyl cinnamate, (*E*)-methyl cinnamate, methyl eugenol, *trans*-α-bergamotene, β-selinene, β-bisabolene limonene, 1,8-cineole representing main components of the essential oils of various reported chemotypes of *O. basilicum* were also subjected to the hierarchical cluster analysis to reflect similarity and differences in the composition of these chemotype of *O. basilicum* [32]. This software computes the hierarchical clustering of a multivariate dataset and the derived dendrogram depicts the grouping of chemical compositions as per their chemical composition based on the content variability of these constituents.

3. Results and discussion

The variations in the essential oil yield of *O. basilicum* during different growth stages in two cropping seasons are presented in Table 1. The essential oil yield was found to vary from 0.28–0.32% and 0.40–0.52% in different growth stages of spring-summer and rain-autumn cropping season, respectively. Results showed that in both cropping season, oil yield increases with plant development upto full bloom stage (maximum oil content), with lowest oil yield in vegetative stage. Developmental/ growth stages of aromatic plants were reported to have a substantial influence on oil yield, which varies plant to plant in diverse geographic regions. A higher essential oil yield at full bloom stage is in agreement with previous reports that showed better accumulation of essential oil during flowering stage in *Ocimum* taxa [7-10]. The hydrodistilled essential oils of the harvested crop in different growth stages of two cropping seasons were analysed and compared by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) methods. Qualitative and quantitative compositions of essential oils from different stages were presented in Table 2. In total, 40 constituents, representing 94.9–98.3% of composition were identified. Phenyl propanoids (42.4–74.0%), represented by (*E*)-methyl cinnamate (upto 66.4%), and monoterpenoids (19.9–46.0%), represented by linalool (upto 43.8%), constitute the main fraction of the composition. In spring-summer seasons, the content of (*E*)-methyl cinnamate and linalool were found to vary from 36.6–42.8% and 39.1–43.8%, respectively. The content of linalool was higher at half full bloom stage (43.8%) as compared to seed setting (42.8%), vegetative stages (42.2%) and full bloom stage (39.1%).

However, the content of (*E*)-methyl cinnamate was maximal at full bloom stage (42.8%) followed by 40.4% in vegetative stage, 39.8% in half bloom stage, and minimal 36.6% in seed setting stage.

Table 1. Essential oil yield of *Ocimum basilicum* at different growth stages

Harvest stages	**Oil yield (%)***
Spring-Summer cropping season	
Vegetative stage	0.28±0.03
Half bloom stage	0.30±0.01
Full bloom stage	0.34±0.02
Seed setting stage	0.32±0.04
Rain-Autumn cropping season	
Vegetative stage	0.38±0.02
Half bloom stage	0.46±0.03
Full bloom stage	0.52±0.01
Seed setting stage	0.40±0.01

*Essential oil yield calculated based on fresh mass of harvested material (v/w)

On the other hand, the content of (*Z*)-methyl cinnamate found to be maximal at full bloom stage (5.9%) and minimal at vegetative stage (5.4%), while the content of γ-eudesmol (1.8%–2.7%) was reported to increase with advancement of crop age during the spring-summer cropping season. The content of (*E*)-methyl cinnamate varies from 39.8% to 66.4% in rain-autumn cropping season, compared to 36.6% to 42.8% of spring-summer season. The content of (*E*)-methyl cinnamate was found be highest (66.4%) in seed setting stage compared to 43.7% in full bloom stage, 41.4% in vegetative stage, 39.8% in half bloom stages. The content of linalool was higher (>40.0%) at vegetative, half bloom and full bloom stages, however it was noticed only up to 11.2% in seed setting stage. Comparison of results showed that the seed setting stage was the best for harvesting *O. basilicum* for higher content of (*E*)-methyl cinnamate in rain-autumn cropping season. Otherwise, the content of linalool and (*E*)-methyl cinnamate in preferential harvesting stage (full bloom) is quite comparable in both cropping seasons. The contents (%) of the major identified constituents [linalool, (*E*)-methyl cinnamate, (*Z*)-methyl cinnamate)] representing 84.9–90.7% of composition in different growth stages of two cropping seasons, were subjected to hierarchical cluster analysis for reflecting similarity or differences in their compositions. The derived dendrogram clearly depicted closeness or distance among the compositions based on the content of these marker constituents. Multivariate analysis, as depicted in Figure 1, clearly revealed a high dissimilarity of the composition of seed setting stage of rain-autumn cropping season compared to other stages.

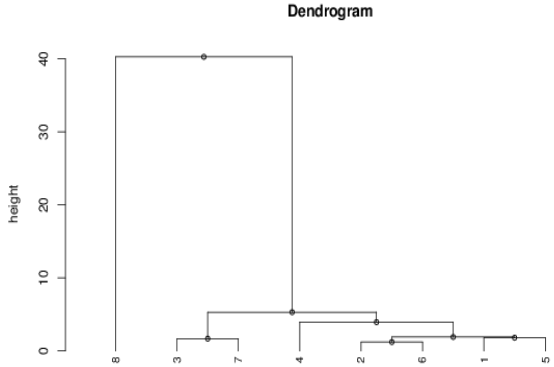

Figure 1. Dendrogram derived from the hierarchical cluster analysis of the essential oil compositions of *O. basilicum* in different maturity stages of the two cropping seasons; [(**1** to **4)**: vegetative, half bloom, full bloom and seed setting in spring-summer cropping season; (**5** to **8)**: vegetative, half bloom, full bloom and seed setting stage in rain-autumn cropping seaso

Table 2. Variation in essential oil constituents of *Ocimum basilicum* L. during different stages of plant growth in two cropping seasons

S.No.	Compounds	RI[a]	RI[b]	Spring-Summer cropping season				Rain-Autumn cropping season			
				VS	HB	FB	SS	VS	HB	FB	SS
1	(*Z*)-3-Hexenol	840	850	0.3	0.2	0.2	0.2	0.1	t	0.1	0.1
2	α-Pinene	939	932	t	t	t	t	t	t	0.1	0.6
3	Sabinene	967	969	0.2	0.1	0.2	0.2	t	t	t	t
4	β-Pinene	980	974	0.4	0.2	0.3	0.3	0.1	0.1	0.1	0.1
5	Myrcene	999	988	0.1	-	t	t	0.2	0.2	0.2	0.1
6	α-Phellandrene	1002	1002	t	t	t	t	t	t	t	t
7	*p*-Cymene	1022	1020	-	-	-	-	t	t	t	0.1
8	Limonene	1023	1024	0.4	0.3	0.4	0.4	t	t	t	0.2
9	1,8-Cineole	1028	1026	0.6	0.4	0.6	0.6	0.6	0.5	0.5	0.7
10	(*E*)-β-Ocimene	1049	1044	t	t	t	-	0.5	0.4	0.4	0.4
11	*cis*-Linalool oxide (furanoid)	1064	1065	0.1	0.1	0.1	0.1	t	t	0.1	0.5
12	*trans*-Linalool oxide (furanoid)	1081	1084	t	0.1	0.1	0.1	t	t	t	t
13	**Linalool**	1097	1095	**42.2**	**43.8**	**39.1**	**42.8**	**43.1**	**43.6**	**40.1**	**11.2**
14	Camphor	1142	1141	t	t	t	t	t	t	t	0.8
15	Terpinen-4-ol	1182	1174	0.1	t	t	0.1	0.1	0.1	0.1	0.1
16	α-Terpineol	1189	1186	0.3	0.4	0.4	0.4	0.4	0.9	0.4	5.0
17	Methyl chavicol	1202	1195	-	t	-	-	t	t	t	t
18	Neral	1232	1235	t	t	t	t	t	t	t	t
19	Linalyl acetate	1247	1254	0.2	0.1	0.2	0.2	0.3	0.2	0.3	0.1
20	Geranial	1266	1264	0.1	t	0.1	0.1	t	t	0.1	t
21	**(*Z*)-Methyl cinnamate**	1297	1299	**5.4**	**5.8**	**5.9±**	**5.5**	**6.6**	**7.0±**	**6.9**	**7.6**
22	Eugenol	1348	1356	t	t	0.1	0.1	t	t	0.1	t
23	**(*E*)-Methyl cinnamate**	1378	1376	**40.4**	**39.8**	**42.8**	**36.6**	**41.4**	**39.8**	**43.7**	**66.4**
24	Methyl eugenol	1390	1403	0.1	0.1	0.1	0.2	t	t	t	t
25	β-Caryophyllene	1418	1417	0.7	0.5	0.7	0.6	0.3	0.5	0.6	0.2
26	β-Copaene	1430	1430	0.3	0.2	0.2	0.4	t	t	t	t
27	*trans*-α-Bergamotene	1434	1432	-	-	-	-	t	t	t	t
28	(*Z*)-β-Farnesene	1436	1440	-	t	0.1	0.1	t	0.1	0.1	0.2
29	α-Humulene	1444	1452	0.6	0.2	0.2	0.3	0.1	0.2	0.2	0.1
30	(*E*)-β-Farnesene	1452	1454	t	0.2	0.2	0.3	t	t	t	t
31	Germacrene D	1482	1484	0.8	0.8	0.8	0.8	0.2	0.3	0.4	t

32	β-Selinene	1492	1489	t	t	-	-	0.1	0.1	0.1	t
33	α-Selinene	1496	1498	0.6	0.4	0.5	0.5	0.3	0.4	0.5	t
34	Bicyclogermacrene	1504	1500	0.9	0.6	0.7	0.9	0.5	0.9	0.9	0.8
35	δ-Cadinene	1521	1522	t	t	0.1	0.1	t	t	t	t
36	Spathulenol	1572	1577	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.1
37	Caryophyllene oxide	1577	1582	t	t	0.1	t	0.1	0.2	0.1	0.3
38	γ-Eudesmol	1630	1630	1.8	2.3	2.4	2.7	1.8	2.1	1.7	1.4
39	α-Cadinol	1655	1652	t	0.1	0.1	0.1	0.1	0.1	0.1	0.1
40	β-Bisabolol	1675	1674	0.1	0.1	0.1	0.1	0.3	0.1	0.2	0.2
	Class compositions										
	Monoterpene hydrocarbons			1.1	0.6	0.9	0.9	0.8	0.7	0.8	1.5
	Oxygenated monoterpenes			43.6	44.9	40.6	44.4	44.5	45.3	41.6	18.4
	Sesquiterpene hydrocarbons			3.9	2.9	3.5	4	1.5	2.5	2.8	1.3
	Oxygenated sesquiterpenes			2	2.7	2.8	3	2.4	2.6	2.3	2.1
	Phenyl propanoids			45.9	45.7	48.9	42.4	48	46.8	50.7	74.0
	Others			0.3	0.2	0.2	0.2	0.1	t	0.1	0.1
	Total identified (%)			**96.8**	**97**	**96.9**	**94.9**	**97.3**	**97.9**	**98.3**	**97.4**

Identification methods: Retention Index (RI), MS (GC-MS), RI[a]: Retention index determined in DB-5 (30 m × 0.32 mm) using *n*-alkanes; RI: Retention index from literature [28]; t=trace (<0.1%); Content of major constituents are average of three replicates ± standard deviation; **VS**: Vegetative stage; **HB**: Half bloom stage; **FB**: Full bloom stage; **SS**: Seed setting stage

O. basilicum is a reputed commercial crop for basil oil of commerce. The presence of essential oil with distinct composition determines the specific aroma and flavour of the O. *basilicum* and its chemotypes. Based on the essential oil composition, methyl chavicol, linalool, methyl eugenol, methyl cinnamate, eugenol, *trans*-β-bergamotene/linalool, menthone/methyl chavicol, 1,8-cineole, camphor, citral, camphor/linalool, geraniol/linalool, (*E*)-anethole chemotypes, along with numerous subtypes marked by mixed composition of diverse proportions of these constituents, were reported in *O. basilicum* from different geographic regions [7,11-18]. Based on their chemical composition and geographic origin, essential oils of *O. basilicum* have been classified into four types: European basil oil (from Italy, France, Bulgaria, Egypt and South Africa) dominated by methyl chavicol (25.0–75.0%) and linalool (25.4–50.0%); Reunion basil (from Comoro Island, Thailand, Madagascar and Vietnam) characterized by a high content of methyl chavicol (≥80%); Tropical basil oil (from India, Guatamala and Pakistan) dominated by methyl cinnamate (upto 65.0%), and basil oil from North Africa and USSR rich in eugenol [33,34]. In our earlier work, we have reported chemotypic variation of *O. basilicum* with composition similar to European basil oil and Reunion basil from foothills of Uttrakhand, India [4, 7-10]. In present analysis, the major constituents identified in the studied cultivar of *O. basilicum* are (*E*)-methyl cinnamate (36.6–66.4%), linalool (11.2–43.8%), and (*Z*)-methyl cinnamate (5.4–7.6%). Moreover, for comparison of the examined essential oil composition with earlier reported representative compositions of various chemotypes of *O. basilicum* from different regions, the content of major constituents were subjected to the hierarchical cluster analysis. The derived dendrogram clearly showed dissimilarity based on the percentages of the constituents present among the different compositions (Figure 2). The derived dendrogram depicts the grouping of chemical compositions as per the chemical compositions of chemotypes based on the content variability of these constituents. The existence of various chemotypes rich in specific essential oil constituents and due to overlapping of various constituents with each other in mixed compositions resulted huge complexity in their chemotaxonomy to correlate the compositional variability of *O. basilicum* cultivars from different geographic regions. Methyl cinnamate is a colorless, crystalline solid with a fruity, sweet-balsamic odour and extensively used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. The global consumption of this molecule is in the range of 10-100 metric tonnes per annum [35,36]. Moreover, linalool one of the most common terpene alcohols, has a fine fresh herbal and floral odor character. Linalool is also used extensively in food and pharmaceutical industry, and as a fragrance ingredient used in many fragrance products as well as in non-cosmetic products [37,38]. Moreover, both these molecules are associated with numerous biological activities such as broad range antibacterial, antifungal, and mosquito repellent properties. The compositional results of studied *O. basilicum* taxa revealed that the essential oil was dominated by (*E*)-methyl cinnamate (upto 66.4%), linalool (upto 43.8%) in different harvesting stage. Moreover, the antibacterial activity of the essential oil of *O. basilicum* from full bloom stage of the rain-autumn cropping season (main season) was subjected to antibacterial and antifungal screening against eight bacterial and three fungal strains. The results of antimicrobial study [in terms of zone of inhibition (ZI) and minimum inhibitory concentration (MIC)] showed that the essential oil of *O. basilicum* possessed varying extent of antimicrobial activity against the tested pathogenic strains (Table 3). The zone of inhibition and MICs for the essential oil against bacterial strains ranged from 2-13 mm and 250-1000 µg/mL. The essential oil showed good activity against *Staphylococcus epidermidis* (ZI: 13 mm; MIC: 250 µg/mL), *Streptococcus mutans* (ZI: 11 mm; MIC: 250 µg/mL), and varying degree of antibacterial activity (250-1000 µg/mL) against other tested strains viz. *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and *Salmonella typhimurium*. In antifungal assay, the zone of growth inhibition and MIC ranged from 5-12 mm and 500-562 µg/mL, respectively. The essential oil of *O. basilicum* showed maximum sensitivity against *Candida albicans* with maximal zone of inhibition (12 mm; MIC: 500 µg/mL) followed by *Candida kefyr* (10 mm; MIC: 562 µg/mL).

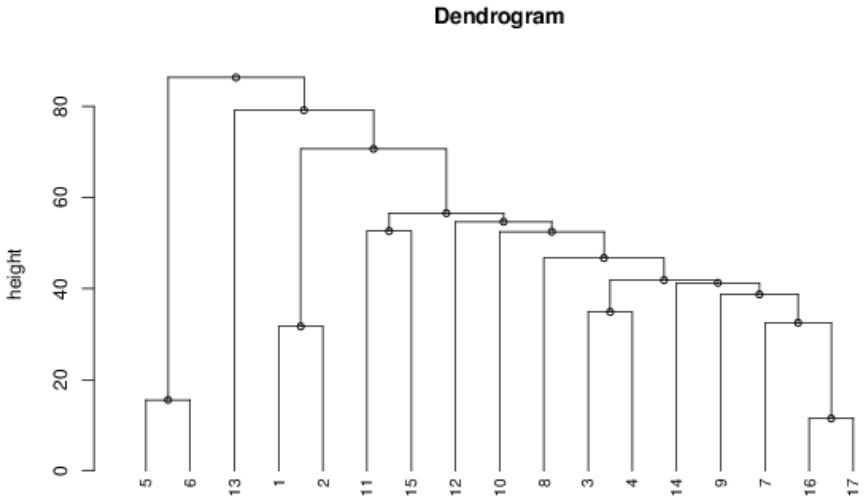

Figure 2. Dendrogram obtained by Hierarchical cluster analysis based on the content of reported major constituents of various representative chemotypes of *Ocimum basilicum* from different geographic region; **1: {**India [7]; Methyl chavicol (88.2%), linalool (1.3%)}; **2**: {India [7]; Methyl chavicol (67.1%), linalool (24.7%)}; **3**: {India [7]; Linalool (74.3%), α-terpineol (3.3%)}; **4**: {Mississippi [11]; Linalool (57.3%), eugenol (29.0%)}; **5: {**Turkey [12]; Methyl eugenol (78.0%)}; **6**: {Mississippi [11]; Methyl eugenol (81.7%)}; **7**: {India [13]; (*E*)-Methyl cinnamate (58.1%), linalool (11.8%)}; **8**: {India [7]; Citral (63.9%; 27.6% neral and 36.3% geranial)}; **9**: {India [7]; Linalool (35.5%, citral (25.0%)}; **10**: {Brazil [14]; Geraniol (46.3%), linalool (17.3%)}; **11**: {Tanzania [15]; 1,8-Cineole (54.3%), α-terpineol (6.6%)}; **12**: {India [16]; Camphor (42.1%), limonene (7.6%)}; **13**: {Togo [17]; *trans*-Anethole (74.7%), linalool (17.3%)}; **14**: {India [18]; (*Z*)-Methyl cinnamate (34.5%), linalool (28.4%)}; **15**: {India [7]; *trans*-α-Bergamotene (25.6%), linalool (17.5%)}; **16**: {India [7]; Linalool (36.3%), (*E*)-methyl cinnamate (33.3%)}, **17:** {India [Present study]: (*E*)-Methyl cinnamate (43.7%), linalool (40.1%)]

Earlier studies on antimicrobial potential of the essential oil of *O. basilicum* rich in chemical constituents (methyl chavicol, linalool, eugenol, methyl eugenol and methyl cinnamate) showed broad antimicrobial activity against numerous pathogenic bacterial and fungal strains ascertaining the potential of basil essential oil as antimicrobial agent in food-flavor and related health care products [20-27]. Most of the earlier studies revealed that Gram-positive strains of bacteria showed higher sensitivity to *O. basilicum* essential oils compared to Gran-negative bacteria [25,39-41]. However, in some studies, basil oil was shown to have high effectiveness on the gram-negatives compared to Gram-positive bacterial strains [23]. It is well known that changes in chemical composition of essential oils have reflective effect on their biological activities. The variation in varying degree of susceptibility of the basil oil against different micro pathogens might be due to the variability in chemical constitution of the essential oils of *O. basilicum* from different geographic regions [25-27]. However, several antimicrobial studies, linked linalool, eugenol, and methyl chavicol for the effective antimicrobial activity of the basil oil against a wide range of microorganisms [20, 39-42]. Hussain et al. (2008) evaluated the antimicrobial efficacy of basil oil, and its constituent linalool, against bacterial strains viz., *S. aureus*, *E. coli*, *B. subtilis*, *P. multocida*, and pathogenic fungi viz., *A. niger*, *M. mucedo*, *F. solani*, *B. theobromae*, *R. solani* [20]. In an another study, the antibacterial properties of the linalool-methyl cinnamate rich basil oil was also investigated reporting inhibitory activity against *S. aureus*, *E. coli*, *S. pyogens*, *B. cereus*, *L. acidophilus*, *A. niger* and *S. cerevisiae* [22,43]. Moreover, the antifungal activity of methyl cinnamate-rich basil oil also showed antagonistic activity against several *Fusarium* spp. [44]. Moreover, the individual principle constituents viz., linalool, methyl chavicol, eugenol, and (*E*)-methyl cinnamate of basil oil also showed to have significant antimicrobial

activity against Gram-negative bacteria (*E. aerogenas* and *P. vulgaris*), Gram-positive bacteria (*S. aureus* and *B. subtilis*), and fungi (*A. flavus*, *A. niger*, *A. ochraceus*, and *P. expansum*) [45]. Hence, the result of present study on antimicrobial activity of the essential oil of *O. basilicum* from India rich in methyl cinnamate and linalool are in agreement with previous studies on antimicrobial efficacy of basil oil for various herbal formulations.

Table 3. Antibacterial and antifungal activity of the studied *O. basilicum* essential oil

Test strains	**Essential oil**		**Standard***	
	ZI (mm)	**MIC (µg/mL)**	**ZI (mm)**	**MIC (µg/mL)**
Bacterial strains				
Staphylococcus aureus	8	500	26	0.39
Staphylococcus epidermidis	13	250	21	3.12
Streptococcus mutans	11	250	29	0.39
Escherichia coli (MTCC 723)	8	500	22	<0.19
Escherichia coli (DH5α)	7	250	24	0.39
Salmonella typhimurium	2	500	27	0.19
Klebsiella pneumoniae	7	500	24	0.19
Pseudomonas aeruginosa	na	1000	20	1.56
Fungal strains				
Candida albicans	5	500	24	0.19
Candida albicans (Clinical isolate)	12	562	26	0.19
Candida kefyr	10	562	32	0.39

*Standard**:** Norfloxacin for antibacterial assay and Ketoconazole for antifungal assay; na: not active

4. Conclusion

Results of the present study clearly indicated that both growth stages and cropping season strongly influence the composition of the essential oil. Results evaluation showed that the seed setting stage was optimum for harvesting (*E*)-methyl cinnamate rich oil in rain-autumn cropping season. Moreover, the content of linalool and (*E*)-methyl cinnamate in preferential harvesting stage (full bloom) is quite comparable in both the cropping seasons. Moreover, the antimicrobial assay showed that the essential oil of studied *O. basilicum* taxa possessed good antibacterial activity against *Streptococcus mutans*, *Staphylococcus epidermidis*, *Escherichia coli*, and antifungal activity against *Candida kefyr* and *Candida albicans*. The essential oil of the studied taxa of *O. basilicum* can be utilized as a good source of chemical products and industrially important aroma constituents, methyl cinnamate and linalool, which could be further, enhanced by varietal and agrotechnology research strategies.

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