

MATURITY-DEPENDENT VARIATIONS IN PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *Ocimum basilicum* L. FROM THE WESTERN HIMALAYAS

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ABSTRACT

Ocimum basilicum L. (Sweet Basil), belonging to the Lamiaceae family, is an important culinary herb with wide industrial and therapeutic uses. The present study investigates how different maturity stages affect the phytoconstituents and bioactive ability of the plant's essential oil. The oil was obtained from three consecutive maturity stages of sweet basil cultivated 30, 60 and 90 days after transplantation by the hydro-distillation method. The phytoconstituents of the volatile oil were analysed through Gas Chromatography and Gas Chromatography–Mass Spectrometry. Methyl chavicol and linalool were the dominant constituents (93–94% of total composition), showing opposite trends: linalool content declined whereas methyl chavicol increased with maturity. Bioassays revealed that the first-stage oil exhibited the highest anti-inflammatory and antibacterial activities, which diminished at later stages. These findings emphasise the ontogenic regulation of phytochemical profiles in *O. basilicum* and suggest the first stage as optimal for harvesting to maximize bioactivity. These results contribute to boosting production, improving financial outcomes, ensuring long-term viability and offering potential uses to provide natural healthcare goods, cosmetics, and food preservation.

Keywords: *Ocimum basilicum*, Essential oil, Hydro-distillation, Anti-inflammatory, Antibacterial.

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INTRODUCTION

The genus *Ocimum*, generally known as ‘basil’ in English and ‘Tulsi’ in Sanskrit, belonging to the Lamiaceae family, has reportedly 150 species of aromatic herbaceous plants widely spread around equatorial and warmer regions all over the world.^{1,2} *Ocimum basilicum* (*O. basilicum*) is the prevalent, affordable and easily available species of this genus. It is commonly known as ‘Sweet basil’ and designated as ‘King of Herbs’ due to its vivid phytoconstituents and therapeutic potential.³ *O. basilicum* exhibits a broad spectrum of activities that justify its various therapeutic potentials. The most documented activities are anti-inflammatory, antimicrobial, antioxidant, analgesic, antihyperlipidemic, antihypertensive, antiulcerative, anticancer, and antiparasitic activities, etc.⁴

Its oil is likely used in the treatment of wasp and snake bites, relieving mental fatigue, curing stomach pain, fever, cough, headache, toothache and earache.^{5,6} As per previous literature, *O. basilicum* contains numerous compounds, namely, linalool, 1,8-cineole, eugenol, estragole, β -ocimene, methyl eugenol, germacrene-D, etc.⁷ Depending on the climate, geographical area, harvesting stage and various other factors, the composition of the volatile oil of the same plant varies.

There are studies that showed linalool as the prime component of the volatile oil of *O. basilicum*, whereas some other studies also reported methyl eugenol, methyl chavicol, 1,8-cineole, and geraniol as the prime components of the oil.⁸⁻¹¹ The extracts and the essential oil of *O. basilicum* possessed good anti-inflammatory and antibacterial activity and seem to be used as a potent product in various industries.^{3,4,12}

Cultivation methods customised for particular regions and growth phases are vital as they harmonise farming operations with distinctive, localised environmental conditions related to agriculture and weather, soil composition and water resources. This alignment is the key for boosting output, enhancing fiscal results and guaranteeing long-term viability. By concentrating on geographical and phase-dependent approaches like choosing appropriate cultivation for an area, agriculture turns out to be more productive and less dependent on common and resource-intensive techniques. This study sought to examine the variation in terpenoids and various bioactivities of the essential oil of *O. basilicum* as the plant proceeds to its maturity state. To our knowledge, there are very few studies that jointly quantify chemical shifts and bioactivities (anti-inflammatory and antibacterial) across defined maturity stages for Western Himalayan *O. basilicum*. Hence, the present study quantified stage-dependent shifts in *O. basilicum* essential-oil composition and linked them to two bioactivity domains (anti-inflammatory and antibacterial) under the middle hill region of Western Himalayan cultivation, providing stage-specific harvest guidance.

EXPERIMENTAL

Collection of Plant Material and its Identification

The collection of sweet basil was done from Pithoragarh district of Uttarakhand, India (at 1646 m, 29.57°N and 80.25°E), which comes under the middle Himalayan region of the Western Himalayas. The plant was grown in an open-field condition in the summer-rainy season. The plant was cultivated consecutively in three different periods of plant maturity at a regular interval of 30 days. The seeds were collected from DIBER, Pithoragarh and sown in the first week of May. The baby plants were transplanted in the whole field at the end of the month. The plants were cultivated in three batches. The first, second and third batches of plants was cultivated after 30, 60 and 90 days of transplantation, respectively and accordingly denoted as the first (I), second (II) and third (III) stage of plant maturity. It was a single-year experiment. The plant specimen was authenticated at (BSD Student Herbarium), Botanical Survey of India, Dehradun, with accession no. 1280.

Extraction of Essential Oil (E.O)

The hydrodistillation method was opted for extracting the E.O. from the freshly harvested aerial part of the plant. The Clevenger apparatus was used for this purpose and was run for 6 hours. Anhydrous sodium sulphate was used to dry the moisture content present in the E.O. It was kept in vials at 4°C until further testing. The E.O. was extracted in three batches at every stage of cultivation by following the same method.

Calculation of E.O yield %

The E.O yield of fresh biomass at each stage was evaluated by using following equation in triplicate:

$$\text{E.O yield (\% v/w, fresh weight basis)} = (\text{mL E.O} / 100 \text{ g fresh biomass}) \times 100 \quad (1)$$

Identification of E.O Components

The E.O sample of each stage was subjected to GC and GC/MS analysis. Shimadzu 2010 GC/MS-QP2010 Ultra gas chromatograph with an Rxi-5 Sil MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness) was used for the GC-MS analysis. Gas chromatography conditions: injector at 260°C, detector at 280°C.

The column was held at 50°C for 2 min, then heated to 210°C at 3°C/min, and finally maintained at 280°C for 18 min. A 2.0 µL sample was injected in split mode (1:10 split ratio). Nitrogen was used as the carrier gas at 1.21 mL/min, with makeup gas supplied to the detector. The ion source was set at 220°C with a solvent cut time of 3.50 min. The scan ranged from 4 min to 69.98 min with an m/z ratio (40.00 - 650.00).

The volatile constituents were identified by comparing their retention indices, which were calculated corresponding to the retention times of the n-alkane (C₇ – C₃₃) series, with those outlined in Adam's literature¹³ and NIST17R, Wiley8 and FFNSC libraries. The area normalisation method was used to determine the concentrations of components irrespective of the response factors. The results were tentative based on MS and RI values only.

Anti-inflammatory Efficacy of E.O

The anti-inflammatory efficacy of E.O samples was tested by the protein denaturation method as described by Rana and colleagues¹⁴ at various concentrations (100-500 µg/mL). Double-distilled water served as the control. Absorbance of the samples was measured at 660 nm. Diclofenac sodium was used as a positive control at the same concentrations. The experiment was performed in triplicate. Following equation was used to calculate the percent inhibition of protein denaturation:

$$\text{Percentage of Inhibition} = (\text{Ao} - \text{At}) / \text{Ao} \times 100 \quad (2)$$

Where, Ao = absorbance taken for blank sample; At = absorbance taken for E.O samples or standards.

Antibacterial Efficacy of the E.O

Microbial Culture

The study was conducted on ten microbial strains having three Gram-positive strains: *Enterococcus faecalis* (MTCC 439), *Staphylococcus aureus* (MTCC 737) and *Bacillus subtilis* (MTCC 121) and seven Gram-negative strains: *Agrobacterium tumefaciens* (MTCC 609) *Dickeya dadantii* (KUMSCC 328), *Klebsiella pneumoniae* (MTCC 109), *Xanthomonas phaseoli* (KUMSCC 327), *Pasteurella multocida* (MTCC 1148), *Salmonella enterica* (MTCC 3223) and *Escherichia coli* (MTCC 443). The bacterial strains were sourced from the Department of Botany, Kumaun University, Nainital, Mycology Laboratory, and CSIR Institute of Microbial Technology, Chandigarh.

Antibacterial Screening

The antibacterial screening of the E.O samples was done by following the disc diffusion method.¹⁵ A fresh nutrient broth was prepared by autoclaving it at 121 °C and 15 lbs pressure. Nutrient broth was used for sub-culturing the colony of each microbial strain. It was incubated for 24 hours at a constant temperature of 37 °C. Nutrient agar was prepared by autoclaving it at 121 °C and 15 lbs pressure. Each petri plate was filled with 20 mL of agar and allowed to rest until it solidified. A radial streaking of freshly prepared nutrient broth of the microbial strain was done on agar plates. The sterile discs of 6 mm diameter containing 5 µl of the E. coli sample were placed on the agar plates using forceps. After that, parafilm was used for sealing the plates to avoid any contamination, and they were incubated for 24 hours in a BOD at 37 °C. After 24 hours, the plates were observed for bacterial growth and the zone of inhibition (ZOI) was measured. At first, the preliminary testing was done at a concentration of 1000 µL/mL prepared in n-hexane by following the above method. For MIC determination, the samples showing greater than 10 mm ZOI in preliminary testing were considered and further diluted at varied concentrations (31.25 µL/mL, 62.5 µL/mL, 125 µL/mL, 250 µL/mL and 500 µL/mL) in triplicate. The lowest concentration that didn't show any microbial growth was marked as the MIC (minimum inhibitory concentration) value. Antibiotics Streptomycin and Erythromycin (30 mcg per disc) were taken as standards, and a hexane disc as a negative control.

Statistical Analysis

MS-Excel and One-way ANOVA with SPSS (16.0) software was used for the statistical evaluation of the data. Each sample was treated as triplicate, and MS-Excel was used to compute the mean and standard deviation (SD). The ultimate outcomes were presented as mean ± SD. Using the Duncan test, all mean value comparisons were explained at a significance level of α=0.05.

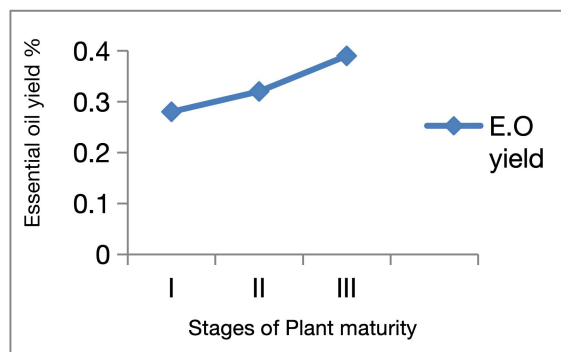
RESULTS AND DISCUSSION

E. O. yield of *O. basilicum*

Figure-1 showed that the E.O. yield of *O. basilicum* increased as the plant advanced to maturity. Essential oil yield (v/w %) of fresh biomass increased by 14.28% and 21.87% on moving from I to II and II to III stage, respectively. As per the literature, the essential oil yield increased with plant ontogeny, which was similar to the present study.¹⁶⁻¹⁹

Terpenoid Composition of E.O. of *O. basilicum*

GC and GC/MS analysis of the E.O. of *O. basilicum* showed variation in the terpenoid composition as the plant advanced toward maturity, as shown in Table-1.

Fig.-1: E.O. Yield % at different Growth Stages of *O. basilicum*

Thirty-one, thirty-two and thirty-four compounds were identified in the first, second and third stages of plant maturity, which represented 99.28%, 99.00% and 99.85% of the total E.O respectively. The analysis revealed that methyl chavicol and linalool were the prime compounds constituting 93-94% of the total E.O. Germacrene-D and trans-bisabolene were detected as the minor compounds present in the E.O.

Table-1: Terpenoid Composition of the *O. basilicum* E.O. at different Stages of Plant Maturity

S. No.	Compound name*	(R _t) _{GC} (min)	RI ^a	RI ^b	Composition of the compound in each stage (in %)		
					I Stage (30 DAT)	II Stage (60DAT)	III Stage (90 DAT)
1.	hex-(3Z)-enol	6.173	859	859	0.22	0.20	0.17
2.	α -pinene	8.716	939	936	0.08	0.15	0.12
3.	sabinene	10.286	975	975	0.04	0.06	0.05
4.	β -pinene	10.549	979	981	0.06	0.03	0.03
5.	myrcene	10.871	990	989	0.07	0.03	0.04
6.	1,8-cineole	12.876	1031	1035	0.10	0.03	0.03
7.	β -ocimene	13.557	1050	1051	0.20	0.09	0.09
8.	linalool oxide	14.695	1072	1075	ND	ND	0.06
9.	fenchone	15.506	1086	1080	0.13	0.05	ND
10.	linalool	16.126	1096	1090	37.28 $\pm$ 1.03	30.28 $\pm$ 1.21	21.89 $\pm$ 0.47
11.	fenchol <exo>	17.151	1126	1121	0.10	0.04	0.01
12.	camphor	17.887	1146	1143	0.01	0.01	0.01
13.	isogeranial	19.930	1185	1186	0.03	0.04	0.03
14.	methyl chavicol	20.705	1196	1201	55.05 $\pm$ 0.90	63.30 $\pm$ 0.53	72.88 $\pm$ 2.07
15.	β -nerol	22.049	1229	1232	0.11	0.22	0.15
16.	neral <Z>	22.392	1238	1240	0.62	0.65	0.40
17.	geraniol	23.017	1252	1254	ND	ND	0.20
18.	geranial	23.804	1267	1271	0.87	0.87	0.42
19.	α -cubebene	27.329	1348	1349	0.35	0.23	0.21
20.	α -copaene	28.547	1376	1377	0.03	0.06	0.08
21.	β -element	29.161	1390	1391	0.03	0.05	0.08
22.	α -gurjunene	29.892	1409	1406	0.02	0.01	0.02
23.	caryophyllene (β , <E>)	30.415	1419	1421	0.37	0.30	0.40
24.	β -gurjunene	30.860	1433	1432	ND	ND	0.02
25.	bergamotene (α , <E>)	31.001	1434	1434	0.18	0.16	0.23
26.	farnesene (β , <Z>)	31.309	1442	1444	ND	ND	0.02
27.	α -humulene	31.913	1454	1457	0.33	0.15	0.17
28.	germacrene-D	33.013	1485	1482	0.89 $\pm$ 0.02	1.37 $\pm$ 0.27	1.75 $\pm$ 0.23
29.	bicyclogermacrene	33.590	1500	1496	0.13	0.11	0.13
30.	germacrene-A	34.092	1509	1509	0.05	0.04	0.06
31.	δ -cadinene	34.524	1523	1519	0.05	0.07	0.10

32.	bisabolene (α , $<E>$)	35.417	1542	1542	0.40 ± 0.02	0.60 ± 0.04	1.15 ± 0.06
33.	nerolidol	36.265	1563	1563	0.07	0.09	0.10
34.	murrolol (α , epi)	39.487	1642	1646	ND	0.07	ND
35.	α -cadinol	39.913	1654	1658	0.10	0.13	0.10
36.	shyobunol	41.028	1689	1686	0.11	0.04	0.06

(Values are in mean $\pm$ S.D) DAT = Days After Transplantation

Rt = Retention time (in min)

RI^a = Retention index as per literature/Adam's literature.

RI^b = Calculated Retention index as per GC data

ND= Not Detected

*Method of identification = RI and MS with Adam's literature, Wiley8, NIST17R and FFNSC libraries.

Monoterpene hydrocarbons (MH): Compounds with serial no. 2-5 and 7. Oxygenated monoterpenes (OS): Compounds with serial no. 6, 8-13 and 15-18. Sesquiterpene hydrocarbons (SH): Compounds with serial nos. 19-32. Oxygenated sesquiterpenes (OS): Compounds with serial no. 33-36. Phenylpropanoids (PP): Compound with serial no. 14. Oxygenated hydrocarbon: Compound with serial no. 1.

Classification of Compounds

As the plant advances toward maturity, a variation was seen in the composition of the class of compounds of E.O. of *O. basilicum* (Table-2).

Table-2: Variation in Percentage of Class of Compounds at Different Stages of Plant's Maturity

Class of Compounds	I Stage (in %) (30 DAT)	II Stage (in %) (60 DAT)	III Stage (in %) (90 DAT)
Monoterpene hydrocarbons	0.45	0.36	0.33
Oxygenated monoterpenes	39.53	32.22	23.58
Sesquiterpene hydrocarbons	2.84	3.00	4.24
Oxygenated sesquiterpenes	0.28	0.33	0.28
Oxygenated hydrocarbon	0.22	0.20	0.17
Phenylpropanoids	55.71	62.83	71.21

DAT = Days After Transplantation

The E.O. of *O. basilicum* had a high content of phenylpropanoids and oxygenated monoterpenes that combinedly covered 94-96 per cent of the whole E.O succeeded by sesquiterpene hydrocarbons (2-5 per cent). The oxygenated monoterpenes decreased, whereas phenylpropanoids and sesquiterpene hydrocarbons increased as the plant attained its maturity state. Furthermore, monoterpene hydrocarbons, though being less in composition, decreased with time. In our study, methyl chavicol and linalool were the prime constituents of the *O. basilicum* E.O in which the former compound increased steadily and had the highest composition in the third stage (71.21%), whereas later compound decreased with plant maturity and had the highest composition in the first stage (37.76%). Numerous factors govern the composition and yield of E.O. of the same plant species depending on their chemotypes, aroma, flowers, leaves, origin of plant, plant ontogeny, plant growth regulators, harvesting methods, oil extraction techniques, seasonal and climatic changes, etc. One study found linalool, eugenol and terpinen-4-ol as the prime constituents of *O. basilicum* E.O where linalool had the highest composition in the flowering stage (39.58%).²⁰ Nurzynska-Wierdak and co-workers conducted the study on two cultivars of *O. basilicum*, namely, Kasia and Wala. Both cultivars possessed a high content of linalool (Kasia- 69.7% and Wala- 68.7%) at the budding stage.²¹ According to Verma and co-workers study, 'Vikrashudha' and 'CIM-Saumya', both cultivars of *O. basilicum*, were a rich source of methyl chavicol that regularly increased with growth stages. In the spring-summer cropping season, Vikrashudha consisted of 93.63%, whereas CIM-Saumya had 76.95% methyl chavicol at the full bloom stage. Linalool was another major composition of the CIM-Saumya cultivar, with the highest composition (34.07%) in its vegetative state and decreased as the plant advanced toward the maturity stage. The major component variation found in the CIM-Saumya cultivar was in agreement with the results of the present investigation. Furthermore, the composition of linalool and methyl chavicol also varied at different segments of the herb: flowers, stem and leaves.²² The E.O. yield increased in both Vikrashudha (0.22-0.61%) and CIM-Saumya (0.18-0.46%)

cultivars of *O. basilicum* with plant ontogeny.¹⁶ In another study, linalool and trans-methyl cinnamate were the key compounds of the E.O. of *O. basilicum*. Both compounds exhibited irregular variation as the plant moved to its maturity state. In the spring-summer cropping season, linalool had the highest composition (43.8%) at the half-bloom stage, and trans-methyl cinnamate (42.8%) had it at the full-bloom stage, whereas in the rainy-autumn cropping season, trans-methyl cinnamate (66.4%) showed the maximum composition at the seed-setting stage. Oxygenated monoterpenes ranged from 18.4% - 44.9%, and phenylpropanoids ranged from 42.4% - 74%, with the maximum composition in the E.O. The E.O. had the maximum yield in the full bloom stage: 0.34% in spring-summer and 0.52% in rainy-autumn cropping season.¹⁷

Variation in Anti-Inflammatory Efficacy as Plant Advanced toward Maturity State

Rheumatoid arthritis and inflammation are known to be caused by denaturation of proteins. Several anti-inflammatory medications have demonstrated concentration-dependent, dose-dependent inhibition of thermally induced protein denaturation.²³ The results showed a positive response in inhibiting protein denaturation at all concentrations of each stage of plant maturity. The anti-inflammatory potency of *O. basilicum* E.O at three different periods of plant cultivation showed variation in inhibition percent from 11.23 ± 0.11 to 83.55 ± 0.12 . Table 3 summaries the IC_{50} values of the E.O at different periods of plant maturity. The findings indicated that the anti-inflammatory potency of the *O. basilicum* E.O decreased as the plant advanced to maturity. The first stage showed the lowest IC_{50} value, which continuously increased as the plant matured over time, reflecting the highest anti-inflammatory potency after 30 days of transplantation.

Table-3: Anti-inflammatory Potency of *O. basilicum* E.O. as the Plant Advanced towards Maturity

Sample	IC_{50} (μ g/ml)
First stage	340.33 ± 0.86
Second stage	462.14 ± 0.63
Third stage	496.01 ± 0.61
Diclofenac sodium (Standard)	<100

Values are in Mean $\pm$ S.D, at $p < 0.05$

Terpenes and terpenoids are physiologically significant for reducing a variety of inflammatory symptoms, most likely by blocking several pathogenic stages of the inflammatory process.²⁴ Methyl chavicol and linalool were the prime components of the E.O. of *O. basilicum* that composed 93-94% of the complete E.O. composition at each stage of cultivation (Table-2). Previous literature revealed that methyl chavicol as well as linalool were potent anti-inflammatory agents.^{12, 25-28} Hence, both major compounds influenced the anti-inflammatory potency of the E.O. However, linalool majorly impacted the anti-inflammatory property of the E.O. As the linalool composition decreased with time (Table-2), the anti-inflammatory potency of the E.O. also decreased. In previous studies, it was also found that the compounds such as linalool, containing a structural hydroxyl group, are the active constituents of the anti-inflammatory potency. As per a study, the anti-inflammatory efficacy of many natural compounds depended on the hydroxyl group present in their chemical structure.²⁹ Therefore, the study suggested that the first stage of *O. basilicum* had the maximum anti-inflammatory property.

Impact of Maturity Stages on Antibacterial Activity of *O. basilicum* E.O

The E.O. of *O. basilicum* showed significant results against various pathogens. It exhibited the maximum ZOI against *Salmonella enterica* (22.00 ± 1.00 mm), followed by *Agrobacterium tumefaciens* (18.66 ± 0.58 mm), *Staphylococcus aureus* (17.66 ± 0.58 mm), *Escherichia coli* (15.00 ± 1.00 mm) and *Dickeya dadantii* (14.00 ± 0.00 mm) in preliminary screening done against ten bacteria. The E.O exhibited minimum inhibitory effect at the lowest concentration (31.25μ L/mL) against *Salmonella enterica* (7.00 ± 0.00 mm) and *Agrobacterium tumefaciens* (7.33 ± 0.58 mm) at the first stage of plant maturity. The hexane disc, which was taken as a negative control, didn't show a zone against any tested bacterial strain. The antibacterial action appeared to diminish as the plant approached maturity, as evidenced by the MIC value at higher concentrations in the second and third stages of the plant maturity. The findings are summarised

in Table-4 and Table-5. Previous literature revealed that E.O. of *O. basilicum* showed good antibacterial properties against various plant and animal pathogens. Linalool and methyl chavicol were both potent components that exhibited a significant antibacterial activity.³⁰ The components of basil E.O. and their solubility play a vital role in deciding the antibacterial potency of the E.O. The components with higher water solubility, such as linalool, exhibit stronger antibacterial potency, whereas methyl chavicol, with lower water solubility, possesses comparatively weaker antibacterial potency. However, the synergistic or antagonistic properties of the various major or minor components present in the complex mixture of the E.O contribute to its antibacterial potency.³¹ Our study also found the similarity to the above factors. Methyl chavicol and linalool were the prime components of the E.O under investigation and constituted 93-94% of the total E.O, with a difference in composition at each stage of plant maturity. Linalool had a higher composition in the first stage that kept on decreasing in further growing stages, as did the antibacterial effect of the E.O. Hence, it may be deduced that linalool had a greater impact in comparison to methyl chavicol on the antibacterial efficacy of the E.O and the first stage of sweet basil was the most promising stage exhibiting the best antibacterial effect.

Table-4: Preliminary Screening of E.O and Antibiotics

Zone of Inhibition (in mm) of Antibiotics and E.O at 1000 μ L/mL				
S. No.	Bacterial strain	Streptomycin	Erythromycin	E.O at 1000 μ L/mL
1.	<i>Bacillus subtilis</i>	21.33 ^a ±1.53	16.66 ^a ±1.53	N.A
2.	<i>Staphylococcus aureus</i>	21.00 ^a ±1.73	23.66 ^f ±1.53	17.66 ^e ±0.58
3.	<i>Enterococcus faecalis</i>	20.33 ^a ±0.58	19.33 ^{b,c} ±1.53	7.66 ^a ±0.58
4.	<i>Xanthomonas phaseoli</i>	22.33 ^{a,b} ±1.53	22.00 ^{e,f} ±1.00	N.A
5.	<i>Dickeya dadantii</i>	24.66 ^c ±0.58	17.66 ^{a,b} ±0.58	14.00 ^c ±0.00
6.	<i>Klebsiella pneumoniae</i>	21.66 ^{a,b} ±1.53	21.66 ^{b,d,e,f} ±1.53	7.00 ^a ±0.00
7.	<i>Escherichia coli</i>	22.00 ^{a,b} ±1.00	16.66 ^a ±0.58	15.00 ^d ±1.00
8.	<i>Pasteurella multocida</i>	22.66 ^{a,b,c} ±0.58	19.66 ^{b,c,d} ±0.58	9.33 ^b ±0.58
9.	<i>Agrobacterium tumefaciens</i>	24.00 ^{b,c} ±1.00	21.00 ^{c,d,e} ±1.00	18.66 ^d ±0.58
10.	<i>Salmonella enterica</i>	22.66 ^{a,b,c} ±1.53	16.66 ^a ±0.58	22.00 ^f ±1.00

All ZOI values are the mean $\pm$ S.D. of triplicates. Depending on the Duncan test ($\alpha=0.05$) and one-way ANOVA analysis, a, b, c, d, and e represent a collection of homogeneous subsets.; N.A = Not Active

Table-5: Zone of Inhibition (in mm) of E.O at all Three Stages of Plant Maturity for Determining MIC Value

Bacterial strain	Stages of maturity	500 μ L/mL	250 μ L/mL	125 μ L/mL	62.5 μ L/mL	31.25 μ L/mL
<i>Staphylococcus aureus</i>	I	13.00 ^b ±0.58	10.33 ^a ±0.58	8.33 ^a ±0.58	7.00 ^a ±0.00*	N.A
	II	12.66 ^c ±0.58	10.00 ^b ±1.00	8.33 ^c ±0.58	7.33 ^a ±0.00*	N.A
	III	12.00 ^b ±1.00	10.66 ^c ±1.15	7.66 ^a ±0.58	7.00 ^a ±0.00*	N.A
<i>Dickeya dadantii</i>	I	12.00 ^a ±1.00	10.33 ^a ±0.58	8.33 ^a ±1.00	7.00 ^a ±0.00*	N.A
	II	10.33 ^a ±1.15	9.00 ^a ±0.58	7.00 ^a ±1.00*	N.A	N.A
	III	11.00 ^b ±1.00	8.66 ^b ±1.15	7.33 ^a ±0.58*	N.A	N.A
<i>Escherichia coli</i>	I	14.00 ^c ±0.00	11.33 ^b ±0.58	8.33 ^a ±0.58	7.00 ^a ±0.00*	N.A
	II	11.33 ^b ±0.58	8.66 ^a ±0.58	7.33 ^{a,b} ±0.58*	N.A	N.A
	III	8.33 ^a ±0.58	7.00 ^a ±1.00*	N.A	N.A	N.A
<i>Agrobacterium tumefaciens</i>	I	16.00 ^d ±0.00	13.33 ^c ±0.58	10.66 ^b ±0.58	9.00 ^b ±0.00	7.33 ^a ±0.58*
	II	11.33 ^b ±0.58	9.00 ^a ±0.00	7.66 ^{a,b,c} ±0.58	7.00 ^a ±0.00*	N.A
	III	10.66 ^b ±0.58	9.00 ^b ±0.00	7.33 ^a ±0.58*	N.A	N.A
<i>Salmonella enterica</i>	I	18.00 ^c ±0.00	14.00 ^c ±0.00	10.66 ^b ±0.58	8.66 ^b ±0.58	7.00 ^a ±0.00*
	II	12.00 ^{b,c} ±0.00	9.33 ^a ±0.58	8.00 ^{b,c} ±0.00	7.00 ^a ±0.00*	N.A
	III	8.66 ^a ±0.58	7.33 ^a ±0.58*	N.A	N.A	N.A

All the ZOI values are represented as mean $\pm$ S.D. of triplicates (in mm); Depending on the Duncan test ($\alpha=0.05$) and one-way ANOVA analysis, a, b, c, d, and e represent groups of homogeneous subsets.; N.A = Not Active; * = Minimum Inhibitory Concentration (MIC)

CONCLUSION

The present study showed high usefulness of the basil cultivar under investigation for both the pharmaceutical and perfume industries as well as for the food industry. *O. basilicum* E.O from the middle Himalayan region of the Western Himalayas is a rich source of methyl chavicol and linalool. The linalool compound possesses a high demand in the flavour and fragrance industries. It is well acknowledged to exhibit numerous bioactivities such as antioxidant, antiviral, anti-inflammatory, anticancer and antimicrobial properties.³² Methyl chavicol is used as a fragrance in perfumes and as a flavour in food and liquor. It is also a prime compound used in the preparation of various aromatic compounds such as anisyl alcohol, anethole, anisaldehyde, etc.³³ To extract methyl chavicol, the third stage (i.e. 90 days after transplantation) is the best stage to cultivate *O. basilicum* plants, whereas for linalool extraction, the first stage (i.e. 30 days after transplantation) is the most prominent stage for cultivation. The anti-inflammatory and antibacterial potential of the E.O. of the *O. basilicum* favoured the first stage of cultivation and hence was suggested as the most appropriate stage for cultivation to attain the maximum potency of these bioactivities. These findings help in optimising crop production, quality, profitability and sustainability in various food and health sectors.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The present study is associated with *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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