Regional Differences in Phytochemical Profiles and Antimicrobial Efficacy of *Ocimum tenuiflorum* L. Essential Oil.

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Abstract

Medicinal plants represent an important source of bioactive compounds with potential applications in antimicrobial therapy. In the present study, the chemical composition and antibacterial activity of essential oil extracted from Ocimum tenuiflorum L. leaves collected from two different geographical regions were investigated. Essential oils were obtained by hydrodistillation using a Clevenger apparatus and analysed by gas chromatography–mass spectrometry to determine their phytochemical profiles. Antibacterial activity was evaluated against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa using the agar well diffusion method, minimum inhibitory concentration determination, and bacterial growth curve analysis under sub-inhibitory conditions. GC–MS analysis revealed distinct variations in chemical composition between the urban and rural samples, with eugenol and methyl eugenol identified as the major constituents. Both essential oil samples exhibited inhibitory effects against all tested bacterial strains, with the rural sample showing comparatively higher antibacterial activity. Growth kinetic studies demonstrated a significant reduction in bacterial proliferation in the presence of essential oils, indicating significant suppression of bacterial growth. The results suggest that geographical origin influences both the phytochemical composition and antibacterial efficacy of Ocimum tenuiflorum L. essential oil. This study highlights the role of environmental factors in determining the antimicrobial potential of plant-derived essential oils and supports their possible application as natural antibacterial agents.

Keywords: *Essential oil, Ocimum tenuiflorum L., Phytochemistry, Antimicrobial efficacy.*

Abbreviation:

EOOT: Essential oil of *Ocimum tenuiflorum* L.

MIC: Minimum inhibitory concentration

GC–MS: Gas chromatography–mass spectrometry

BHI: Brain heart infusion broth

PC: Positive control

DMSO: Dimethyl sulfoxide

OD: Optical density

AUC: Area under the curve

1. INTRODUCTION

Medicinal plants are recognised as an important source of bioactive compounds with established therapeutic relevance. In recent decades, the increasing prevalence of antimicrobial resistance has reduced the effectiveness of conventional antibiotics and intensified interest in alternative antimicrobial agents derived from natural sources [1], [3]. Plant secondary metabolites such as phenolics, terpenoids, alkaloids, and flavonoids exhibit antimicrobial activity through multiple mechanisms, including disruption of cell membranes, inhibition of enzymatic pathways, and interference with cellular metabolism [3], [10]. Essential oils represent a concentrated form of these secondary metabolites and have therefore been widely investigated for their broad-spectrum antimicrobial potential.

The genus Ocimum (family Lamiaceae) comprises several medicinally important species known for their essential oil content and diverse biological activities. Among them, *Ocimum tenuiflorum* L., commonly known as holy basil or tulsi, has been extensively used in traditional medicine systems for the treatment of microbial, inflammatory, and respiratory disorders [7], [11]. The pharmacological properties of O. tenuiflorum are primarily attributed to its essential oil, which is rich in phenylpropanoids and terpenoids such as eugenol, methyl eugenol, and sesquiterpenes [8], [11]. Previous studies have demonstrated that essential oils derived from Ocimum species exhibit pronounced antibacterial activity, particularly against Gram-positive bacteria, while moderate inhibitory effects against Gram-negative strains have also been reported [15], [16].

The chemical composition of essential oils is not constant and is strongly influenced by environmental and geographical factors. Variations in climate, soil composition, water availability, and cultivation conditions significantly affect the biosynthesis and accumulation of secondary metabolites in medicinal plants [5], [6]. In Ocimum species, such environmental influences have been associated with changes in essential oil yield and the relative abundance of key constituents, leading to variations in biological activity [8], [11]. As a result, essential oils obtained from plants grown in different regions may exhibit inconsistent antimicrobial performance if geographical origin is not taken into consideration.

1.1 Secondary Metabolites and Antimicrobial Mechanisms

Secondary metabolites play a crucial role in plant defence and contribute significantly to antimicrobial activity. Phenylpropanoids and terpenoids, which are dominant constituents of Ocimum essential oils, exert antibacterial effects primarily by altering bacterial cell membrane integrity and increasing permeability, leading to leakage of intracellular contents [3], [10]. Eugenol, a major phenylpropanoid present in O. tenuiflorum essential oil, has been reported to inhibit bacterial growth by disrupting membrane structure and impairing essential cellular functions [7], [15].

The antibacterial efficacy of essential oils depends not only on the concentration of individual compounds but also on their combined and synergistic effects. Oils containing higher proportions of biologically active constituents often exhibit lower minimum inhibitory concentrations and enhanced suppression of bacterial growth [9], [16]. Therefore, detailed chemical characterisation is essential for correlating phytochemical composition with antimicrobial performance.

1.2 Aim of the Study

The present study aims to evaluate the influence of geographical origin on the chemical composition and antibacterial activity of *Ocimum tenuiflorum* L. essential oil. Essential oils extracted from leaves collected from urban and rural regions were analysed using gas chromatography–mass spectrometry. Their antibacterial efficacy was assessed against selected Gram-positive and Gram-negative bacterial strains using agar well diffusion, minimum inhibitory concentration determination, and bacterial growth curve analysis. The study seeks to establish a correlation between regional variation in phytochemical composition and observed antibacterial activity.

2. MATERIALS AND METHODS

2.1 Plant Sample Collection

Fresh leaves of *Ocimum tenuiflorum* L. were collected from two geographically distinct locations to evaluate the influence of regional variation on essential oil composition and antibacterial activity. The urban sample was collected from the Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India, while the rural sample was collected from Killa Pardi village, Valsad district, Gujarat, India. Leaves were washed thoroughly with tap water followed by distilled water to remove dust and surface contaminants, following standard practices reported for Ocimum species used in essential oil studies [18], [19]. The cleaned plant material was used immediately for essential oil extraction to minimise loss of volatile constituents.

2.2 Extraction of Essential Oil (Hydrodistillation)

Essential oil extraction was performed using the hydrodistillation method with a Clevenger-type apparatus. Fresh leaves (350 g) were subjected to hydrodistillation for six hours. The essential oil was separated from the aqueous layer, dried over anhydrous sodium sulphate to remove residual moisture, and stored in airtight amber-coloured vials at 4 °C until further analysis. Hydrodistillation using a Clevenger-type apparatus is a widely accepted and standardized method for essential oil extraction from aromatic and medicinal plants and has been extensively reported in the literature [18–20].

2.3 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The chemical composition of the extracted essential oils was analysed using gas chromatography–mass spectrometry. Individual constituents were identified by comparing their retention times and mass spectral fragmentation patterns with those reported in the literature and standard mass spectral libraries. GC–MS is a reliable and extensively used analytical technique for qualitative and semi-quantitative analysis of volatile compounds in essential oils [21–23].

2.5 Agar Well Diffusion Assay

The antibacterial activity of essential oils was assessed using the agar well diffusion method, which is widely employed for screening antimicrobial activity of plant derived essential oils [22], [23]. Bacterial suspensions were adjusted to 0.5 McFarland standard and uniformly spread onto nutrient agar plates using sterile cotton swabs. Wells of 5 mm diameter were prepared using a sterile cork borer, and measured volumes of essential oil were introduced into the wells. Dimethyl sulfoxide was used as the solvent control, while a standard antibiotic served as the positive control. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimetres.All antibacterial assays were performed in triplicate, and results are expressed as mean ± standard error (SE).

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of essential oils was determined using a concentration-dependent growth inhibition method. Serial dilutions of essential oils were prepared and tested against the selected bacterial strains. The MIC was defined as the lowest concentration that inhibited visible bacterial growth after incubation. MIC determination was performed according to standard antimicrobial susceptibility testing guidelines and previously reported protocols [27–29]. All experiments were conducted in triplicate, and results are expressed as mean ± standard error.

2.7 Bacterial Growth Curve Analysis

Bacterial growth kinetics were analysed to evaluate the effect of essential oils under sub-inhibitory conditions. Bacterial suspensions were adjusted to approximately 10^6 CFU mL^{-1} and treated with essential oil at half of the determined MIC value. Optical density was measured at 630 nm at regular time intervals over a period of 18 to 24 hours, depending on the bacterial strain. Growth curves were constructed, and bacterial growth was quantified by calculating the area under the curve, following established kinetic analysis methods [30,31]. Each experiment was conducted using six independent replicates.

3. Results & Discussion

3.1 Chemical insights into *Ocimum tenuiflorum* L. by GC-MS

Gas chromatography–mass spectrometry analysis revealed clear variations in the chemical composition of essential oils extracted from *Ocimum tenuiflorum* L. leaves collected from urban and rural regions. The identified compounds, their retention times, and relative area percentages are presented in Tables 1 and 2.

The essential oil obtained from the urban sample exhibited a comparatively diverse phytochemical profile, with more than twenty compounds detected. The major constituents included bicyclo[7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene, 3-allyl-6-methoxyphenol, humulene, germacrene D, and caryophyllene oxide. These compounds belong primarily to the terpenoid, sesquiterpene, and phenylpropanoid classes, which are commonly reported in Ocimum species.

In contrast, the rural sample essential oil contained fewer compounds but showed a higher proportion of phenylpropanoids. Eugenol and methyl eugenol were identified as the dominant constituents, together accounting for a substantial percentage of the total oil composition. The predominance of these compounds is consistent with earlier reports on O. tenuiflorum essential oil and is known to influence its biological activity.

The observed compositional differences indicate that geographical and environmental factors play a significant role in determining the phytochemical profile of O. tenuiflorum essential oil. Variations in temperature, soil composition, and cultivation conditions may contribute to differences in secondary metabolite synthesis.

GC-MS Analysis of essential oils extracted from samples of leaves taken from Urban Ocimum tenuieflorum L.

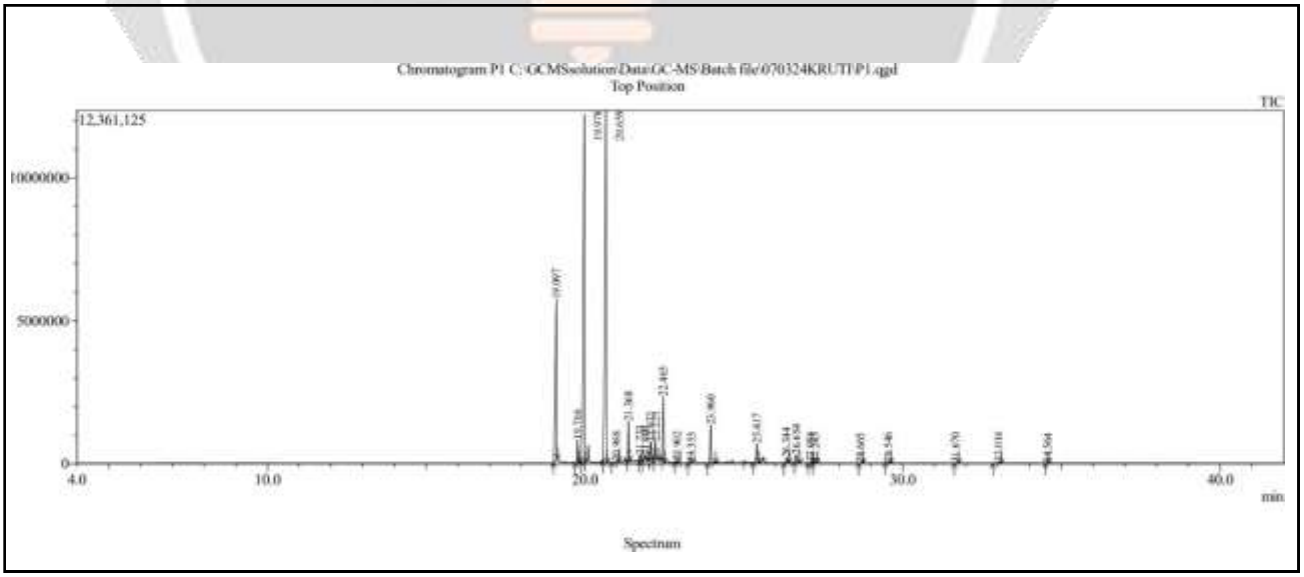

Figure-1 Graphical Representation of Peak detected by GC-MS Analysis.

Table No-1 Characterization of the main components of EOOT extracted from Sample collected from pot by GC-MS Analysis.

Peak	R. Time	Area%	Name
1	19.097	15.05	3-Allyl-6-methoxyphenol
2	19.766	1.52	Cyclohexane, 1-ethenyl-1-methyl-2,4- bis (1-Methyl ethenyl)
3	19.978	33.66	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-Methyl ethenyl)
4	20.659	34.53	Bicyclo [7.2.0] undec-4-ene, 4,11,11-trimethyl-8-methylene
5	20.988	0.07	(1R,5R)-1,8-Dimethyl-4-(propan-2-ylidene) spiro [4.5] dec-7-ene
6	21.368	2.43	Humulene
7	21.733	0.33	4a,8-Dimethyl-2-(prop-1-en-2-yl)
8	21.900	0.28	Germacrene D
9	22.072	1.01	Naphthalene, decahydro-4a-methyl-1-methylene
10	22.221	1.24	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl
11	22.465	4.20	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)
12	22.902	0.00	(3R,3aR,3bR,4S,7R,7aR)-4-Isopropyl-3,7-Di methyl octahydro-
13	23.960	2.81	Caryophyllene oxide
14	25.417	1.47	Neo intermedeol
15	26.344	0.29	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol
16	28.665	0.03	2-Pentadecanone, 6,10,14-trimethyl-
17	29.546	0.01	1,8,11,14-Heptadecatetraene, (Z, Z, Z)-
18	31.670	0.04	Isopropyl palmitate
19	33.016	0.13	Phytol
20	34.564	0.01	Neophytadiene
		100.00	

🟨	Antimicrobial activity
🟥	Highly anti-inflammatory and anti-cancerous activity
🟦	Anti-inflammatory and pesticide protection
🟩	Suppress various types of harmful micro-organisms
⬜	Antibacterial efficacy

GC-MS Analysis of essential oils extracted from samples of leaves taken from farms.

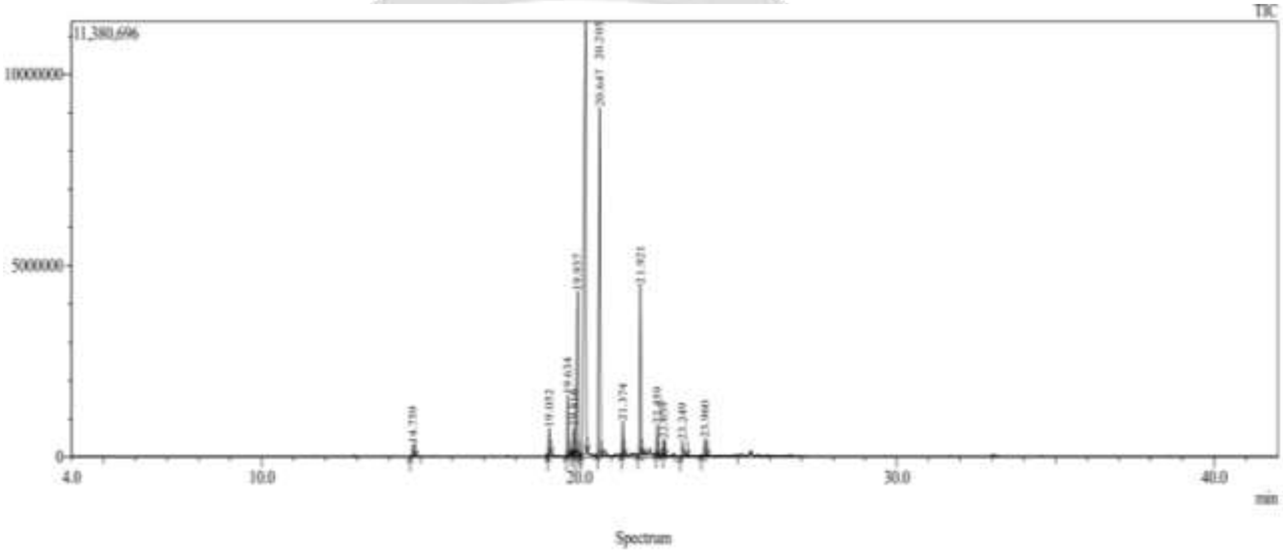

Figure-2 Graphical Representation of Peak detected by GC-MS Analysis.

Table No-2 characterization of the main components of EOOT extracted from Sample collected from farms by GC-MS Analysis.

Peak	R. Time	Area %	Name
1	14.759	0.55	Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl-, (1S- endo)
2	19.052	1.31	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene
3	19.634	2.83	Copaene
4	19.816	1.08	(-)-. Beta. -Bourbonene
5	19.937	9.56	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)
6	20.205	49.20	Methyl eugenol
7	20.647	21.60	Eugenol
8	21.374	1.54	Humulene
9	21.921	8.73	Germacrene D21
10	22.459	1.40	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)
11	22.659	0.60	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthale
12	22.249	0.80	3,7-Cyclodecadiene-1-methanol, alpha.,. alpha.,4,8-tetramethyl-, [s-(Z, Z)]
13	23.960	0.88	Caryophyllene oxide
		100.00	

🟨	Antimicrobial activity
🟪	Highly anti-inflammatory and anti-cancerous activity
🟦	Anti-inflammatory and pesticide protection
🟩	Suppress various types of harmful micro-organisms
⬜	Antibacterial efficacy

3.2 Antimicrobial Efficacy

The antibacterial activity of essential oils extracted from both regions was evaluated against selected Gram-positive and Gram-negative bacterial strains using the agar well diffusion method. Both essential oils demonstrated inhibitory activity against all tested microorganisms. However, differences in the extent of inhibition were observed between the urban and rural samples.

The essential oil extracted from the rural region exhibited comparatively larger zones of inhibition against Bacillus subtilis and Staphylococcus aureus, indicating stronger antibacterial activity against Gram-positive bacteria. Moderate inhibition was observed against Escherichia coli and Pseudomonas aeruginosa. The urban sample also showed antibacterial activity but with relatively lower inhibition zones.

These results suggest that the higher concentration of phenylpropanoid compounds, particularly eugenol and methyl eugenol, in the rural sample may contribute to enhanced antibacterial efficacy. Gram-positive bacteria appeared more susceptible to the essential oils than Gram-negative bacteria, which may be attributed to differences in cell wall structure.

Antibacterial Activity

Bacterial Strains Employed in the Study: The EOOT were tested for their in-vitro antimicrobial properties against bacterial isolates of Staphylococcus aureus, Bacillus Subtilis, Escherichia coli and Pseudomonas aeruginosa. For that we have performed well diffusion method.

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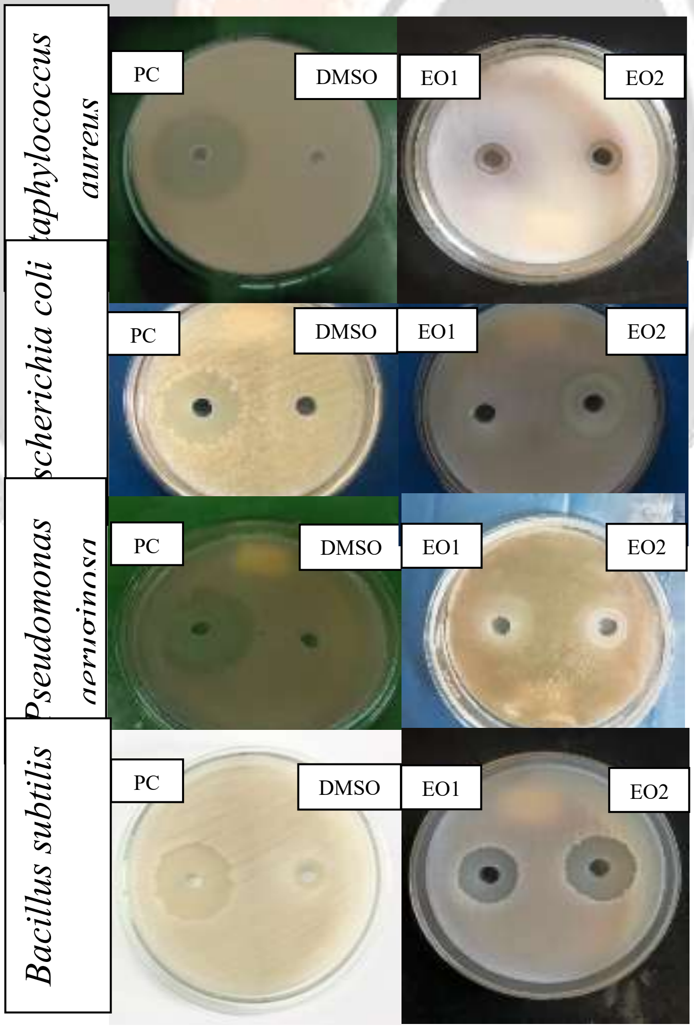

Figure-3 Antibacterial activity of EO1 and EO2 against selected bacterial strains determined by the agar well diffusion assay.

Notes: Standard Antibiotics as Positive Control (PC), DMSO: Solvent used for essential oil

EO1: Urban Sample, EO2: Rural Sample of *Ocimum tenuiflorum* L.

The antibacterial activity of essential oils extracted from *Ocimum tenuiflorum* L. leaves collected from two geographical regions was evaluated using the agar well diffusion method, and the results are presented in Figure 3 as mean ± standard error. Both essential oil samples exhibited measurable inhibitory effects against all tested bacterial strains.

The essential oil obtained from the rural region (EO2) showed slightly higher inhibitory activity against Pseudomonas aeruginosa compared to EO1, while EO1 exhibited marginally higher inhibition against Escherichia coli and Staphylococcus aureus. Among the tested microorganisms, Bacillus subtilis showed relatively higher sensitivity to both essential oil samples. The low standard error values indicate good reproducibility of the antibacterial measurements.

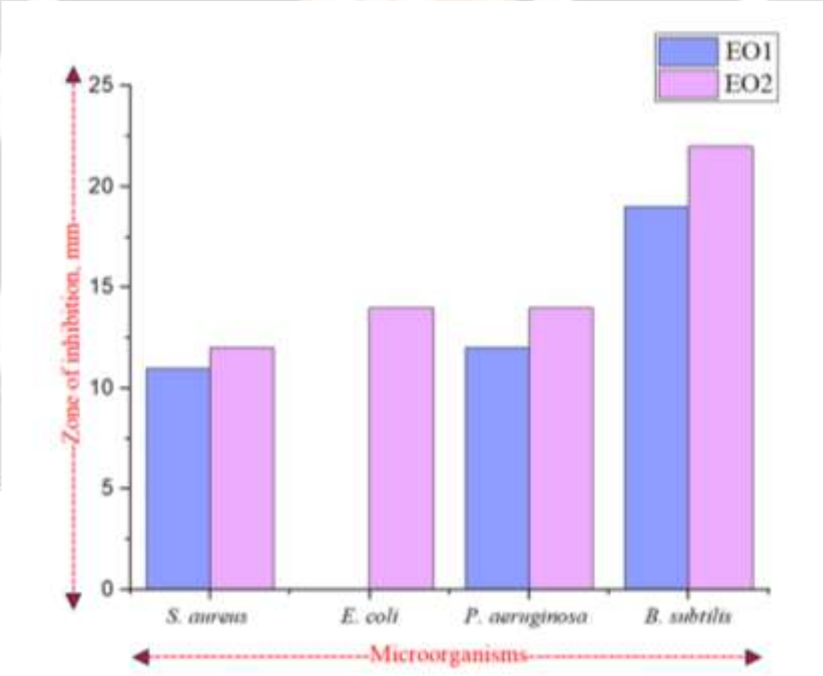

Figure-4 Graphical representation of two different essential oil showing Zone of inhibition on different Microorganism.

3.3 MIC: Minimum Inhibitory Concentration

The experiment began by comparing the killing activities tested against a range of bacteria and estimated them based on the lowest dose required to be fatal. They were evaluated at varied concentrations and According to the data, which demonstrated adequate growth-inhibitory activity.

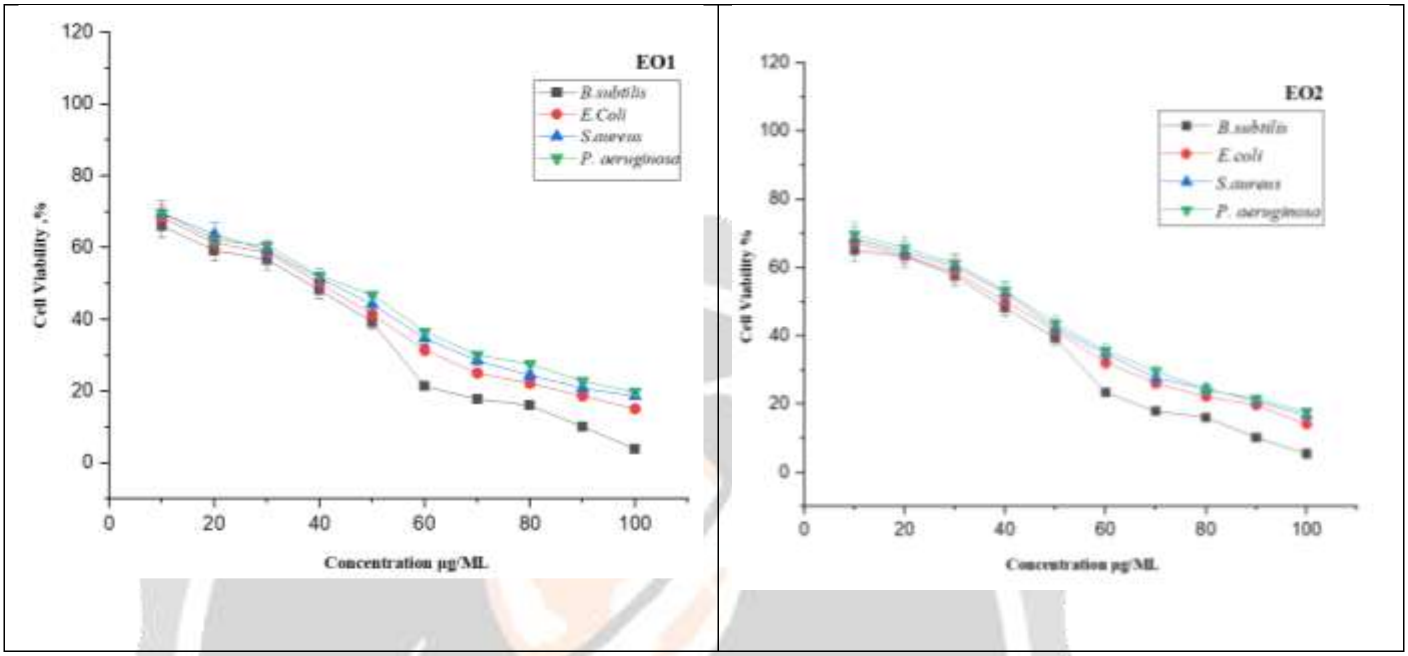

Figure-4 Graphical representation of Dose response curves reflecting the antibacterial effect of EO in cell viability on different Bacterial cell. Data are presented as mean ±SE.

Table No-3 Minimal inhibitory Concentration (MIC) of both the samples EO1 and EO2 for different microorganisms.

Sample	***B. subtilis***	***E. coli***	***S. aureus***	***P. aeruginosa***
EO1	24.20 ± 0.30	35.08 ± 0.30	39.16 ± 0.30	40.53 ± 0.30
EO2	23.82 ± 0.30	33.25 ± 0.30	38.61 ± 0.30	43.21 ± 0.30

The minimum inhibitory concentration values of essential oils extracted from *Ocimum tenuiflorum* L. leaves collected from two geographical regions are presented in Table X. MIC values are expressed as mean ± standard error, calculated from triplicate measurements.

Both essential oil samples exhibited inhibitory activity against all tested bacterial strains. The essential oil obtained from the rural region (EO2) demonstrated lower MIC values against Bacillus subtilis (23.82 ± 0.30 µg mL^{-1}), Escherichia coli (33.25 ± 0.30 µg mL^{-1}), and Staphylococcus aureus (38.61 ± 0.30 µg mL^{-1}) compared to the urban sample (EO1), indicating higher antibacterial potency. In contrast, EO1 showed a slightly lower MIC value against Pseudomonas aeruginosa (40.53 ± 0.30 µg mL^{-1}) relative to EO2 (43.21 ± 0.30 µg mL^{-1}).

Among the tested bacterial strains, Bacillus subtilis exhibited the lowest MIC values for both essential oil samples, suggesting higher susceptibility. Gram-positive bacteria were generally more sensitive to the essential oils than Gram-negative bacteria, which may be attributed to structural differences in the bacterial cell envelope. The relatively higher MIC values observed for Pseudomonas aeruginosa indicate its intrinsic resistance, which is consistent with its well known permeability barrier.

The small standard error values associated with the MIC measurements indicate good reproducibility and consistency of the experimental data. Overall, the MIC results confirm that geographical variation influences the antibacterial efficacy of *Ocimum tenuiflorum* L. essential oil, with the rural sample exhibiting enhanced inhibitory activity against most tested strains.

3.4 Proposed Mode of Antibacterial Action

The antibacterial activity of *Ocimum tenuiflorum* L. essential oils observed in the present study can be attributed to the combined effects of their major phytochemical constituents. GC–MS analysis confirmed the presence of phenylpropanoids and terpenoid compounds, particularly eugenol and methyl eugenol, which are widely reported to exhibit antibacterial properties. Although the present work does not include direct mechanistic experiments, the observed antibacterial effects may be explained based on previously reported interactions of these compounds with bacterial cells.

Phenylpropanoids such as eugenol are known to interact with bacterial cell membranes, leading to increased membrane permeability and leakage of intracellular components. This disruption can interfere with essential cellular processes, resulting in growth inhibition. The higher susceptibility of Gram-positive bacteria observed in the present study may be related to the absence of an outer membrane, allowing easier penetration of hydrophobic essential oil components.

The inhibition of bacterial growth at sub-inhibitory concentrations, as evidenced by growth curve analysis, further suggests interference with normal cellular metabolism rather than immediate bactericidal action. The combined and potentially synergistic action of multiple constituents present in the essential oil is likely responsible for the overall antibacterial efficacy rather than the effect of a single compound.

These interpretations are consistent with the antibacterial trends observed in agar diffusion and minimum inhibitory concentration assays. However, further studies involving membrane integrity assays or molecular level investigations would be required to confirm the exact mechanism of antibacterial action.

3.5 Bacterial Growth Curve Analysis

The effect of *Ocimum tenuiflorum* L. essential oils on bacterial growth kinetics was evaluated using growth curve analysis at sub-inhibitory concentrations. The growth curves of untreated control cultures exhibited a typical bacterial growth pattern, characterised by a short lag phase followed by exponential growth and a stationary phase.

In contrast, cultures treated with essential oils showed a noticeable alteration in growth behaviour. Both EO1 and EO2 caused an extension of the lag phase and a reduction in the exponential growth rate across all tested bacterial strains. A lower optical density was observed during the stationary phase, indicating reduced bacterial proliferation in the presence of essential oils.

The inhibitory effect was more pronounced in cultures treated with EO2, which is consistent with its lower minimum inhibitory concentration values and higher antibacterial activity observed in agar diffusion assays. Gram-positive bacteria showed a greater reduction in growth compared to Gram-negative strains, reflecting their higher susceptibility to essential oil components.

The observed growth suppression at sub-inhibitory concentrations suggests that the essential oils interfere with normal cellular metabolism rather than causing immediate bactericidal effects. These results support the antibacterial trends obtained from diffusion and MIC studies and further confirm the influence of geographical variation on the antibacterial efficacy of *Ocimum tenuiflorum* L. essential oil.

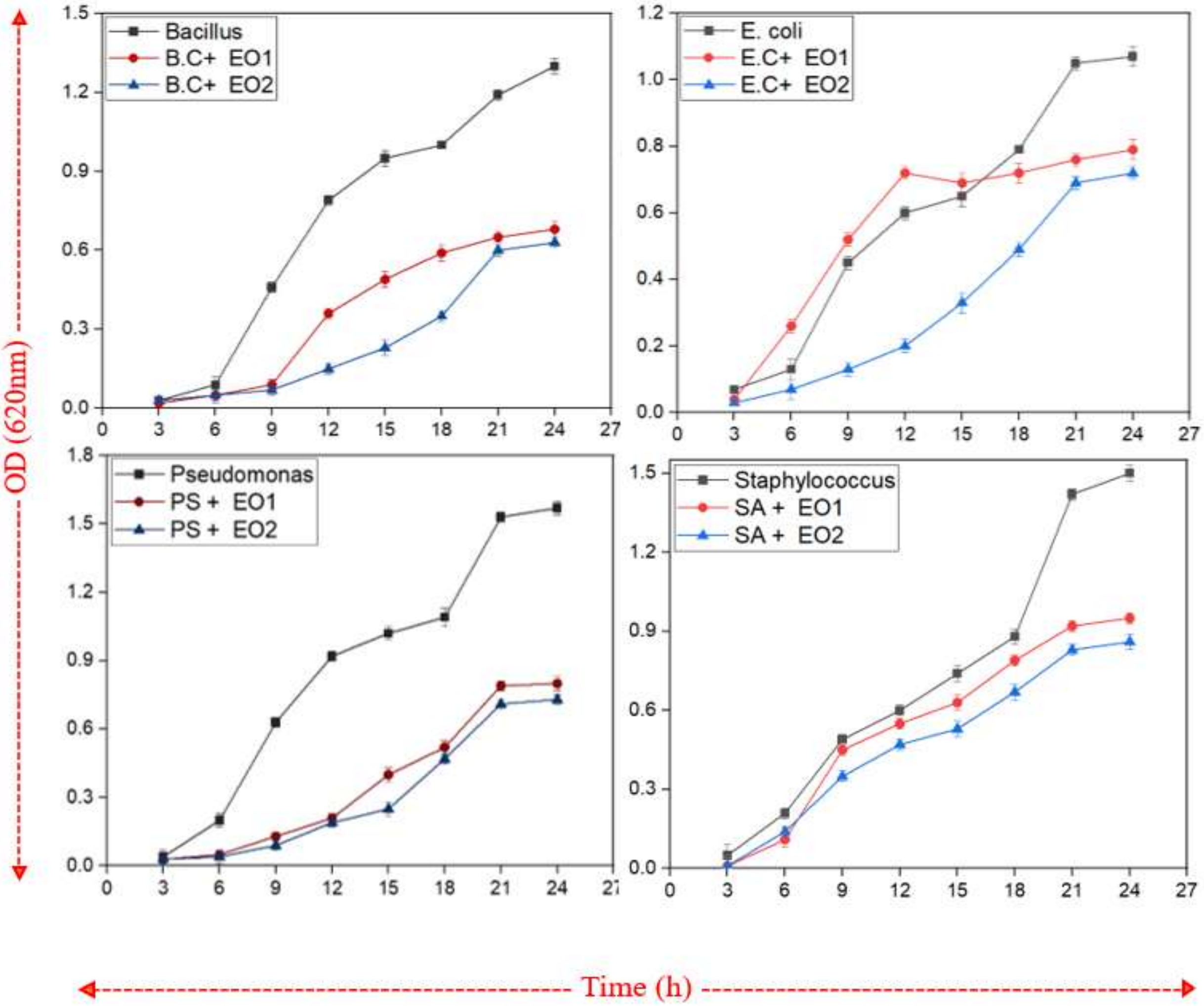

Figure-4 Graphical representation of Bacterial Growth curve.

(In order to assess the bacterial growth curve activity, the microorganisms were treated with extracted essential oil of two different Essential oil of *Ocimum tenuiflorum* L. Which is EO1 & EO2., Where EO1- sample which is collected from urban region, EO2 – Sample which is collected from Rural aera.)

The study examined the growth pattern of Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus in the presence and absence of two different sample of EOOT (a compound of interest).

4. CONCLUSION

The present study demonstrates that the geographical origin of *Ocimum tenuiflorum* L. significantly influences the yield, chemical composition, and antibacterial activity of its essential oil. Essential oil extracted from plants grown in the rural region exhibited a higher yield and stronger antibacterial activity compared to the urban sample, despite the urban oil showing a more diverse phytochemical profile. Gas chromatography–mass spectrometry analysis confirmed the dominance of phenylpropanoid compounds, particularly eugenol and methyl eugenol, in the rural sample, which likely contributes to its enhanced antibacterial efficacy.

Both essential oil samples exhibited inhibitory effects against Gram-positive and Gram-negative bacterial strains, with Bacillus subtilis showing the highest sensitivity. Growth kinetic studies further revealed that essential oils interfere with bacterial proliferation by prolonging the lag phase and reducing overall growth, even at Sub-inhibitory concentrations. These findings indicate that environmental and geographical factors play a critical role in determining the antimicrobial potential of O. tenuiflorum essential oil.

Overall, the study highlights the importance of regional variation in evaluating the biological activity of medicinal plants and supports the potential application of *Ocimum tenuiflorum* L. essential oil as a natural antibacterial agent.

5. ACKNOWLEDGEMENT

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