

RESEARCH ARTICLE

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GC-MS Characterization of *Eupatorium odoratum* (L.) Leaves Essential Oil and Evaluation of *In vitro* Antimicrobial and Antioxidant Activity

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Abstract

The leaves essential oil of *Eupatorium odoratum* (L.) obtained by the hydro-distillation method was light yellowish in colour, having pungent aromatic fragrance with the yield of 0.010%. Fifteen secondary metabolites were identified via the GC-MS analysis of the essential oil and α -Cadinol (20.10%) was found to be the major secondary metabolite in the essential oil followed by α -Bisabolol (15.33%), cis-muurolo-4 (14) 5- diene (10.79%), Isobornyl acetate (6.40%), and Isocaryophyllene (5.39%). The antimicrobial efficacy was investigated via agar well diffusion technique and essential oil was most effective against *P. aeruginosa* with inhibition zone 22.00 \pm 0.57 mm, which was followed by *E. coli* (19.00 \pm 0.57 mm), *S. aureus* (18.33 \pm 0.33 mm), *K. pneumoniae* (16.50 \pm 0.33 mm), and *S. pyogenes* (11.00 \pm 0.33 mm). MIC and MBC values were observed lowest against *P. aeruginosa* i.e., 3.12 μ l/ml and 6.24 μ l/ml respectively. Antioxidant activity of essential oil was investigated by DPPH and H₂O₂ scavenging assay and IC₅₀ value was found very low, i.e. 10.58 μ l/ml and 11.76 μ l/ml, respectively, so the essential oil was considered as a potential antioxidant agent. The results validate scientifically the traditional utilization of leaves essential oil of *E. odoratum* in traditional medicinal system and confirmed that it can be used as modern phytomedicines as an antimicrobial as well as antioxidant agent.

Keywords: *Eupatorium odoratum*, Essential Oil, GC-MS, Antimicrobial Activity, Antioxidant Activity

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INTRODUCTION

Medicinal plant produces a variety of bioactive phytochemicals, offering a valuable source of various herb-based medicines. Medicinal and aromatic plants have long been served as vital therapeutic agents with pharmacological and economic significance. Due to economic considerations, approximately 80% of the population in developing nations still uses medicinal plants as a source of their treatments.¹ Herbal drugs have several advantages, such as low cost, fewer side effects, various molecular targets, etc.; due to these benefits, these are considered as good and safe alternatives of synthetic drugs.²

The essential oils are the composite of aromatic, volatile, and lipophilic secondary plant metabolites and the major constituents include mono and sesquiterpenes that are synthesised from isoprenoid pathway as well as their oxygenated derivatives as alcohols, oxides, aldehydes, ketones, esters, and phenols. Aromatic medicinal plants are thoroughly used in conventional medicinal system due to the bioactivity of their essential oils. From the last few years, it was observed that the use of aromatic medicinal herbs and their essential oils has been increased in the field of scientific research as well as in industries such as pharmaceutical, nutritional, and cosmetics.³ Essential oils and their constituents are used in sanitary products, cosmetics, medicines, aromatherapy, agriculture, food additives, food preservation, and natural medicine. As a result, with yearly earnings in the billions, essential oils became a far more interesting topic for both study and business.⁴

Genus *Eupatorium* belongs to Asteraceae family is perennial weed, native to America and distributed to different parts of the world. It includes approximately 1200 species out of which 7 are reported in India. *E. odoratum* is synonymously well recognized as *Chromolaena odorata* (L.), commonly called Siam weed, etc. and known for its pharmacological and ethnobotanical properties.⁵ Pungent aromatic fragrance of crushed leaves, colour of flower represents its signature morphology. Decoction of this plant is very useful for skin disorders,

curing cough, and cold, used as wound healing agent around the globe. As this plant is highly diverged, its essential oil is used in several pharmacological purposes such as antimicrobial, antidepressant, sedative, analgesic etc., these properties are probably because of the presence of flavonoids, alkaloids, phenols, and tannins, etc.⁶ It has hepatoprotective, anti-diabetic, anti-hypertensive, and immunomodulatory properties. Mainly terpenoids and flavonoids present in essential oils are responsible for these broad-spectrum bioactivities.⁷ According to Amaliah et al.⁸ leaf extract at dosage concentrations of 5%, 10%, and 20% can lower blood glucose levels in hyperglycemic mice, with 20% being the most effective.

The objective of the present investigation is to extract essential oil from leaves of *E. odoratum* and characterize its phytoconstituents via GC-MS, along with the evaluation of in vitro antimicrobial activity against some wound infection causing bacteria.

METHODOLOGY

Plant material

For the selection of sampling area, firstly the field survey was done for the identification of plant habitat. Then the plant material (leaves) was collected from Sahastradhara road, Dehradun in the month of December when the plant was in flowering period. The leaves were then packed, brought to laboratory, and left to dry in the shade then crushed and stored till further processing. Plant material was authenticated in Botanical Survey of India, Dehradun, Uttarakhand and the herbarium sheet was submitted with the accession number 911.

Essential oil extraction

Freshly dried leaves of *E. odoratum* were hydro-distilled for approximately 3 hours by using Clevenger-type appliance. The distillate has been saturated and n-hexane was used for the oil extraction and dehydrate with the help of anhydrous sodium sulphate to remove moisture content and stored in glass vials with tight lid in refrigerator at 4°C for further use.⁹ The formula was used to calculate the yield of *E. odoratum* leaves essential oil:

$$\text{Yield (\%)} = \frac{(\text{Weight of the essential oil})}{(\text{Weight of initial sample taken})} \times 100$$

GC-MS characterization

The analysis was performed in Sophisticated Analytical Instrumentation Facility, SAIF Panjab University, Chandigarh India. GC-MS characterization of *E. odoratum* leaves essential oil was performed on Trace 1300 GC coupled with Thermo TSQ8000 Triple Quadrupole MS, Thermo Scientific, US. Column was consist of triple quadrupole 30 mm 0.25 mm ID 0.25 m dimensions film of a DB 35-MS Capillary Standard non-polar column and Helium carrier gas (flow rate: 1.0 ml/min) was used. Ion Source temperature is 350°C and its mass range is 2.1100amu. It maintained temperature of oven at 60°C-280°C for approximately 15-30 min. The constituents were analysed by comparing their retention time and mass spectra of peaks to that of standard computational database of NIST and Willey libraries.¹⁰

Antimicrobial studies

The antimicrobial activity was assessed by using two Gram-positive human pathogenic bacteria, i.e. *Staphylococcus aureus* (MTCC 1144), *Streptococcus pyogenes* (MTCC 442) and three Gram-negative bacteria, i.e. *Klebsiella pneumoniae* (MTCC 4030), *Escherichia coli* (MTCC 40), and *Pseudomonas aeruginosa* (MTCC2474). The agar well diffusion technique was utilized to assess the antimicrobial potency of the essential oil. Essential oil was diluted with dimethyl sulfoxide (DMSO) to prepare final concentration (100µl/ml) for the in vitro assay and the bacterial inoculum turbidity was maintained at 1.5×10^8 CFU/ml (0.5 McFarland standards).¹¹ The diameter of well was 6 mm and 50µl of essential oil dilution was suspended into the well for the antimicrobial assay. Plates were incubated for 24 h in a BOD incubator at 37°C and experiment was conducted in triplicates.¹² DMSO was used as negative control and three antibiotic disks [streptomycin (10µg), clindamycin (2µg) and ciprofloxacin (10µg)] were taken as positive control to compare the antibacterial efficacy of the essential oil by disk diffusion method.

MIC and MBC evaluation

Minimum inhibitory concentration (MIC) of the essential oil was carried out using the broth micro dilution method using 96 well microtiter plate method.¹³ For the preparation of stock solution essential oil suspended into the DMSO and different oil concentrations were made ranges from 100, 50, 25, 12.5, 6.25 and 3.12 µl/ml. The smallest concentration of the essential oil that can inhibit the bacterial growth is noted as MIC. For Minimum bactericidal concentration (MBC) calculation, 10µl inoculum from MIC plate with no visible growth was transferred to the nutrient agar plates and incubated. The concentration, that resulted in no apparent bacterial growth on the plate was noted as MBC. DMSO and Ciprofloxacin were utilized as negative and positive control respectively for the experiment.

Antioxidant activity

DPPH free radical scavenging assay

To evaluate the antioxidant potential of the essential oil 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out.¹⁴ 4 mg of DPPH was dissolved in 100 ml of ethanol to prepare 0.004% w/v fresh DPPH working solution. 2 ml of different concentrations of essential oil (15.62, 31.25, 62.5, 125, 250, 500, 1000 µg/ml) was suspended in 3 ml aliquot of working solution and incubated for 30 min in dark at room temperature. 2 ml ethanol + 3 ml DPPH working solution was taken as negative control and ascorbic acid was used as positive control. The absorbance of essential oil was compared to the corresponding absorbance of ascorbic acid at 517 nm in triplicates. The percentage inhibition of DPPH was measured by the formula:

$$\text{Percentage Inhibition} = \frac{((\text{OD of Control} - \text{OD of Sample}))}{(\text{OD of Control})} \times 100$$

Hydrogen peroxide scavenging assay

The method was carried out to assess the capacity of essential oil to scavenge hydrogen peroxide (H₂O₂) according to Bhatti et al.¹⁵ Aliquot of 0.1 mL of oil (25-400µg/mL) was transferred into the Eppendorf tubes and their volume was made

up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by the addition of 0.6 mL of H₂O₂ solution (2 mM). After 10 minutes of reaction time, the reaction mixture was vortexed, and its absorbance at 230 nm was determined. Ascorbic acid was served as positive control. Each experiment was performed out in triplicates. The following equation was used to determine the capacity of extracts to scavenge H₂O₂:

$$\text{Percentage of H}_2\text{O}_2 \text{ scavenging activity} = ((A_0 - A_1)) / A_0 \times 100$$

Where: A₀ = Absorbance of control, A₁ = Absorbance of sample.

Statistical analysis

All the *in vitro* biological experiments were completed in triplicates, and results were evaluated as mean standard error. Data was statistically checked using the one-way analysis of variance (ANOVA) by using Microsoft Excel 2010 and differences between the means were checked for the significance at P ≥ 0.05.

RESULTS

Yield and GC-MS characterization of essential oil

The physical characteristics of essential oil obtained by the hydro-distillation method was light yellowish in colour, having pungent aromatic fragrance with the yield of 0.010%.

Fifteen bioactive phyto-constituents were identified in the GC-MS analysis of *E. odoratum* leaves essential oil and displayed in Table 1. The chromatogram of GC-MS is shown in Figure 1. Tau-Cadinol was found to be the major

phytochemical with 20.10% peak area followed by α Bisabolol (15.33%); cis-muurolo-4 (14), 5-diene (10.79%); Isobornyl acetate (6.40%); Isocaryophyllene (5.39%); 2,6 Dimethyl 10 methylene 12 oxatricyclo [7.3.1.0(1,6)] tridec2ene (3.53%); Cedrene (3.26%); Cyclohexane, 1 ethenyl 1 methyl 2, 4 bis (1 methyl ethenyl), [1S(1α,2α,4α)] (2.80%); Tricyclo [6.3.0.0(1,5)] undecan4one, 5, 9 dimethyl (2.46%); Cyclobutane, tetrakis (1 methyl ethylidene) (2.14%), and others were present in traces.

Antimicrobial activity

The antimicrobial activity data of *E. odoratum* leaves essential oil and standard reference antibiotics are shown in Table 2. Essential oil was most effective against *P. aeruginosa* among all the test bacterial strains with an inhibition zone of 22.00±0.57 mm which was followed by *E. coli* (19.00±0.57 mm), *S. aureus* (18.33±0.33 mm), *K. pneumoniae* (16.50±0.33 mm), and *S. pyogenes* (11.00±0.33 mm). The reference antibiotic Ciprofloxacin showed the activity against *P. aeruginosa* with a 19.66±0.33 mm zone of inhibition, i.e. lesser than the zone of inhibition exerted by *E. odoratum* leaves essential oil.

MIC/MBC evaluation

The MIC of *E. odoratum* leaves essential oil ranges between 3.12-50µl/ml and MBC ranges between 6.25-100µl/ml against all the test microorganisms. The MIC was observed lowest against *P. aeruginosa*, i.e. 3.12µl/ml which was followed by *E. coli* (6.25µl/ml), *S. aureus* (6.25µl/ml), *K. pneumoniae* (12.5µl/ml), and *S. pyogenes* (50.0µl/ml). The MBC was reported lowest against

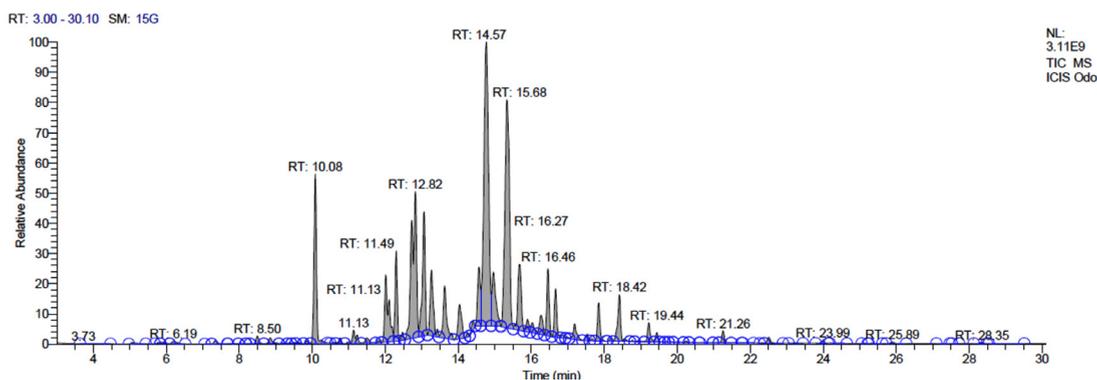
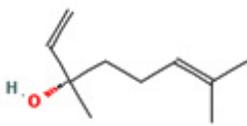
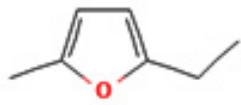
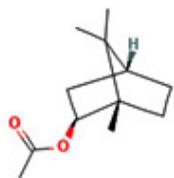
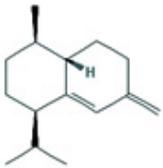
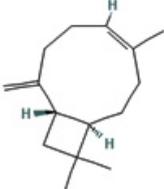
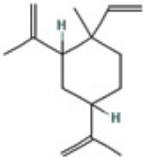
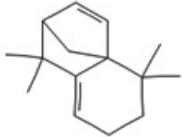
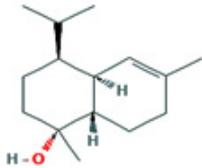
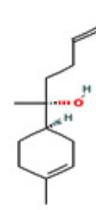
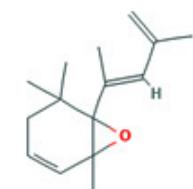
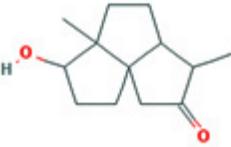
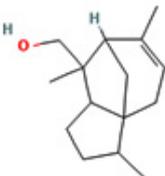


Figure 1. GC-MS analysis of secondary metabolites of leaves essential oils of *E. odoratum*

Table 1. Phytoconstituents of leaves essential oil of *E. odoratum* identified by GC-MS

No.	Retention time	% Peak Area	Compound Name	Molecular Structure
1.	7.34	0.16	1,6 Octadien3ol,3,7dimethyl	
2.	8.50	0.34	2-Ethyl-5-methylfuran	
3.	10.08	6.40	Isobornyl acetate	
4.	11.13	0.78	Bicyclo[2.2.1] heptane, 7,7dimethyl2methylene	
5.	12.82	10.79	cis-Muurola-4(14),5-diene	
6.	13.07	5.39	Isocaryophyllene	
7.	13.27	3.26	Cedrene	

8.	13.63	2.80	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl),[1S(1 α ,2 α ,4 α)]	
9.	14.57	2.39	Isolongifolene, 4, 5dehydro	
10.	14.77	20.10	tau.Cadinol	
11.	15.33	15.33	α Bisabolol	
12.	15.68	3.53	6-(1,3-Dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0]hept-2-ene	
13.	16.46	2.46	Tricyclo[6.3.0.0(1,5)]undecan4one, 5,9dimethyl	
14.	18.42	2.14	Cyclobutane, tetrakis(1-methylethylidene)	
15.	21.26	0.46	8- Cedren-13-ol,	

P. aeruginosa, i.e. 6.25µl/ml which was followed by *E. coli* (12.5µl/ml), *S. aureus* (12.5µl/ml), *K. pneumoniae* (50.0µl/ml), and *S. pyogenes* (100µl/ml) (Table 3).

Antioxidant activity

DPPH free radical scavenging assay

The antioxidant activity of *E. odoratum* leaves essential oil is shown in Figure 2. At 1000 µl/ml concentration of ascorbic acid showed 99.57±0.57% inhibition of DPPH free radical, and at the same concentration, *E. odoratum* essential oil showed 95.93±0.33% inhibition, which was almost comparable to that of ascorbic acid. The

IC₅₀ value of essential oil was 10.58 µl/ml that is very low, so the essential oil was considered as a potential antioxidant agent.

Hydrogen peroxide scavenging assay

Antioxidant activity evaluated by H₂O₂ scavenging assay is shown in Figure 3 where ascorbic acid at 1000 µl/ml concentration showed 98.12±0.57% inhibition, whereas *E. odoratum* essential oil showed 95.12±0.33% inhibition at the same concentration, which was nearly as effective as ascorbic acid. The IC₅₀ value was reported very low, i.e. 11.76 µl/ml, that suggests remarkable antioxidant activity.

Table 2. Zone of inhibitions of leaves essential oils of *E. odoratum* against some human bacterial pathogens

Pathogens	Diameter of zone of inhibition (mm)				
	Essential oil	Negative control (DMSO)	Positive control		
			Clindamycin	Streptomycin	Ciprofloxacin
<i>S. aureus</i> (MTCC 1144)	18.33±0.33	-	11.66±0.33	20.00±0.57	14.00±0.57
<i>S. pyogenes</i> (MTCC 442)	11.00±0.33	-	21.00±0.57	29.33±0.33	24.33±0.33
<i>K. pneumoniae</i> (MTCC 4030)	16.50±0.33	-	-	14.50±0.28	-
<i>E. coli</i> (MTCC 40)	19.00±0.57	-	19.33±0.33	19.66±0.33	26.00±0.57
<i>P. aeruginosa</i> (MTCC 2474)	22.00±0.57	-	-	-	19.66±0.33

DMSO: Dimethyl Sulphoxide, Significant at $P \leq 0.05$ level; Diameter of well: 6 mm; - : No zone of inhibition; MTCC: Microbial Type Culture Collection

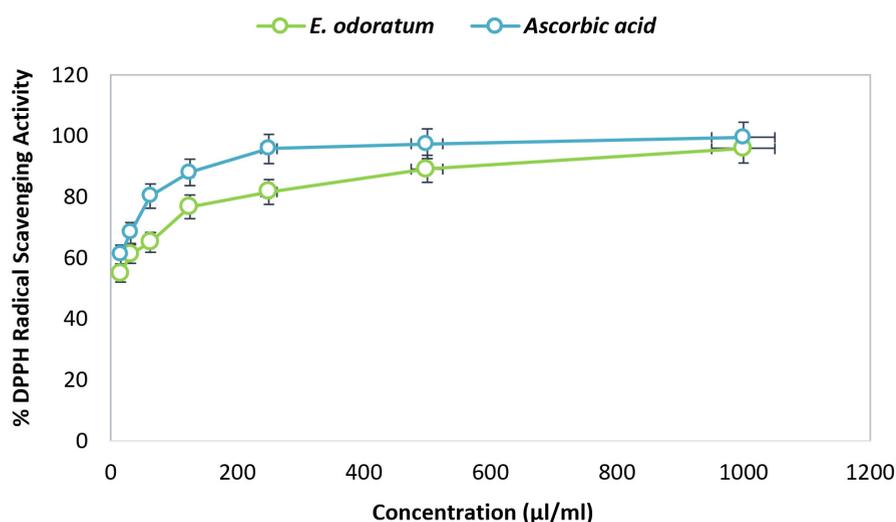


Figure 2. Percentage of antioxidant activity of *E. odoratum* leaves essential oil by DPPH method

DISCUSSION

Plants are recognized as a potential source of medicinally important bioactive phytoconstituents which leads to the development of new eco-friendly, having less cost medicines with negligible side effects to that of synthetic drugs. In modern medicine, essential oils, volatile aromatic compounds, and their derivative are thoroughly used in traditional medicines.^{16,9}

In the present study, the major compound identified in the leaves essential oil of *E. odoratum* were tau. Cadinol followed by α Bisabolol; cis-muurolo-4(14) 5- diene, Isobornyl acetate; Isocaryophyllene; 2,6 Dimethyl10methylene12oxatricyclo[7.3.1.0(1,6)] tridec2ene; Cedrene; Cyclohexane, 1ethenyl1methyl2,4bis

(1 methylethenyl), [1S(1 α , 2 α , 4 α)] ; Tricyclo[6.3.0.0(1,5)]undecan4one, 5,9dimethyl; Cyclobutane, tetrakis (1 methyl ethylidene) and others were present in very small amount.

Sharma et al.,⁵ identified approximately 50 phytocompounds from *E. odoratum* essential oil that include α -pinene (9.64 %), germacrene D (20.27 %), trans- β -caryophyllene (10.04 %), geijerene (25.10 %) as major components while other were present in trace elements. Another study was performed by Dougnon and Ito,⁶ in which 15 essential oil components were reported in which caryophyllene oxide with area percent 43.75 present as major compounds.

In other investigation α -pinene, β -pinene, germacrene D were present with area percent 42.2%, 10.6%, 9.7% respectively while others main compounds were β -copaen-4 α -ol (9.4%); (E)-

Table 3. MIC and MBC values of leaves essential oils of *E. odoratum*

Pathogens	Essential oil (μ l/ml)		Ciprofloxacin (μ g/ml)	
	MIC	MBC	MIC	MBC
<i>Staphylococcus aureus</i> (MTCC 1144)	6.25	12.5	5.0	10.0
<i>Streptococcus pyogenes</i> (MTCC 442)	50.0	100	0.62	1.25
<i>Klebsiella pneumoniae</i> (MTCC 4030)	12.5	50.0	-	-
<i>Escherichia coli</i> (MTCC 40)	6.25	12.5	0.62	1.25
<i>Pseudomonas aeruginosa</i> (MTCC 2474)	3.12	6.25	2.50	5.0

Microbial Type Culture Collection; - : No activity

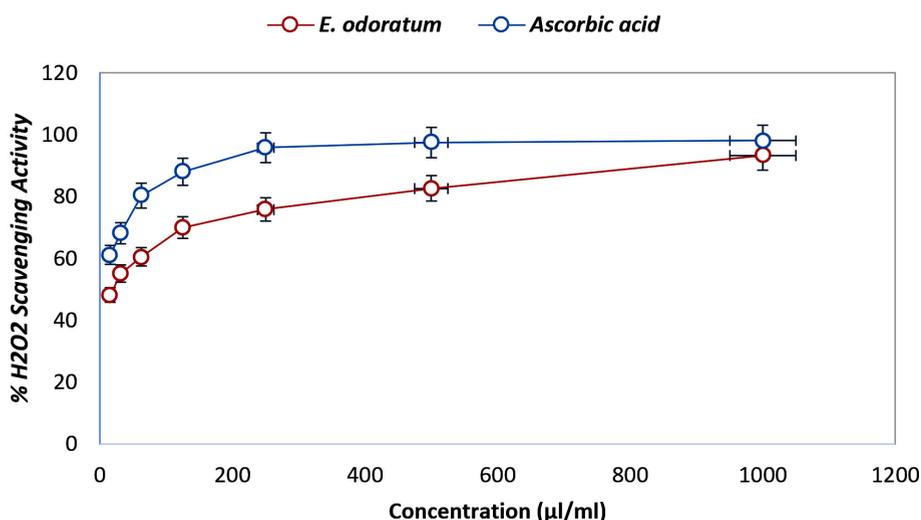


Figure 3. Percentage of antioxidant activity of *E. odoratum* leaves essential oil by H₂O₂ method

caryophyllene (5.4%); and geijerene/pregeijerene (7.5%).¹⁷ In case of leaves essential oil, (+)-camphor (15.46%); α -pinene (19.32%); cadinene (19.09%); cadinol isomer (6.36%); limonene (10.22%); and β -caryophyllene (7.05%) as major compounds by GC/MS analysis.¹⁸ *E. odorata* leaves essential oils were analysed by GC/MS where 20 compounds were detected and include Pregeijerene, dauca-5 (40.60%); 8-diene (16.75%); α -pinene (9.67%); (E)-caryophyllene (6.11%); and β -pinene (5.37%) were found as major components of essential oil.¹⁹

The chemical components and yield of leaves essential oil of *E. odoratum* were reported some how different from that of previous studies. The difference observed may be due to the plant part that is used for extraction, habitat, distribution, climatic conditions, type of soil, plant age or may be the method of the extraction.

Present investigation reported the *E. odoratum* leaves essential oil has antibacterial action against a variety of Gram-negative as well as Gram-positive pathogenic bacteria. *P. aeruginosa* was very well inhibited by the essential oil with a zone of inhibition 22.00 ± 0.57 mm followed by *E. coli*, *S. aureus*, *S. pyogenes* and *K. pneumoniae*.

S. aureus, *E. coli*, *P. aeruginosa*, and *B. cereus*, were screened for the antimicrobial activity using broth microdilution technique and *B. cereus* was observed most sensitive strain to the leaves essential oil of *E. odoratum*.¹⁷

Inya-Agha et al.¹⁸ reported the antimicrobial efficacy of *E. odoratum* essential oil against bacterial pathogens, namely *Bacillus subtilis*, *E. coli*, *S. aureus*, and *K. aerogenes*. According to their results, *E. coli* and *S. aureus* were noted to be the most sensitive strain that is comparable to the present research work. A recent study reported the remarkable antimicrobial activity of methyl cellulose encapsulated *E. odorata* methanol extract against *E. coli* and *S. aureus*.²⁰

The MIC/MBC values showed that leaves essential oil of *E. odoratum* inhibited and eradicated the bacterial growth with least concentrations, and this might be because of the biologically active secondary metabolites present in the essential oils.

Antioxidant compounds reduce the detrimental effects of harmful free radicals before

they attack the cell or tissue preventing the damage to proteins, enzymes, lipids, DNA carbohydrates. Phytochemicals from medicinal herbs are a potentially vast source of biologically active phyto-components serving as antioxidant agents having negligible side effects in comparison to synthetic chemical drugs.^{21,22} Present investigation reported the potential antioxidant activity of leaves essential oil of *E. odoratum* with IC₅₀ value 10.58 μ l/ml. Very low IC₅₀ value of essential oil supports the significance of *E. odoratum* leaves as a promising herbal source of antioxidant compounds, hence can be utilized in nutritional as well as pharmaceutical in industries. Raman et al.,²³ reported the DPPH scavenging activity of aqueous and methanol extracts of *E. odoratum* having IC₅₀ values 10.5 μ g/ml and 10.2 μ g/ml, respectively, that are comparable to the present study. Tahir et al.,²⁴ performed antioxidant activity of the leaf extract by DPPH method, and reported n-butanol fraction have maximum activity with IC₅₀ value at 33.535 μ g/ml. Another study revealed that the DPPH assay of ethyl acetate fraction had the highest antioxidant activity, with IC₅₀ value of 10.05 \pm 0.06 mg/ml.²⁵ Amatya and Tuladhar²⁶ reported the antioxidant activity of ethanolic extracts of various parts of *E. odoratum* (stem, root, leaf, defatted flowers). Leaf and flower extracts showed the lowest IC₅₀, i.e. 44.5 and 50.9 μ g/ml, respectively, that suggests *E. odoratum* could be used pharmaceutically.

CONCLUSION

The present investigation conclusively demonstrates the efficacy of secondary metabolites of leaves essential oil of *E. odoratum* as a promising antimicrobial and antioxidant agent. Gram-positive and gram-negative pathogenic microorganisms examined showed good antibacterial activity. The leaves of *E. odoratum* have very good amount of essential oil in which approximately 15 compounds were identified with the help of GC-MS analysis, showing very good biological efficacy against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. pyogenes*, and *S. aureus*. In DPPH and H₂O₂ assay, the IC₅₀ value of leaves essential oil of *E. odoratum* was found almost equal to the ascorbic acid that indicates it as potential natural antioxidant agent. Hence, this study scientifically concluded and validated the

utilization of leaves essential oil of *E. odoratum* in traditional medicine and suggests its use as a resource material in the pharmaceutical industry. In future, some more in vivo experimentations and clinical investigations would be required to validate the potential of essential oil.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

NB conceptualized and supervised the study. EC designed and conducted all the experiments. MR evaluated the data. MR wrote the manuscript. TS and PS edited the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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