Indian Journal of Natural Products and Resources
Vol. 17(1), March 2026, pp. 144-154
DOI: 10.56042/ijnpr.v17i1.21499

Post-harvest handling effect on chemical composition and antibacterial properties of rose-scented geranium essential oil cultivated in the Western Himalayas

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Received 10 July 2025; revised received 28 November 2025; accepted 01 January 2025

The present investigation aimed to evaluate the effect of various post-harvest drying intervals on the essential oil yield and chemical composition of rose-scented geranium. This study also assessed the antibacterial efficacy of essential oil extracted from rose-scented geranium (*Pelargonium graveolens* L'Her.) cultivated under mid-hill agro-climatic conditions of the Western Himalayas. In this study, four post-harvest drying intervals were evaluated: T1 (0 h), T2 (24 h), T3 (48 h), and T4 (72 h). The essential oil extraction was conducted by hydro-distillation, and the chemical profiling was performed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC-MS). The principal constituents identified in the essential oil included citronellol (44.2–49.4%), geraniol (17.0%), linalool (2.1–3.3%), iso-menthone (6.3–7.14%), and citronellyl propanoate (4.3-6.3%). The highest essential oil yield was observed after 24 hours of drying (T2), accompanied by a desirable citronellol to geraniol (C: G) ratio of approximately 3:1 (citronellol 49.4%; geraniol 17.4%). The antibacterial activity of the essential oil was evaluated against six bacterial strains: three Gram-negative (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*) and three Gram-positive (*Bacillus subtilis*, *Micrococcus luteus*, and *Staphylococcus aureus*). The minimum inhibitory concentrations (MICs) of the essential oil ranged from 0.625% to 10% (v/v), indicating significant antimicrobial potential. In conclusion, a post-harvest drying duration of T2 (24 h) was found to optimise essential oil yield, maintain a favourable compositional profile and enhance its antibacterial efficacy. These findings suggest that properly timed post-harvest processing can enhance the pharmaceutical applicability of *P. graveolens* essential oil.

Keywords: Bacterial strains, Citronellol, Essential oil, Geraniol, Gram-negative, Hydro-distillation

IPC code; Int. cl. (2021.01)– A61K 36/00, A61P 31/00

Introduction

The genus *Pelargonium* encompasses 283 accepted species, among which *Pelargonium graveolens* L'Her. is one of the most commercially significant species for essential oil production[1]. *P. graveolens*, also referred to as rose-scented geranium, is a perennial aromatic bushy shrub (family Geraniaceae) cultivated throughout the world, particularly in China, Algeria, Russia, Egypt, Reunion Island, Israel, South Africa, Morocco and India[2]. In India, rose-scented geranium is cultivated in the Pulney hills and the Nilgiri range of Tamil Nadu and Karnataka, as well as in some plains of Andhra Pradesh. Recently, commercial cultivation has also commenced in the Northern plains of India[3].

The essential oil of rose-scented geranium is obtained from the foliage, which contains the entire aerial portion of the plant[4]. Usually, it has been esteemed for its unique rose-like aroma and has been widely used in aromatherapy, flavouring, cosmetics, perfumery, and pharmaceutical industries[5]. *P. graveolens* essential oil exhibits a broad spectrum of bioactivities, including antioxidant[6], antimicrobial[7], insect-repellent[8], and anti-cancerous properties[9]. Owing to these properties, it also has significant potential for application in culinary items, particularly for extending the shelf life of fresh and processed food products[10]. Furthermore, the essential oil is prominently a rich source of flavones, flavonoids, tannins, phenolic acids, monoterpenes, sesquiterpenes, coumarins, and flavanol derivatives[11]. The primary constituents of the commercial geranium oil are citronellyl and geranyl formate, geraniol, citronellol, isomenthone, and linalool. The essential oil contains a higher percentage of citronellol (>45%), a lower proportion of geraniol (<24%), and linalool (<14%)[10].

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The global demand for rose-scented geranium essential oil was estimated at approximately USD 73.80 million in 2020 and is expected to grow at a 6% compound annual growth rate (CAGR) between 2022 and 2031, potentially reaching USD 136.60 million by the end of the forecast period[12]. In India, however, the annual production remains limited to around 20 metric tons, while the domestic demand is estimated at nearly 200 metric tons[13]. This production gap necessitates substantial imports to meet national requirements. To fill this import gap, both researchers and growers aim to maximise the yield of green herbage to ensure the optimal possible recovery of essential oil[3]. However, various factors, including cultivar, distillation method, temperature, light intensity, climate, transplantation, harvesting, and post-harvest drying period, can alter the proportion, composition, and content of essential oil[2]. Similarly, to extract all their active ingredients, essential oils must be distilled for an appropriate amount of time. Therefore, different post-harvest drying periods for subsequent essential oil extraction are vital for assessing the quality and quantity of rose-scented geranium and unlocking its full potential.

Recent studies indicate that different post-harvest drying periods significantly influence quality indicators, including essential oil content, composition, and sensory and organoleptic characteristics, across various medicinal and aromatic plants. In various crops, previous studies reported that allowing harvested biomass to dry in the field for 24 to 48 hours before essential oil extraction enhances the evaporation of essential oil during hydro-distillation and facilitates packing more biomass into the oil extraction vessel[14]. Although the production of geranium oil is widely documented, studies focus more on distillation parameters, agronomic practices, and genotypes than on the drying duration of the biomass before distillation. In contrast, studies in aromatic crops highlight that drying duration significantly affects moisture content and essential oil yield due to alterations in cellular structures (oil glands) and volatile retention, and hence the influence of different drying periods before distillation has not been systematically evaluated in rose-scented geranium[15]. This drying process is essential for reducing energy costs, shortening the distillation process, and improving the efficiency of oil recovery[16].

Given these facts, the current research focuses on the impact of post-harvest conditions, particularly drying time, on the percentage of essential oil, the biochemical profile, and the antimicrobial activity of rose-scented geranium essential oil in the Western Himalayas. We aim to determine the optimal time to extract essential oil from the crop, identify the peak composition of the essential oil, and determine when the composition of various constituents begins to decline.

Material and Method

Experimental details

P. graveolens (IC-IHBT-PG-01) was utilised as the model crop in this experimental study. The experimental plants were grown under controlled agronomic conditions during 2023-24 at the agronomy experimental field of CSIR-Institute of Himalayan Bioresource Technology, Palampur, India, situated at an altitude of 1386 m amsl, with geographic coordinates of 32°11'52" N latitude and 76°5'65" E longitude. To support optimal establishment of the crop post-transplantation, freshwater irrigation was administered at regular intervals. Following initial establishment, no additional organic or chemical fertilisers were applied, as the plants exhibited satisfactory adaptation to the local climatic conditions. The soil at the experimental site was predominantly silty clay in texture, containing sand (10.0%), silt (52.0%), clay (38.0%), and other physicochemical characteristics of the soil.

The fresh biomass was harvested in September of 2023 and 2024, and subjected to shade drying (20-25 °C) under four distinct post-harvest durations: 0 hours (T1), 24 hours (T2), 48 hours (T3), and 72 hours (T4). The statistical design used in the experiment was a randomised block design (RBD) to compare treatment means ($p \leq 0.05$). Following these post-harvest treatments, the biomass (1 kg) was hydro-distilled with 2000 mL of distilled water in a glass Clevenger apparatus for 3 hours to extract essential oil. The essential oil obtained was dried over Na_2SO_4 (Merck, AR grade) to remove traces of water droplets. The isolated essential oil was stored in an amber tube at 4°C until further analysis. The essential oil content was computed on a fresh-weight (v/w) basis in T1 and on a dry-weight basis in the remaining treatments (T2, T3, and T4).

GC analysis

The fractions of essential oil components were determined by gas chromatography using Shimadzu

GC-2010 (Tokyo, Japan) with a flame ionisation detector (FID). The SH-RX-5Si/MS capillary column (30 m x 0.25 mm x 0.25 μm) from Shimadzu Asia Pacific, USA, was used for GC. An auto-injection system was employed, injecting 2-3 μL essential oil diffused in 1.8-1.7 mL of dichloromethane (Sigma-Aldrich, GC grade). Nitrogen gas was used as the mobile phase, maintaining a continuous flow rate of 1.05 mL/min. The column temperature was first set to 70°C for 3 minutes, then increased to 220°C at 4°C/min for 5 minutes. The initial pressure was set to 65.30 kPa, resulting in a straight rate of 37.60 cm/s.

GC-MS analysis

Gas chromatography-mass spectrometry (GC-MS) investigation of essential oil compounds was conducted using a GC-MS-QP2010 (Shimadzu, Tokyo, Japan), equipped with an AOC 5000 and a SH-RX-5Si/MS capillary column of 30 m x 0.25 mm x 0.25 μm (Shimadzu Asia Pacific, USA). The column temperature was initially set to 70°C for 3 min, then increased to 220°C at 4°C/min over 5 min. The MS source and interface temperatures were maintained at 240 and 250°C, respectively. A sample inoculation volume of 2 μL was used, with a divided ratio of 10, and mass scans were performed over the range of 40 to 800 atomic mass units. Nitrogen gas served as the carrier gas at a flow rate of 1.05 mL/min, while the electron energy was fixed at 0.85kV.

Identification of compounds

Retention index values for each peak in the GC-MS spectra have been calculated to identify the chemical compounds. These calculated retention index values were compared with the RI values, allowing for a tolerance of ±10, Adams 1995 and National Institute of Standards and Technology (NIST), New York, 2005. Additionally, the mass spectral peak patterns of the identified compounds were cross-referenced with retention index values reported in recent literature.

Determination of antimicrobial activity

Microorganisms' strains

The antibacterial properties of *P. graveolens* essential oils were examined against six bacterial species, comprising three Gram-negative and three Gram-positive strains. The bacterial strains tested in this study comprised Gram-positive species *S. aureus* (MTCC 96), *B. subtilis* (MTCC 121), *and M. luteus* (MTCC 2470), and Gram-negative species *P. aeruginosa* (MTCC 2453), *K. pneumoniae* (MTCC 109), and *E. coli* (MTCC 43). The bacteria were grown in Mueller-Hinton broth (MHB) (Oxoid, UK) and subsequently again transferred to MHA media to establish working cultures. A bacterial suspension was created using sterile normal saline by selecting colonies from an overnight culture plate. Additionally, all cultures were kept in a culture room at 37°C for 24 hours for further study.

Antibacterial activity assay

The antibacterial properties of the *P. graveolens* essential oil extract were evaluated by agar well diffusion method, based on previously published protocols with minor modifications[17,18]. Cultures were grown at 37°C, and the inoculum concentration was maintained at 1.0×10^5 CFU/mL. Subsequently, 100 μL of the cultured strains were consistently spread across the surface of Mueller-Hinton agar using a sterile cotton swab, then allowed to air-dry at ambient temperature in a laminar chamber. Wells with a diameter of 5 mm were then formed, into which 50 μL of each test sample was loaded. The petri plates were stored at 4°C for 2 h under aerobic conditions to expedite the diffusion of essential oil. Furthermore, the samples were incubated for 24 h at 37°C. Antibacterial activity was computed by measuring the diameter of clearly visible zones. As a positive control, streptomycin (10 mg/mL) was utilised.

Minimum Inhibitory Concentration (MIC)

The MIC of each essential oil against the bacterial strains was assessed using the broth microdilution method, according to the procedure outlined by Rathore *et al.*[19]. The MIC was determined as the minimum concentration at which no colour shift occurred. The wells in the first row serve as the negative control, containing 50 μL of broth medium along with the bacterial culture. The wells in the second row act as the positive control, containing 50 μL of broth medium with streptomycin. The samples were serially diluted in Mueller-Hinton broth (Himedia, India) supplemented with 0.5% (v/v) Tween-20 as an emulsifying agent, resulting in a concentration gradient from 10% (v/v) to 0.0195% (v/v) in a 96-well plate. Subsequently, 50 μL of the prepared bacterial suspension was poured into all 96-well plates and incubated for 24 h at 37°C. Furthermore, after incubation, 30 μL of Resazurin sodium salt solution (0.015% w/v) was poured into each well and incubated for 2-4 h. A visible colour

shift from blue to pink was perceived, indicating bacterial growth.

Statistical analysis

The current investigation was conducted in triplicate, and the average was computed using MS Excel 2016. The mean ± standard error (SE) is used to display the results. One-way analysis of variance (ANOVA) was performed using SPSS (version 16.0, USA), and mean values were compared employing DMRT (Duncan's multiple range test) at a significance threshold of $p \leq 0.05$. Additionally, Ward's method in Past 4.03 was used to perform Principal Component Analysis (PCA) biplot and hierarchical cluster analysis of the essential oil[20]. To enhance data interpretation, a cluster map illustrating the relationships between treatments and chemical compounds was generated in MS Excel 2016. Furthermore, the inhibition zones on Mueller-Hinton agar plates were quantified to evaluate antimicrobial properties. The outcomes were expressed as mean values ± standard error (SE), built on three autonomous copies and statistical analysis using Sigma Stat 3.5.

Results and Discussion

Effect of Post-harvest drying duration on Biomass and essential oil content

Post-harvest drying duration significantly affected the fresh biomass retention of *P. graveolens*, as shown in Table 1; oil yield progressively declined with extended drying intervals. The initial biomass of 1000 g at harvest (0 h) was reduced to 858 g, 760 g, and 650 g after 24, 48, and 72 h of shade drying, respectively. The highest reduction in moisture content (35.00±1.20%) was recorded after 72 h of shade drying (20-25°C), whereas the 24 h treatment resulted in the least moisture loss (14.02±0.44%) compared to fresh biomass (T1).

Remarkably, the essential oil yield followed a non-linear trend, initially increasing with short-term drying but decreasing with prolonged desiccation. The 24 h drying treatment resulted in the highest percentage of essential oil content (0.16±0.01%), indicating an optimal balance between moisture reduction and preservation of volatile constituents. In contrast, both extended drying periods (48 and 72 h) were associated with a notable decline in essential oil yield, likely due to the deterioration or evaporation of thermolabile aromatic constituents as post-harvesting drying time increased. Maltova *et al*. reported the highest essential oil content of 0.22% after a 17 h post-harvest drying period[16].

Essential oil composition

The essential oil isolated from the whole vegetative portions of *P. graveolens* appears pale yellow to greenish yellow and carries a pleasant fragrance. As a result, the process of isolating essential oil (EO) by hydro-distillation with a Clevenger-type apparatus demonstrated notable differences in EO compounds at different drying periods after harvesting. The essential oil analysis revealed 15 chemical constituents (Table 2). The study confirmed that there is no remarkable outcome on the prevalence of oil constituents. However, there is an important impact on the fractions of major and minor components during distillation (Fig. 1). This could be due to the differing arrangement and bonding of molecular components of essential oil at the time of extraction[19]. Statistically, it unveiled the proportions of oil components, *viz*., citronellol (44.24 to 49.44%), geraniol (17.01 to 17.70%), isomenthone (6.37 to 7.14%), citronellyl propanoate (3.58 to 6.31%), linalool (2.12 to 3.30%), cis-β-guaiene (1.19 to 1.73%), geranyl formate (0.38 to 0.93%), citronellyl tiglate (1.34 to 1.74%), geranyl tiglate (1.32 to 1.81%), germacrene (0.84 to 1.27%), γ-cadinene (0.56 to 1.22%), citronellyl butyrate (0.95 to 1.34%), phenyl ethyl tiglate (0.75 to 1.05%) and 10-epiγ-eudesmol (0.46 to 1.46%). The graphical representation of major compounds present in rose-

Table 1 — Effect of different drying periods under shade condition before extraction of essential oil on different parameters

Drying period	Fresh biomass (g)	Biomass after drying (g)	Moisture content (%)	Essential oil (%)
T1 (0 h)	1000.0±0.00	1000.00^{a}±0.00	00.00^{d}±0.00	0.14^{b}±0.07
T2 (24 h)	1000.0±0.00	858.00^{b}±11.62	14.02^{c}±0.44	0.16^{a} ±0.01
T3 (48 h)	1000.0±0.00	760.00^{c}±5.77	24.00^{b}±1.20	0.13^{c}±0.09
T4 (72 h)	1000.0±0.00	650.00^{d}±10.41	35.00^{a}±1.20	0.12^{d}±0.03
SE±(m)	-	11.38	0.96	0.08
CD ($P \leq 0.05$)	-	45.88	3.88	0.03

*T: treatment of post-harvest drying periods; g: gram

Table 2 — Effect of different drying periods after harvesting on essential oil composition (area%) of rose-scented geranium

S. No.	Compounds	RI (Exp.)	RI (Lit.)	Area (%) ± SE(m) Drying periods			
				T1 (0 h)	T2 (24 h)	T3 (48 h)	T4 (72 h)
1	Linalool	1108	1098	2.12±0.02	2.56±0.01	3.30±0.03	3.02±0.03
2	Isomenthone	1166	1158	7.14±0.06	7.06±0.02	6.37±0.11	7.13±0.07
3	Citronellol	1234	1223	49.44±0.27	49.09±0.10	44.84±0.31	44.24±0.02
4	Geraniol	1242	1249	17.35±0.85	17.70±0.10	17.12±0.13	17.01±0.68
5	Geranyl formate	1305	1298	0.57±0.01	0.93±0.01	0.38±0.19	0.90±0.02
6	(E)-Caryophyllene	1427	1417	0.62±0.01	0.62±0.02	0.57±0.28	0.74±0.10
7	Citronellyl propanoate	1448	1444	4.36±0.05	3.58±1.10	6.31±0.26	6.07±0.20
8	Germacrene D	1480	1480	0.91±0.15	0.84±0.02	1.15±0.28	1.27±0.28
9	cis-β-Guaiene	1489	1492	1.19±0.18	1.31±0.01	1.42±0.14	1.73±0.23
10	γ-Cadinene	1498	1513	1.22±0.17	0.56±0.28	0.88±0.23	0.85±0.42
11	Citronellyl butyrate	1532	1530	1.31±0.25	1.12±0.13	0.95±0.37	1.34±0.16
12	Phenyl ethyl tiglate	1592	1584	1.05±0.24	1.05±0.21	0.86±0.09	0.75±0.00
13	10-epiγ-Eudesmol	1627	1622	1.30±0.31	0.46±0.46	1.24±0.27	1.46±0.00
14	Citronellyl tiglate	1671	1666	1.59±0.09	1.34±0.15	1.74±0.03	1.49±0.01
15	Geranyl tiglate	1706	1696	1.44±0.06	1.32±0.04	1.81±0.04	1.66±0.03
	C/G ratio			2.84	2.77	2.61	2.60
	Total (%)			91.61	89.54	89.94	89.66
Classes of Compounds							
Monoterpene alcohol				77.37±0.84	77.53±0.28	72.55±0.51	72.72±0.72
Bicyclic sesquiterpene				0.62±0.01	0.62±0.02	0.57±0.28	0.74±0.10
Monoterpenoid acetate ester				2.02±0.07	2.24±0.05	2.19±0.23	2.56±0.05
Acyclic monoterpenoids				4.36±0.05	3.58±1.10	6.31±0.26	6.07±0.20
Sesquiterpene				4.63±0.18	3.17±0.66	4.69±0.17	5.32±0.38
Monoterpenoid alcohol ester				3.95±0.08	3.51±0.33	3.55±0.27	3.58±0.15

RI(Lit.): Retention Index Literature, RI(Exp): Retention Index Experimental, SE(m) ±: Standard error mean, T: Treatments, h: hours

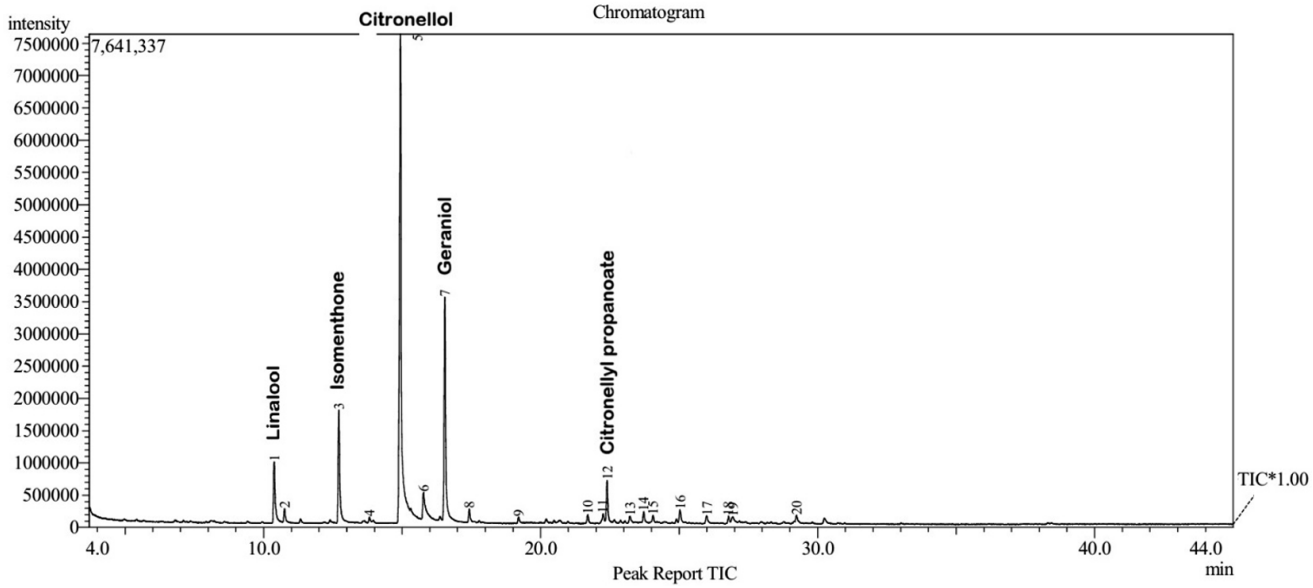

Fig. 1 — Representative chromatogram of gas chromatography analysis of rose-scented geranium using DB-5 capillary column. Major peaks: (1) Linalool (3) Isomenthone (5) Citronellol (7) Geraniol (13) Citronellyl propanoate.

scented geranium essential oil is shown in Fig. 2. Araya *et al.* reported geraniol and citronellol as the major and most significant compounds of rose-scented geranium[21]. Essential oils demonstrate notable compositional differences relative to those produced in other countries. These variations are primarily attributed to factors such as climatic conditions, post-harvest management practices and the specific types and methods utilised in the distillation process[22,23].

Citronellol and geraniol accounted for 70% of the entire essential oil percentage, with the remaining percentage derived from minor constituents. A substantially elevated percentage of citronellol (49.44%±0.27) was recorded in T1 (0 h), which was statistically comparable to T2 (24 h); however, the content of geraniol (17.70%±0.10) was higher in T2 (24 h) than in other treatments. The levels of linalool and citronellyl propanoate were considerably higher in T2 than in other treatments. The percentage of Isomenthone was notably higher at T1 (0 h), 7.14% compared to the other treatments. Similarly, the study on *Lippia thymoides* also reported variance in the configuration of essential oil at different drying conditions[24]. The data clearly show that hydro-distillation of different post-harvest drying periods significantly affects the essential oil composition, likely due to the optimal moisture loss during the drying period[25]. The essential oil had a suitable C:G ratio (3:1) up to 24h (T2), which declined with increasing drying period. A notable increase in the essential oil constituents was noted after drying the biomass for up to 24 h (T2). However, it declined significantly thereafter. This study signifies that the different drying periods had a positive impact on the composition of the essential oil. In prior research on *P. graveolens,* the proportions of citronellol, linalool and geraniol were higher under shade drying for 17 to 48 h[16,25].

The outcomes of the present research indicate that post-harvest drying duration enhanced essential oil yield, particularly during the first 24 hours of drying. Monoterpene alcohols constitute the major fraction across all treatments, maintaining a proportion of approximately 70–75% (Table 2). These compounds, including citronellol and geraniol, are the primary bioactive constituents of rose-scented geranium essential oil and are responsible for its characteristic fragrance and therapeutic properties. The stability of monoterpene alcohols during drying periods suggests that short-term drying (24–48 h) does not significantly degrade these compounds, whereas prolonged drying (72 h) results in a slight decline. Sesquiterpenes are present in low concentrations across all treatments but show a slight increase with drying time (T3 and T4). Bicyclic sesquiterpenes remain at minimal levels, with a slight reduction after 72 h of drying. Since sesquiterpenes have a higher molecular weight and lower volatility compared to monoterpenes, they are less prone to evaporation and may even become more concentrated as lighter compounds are lost during drying[25]. Monoterpenoid alcohol esters and monoterpenoid acetate esters increase progressively with drying duration[26]. This suggests that oxidation and esterification reactions occur over time, converting monoterpene alcohols into their ester derivatives, which are more stable and confer a distinct olfactory profile. Such changes are consistent with previous studies indicating that prolonged drying

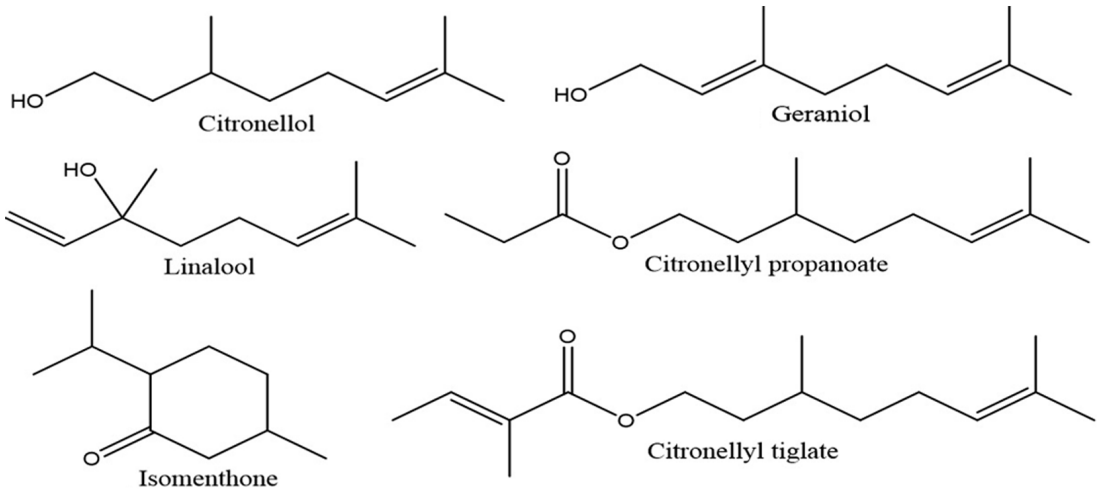

Fig. 2 — Structural representation of major volatile compounds of *P. graveolens* L'Her.

enhances ester formation, particularly in aromatic plants used in perfumery[27]. Acyclic monoterpenoids increase slightly with drying, particularly after 48–72 h. This study highlights the importance of optimising drying duration to balance essential oil yield, chemical stability and application-specific needs. To preserve high monoterpene alcohol content, minimal drying (T1–T2) is recommended, whereas longer drying times (T3–T4) favour esterification and sesquiterpene enrichment, potentially enhancing fragrance complexity.

Principal component analysis (PCA) and clustering analysis

It was sequentially employed to evaluate variations among various treatments by examining the well-known compounds in the extracted essential oil under different treatments (Fig. 3). To visually analyse the variance in compound classes associated with different drying durations, a biplot of Principal Component Analysis (PCA) was developed, integrating both the drying periods and the respective compound classes. This method was proposed by Yan and Rajcan to examine variations across multiple studies utilising multi-trait data[28]. In the biplot, the close relationship between two compound classes is determined by the cosine angle between the vectors. Right angles indicate the absence of correlation; acute angles denote a positive correlation, and obtuse angles reveal a negative association between the two classes. Furthermore, each vector's span illustrates the discriminative capacity of the compound class; shorter vectors suggest a lack of association with other classes, limited variation, or reduced effectiveness in distinguishing between drying methods[29].

In the present study, the biplot captured 97.70% of the total variability within different post-harvesting drying periods by classes of compound group interactions. A rhomboid was formed to estimate the drying period in the biplot, and compound classes were accessible with vectors. The Monoterpenes alcohol (MA) and Monoterpenoid acetate ester (MAE) showed a progressive association with the drying period T1 (0h) and aligned with the positive quadrant of PC1 and PC2. This suggests that these variables had a strong positive influence on the T1 and T2 treatments. In contrast, Bicyclic sesquiterpene (BS) and MAE were associated with the negative quadrant of PC1, indicating their increased influence at T3 and T4 treatments (Fig. 3a).

Based on the predominant compounds (≥5.00%) present in the essential oil of *P. graveolens* collected during the 2023-24 period, two distinct clusters were formed using Hierarchical cluster analysis (Fig. 3b). Cluster I included samples T1 (0h) and T2 (24h), while Cluster II comprised samples T3 (48h) and T4 (72h). In a related study, Kanyal *et al*. classified the essential oil of *Cinnamomum camphora* L. into four clusters across seven different drying durations[30]. The clusters and their corresponding compositions identified in the current study are as follows: Cluster I (T1 and T2) Isomenthone (7.14 and 7.06%), Citronellol (49.44 and 49.09%) and Geraniol (17.35 and 17.70%). Cluster II (T3 and T4) Isomenthone (6.37 and 7.13%), Citronellol (44.84 and 44.24%) and Geraniol (17.12 and 17.01%).

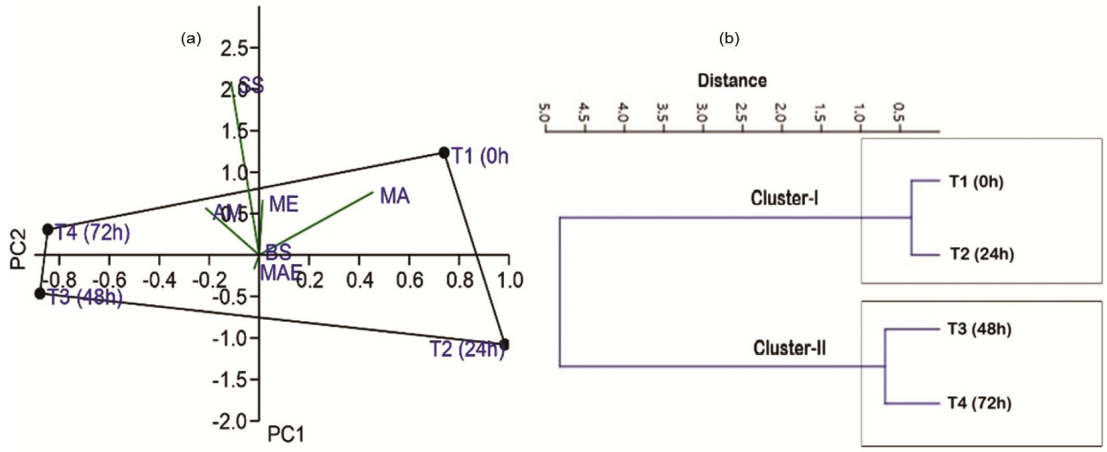

Fig. 3 — *P. graveolens* L'Her. essential oil analysis, a) PCA biplot of the changes in active ingredient groups according to different drying periods and the relations between classes; MA=Monoterpenes alcohol, BS=Bicyclic sesquiterpene, MAE=Monoterpenoid acetate ester, SS=Sesquiterpene, AM=Acyclic monoterpenoids, ME=Monoterpenoid alcohol ester, and b) Hierarchical Clustering analysis of samples at different drying periods.

Antimicrobial activity

The antibacterial properties of an essential oil derived from rose-scented geranium revealed promising results against selected bacterial strains (Table 3). The highest inhibition zones were recorded by T_3 against *P. aeruginosa* MTCC 2453 (15.66±0.94), as well as T_1 against *S. aureus* MTCC 96 (14.66±0.47). T_1 showed an inhibition zone against all tested bacteria; however, it showed less sensitivity against *P. aeruginosa* MTCC 2453 (5.66±0.47). Samples T_2, T_3, and T_4 were not resistant to *E. coli* (MTCC 43) and *K. pneumoniae* (MTCC 109) as presented in Table 3. The sensitivity of essential oil against various bacterial strains is apparently due to the higher concentration of monoterpenoids containing hydroxyl groups, predominantly linalool and geraniol[31].

Minimum inhibitory concentration (MIC) of essential oil

The MIC of the essential oil against each of the studied bacterial strains was calculated using the broth microdilution at 10% (v/v). The MIC values for rose-scented geranium essential oil are presented in Table 4. According to the MIC results obtained from the microplate tests, rose-scented geranium essential oil demonstrates promising antibacterial properties, as all samples exhibited bacterial growth (Fig. 4). All the tested samples, T_1, T_2, T_3, and T_4, showed potential MIC in the case of all the tested bacteria, with values lying between 0.63-10.00% (v/v). Sample T1 showed the maximum potential with MIC values of 0.625% (v/v) against *M. luteus* MTCC 2470 *and E. coli* MTCC 43. However, all the samples (T_1, T_2, T_3, and T_4) were less sensitive to *P. aeruginosa* MTCC 2453 and *K. pneumoniae* MTCC 109, with MIC values ranging from 1.25 to 10% (v/v), as shown in Table 4.

Moreover, Radulovic *et al.* observed inhibitory and bactericidal effects of rose-scented geranium sp. essential oil on *S. aureus*, *B. subtilis*, and *E. coli* at concentrations ranging from 5 to 10 mg/mL, which supports our current findings (Fig. 4)[32]. We observed the antibacterial effects of linalool in case of *E. coli*, *P. aeruginosa* and *S. aureus* which has also been previously proclaimed by Herman *et al.*[33]. Moreover, linalool and α-terpineol essential oil showed antibacterial activity against some pathogens within a concentration range (0.1-0.8 mg/mL)[34] and geraniol was reported to suppress the growth of *S. aureus* and *E. coli*[35]. These reports align with the results of the samples studied, suggesting that the plant could serve as a valuable source of bioactive compounds with latent or potential broad-spectrum antibacterial activity.

Correlation analysis

The correlation heatmap analysis clearly demonstrates that variations in post-harvest drying methods significantly influence the essential oil (EO) yield, chemical composition, and antimicrobial activity of rose-scented geranium (Fig. 5). Among the

Table 3 — Zone of inhibition under different treatments of *P. graveolens* essential oil against Gram-positive and Gram-negative bacteria

Treatments	Inhibition zone (mm), 50 µL essential oils					
	Gram-positive			Gram-negative		
	B. subtilis MTCC 121	S. aureus MTCC 96	M. luteus MTCC 2470	P. aeruginosa MTCC 2453	E. coli MTCC 43	K. pneumonia MTCC 109
T1 (0 h)	14.33±0.47	14.66±0.47	14.33±0.47	5.66±0.47	11.0±0.81	11.0±0.81
T2 (24 h)	11.66±0.47	10.66±0.81	8.00±0.81	10.66±0.94	-	-
T3 (48 h)	11.33±0.94	13.0±0.94	10.66±0.94	15.66±0.94	-	-
T4 (72 h)	13.00±0.81	12.33±1.24	10.33±0.47	14.33±0.47	-	-
Streptomycin (control)	9.33±0.94	8.00±0.81	11.66±0.47	10.33±0.47	9.33±0.94	9.66±0.47

Table 4 — Minimum inhibitory concentration (% v/v) of different essential oil against different bacterial strains

Treatments	Minimum inhibitory concentration (% v/v)					
	Gram-positive			Gram-negative		
	B. subtilis MTCC 121	S. aureus MTCC 96	M. luteus MTCC 2470	P. aeruginosa MTCC 2453	E. coli MTCC 43	K. pneumoniae MTCC 109
T_1 (0 h)	1.25	1.25	0.63	1.25	0.63	2.50
T_2 (24 h)	0.63	2.50	1.25	5.00	1.25	2.50
T_3 (48 h)	2.50	5.00	1.25	2.50	1.25	5.00
T_4 (72 h)	0.63	2.50	2.50	10.00	2.50	10.00

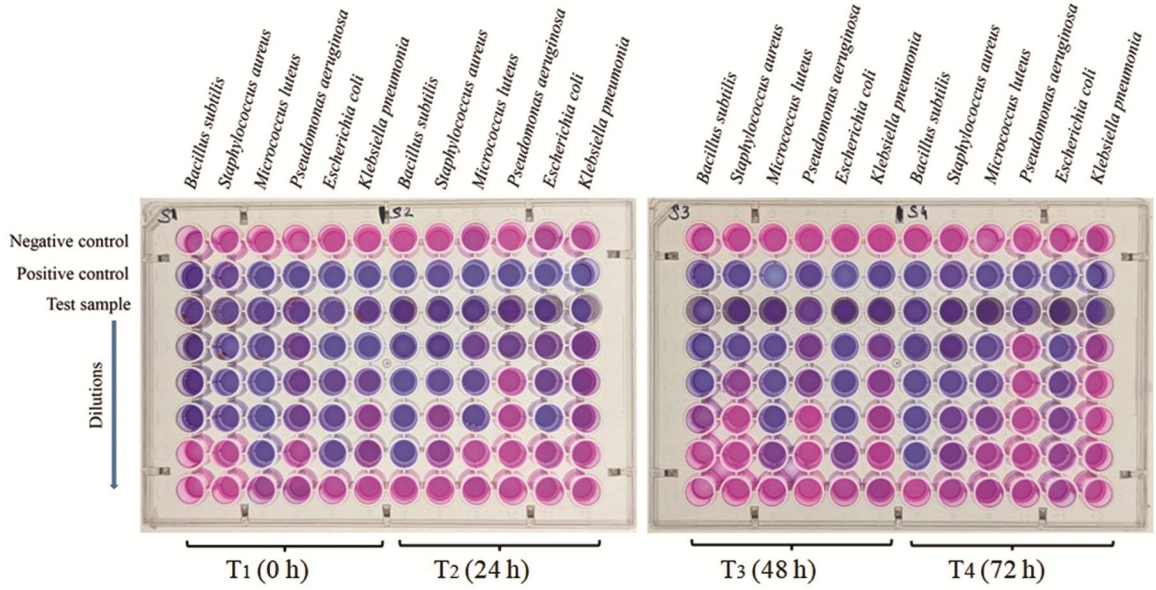

Fig. 4 — Minimum inhibitory concentration of essential oil against different bacterial strains.

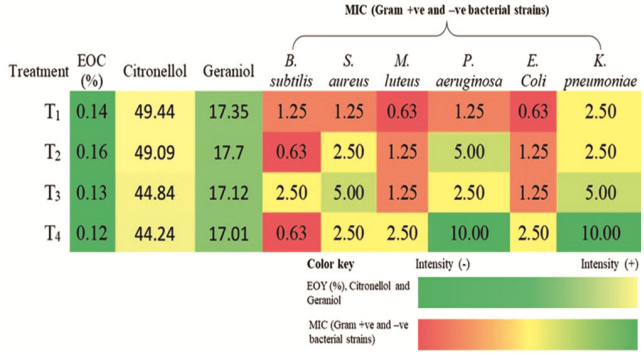

Fig. 5 — Correlation heatmap of different post-harvesting drying variations in EO content, main commercially economic compounds (Citronellol and geraniol) compound of rose-scented geranium and its MIC (Minimum inhibitory concentration) for each bacterial strain.

tested treatments, T2 exhibited the highest EO content (0.16%), closely followed by T1, indicating that these drying approaches are more effective in preserving or enhancing oil yield. Both treatments also maintained elevated levels of citronellol and geraniol, the primary commercially valuable and bioactive compounds. In contrast, T4 displayed the lowest concentrations of EO and key constituents, highlighting the detrimental effect of less optimal drying conditions on oil quality.

A clear positive correlation was observed between the concentration of these bioactive compounds and antimicrobial performance. T1 demonstrated the strongest antibacterial activity, marked by the lowest minimum inhibitory concentrations (MICs) against *B. subtilis*, *M. luteus*, and *E. coli*. This suggests that higher levels of citronellol and geraniol enhance antimicrobial efficacy. Conversely, T4 exhibited the weakest activity, with the highest MIC values, particularly against *P. aeruginosa* and *K. pneumoniae*, indicating reduced biological potency. These results underlined the crucial role of drying methods in shaping both the chemical integrity and functional properties of geranium essential oils. T1 and T2 emerged as the most effective treatments, offering an optimal balance between EO yield, compound concentration, and antimicrobial effectiveness, thereby maximising both commercial and therapeutic potential.

Conclusion

This research concludes that various drying methods influence the physicochemical and antimicrobial characteristics of essential oils. The length of post-harvest drying is a vital element that significantly affects the yield, chemical makeup and antibacterial efficacy of essential oil derived from *P. graveolens*. An increase in drying duration led to changes in monoterpenoid components, including citronellol (44.2–49.4%) and geraniol (17.0–17.4%), which are essential for the oil's antimicrobial activity. Furthermore, the C:G ratio decreases as the post-harvest drying time of the biomass increases. These findings emphasise that rose-scented geranium essential oil is an effective natural antibacterial agent against *P. aeruginosa* (15.66±0.94) and *S. aureus*

(14.66±0.47) when distilled within 24-48 hours of harvesting. A drying time of 24 hours (T_2) was identified as the most effective duration for achieving an optimal balance between essential oil yield and bioactive compound preservation. Conversely, extending the drying period beyond this timeframe led to a decline in oil quality and reduced antibacterial effectiveness due to the volatilisation or degradation of compounds. Future research should focus on exploring the biochemical mechanisms that contribute to compound loss during prolonged drying, examining the influence of environmental factors on drying efficiency, and investigating the interactive effects of lesser-known constituents. By highlighting the previously overlooked significance of drying duration prior to distillation, this study offers important insights for enhancing the post-harvest management of rose-scented geranium. This could lead to improved practical guidelines for sustainable essential oil production and boost its commercial and therapeutic uses, particularly in areas distant from distillation facilities.

Acknowledgement

The authors are also grateful to Dr Arvind Verma and Mrs Vijay Lata Pathania (Technical Officers) for providing technical support in conducting the research work under field and lab conditions. Financial grant from the Council of Scientific and Industrial Research, New Delhi, India, under the CSIR Aroma Mission Phase-III project (HCP-0007) is also acknowledged.

Conflict of interest

The authors stated that they had no potential conflicts of interest.

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