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GC–MS Characterization and In Vitro Antibacterial Activity of *Pelargonium graveolens L'Hér.* Essential Oil from Tajoura, Libya

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ABSTRACT

Background: *Pelargonium graveolens* L'Hér. essential oil is valued for its chemical constituents and antimicrobial potential. To our knowledge, no previous study has combined GC–MS characterization with antibacterial screening of Libyan-cultivated *P. graveolens*.

Aims: This study investigated its chemical composition, antibacterial activity, and relationship to ISO 4731:2012 and published North African profiles.

Methods: Essential oil was extracted from fresh aerial parts collected in Tajoura, Libya, by Clevenger hydrodistillation. Chemical composition was analyzed using GC–MS, while antibacterial activity was evaluated against five reference bacterial strains using disc diffusion (4 µL/disc).

Results: The oil yield was 0.8% (v/w). GC–MS recorded 52 peaks, dominated by citronellol (26.74%), citronellyl formate (11.79%), isomenthone (10.50%), geraniol (8.97%), and linalool (5.44%). Partial numerical agreement with ISO 4731:2012 was observed. The strongest antibacterial activity was recorded against *Staphylococcus aureus* (21.00 ± 1.00 mm) and *Staphylococcus epidermidis* (20.00 ± 1.00 mm).

Conclusion: Libyan *P. graveolens* essential oil exhibited a citronellol-dominant profile and measurable antibacterial activity. These findings provide preliminary evidence supporting further quality assessment and quantitative antimicrobial investigations.

Keywords*: Pelargonium graveolens; citronellol; GC–MS; Libya; antibacterial activity*

1. Introduction

Pelargonium graveolens L'Hér. (Geraniaceae), commonly known as rose geranium, is an economically important aromatic plant widely cultivated for its essential oil, which is extensively used in the perfumery, cosmetic, and fragrance industries and has attracted considerable scientific interest due to its potential biological activities. The essential oil is predominantly characterized by oxygenated monoterpenes, particularly citronellol, geraniol, and their corresponding esters, although its chemical composition and the relative abundance of individual

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constituents may vary substantially depending on geographical origin, environmental conditions, plant organs, and cultivation practices. Previous investigations of Tunisian *P. graveolens* have demonstrated such compositional variability. Boukhris et al. reported an essential oil characterized by citronellol, citronellyl formate, and geraniol as major constituents, whereas Ben ElHadj Ali et al. documented differences in essential-oil composition between leaves and flowers [1,2]. These findings highlight the importance of investigating the chemical composition of *P. graveolens* essential oils from different geographical origins to better understand their phytochemical variability and potential biological properties.

The North African literature provides an unusually informative comparative setting. A Tunisian study of four growing biotopes documented variation in oil yield and antibacterial performance while identifying citronellol and geraniol as principal oxygenated monoterpenes [3]. Another Tunisian investigation contrasted two sites in Chebba and reported an ester- and rose-oxide-dominant pattern under different cultivation environments [4]. Algerian evidence includes earlier citronellol-rich oils [5,6] and a geraniol-rich sample from Tebessa [7], whereas a 2026 Edough investigation reported high citronellol, comparatively low geraniol and several distinctive ester and sesquiterpene constituents [8]. Moroccan crude oil was comparatively enriched in epi-γ-eudesmol [9]. More recent Egyptian Gas Chromatography–Mass Spectroscopy (GC-MS) studies also demonstrate pronounced differences in geraniol, menthone and sesquiterpene patterns despite prominent citronellol peaks [10,11].

ISO 4731:2012 provides geographically differentiated chromatographic specifications for commercial geranium essential oil, including a North African reference profile [12]. These specifications offer a useful basis for comparing major chemical constituents. However, the standard applies to steam-distilled oil, whereas the present study used hydrodistillation of fresh *P. graveolens* aerial parts. Therefore, comparisons are intended for phytochemical interpretation rather than establishing ISO compliance.

Although the chemical composition and biological activities of *P. graveolens* essential oils have been investigated across North Africa, corresponding evidence from Libya remains limited. A recent Libyan study evaluated the direct-contact and vapor-phase antibacterial activities of *P. graveolens* essential oil collected from Tajoura against *Proteus mirabilis* ATCC 14153 but did not characterize its chemical composition [13]. Consequently, integrated data linking the GC–MS chemical profile of locally cultivated *P. graveolens* with its antibacterial activity against different reference strains remain insufficiently documented. Addressing this gap would provide a locally sourced phytochemical reference and support comparisons with previously characterized North African essential oils.

Accordingly, the present study aimed to characterize the chemical composition and evaluate the in vitro antibacterial activity of essential oil hydrodistilled from fresh aerial parts of *P. graveolens* cultivated in Tajoura, Libya. Specifically, the study determined the essential-oil yield, investigated its volatile constituents and major chemical classes using GC–MS, and assessed its antibacterial activity against five reference bacterial strains using the disc-diffusion method. The resulting chemical profile was further compared with published North African studies and the regional chromatographic specifications of ISO 4731:2012 to provide a comparative phytochemical context for the Libyan essential oil.

2. Materials and Methods

2.1. Study material, locality and authentication

Cultivated *P. graveolens* plant material was collected in Tajoura, northwestern Libya, in June 2025. Botanical identification was performed by Prof. Mohammed Makhlouf, Department of Botany, Faculty of Science, University of Tripoli. Fresh, aerial plant material was used for essential-oil extraction.

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2.2. Essential-oil extraction and yield

The essential oil was extracted from fresh aerial parts of *P. graveolens* by hydrodistillation using a Clevenger-type apparatus. Briefly, 400 g of fresh plant material was placed in a 4,000-mL round-bottom flask containing 2,000 mL of distilled water. Hydrodistillation was carried out for 3 h, after which the essential oil was carefully separated from the aqueous phase. The recovered oil was dried over anhydrous sodium sulfate to remove residual moisture, transferred into an amber glass bottle, and stored at 4 °C until further analysis. The essential-oil yield was calculated as the volume of oil recovered (mL) per 100 g of fresh plant material and expressed as a percentage volume per weight (% v/w) using the following equation:

Essential-oil yield (% v/w) = [Volume of essential oil (mL) / Fresh weight of plant material (g)] × 100

The essential-oil yield was 0.8% (v/w, fresh-weight basis).

2.3. GC–MS conditions

The chemical composition of *P. graveolens* essential oil was analyzed using a TRACE GC–TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). The oven temperature was initially maintained at 50 °C, increased to 250 °C at a rate of 5 °C/min and held for 2 min, followed by an increase to 300 °C at 30 °C/min with a final holding time of 2 min. The injector and MS transfer-line temperatures were maintained at 270 °C and 260 °C, respectively. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. A 1-µL aliquot of diluted essential oil was automatically injected in split mode. Mass spectra were acquired using electron ionization (EI) at 70 eV, with an ion-source temperature of 200 °C and a mass scanning range of *m/z* 50–650. The volatile constituents were tentatively identified by comparing their mass spectra with reference spectra in the NIST 19 and Wiley 09 mass spectral libraries.

2.4. ISO reference comparison and North African literature selection

The chemical composition of *P. graveolens* essential oil was compared with the 15 constituent ranges specified for North African commercial geranium oil in Table 1 of ISO 4731:2012 [12]. The relative GC–MS peak-area percentages of the Libyan sample were compared with the corresponding reference intervals to evaluate compositional similarities and differences.

For regional comparison, original studies reporting GC–MS or GC–FID chemical profiles of *P. graveolens* essential oils from Tunisia, Algeria, Morocco, and Egypt were selected. Priority was given to studies with verifiable bibliographic information, clearly defined botanical origins, and reported constituent percentages. Chemical nomenclature was harmonized where appropriate, and results from different geographical locations or plant organs were retained separately. Unreported or unverifiable values were designated as not reported (NR). Studies involving other *Pelargonium* species, isolated fractions, or non-distilled headspace volatiles were excluded from direct quantitative comparisons.

2.5. Antibacterial screening

Reference strains comprised *Escherichia coli* ATCC 8739, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 29213, *Salmonella enterica* ATCC 14028 and *Shigella flexneri* ATCC 12022. Inocula were visually adjusted to a 0.5 McFarland standard and spread over Mueller–Hinton agar at approximately 4 mm depth in 90-mm Petri dishes. Sterile 6-mm Whatman paper discs were loaded with 4 µL of undiluted essential oil per disc. Gentamicin (10 µg/disc) served as the positive reference, and unloaded sterile discs as the negative control. The plates were incubated at 37°C for 24 h. Oil and antibiotic inhibition zones were measured as total diameters, including the 6-mm disc.

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Three independent experimental runs were available for each essential-oil and gentamicin condition. CLSI M02 informed general disc-testing practice [14].

2.6. Statistical analysis

Antibacterial activity was expressed as the mean ± standard deviation (SD) of three replicate inhibition-zone measurements (n = 3) for each bacterial strain. GC–MS results were presented as relative peak-area percentages. Published comparisons were descriptive and were not treated as a factorial regional experiment.

1. 3. Results and Discussion

3.1. Extraction yield and complete chromatographic signature

Hydrodistillation of fresh aerial parts of *P. graveolens* collected in Tajoura, Libya, yielded 0.8% (v/w) essential oil on a fresh-weight basis. The reported GC–MS profile comprised 52 peaks meeting the study's library-reporting criterion, with listed relative peak areas summing to 99.84% (Figure 1; Table 1). Fifteen peaks individually represented at least 1.0% of the chromatographic signal and together contributed 85.05%. The five largest tentative assignments were citronellol (26.74%; RT 12.43 min), citronellyl formate (11.79%; RT 13.50 min), isomenthone (10.50%; RT 10.14 min), geraniol (8.97%; RT 13.05 min) and linalool (5.44%; RT 8.72 min), collectively contributing 63.44%. Other peaks with areas ≥2% were furopelargone A (3.69%), geranyl formate (2.61%), β-caryophyllene (2.52%), geranyl tiglate (2.34%), germacrene A (2.20%), (−)-aristolene (2.13%), (Z)-rose oxide (2.04%) and δ-cadinene (2.01%).germacrene A (2.20%), (−)-aristolene (2.13%), (Z)-rose oxide (2.04%), and δ-cadinene (2.01%).

On the basis of the provisional assignments, oxygenated monoterpenes represented 55.99% of the reported chromatographic area, followed by terpenoid esters (22.42%; monoterpenoid esters 21.19% and sesquiterpenoid esters 1.23%), sesquiterpene hydrocarbons (14.22%), oxygenated sesquiterpenes other than esters (6.59%) and monoterpene hydrocarbons (0.62%). Oxygenated monoterpenes together with terpenoid esters accounted for 78.41% of the reported peak areas. The peak at RT 20.10 min (0.16%; maximum match factor 726) was excluded from the 52-entry reported table because it did not meet the stated MF ≥800 threshold.

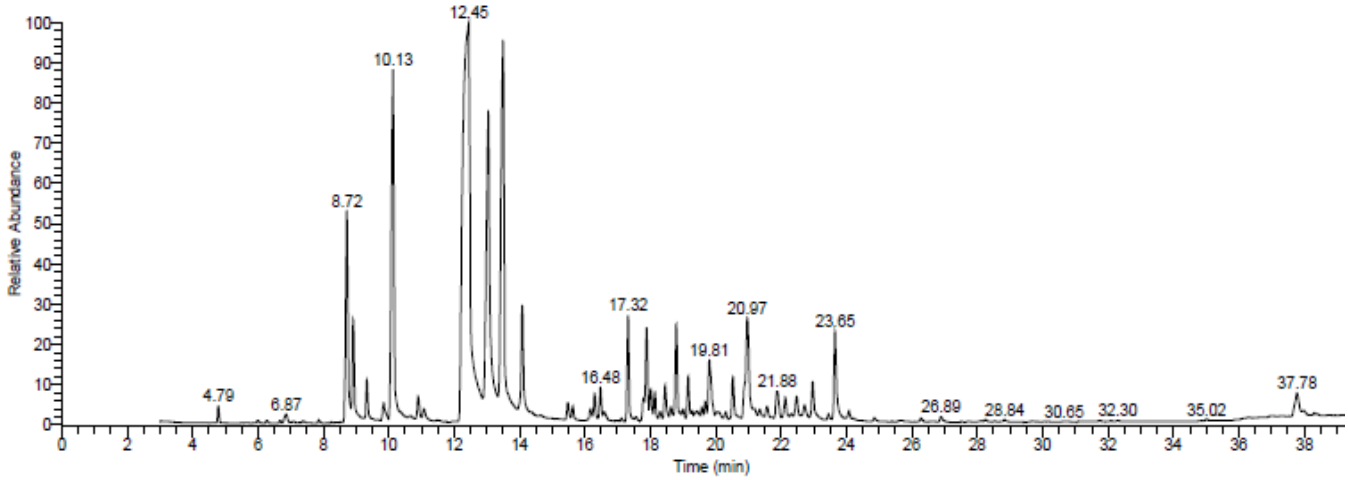


Figure 1. GC-MS chromatogram of *Pelargonium graveolens* oil from Tajoura, Libya.

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Table 1. GC–MS chemical profile of *Pelargonium graveolens* essential oil from Tajoura, Libya with chemical-class totals

Peak	RT (min)	Tentative constituent	Chemical class	Molecular formula	Peak area (%)	MF
1	4.79	α-Pinene	Monoterpene hydrocarbon	C10H16	0.35	941
2	6.87	Limonene	Monoterpene hydrocarbon	C10H16	0.27	910
3	8.72	Linalool	Oxygenated monoterpene	C10H18O	5.44	953
4	8.92	(Z)-Rose oxide	Oxygenated monoterpene	C10H18O	2.04	953
5	9.33	(E)-Rose oxide	Oxygenated monoterpene	C10H18O	0.88	957
6	9.85	trans-Menthan-3-one	Oxygenated monoterpene	C10H18O	0.57	941
7	10.14	Isomenthone	Oxygenated monoterpene	C10H18O	10.50	956
8	10.90	Neoisomenthol	Oxygenated monoterpene	C10H20O	0.57	943
9	11.08	α-Terpineol	Oxygenated monoterpene	C10H18O	0.28	868
10	12.43	Citronellol	Oxygenated monoterpene	C10H20O	26.74	938
11	13.05	Geraniol	Oxygenated monoterpene	C10H18O	8.97	942
12	13.50	Citronellyl formate	Monoterpenoid ester	C11H20O2	11.79	930
13	14.09	Geranyl formate	Monoterpenoid ester	C11H18O2	2.61	924
14	15.48	Citronellyl acetate	Monoterpenoid ester	C12H22O2	0.40	931
15	15.63	Germacrene D	Sesquiterpene hydrocarbon	C15H24	0.28	889
16	16.17	Geranyl acetate	Monoterpenoid ester	C12H20O2	0.28	865
17	16.30	α-Copaene	Sesquiterpene hydrocarbon	C15H24	0.63	929
18	16.48	β-Bourbonene	Sesquiterpene hydrocarbon	C15H24	0.76	937
19	16.60	β -cubebene	Sesquiterpene hydrocarbon	C15H24	0.25	920
20	17.32	β-Caryophyllene	Sesquiterpene hydrocarbon	C15H24	2.52	956
21	17.79	Citronellyl propionate	Monoterpenoid ester	C13H24O2	0.47	929
22	17.89	(-)-Aristolene	Sesquiterpene hydrocarbon	C15H24	2.13	947
23	18.02	γ-Muurolene	Sesquiterpene hydrocarbon	C15H24	0.56	918
24	18.14	α-Humulene	Sesquiterpene hydrocarbon	C15H24	0.54	948
25	18.30	β-Gurjunene	Sesquiterpene hydrocarbon	C15H24	0.18	947
26	18.45	Geranyl propionate	Monoterpenoid ester	C13H22O2	0.85	916
27	18.63	Aromandendrene	Sesquiterpene hydrocarbon	C15H24	0.21	895
28	18.80	Germacrene A	Sesquiterpene hydrocarbon	C15H24	2.20	964
29	19.01	Bicyclogermacrene	Sesquiterpene hydrocarbon	C15H24	0.17	913
30	19.16	γ-Elemene	Sesquiterpene hydrocarbon	C15H24	1.02	917
31	19.28	α-Muurolene	Sesquiterpene hydrocarbon	C15H24	0.15	939
32	19.37	β-Elemene	Sesquiterpene hydrocarbon	C15H24	0.08	909
33	19.44	Farnesol	Oxygenated sesquiterpene	C15H26O	0.15	860
34	19.57	γ-Muurolene	Sesquiterpene hydrocarbon	C15H24	0.16	928
35	19.68	cis-Calamenene	Sesquiterpene hydrocarbon	C15H22	0.28	929
36	19.80	δ-Cadinene	Sesquiterpene hydrocarbon	C15H24	2.01	962
37	20.02	α-Ylangene	Sesquiterpene hydrocarbon	C15H24	0.09	861
38	20.31	Caryophyllene oxide	Oxygenated sesquiterpene	C15H24O	0.17	868
39	20.52	Geranyl butyrate	Monoterpenoid ester	C14H24O2	1.05	937
40	20.97	Furopelargone A	Oxygenated sesquiterpene	C15H22O2	3.69	861
41	21.34	Epiglobulol	Oxygenated sesquiterpene	C15H26O	0.19	855
42	21.57	β-caryophyllene oxide	Oxygenated sesquiterpene	C15H24O	0.32	856
43	21.87	Di-epi-1,10-cubenol	Oxygenated sesquiterpene	C15H26O	0.94	925
44	22.13	1-epi-Cubebol	Oxygenated sesquiterpene	C15H26O	0.56	924
45	22.48	Cubenol	Oxygenated sesquiterpene	C15H26O	0.57	909
46	22.72	Nerolidol tiglate	Sesquiterpenoid ester	C20H32O2	0.36	806
47	22.97	Citronellyl tiglate	Monoterpenoid ester	C15H26O2	0.89	944
48	23.45	Citronellyl 4-methylpentanoate	Monoterpenoid ester	C16H30O2	0.15	892

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Table 1 (continue)						
Peak	**RT (min)**	**Tentative constituent**	**Chemical class**	**Molecular formula**	**Peak area (%)**	**MF**
49	23.65	Geranyl tiglate	Monoterpenoid ester	C15H24O2	2.34	965
50	24.07	Geranyl angelate	Monoterpenoid ester	C15H24O2	0.20	910
51	26.89	Linalyl formate	Monoterpenoid ester	C11H18O2	0.16	871
52	37.78	E-Nerolidyl acetate	Sesquiterpenoid ester	C17H28O2	0.87	892
CHEMICAL-CLASS TOTALS (reported 52-peak profile)						
Monoterpene hydrocarbons (n = 2)					0.62	
Oxygenated monoterpenes (n = 9)					55.99	
Monoterpenoid esters (n = 12)					21.19	
Sesquiterpene hydrocarbons (n = 19)					14.22	
Oxygenated sesquiterpenes (excluding esters) (n = 8)					6.59	
Sesquiterpenoid esters (n = 2)					1.23	
Combined terpenoid esters (n = 14)					*22.42*	
TOTAL REPORTED PEAKS (n = 52)					**99.84**	

RT, retention time; MF, maximum mass spectral library match factor; n, number of peaks.

3.2. Comparison with ISO 4731:2012 North African Marker Intervals

Comparison with the 15 constituent intervals in the North African chromatographic specification of ISO 4731:2012 [12] showed partial numerical agreement (Table 2; Figure 2). Of the 15 constituents, 12 had corresponding provisional assignments in the Libyan profile. Citronellol (26.74% versus 25.0–36.0%), linalool (5.44% versus 4.0–8.5%), geranyl formate (2.61% versus 2.0–7.0%) and geranyl butyrate (1.05% versus 0.7–2.0%) were within their respective intervals. The tentatively mapped menthone-related peak (0.57%) was numerically within the ISO upper limit of 2.1%. By contrast, geraniol (8.97%) was below the specified 10.0–18.0% interval and citronellyl formate (11.79%) exceeded the 4.0–8.0% interval. The citronellol-to-geraniol TIC peak-area ratio was 2.98, reported solely as a descriptive chromatographic ratio.

On the current study, isomenthone (10.50% versus 4.0–8.0%), (Z)-rose oxide (2.04% versus 0.7–1.5%), (E)-rose oxide (0.88% versus 0.3–0.6%) and geranyl tiglate (2.34% versus 0.9–2.0%) were numerically above their reference intervals, whereas α-terpineol (0.28% versus 0.3–0.6%) was marginally below. Guaia-6,9-diene, 10-epi-γ-eudesmol and β-phenylethyl tiglate were not specifically assigned and were therefore designated NR rather than assumed absent

Table 2. Numerical comparison of 15 ISO 4731:2012 North African geranium-oil reference constituents with Present: Tajoura, Libya GC–MS peak-area profile.

Reference component	ISO North Africa (%)	Libyan (%)	Numerical position
(Z)-Rose oxide	0.7–1.5	2.04	Above*
(E)-Rose oxide	0.3–0.6	0.88	Above*
Menthone	n.d.–2.1	0.57	Provisionally within
Isomenthone	4.0–8.0	10.50	Above*
Linalool	4.0–8.5	5.44	Within
Guaia-6,9-diene	n.d.–0.5	NR	Not evaluated
Citronellyl formate	4.0–8.0	11.79	Above
α-Terpineol	0.3–0.6	0.28	Below
Geranyl formate	2.0–7.0	2.61	Within

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Table 2 (Continue)

Reference component	ISO North Africa (%)	Libyan (%)	Numerical position
Citronellol	25.0–36.0	26.74	Within
Geraniol	10.0–18.0	8.97	Below
Geranyl butyrate	0.7–2.0	1.05	Within
10-epi-γ-Eudesmol	3.0–6.2	NR	Not evaluated
Geranyl tiglate	0.9–2.0	2.34	Above*
β-Phenylethyl tiglate	0.5–1.2	NR	Not evaluated

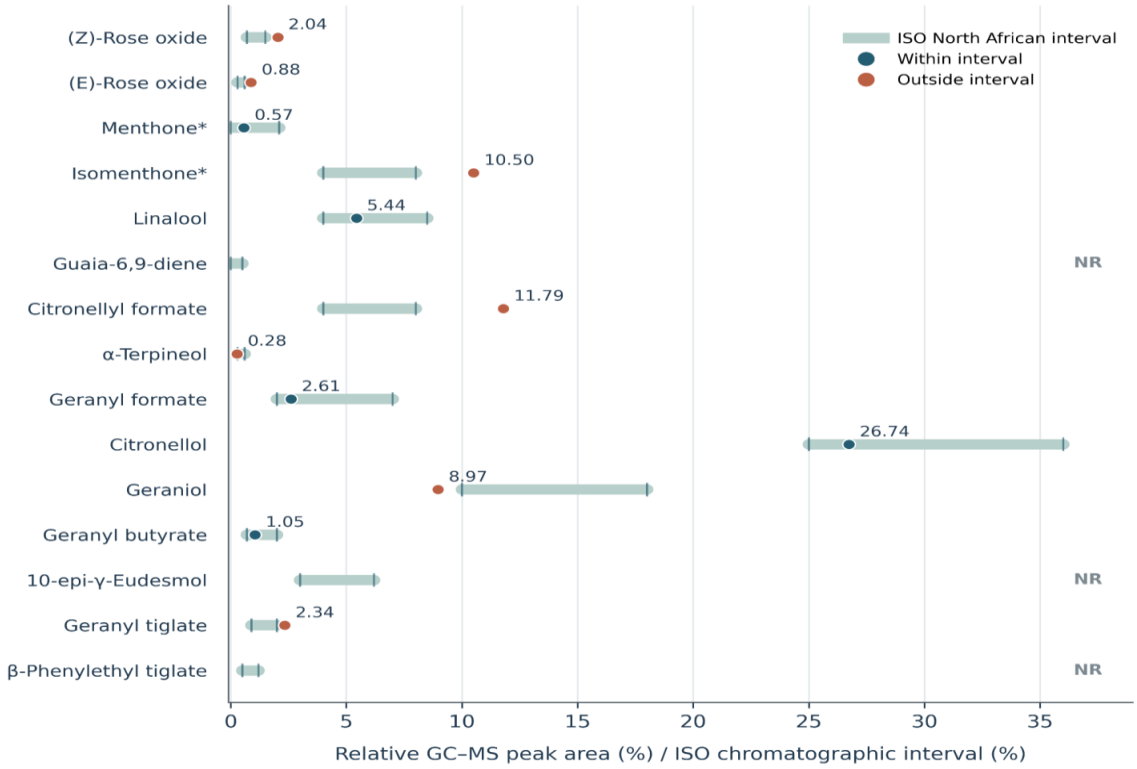


Figure 2. Selected Libyan GC–MS peak-area percentages compared numerically with ISO 4731:2012 North African reference intervals

3.3. Comparison with Published North African Essential-Oil Profiles

Comparison with previously published North African studies revealed both similarities and substantial differences in the chemical composition of *P. graveolens* essential oils. Citronellol was frequently reported among the predominant constituents, although its relative abundance and the proportions of accompanying monoterpene alcohols, esters, and sesquiterpenes varied across investigated samples (Table 3; Figure 3) [1–11]. The citronellol content of the Tajoura oil (26.74%) was comparable to values reported for several Tunisian, Algerian, and Egyptian oils, whereas its relatively low geraniol content (8.97%) distinguished it from many of these profiles.

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Table 3. Selected study-specific chemical markers (%) reported for original North African *P. graveolens* essential oils.

Study / origin	Year	Citronellol	Geraniol	Citronellyl. formate	Linalool	Menthone/iso-menthone
Present: Tajoura, Libya	2025	26.74	8.97	11.79	5.44	10.50*
Boukhris, Tunisia [1]	2013	21.90	11.10	13.20	5.60	NR
Ben ElHadj Ali, Tunisia leaves [2]	2020	26.98	20.65	NR	NR	NR
Atailia, Algeria [5]	2015	19.22	14.03	10.02	5.60	NR
Boukhatem, Algeria [6]	2013	30.20	7.60	9.30	NR	NR
Mokhati, Algeria [7]	2025	24.86	20.50	8.30	2.91	5.26**
Benzaid, Algeria [8]	2026	28.68	5.35	0.76	3.15	5.44*
M'hamdi, Morocco [9]	2024	12.34	12.54	7.70	4.06	NR
Haikal, Egypt [10]	2025	26.74	20.28	9.30	NR	7.03**
Abd El-Kareem, Egypt [11]	2020	27.67	NR	NR	10.05	10.23**

NR = constituent percentage not verified from the original report, not analytical absence.

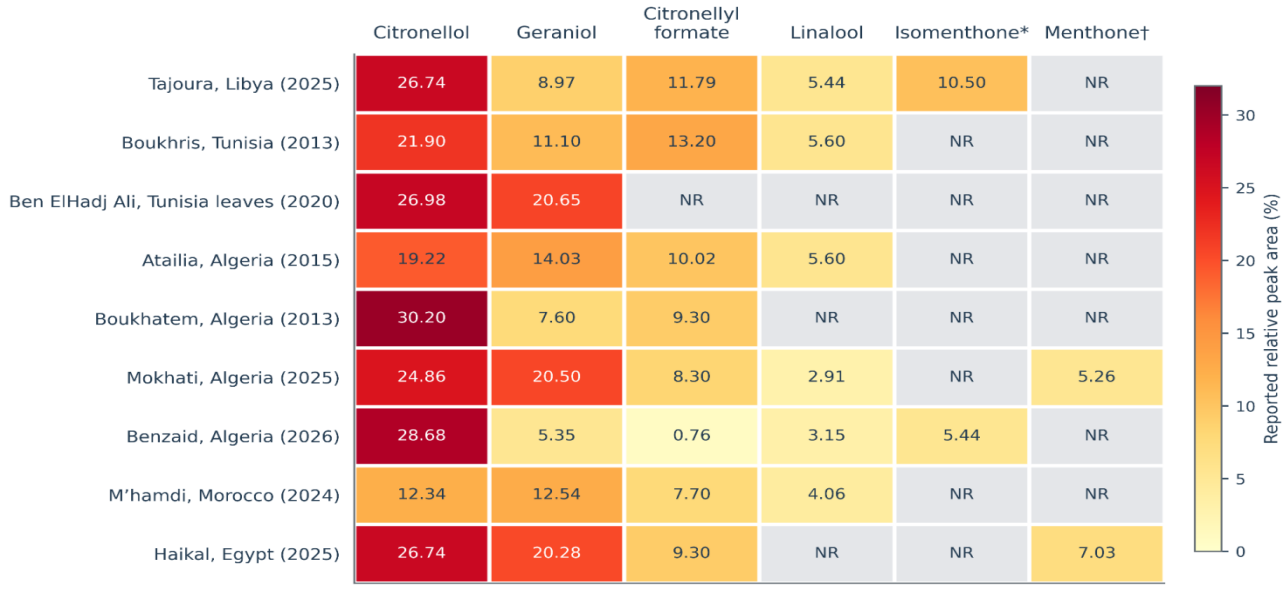


Figure 3. Heatmap of selected major volatile constituents reported for Libyan and original North African *Pelargonium graveolens* oils. Yellow represents a lower relative peak area and red represents a higher peak area; gray (NR) represents unreported values, not measured zero.

Among Tunisian investigations, Boukhris et al. [1] reported a chemical composition resembling the present oil, particularly in citronellyl formate (13.20% versus 11.79%) and linalool (5.60% versus 5.44%). However, geranyl formate was more abundant in the Tunisian oil (6.20% versus 2.61%). Ben ElHadj Ali et al. [2] reported comparable citronellol levels in leaf and flower oils (26.98% and 24.30%, respectively), but substantially higher geraniol proportions (20.65% and 21.81%) than those observed in Tajoura. Further compositional variability was documented by Reziga et al. [3] across four Tunisian growing biotopes. Notably, Lahmar et al. [4] identified chemically contrasting oils from two cultivation environments in Chebba, characterized by relatively high proportions of geranyl acetate, cis-ocimene, and trans-rose oxide rather than the citronellol-dominant profile observed in the present study. Collectively, these findings demonstrate substantial variation among Tunisian samples, including differences associated with plant organs and cultivation environments.

A similar pattern of chemical variability was evident among Algerian oils. Atailia and Djahoudi [5] reported citronellol (19.22%), geraniol (14.03%), and citronellyl formate (10.02%), whereas Boukhatem et al. [6] identified citronellol (30.20%), citronellyl formate (9.30%), and

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geraniol (7.60%) as the major constituents. The latter profile showed a similar ordering of these principal markers to the Tajoura oil. In contrast, Mokhati et al. [7] reported a substantially higher geraniol proportion (20.50%) in an oil from Tebessa, despite a comparable citronellol level (24.86%). Benzaid et al. [8] identified another distinctive Algerian profile containing citronellol (28.68%) and geraniol (5.35%), but considerably less citronellyl formate (0.76%) than Libyan oil (11.79%). These differences highlight considerable chemical heterogeneity within Algeria and suggest that individual oil profiles cannot be reliably generalized at the country level.

Additional contrasts were observed in Morocco and Egypt. M'hamdi et al. [9] characterized a Moroccan oil containing relatively high proportions of epi-γ-eudesmol (16.67%) and geraniol (12.54%), alongside a lower citronellol percentage (12.34%). In Egypt, Haikal et al. [10] reported the same numerical citronellol abundance as the Tajoura sample (26.74%), but substantially higher proportions of geraniol (20.28%) and 10-epi-γ-eudesmol (17.14%). Another Egyptian investigation by Abd El-Kareem et al. [11] described a citronellol-rich composition, although discrepancies between its reported constituent assignments and percentages limit reliable quantitative comparison.

Overall, the available evidence indicates that citronellol-rich profiles occur across several North African regions, while substantial differences exist in geraniol, citronellyl formate, and oxygenated sesquiterpene distributions. Such variability may reflect differences in plant material, developmental stage, growing conditions, extraction procedures, and analytical methods. However, the relative contributions of these factors cannot be established from comparisons among independent studies. Furthermore, differences between GC–MS peak-area percentages and GC-FID-derived relative compositions, together with varying identification criteria, limit direct quantitative interpretation. Accordingly, the present results should be considered a preliminary chemical fingerprint of a specific Libyan oil batch rather than evidence of a geographically distinct chemotype. Independent sampling and standardized comparative analyses are needed to clarify the reproducibility and determinants of these compositional patterns.

3.4. Antibacterial screening and analytical interpretation

At a loading of 4 µL of undiluted oil per 6-mm paper disc, *P. graveolens* essential oil produced inhibition zones against all five reference strains (Table 4; Figure 4). The largest mean zone was obtained against *S. aureus* ATCC 29213 (21.00 ± 1.00 mm), followed by *S. epidermidis* ATCC 12228 (20.00 ± 1.00 mm), *Sh. flexneri* ATCC 12022 (12.67 ± 0.58 mm), *S. enterica* ATCC 14028 (8.67 ± 0.58 mm) and *E. coli* ATCC 8739 (7.50 ± 0.50 mm). All reported diameters include the 6-mm disc; consequently, the *E. coli* response corresponded to only approximately 0.75 mm of radial clearance beyond the disc edge. Unloaded-disc controls showed no visible halo beyond the paper disc (0 mm additional clearance). The reference gentamicin discs (10 µg/disc) produced mean zones of 17–26 mm across the five strains.

Table 4. Disc-diffusion inhibition-zone diameters (mm, mean ± sample SD, n = 3) for *P. graveolens* essential oil and gentamicin

Reference strain	EO, 4 µL/disc	Gentamicin, 10 µg/disc
S. aureus ATCC 29213	21.00 ± 1.00	17.00 ± 0.00
S. epidermidis ATCC 12228	20.00 ± 1.00	26.00 ± 0.00
S. flexneri ATCC 12022	12.67 ± 0.58	17.00 ± 0.00
S. enterica ATCC 14028	8.67 ± 0.58	18.00 ± 0.00
E. coli ATCC 8739	7.50 ± 0.50	21.00 ± 0.00

Measured diameters include the 6-mm paper disc. Gentamicin (10 µg/disc) was an assay reference; oil (4 µL/disc).

The observed larger responses among the two Gram-positive staphylococci are compatible with the broad literature describing restricted

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penetration of many hydrophobic volatiles through the Gram-negative outer membrane and the complex membrane interactions of essential-oil mixtures [15]. Nonetheless, outer-membrane differences alone do not account for all observed variability: inoculum biology, oil volatility, exposure dose, bacterial species, sorption to paper, and diffusion through agar each influence inhibition zones. Because constituent-specific inhibitory experiments were not conducted, activity cannot be assigned causally to citronellol, geraniol, isomenthone or furopelargone A, nor can synergistic effects be claimed.

Previous Libyan studies provide additional context for the antibacterial activity of *P. graveolens* essential oil. Abushiba et al. [13] reported an inhibition zone of 12 ± 1 mm against *Proteus mirabilis* ATCC 14153 using 10 µL of essential oil under direct-contact and vapor-phase assay conditions, although the chemical composition of the tested oil was not characterized by GC–MS. These findings provide preliminary evidence of antibacterial activity in locally sourced *P. graveolens* essential oil. However, direct quantitative comparison with the present findings is limited by differences in collection periods, bacterial strains, oil loading, and assay conditions. Similarly, a previous Libyan investigation highlighted the influence of essential-oil application methods on the observed antibacterial responses [16].

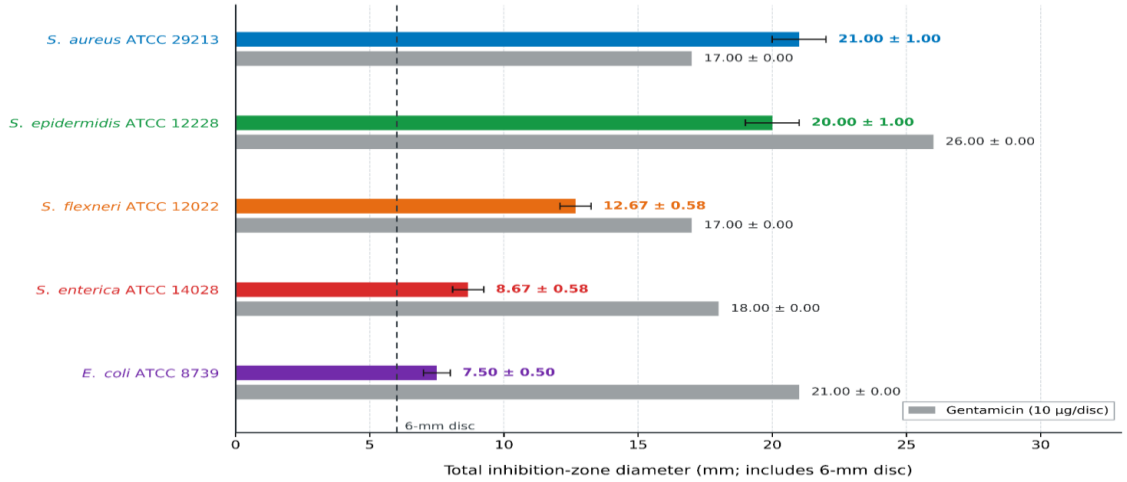


Figure 4. Disc-diffusion inhibition-zone diameters for *P. graveolens* oil (4 µL/disc) and gentamicin reference discs (10 µg/disc) across five bacterial strains (Error bars indicate sample SD from three experimental runs)

Previous Algerian investigations provide additional context for the antibacterial activity of *P. graveolens* essential oil, although methodological differences limit direct comparisons. Atailia and Djahoudi [5] reported minimum inhibitory concentration (MIC) values against clinical bacterial isolates, whereas Boukhatem et al. [6] evaluated antimicrobial activity against food-associated microorganisms using direct-contact and vapor-phase assays. These methodological differences prevent direct quantitative comparison with the inhibition-zone measurements obtained in the present study. Although the essential oil produced a larger inhibition zone against *Staphylococcus aureus* than gentamicin (21.00 versus 17.00 mm), differences in applied doses and agar-diffusion characteristics preclude interpreting this observation as evidence of superior antibacterial potency.

3.5. Study Limitations and Future Research Directions

Although the present study provides preliminary evidence of the chemical composition and antibacterial activity of *P. graveolens* essential oil from Tajoura, Libya, several limitations warrant consideration. The investigation was restricted to a single collection batch, and the

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identification of volatile constituents was based primarily on mass-spectral library matching without experimental retention-index confirmation. Furthermore, antibacterial activity was evaluated using agar-disc diffusion, which does not establish minimum inhibitory or bactericidal concentrations.

Future investigations should prioritize the collection of authenticated plant material from different Libyan cultivation areas, seasons, and phenological stages to assess chemical variability and reproducibility. Additional chromatographic characterization, including experimental retention indices and authentic reference standards, would improve compound-identification confidence. Comprehensive quality assessment using the relevant analytical and physicochemical requirements of ISO 4731:2012 would also clarify the suitability of locally produced oil for standardized applications. Moreover, broth microdilution assays, MIC and MBC determination, and evaluation of selected major constituents would provide a stronger basis for interpreting antibacterial activity. These investigations could support future quality-control studies and more comprehensive assessment of the antimicrobial potential of Libyan *P. graveolens* essential oil.

4. Conclusions

GC–MS analysis of the essential oil obtained from *P. graveolens* cultivated in Tajoura, Libya, revealed a citronellol-dominant chemical profile, characterized mainly by citronellol (26.74%), citronellyl formate (11.79%), isomenthone (10.50%), geraniol (8.97%), and linalool (5.44%). The essential oil, extracted from fresh aerial parts by hydrodistillation, yielded 0.8% (v/w), and its chromatographic profile comprised 52 recorded peaks. Comparison with ISO 4731:2012 showed partial agreement with the North African reference composition, although variations in selected constituents, particularly geraniol and citronellyl formate, were observed. These findings highlight the chemical characteristics of the investigated Libyan oil and its compositional differences from previously reported North African samples.

The essential oil exhibited antibacterial activity against all five tested bacterial strains, with the strongest inhibition observed against *S. aureus* and *S. epidermidis*, followed by *S. flexneri*, *S. enterica*, and *E. coli*. These preliminary findings suggest that Libyan *P. graveolens* essential oil represents a promising source of naturally occurring compounds for further antimicrobial investigation. However, additional studies involving MIC and MBC determination, confirmation of major chemical constituents, and evaluation of biological activity under practical application conditions are necessary before its potential use as a natural antimicrobial agent can be established.

ETHICAL STATEMENT

Ethical approval was not required, as the study involved only plant material and in vitro testing of reference bacterial strains.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the research and manuscript preparation, reviewed the final version, and approved its submission.

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DATA AVAILABILITY

The GC–MS data and antibacterial results are available from the corresponding author upon reasonable request.

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