

Comparative Direct Contact- and Vapor-Phase Antibacterial Activity of *Eucalyptus camaldulensis* and *Pelargonium graveolens* Essential Oils against *Proteus mirabilis* ATCC 14153

Mahmuod A. Abushiba¹, Mhmuod Zughdani², Abdulaziz Sh. Suwaydan², Mohamed S. Altraiki³, Abdulkarem Rajab Tamer⁴

¹ Department of Microbiology and Immunology, Faculty of Medicine, University of Azzaytuna, Libya

² Department of Pharmacognosy, Faculty of Pharmacy, Elmergib University, Al-Khoms, Libya

³ Libyan Center for Sustainable Development Research, Libyan Authority for Scientific Research, Tripoli, Libya.

⁴ Department of Microbiology and Immunology, Faculty of Pharmacy, University of Tripoli, Libya

Correspondence: Mahmuod A. Abushiba; m.abushiba@azu.edu.ly

Abstract

Background: *Proteus mirabilis* (*P. mirabilis*) is a clinically important uropathogen, and Essential oils (EOs) are of continued interest as complementary antibacterial agents. Because EOs are volatile and hydrophobic in nature, their apparent antibacterial activity can depend on the exposure mode used to test them. **Objective:** to compare the direct-contact and non-contact vapor phase antibacterial activity of hydrodistilled EOs from *Eucalyptus camaldulensis* (*E. camaldulensis*) and *Pelargonium graveolens* (*P. graveolens*) in vitro against *P. mirabilis* ATCC 14153. **Methods:** EOs were obtained by hydrodistillation of leaves of *E. camaldulensis* and *P. graveolens* were collected from Tajoura, Libya. Antibacterial activity was assessed by disc diffusion for direct-contact exposure and disc volatilization for non-contact vapor exposure, using 10 µL of undiluted EO per assay. **Results:** EOs yield $1.6 \pm 0.05\%$ and $1.4 \pm 0.05\%$ (v/w, dry-weight basis) for *E. camaldulensis* and *P. graveolens*, respectively. Each antibacterial condition was tested in triplicate (n=3). *E. camaldulensis* produced inhibition zones of 20 ± 1 mm by disc diffusion and 22 ± 1 mm by disc volatilization, whereas *P. graveolens* produced 12 ± 1 mm in both assays. **Conclusion:** Both EOs are active against *P. mirabilis* ATCC 14153 under direct-contact and non-contact vapor phase conditions, with *E. camaldulensis* consistently more active. As chemical composition was not determined, the results describe the whole of the EOs as tested rather than specific constituents. These findings support further standardized evaluation of EO vapor-phase antibacterial activity.

Keywords: Essential oil; Disc diffusion; Disc volatilization; Vapor phase; *Proteus mirabilis*; *Eucalyptus camaldulensis*; *Pelargonium graveolens*; Bioactive volatiles; Uropathogens.

النشاط المضاد للبكتيريا بمقارنة طريقتي التماس المباشر وطور البخار للزيوت العطرية لنباتي *Proteus mirabilis* و *(Eucalyptus camaldulensis)* ضد بكتيريا *(Pelargonium graveolens)*

الملخص

الخلفية: تُعد بكتيريا *Proteus mirabilis* من ضمن مسببات عدوى المسالك البولية المهمة سريريًا، وحيث لا تزال الزيوت العطرية تحظى باهتمام متواصل بوصفها عوامل مضادة للبكتيريا يمكن استخدامها في العديد من المجالات. ونظرًا للطبيعة المتطايرة والكارهة للماء للزيوت العطرية، فإن فعاليتها الظاهرية المضادة للبكتيريا قد تعتمد على نمط التعرض المستخدم في اختبارها. **الهدف:** مقارنة الفعالية المضادة للبكتيريا مخبريًا بطريقتي التماس المباشر وطور البخار للزيوت العطرية المستخلصة بالتقطير المائي من نباتي *(Eucalyptus camaldulensis)* و *(Pelargonium graveolens)* ضد بكتيريا *(Proteus mirabilis)* ATCC 14153. **الطريقة:** تم الحصول على الزيوت العطرية عن طريق التقطير المائي لأوراق *(Eucalyptus camaldulensis)* و *(Pelargonium graveolens)* التي جُمعت من تاجوراء، ليبيا. تم تقييم النشاط المضاد للبكتيريا باستخدام طريقة انتشار الأقراص للتعرض المباشر بالتلامس، وطريقة تطاير الزيوت من القرص للتعرض لبخار الزيت دون تلامس، باستخدام 10 ميكرو لتر من الزيت العطري غير المخفف في كل اختبار. **النتائج:** بلغت نسبة استخلاص الزيوت العطرية $1.6 \pm 0.05\%$ لزيت نبات *(Eucalyptus camaldulensis)* و $1.4 \pm 0.05\%$ (حجم/وزن، على أساس الوزن الجاف) لزيت نبات *(Pelargonium graveolens)*. تم اختبار كل حالة من حالات النشاط المضاد للبكتيريا في ثلاث مكررات (n=3). أظهر زيت نبات *(Eucalyptus camaldulensis)* مناطق تثبيط بقطر 1 ± 20 مم باستخدام طريقة انتشار الأقراص، و 1 ± 22 مم باستخدام طريقة تطاير الزيت من القرص، في حين أظهر زيت نبات *(Pelargonium graveolens)* مناطق تثبيط بقطر 1 ± 12 مم في كلتا الطريقتين. **الاستنتاج:** أظهر كلا الزيتين العطريين نشاطًا مضادًا للبكتيريا ضد بكتيريا *(Proteus mirabilis)* ATCC 14153 في ظروف التلامس المباشر وظروف التعرض لطور البخار دون تلامس، مع استمرار تفوق *(Eucalyptus camaldulensis)* في النشاط المضاد للبكتيريا. ونظرًا لعدم تحديد التركيب الكيميائي للزيوت، فإن النتائج تصف فعالية الزيوت العطرية ككل كما تم اختبارها، وليس فعالية مكونات كيميائية محددة منها. وتدعم هذه النتائج إجراء المزيد من التقييمات المعيارية للنشاط المضاد للبكتيريا للزيوت العطرية في طور البخار.

الكلمات المفتاحية: الزيت العطري؛ انتشار الأقراص؛ تطاير الزيت من القرص؛ طور البخار؛ *(Proteus mirabilis)*؛ *(Eucalyptus camaldulensis)*؛ *(Pelargonium graveolens)*؛ المركبات المتطايرة النشطة حيويًا؛ مسببات عدوى المسالك البولية.

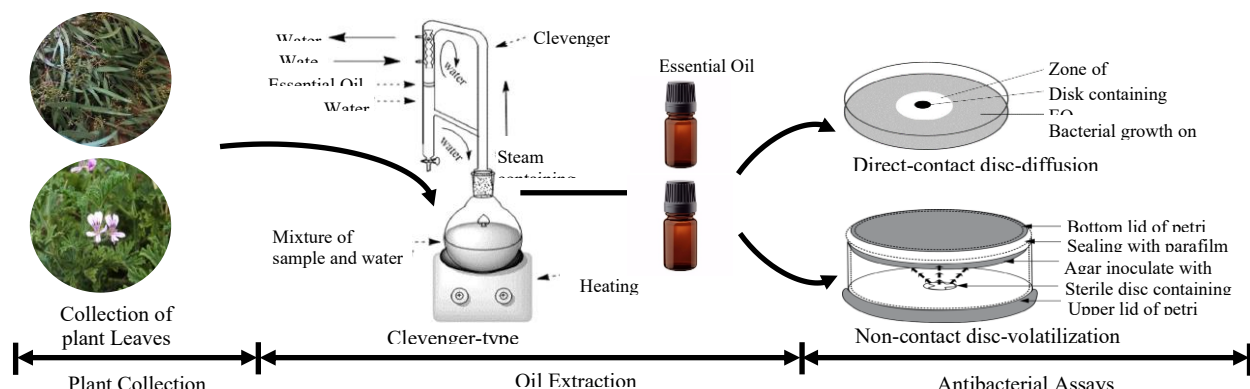


Figure 1. Graphical abstract illustrating essential oil extraction and comparative contact- and vapor-phase antibacterial assays of *E. camaldulensis* and *P. graveolens* against *P. mirabilis* ATCC 14153.

1. Introduction

Proteus mirabilis (*P. mirabilis*) is a Gram-negative facultative anaerobic bacterium, a member of the Enterobacterales, and an important opportunistic pathogen, particularly in complicated and catheter-associated urinary tract infections. Its clinical importance is reinforced by biofilm formation, persistence in urinary environments, and the acquisition of antimicrobial-resistance determinants (Schaffer & Pearson, 2015; Wasfi *et al.*, 2020; Girlich *et al.*, 2020; Kynshi *et al.*, 2025). These characteristics sustain interest in complementary antimicrobial strategies and in vitro screening of natural products against well-defined reference strains.

EOs are complex mixtures of volatile plant metabolites with documented antibacterial activity. Their hydrophobic and volatile nature, however, complicates conventional antimicrobial testing because assay outcomes can depend strongly on diffusion through agar, dispersion in aqueous media, dose, and exposure geometry (Nazzaro *et al.*, 2013; Reyes-Jurado *et al.*, 2015; Wińska *et al.*, 2019). Antibacterial activity has previously been reported for *Eucalyptus* and *Pelargonium* EOs, including *E. camaldulensis* and *P. graveolens* (Gilles *et al.*, 2010; Hsouna *et al.*, 2011; Boukhatem *et al.*, 2013; Knezevic *et al.*, 2016).

Vapor-phase assays provide a complementary exposure model in which volatile EO components reach microorganisms without direct liquid contact with the inoculated agar. Disc-volatilization and related gaseous-contact methods have therefore been used alongside conventional contact assays to examine method-dependent antimicrobial responses (Inouye *et al.*, 2001; Tyagi & Malik, 2011; Klūga *et al.*, 2021; Ignatiuk *et al.*, 2023; Łyczko *et al.*, 2023). Because contact and vapor assays involve different mass-transfer processes, their inhibition-zone diameters should not automatically be interpreted as quantitatively interchangeable measures of intrinsic antimicrobial potency.

The present study therefore compared the in vitro antibacterial response of *P. mirabilis* ATCC 14153 to EOs obtained from *E. camaldulensis* and *P. graveolens* collected in Tajoura, Libya, under two exposure modes: direct-contact disc diffusion and non-contact disc volatilization. The objective was to determine whether each oil produced measurable inhibition under both assay conditions and to compare the resulting response patterns descriptively using the same nominal EO volume per assay.

2. Materials and methods

2.1. Plant material and botanical identification

Leaves of *E. camaldulensis* and *P. graveolens* were collected in July 2026 from plants growing in the Tajoura region, Tripoli, Libya; and allowed to dry in shade. The plant material was authenticated and identified by Prof.Mohammed Makhlof, Department of Botany, Faculty of Science, University of Tripoli, Libya (Figure 2).



Figure 2. Shows the Leaves of the aerial parts of *E. camaldulensis* (left) and *P. graveolens* (right).

2.2. EO extraction and yield determination

EOs were obtained by hydrodistillation using a Clevenger-type apparatus, following the approach described by Suwaydan and Altraiki (2025) with the experimental conditions available in the present study. For each species, 100 g of dry leaves were cut into small pieces and subjected to hydrodistillation for 3 h. Three independent extractions were performed for each plant species (n=3). The recovered EO was dried over anhydrous sodium sulfate, transferred to tightly sealed amber glass vials, and stored at 4 °C until antibacterial testing. EOs yield were expressed as percentage volume per dry mass (v/w) and calculated as:

$$EO \text{ yield (\%)} = \frac{EO \text{ volume (mL)}}{\text{dry plant material (g)}} \times 100$$

2.3. Bacterial strain and culture conditions

The study used the reference strain *P. mirabilis* ATCC 14153, supplied by the Center for Disease Control, Tripoli, Libya, and originally obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The strain was stored in 20% (v/v) glycerol at -20 °C. Working cultures were maintained on nutrient agar and subculture onto fresh nutrient agar plates at 37 °C for 18-24 h before each assay to confirm viability and purity. All microbiological procedures were performed aseptically.

2.4. Direct-contact disc-diffusion assay

The direct-contact antibacterial assay used the agar disc-diffusion principle. Overnight bacterial growth was suspended in sterile saline and adjusted to a 0.5 McFarland turbidity standard, corresponding to approximately 1.5×10^8 CFU/mL. A 100 µL aliquot of the standardized suspension was distributed over Mueller-Hinton agar (MHA). Sterile 6-mm filter-paper discs were loaded with 10 µL of undiluted EO and placed on the inoculated agar surface. Gentamicin (10 µg/disc) was included as a positive reference for the direct-contact assay. Plates were incubated aerobically at 37 °C for 24 h, after which inhibition-zone diameters were measured in millimeters. Inoculum standardization, MHA use, and general disc-test conditions were based on CLSI M02 principles, with modifications appropriate for EO screening (CLSI, 2024). CLSI does not provide susceptibility breakpoints for these EOs; therefore, EO inhibition zones were interpreted descriptively and were not classified as susceptible, intermediate, or resistant.

2.5. Non-contact disc-volatilization assay

Vapor-phase antibacterial activity was evaluated by a disc-volatilization assay adapted from published EO vapor-testing approaches (Ignatiuk *et al.*, 2023; Swedan, 2025). A 100 µL aliquot of the standardized bacterial suspension (approximately 1.5×10^8 CFU/mL) was spread over MHA. A sterile blank paper disc was positioned centrally on the inner surface of the Petri-dish lid and loaded with 10 µL of undiluted EO. The inoculated agar-containing plate base was then inverted over the lid so that the EO did not contact the agar directly. The assembled plates were sealed with Parafilm and incubated for 24 h at 37 °C. After incubation, the diameter of the clear growth-inhibition zone on the agar surface opposite the lid-mounted EO disc was measured in millimeters. A growth-control plate with a sterile paper disc without EO was included in triplicate. The nominal vapor-phase exposure is reported as 10 µL EO per plate because the internal Petri-dish headspace volume was not quantified in the experimental record.

2.6. Data analysis

EO yield and each antibacterial assay condition were evaluated in triplicate (n=3). Results are reported as mean ± standard deviation (SD). Because only three replicate measurements were available per condition and the contact and vapor assays represent different transport environments,

no inferential test was used to claim superiority of one exposure mode over the other. Differences between assay modes were therefore interpreted descriptively. Gentamicin results were used only as a positive reference for the direct-contact disc-diffusion assay and were not treated as a comparator for vapor-phase activity.

3. Results

3.1. EO yield

Hydrodistillation produced an EO yield of $1.6 \pm 0.05\%$ (v/w, dry-weight basis) from *E. camaldulensis* leaves and $1.4 \pm 0.05\%$ from *P. graveolens* leaves (Table 1).

Table 1. EO yields from dried leaves of the two plant species.

Plant species	Extraction replicates, n	EO yield (% v/w, dry-weight basis)
<i>Eucalyptus camaldulensis</i>	3	1.6 ± 0.05
<i>Pelargonium graveolens</i>	3	1.4 ± 0.05

3.2. Direct-contact and vapor-phase antibacterial activity

Both EOs inhibited *P. mirabilis* ATCC 14153 under direct-contact and vapor-phase conditions (Table 2; Figure 3). *E. camaldulensis* produced a mean inhibition zone of 20 ± 1 mm in the disc-diffusion assay and 22 ± 1 mm in the disc-volatilization assay. The 2-mm difference is reported as a descriptive numerical difference only and was not interpreted as evidence of greater intrinsic vapor-phase potency. *P. graveolens* produced 12 ± 1 mm inhibition zones in both assay modes. Across both methods, the inhibition zones produced by *E. camaldulensis* were larger than those produced by *P. graveolens*. In the direct-contact assay, the gentamicin positive reference produced a 23 ± 1 mm inhibition zone.

Table 2. Inhibition-zone diameters of EOs against *Proteus mirabilis* ATCC 14153 under direct-contact and vapor-phase exposure.

EO	n	Disc diffusion (mm)	Disc volatilization (mm)
<i>E. camaldulensis</i>	3	20 ± 1	22 ± 1
<i>P. graveolens</i>	3	12 ± 1	12 ± 1

Note. Values are mean $\pm$ SD (n=3). Gentamicin ($10 \mu\text{g}/\text{disc}$) produced 23 ± 1 mm in the direct-contact disc-diffusion assay and was not used as a vapor-phase comparator.

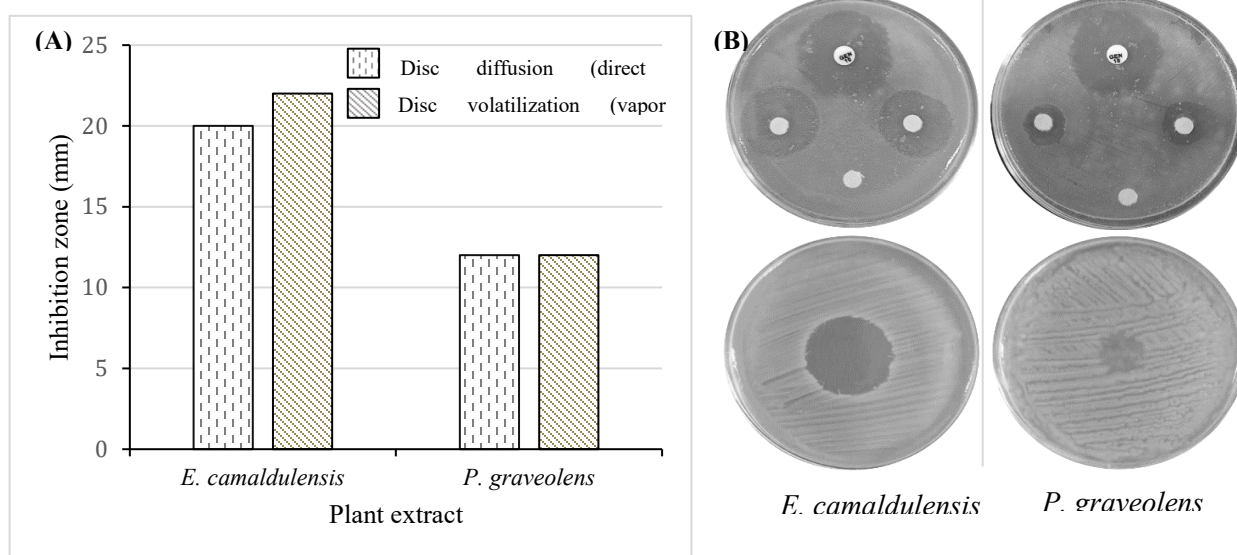


Figure 3. (A) Mean inhibition-zone diameters ($\pm$ SD; $n=3$) produced by *E. camaldulensis* and *P. graveolens* essential oils in direct-contact disc-diffusion and non-contact disc-volatilization assays against *P. mirabilis* ATCC 14153. No inferential comparison between assay modes was performed. (B) The zone of inhibition using direct contact (top) and volatile-phase methods (bottom) against *Proteus mirabilis* ATCC 14153.

4. Discussion

The present study provides a matched in vitro screening comparison of two locally collected medicinal and aromatic plant EOs against the single reference strain *P. mirabilis* ATCC 14153 using direct-contact and vapor-phase exposure. *E. camaldulensis* showed larger inhibition zones than *P. graveolens* under both assay conditions. Importantly, both oils also produced measurable inhibition when the EO-containing disc was physically separated from the inoculated agar, confirming antibacterial activity under the non-contact vapor-exposure conditions used in this experiment.

The observation of EO activity in both assay modes is consistent with the broader literature showing that EOs can exert measurable antimicrobial effects when applied directly to agar and when delivered via the vapor phase (Inouye *et al.*, 2001; Tyagi & Malik, 2011; Klūga *et al.*, 2021; Ignatiuk *et al.*, 2023). However, the numerical inhibition-zone values generated by the two methods should not be regarded as directly equivalent. In disc diffusion, antibacterial compounds must partition from the disc into the agar and diffuse laterally through the hydrated medium. In disc volatilization, volatile material must first evaporate into the headspace and subsequently reach the inoculated agar surface. Differences in volatility, agar partitioning, headspace geometry, sealing, temperature, and diffusion can therefore influence the apparent zone size independently of intrinsic antimicrobial potency (Cos *et al.*, 2006; Reyes-Jurado *et al.*, 2015; Łyczko *et al.*, 2023). For this reason, the 22 ± 1 mm vapor-phase zone observed with *E. camaldulensis*, compared with 20 ± 1 mm by disc

diffusion, is interpreted only as a numerically larger response under the vapor-test conditions rather than evidence that the vapor phase was significantly or intrinsically more potent.

Previous studies have documented antibacterial activity of *E. camaldulensis* and *P. graveolens* EOs (Gilles *et al.*, 2010; Hsouna *et al.*, 2011; Boukhatem *et al.*, 2013; Knezevic *et al.*, 2016). The present findings are broadly compatible with that literature, but direct quantitative comparison is limited because EO activity is strongly affected by plant origin, chemical profile, test organism, inoculum, EO dose, agar conditions, and assay design. In the present work, GC-MS or GC-FID characterization was not available. Accordingly, no attempt should be made to attribute the observed antibacterial activity to 1,8-cineole, citronellol, geraniol, alpha-pinene, or any other individual constituent. The data support the activity of the whole EOs as tested, not a compound-specific mechanism.

Within these boundaries, the study demonstrates the practical value of examining EOs under more than one exposure mode. Conventional disc diffusion alone characterizes activity after direct contact and agar diffusion, whereas disc volatilization can reveal whether a tested EO also exerts inhibitory effects without direct oil-agar contact. Future work should standardize headspace geometry and vapor dose and should incorporate chemical profiling and broth-based quantitative endpoints when these resources are available.

5. Conclusions

Under the conditions of this in vitro screening study, *E. camaldulensis* EO produced larger inhibition zones against *P. mirabilis* ATCC 14153 than *P. graveolens* EO in both direct-contact disc diffusion and non-contact disc volatilization assays. *E. camaldulensis* produced inhibition zones of 20 ± 1 and 22 ± 1 mm in the two assays, respectively, whereas *P. graveolens* produced 12 ± 1 mm in both. The numerical difference between the two *E. camaldulensis* assay results should not be interpreted as proof of greater vapor-phase potency because the two methods represent different exposure and mass-transfer systems and were not compared inferentially. The measurable non-contact inhibition observed for both oils supports continued investigation of EO vapor-phase antibacterial activity using more standardized quantitative methods. Because chemical profiling was not performed, the present results apply to the whole oils as tested and cannot be assigned to specific constituents.

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