

Chemical Composition and In Vitro Antioxidant and Antibacterial Activities of *Eucalyptus camaldulensis* Leaf Essential Oil from Northwestern Libya

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التركيب الكيميائي والأنشطة المضادة للأكسدة والمضادة للبكتيريا في المختبر للزيت العطري لأوراق
شجر الكافور (*Eucalyptus camaldulensis*) من شمال غرب ليبيا

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Abstract:

This study investigates the phytochemistry, antioxidant capacity, and antibacterial activity of *Eucalyptus camaldulensis* essential oil (EEO) cultivated in northwestern Libya. The volatile profile was analyzed via gas chromatography-mass spectrometry (GC-MS). The analysis identified 1,8-cineole (33.61%) and α -pinene (29.90%) as the principal constituents of the observed chemical profile. Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, which demonstrated a weak antioxidant potential ($IC_{50} > 10,000 \mu\text{g/mL}$). Conversely, preliminary antibacterial screening via the disc diffusion method demonstrated variable, strain-dependent growth-inhibitory effects across five standard reference strains. The most pronounced susceptibility was observed against the Gram-positive strain *Staphylococcus aureus* (20 mm zone of inhibition). These findings successfully fill a localized geographic evidence gap by providing foundational phytochemical data for North African *E. camaldulensis*. Furthermore, this study highlights a distinct monoterpene-dominant profile that contrasts sharply with the chemical polymorphism frequently observed in other widely cultivated regional species such as *E. globulus*, providing valuable chemotaxonomic context without implying strict geographic causation or direct pharmaceutical efficacy.

Keywords: *Eucalyptus camaldulensis*, Essential Oil, GC-MS, 1,8-cineole, α -pinene, Antibacterial Activity, Libya.

الملخص

تهدف هذه الدراسة إلى فحص التركيب الكيميائي، وتقييم القدرة المضادة للأكسدة، والنشاط المضاد للبكتيريا للزيت العطري لمستخلص نبات الكافور (*Eucalyptus camaldulensis*) المزروع في شمال غرب ليبيا. أظهر تحليل الاستشراب الغازي المزود بمطياف الكتلة (GC-MS) أن المكونات الأساسية للزيت الطيار تتمثل في مركب 1,8-سينيول بنسبة (33.61%)، ومركب ألفا-بينين بنسبة (29.90%). أما النشاط المضاد للأكسدة باستخدام اختبار DPPH، فقد أظهرت قدرة مضادة للأكسدة ضعيفة ($IC_{50} > 10,000 \mu\text{g/mL}$). وبالعكس، فالتحليل الأولي للنشاط المضاد للبكتيريا باستخدام طريقة انتشار القرص أظهرت تأثيرات مثبطة للنمو متغيرة، تعتمد على السلالة، عبر خمسة سلالات مرجعية قياسية. كانت الحساسية الأكثر وضوحاً ملاحظة ضد السلالة موجبة الجرام *Staphylococcus aureus* (منطقة تثبيط 20 ملم). هذه النتائج تملأ فجوة أدلة جغرافية محلية بنجاح من خلال توفير بيانات أساسية كيميائية لليبيا الشمالية الغربية *E. camaldulensis*. علاوة على ذلك، فإن هذه الدراسة تبرز ملفاً مميزاً غنياً بالمواد الحلقية الأحادية، يتناقض حاداً مع التنوع الكيميائي الشائع في أنواع إقليمية أخرى شائعة زراعتها مثل *E. globulus*، مما يوفر سياقاً كيميائياً تصنيفياً ذا قيمة دون أن يفترض سبباً جغرافياً صارماً أو فعالية دوائية مباشرة.

1- بيكرابل هيدرازابل (DPPH) بيّن أن الزيت العطري يمتلك قدرة ضعيفة كمضاد للأكسدة ($IC_{50} > 10,000 \mu\text{g/mL}$). وبالمقابل أظهر الفحص الأولي المضاد للبكتريا عبر طريقة نشر القرص (disc diffusion method) أن للزيت تأثيرات متفاوتة في تثبيط نمو خمس سلالات بكتيرية مرجعية؛ حيث سُجل التأثير الأقوى والأكثر فعالية ضد بكتيريا المكورات العنقودية الذهبية (*Staphylococcus aureus*) الموجبة لصبغة جرام، بقطر تثبيط بلغ 20 ملم. توفر هذه النتائج بيانات علمية أساسية تسهم في اضافة علمية حول تواجد نبات الكافور (*E. camaldulensis*) في منطقة شمال إفريقيا متمثلا في غرب ليبيا. كما تُبرز هذه الدراسة وجود تركيبة كيميائية مميزة يغلب عليها صنف المونوتيربين (Monoterpenes)، والتي تختلف بوضوح عن التباين الكيميائي للأنواع الإقليمية الأخرى واسعة الانتشار.

الكلمات المفتاحية: نبات الكافور، زيت عطري، الاشتراب الغازي- مطياف الكتلة، 8-سينيول، ألفا-بينين، النشاط المضاد للبكتريا، ليبيا.

Introduction

Plants and their essential oils have been utilized for centuries as natural medicines to treat and prevent a wide array of diseases. Medicinal plants are often preferred for therapeutic use due to their diverse secondary metabolites and their generally low risk of causing mutagenic, carcinogenic, or teratogenic side effects (1). Aromatic plants remain deeply integrated into traditional medicine practices and continue to serve as a vital component of primary healthcare in rural communities worldwide (2).

The Myrtaceae family encompasses approximately 100 genera and 3,000 species of trees and shrubs. Within this family, the genus *Eucalyptus* stands out as one of the most economically and scientifically significant groups. Comprising over 900 species natively distributed from Australia to the Philippines, *Eucalyptus* has been successfully introduced to tropical and warm-temperate regions globally (3). *Eucalyptus* trees were first introduced to Libya during the 1920s and 1930s as an ecological strategy to combat desertification and stabilize the arid environment (4). Extensively planted for shelterbelts, wind protection, landscaping, and ecological stabilization, these trees demonstrated a remarkable ability to adapt to local climatic conditions, leading to extensive afforestation across the country. Beyond their ecological benefits, *Eucalyptus* trees are critical nectar-producing plants; their unifloral honey is highly valued worldwide for its unique health-promoting properties (5). The scientific community continues to explore the genus *Eucalyptus*, with a particular focus on the phytochemical characterization of its essential oils. These oils are renowned for their antioxidant and antimicrobial properties, making them highly valuable to the pharmaceutical, agricultural, and cosmetic industries (6). However, previous studies emphasize that *Eucalyptus* species exhibit a high degree of chemical polymorphism. The chemical profiles of these plants are heavily influenced by geographic location, environmental conditions, harvesting times, drying processes, and the specific extraction techniques utilized. Recent assessments of the same species across North Africa, such as in Tunisia, Algeria, and Egypt, demonstrate that this variability results in diverse regional chemical profiles, ranging from 1,8-cineole dominance to high oxygenated sesquiterpene concentrations.

In Libya, the Myrtaceae family is represented by five genera, with several *Eucalyptus* species reported, including *Eucalyptus camaldulensis* (7). Nevertheless, integrated studies that connect authenticated Libyan *E. camaldulensis* material with pharmacognostic characters, essential oil composition, and preliminary biological activity remain limited. Consequently, the present study aimed to: (i) standardize the taxonomic identity of the collected plant using the current Kew nomenclatural framework; (ii) document diagnostic macroscopic pharmacognostic characters visible in the collected reference material; (iii) characterize the leaf essential oil of *E. camaldulensis* cultivated in the coastal climate of Tajoura via GC-MS; and (iv) evaluate DPPH radical-scavenging activity alongside exploratory antibacterial responses against four reference bacterial strains. Ultimately, this research is framed as a locality-specific botanical and phytochemical baseline, contributing to the broader North African chemotaxonomic mapping of this species, rather than serving as proof of therapeutic efficacy, mechanism of action, or a definitive Libyan chemotype.

Material and methods

1. Plant Material, Taxonomic Standardization, and Botanical Authentication

Fresh leaves of *E. camaldulensis* Dehnh. (Myrtaceae) were collected during July 2025 from Tajoura, situated in northwestern Libya along the Mediterranean coast near Tripoli at an elevation of approximately 6 m above sea level (Figure 1). Preliminary taxonomic identification was conducted in the field and subsequently verified by Prof. Mohammed Makhoulf using the diagnostic criteria in the Flora of Libya (8). A representative portion of the collected plant material was shade-dried at room temperature in the dark to prepare herbarium sheets; a verified voucher specimen was formally deposited in the Herbarium of the Faculty of Science, University of Tripoli (Voucher No: 76.122.4.6). The remaining fresh leaves were subjected immediately to essential oil extraction.

To support the proper documentation and authentication of the sample, a fundamental macroscopic pharmacognostic examination was conducted following the general principles of visual and organoleptic assessment for herbal materials (W.H.O, 2011). This evaluation focused on the primary diagnostic characters

that could be documented from the supplied material, such as plant part, leaf type and shape, margin, apex, base, surface appearance, color, venation, inflorescence arrangement, bud morphology, and fruit morphology. This targeted macroscopic assessment sufficiently confirmed the sample's identity against standard taxonomic references, establishing a reliable botanical baseline for the subsequent phytochemical extraction and profiling without necessitating exhaustive microscopic or quantitative histological analyses.

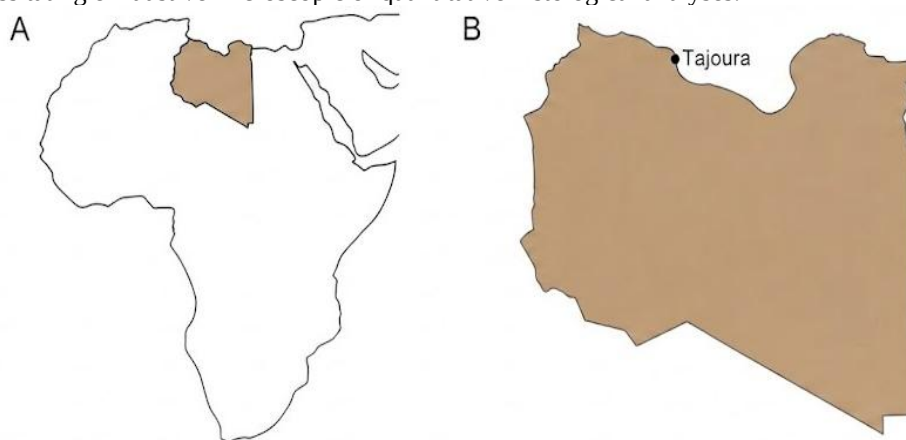


Figure 1. Libya geographical location (A) and collection site (B) of *Eucalyptus camaldulensis* – Tajoura

2.2. Extraction of *Eucalyptus* Essential Oil (EEO)

The essential oils were isolated from the fresh leaves of *E. camaldulensis* by hydrodistillation at the Faculty of Pharmacy, Elmergib University, Al-Khums, Libya, in accordance with the European Pharmacopoeia (9). Briefly, 150 g of sectioned leaves were placed in a 2000 mL round-bottom flask, submerged in a sufficient volume of water, and connected to a Clevenger apparatus. The mixture was heated under reflux for three hours. The resulting essential oil was collected, dried, and stored in an Eppendorf tube at 4 °C until further analysis.

2.3. GC–MS Analysis

The chemical composition of the essential oil was analyzed using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a TG-5MS direct capillary column (30 m x 0.25 mm x 0.25 µm film thickness). The column oven temperature was initially held at 50 °C, then increased at a rate of 5 °C/min to 250 °C (held for 2 min), and finally raised to 300 °C at 30 °C/min (held for 2 min). The injector and MS transfer line temperatures were maintained at 270 °C and 260 °C, respectively. Helium served as the carrier gas at a constant flow rate of 1 mL/min. Diluted samples (1 µL) were injected automatically in split mode. Electron ionization (EI) mass spectra were collected at 70 eV over a mass range of m/z 50–650, with the ion source temperature set at 200 °C. Due to the unavailability of an *n*-alkane homologous series for retention index calculation, all components were tentatively identified based strictly on the comparison of their mass spectra with the WILEY 09 and NIST 14 mass spectral libraries. Only compounds yielding a library Match Factor (MF) of ≥ 800 were accepted and reported.

2.4. DPPH Antioxidant Assay

The DPPH radical scavenging assay was performed in a 96-well microplate format. A stock solution of the EEO was prepared in methanol and serially diluted to yield a concentration range from 156.25 µg/mL to 10,000 µg/mL. Trolox was utilized as the standard positive control and tested across a concentration series ranging from 3.125 µg/mL to 100 µg/mL. For the assay, 100 µL of a freshly prepared dark violet DPPH solution in methanol (2 mg/50 mL) was combined with 100 µL of each test sample or standard concentration. A negative control consisted of 100 µL DPPH solution mixed with 100 µL of methanol, while a sample blank (100 µL EEO sample + 100 µL methanol) was prepared to correct for any background absorbance native to the essential oil. All procedures were performed in independent technical triplicates. The reaction mixtures were incubated in the dark at room temperature for 30 minutes. Absorbance was subsequently measured at 517 nm using a microplate reader. The percentage of free radical inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = [(\text{Absorbance of Control} - \text{Absorbance of Sample}) / \text{Absorbance of Control}] \times 100.$$

2.5. Antibacterial Activity (Disc Diffusion)

The antibacterial potential of the *Eucalyptus* essential oil was evaluated against four pathogenic bacterial strains—specifically *Escherichia coli* (ATCC [8739]), *Staphylococcus epidermidis* (ATCC [12228]), *Staphylococcus aureus* (ATCC [29213]) and *Salmonella enterica* (ATCC [14028]) using the disc diffusion method. Bacterial inocula were prepared by suspending isolated colonies in sterile saline and adjusting the

turbidity to visually match a 0.5 McFarland standard. The bacterial suspensions were uniformly swabbed onto standard Mueller-Hinton agar (MHA) poured to a depth of 4 mm in standard 90 mm petri dishes. For the assay, uniform 6 mm diameter discs were cut from Whatman filter paper, sterilized, and fixed onto the surface of the inoculated agar. Next, a single 10 μ L volume of the undiluted essential oil (100% concentration) was carefully dispensed onto each attached disc. A sterile disc infused with sterile distilled water was used as a negative control, while a standard gentamicin disc (25 μ g) served as a positive control. As an initial targeted bioactivity screening, the assay was performed as a single independent run ($n = 1$). After incubating the petri dishes for 24 hours at 37 $^{\circ}$ C, the antimicrobial activity was determined by measuring the diameter of the zones of inhibition in millimeters (mm).

Results and discussion

1. Taxonomic Identity and Macroscopic Pharmacognostic Characters

Macroscopic examination of the Libyan material revealed diagnostic vegetative and reproductive features that are highly characteristic of *E. camaldulensis* Dehnh. (Myrtaceae) (Figure 2). The adult leaves were simple, petiolate, and predominantly narrow-lanceolate to lanceolate, frequently appearing slightly falcate (curved). The leaf margins were entire, with acute to long-acuminate apices and bases that narrowed gradually toward the petiole. The leaf surfaces were green to grey-green, apparently glabrous, and largely concolorous, featuring a prominent midrib, fine lateral venation, and a visible near-marginal vein



Figure 2. Macroscopic morphological features of the collected *Eucalyptus camaldulensis* specimen from Tajoura, Libya, detailing the lanceolate adult leaves, rostrate flower buds, and mature capsular fruits.

Regarding the reproductive structures, the specimen exhibited axillary umbelliform clusters on slender stalks. The green flower buds were ovoid to rostrate, featuring a conspicuously beaked (conical) operculum, which serves as a key taxonomic identifier for this species. Upon maturity, the green immature fruits developed into brown, woody, hemispherical-to-ovoid capsules displaying prominent, exerted apical valves. Together, these observed morphological features closely align with standard floristic descriptions of *E. camaldulensis* (verified via Plants of the World Online, Kew). This visual and organoleptic assessment effectively confirms the sample's identity at the species level, establishing a reliable botanical baseline for the subsequent phytochemical extraction and profiling.

2. Essential Oil Yield, Volatile Composition, and Regional Context

Hydro distillation of the fresh leaves yielded 1.6% (v/w) of essential oil. GC-MS analysis tentatively assigned 26 volatile constituents, representing 99.98% of the total detected chromatographic profile (Figure 3, Table 1). Grouped by chemical class, the oil is exceptionally monoterpene-dominant, with monoterpene hydrocarbons and oxygenated monoterpenes accounting for 46.33% and 45.52% of the extract, respectively. In contrast, the sesquiterpene fractions were minor, comprising only 5.51% oxygenated sesquiterpenes and 2.24% sesquiterpene hydrocarbons. Because authentic standards and retention indices were not utilized during the analysis, this profile is conservatively described as a tentative pattern rather than a formally established chemotype.

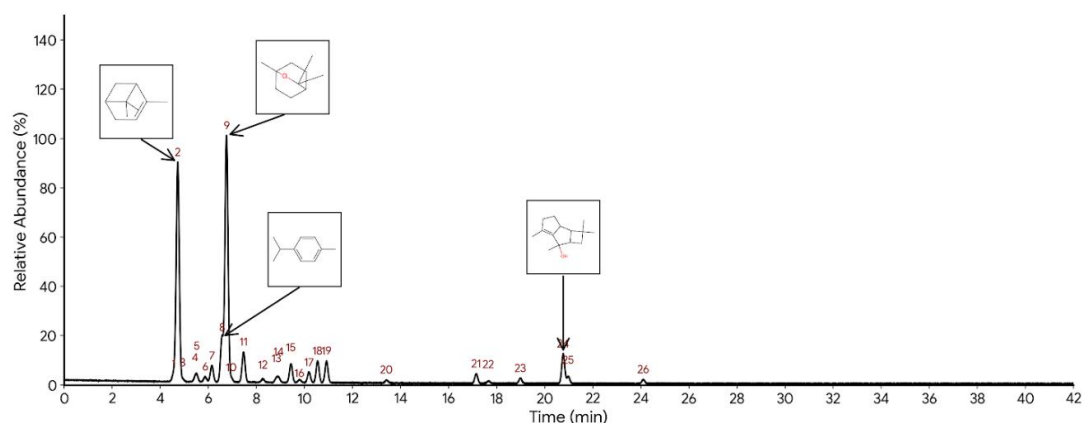


Figure 3. Typical GC-MS Total Ion Chromatogram (TIC) of *Eucalyptus camaldulensis* essential oil cultivated in northwestern Libya. Numbered peaks correspond directly to the chemical constituents identified in Table 1

The chemical profile was heavily defined by two principal constituents: 1,8-cineole (33.61%) and α -pinene (29.90%), which together accounted for a remarkable 63.51% of the detected area. These were followed by smaller contributions from p-cymene (6.08%), γ -terpinene (4.10%), spathulenol (4.04%), terpinen-4-ol (2.96%), α -terpineol (2.95%), and trans-pinocarveol (2.55%). This specific 1,8-cineole and α -pinene dominant profile is highly noteworthy within the broader North African context, where *E. camaldulensis* exhibits significant chemical polymorphism (Table 2).

A somewhat similar monoterpene-rich phenotype was previously reported in Egypt (10), which contained 1,8-cineole (21.75%) and β -pinene (20.51%). While this indicates that a cineole/pinene-associated profile is not unprecedented in the region, the exceptionally high accumulation of α -pinene clearly distinguishes the Tajoura sample from that Egyptian dataset. Conversely, several other regional *E. camaldulensis* oils are fundamentally different, being dominated by oxygenated sesquiterpenes rather than monoterpenes. For instance, studies from Tunisia have repeatedly shown a sesquiterpene-rich pattern, with researchers identifying spathulenol as the major constituent alongside p-cymene and cryptone, accompanied by low levels of 1,8-cineole (11, 12). This contrast is even sharper in recent Algerian material, which yielded a heavily spathulenol-dominant (58.24%) profile (13, 14). Furthermore, recent alternative Egyptian assessments also emphasized a spathulenol and p-cymene profile over cineole (15, 16). While interspecific comparisons such as highly cineole-dominant *E. globulus* from Morocco demonstrate that the *Eucalyptus* genus can readily produce such profiles in North Africa, they cannot be used to infer *E. camaldulensis* chemotypes directly.

Table 1. Chemical composition of *Eucalyptus camaldulensis* leaf essential oil analyzed by GC-MS.

No.	Compound Name	Structure	Molecular Formula	MW	RT (min)	Relative Area (%)	Chemical Class
1	α -Thujene		C ₁₀ H ₁₆	136	4.56	1.11	Monoterpene hydrocarbon
2	α -Pinene		C ₁₀ H ₁₆	136	4.73	29.9	Monoterpene hydrocarbon
3	Camphene		C ₁₀ H ₁₆	136	4.94	0.37	Monoterpene hydrocarbon
4	Sabinene		C ₁₀ H ₁₆	136	5.44	0.28	Monoterpene hydrocarbon
5	β -Pinene		C ₁₀ H ₁₆	136	5.51	1	Monoterpene hydrocarbon
6	β -Myrcene		C ₁₀ H ₁₆	136	5.87	0.69	Monoterpene hydrocarbon

No.	Compound Name	Structure	Molecular Formula	MW	RT (min)	Relative Area (%)	Chemical Class
7	α -Phellandrene		C ₁₀ H ₁₆	136	6.15	2.22	Monoterpene hydrocarbon
8	p-Cymene		C ₁₀ H ₁₄	134	6.57	6.08	Monoterpene hydrocarbon
9	1,8-Cineole		C ₁₀ H ₁₈ O	154	6.76	33.61	Oxygenated monoterpene
10	(E)- β -Ocimene		C ₁₀ H ₁₆	136	6.96	0.58	Monoterpene hydrocarbon
11	γ -Terpinene		C ₁₀ H ₁₆	136	7.47	4.1	Monoterpene hydrocarbon
12	cis-Verbenol		C ₁₀ H ₁₆ O	152	8.27	0.46	Oxygenated monoterpene
13	endo-Fenchol		C ₁₀ H ₁₈ O	154	8.83	0.51	Oxygenated monoterpene
14	α -Campholenal		C ₁₀ H ₁₆ O	152	8.93	0.64	Oxygenated monoterpene
15	trans-Pinocarveol		C ₁₀ H ₁₆ O	152	9.44	2.55	Oxygenated monoterpene
16	Pinocarvone		C ₁₀ H ₁₄ O	150	9.8	0.4	Oxygenated monoterpene
17	Borneol		C ₁₀ H ₁₈ O	154	10.19	1.44	Oxygenated monoterpene
18	Terpinen-4-ol		C ₁₀ H ₁₈ O	154	10.55	2.96	Oxygenated monoterpene
19	α -Terpineol		C ₁₀ H ₁₈ O	154	10.92	2.95	Oxygenated monoterpene
20	Bornyl acetate		C ₁₂ H ₂₀ O ₂	196	13.42	0.38	Monoterpenoid ester
21	(E)-Caryophyllene		C ₁₅ H ₂₄	204	17.15	1.27	Sesquiterpene hydrocarbon
22	Aromadendrene		C ₁₅ H ₂₄	204	17.66	0.3	Sesquiterpene hydrocarbon
23	γ -Elemene		C ₁₅ H ₂₄	204	18.99	0.67	Sesquiterpene hydrocarbon
24	Spathulenol		C ₁₅ H ₂₄ O	220	20.77	4.04	Oxygenated sesquiterpene

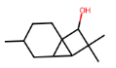
No.	Compound Name	Structure	Molecular Formula	MW	RT (min)	Relative Area (%)	Chemical Class
25	Globulol		C ₁₅ H ₂₆ O	222	20.98	0.96	Oxygenated sesquiterpene
26	Ylangenal		C ₁₅ H ₂₂ O	218	24.1	0.51	Oxygenated sesquiterpene
Compound identification is tentative and based solely on mass spectral library matching (WILEY 09 and NIST 14) without the confirmation of calculated retention indices or authentic standards.							

Table 2: Comparative analysis of *Eucalyptus camaldulensis* essential oil profiles across regional studies.

Species*	Country	Plant Organ	Harvest Season	State	Major Constituents
<i>E. camaldulensis</i>	Libya/Tajura	Leaves	July	Fresh	1,8-Cineole, α -Pinene
<i>E. camaldulensis</i>	Egypt	Leaves	Not specified	Dried (Shade)	Spathulenol, p-Cymene
<i>E. globulus</i>	Tunisia	Leaves	Feb / Mar / May	Freeze-dried	p-Cymene, 1,8-Cineole
<i>E. globulus</i>	Algeria	Leaves	April	Dried (Shade)	p-Cymene, 1,8-Cineole
<i>E. globulus</i>	Morocco	Leaves	April	Dried (Shade)	1,8-Cineole, Limonene

*: All species are extracted by distillation and analyzed by GC-MS

Collectively, these studies demonstrate that North African *E. camaldulensis* does not possess a single, fixed volatile pattern. The observed heterogeneity is multifactorial, shaped by a complex interaction of genetic provenance, local environmental conditions such as the coastal climate of Tajoura, and harvest season. Furthermore, methodological differences—including whether the leaves were extracted fresh or dried, alongside variations in distillation and chromatographic identification are inseparable from geographic differences. Consequently, the safest and most accurate interpretation is that the Tajoura material represents a distinct, locality-specific 1,8-cineole/ α -pinene-dominant profile, rather than definitive proof of a geographically fixed Libyan chemotype.

3. Antioxidant Activity

The antioxidant capacity of the EEO was evaluated utilizing the DPPH radical scavenging assay. The essential oil exhibited a relatively weak free radical scavenging potential, failing to reach 50% inhibition even at the highest tested concentration of 10,000 μ g/mL (recording a maximum inhibition of only 27.63%). Consequently, an exact IC₅₀ could not be experimentally estimated, and the IC₅₀ was greater than the highest tested concentration. This was compared to the highly potent standard reference Trolox (IC₅₀ = 10.26 μ g/mL). This modest antioxidant activity is directly attributable to the specific chemotype of the oil. Strong free radical scavenging in essential oils is typically driven by phenolic compounds (such as thymol or carvacrol), which readily donate hydrogen atoms to neutralize DPPH radicals (17). As demonstrated by our GC-MS analysis, the Libyan EEO is entirely devoid of these phenolic constituents, being dominated instead by the monoterpenes 1,8-cineole and α -pinene. Previous studies have consistently confirmed that 1,8-cineole and α -pinene possess remarkably weak antioxidant capacities due to the absence of hydrogen-donating hydroxyl groups in their chemical structures (18). Consequently, while this specific monoterpene-rich profile is highly effective for antimicrobial applications, its utility as a primary free-radical scavenger is inherently limited.

4. Antimicrobial Activity

In the exploratory disc-diffusion screening, the *Eucalyptus* essential oil (EEO) exhibited variable, strain-dependent antibacterial activity. The growth-inhibitory effect was most pronounced against the tested Gram-positive bacteria. Specifically, the largest inhibition zone was observed against *Staphylococcus aureus* (20 mm), followed by *Staphylococcus epidermidis* (16 mm). The essential oil demonstrated moderate to weak activity against the Gram-negative strains, yielding zones of 10 mm for *Escherichia coli* and 7 mm for *Salmonella*.

enterica (Figure 4). These results were observed alongside a standard gentamicin positive control, which served strictly as a methodological comparator to validate assay performance rather than a dose-equivalent benchmark.

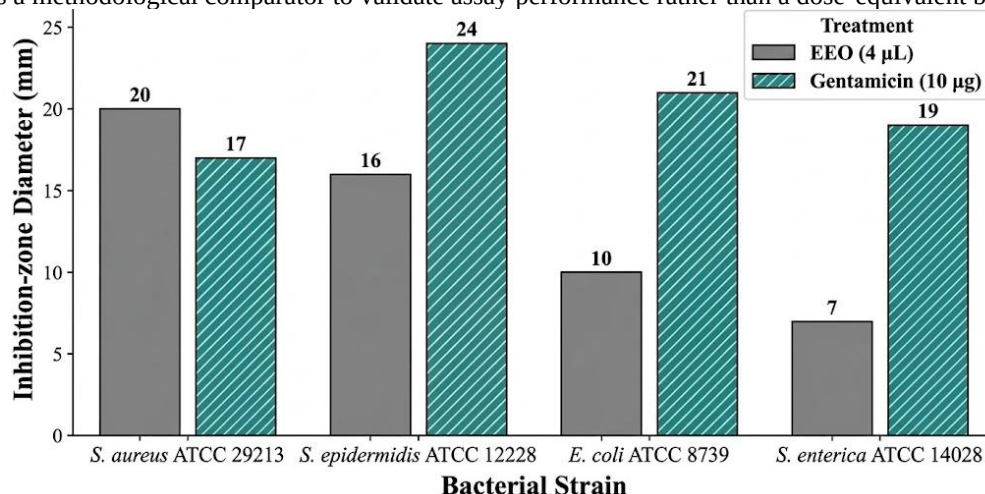


Figure 4. Descriptive antibacterial disc-diffusion profile. Solid grey bars represent the inhibition-zone diameter of *Eucalyptus* essential oil (EEO; 4 µL/disc), whereas hatched teal bars represent the reference antibiotic, gentamicin (10 µg/disc). Values reflect single-run observations (n = 1); therefore, error bars and inferential statistics are not shown.

This observed pattern is compatible with the frequent susceptibility of Gram-positive bacteria to hydrophobic plant volatiles. The robust inhibitory effect, particularly against *S. aureus*, is largely attributed to the high concentration of highly lipophilic monoterpenes—specifically α -pinene and 1,8-cineole—present in the Libyan EEO. Previous literature suggests that the highly lipophilic nature of these monoterpenes allows them to interact with and penetrate the lipid bilayer of the bacterial cell membrane, potentially disrupting the thick peptidoglycan layer of Gram-positive bacteria and altering overall cellular permeability (19). However, Gram status alone does not dictate essential oil sensitivity; factors such as outer-membrane permeability, efflux mechanisms, oil volatility, and agar diffusion characteristics also heavily influence the measured zones.

Placing these findings within the broader North African literature provides valuable context, though it also demonstrates the complexity of cross-study comparisons. While the monoterpene-rich Libyan EEO showed distinct activity against *S. aureus*, regional studies demonstrate that oils with entirely different profiles can also exhibit significant antimicrobial effects. For instance, research on Algerian *E. camaldulensis* reported measurable inhibition against *S. aureus* using a chemically distinct, spathulenol-dominant oil (14). Similarly, Tunisian studies have identified *E. camaldulensis* as a highly bioactive oil, emphasizing through multivariate analysis that antibacterial behavior typically tracks complex combinations of constituents rather than a single universal marker compound (11). Further genus-level context from Morocco supports the broader proposition that highly cineole-dominant *Eucalyptus* oils can deliver potent antimicrobial effects, though direct cross-study potency comparisons remain challenging due to differing testing methodologies and quantitative endpoints (20). While these initial observations highlight the oil's antibacterial potential, the current zone diameters represent exploratory screening observations rather than intrinsic potency estimates. The assay does not directly confirm the mechanism of action, meaning the proposed membrane-associated effects and cellular leakage remain mechanistic hypotheses for this specific oil. To fully quantify the pharmacological efficacy of the Libyan *E. camaldulensis* essential oil and conclusively determine whether its biological action is bacteriostatic or bactericidal, future investigations must prioritize targeted Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determinations.

Conclusion

The present study provides locality-specific information on the volatile composition and in vitro biological properties of *Eucalyptus camaldulensis* leaf essential oil collected in northwestern Libya. The oil exhibited a 1,8-cineole/ α -pinene-dominant profile, with 1,8-cineole and α -pinene accounting for 33.61% and 29.90% of the detected volatile fraction, respectively. The EO showed weak DPPH radical-scavenging activity under the tested conditions but measurable strain-dependent antibacterial activity, with the largest inhibition zone observed against *Staphylococcus aureus*. These findings expand the available phytochemical evidence for North African *E. camaldulensis*, establishing a baseline that sharply contrasts with the chemotypes of other regional *Eucalyptus* species. Further broader geographical sampling, retention-index-supported compound identification, and quantitative MIC/MBC testing are required before regional chemotype differentiation or pharmaceutical antimicrobial potential can be fully established.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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