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Antibacterial and anti-biofilm properties of *Cymbopogon martini* (Roxb.) essential oil and geraniol against *Staphylococcus aureus* strains

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ABSTRACT

Cymbopogon martini (Roxb.) Wats, known as palmarosa, a perennial aromatic grass cultivated for its essential oil, represents a promising industrial crop widely used in perfumery, cosmetics, self-care products, and aromatherapy, with emerging applications in pharmaceutical formulations. This study investigates the chemical composition and antimicrobial potential of *Cymbopogon martini* essential oil and one of its main components, geraniol, against *Staphylococcus aureus* from clinical isolates and commercial sources. The effect upon planktonic state was assessed obtaining the MIC and MBC values. Biofilm viability and biomass were quantified using resazurin and crystal violet assays, respectively. Additionally, membrane disruption was explored as a potential mechanism of action. Potential toxicity was evaluated in A549 and HaCaT cell lines and in adult *Artemia* spp. GC-MS analysis revealed the presence of geraniol as the major compound (75.29 %) but also other terpenes like geranyl acetate (10.39 %), (*E*)-β-caryophyllene (3.14 %), linalool (2.90 %) and trans-β-ocimene (1.36 %). The essential oil displayed lower MIC and MBC values (MIC: 300–350 μg ml^{-1}; MBC: 400 μg ml^{-1}-450 μg ml^{-1}) than the pure geraniol (MIC: 500 μg ml^{-1}; MBC: 550–600 μg ml^{-1}). Results showed nearly 100 % inhibition of biofilm formation across tested concentrations (500 μg ml^{-1} to 1000 μg ml^{-1}). Unlike vancomycin, the essential oil remained active in disrupting established *S. aureus* biofilms (IC_{50} = 554.47 μg ml^{-1}). No cytotoxicity was observed in human cells at the range of concentrations from 250 μg ml^{-1} to 1000 μg ml^{-1}. These findings highlight *Cymbopogon martini* as a valuable industrial crop with potential as a plant-derived antibiofilm agent.

1. Introduction

Essential oils (EOs) are liquid complex substances of natural origin mainly obtained through steam, hydro-distillation or other accepted techniques, from different organs and parts of plants. In a single oil, up to 400 substances can be identified, with 2 or 3 major components being responsible for their aromatic and bioactive properties, being terpenes the most prevalent chemical compounds found in EOs (De Matos et al., 2019; Ramsey et al., 2020). Some EOs have a long history of traditional use in pharmaceutical, cosmetic, and healthcare products and have been characterized in various studies as antimicrobial agents.

Cymbopogon martini (Roxb.) Will.Watson (*C. martini*) from the Poaceae family commonly known as palmarosa or rosagrass is a perennial aromatic grass cultivated for its EO. It thrives in tropical and subtropical climates with well-drained loamy soils and adequate fertility and moisture. India remains the leading global producer, although cultivation is also expanding in Indonesia, East Africa, and Latin America. Improved agro-technological practices such as the use of high-yielding cultivars, balanced fertilization, and effective weed and pest management are essential for maximizing oil yield and quality (Kumar et al., 2024). Due to its scent, the essential oil is used for flavouring tobacco and soap, and it is additionally recognized for its geraniol content, which can account for approximately 65–85 % of its composition. Due to its role as both a key fragrance component and a biosynthetic precursor to more complex molecules, such as geranyl esters with a persistent rose-like aroma, geraniol holds significant commercial and industrial value (Kumar et al., 2024).

Bacterial biofilms are a form of bacterial life that holds a high level of

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organization and provides protection to the bacteria in adverse conditions, being responsible for a substantial proportion of bacterial chronic infections (Campoccia et al., 2019). These communities of microorganisms can be adhered to a biotic or abiotic surface, meaning they can be form in living tissues, prosthetics, and even on food products, leading to conditions that pose a human and economic cost to society (Arciola et al., 2018). Biofilms represent a significant challenge in the management of bacterial infections and are a key factor of their chronicity and persistence (Campoccia et al., 2019; Zhao et al., 2023), being also relevant in industrial, environmental and agricultural settings. Some industrial environments represent a suitable scenario for biofilm growth, due to the complexity of the plants, temperature, long production periods or mass product generation among other factors (Lindsay and Holy, 2006). Biofilms seem to be responsible for damaging equipment, economic loss, outbreaks, and food spoilage, exacerbated by the resistance to disinfectants (Carrascosa et al., 2021; Shi and Zhu, 2009). Therefore, the pressing need to develop new strategies for biofilm cleaning and disinfection aligned the One Health strategy (Fernandes et al., 2024).

Given the established industrial relevance of palmarosa EO as a natural source of geraniol, production of perfumes, cosmetics, and soaps, this study aims to comprehensively evaluate the potential of *C. martini* EO and geraniol for expanded antibiofilm applications. Specifically, the chemical composition of *C. martin* EO was investigated, with particular focus on its geraniol content. The antibacterial activity of both the EO and geraniol against *Staphylococcus aureus* (*S. aureus*) clinical isolates and biofilms was also evaluated. Additionally, other aspects such the mechanism of action and its impact on cellular models and *Artemia* were also assessed to better understand the activity of the EO and establish preliminary data on safety for human use and the impact upon the environment.

2. Material and methods

2.1. Essential oils and geraniol

Palmarosa (*C. martini)* essential oil was supplied from Pranarôm (Ghislenghien, Belgium); batch number: OF005923; country of origin: India; part of the plant used: aerial parts. The EO was obtained by industrial steam distillation of the aerial parts of *C. martini,* cultivated in India. After distillation, it was packed and stored at room temperature in amber glass vial and protected from light exposure. Stock solutions were freshly prepared each day immediately before use. Geraniol was purchased from Vidrafoc (Barcelona, Spain).

2.2. Chemical composition

The chemical constituents of the EO were identified using gas chromatography coupled with mass spectrometry (GC-MS). The analysis was carried out on an Agilent Tecnologies CG 6890 MS 5973 system equipped with a HP-5MS-IU (30 m × 0,25 mm ×0,25 μm) (Agilent Technologies). The injection was performed in split mode (split ratio 100:1), with an injection volume of 0.2 μL.

The oven temperature was initially set at 60 °C and increased to 240 °C at a rate of 3 °C/min. Helium was used as the carrier gas at a constant flow rate of 1.0 ml/min. The MS detector was operated in electron impact mode at 70 eV, scanning a mass range from *m/z* 41–250. Volatile compounds were identified by comparing their retention times and mass spectra with those in the NIST mass spectral database, as well as with literature data (Adams, 2009). In addition, linear retention indices (LRIs) were calculated using a homologous series of n-alkanes (C8–C30) analysed under identical chromatographic conditions. These experimental LRIs were then compared with published values to support the tentative identification of the compounds. When available, identification was further confirmed by matching both the mass spectral fragmentation patterns and the LRI values with entries reported by Wiley275 and Adams (2009).

2.3. Antimicrobial activity: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

S. aureus isolates were obtained from clinical specimens, including non-surgical wound swabs, exudates, broncho aspirates, and sputum samples from the Microbiology Section at the Hospital Clínico Lozano Blesa from Zaragoza (Spain). The specimens were cultured on appropriate selective and differential media; bacterial identification being carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) with a MALDI Biotyper Sirius system (Bruker Daltonics). A total of four clinical isolates of *S. aureus* were selected for the study. The isolates were coded as SA01–SA04. Information on the clinical source of each isolate and the corresponding antibiograms is provided in the Supplementary Material.

Broth microdilution method in 96-well plates was used to determine the MIC. A bacterial preculture was then incubated to an optical density (OD) of 0.3 corresponded to 1 × 10^8 colony-forming unit (CFU)/ml. A 1:10 dilution in tryptic soy broth (TSB; Thermo Scientific) was performed to obtain a bacterial suspension of 1 × 10^7 CFU/ml. From this suspension, 10 μL were added to a 96 well plate containing 190 μL of TSB in which the sampled had been previously dissolved with DMSO (1 %) as a cosolvent. Blanks and negative controls consisting only of TSB and bacterial suspension were also prepared, as well as and solvent controls with 1 % DMSO to ensure no interference with the assay. The plate was incubated overnight at 37°C. All experiments were conducted with a minimum of three replicates.

MIC was determined as the lowest concentration of the antimicrobial that prevented visible bacterial growth, indicated by the absence of turbidity. To assess the MBC, aliquots from the wells were diluted in TSB and plated on tryptic soy agar (TSA; Neogen) or in Mueller-Hinton agar. The plates were incubated overnight at 37°C and colonies were counted to calculate the MBC, defined as the lowest concentration that resulted in a 99.9 % reduction in bacterial growth.

2.4. Anti-biofilm activity

Gram-positive *S. aureus* ATCC 25923 strain was used to determine the anti-biofilm potential of the EO. The bacteria were stored in TSB and 20 % glycerol (Thermo Scientific). Prior to the determination of the antibiofilm potential, the MIC and MBC of the commercial strain were also determined as described in 2.3.

Before the experiments, a streak plate of *S. aureus* was prepared in TSA and was incubated 18 h at 37 °C, after which, 2–3 colonies of *S. aureus* were dispersed in 5 ml of TSB. The bacterial preculture was then incubated (37 °C, 220 rpm), until a mid-exponential phase of growth, which corresponded to an OD of 0.3 measured in a Multiskan GO spectrophotometer (Thermo Scientific, Waltham, MA, US). This OD corresponded to 1 × 10^8 colony-forming unit (CFU)/ml.

The biofilm inhibitory activity was determined following the protocol of Skogman, M. E., et al. (2012). The activity was assessed in terms of their capacity preventing the formation of a biofilm (pre-exposure) and their capacity disrupting a pre-stablished biofilm (post-exposure) (Skogman et al., 2012).

For the experiments, the bacterial suspension was diluted to a concentration of 1 × 10^6 CFU/ml prior to initiating the experiment. Negative controls consisted solely of the bacterial suspension. Solvent-treated controls were also prepared, with a 1 % DMSO. Vancomycin was used as a positive control. All experiments were conducted with a minimum of three replicates.

2.4.1. Pre-exposure

The bacteria and the EOs, solved in DMSO (final concentration of 1 %), were plate in a 96 well plate (Nunc™ MicroWell™ 96-Well, Nunclon Delta-Treated, Flat-Bottom Microplate, Thermo Scientific,

Waltham, MA, US) and incubated for 18 h (37 °C, 220 rpm). After incubation, the planktonic solution was removed, and the plate containing the biofilm was preserved for further use. Each well was washed twice with 200 μL of PBS, after which 200 μL of resazurin at 20 μM were added. The plate was incubated for 30 min (220 rpm, room temperature, in the dark), after which the fluorescence was measured at $\lambda_{ex} = 560$ nm and $\lambda_{em} = 590$ nm. To measure the biofilm biomass, resazurin was removed, and the biofilm was fixed with 200 μL/well of methanol (Sigma-Aldrich) for 15 min. After fixation, biofilms were air-dried and stained by adding 190 μL/well of a 0.01 % v/v crystal violet (Sigma-Aldrich) solution in MQ-water. The biofilms were stained for 5 min after which they were washed three times with 200 μL/well of MQ-water. After the last wash, the wells were allowed to dry completely, and the staining was dissolved in 96 % ethanol (Sigma-Aldrich). The absorbance was measured after a 1-hour incubation at 595 nm with a in a Multiskan GO spectrophotometer.

2.4.2. Post-exposure

For the post exposure, the bacteria were grown for 18 h in a 96-well plate, without the addition of the sample. Following this incubation period, the media was replaced by different concentrations of the EO as described in Section 2.3 and further incubated for 24 h (37 °C, 220 rpm). After the incubation, the viability and total biomass of the biofilm were determined following the protocol described in Section 2.4.1.

2.5. Bacterial membrane assays

2.5.1. Membrane permeabilization assay

A bacterial pre-culture was left to grow overnight as described in 2.4.

After 16 h of incubation, the pre-culture was centrifuged at 4000 rpm for 10 min. The pellet was washed twice with Hepes buffer (5 mM Hepes, 5 mM glucose, pH 7.2) and resuspended to an OD_{600} of 0.15. 10 ml of the suspension were mixed with propidium iodide to a final concentration of 20 μM and the mixture was incubated in the dark for 30 min at 37°C. 100 μL of the suspension were plated into a 96-well plate, and fluorescence was measured for 10 min until stabilized. 10 μL microliters of the compounds were added to the wells, and fluorescence was then measured at 535 nm and 617 nm for 1 h. The concentration of EO studied was 16 times higher than the calculated MIC. A minimum of three replicates were conducted.

2.5.2. Membrane depolarization assay

The procedure closely followed the method used for membrane permeabilization assays in 2.5.1. The bacterial pre-culture was washed with Hepes buffer, consisting in this case 5 mM Hepes, 5 mM glucose, and 100 mM KCl, adjusted to pH 7.2. The preculture was resuspended to an OD_{600} of 0.15, mixed with 0.4 μM of DISC35 and incubated for 30 min. Following this, 190 μL of the suspension were transferred to a 96-well plate, and fluorescence was measured for 10 min until stable. 10 μL of the test compounds were added to the wells, and fluorescence was measured at 622 nm and 670 nm for 1 h. Hepes buffer and DMSO were used as negative controls, while Triton X was included as a positive control. All experiments were conducted with a minimum of three replicates.

2.6. Cytotoxicity assessment

2.6.1. Cell maintenance

A549 cells (Epithelial cell *Homo sapiens* (human) CCL-185) were maintained in Dulbecco's modified Eagle's medium (DMEM; Cytiva), supplemented with 20 μg/ml gentamicin (Sigma-Aldrich) and 10 % heat-inactivated foetal bovine serum (FBS; Invitrogen, Thermo Scientific). The cells were kept at 37 °C, 5 % CO_2 in a humidified incubator (Heracell™ 240i, Thermo Scientific, Waltham, MA, US). HaCaT cells (Keratinocytes *Homo sapiens* (human)) were cultured in DMEM medium (Sigma Aldrich), 10 % FBS (Sigma Aldrich) and 1 % penicillin-streptomycin (Sigma Aldrich) in the presence of 5 % CO2 at 37°C.

2.6.2. Cell viability Assay

One day before the experiment A549 and HaCaT cells were seeded at a density of 1 × 10^4 cells/well on a 96-well plate. On the day of the experiment, the media was replaced with DMEM containing different concentrations of the EO (ranging from 250 to 1000 μg ml^{-1}) and further incubated for 24 h.

After the incubation period, the media was removed, and the cells washed twice with 200 μL/ well of PBS. Subsequently, resazurin (20 μM) was added (200 μL/ well), and the plate was further incubated for 2 h. After incubation, the fluorescence was measured at λex = 560 nm and λem = 590 nm (Varioskan LUX Multimode microplate reader). A minimum of three replicates were conducted.

2.7. Brine shrimp assay

The ecotoxicity of the EO was evaluated through the assessment of ~~the~~ toxicity on adult *Artemia* sp. The assay was conducted in 6-wells plates, placing five adults and 10 ml of sea water in each well. Each plate was exposed to a different concentration of EO, ranging from 1000 to 62.5 μg ml^{-1}. The plates were then incubated at room temperature for 24 h before assessing the mortality. All experiments were conducted with a minimum of three replicates.

2.8. Statistical analysis

GraphPad Prism 10 was used to perform the statistical analysis. Grubbs' test was utilized to identify significant outliers. To compare the different concentrations studied with the untreated conditions or controls, a One-way analysis of variance (ANOVA) with Dunnett as *post hoc* test was performed. Significance level was obtained by One-way ANOVA with Bonferroni's post-test. The different values of IC_{50} and LD_{50} for the essential oils were also calculated with GraphPad Prism 10.

3. Results

3.1. Chemical composition

The GC-MS analysis revealed a complex phytochemical profile with 18 compounds identified representing the 95.772 % of the EO. The chemical analysis trough GC-MS revealed E-geraniol as the main compound found in the EO, constituting the 75.289 % of the product while geranyl acetate represented the 10.390 % of the EO. (*E*)-β-caryophyllene, linalool and trans-β-ocimene were also characterized in the EO, representing a 3.145 %, 2.906 % and 1.359 % respectively. Other compounds identified in the EO accounted for less than the 1 % of the total product. The complete list of identified constituents is provided in Table 1. Chemical structure of the main constituents is presented in the Supplementary material.

3.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values for both the EO and geraniol, the main compound, against *S. aureus* clinical isolates are presented in Table 2. For the four clinical isolates used, the EO showed lower MIC and MBC values than the pure compound (Table 2).

3.3. Anti-biofilm activity

Since *C. martini* EO exhibited lower MIC and MBC values compared to its major component geraniol (Table 2), subsequent antibiofilm assays were performed exclusively with the EO. Prior to the biofilm evaluation, MIC and MBC values for *C. martini* EO were determined against the biofilm-forming strain *S. aureus* ATCC 25923, being those values

Table 1
Chemical composition of the volatile compounds identified in *C. martini* essential oil by GC-MS, listed in descending order of abundance (%).

Compound	CAS number	IA	%
E-Geraniol	106–24–1	1256	75.289
Geranyl acetate	105–87–3	1388	10.390
(*E*)-beta-Caryophyllene	87–44–5	1417	3.145
Linalool	78–70–6	1103	2.906
trans-beta-Ocimene	3779–61–1	1047	1.359
trans-Citral (geranial)	141–27–5	1271	0.522
cis-beta-Ocimene	3338–55–4	1034	0.377
Caryophyllene oxide	1139–30–6	1584	0.365
Nerolidol	142–50–7	1566	0.273
alfa-Humulene	6753–98–6	1452	0.270
Nerol (Z-geraniol)	106–25–2	1231	0.170
Myrcene	123–35–3	992	0.162
beta- Selinene	17066–67–0	1485	0.138
Limonene	138–86–3	1028	0.136
cis-Citral (neral) (beta-citral)	106–26–3	1241	0.105
Valencene	4630–07–3	1494	0.099
alfa-Terpineol	98–55–5	1179	0.053
Camphene	79–92–5	947	0.011
Total			**95.772 %**

IA: Kovats index

Table 2
Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *C. martini* for *S. aureus* clinical isolates.

S. aureus isolate	*C. martini* oil		Geraniol	
	MIC	MBC	MIC	MBC
SA01	300 μg ml^{-1}	400 μg ml^{-1}	500 μg ml^{-1}	600 μg ml^{-1}
SA02	300 μg ml^{-1}	400 μg ml^{-1}	500 μg ml^{-1}	550 μg ml^{-1}
SA03	350 μg ml^{-1}	450 μg ml^{-1}	500 μg ml^{-1}	600 μg ml^{-1}
SA04	350 μg ml^{-1}	450 μg ml^{-1}	500 μg ml^{-1}	600 μg ml^{-1}

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; SA01: Clinical isolate of *S. aureus* coded as SA01, obtained from a non-surgical wound, resistant to oxacillin; SA02: Clinical isolate of *S. aureus* coded as SA02, obtained from an exudate, sensitive to oxacillin; SA03: Clinical isolate of *S. aureus* coded as SA03, obtained from a sputum, resistant to oxacillin; SA04: Clinical isolate of *S. aureus* coded as SA04, obtained from a broncho aspirate, sensitive to oxacillin.

350 μg ml^{-1} and 400 μg ml^{-1}, respectively.

Fig. 1 shows the activity of the EO and vancomycin against *S. aureus* biofilms in both pre-exposure and post-exposure conditions. Fig. 1A shows the activity of the EO in pre-exposure measuring both the inhibition of bacteria viability within the biofilm and the total biomass, reaching values close to 100 % inhibition at all concentrations tested ($p < 0.0001$) and displaying an IC_{50} lower than 500 μg ml^{-1} in both cases. Vancomycin (Fig. 1B), the positive control, also inhibited biofilm formation at all the conditions and concentrations studied. For the viability within the biofilm in the post-exposure, the EO was significantly active at all concentrations tested (1000–500 μg ml^{-1}) displaying an IC_{50} of 554.47 (Fig. 1C). For the total biomass, the EO showed significant differences when compared to the control at all concentrations tested however, the activity did not seem to be concentration dependant (Fig. 1C). Vancomycin despite having significant inhibition at the two highest concentrations (10 μM and 20 μM), reduced its activity in the post-exposure reaching only 33.81 % inhibition for the bacterial viability at the highest concentration (Fig. 1D).

3.4. Bacterial membrane assays

To elucidate whether *C. martini* EO activity was related to membrane alteration, the impact of the EO on membrane permeabilization and membrane depolarization was determined. Fig. 2A illustrates the membrane permeabilization activity of the EO and the positive control, Triton X. Initially, both samples exhibited low fluorescence signals, starting at 117.99 % and 135.51 % for *C. martini* and Triton X, respectively. Over time, the fluorescence signals increased, reaching 435.67 % and 394.72 %, while the negative control remained unchanged.

A similar trend was observed in the membrane depolarization assay (Fig. 2B), where the EO increased the signal from 138.86 % to 264.18 %. In this case, the EO did not induce as significant increase as the positive control (from 200.63 % to 424.13 %), but still showed a higher signal compared to the negative control.

3.5. Cytotoxicity assessment

To evaluate the effects of the EO on mammalian cells, two human cell lines were selected, HaCaT, a keratinocyte line derived from adult epidermal cells, and A549, an alveolar human epithelial cell line. As shown in Table 3, the EO demonstrated safety for both cell lines across a concentration range from 1000 μg ml^{-1} to 250 μg ml^{-1}, which included the MIC and MBC values and concentrations tested in the antibacterial/antibiofilm assays. No significant differences were observed with the non-treated control. At all concentrations, cell viability remained close to 100 %.

3.6. Toxicity assessment in brine shrimp

Fig. 3 illustrates the impact of *C. martini* EO upon *Artemia salina*, expressing the results as a reduction in viability. *C. martini* exhibited slight toxicity towards adult *Artemia*, with a calculated LC_{50} 772.45 ± 128.85 μg ml^{-1}. Among all the concentrations tested, only 1000 μg ml^{-1} was found to be statistically significantly ($p < 0.01$), reducing the viability of *Artemia* to a 41.31 %. Despite not showing significant differences with the untreated control, the concentration of 500 μg ml^{-1}, reduced the viability to a 74.67 % while the viability at the other concentrations remained close to the 100 %.

4. Discussion

Palmarosa is a hardy, drought-tolerant aromatic grass that can be established even on marginal or moderately saline soils (up to pH 9). It requires minimal irrigation and low input costs, making it suitable for cultivation in resource-limited settings (Smitha and Rana, 2015). It serves as a key botanical source of EOs extensively employed in the global production of perfumes, cosmetics, and soaps. Several works have described geraniol as the principal constituent of palmarosa oil, which typically represents between 65 % and 85 % of its total composition (Dangol et al., 2023; Kumar et al., 2024; Vella et al., 2020). The concentration of geraniol and other compounds can vary depending on several factors, including the plant part and the stage of development (Smitha and Rana, 2015). In addition, agronomic factors such as harvest time have been reported to significantly influence geraniol content (Kakaraparthi et al., 2015). In the sample herein studied geraniol accounted for 75.289 % of the total EO content (Table 1). Other compounds found in high amounts were geranyl acetate (10.390 %) and linalool (2.906 %) which concentration is consistent with previous reports (Kumar et al., 2024). However, (*E*)-β-caryophyllene (3.145 %) and trans-β-ocimene (1.359 %) were found in higher amounts than in other works (Dangol et al., 2023; Vella et al., 2020).

Geraniol is a widely used fragrance ingredient in self-care consumer products, due to its sweet and floral scent (Maczka et al., 2020). In addition, this compound has been previously documented as an antibiofilm agent. In fact, geraniol has previously demonstrated antimicrobial activity against *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus gordonii*, inhibiting biofilm formation and disrupting pre-formed biofilms (Nemoda et al., 2024). This monoterpenic alcohol is characterized by a largely hydrophobic carbon chain and a polar hydroxyl group (see structure in Supplementary material). A previous study showed that terpenes bearing free hydroxyl groups exhibit

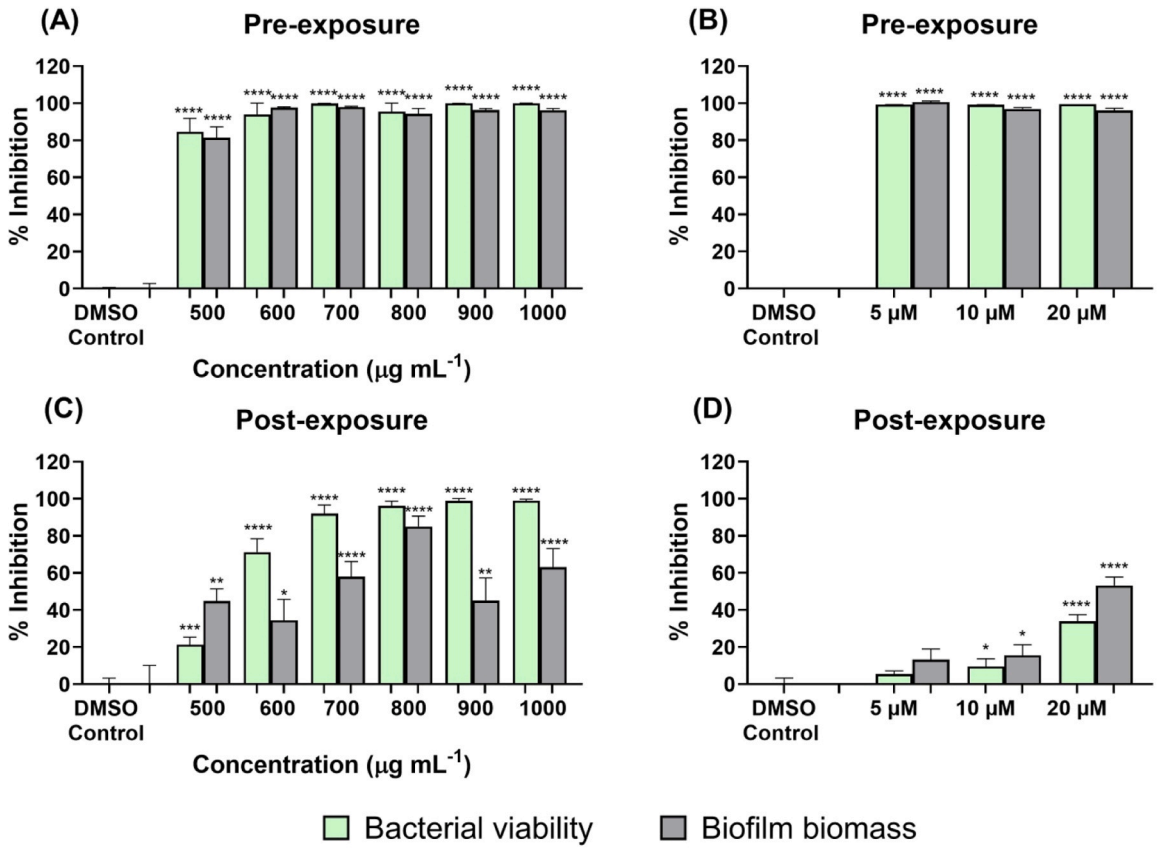

Fig. 1. *C. martini* essential oil (A) (C) and vancomycin (positive control) (B) (D) activity against *S. aureus* biofilm formation (pre-exposure) (A) (B) and biofilm dispersion (post-exposure) (C) (D). The activity was studied as the inhibition of viability within the biofilm and total biofilm biomass. All the data were normalized to the control Results correspond to the mean ± SEM. Statistical analysis via ANOVA revealed significant differences at all concentrations between the samples the non-represented untreated control (statistical significance: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

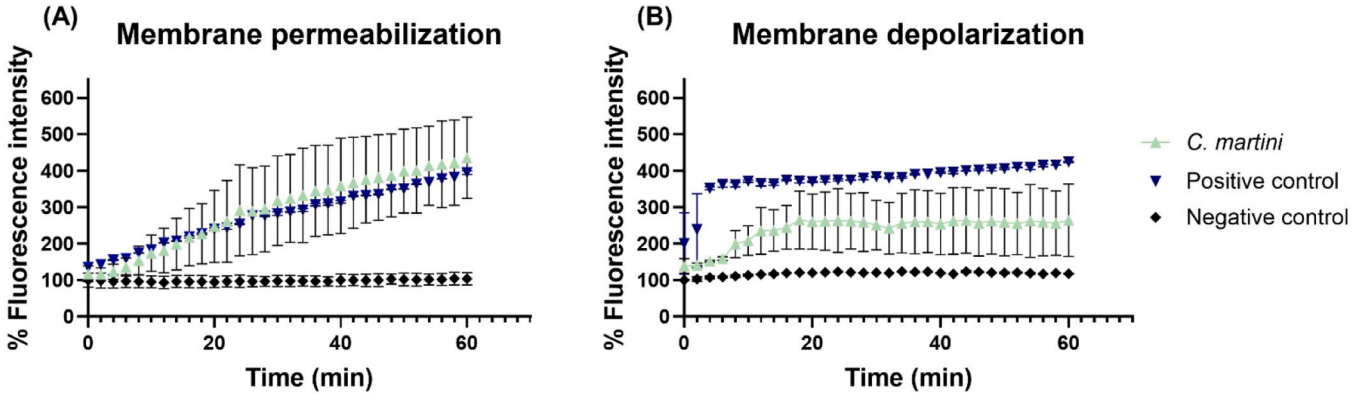

Fig. 2. *C. martini* EO and Triton X (positive control) activity as membrane permeabilizing (A) and membrane depolarizing (B) agents in *S. aureus*. Results correspond to the percentage of increment in fluorescent signal in a 60 min kinetic. Results correspond to the mean ± SEM.

Table 3
Cell viability of HaCaT and A459 cells exposed to *C. martini* oil for 24 h. Results correspond to the mean cell viability obtained across the different replicates. Statistical analysis via ANOVA revealed no significant differences between the viability of the different concentrations and the non-represented untreated control.

	***C. martini* essential oil concentration (μg ml^{-1})**				
	1000	750	500	375	250
% Cell viability HaCaT	93.31	99.38	101.39	93.31	99.37
% Cell viability A549	93.69	100.34	95.13	105.48	98.35

stronger antimicrobial activity since they can form hydrogen bonds with the active sites of target enzymes. This leads to the inactivation of those enzymes and ultimately causes dysfunction or rupture of the cell membrane. For instance, compounds such as carvacrol and eugenol exhibit rapid bactericidal action, in contrast to purely hydrocarbon monoterpenes such as pinene or limonene, which are generally less effective (Guimarães et al., 2019). Nevertheless, the long hydrocarbon tail, also present in geraniol, confers high lipophilicity, enabling its efficient partitioning into bacterial membranes. According to previous reports, geraniol can disrupt cytoplasmic membranes and induce intracellular leakage by solvating membrane phospholipids. In addition, it

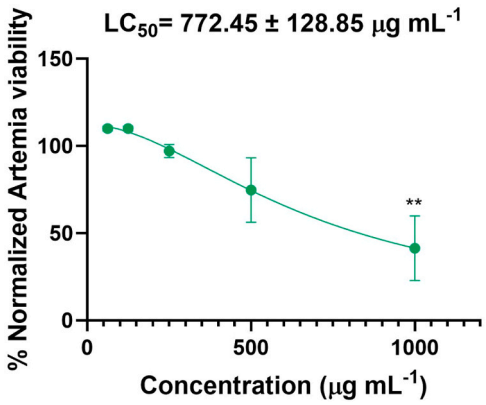

Fig. 3. *Artemia* viability exposed to *C. martini* EO for 24 h. Results correspond to the mean ± SEM and a non-linear regression. All the data were normalized to the non-treated control (non-represented), which displayed a 100 % viability. Statistical analysis via ANOVA revealed significant differences between the highest concentration and the non-represented untreated control. (statistical significance: * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

may impair the structural organization of the cytoplasmic membrane prior to the onset of protein denaturation within the cytoplasm (Gutiérrez-Pacheco et al., 2023). This membrane-targeting behaviour is consistent with the results obtained in this work (Fig. 2), in which the geraniol-containing EO visibly compromises membrane integrity. Other essential oils from aromatic plants have also been described to destabilize the cellular architecture of the bacteria, leading to the breakdown of membrane integrity and increased permeability, which disrupts many cellular activities, including energy production, membrane transport, and among other functions (Swamy et al., 2016). In view of this, to determine if *C. martini* oil followed a similar activity, membrane permeabilization and depolarization assays were performed. As shown in Fig. 2, the EO herein studied showed activity as membrane permeabilizing and membrane depolarizing agent, indicating that the oil disrupts the integrity of the cell membrane, leading to increased permeability and loss of membrane potential, which are critical factors in its antimicrobial efficacy. This disruption causes the bacteria to lose their basic structural functions and eventually leads to bacterial cell death at certain concentrations (Wang et al., 2020). However, essential oils usually contain various bioactive compounds that act on different parts of bacteria (Sharifi et al., 2021), therefore, although this study focused on membrane disruption, it is also possible that other mechanisms are involved. For instance, in previous works geraniol was described as an inhibitor of the secretion of eDNA and the polysaccharide intercellular adhesin in this bacterium, while also reducing staphyloxanthin production in *S. aureus* (Gu et al., 2022; Hartman et al., 2025). Geranyl acetate has also been described as a cell wall synthesis proteins inhibitor in the same bacteria (Manjunath et al., 2023), while linalool and β-caryophyllene have also shown potential in recent studies to eradicate *S. aureus* (Almeida-Bezerra et al., 2025; Long et al., 2025). Therefore, the biofilm inhibitory activity shown in Fig. 1 and the lower MIC values obtained for the EO could result from a synergistic interaction between these components and their distinct mechanisms of action.

As shown in Table 2, both the EO and geraniol showed activity against the *S. aureus* human isolates with heterogeneous resistance patterns (Supplementary material). This is relevant since *S. aureus* is a common cause of morbidity due to the range of diseases it can cause, ranging from skin conditions to pneumonia and sepsis (Cheung et al., 2021). In fact, antibiotic-resistant *S. aureus* has been designated as high-priority pathogen in the latest update of the WHO Bacterial Priority Pathogens List (World Health Organization, 2024). In addition, *C. martini* oil significantly inhibited biofilm formation at all the concentrations tested while acting as a biofilm dispersant at most of them. When compared to the results obtained for vancomycin, this compound losses effectivity in the post-exposure scenario while the activity of the EO remains (Fig. 1). In general, most antimicrobial agents perform well in pre-exposure conditions, but very few are effective at dispersing established biofilms (Del Pozo, 2018). Notably, palmarosa exhibited only a slight reduction in activity when comparing its effects in pre-exposure (biofilm inhibition) and post-exposure (biofilm dispersion) assays. A previous study demonstrated the antibiofilm activity of palmarosa oil against *P. aeruginosa* and *Staphylococcus* spp., focusing on biofilm inhibition and measuring biomass reduction (Sezener Kabay et al., 2024). The present study builds upon these findings by evaluating both biofilm biomass and bacterial viability under both pre- and post-exposure conditions, contributing to a better understanding of the antibiofilm properties of palmarosa, adding further evidence to support its potential application. Some studies have also observed that when palmarosa oil is combined with amphotericin B (AmB), significantly enhancing antifungal activity against *Candida albicans* during its active growth phase (Yuan et al., 2024); in this sense, further studies should explore the potential of this oil in synergy with other antibiotics as an interesting strategy to enhance the antibacterial activity of commonly used antibiotic substances.

The EO herein tested showed activity against the biofilm matrix production (Fig. 1). This is particularly relevant since a defining feature of biofilms is their extracellular matrix, excreted by the bacteria within the biofilm. This matrix is composed by extracellular polymeric substances, namely polysaccharides, exogenous DNA, proteins, and lipids. Moreover, the matrix provides the biofilm physical and chemical properties enabling the protection of the bacteria in the biofilm (Zhao et al., 2023).

An important aspect to consider when studying industrial products is their impact upon the environment, therefore the use of adult *Artemia salina* (Thirumalaivasan et al., 2024). While slight toxicity was observed at the highest concentrations, still, the oil remained safe at most of the concentrations where it exhibited antibiofilm activity. Therefore, the results suggest a relatively low toxicity profile, implying limited potential for adverse environmental impact.

Another fundamental aspect to contemplate is to evaluate their effects on mammalian cells, thus, their potential cytotoxicity. *C. martini* proved to be safe for the A549 and HaCaT cells at the concentrations tested for the biofilm assays. This fact indicates that the concentrations determined as effective against biofilms are safe *in vitro* for both human alveolar epithelial cells and keratinocytes, bringing the results closer to a potential application.

These findings add a new dimension to the commercial value of palmarosa oil. In addition to its established role as a promising industrial crop, widely used in perfumery, cosmetics, soaps, and aromatherapy, the growing evidence of its antimicrobial efficacy reinforces its potential in the development of new products with enhanced antibiofilm properties.

5. Conclusions

This study highlights the promising antibacterial potential of palmarosa (*C. martini*) essential oil against *S. aureus* resistant strains, demonstrating activity at lower concentrations than those determined for geraniol, its major compound (75.3 %). Besides, its capacity to prevent biofilm formation or disperse established biofilms underscores its relevance as an alternative strategy to target persistent bacterial communities. Although the membrane appears to the main target of the EO, the contribution of additional mechanisms cannot be dismissed and warrants further investigation.

The low toxicity demonstrated in human alveolar and epithelial cells and also in adult brine shrimp supports the suitability of palmarosa for applications where safety is a key aspect. These findings expand the value of *C. martini* EO and geraniol beyond their use in perfumery, cosmetics, and aromatherapy, positioning it as an attractive candidate for the development of new antimicrobial and antibiofilm formulations.

Nevertheless, the complexity of its chemical profile suggests that its activity may arise from synergistic interactions among its constituents and their different mechanisms of action. Future studies should therefore prioritize evaluating such synergistic effects and investigating combinations of plamarosa with conventional antibiotics and disinfectants.

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CRediT authorship contribution statement

Víctor López: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Leena Hanski:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Cristina Seral:** Resources, Methodology. **Maarit Ylätalo:** Supervision. **Candela Geredíaga:** Formal analysis. **Elena Alvarado:** Investigation. **Inés Reigada:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Pilar Cebollada:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2025.122479.

Data Availability

Data will be made available on request.

References

Adams, R.P., 2009. Identification of essential oil components by gas chromatography/mass spectrometry, 4th ed. Allured Publishing Corporation, Carol Stream, IL.

Almeida-Bezerra, J.W., da Costa Silva, J.T., Morais-Braga, M.F.B., da Cruz, R.P., Alencar, G.G., Alves, D.S., de Sousa Rodrigues, E.Y., de Sousa, S.G., de Menezes, I.R. A., Rocha, J.E., Filho, J.M.B., Leite dos Santos, C.A., Costa, A.R., Domiciano, C.B., de Lima, L.R., Coutinho, H.D.M., 2025. ADME/Tox study and the effect of β-Caryophyllene on the resistant strain of Staphylococcus aureus carrying the QacA/B efflux pump gene. Toxicol. Rep. 14. https://doi.org/10.1016/j.toxrep.2025.101929.

Arciola, C.R., Campoccia, D., Montanaro, L., 2018. Implant infections: adhesion, biofilm formation and immune evasion. Nat. Rev. Microbiol 16, 397–409. https://doi.org/10.1038/S41579-018-0019-Y.

Campoccia, D., Mirzaei, R., Montanaro, L., Arciola, C.R., 2019. Hijacking of immune defences by biofilms: a multifront strategy. Biofouling 35, 1055–1074. https://doi.org/10.1080/08927014.2019.1689964.

Carrascosa, C., Raheem, D., Ramos, F., Saraiva, A., Raposo, A., 2021. Microbial Biofilms in the Food Industry—A Comprehensive Review. Int J. Environ. Res Public Health 18, 1–31. https://doi.org/10.3390/IJERPH18042014.

Cheung, G.Y.C., Bae, J.S., Otto, M., 2021. Pathogenicity and virulence of Staphylococcus aureus. Virulence 12, 547–569. https://doi.org/10.1080/21505594.2021.1878688.

Dangol, S., Poudel, D.K., Ojha, P.K., Maharjan, S., Poudel, A., Satyal, R., Rokaya, A., Timsina, S., Dosoky, N.S., Satyal, P., Setzer, W.N., 2023. Essential Oil Composition Analysis of Cymbopogon Species from Eastern Nepal by GC-MS and Chiral GC-MS, and Antimicrobial Activity of Some Major Compounds. Molecules 28, 543. https://doi.org/10.3390/MOLECULES28020543/S1.

De Matos, S.P., Teixeira, H.F., De Lima, Á.A.N., Veiga-Junior, V.F., Koester, L.S., 2019. Essential oils and isolated terpenes in nanosystems designed for topical administration: a review. Biomolecules 9. https://doi.org/10.3390/BIOM9040138.

Del Pozo, J.L., 2018. Biofilm-related disease. Expert Rev. Anti Infect. Ther. 16, 51–65. https://doi.org/10.1080/14787210.2018.1417036.

Fernandes, S., Gomes, I.B., Simões, M., Simões, L.C., 2024. Novel chemical-based approaches for biofilm cleaning and disinfection. Curr. Opin. Food Sci. 55, 101124. https://doi.org/10.1016/J.COFS.2024.101124.

Gu, K., Ouyang, P., Hong, Y., Dai, Y., Tang, T., He, C., Shu, G., Liang, X., Tang, H., Zhu, L., Xu, Z., Yin, L., 2022. Geraniol inhibits biofilm formation of methicillin-resistant Staphylococcus aureus and increase the therapeutic effect of vancomycin in vivo. Front Microbiol 13. https://doi.org/10.3389/FMICB.2022.960728/FULL.

Guimarães, A.C., Meireles, L.M., Lemos, M.F., Guimarães, M.C.C., Endringer, D.C., Fronza, M., Scherer, R., 2019. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils, 24, 2471 Molecules 2019 24, 2471. https://doi.org/10.3390/MOLECULES24132471.

Gutiérrez-Pacheco, M.M., Torres-Moreno, H., Flores-Lopez, M.L., Velázquez Guadarrama, N., Ayala-Zavala, J.F., Ortega-Ramírez, L.A., López-Romero, J.C., 2023. Mechanisms and Applications of Citral's Antimicrobial Properties in Food Preservation and Pharmaceuticals Formulations, 12, 1608 Antibiotics 2023 12, 1608. https://doi.org/10.3390/ANTIBIOTICS12111608.

Hartman, K., Świerczyńska, M., Wieczorek, A., Baszuk, P., Wojciechowska-Koszko, I., Garbacz, K., Sienkiewicz, M., Kwiatkowski, P., 2025. Preliminary evaluation of the synergistic antibacterial effects of selected commercial essential oil compounds against methicillin-resistant staphylococcus aureus ATCC 43300. Antibiot. (Basel) 14, 733. https://doi.org/10.3390/ANTIBIOTICS14070733.

Kakaraparthi, P.S., Srinivas, K.V.N.S., Kumar, J.K., Kumar, A.N., Rajput, D.K., Anubala, S., 2015. Changes in the essential oil content and composition of palmarosa (Cymbopogon martini) harvested at different stages and short intervals in two different seasons. Ind. Crops Prod. 69, 348–354. https://doi.org/10.1016/J.INDCROP.2015.02.020.

Kumar, A., Sharma, N., Gupta, A.K., Chanotiya, C.S., Lal, R.K., 2024. The aromatic crop rosagrass (Cymbopogon martinii (Roxb.) Wats. Var. motia Burk.) its high yielding genotypes, perfumery, and pharmacological potential: A review. Ecol Genet Genom 32, 100280. https://doi.org/10.1016/J.EGG.2024.100280.

Lindsay, D., Holy, A.Von, 2006. What food safety professionals should know about bacterial biofilms. Br. Food J. 108, 27–37. https://doi.org/10.1108/00070700610637616/FULL/XML.

Long, N., Qiu, M., Zuo, Y., Deng, H., 2025. Antimicrobial Activity and Metabolomic Analysis of Linalool Against Pathogenic Bacteria Methicillin-Resistant Staphylococcus aureus. Infect. Drug Resist 18, 731. https://doi.org/10.2147/IDR.S491358.

Maczka, W., Winska, K., Grabarczyk, M., 2020. One hundred faces of geraniol. Molecules 25. https://doi.org/10.3390/MOLECULES25143303.

Manjunath, A., Chinmayi, G.V.A., Renganathan, S., Chandramohan, V., Sabat, S., 2023. Antimicrobial activity of Geranyl acetate against cell wall synthesis proteins of P. aeruginosa and S. aureus using molecular docking and simulation. J. Biomol. Struct. Dyn. https://doi.org/10.1080/07391102.2023.2212060.

Nemoda, M., Veljković, F., Nikolić, B., Brkić, S., Marković, D., Momčilović, M., Lal, M., Živković, L., Marinković, J., 2024. Geraniol in vitro and geraniol-based emulsion ex vivo potential against four-species Streptococcus spp. biofilm relevant for dentistry. Ind. Crops Prod. 222, 119827. https://doi.org/10.1016/J.INDCROP.2024.119827.

Ramsey, J.T., Shropshire, B.C., Nagy, T.R., Chambers, K.D., Li, Y., Korach, K.S., 2020. Focus: Plant-based Medicine and Pharmacology: Essential Oils and Health. Yale J. Biol. Med 93, 291.

Sezener Kabay, M.G., Inal, S., Gökmen, S., Ergüden, V.E., Fındık, A., Güvenç, T., Kayhan, H., Güvenç, D., 2024. Antibacterial Effects of Essential Oils on P. aeruginosa, Methicillin-Resistant S. aureus, and Staphylococcus spp. Isolated from Dog Wounds. Pharmaceuticals 17. https://doi.org/10.3390/PH17111494.

Sharifi, A., Mohammadzadeh, A., Salehi, T.Z., Mahmoodi, P., Nourian, A., 2021. Cuminum cyminum L. Essential Oil: A Promising Antibacterial and Antivirulence Agent Against Multidrug-Resistant Staphylococcus aureus. Front Microbiol 12. https://doi.org/10.3389/FMICB.2021.667833.

Shi, X., Zhu, X., 2009. Biofilm formation and food safety in food industries. Trends Food Sci. Technol. 20, 407–413. https://doi.org/10.1016/j.tifs.2009.01.054.

Skogman, M.E., Vuorela, P.M., Fallarero, A., 2012. Combining biofilm matrix measurements with biomass and viability assays in susceptibility assessments of antimicrobials against Staphylococcus aureus biofilms, 65 J. Antibiot. 2012 65 (9), 453–459. https://doi.org/10.1038/ja.2012.49.

Smitha, G.R., Rana, V.S., 2015. Variations in essential oil yield, geraniol and geranyl acetate contents in palmarosa (Cymbopogon martinii, Roxb. Wats. var. motia) influenced by inflorescence development. Ind. Crops Prod. 66, 150–160. https://doi.org/10.1016/J.INDCROP.2014.12.062.

Swamy, M.K., Akhtar, M.S., Sinniah, U.R., 2016. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated

Review. Evid. Based Complement Altern. Med 2016, 3012462. https://doi.org/10.1155/2016/3012462.

Thirumalaivasan, N., Mahapatra, S., Ramanathan, G., Kumar, A., Raja, T., Muthuramamoorthy, M., Pandit, B., Pandiaraj, S., Prakash, S., 2024. Exploring antimicrobial and biocompatible applications of eco-friendly fluorescent carbon dots derived from fast-food packaging waste transformation. Environ. Res 244, 117888. https://doi.org/10.1016/J.ENVRES.2023.117888.

Vella, F.M., Calandrelli, R., Cautela, D., Fiume, I., Pocsfalvi, G., Laratta, B., 2020. Chemometric Screening of Fourteen Essential Oils for Their Composition and Biological Properties. Molecules 25, 5126. https://doi.org/10.3390/MOLECULES25215126.

Wang, X., Shen, Y., Thakur, K., Han, J., Zhang, J.G., Hu, F., Wei, Z.J., 2020. Antibacterial activity and mechanism of ginger essential oil against escherichia coli and staphylococcus aureus. Molecules 25. https://doi.org/10.3390/MOLECULES25173955.

World Health Organization, 2024. WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. https://www.who.int/publications/i/item/9789240093461(accessed 9 June 2025).

Yuan, X., Cao, D., Xiang, Y., Jiang, X., Liu, J., Bi, K., Dong, X., Wu, T., Zhang, Y., 2024. Antifungal activity of essential oils and their potential synergistic effect with amphotericin B. Sci. Rep. 14. https://doi.org/10.1038/S41598-024-82380-0.

Zhao, A., Sun, J., Liu, Y., 2023. Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect. Microbiol 13. https://doi.org/10.3389/FCIMB.2023.1137947/FULL.