

#### **Research Article**



# Antibacterial properties of commercial lavender essential oil against some Gram-positive and Gram-negative bacteria

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Herbs and essential oils (EOs) have been used in medicine and veterinary, agriculture, the food industry, and cosmetology. Many EOs possess various biological properties, i.e. antibacterial, analgesic, anti-inflammatory properties, antioxidant, fungicide, larvicidal, antitumor activities, etc. Lavender oil is one of the most valuable aromatherapy oils. Its antibacterial and antifungal activities have been revealed in many studies. In the current study, the antibacterial properties of commercial lavender EO against some Gram-positive and Gram-negative bacteria were studied. To this intent, the antimicrobial susceptibility test was used (the Kirby-Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition). In the current study, Gram-negative strains such as Escherichia coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), Escherichia coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>), Pseudomonas aeruginosa (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>) and Gram-positive strains such as Staphylococcus aureus subsp. aureus Rosenbach (ATCC® 29213™), methicillin-resistant (MRSA), mecA positive Staphylococcus aureus (NCTC® 12493), Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) were used. Results of this study revealed that resistant to the lavender EO were Gram-negative bacterial strains, such as E. coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), E. coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>), *P. aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>) strains. The diameters of inhibition zones after the application of lavender EO were similar to control samples (96% ethanol). On the other hand, Gram-positive strains such as S. aureus subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>), methicillinresistant S. aureus (NCTC<sup>®</sup> 12493), E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) were sensitive to lavender EO. The highest diameters of inhibition zones after the application of lavender EO were observed for *E. faecalis* strains. This study demonstrates the potential of commercial lavender essential oil as an antibacterial agent and for use in the treatment of MRSA infection. The data contributes to the ongoing scientific investigation regarding the application of essential oils as natural antibacterial agents.

Keywords: commercial lavender essential oil, antibacterial activity, inhibition zones, Kirby-Bauer disc diffusion technique

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## Introduction

Lavender essential oil (EO) has been used both cosmetically and therapeutically for centuries (Cavanagh and Wilkinson, 2002). It has been used as an anxiolytic drug, a mood stabilizer, a sedative, spasmolytic, antihypertensive, antimicrobial, and analgesic agent as well as a wound healing accelerator (Sasannejad et al., 2012). Several studies have investigated the antinociceptive, immunomodulatory and anti-inflammatory properties of compounds found in lavender EO (Silva et al., 2015). It is traditionally used in herbal medicine to relieve stress and anxiety confirmed by positive results in models of anxiety and depression using some animal and clinical studies (López et al., 2017). The two primary terpenoid constituents of lavender EO, linalool and linalyl acetate, may produce an anxiolytic effect in combination with inhibition of voltage-gated calcium channels, reduction of 5HT1A receptor activity, and increased parasympathetic tone (Malcolm and Tallian, 2018). Sasannejad et al. (2012) have studied the efficacy of lavender EO inhalation for the treatment of migraine in a placebo-controlled clinical trial. That study suggests that inhalation of lavender EO may be an effective and safe treatment modality in the acute management of migraine headaches (Sasannejad et al., 2012). Also, the current body of literature suggests apotential therapeutic benefit of lavender EO in wound healing. The studies of Samuelson et al. (2020) have demonstrated a faster rate of wound healing, increased expression of collagen, and enhanced activity of proteins involved in the tissue remodelling process in wounds treated with lavender EO.

The Lavandula genus includes about 40 different species and hundreds of varieties and hybrids. The three species most commonly grown are L. angustifolia Mill. (narrow-leaved lavender, usually medical), a species with the most significant industrial importance, L. stoechas (French lavender), L. latifolia, and their hybrids (Wińska et al., 2019). The composition of lavender EO is described in the review article written by Wińska et al. (2019). These researchers noted that the main components of lavender EO are R enantiomers of linalool (20–45%) and linalyl acetate (25 to 46%). The high content of these ingredients determines the quality of the oil. The content of other ingredients should be in the following ranges: limonene (>1.0%), eucalyptol (<2.5%), camphor (>1.2%), terpin-4-ol (0.1-6.0) %), lawandulol (<0.1%), lavandulyl acetate (<0.2%), and  $\alpha$ -terpineol (>2.0%). Due to the incalculable influence on the scent, lavender oil should not contain too much ocymen, cineole, camphor, or terpin-4-ol (Wińska et

al., 2019). The essential oil composition obtained from fresh flowers of thirteen new Ukrainian cultivars of *L. angustifolia* was analyzed by Pokajewicz et al. (2021). Eighty-two components were identified. Linalool and linalyl acetate were principal constituents of all of the samples and ranged from 11.4% to 46.7% and 7.4% to 44.2%, respectively (Pokajewicz et al., 2021).

The long-known antimicrobial actions of essential oils are now being extensively scientifically reviewed and applied in health and industry fields (Sienkiewicz et al., 2011, 2014). The antimicrobial activity of lavender EO against bacteria and fungi has long been established. Its anti-bacterial and anti-fungal activities can be explained by the presence of a main components such as linalool, linalyl acetate, lavandulol, geraniol, or eucalyptol (Białoń et al., 2019). Lavender EO also has antibacterial activity against clinical strains of bacteria isolated from patients with respiratory tract infections (Sienkiewicz et al., 2011). Moreover, the antibacterial activity of lavender EO may be accompanied by an immunostimulatory effect reducing the incidence of infections in patients with bacterial respiratory infections (Roller et al., 2009). Also, few studies have been carried out to elucidate the mechanism of its action to capitalize on its application in clinical settings (Yang et al., 2020).

In the current study, the antibacterial properties of commercial lavender EO provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Kłaj, Poland) against some Gram-positive and Gram-negative bacteria were studied. To this intent, the antimicrobial susceptibility test was used (the Kirby–Bauer disk diffusion test for measuring zone diameters of bacterial growth inhibition).

## Material and methodology

### Lavender essential oil

The lavender EO was provided by Polish essential oil manufacturers (Naturalne Aromaty sp. z o.o., Kłaj, Poland). The investigated sample did not contain additives or solvents and was confirmed to be natural by the manufacturers. The samples were stored in resalable vials at 5 °C in the dark but were allowed to adjust to room temperature prior to investigation. Geographical origins were excluded as information was mostly not available.

### Determination of the antibacterial activity of plant extracts by the disk diffusion method

The testing of the antibacterial activity of lavender EO was carried out *in vitro* by the Kirby-Bauer disc

diffusion technique (Bauer et al., 1966). In the current study, Gram-negative strains such as Escherichia coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), Escherichia coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>), *Pseudomonas aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>) and Gram-positive strains such as Staphylococcus aureus subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>), methicillin-resistant (MRSA), mecA positive Staphylococcus aureus (NCTC<sup>®</sup> 12493), Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and Enterococcus faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) were used.

The strains were inoculated onto Mueller-Hinton (MH) agar dishes. Sterile filter paper discs impregnated with lavender EO were applied over each of the culture dishes. Isolates of bacteria with lavender EO were then incubated at 37 °C for 24 h. The Petri dishes were then observed for the zone of inhibition produced by the antibacterial activity of lavender EO. A control disc impregnated with 96% ethanol was used in each experiment. At the end of the 24-h period, the inhibition zones formed were measured in millimetres using the vernier. For each strain, eight replicates were assayed (n = 8). The Petri dishes were observed and photographs were taken. The susceptibility of the test organisms to the lavender EO was indicated by a clear zone of inhibition around the discs containing the lavender EO and the diameter of the clear zone was taken as an indicator of susceptibility. Zone diameters were determined and averaged. The following zone diameter criteria were used to assign susceptibility or resistance of bacteria to the phytochemicals tested: Susceptible (S)  $\geq$ 15 mm, Intermediate (I) = 10–15 mm, and Resistant (R) ≤10 mm (Okoth et al., 2013; Truchan et al., 2019).

### Statistical analysis

Zone diameters were determined and averaged. Statistical analysis of the data obtained was performed by employing the mean ± standard error of the mean (S.E.M.). All variables were randomized according to the phytochemical activity of the lavender EO tested. All statistical calculation was performed on separate data from each strain. The data were analyzed using a one-way analysis of variance (ANOVA) using Statistica v. 13.3 software (TIBCO Software Inc., Krakow, Poland) (Zar, 1999).

# **Results and discussion**

The antibacterial activity induced by lavender essential oil estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains was presented in Figure 1 and 2.

Results of the current study revealed that Gram-negative strains, such as E. coli and P. aeruginosa were resistant to the lavender EO. The diameters of inhibition zones for E. coli (Migula) Castellani and Chalmers (ATCC® 25922<sup>™</sup>) strain after the application of lavender EO were similar (7.98 ±0.81 mm) compared to the 96% ethanol as control samples (8.85 ±0.91 mm). Similar results were obtained for E. coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>) strain. The diameters of inhibition zones after the application of lavender EO were  $(8.56 \pm 0.76 \text{ mm})$  compared to the 96% ethanol as control samples (8.98 ±0.88 mm). P. aeruginosa (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>) strain was also resistant to the lavender EO. The diameters of inhibition zones after the application of lavender EO were (7.12 ±0.81 mm) compared to the 96% ethanol as control samples (7.78 ±0.91 mm) (Figure 1).

Gram-positive strains were sensitive to the lavender EO compared to the Gram-negative strains. S. aureus strains exhibited *intermediate* activity to the lavender EO. S. aureus subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>) strain was less sensitive then *S. aureus* (NCTC<sup>®</sup> 12493). Diameters of inhibition zones after application of lavender EO were (13.44  $\pm 0.56$  mm) compared to the 96% ethanol as control samples ( $8.56 \pm 0.75$  mm) for S. aureus subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>) strain and (16.10 ±0.69 mm) compared to the 96% ethanol as control samples (9.12 ±0.95 mm) for S. aureus (NCTC® 12493) strain. The increase of diameters of inhibition zones after the application of lavender EO was 57% (p <0.05) and 76.5% (p <0.05) for *S. aureus* subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>) and *S. aureus* (NCTC<sup>®</sup> 12493) strains, respectively compared to the control samples (96% ethanol) (Figure 1).

*E. faecalis* strains were more sensitive to lavender EO (Figure 1). Diameters of inhibition zones after application of lavender EO were (21.78 ±0.71 mm) compared to the 96% ethanol as control samples (9.15±0.99 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>TM</sup>) strain and (23.15±0.98 mm) compared to the 96% ethanol as control samples (8.92 ±0.91 mm) for *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>TM</sup>) strain. The increase of diameters of inhibition zones after the application of lavender EO was 138% (p <0.05) and 159.5% (p <0.05) for

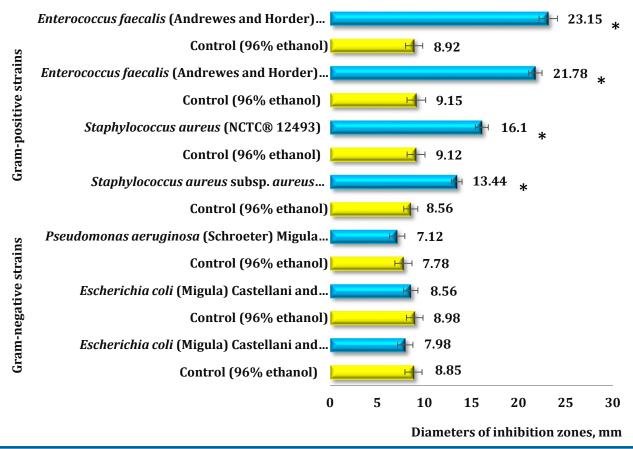


Figure 1 The antibacterial activity induced by lavender essential oil estimated as diameters of growth inhibition zones of examined Gram-positive and Gram-negative strains. The data were presented as the mean ± the standard error of the mean (S.E.M.)

\* denote significant differences between the control (96% ethanol) and lavender EO (p <0.05)

*E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) strains, respectively (Figure 1).

Detailed photos regarding the zones of inhibition by the lavender EO against Gram-positive and Gramnegative bacterial strains were recorded and presented in Figure 2.

In line with our previous studies according to the antibacterial potential of different plant extracts and EOs, in the current study, we examined the antibacterial potential of commercial lavender EO against Grampositive and Gram-negative bacterial strains. Resistant to the lavender EO were Gram-negative bacterial strains, such as *E. coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), *E. coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>), *P. aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>) strains. The diameters of inhibition zones after the application of lavender EO were similar to control samples (96% ethanol). On the other hand, Gram-positive strains such as *S. aureus* subsp. *aureus* 

Rosenbach (ATCC<sup>®</sup> 29213<sup>m</sup>), methicillin-resistant *S. aureus* (NCTC<sup>®</sup> 12493), *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>m</sup>) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>m</sup>) were sensitive to lavender EO. The highest diameters of inhibition zones after the application of lavender EO were observed for *E. faecalis* strains (Figure 1 and 2).

Lavender EO has antibacterial activity against bacterial strains, as reported in many studies. For example, Roller et al. (2009) have compared the antimicrobial efficacy of several lavender oils from *Lavandula angustifolia*, *L. latifolia*, *L. stoechas*, and a necrodanerich *L. luisieri*, used singly and in combination, on methicillin-sensitive and methicillin-resistant *S. aureus* (MSSA and MRSA). All four lavender oils inhibited the growth of both MSSA and MRSA by direct contact but not in the vapour phase. Inhibition zones ranged from 8 to 30 mm in diameter at oil doses ranging from 1 to 20  $\mu$ L, respectively, demonstrating a dose-response. At any single dose, the extent of inhibition zones ranged rom similar irrespective of the chemical composition

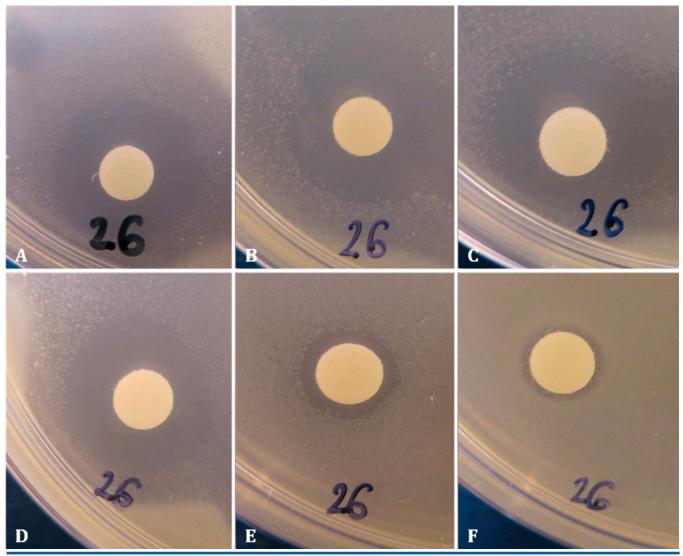


Figure 2 Inhibition growth zones induced by lavender essential oil against *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) (A), *Enterococcus faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (B), *Staphylococcus aureus* subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>) (C), *Staphylococcus aureus* (NCTC<sup>®</sup> 12493) (D), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>) (E), *Escherichia coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>) (F)

of the oils or the strain of *S. aureus* used. Several binary combinations of the oils were tested, and the results showed that the necrodane-rich *L. luisieri* oil interacted synergistically with *L. stoechas* (high in 1,8-cineole, fenchone, and camphor) and *L. angustifolia* (rich in linalool and linalyl acetate) to produce larger inhibition zones than those produced using each oil individually (Roller et al., 2009).

Lavender EO could be a promising candidate for an efficient enhancer of conventional antiseptics. Kwiatkowski et al. (2019) have investigated the impact of lavender EO on octenidine dihydrochloride (OCT) efficiency towards methicillin-resistant *S. aureus* strains (MRSA). The lavender EO increased the OCT's susceptibility against MRSA strains. Subsequent FTIR analysis revealed cellular wall modifications in MRSA strain cultured in media supplemented with OCT or lavender EO/OCT (Kwiatkowski et al., 2019).

The chemical composition of lavender (*Lavanda angustifolia* L.) EO and some by-products derived from its production (residual water, residual herbs), as well as their *in vitro* antimicrobial activity, were assessed by Ciocarlan et al. (2021). The main constituents of EOs are monoterpenes (84.08–92.55%), followed by sesquiterpenes (3.30–13.45%), and some aliphatic compounds (1.42–3.90%). The high-performance liquid chromatography analysis allowed the quantification of known triterpenes, ursolic, and oleanolic acids, in freshly dried lavender plants and the residual by-products after hydrodistillation

of the essential oil. The lavender essential oil showed good antibacterial activity against Bacillus subtilis, Pseudomonas fluorescens, Xanthomonas campestris, Erwinia carotovora at 300 µg.mL<sup>-1</sup> concentration, and Erwinia amylovora, Candida utilis at 150 µg.mL<sup>-1</sup> concentration, respectively. Lavender plant material but also the residual water and ethanolic extracts from the solid waste residue showed high antimicrobial activity against Aspergillus niger, Alternaria alternata, Penicillium chrysogenum, Bacillus sp., and Pseudomonas aeroginosa strains, at 0.75-6.0 µg.mL<sup>-1</sup>, 0.08–0.125 μg.mL<sup>-1</sup>, and 0.05–4.0 μg.mL<sup>-1</sup>, respectively (Ciocarlan et al., 2021).

Lavender EOs could find potential applications in food biopreservation and surface decontamination, even in hospitals. Tardugno et al. (2019) evaluated the antimicrobial activity of EOs of four cultivars (cv) of Lavandula × intermedia (Abrialis, Alba, Rinaldi Ceroni (R.C.) and Sumiens) against Listeria monocytogenes (24 strains) and Salmonella enterica (10 food strains). Minimal inhibitory concentrations (MIC)  $\geq 10.0 \ \mu L.mL^{-1}$ inhibited Salmonella (cv. R.C. was the most active); MIC of 0.3 µL.mL<sup>-1</sup> for cv. Abrialis and cv. R.C. inhibited L. monocytogenes, revealing noticeable activity, especially on clinical strains. Particularly cv. Abrialis and cv. R.C. showing the highest antimicrobial activity, were rich in the specific constituents: linalool (38.17 and 61.98%), camphor (8.97 and 10.30%), 1,8-cineole (6.89 and 8.11%, respectively) (Tardugno et al., 2019).

The anti-microbial effects of two different lavender oils such as commercial lavender oil and essential lavender oil from the Crimean Peninsula on a mixed microbiota from facial skin were assessed by Białoń et al. (2019). The composition and properties of the studied oils were significantly different. The commercial lavender oil (Etja, Poland) contained 10% more linalool and linalyl acetate than the Crimean lavender oil. Both oils also had different effects on the mixed facial skin microbiota. The Gram-positive bacilli were more sensitive to commercial lavender EO, and Gram-negative bacilli were more sensitive to Crimean lavender EO. However, neither of the tested oils inhibited the growth of Grampositive cocci. The tested lavender oils decreased the cell number of the mixed microbiota from facial skin, but commercial lavender EO showed higher efficiency, probably because it contains higher concentrations of monoterpenoids and monoterpenes than Crimean lavender oil does (Białoń et al., 2019).

The laboratory and clinical efficacy of lavender oil in the treatment of recurrent aphthous ulceration were revealed by Altaei (2012). Animals treated with lavender oil showed a significant ulcer size reduction, increased rate of mucosal repair, and healing within 3 days of treatment compared to baseline and placebo groups (2–3 days (90%), 4 days (10%)). Lavender oil showed a broad antibacterial activity against all tested strains; it exhibited significant inhibition on tested bacteria where the value of zone of inhibition ranged from 14.5–24.0 mm vs. Streptomycin (25  $\mu$ g.disc<sup>-1</sup>) 12–22±0.5 mm; MIC was >6.4–36 mg.ml<sup>-1</sup>. Patients with recurrent aphthous ulceration treated with lavender oil showed a significant reduction in inflammation level, ulcer size, and healing time, from 2–4 days (2 days (40%), 3 days (50%), 4 days (10%)), and pain relief mostly from the first dose, compared to baseline and placebo (Altaei, 2012).

The combined use of two naturally derived compounds, sodium alginate and lavender essential oil, for the production of bioactive nanofibrous dressings by electrospinning, and their efficacy for the treatment of skin burns induced by midrange ultraviolet radiation (UVB) was demonstrated by Hajiali et al. (2016). These researchers have demonstrated that the engineered dressings reduce the risk of microbial infection of the burn since they stop the growth of *Staphylococcus aureus*. Furthermore, they can control and reduce the inflammatory response that is induced in human foreskin fibroblasts by lipopolysaccharides, and in rodents by UVB exposure (Hajiali et al., 2016).

The products derived from the Lavandula pubescens Decne (LP), including the EO, have been used in Traditional Arabic Palestinian Herbal Medicine for centuries as therapeutic agents. The EO is traditionally believed to have sedative, anti-inflammatory, antiseptic, anti-depressive, anti-amnesia, and anti-obesity properties. Ali-Shtayeh et al. (2020) have assessed the in vitro bioactivities associated with the aerial parts of LP plants and analyzed them for their antioxidant, antimicrobial, anticholinesterase, and anti-lipase activities. The EO also demonstrated high antibacterial activity with the highest susceptibility observed for S. aureus with 95.7% inhibition. The EO was shown to exhibit strong inhibitory activity against Candida albicans (MIC 0.47 µL.mL<sup>-1</sup>). The EO was also shown to possess strong anti-dermatophyte activity against Microsporum canis, Trichophyton rubrum, Trichophyton mentagrophytes, and Epidermophyton floccosum ( $EC_{50}$ 0.05–0.06  $\mu$ L.mL<sup>-1</sup>). The high antioxidant, enzyme inhibitory, and antimicrobial potentials of the EO can, therefore, be correlated with its high content of monoterpenes, especially carvacrol, as shown by its comparable bioactivities indicators results (Ali-Shtayeh et al., 2020).

The chemical composition of Lavandula angustifolia Mill. EO collected during four consecutive years of growth were the aims of the work of Najar et al. (2022). The antibacterial activities of the EOs were assessed on three Gram-positive bacteria strains: Staphylococcus aureus ATCC 6538, Enterococcus faecalis VAN B V 583 E, and Listeria monocytogenes, together with three Gram-negative bacteria strains: Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 15325, and Salmonella enterica ser. Typhimurium ATCC 14028. Gram-negative bacteria were found to be less inhibited by oils derived from plants each year with maximum MIC values of 1:32 for Escherichia coli for fourth-year plants. The most sensitive bacterium was Listeria monocytogenes (a maximum MIC of 1:128 and MBC of 1:64 for the EOs of fourth-year plants), while the most resistant was *Pseudomonas aeruginosa* (a maximum MIC of 1:16 and MBC of 1:8 for the EO of fourth-year plants). Considering the age of the plants from which the oil was extracted, the ones that showed a higher inhibitory and bactericidal activity were those of the fourth year, followed by (in decreasing order) those of the third year, the second year, and finally the first year. Comparing these data with the GC-MS analyses, the trend of increased inhibitory efficacy against bacteria demonstrated as the plants aged is superimposed by the trend of an increased relative percentage of linalool (19.3, 23.1, 27.6, and 34.2 for each year), which was highly correlated with the Escherichia coli ATCC 15325 activity (correlation coefficient: 0.9). Linalool has shown a significant effect on *P. fluorescens* with 1.25 and 2.5 µL.mL<sup>-1</sup> of the MIC and MBC, respectively. The EO extracted from the oldest plants evidenced higher activity on the studied strains, with more sensitivity on the Gram-positive ones. Tuscan lavender EO, especially that obtained from four-year-old plants, is of great interest for its potential industrial applications and constitutes an example of the valorization of marginal Tuscan land and good-quality production (Najar et al., 2022).

*L. angustifolia* EO can stimulate the human innate macrophage response to bacteria that are responsible for one of the most important nosocomial infections and might suggest the potential development of this plant extract as an anti-inflammatory and immune regulatory coadjutant drug. Giovannini et al. (2016) have investigated, by transcriptional analysis, how an *L. angustifolia* EO treatment influenced the macrophage response to *Staphylococcus aureus* infection. The results of these researchers showed that the treatment increased the phagocytic rate and stimulates the containment of intracellular bacterial replication by

macrophages. This stimulation is coupled with the expression of genes involved in reactive oxygen species production. Moreover, the EO treatment balanced the inflammatory signaling induced by *S. aureus* by repressing the principal pro-inflammatory cytokines and their receptors and inducing the heme oxygenase-1 gene transcription (Giovannini et al., 2016). The lavender EO extracted at the beginning of the flowering period is a potent inhibitor of the synthesis of four pro-inflammatory cytokines IL-6, IL-8, IL- $\beta$ , and TNF $\alpha$  of THP-1 macrophages (Pandur et al., 2021). This supports the relevance of the collection of lavender flowers from the early blooming period for essential oil production and for utilization as an anti-inflammatory treatment (Pandur et al., 2021).

## Conclusions

In summary, this study provides insight into the in vitro antibacterial activity of commercial lavender EO against Gram-negative strains such as *E. coli* (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 25922<sup>™</sup>), E. coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>), *P. aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>) and Gram-positive strains such as S. aureus subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>), methicillinresistant (MRSA) S. aureus (NCTC® 12493), E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) (resistant to vancomycin; sensitive to teicoplanin) and *E. faecalis* (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>). Results of the current study revealed that resistant to the lavender EO were Gram-negative bacterial strains, such as E. coli (Migula) Castellani and Chalmers (ATCC® 25922<sup>™</sup>), E. coli (Migula) Castellani and Chalmers (ATCC<sup>®</sup> 35218<sup>™</sup>), *P. aeruginosa* (Schroeter) Migula (ATCC<sup>®</sup> 27853<sup>™</sup>) strains. The diameters of inhibition zones after the application of lavender EO were similar to control samples (96% ethanol). On the other hand, Gram-positive strains such as S. aureus subsp. aureus Rosenbach (ATCC<sup>®</sup> 29213<sup>™</sup>), methicillin-resistant S. aureus (NCTC® 12493), E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 51299<sup>™</sup>) and E. faecalis (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC<sup>®</sup> 29212<sup>™</sup>) were sensitive to lavender EO. The highest diameters of inhibition zones after the application of lavender EO were observed for E. faecalis strains. This study demonstrates the potential of commercial lavender essential oil as an antibacterial agent and for use in the treatment of MRSA infection. The data contributes to the ongoing scientific investigation regarding the application of essential oils as natural antibacterial agents.

#### **Conflicts of interest**

The authors declare no conflict of interest.

#### **Ethical statement**

This article doesn't contain any studies that would require an ethical statement.

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