From sustainable harvesting to antibacterial activity: Yield optimization, chemical composition, and foodborne pathogen inhibition of *Juniperus communis* L. and *Pistacia lentiscus* L. essential oils

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ABSTRACT

The present study evaluated the chemical composition and antibacterial activity of *Juniperus communis* L. and *Pistacia lentiscus* L. essential oils (EOs) and their 50:50 mixture against clinically relevant Gram-positive and Gram-negative bacteria. Antibacterial activity was assessed using agar diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and fractional inhibitory concentration index (FICI) assays, while the chemical profiles were characterized by GC-MS (Gas Chromatography-Mass Spectrometry) and FTIR (Fourier-Transform Infrared Spectroscopy) analyses. Both EOs exhibited antibacterial activity, with Gram-positive bacteria showing greater susceptibility than Gram-negative strains. The MBC/MIC ratios were ≤ 4 for all tested samples, indicating a predominantly bactericidal effect. Among the evaluated formulations, the 50:50 mixture displayed the highest antibacterial activity, particularly against *Staphylococcus aureus* ATCC 6538, with a MIC value of 6.25% (v/v) and a synergistic interaction against *S. aureus* (FICI = 0.49). GC–MS analysis showed that the mixture was predominantly composed of monoterpene hydrocarbons (71.42%), followed by sesquiterpene hydrocarbons (14.71%), oxygenated monoterpenes (7.11%), and oxygenated sesquiterpenes (1.14%). The major constituents identified were α-pinene, limonene, β-pinene, myrcene, and terpinen-4-ol. FTIR analysis confirmed the terpene-rich nature of the oils and supported the GC-MS results. The enhanced antibacterial activity of the mixture is likely associated with additive and synergistic interactions among its bioactive compounds, leading to increased disruption of bacterial cellular functions. These findings suggest that the combination of *J. communis* and *P. lentiscus* EOs represents a promising natural antibacterial agent, particularly against Gram-positive pathogens.

Keywords: *Juniperus communis*; *Pistacia lentiscus*; essential oils; antibacterial activity; GC-MS; FTIR.

INTRODUCTION

The emergence and rapid spread of antimicrobial resistance (AMR) represent one of the most serious global public health threats, underscoring the urgent need to discover innovative antibacterial agents, particularly in light of the increasing resistance observed among bacterial strains in the treatment of many diseases (Benmoumou et al., 2025). The excessive and inappropriate use of antibacterial agents in human medicine, livestock production, and agriculture has significantly contributed to the development and dissemination of resistant bacterial strains (Ventola, 2015). In parallel, food safety concerns are strongly associated with the presence of pathogenic microorganisms in food products, which are responsible for a wide range of foodborne diseases in humans, as highlighted by FAO (Food and Agriculture Organization of the United Nations) and WHO (World Health Organization) reports (FAO & WHO, 2022).

Foodborne pathogens are a major cause of morbidity worldwide. Among them, *Escherichia coli* and *Staphylococcus aureus* are frequently implicated, and several strains have developed multidrug-resistant profiles (Poolman and Anderson, 2018). *E. coli*, a Gram-negative member of the *Enterobacteriaceae* family, is commonly found in the gastrointestinal tract of animals and in the environment, including soil, water, and plants. While many strains are harmless, pathogenic variants can cause diseases ranging from mild gastrointestinal infections to severe systemic conditions (Nazzaro et al., 2013). *S. aureus*, a Gram-positive member of the family *Staphylococcaceae*, is commonly found as a commensal colonizer of the skin and mucous membranes, particularly the anterior nares, in approximately 20-30% of the human population. While many colonizing strains are asymptomatic, pathogenic variants can cause a wide spectrum of infections, ranging from mild skin and soft tissue infections to severe, life-threatening conditions, including pneumonia, endocarditis, osteomyelitis, and sepsis. Additionally, the

77 emergence of antibiotic-resistant strains, particularly methicillin-resistant *S. aureus* (MRSA), has
78 made this pathogen a major public health concern worldwide.
79 According to the WHO, consumption of contaminated food containing bacteria, viruses, or
80 chemical hazards is responsible for more than 600 million cases of illness and approximately
81 420,000 deaths annually worldwide (WHO, 2022). In this context, *E. coli* is considered a key
82 indicator of antimicrobial resistance among Gram-negative bacteria (Lakhdari et al., 2020;
83 Loayza et al., 2020), while MRSA remains a major concern in food-producing animals and
84 public health surveillance programs (Cain, 2013) The spread of these multidrug-resistant
85 pathogens therefore represents a significant risk of interspecies transmission, including
86 transmission to humans (Wieler et al., 2011; Nait Irahal et al., 2020).
87 Increasing attention has been given to natural alternatives to synthetic antimicrobial agents in the
88 food and pharmaceutical industries. Essential oils (EOs) and their bioactive constituents have
89 emerged as promising candidates due to their broad-spectrum antimicrobial activity and their
90 ability to inhibit both food-spoilage organisms and pathogenic bacteria (Bakkali et al., 2008;
91 Swamy et al., 2016; Sateriale et al., 2022; Laassami et al., 2024).
92 The present study aimed to (i) characterize and compare the chemical composition of *Juniperus*
93 *communis* L. and *Pistacia lentiscus* L. EOs, as well as their 1:1 (v/v) binary mixture, using GC-
94 MS and FTIR analyses, and (ii) investigate their antibacterial activity against a panel of
95 foodborne pathogens, including four reference strains and two bovine mastitis clinical isolates of
96 both Gram-positive and Gram-negative bacteria. We formulated the hypothesis that the binary
97 mixture would demonstrate synergistic antibacterial efficacy greater than that of the individual
98 oils.
99

MATERIALS AND METHODS

Plant Material

Fresh leaves of *J. communis* L. and *P. lentiscus* L. were collected from the Médéa region (Tamezguida, 635 m; 36.21°N, 3.12°E, north-west of Algiers, Algeria) in March 2025. The study area is characterized by a Mediterranean climate and rich biodiversity favorable to aromatic and medicinal plants. The plant materials were identified and authenticated by Dr. Faiza Baali. Representative voucher specimens were deposited at the Department of Biology, University of Ghardaïa (Algeria), under the following reference numbers: *P. lentiscus* L. (MP-2024-6) and *J. communis* L. (MP-2024-7).

Essential Oil Extraction

Essential oils (EOs) were extracted by hydrodistillation using a Clevenger-type apparatus in accordance with the European Pharmacopoeia method (European Pharmacopoeia, 2019). Briefly, 100 g of dried aerial parts of *J. communis* and *P. lentiscus* were mixed with 500 mL of distilled water and subjected to hydrodistillation for 3 h. The obtained oils were separated, dried over anhydrous sodium sulfate, and stored in amber glass vials at 4 °C until analysis.

Preparation of the Essential Oil Mixture

In addition to the individual EOs of *J. communis* L. and *P. lentiscus* L., a binary mixture was prepared by mixing equal volumes of both oils (1:1, v/v). The mixture was freshly prepared immediately before analysis in order to assess possible synergistic, additive, or antagonistic effects arising from the combination of the two EOs. Subsequently, the individual oils and their mixture were characterized by GC-MS and FTIR and tested for their antibacterial activity.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The chemical composition of the EOs was analyzed using an Agilent 6890N gas chromatograph coupled to an Agilent 5973N mass-selective detector (MSD). Separation was achieved on an HP-5MS capillary column (5% phenyl methyl polysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA).

The oven temperature was programmed from 60 °C (held for 5 min) to 220 °C at a rate of 4 °C/min, followed by an increase to 280 °C at 11 °C/min and maintained for 15 min. The total analysis time was 65 min. Injector and transfer-line temperatures were maintained at 280 °C. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min, with a split ratio of 1:50. Mass spectra were acquired in electron impact (EI) mode at 70 eV over a mass range of *m/z* 29-400.

Identification of the volatile constituents was based on comparison of their mass spectra with those stored in the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) mass spectral library and by comparison of their retention indices with literature data whenever available.

Identification of Compounds

For chemical characterization, EO samples were diluted (1:100, v/v) in ethyl acetate and filtered before analysis. Then, 1 µL of each diluted sample was injected into the GC-MS system. Volatile constituents were identified by comparing their calculated linear retention indices (LRIs) and mass spectral similarity indices with data reported in the literature (Costa et al., 2007), and also with the NIST Mass Spectral Library (NIST, 2017). Retention indices were further confirmed using the data of Adams (2001), ensuring reliable compound identification. The LRIs were determined under temperature-programmed conditions and calculated according to the van Den

Dool and Kratz equation (van Den Dool and Kratz, 1963), using a homologous series of *n*-alkanes (C10-C40) analyzed under identical chromatographic conditions. Mass spectral similarity indices (SI/MS) were computed following the method described by Koo et al. (2013). The relative abundance of each identified compound was expressed as a percentage of the total ion chromatogram peak area, without the application of correction factors.

FTIR (Fourier-Transform Infrared Spectroscopy) analysis

FTIR was employed to characterize the principal functional groups present in the EOs. Spectra were recorded in the range of 4000-400 cm^{-1} using an attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectrometer at room temperature. A small aliquot of each EO was directly deposited onto the ATR crystal surface and analyzed according to the procedure described by Stuart (2004). The obtained spectra were interpreted based on the characteristic absorption bands corresponding to the major chemical constituents of the samples.

Bacterial strains

The antibacterial activity of the EOs was evaluated against four reference bacterial strains, including two Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538 and *Bacillus cereus* ATCC 6633, and two Gram-negative bacteria: *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027. In addition, two clinical isolates, namely, *E. coli* and *S. aureus*, were included in this study. These two strains were kindly provided by the Bacteriology Laboratory of the Institute of Veterinary Sciences, University of Blida 1, Algeria. The clinical isolates were recovered from milk samples collected from cows with mastitis. Milk samples were first screened using the California Mastitis Test (CMT). Bacterial isolates were subsequently characterized based on colony morphology and Gram staining. Species identification was confirmed using

commercial biochemical identification systems (API 20E and API Staph; bioMérieux, Marcy-l'Étoile, France).

All bacterial strains were stored at −20 °C in Tryptic Soy Broth (TSB) supplemented with 20% (v/v) glycerol. Prior to antimicrobial testing, cultures were reactivated by inoculating 200 µL of stock culture into 3 mL of TSB and incubating at 37 °C for 24 h. The bacterial inocula were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) and then diluted to approximately 10^6 CFU/mL for antimicrobial assays, according to the Clinical and Laboratory Standards Institute guidelines (NCCLS 1997, 1999). All assays were performed in triplicate.

Antibacterial assay and controls

The antibacterial activity of *J. communis* and *P. lentiscus* EOs, as well as their 50:50 mixture, was evaluated using the disk diffusion method according to Laassami et al. (2024), with slight modifications. Bacterial suspensions were adjusted to 0.5 McFarland standard and uniformly spread onto Mueller-Hinton Agar (MHA) plates using sterile cotton swabs. Sterile filter paper discs (6 mm diameter, Whatman No. 1) were impregnated with 10 µL of pure EO or diluted samples prepared in DMSO and placed on the inoculated agar surface. Plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring inhibition zone diameters (mm), including the disc diameter. All assays were performed in triplicate, and results were expressed as mean ± standard deviation. Gentamicin (40 mg/mL) was used as a positive control, while dimethyl sulfoxide (DMSO) served as a negative control in all experiments.

Broth microdilution assay (MIC and MBC)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the broth microdilution method according to Moghimi et al. (2010) with minor modifications. EOs were emulsified in Mueller-Hinton Broth (MHB) containing 1% (v/v)

DMSO. Serial two-fold dilutions were prepared to obtain final concentrations ranging from 50% to 0.3125% (v/v). Each well was inoculated with a standardized bacterial suspension, and all tests were performed in triplicate.

Growth control wells contained bacterial suspension in MHB, whereas sterility control wells contained MHB only. Gentamicin was tested under the same conditions as the reference antibiotic control. Plates were incubated at 37 °C for 24 h.

The MIC was defined as the lowest concentration at which no visible bacterial growth was observed. For MBC determination, aliquots from non-turbid wells were subcultured onto Mueller-Hinton Agar and incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration showing no colony growth.

Checkerboard Assay and Fractional Inhibitory Concentration Index (FICI)

The interaction between *J. communis* and *P. lentiscus* EOs was evaluated using the checkerboard microdilution method (Odds, 2003; Bassolé and Juliani, 2012). Serial two-fold dilutions of each EO were prepared in Mueller-Hinton Broth and combined in varying concentration ratios in 96-well microplates to assess potential synergistic, additive, or antagonistic effects.

Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical comparisons among treatments were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. Analyses were carried out using IBM SPSS Statistics® version 25 (IBM Corp, Armonk, NY, USA).

Pearson correlation analysis was applied to assess relationships between the chemical composition of the EOs and their antibacterial activity. In addition, principal component analysis

(PCA) was performed to explore multivariate relationships among samples and variables and to visualize clustering patterns.

RESULT AND DISCUSSION

Essential oil yield

The essential oil yields obtained from the leaves of *J. communis* and *P. lentiscus* collected from Tamezguida region (Médéa Province, a mountainous area of the Blidéen Atlas, Algeria) were 0.94% and 0.54% (w/w, dry weight basis), respectively. These yields were higher than those reported for some Algerian populations, including 0.26% for the EO obtained from dried needles of *J. communis* (Dahmane et al., 2016) and 0.22% for the EO obtained from leaves of *P. lentiscus* collected in El Kala, Algeria (Souilah et al., 2023). For *P. lentiscus*, a yield of 0.125% was also reported for leaves collected in the Tizi-Ouzou region, further illustrating the variability among Algerian populations (Sehaki et al., 2022; Djenad et al., 2026). Such differences may be attributed to geographical origin, environmental conditions, phenological stage, plant material, and extraction procedures and parameters, all of which can influence essential oil yield.

Chemical composition of the essential oil

The chemical profiles of *J. communis* and *P. lentiscus* EOs were characterized by GC-MS analysis, and the identified constituents are summarized in Table 1. The GC-MS analysis revealed distinct chemical profiles for the EOs of *J. communis*, *P. lentiscus*, and their 50:50 mixture. Overall, the EOs were predominantly characterized by monoterpene hydrocarbons, which accounted for 44.89%, 60.39%, and 71.42% of the total identified compounds in *J. communis*, *P. lentiscus*, and their 50:50 mixture, respectively. Oxygenated monoterpenes represented 1.03%, 12.73%, and 7.11%, respectively, whereas sesquiterpene hydrocarbons accounted for 17.64%, 7.69%, and 14.71%, and oxygenated sesquiterpenes represented 9.08%,

0.64%, and 1.14%, respectively. Other compounds accounted for 0.13%, 0.28%, and 2.20%, respectively. Overall, the identified compounds represented 72.77%, 81.73%, and 96.58% of the total composition of the *J. communis*, *P. lentiscus*, and mixed essential oils, respectively (Table 1).

α-Pinene was the predominant constituent among the identified compounds in the present study, accounting for 20.44% of the J. communis oil, 13.16% of the P. lentiscus oil, and 27.17% of their 50:50 mixture. The predominance of monoterpene hydrocarbons observed in J. communis is consistent with previous and recent studies of Algerian Juniperus essential oils. The essential oil of J. communis from Mostaganem (western Algeria), obtained by hydrodistillation, contained 74 identified compounds, with α-pinene (14.2%), terpinen-4-ol (14.1%), and sabinene (12.4%) as the major constituents (Dahmane et al., 2016). Similarly, Malti et al. (2025) reported α-pinene-rich leaf essential oils of J. oxycedrus from northwestern Algeria, although substantial chemical variability was observed among populations. More recently, Medjekane et al. (2026) reported a monoterpene-rich essential oil of J. phoenicea from El Bayadh, Algeria, in which α-terpinolene, limonene, and terpinen-4-ol, rather than α-pinene, were the major constituents.

The chemical profile of the P. lentiscus essential oil obtained in the present study is also broadly consistent with previous investigations of Algerian populations. Mecherara-Idjeri et al. (2008) analyzed nine samples of fruit oil from wild P. lentiscus L. collected from different regions of Algeria and found that the oils were dominated by monoterpene hydrocarbons, with α-pinene (37.9–51.5%) and myrcene (27.0–69.7%) as the major constituents. Hamiani et al. (2016) reported an essential oil yield of 1.26% from P. lentiscus collected in Oran, Algeria, and identified 20 compounds, among which terpinen-4-ol (41.24%), β-caryophyllene (12.62%), β-myrcene (10.5%), α-pinene (9.48%), and limonene (9.11%) were the major constituents.

Similarly, Medjkane et al. (2016) reported an essential oil yield of 1.28% (w/w) from dried P. lentiscus leaves. Their oil was predominantly composed of monoterpene hydrocarbons (65.56%), with α-pinene (15.47%), limonene (14.70%), β-myrcene (9.93%), and β-pinene (7.31%) as the major constituents. Dob et al. (2006), however, reported that longifolene was the predominant constituent of P. lentiscus oils from Algiers (12.8%) and Tizi-Ouzou (16.4%), whereas α-pinene was the major constituent of the oil from Oran (19.0%). More recently, Nouar et al. (2025) reported that the essential oil of P. lentiscus from Taougrite, Chlef (northwestern Algeria), contained 39 identified compounds, with limonene (17.7%) and α-pinene (15.8%) as the major constituents. EOs from P. lentiscus collected in El Kala, Algeria, were also analyzed by Souilah et al. (2023), who identified 27 aroma compounds, predominantly monoterpene and sesquiterpene hydrocarbons. Collectively, these studies indicate that α-pinene and other monoterpene hydrocarbons can be important constituents of P. lentiscus essential oils, although their relative abundance varies considerably among Algerian populations.

Such variability may be related to geographical origin, environmental conditions, seasonal and phenological variation, plant material, and extraction procedures and parameters. Indeed, recent Algerian studies have demonstrated considerable geographical, seasonal, and processing-related variability in the chemical composition of P. lentiscus essential oils (Sehaki et al., 2022; Benterrouche et al., 2023; Nouar et al., 2025; Djenad et al., 2026). Therefore, the predominance of monoterpene hydrocarbons observed in the present P. lentiscus oil should be regarded as one of the chemical profiles occurring among Algerian populations rather than as evidence of a uniform Maghrebian chemotype.

The major constituents identified across the three oils in the present study included α-pinene, β-caryophyllene, limonene, β-myrcene, terpinen-4-ol, and Δ-3-carene, although their relative

abundances differed among the individual oils. The 50:50 mixture was characterized by relatively high levels of α-pinene, limonene, β-myrcene, and α-terpinene, reflecting the combined chemical contributions of the two parent oils. In contrast, P. lentiscus exhibited a higher proportion of oxygenated monoterpenes, particularly terpinen-4-ol (7.71%), whereas J. communis was richer in sesquiterpene hydrocarbons and oxygenated sesquiterpenes, including β-caryophyllene, α-humulene, cedrol, and caryophyllene oxide.

The high abundance of α-pinene in the present oils is also of biological interest because this monoterpene exhibits a broad range of reported biological activities. In bone metabolism, α-pinene promotes osteoblast differentiation and mineralization while reversing TNFα-induced inhibition (Min et al., 2020). Mechanistically, it suppresses pro-inflammatory signaling through the MAPK and NF-κB pathways (Kim et al., 2015) and enhances total antioxidant capacity in human blood cells (Turkez and Aydin, 2016). In addition, α-pinene has demonstrated promising antimicrobial and anticancer properties (Salehi et al., 2019), as well as potential applications in cosmetic formulations for improving skin health (Grzeszczak et al., 2024).

To further explore the chemical relationships among samples, hierarchical cluster analysis (HCA) was performed based on the relative abundances of identified compounds (Figure 1). The dendrogram revealed two main clusters: *P. lentiscus* grouped with the 50:50 mixture, indicating a higher chemical similarity between these samples. This clustering is mainly associated with their relatively higher proportions of monoterpene hydrocarbons and oxygenated monoterpenes, including limonene, β-myrcene, γ-terpinene, and terpinen-4-ol. In contrast, *J. communis* formed a distinct cluster, reflecting its higher content of sesquiterpenes and oxygenated sesquiterpenes.

These results suggest that the chemical profile of the mixture is more strongly influenced *by P. lentiscus*, although characteristic constituents from both oils contribute to its final composition.

The predominance of monoterpene hydrocarbons in the mixture may enhance its biological activity due to the well-documented antimicrobial properties of compounds such as α-pinene and limonene. Moreover, the coexistence of monoterpenes and sesquiterpenes may promote synergistic interactions, potentially contributing to the improved antibacterial activity observed in subsequent bioassays. Basa et al. (2022) analyzed the EOs of *J. oxycedrus* subsp. *macrocarpa* from Oum El Bouaghi (Algeria), identifying 57 compounds, with 5-tetradecen-1-ol acetate and muurolene among the major constituents.

FTIR analysis of the essential oils

The FTIR spectra of the 50:50 essential oil mixture (top), *P. lentiscus* (middle), and *J. communis* (bottom) are presented in Figure 2. The corresponding absorption bands, along with their respective wavenumbers and functional group assignments, are summarized in Table 2.

The spectra exhibited characteristic absorption bands consistent with the terpene-rich nature of the EOs, confirming the presence of functional groups such as alkenes, alcohols, and esters, as previously identified by GC-MS analysis.

The FTIR spectra of *J. communis*, *P. lentiscus*, and their 50:50 mixture showed comparable profiles, confirming the predominance of terpene-based compounds. The main absorption bands were attributed to aliphatic C–H stretching vibrations (~2920 cm^{-1}), carbonyl groups (1738–1739 cm^{-1}), and C–O stretching vibrations (1260-1000 cm^{-1}), which are characteristic of EOs rich in mono- and sesquiterpenes (Bakkali et al., 2008; Dhifi et al., 2016). A more intense O–H stretching band was observed in *P. lentiscus*, suggesting a higher proportion of oxygenated constituents, particularly alcohol-type compounds. The mixture exhibited an intermediate spectral profile, reflecting the combined contribution of both EOs.

The predominance of bioactive monoterpenes and sesquiterpenes, particularly α-pinene, limonene, terpinen-4-ol, and β-caryophyllene, suggests a significant role of these compounds in the antibacterial activity observed for both individual EOs and their mixture. These constituents have been widely reported for their membrane-disrupting and antimicrobial properties (Bakkali et al., 2008; Hyldgaard et al., 2012).

Antibacterial activity

The antibacterial activity of *J. communis*, *P. lentiscus* EOs, and their 50:50 mixture was evaluated against clinical and reference bacterial strains, and the results are presented in Table 3 and Figure 3.

The mixture (50:50) was evaluated against six bacterial strains and compared with gentamicin. Results are expressed as inhibition zone diameters (mm). The mixture showed strong activity against *S. aureus*, while Gram-negative bacteria were less sensitive. Gentamicin exhibited overall higher antibacterial activity, as expected for a broad-spectrum antibiotic (Wiegand et al., 2008; EUCAST, 2023).

The antibacterial activity of *J. communis*, *P. lentiscus* EOs, and their 50:50 mixture was evaluated against clinical and reference bacterial strains. Overall, all tested samples exhibited measurable inhibitory effects, with clear variations depending on the bacterial species. In general, Gram-positive bacteria were more susceptible than Gram-negative bacteria, which is commonly attributed to structural differences in the cell envelope and the presence of an outer membrane that limits the penetration of hydrophobic compounds (Nikaido, 2003; Trombetta et al., 2005; Bakkali et al., 2008; Benmoumou et al., 2025).

Among all tested strains, *S. aureus* showed the highest sensitivity, with the greatest inhibition zone observed for the EO mixture (28.0 ± 1.4 mm), followed by *B. cereus* (24.0 ± 1.4 mm) and *S.*

aureus ATCC 6538 (22.5 ± 2.1 mm). In contrast, Gram-negative bacteria exhibited lower inhibition zones (14-15 mm), indicating reduced susceptibility. Similar antibacterial activity has been reported for other *Juniperus* species, including *J. phoenicea*, whose EO exhibited inhibitory effects against several Gram-positive and Gram-negative bacteria (Ait-Ouazzou et al., 2012). EOs exert their antimicrobial effects through multiple mechanisms, including the inhibition of efflux pumps, which reduces bacterial drug resistance, and the scavenging of reactive oxygen species (ROS), thereby mitigating oxidative stress in target cells (Iskandar et al., 2025). The mixture generally showed higher or comparable activity compared to individual EOs, suggesting potential interactions between their bioactive constituents. However, this improvement was strain-dependent rather than uniform across all bacteria, which is consistent with previous reports on the variable synergistic effects of EO combinations (Bassolé and Juliani, 2012; Hyldgaard et al., 2012; Sateriale et al., 2022). The activity of a complex mixture is not easily attributed to a unique or specific constituent. Major or minor compounds present in the EO may cause antimicrobial activity. Possible synergistic and antagonistic interactions between compounds in the oil should be considered (Lopes-Lutz et al., 2008; Soltani et al., 2023). Basa et al. (2022) reported that the essential oils of *J. oxycedrus* subsp. *macrocarpa* exhibited potential antibacterial activity against a range of bacterial species.

MIC and MBC determination

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *J. communis*, *P. lentiscus* EOs, and their 50:50 mixture against the tested bacterial strains are presented in Table 4.

MIC and MBC results confirmed the antibacterial activity observed in the diffusion assay. Variations in MIC and MBC values were observed depending on both the bacterial strain and the

tested sample. In all cases, the MBC/MIC ratio was ≤ 4, indicating that all EO formulations exerted a bactericidal effect. According to Pankey and Sabath (2004), antimicrobial agents are generally considered bactericidal when the MBC/MIC ratio is ≤ 4, whereas ratios > 4 indicate a bacteriostatic effect. Therefore, the tested EOs, whether used individually or in combination, demonstrated bactericidal activity against all bacterial strains evaluated (Pankey and Sabath, 2004; Wiegand et al., 2008).

The EO mixture generally exhibited lower MIC and MBC values than the individual oils, suggesting enhanced antibacterial efficacy. This improvement may result from complementary or synergistic interactions among bioactive constituents, which can increase their overall antimicrobial effectiveness (Bassolé and Juliani, 2012; Yap et al., 2014). The observed activity may be attributed to the high content of terpenes and oxygenated terpenoids, which are known to disrupt bacterial cell membranes, increase membrane permeability, cause leakage of intracellular constituents, and ultimately lead to cell death (Cox et al., 2000; Trombetta et al., 2005; Bakkali et al., 2008).

FICI analysis

The checkerboard assay revealed predominantly additive interactions between *J. communis* and *P. lentiscus* EOs, with FICI values ranging from 0.60 to 0.75 (Table 5, Figure 4). A synergistic interaction was observed only against *S. aureus* ATCC 6538 (FICI = 0.49), while no antagonistic effects were detected.

These findings indicate that the combination of both oils does not systematically enhance antibacterial activity but may improve efficacy in specific bacterial contexts. Similar results have been reported in previous studies, where EO combinations frequently produce additive rather

than synergistic effects depending on chemical composition and microbial susceptibility (Bassolé and Juliani, 2012; Yap et *al.*, 2014).

The observed additive effects against *B. cereus*, *E. coli* (clinical and ATCC 8739 strains), and *P. aeruginosa* suggest that both oils likely act through similar mechanisms, primarily by disrupting bacterial cell membranes. EO constituents, particularly monoterpenes, exert antibacterial effects by altering membrane integrity, increasing permeability, and causing leakage of intracellular components, which often results in additive interactions when mechanisms overlap (Cox et al., 2000; Trombetta et al., 2005).

The synergistic effect observed against *S. aureus* ATCC 6538 may be explained by enhanced membrane permeability in Gram-positive bacteria, which lack an outer membrane and therefore allow easier penetration of hydrophobic compounds. This is consistent with Bassolé and Juliani (2012), who reported that synergism may occur when one component enhances the cellular uptake or activity of another.

GC-MS analysis revealed that the oils are rich in monoterpene hydrocarbons (α-pinene, β-pinene, and camphene) alongside minor oxygenated compounds. As reported by Bakkali et al. (2008) and Hyldgaard et al. (2012), the bioactivity of EOs typically arises from additive and synergistic interactions among multiple constituents, rather than from a single dominant compound.

Taken together, the present findings demonstrate that the antibacterial interactions between *J. communis* and *P. lentiscus* EOs depend on both the bacterial species and the susceptibility of the tested strains. While additive effects predominated, the synergistic interaction observed against *S. aureus* ATCC 6538 highlights the potential of specific EO combinations to improve antimicrobial efficacy. These observations emphasize the importance of considering the complex interactions among EO constituents when developing natural antimicrobial formulations. Further

418 investigations are needed to elucidate the molecular mechanisms underlying these interactions
419 and to assess their efficacy and safety *in vivo*.

420 **CONCLUSION**

421 This study demonstrated that the EOs of *J. communis* and *P. lentiscus*, individually and in a 1:1
422 combination, possess significant antibacterial activity against both clinical and reference bacterial
423 strains. The EO mixture exhibited enhanced inhibitory effects, particularly against *S. aureus*, with
424 antibacterial activity approaching that of gentamicin in some cases. MIC and MBC values,
425 together with MBC/MIC ratios ≤ 4, confirmed the bactericidal nature of all tested formulations.
426 Furthermore, FICI analysis indicated mainly additive interactions and revealed a synergistic
427 effect against *S. aureus* ATCC 6538 (FICI = 0.49). These findings suggest that combining *J.*
428 *communis* and *P. lentiscus* EOs may represent an effective natural antimicrobial approach,
429 especially against Gram-positive bacteria. Further studies are warranted to elucidate their
430 mechanisms of action and evaluate their potential applications in pharmaceutical and food
431 preservation systems.

432

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437 Nacéra Tadjine: Conceptualisation, Data curation, Formal analysis, Investigation, Methodology,
438 Software, Resources, Supervision, Validation, Visualisation, writing – original draft, Writing –
439 review & editing. Atika Benrima: Investigation, Methodology, Resources, Supervision,
440 Validation. Noureddine Bouras: Supervision, Validation, Visualisation, Writing – review &

441 editing. Fawzi Rostane Meklati: Supervision, Validation, Visualisation, Writing – review &
442 editing.

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444 The authors declare that they have no known competing financial interests or personal
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453 We declare that we used AI-based tools to assist in language correction and sentence refinement.

454

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Table 1. Chemical composition of the essential oils from *J. communis* L., *P. lentiscus* L., and their 50:50 mixture (Only compounds ≥ 0.1% are presented)

Compound	Retention time (min)	*J. communis* (%)	*P. lentiscus* (%)	Mixture 50:50 (%)
Tricyclene	10.14	0	0.80	0
Bicyclic monoterpene	10.68	0	0.99	0
α-Pinene	11.46	20.44	13.16	27.17
Camphene	11.88	0	2.72	1.77
β-Pinene	13.94	0	6.59	5.64
β-Myrcene	15.37	3.31	8.42	8.22
α-Phellandrene	16.07	3.08	3.31	1.94
Sabinene	15.97	0.15	0	0
Δ-3-Carene	16.58	9.37	0.10	0.13
α-Terpinene	16.89	0.59	0.67	5.85
p-Cymene	17.46	1.22	4.99	2.97
Limonene	17.79	2.85	9.73	9.22
β-Ocimene	19.49	0	0	0.62
γ-Terpinene	20.01	0.68	5.78	3.87
β-Phellandrene	20.85	0	0.17	1.32
L-Fenchone	21.87	0	0.20	0.07
α-Terpinolene	22.18	3.20	2.96	2.70
n-Amyl isovalerate	23.85	0	0.28	0.54
Dihydrocarvone/Cyclohexanone	26.88	0.13	0	0.12
Terpinen-4-ol	28.48	0.73	7.71	5.00
α-Terpinyl acetate	29.58	0	0.54	1.43
3-Cyclohexene-1-methanol	29.74	0	2.9	0
Thymyl methyl ether	33.43	0.30	0	0
Bornyl acetate	35.99	0	1.38	0.61
α-Cubebene	41.75	0	0	0.19
Bicyclopentylidene	42.90	0	0	0.12
β-Caryophyllene	44.53	9.83	3.74	6.74
α-Caryophyllene	46.48	0	0	1.40
α-Humulene	46.61	3.08	0.96	1.94
epi-bicyclosesquiphellandrene	47.08	1.22	0.3	0.30
Germacrene D	48.29	1.74	2.33	1.53
Butanoic acid	49.19	0	0	1.42
α-Muurolene	49.53	0	0	0.89
δ-Cadinene	50.98	1.77	0.36	1.72
Caryophyllene oxide	54.19	3.92	0	0
Cedrol	55.18	4.13	0	1.14
t-Cadinol	58.28	1.03	0.64	0
Monoterpene hydrocarbons		44.89	60.39	71.42
Oxygenated monoterpenes		1.03	12.73	7.11
Sesquiterpene hydrocarbons		17.64	7.69	14.71
Oxygenated sesquiterpenes		9.08	0.64	1.14
Other compounds		0.13	0.28	2.20
Total identified compounds		72.77	81.73	96.58

Table 2. Correlation between GC-MS-identified compounds and FTIR absorption bands (FTIR-GC-MS correlation of major functional groups and volatile constituents)

GC-MS compound (examples)	Functional group/chemical class	FTIR band (cm^{-1})	Vibrational assignment
α-Pinene/β-Pinene	Monoterpene hydrocarbons (alkene + aliphatic C–H)	2915–2920	C–H stretching (CH_3, CH_2)
Limonene	Unsaturated monoterpene hydrocarbon	2833–2877	Symmetric/asymmetric C–H stretching
Myrcene	Conjugated diene (acyclic monoterpene)	1640	C=C stretching vibration
Terpinene/Terpinolene	Unsaturated monoterpenes	1600–1650	C=C stretching vibrations
Terpinen-4-ol	Oxygenated monoterpene (alcohol)	1218–1219	C–O stretching (alcohol)
Carvone/Camphor	Oxygenated monoterpene (ketone)	1738–1739	C=O stretching (carbonyl group)
Sesquiterpenes (e.g., β-caryophyllene)	Sesquiterpene hydrocarbons	1443–1366	CH_2 / CH_3 bending vibrations
Unsaturated terpenes	General terpene skeleton	989–787	=C–H out-of-plane bending

665 **Table 3.** Antibacterial activity (inhibition zone diameters in mm) of *J. communis*, *P. lentiscus*,
666 and their mixture against reference strains and clinical isolates from bovine mastitic milk
667 (Clinical isolates were recovered from bovine mastitic milk samples, whereas ATCC strains were
668 used as reference and quality control microorganisms to ensure reproducibility and allow
669 standardized comparison of antimicrobial susceptibility)

Microorganism	Origin	*J. communis*	*P. lentiscus*	Mixture	Gentamicin
S. aureus (clinical isolate)	Mastitic milk	19.0 ± 2.4	22.0 ± 1.3	28.0 ± 1.4	27.0 ± 0.5
S. aureus ATCC 6538	Reference strain	15.0 ± 0.7	17.5 ± 1.6	22.5 ± 2.1	24.0 ± 0.1
B. cereus ATCC 6633	Reference strain	17.0 ± 1.4	14.2 ± 1.2	24.0 ± 1.4	29.0 ± 0.2
E. coli (clinical isolate)	Mastitic milk	11.0 ± 1.6	12.0 ± 1.4	15.0 ± 1.8	14.5 ± 0.2
E. coli ATCC 8739	Reference strain	12.5 ± 1.4	11.0 ± 1.4	14.0 ± 1.2	17.0 ± 0.1
P. aeruginosa ATCC 9027	Reference strain	11.0 ± 2.3	12.5 ± 1.9	14.0 ± 1.3	15.0 ± 0.5

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673 **Table 4.** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration
674 (MBC) values (%) of *J. communis*, *P. lentiscus* essential oils, and their 50:50 mixture against the
675 tested bacterial strains (MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal
676 concentration. Values are expressed as percentages (% v/v))
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Bacterial strain	*J. communis*		*P. lentiscus*		Mixture (50:50)	
	MIC (%)	MBC (%)	MIC (%)	MBC (%)	MIC (%)	MBC (%)
S. aureus (clinical isolate)	6.25	12.50	3.12	6.25	3.12	3.12
S. aureus ATCC 6538	15.00	20.00	12.50	15.00	6.25	6.25
B. cereus ATCC 6633	6.25	12.50	12.50	20.00	3.12	6.25
E. coli (clinical isolate)	20.00	25.00	20.00	25.00	6.25	10.00
E. coli ATCC 8739	20.00	25.00	25.00	25.00	6.25	12.50
P. aeruginosa ATCC 9027	25.00	25.00	20.00	25.00	15.00	20.00

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683 **Table 5.** Fractional Inhibitory Concentration Index (FICI) of *J. communis* and *P. lentiscus*
684 essential oils used in combination against tested bacterial strains [FICI = (MIC of *J. communis* in
685 combination/MIC of *J. communis* alone) + (MIC of *P. lentiscus* in combination/MIC of *P.*
686 *lentiscus* alone). FICI values ≤ 0.50 indicate synergism, whereas values between 0.50 and 1.00
687 indicate an additive effect.]
688

Bacterial strain	MIC *J. communis* (%)	MIC *P. lentiscus* (%)	MIC *J. communis* in combination (%)	MIC *P. lentiscus* in combination (%)	FICI	Interaction
S. aureus (clinical isolate)	6.25	3.12	0.75	1.50	0.60	Additive
S. aureus ATCC 6538	15.00	12.50	3.70	3.00	0.49	Synergistic
B. cereus ATCC 6633	6.25	12.50	1.50	6.25	0.75	Additive
E. coli (clinical isolate)	20.00	20.00	5.00	10.00	0.75	Additive
E. coli ATCC 8739	20.00	25.00	5.00	12.50	0.75	Additive
P. aeruginosa ATCC 9027	25.00	20.00	6.25	10.00	0.75	Additive

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693 **FIGURE CAPTIONS**

694 **Fig. 1.** Hierarchical clustering heatmap based on GC-MS profiles of *J. communis* L., *P. lentiscus*
695 L., and their 50:50 mixture

696 **Fig. 2.** FTIR spectra of *J. communis* L., *P. lentiscus* L., and their 50:50 mixture

697 **Fig. 3.** Antibacterial activity of essential oils, their mixture, and gentamicin

698 **Fig. 4.** Fractional Inhibitory Concentration Index (FICI) values for the combination of *J.*
699 *communis* L. and *P. lentiscus* L. essential oils against the tested bacterial strains

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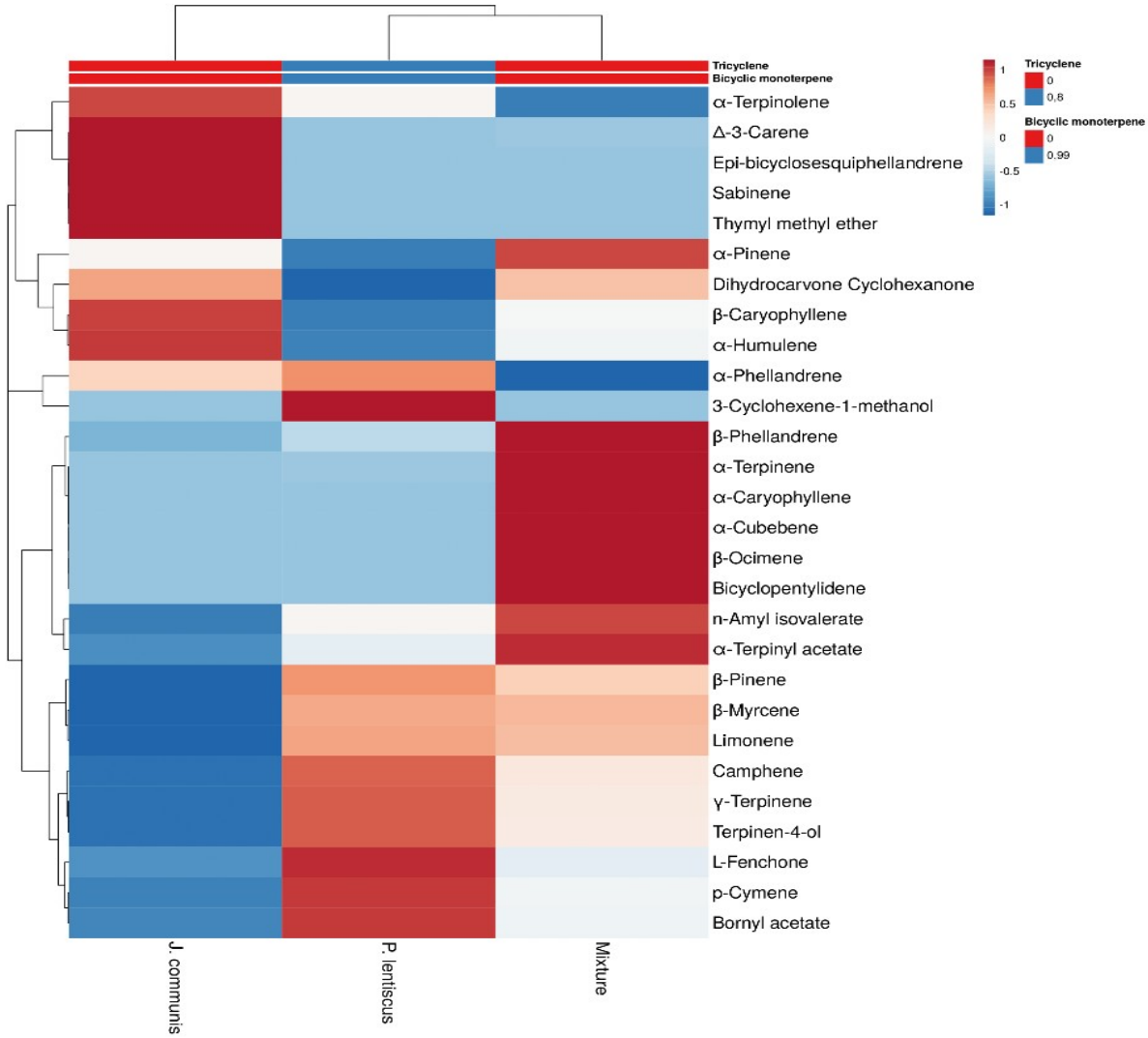

Figure 1. Hierarchical clustering heatmap based on GC-MS profiles of *J. communis* L., *P. lentiscus* L., and their 50:50 mixture

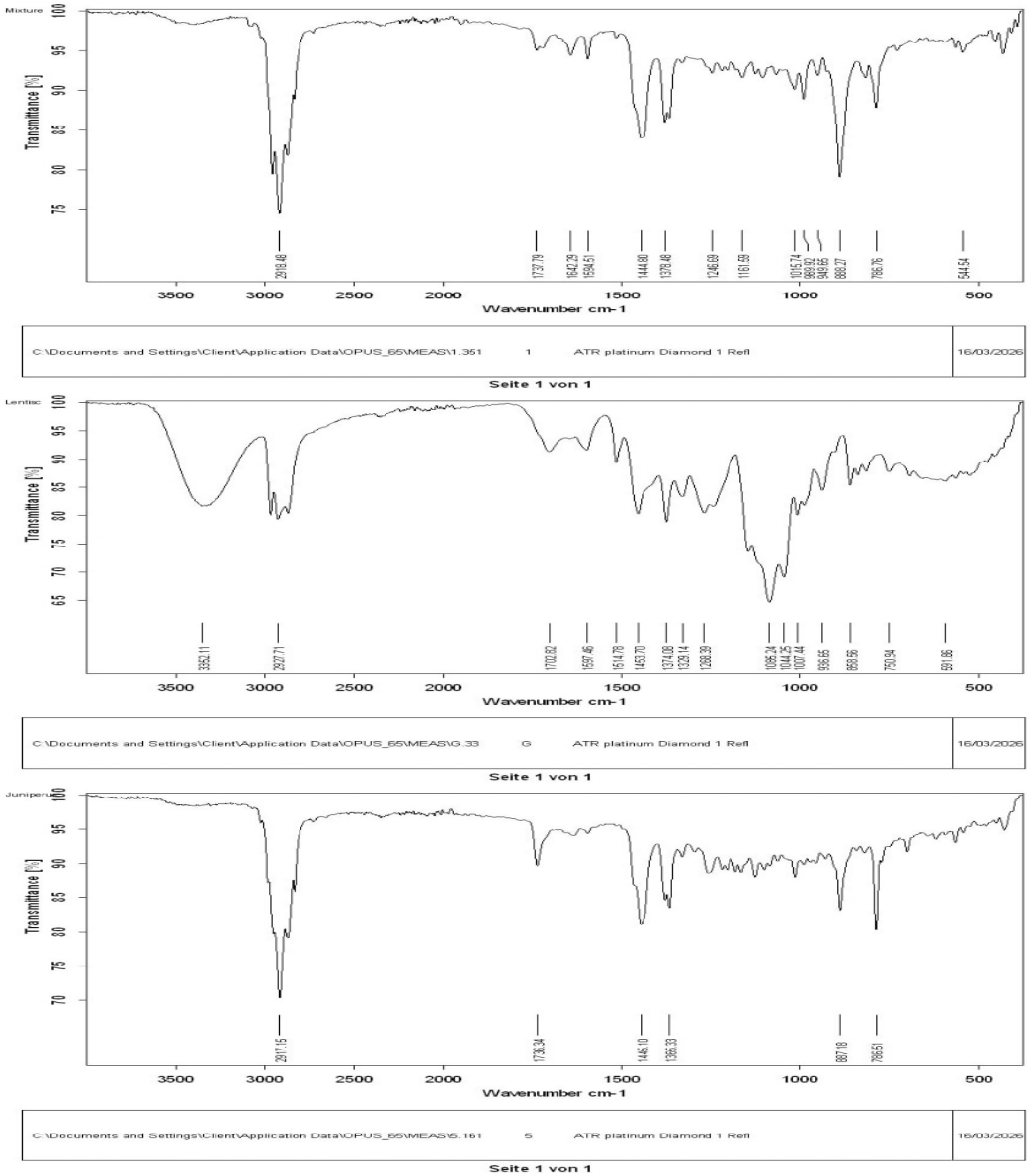

Figure 2. FTIR spectra of *J. communis* L., *P. lentiscus* L., and their 50:50 mixture

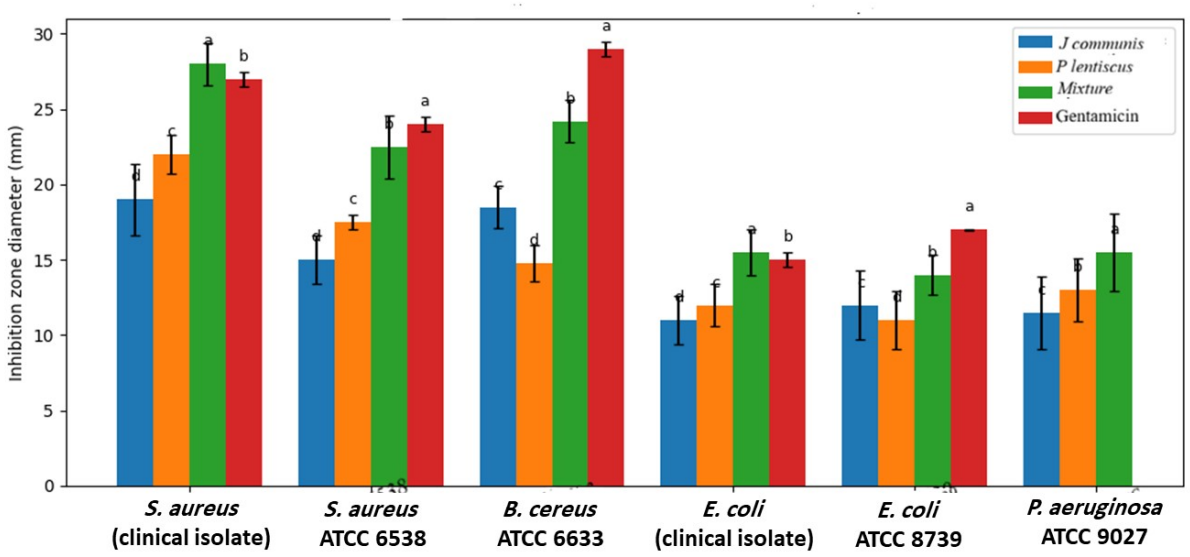

Figure 3. Antibacterial activity of essential oils, their mixture, and gentamicin

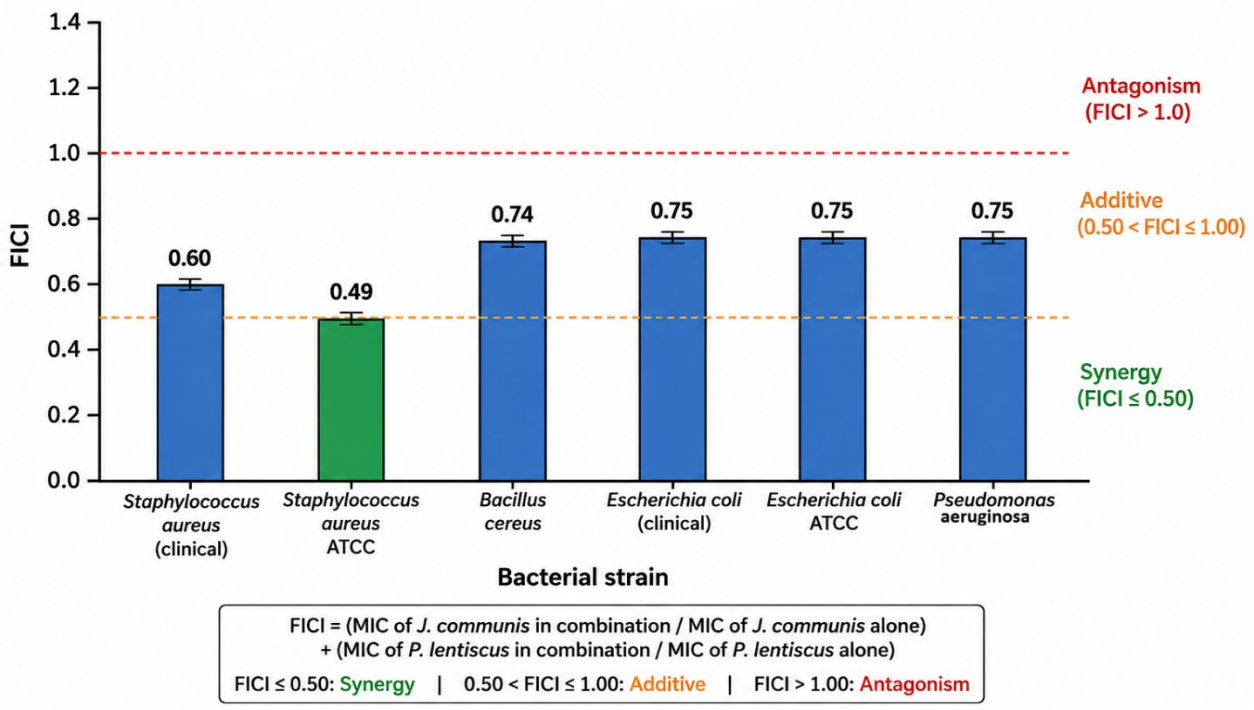

Figure 4. Fractional Inhibitory Concentration Index (FICI) values for the combination of *J. communis* L. and *P. lentiscus* L. essential oils against the tested bacterial strains