Original Article

Antibacterial and Antibiofilm Activity of Lavandula angustifolia Essential Oil for Inhibiting Primary Biofilm Colonizers: An In Vitro Study

Lara S. Mohammed Raouf[*1], *Faraedon M. Zardawi*[2]

Abstract

Objective: The present study aimed to assess the effectiveness of Lavandula angustifolia (L. angustifolia) essential oil (EO) in inhibiting bacterial growth and biofilm formation of primary colonizers, such as Streptococcus sanguinis (S. Sanguinis), Streptococcus mitis (S. mitis), and Streptococcus oralis (S. oralis), in an in vitro setting.

Methods: Gas chromatography-mass spectrometry (GC–MS) analysis was used to examine the oil extracted from L. angustifolia EO by hydrodistillation. Agar well diffusion and broth dilution techniques were performed to evaluate antibacterial activity and Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs), respectively, against the three American Type Culture Collection (ATCC) strains. In contrast, the qualitative tube method was performed to assess the antibiofilm effect. Chlorhexidine 0.12% was used as a positive control, and all experiments were performed in triplicate (n = 3). Data were analyzed using SPSS (version 26), with $p \leq 0.05$ considered statistically significant.

Results: GC–MS identified 25 constituents in L. angustifolia EO, with linalool (20.99%) predominant. The EO (5–20%) showed dose-dependent antibacterial activity, producing inhibition zones up to 16.2 mm compared with 16–18 mm for chlorhexidine. MIC/MBC values (µL/mL) were 1.56/3.125 for S. mitis, 0.39/0.781 for S. oralis, and 0.156/3.12 for S. sanguinis. For the mixed-species consortium, MIC and MBC were 0.156 and 3.12 µL/mL, respectively. The EO demonstrated moderate to weak antibiofilm activity.

Conclusions: L. angustifolia EO showed concentration-dependent antibacterial activity against primary oral colonizers. While chlorhexidine 0.12% produced larger inhibition zones overall, L. angustifolia EO at higher concentrations, especially 15%, demonstrated comparable efficacy against S. mitis. These findings suggest its potential as a supplementary therapeutic natural adjunct for preventing early plaque formation.

Keywords: *Lavandula angustifolia, Antibacterial effect, Antibiofilm activity, Primary biofilm colonizers.*

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1. *Periodontics Department, College of Dentistry, University of Sulaimani, Sulaimani, Iraq.*
2. *Dean of Faculty of Dentistry, Qaiwan International University, Sulaymaniyah 46001, Iraq.*

** Corresponding author*: *Lara.mohammedraouf@univsul.edu.iq.*

Introduction

Periodontal diseases, such as gingivitis and periodontitis, are chronic, multifactorial inflammatory diseases that primarily result from an immune response that is exacerbated by dysbiotic, diverse bacterial biofilms. These diseases and conditions affect the supporting structures of the teeth[1]. Investigations of the oral cavity, especially the dental biofilm, have found more than 700 different species of bacteria living in a very organized yet very diverse microbial community that plays a crucial role in periodontal health and disease[2,3].

Initial biofilm colonizers, including *Streptococcus oralis, Streptococcus mitis,* and *Streptococcus sanguinis*, which make up the yellow complex of the typical oral flora, are proposed to be crucial in forming the initial biofilm matrix and account for around 80% of the nascent biofilm. These species form an attachment framework for later colonizers such as *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Porphyromonas gingivalis* to colonize gingival pockets and firmly establish themselves, ultimately causing disease. These streptococci facilitate bacterial adherence and enhance biofilm stability through the production of extracellular polysaccharides, making them key targets for strategies aimed at preventing biofilm-induced periodontal diseases[4, 5].

Although mechanical methods such as brushing and flossing are effective for removing biofilms, they are often insufficient, particularly in inaccessible areas. Furthermore, individual differences in oral hygiene practices, dexterity, and compliance often lead to the persistence of pathogenic biofilms, necessitating antimicrobial agents as an adjunct[6,7].

Chlorhexidine mouthwash has long been considered the benchmark for chemical plaque control because of its broad-spectrum antimicrobial action. Its cationic molecules bind to bacterial cell walls, alter membrane permeability, and cause leakage of intracellular components, ultimately leading to cell death. However, prolonged use is associated with undesirable side effects, such as dental staining, taste alteration, mucosal irritation, and the risk of bacterial resistance, prompting continuous interest in safer and more biocompatible alternatives[7-9].

In this context, natural plant-derived products, particularly essential oils, have attracted considerable attention due to their diverse bioactive components, favorable safety profile, and lower cost.

Several *in vitro* investigations have indicated that herbal mouthrinses can achieve antibacterial effects comparable to chlorhexidine. Among these, *Lavandula angustifolia* (lavender) essential oil (EO) has been recognized for its analgesic, anti-inflammatory, and antimicrobial activities against both Gram-positive and Gram-negative organisms, primarily attributed to its key constituents, linalool and linalyl acetate[10-12].

The chemical composition of *L. angustifolia* EO varies widely depending on factors such as chemotype, climate, geography, and harvest conditions, as reflected in GC–MS analyses from different regions, including studies from Iraq and the Kurdistan Region. Reported profiles often show notable shifts in the relative abundance of major constituents such as linalool, linalyl acetate, and terpinen derivatives, emphasizing the importance of chemical characterization when interpreting biological activity[13-15].

In studies, lavender EO has shown antibacterial properties against periodontal pathogens like *P. gingivalis, P. intermedia,* and *F. nucleatum*[16,17]. However, they examined oils from different regions, cultivars, or chemotypes, making direct comparisons difficult. Moreover, these studies have focused on established periodontal pathogens rather than early oral streptococcal colonizers. The antibacterial and antibiofilm properties of *L. angustifolia* EO against *S. mitis*, *S. oralis*, and *S. sanguinis* have not been investigated.

Understanding its capacity to suppress bacterial growth and impede early biofilm development could introduce a promising natural approach to periodontal disease prevention. Accordingly, this study aimed to evaluate the antibacterial and biofilm-inhibiting effects of *L. angustifolia* EO on *S. mitis, S. oralis,* and *S. sanguinis* in an *in vitro* setting. These findings could support the development of plant-based antimicrobial formulations aimed at preventing initial biofilm formation and minimizing the onset of biofilm-associated oral diseases.

Materials and Methods

Study design and setting

This experimental study was conducted in the Microbiology Laboratory of the College of Dentistry at the University of Sulaimani, College of Agricultural Engineering Sciences for plant identification, and the Bahar factory for essential oil extraction.

Plant collection and identification

Fresh stems and flowers of *L. angustifolia* were supplied by Bahar Factory (Sulaymaniyah, Kurdistan Region of Iraq), which collected the plants in March from cultivated lavender fields in the Sulaymaniyah governorate (Figure 1). Assistant Professor Dr. Lanja

Hewa Khal (PhD, Plant Taxonomist, College of Agricultural Engineering Sciences, University of Sulaimani) confirmed the plant species and its cultivation status based on standard morphological characteristics. The Ethical Committee at the College of Dentistry, University of Sulaimani, approved the research project with Code No. (COD-EC-24-0020) on December 16, 2024.

Essential oil extraction

The EO was extracted using the hydrodistillation method at the Bahar factory in Sulaymaniyah, following the standard process[18,19]. The aerial parts of *L. angustifolia* were collected in mid-July and air-dried under shade at ambient indoor conditions until constant weight was achieved. Approximately 1 kg of the dried plant material was loaded into a Novin stainless-steel hydrodistillation unit (Tehran, Iran). For each kilogram of dried plant material, 5 L of water was added, and hydrodistillation was performed for 8 hours. During distillation, steam carried the volatile compounds through the plant matrix, after which the vapor was condensed and collected in a separation vessel. Because of density differences, the essential oil separated naturally from the aqueous phase and was collected from the upper layer, yielding approximately 2-3% EO per 100g of dried plant material. The extracted oil was immediately transferred into tightly sealed amber-glass bottles and stored in the dark at a controlled room temperature of 20-25 °C. The interval between extraction and subsequent antimicrobial testing did not exceed four weeks (Figure 1).

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

Gas Chromatography–Mass Spectrometry (GC-MS) analysis revealed the unique chemical profiles of the EO. The volatile compounds were separated and identified using an Agilent (7890A) gas chromatography-mass spectrometry system (Santa Clara, CA, USA) with high resolution and sensitivity. All analyses were conducted at the Central Laboratory – Chemistry Division, Islamic Azad University (Sanandaj Branch).

The essential oil was diluted 1:100 in n-hexane, and 1 µL of the solution was injected in split mode (1:50) onto a DB-35MS capillary column (30 m × 0.32 mm). Helium was used as carrier gas at a flow rate of 2 mL/min. The oven program began at 35 °C (5 min hold), increased to 280 °C at 10 °C/min, with a final 5-min hold at 280 °C. The injector temperature was 250 °C, and the MS detector operated in electron-ionization mode (70 eV) with a scan range of 50–500 m/z[20].

Compound identification was achieved by comparing mass spectra with the NIST 11 library, supported by Kovats retention indices calculated from a homologous series of n-alkanes run under identical conditions. Only compounds meeting all three criteria (high-quality library match, RI agreement with published data for *L. angustifolia*, and characteristic fragmentation patterns) were accepted. Relative abundances were reported as percentages of the total ion chromatogram (TIC)[21].

Bacterial strains preparation

The three bacterial strains were reference strains of *S. mitis* (ATCC 49456), *S. oralis* (ATCC 35037), and *S. sanguinis* (ATCC 10556), purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Lyophilized cultures were reanimated under sterile conditions using the features described in ATCC protocols. Subsequently, each strain was plated and cultured onto Blood Agar and Brain Heart Infusion (BHI) broth (HiMedia, India) and incubated at 37 °C for 24 hours under CO_2-enriched microaerophilic conditions generated using a candle jar. Stock cultures were preserved in 20% glycerol (Unimedic Pharma, Sweden) at −80 °C for subsequent use. All experimental procedures were conducted using fresh subcultures from the frozen stocks to ensure consistency and viability (Figure 2). Gram staining confirmed the presence of Gram-positive cocci in chains[22].

Preparation of essential oil solutions

Owing to the limited water solubility of EOs, a single standardized vehicle composed of 0.5% Tween 80 (Biochem-France) and 10% dimethyl sulfoxide (DMSO) (Merck, Germany) was employed consistently for all assays, including the agar well diffusion test, MIC, and MBC to facilitate their dissolution while maintaining methodological uniformity. This solution allowed for antimicrobial testing of various EO concentrations against the three streptococcal strains[23]. Vehicle controls (10% DMSO alone, 0.5% Tween 80 alone, and the combined 0.5% Tween 80 + 10% DMSO mixture) were included in all assays to verify that the solvent system had no intrinsic antimicrobial activity.

Antibacterial efficacy evaluation

Mueller–Hinton agar (MHA) without blood supplementation was used for the agar well diffusion assay, a qualitative method for assessing the sensitivity of bacterial strains to the tested essential oil. This medium provides standardized conditions that allow reliable diffusion of hydrophobic compounds and is widely applied in essential-oil antimicrobial testing. Although viridans streptococci are clinically fastidious, all ATCC strains showed consistent, reproducible growth on MHA[24-26].

Suspensions of bacteria were standardized to ≈1.5×10^8 CFU/mL through comparison with the 0.5 McFarland

standard in phosphate-buffered saline (PBS) (Central Drug House, India). From this suspension, 100 µL of the bacterial inoculum was applied to Mueller–Hinton agar plates (HiMedia, India) and evenly spread using a sterile cotton swab, followed by a drying period of approximately 5 minutes.

Wells of 6 mm diameter were aseptically created using a sterile stainless-steel borer. Essential oil concentrations (5%, 10%, and 15% for *S. mitis* and *S. oralis*; 10%, 15%, and 20% for *S. sanguinis*) were prepared by making 5 mL working solutions in which the required percentage of EO was added, followed by 10% DMSO and 0.5% Tween 80, and the remaining volume completed with sterile distilled water. This standardized vehicle ensured complete solubilization of the EO and consistent preparation across all assays. From each prepared EO dilution, 20 µL was dispensed into the wells. Chlorhexidine gluconate 0.12% (CHX) served as the positive control.

Plates were left at room temperature for 15 minutes to facilitate diffusion of the test agents and then incubated at 37 °C for 24 h in a candle jar. Inhibition zones were measured using a digital Vernier caliper, recording the total diameter (clear zone + 6-mm well), a commonly used method in essential-oil diffusion assays where hydrophobic compounds exhibit variable diffusion patterns[24] (Figure 3). All tests were performed in triplicate across three independent experiments (n = 9 per concentration per strain).

Determination of the MIC and MBC

The macrodilution broth method was utilized to determine the MIC of the EO tested[27]. Stock solutions were prepared by dissolving the EO in 10% dimethyl sulfoxide (DMSO; Merck, Germany) and 0.5% Tween 80 (Biochem-France). Positive (bactericidal) control was chlorhexidine gluconate 0.12% (CHX; KIN, SA, Spain), while Mueller–Hinton broth (MHB; HiMedia, India) served as the negative control.

The stock concentrations for MIC testing were selected based on the agar well diffusion results. For *S. mitis* and *S. oralis*, antibacterial activity began at 5% EO; therefore, a 50 µL/mL stock solution was prepared, and two-fold serial dilutions using 10 glass tubes were generated to obtain final test concentrations ranging from 25 to 0.048 µL/mL, using the same vehicle system (10% DMSO + 0.5% Tween 80), which showed no intrinsic antimicrobial activity in control assays. Because *S. sanguinis* demonstrated inhibition zones at 10% EO, a 100 µL/mL stock solution was used to prepare serial dilutions ranging from 50 to 0.0975 µL/mL. For each MIC tube, 900 µL of Mueller–Hinton broth (MHB; HiMedia, India) was dispensed into sterile glass tubes, followed by 100 µL of the standardized bacterial inoculum (0.5 McFarland ≈ 1.5×10^8 CFU/mL), resulting in a final test volume of 1 mL. The essential oil concentrations described above were incorporated into the tubes via the prepared dilution series according to standard macrodilution methodology. Tubes were gently vortexed, sealed with cotton plugs, and incubated for 24 h at 37 °C in a candle jar. After incubation, tubes were examined for visible growth, manifested as turbidity or pellet formation[28]. Figure 4 illustrates this process. The MIC was defined as the lowest EO concentration that inhibited visible bacterial growth, as indicated by the absence of cloudiness or particle formation.

During MIC evaluation, the concentration showing no bacterial growth was judged to be the MBC. In accordance with standard CLSI macrodilution procedures, only the tubes that showed no visible growth at the MIC reading (i.e., absence of turbidity or pellet formation) were selected for subculture. A sample was gathered from the contents of these growth-negative tubes using a sterile wire loop, then plated onto agar plates and incubated at 37 °C within a candle jar for 24 hours. After incubation, plates were examined for the presence or absence of bacterial colonies, and the MBC was defined as the lowest concentration that yielded no growth. All assays for MIC and MBC determination were performed in triplicate[29].

Antibiofilm activity assay

This test was carried out using a standardized tube adhesion assay to assess the anti-biofilm activity of the extracts[30]. After the MIC of the EO was determined, the inoculated culture medium was removed aseptically. The test tubes were washed three times with phosphate-buffered saline (PBS, pH 7.3) to remove any planktonic and non-adherent cells, then inverted and left to dry for 45 minutes. Then, for each tube, 1 mL of 1% crystal violet solution was added and left at room temperature for 15 minutes. Subsequently, the tubes were washed three times with sterile distilled water to remove excess stain. Crystal violet staining of the tubes was used to assess biofilm formation by the presence of a violet-stained layer on the inner walls and base of the tubes. Staining intensity was visually evaluated qualitatively (examiner's vision) according to standard interpretive categories: weak (+), moderate (++), or strong (+++) biofilm formation, based on the density and uniformity of the violet film on the wall and bottoms of the tubes[31,32]. A negative control (bacterial inoculum in MHB without EO) and a positive control (0.12% chlorhexidine) were included in every run. All

procedures were conducted in triplicate, and the experimental protocol was consistently applied across the three bacterial species tested.

Statistical analysis

The statistical analyses were performed with SPSS software version 26.0 (IBM Corp., Armonk, NY, USA). Kolmogorov–Smirnov and Shapiro–Wilk tests were used to assess the normality of the data distribution. An independent sample t-Test was used to compare the antibacterial and antibiofilm activities of L. angustifolia essential oil (0.05, 0.1, and 0.2 mg/mL) and chlorhexidine mouthwash (0.01, 0.02, 0.04, and 0.08mg/mL). Results are expressed as mean ± SD (standard deviation) and with $p \leq 0.05$ considered statistically significant.

Results

Bacterial strains assessment and identification

The bacterial cultures were obtained from the ATCC (Manassas, VA, USA). The initial assessment involved visual examination of colony attributes under magnification, staining methods to discern cell structure and arrangement, growth characteristics, and Gram staining. After 24 hours of incubation, the S. mitis and S. oralis colonies displayed a greenish halo, indicating alpha-hemolysis with red blood cells, distinguishing them as streptococci. Separately, *S. sanguinis* colonies emerged as tiny grayish or colorless microscopic clusters (Figure 2).

Analysis of bioactive compounds

GC-MS analysis of L. angustifolia

Gas chromatography–mass spectrometry (GC–MS) analysis of *L. angustifolia* EO using the Agilent 7890A system revealed twenty-five phytochemical constituents, with six major compounds dominating the profile.

Linalool (20.99%) and linalyl anthranilate (14.20%) were the most abundant, followed by 1,3,6-octatriene, 3,7-dimethyl-, (E) (7.06%), L-caryophyllene (5.80%), 4-terpineol (5.65%), and 1,6,10-dodecatriene, 7,11-dimethyl-3-methylene-, (E) (5.11%), as presented in Table 1. The essential oil used in this study was tested within 4 weeks of extraction to ensure chemical stability.

The Antibacterial Activity

The Antibacterial Activity of L. angustifolia. EO

The sensitivity of the tested oral streptococcal strains to *L. angustifolia* essential oil was assessed using the agar well diffusion method, and the results are summarized in Table 2. In this study, inhibition zones greater than the 6-mm well diameter were interpreted as evidence of antibacterial activity, consistent with the essential-oil diffusion assay methodology, in which hydrophobic compounds often diffuse minimally beyond the well. The tested bacterial strains, *S. mitis*, *S. sanguinis*, *S. oralis*, and a mixed-species consortium, exhibited varying degrees of sensitivity to *L. angustifolia* EO across concentrations of 5%, 10%, 15%, and 20%. Vehicle controls (10% DMSO, 0.5% Tween 80, and their mixture) consistently produced 0-mm inhibition zones, confirming the absence of intrinsic antimicrobial activity.

For *S. mitis*, inhibition zones ranged from 9.33 ± 1.15 mm at 5% to 15.77 ± 1.39 mm at 15%, with statistically significant differences compared to CHX 0.12% at lower concentrations ($p < 0.05$), but not at 15% ($p = 0.487$). *S. oralis* showed a concentration-dependent response, with inhibition zones increasing from 8.21 ± 1.02 mm at 5% to 16.21 ± 1.02 mm at 15%, all significantly lower than CHX ($p \leq 0.05$).

S. sanguinis demonstrated modest susceptibility, with inhibition zones of 8.00 ± 0.00 mm, 9.55 ± 0.39 mm, and 11.00 ± 0.58 mm at 10%, 15%, and 20% EO concentrations, respectively. These were significantly smaller than those produced by CHX 0.12% ($p < 0.05$). The mixed-species group showed minimal inhibition at 5% EO (0.00 ± 0.00 mm), but responded at higher concentrations, reaching 12.00 ± 0.00 mm at 15% ($p < 0.05$).

Across all bacterial strains and concentrations, CHX 0.12% consistently produced larger inhibition zones, ranging from 14.22 ± 0.38 mm to 18.00 ± 0.67 mm, with statistically significant superiority over *L. angustifolia* EO ($p < 0.05$) as shown in Table 2. All results represent median (IQR) values from n = 3 replicates × 3 independent experiments.

MIC and MBC of L. angustifolia EO against the bacterial strain

The MICs of *L. angustifolia* EO against the tested bacterial strains, as measured by the broth macro dilution method, were 1.56 µL/mL and 0.39 µL/mL for *S. mitis* and *S. oralis*, respectively, and 0.156 µL/mL for both *S. sanguinis* and the mixed-species consortium, as shown in Table 3.

Corresponding MBC values, as seen in Figure 3, were 3.125 µL/mL *for S. mitis*, 0.781 µL/mL for *S. oralis*, and 3.120 µL/mL for both *S. sanguinis* and the mixed-species group. The larger MIC–MBC gap observed for S. sanguinis (0.156 vs. 3.120 µL/mL) is consistent with essential oils, in which bacteriostatic inhibition often occurs at much lower concentrations than bactericidal killing due to hydrophobicity, slower kill kinetics, and

reduced penetration into cellular structures. Additionally, the loop-based subculture method used for MBC determination has limited plating sensitivity, which may contribute to the wider separation between inhibitory and bactericidal concentrations[24,27].

The relatively low MIC and MBC values observed for *S. oralis* suggest that L. angustifolia EO exhibits potent bacteriostatic and bactericidal activity against this strain. In contrast, *S. mitis* required higher concentrations to achieve complete bacterial eradication, reflecting its comparatively reduced susceptibility.

Antibiofilm activity of L. angustifolia EO

The antibiofilm activity of *L. angustifolia E. oil* was evaluated using the test-tube adherence method, wherein biofilm formation was qualitatively assessed based on the intensity of crystal violet staining on the inner walls and bottoms of the tubes. Compared to the negative control (Mueller–Hinton broth with bacterial inoculum), which exhibited strong biofilm formation (+++), *L. angustifolia* EO demonstrated variable inhibitory effects across the tested strains.

As shown in Table 4, *L. angustifolia* EO exhibited weak biofilm formation in *S. mitis* (+) and S. *sanguinis* (+), moderate adherence in *S. oralis* (++), and strong biofilm persistence in the mixed-species consortium (+++). These results suggest that *L. angustifolia* EO possesses strain-specific antibiofilm properties, with greater efficacy against individual strains than against polymicrobial communities.

In contrast, the positive control (0.12% chlorhexidine mouthwash) completely inhibited visible biofilm formation in *S. oralis*, *S. sanguinis*, and the mixed-species group, while *S. mitis* remained weakly adherent (+). These findings highlight the partial antibiofilm potential of *L. angustifolia E. oil* and support its role as a natural adjunct in biofilm management, particularly in mono-species oral infections.

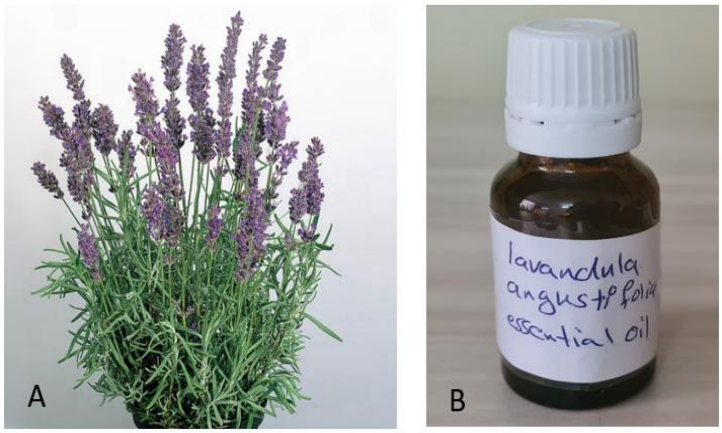

Figure 1: (A) *L. angustifolia* plant, (B) *L. angustifolia* essential oil.

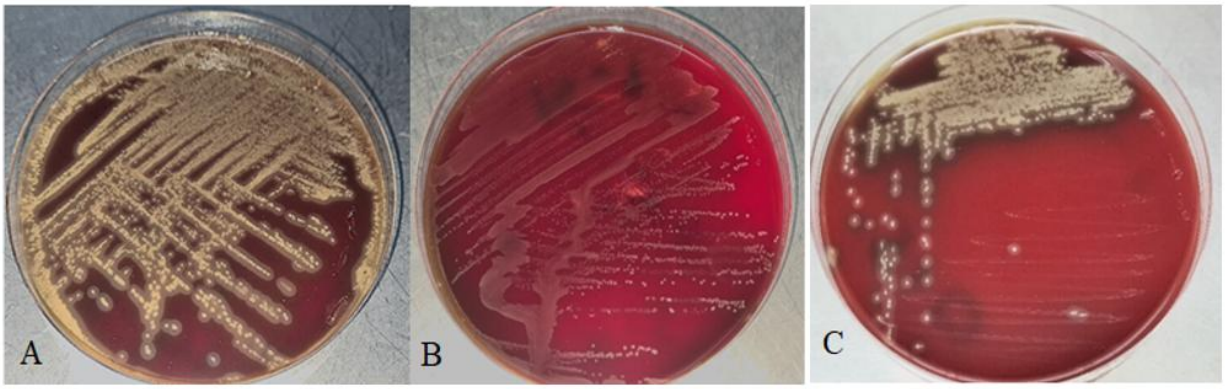

Figure 2: Presents the colony morphology of alpha-hemolytic streptococcal strains cultured on blood agar: (A) *Streptococcus mitis* shows dense, brownish colonies with diffuse spread; (B) *Streptococcus sanguinis* exhibits less dense, reddish colonies with focal growth zones; and (C) *Streptococcus oralis* displays moderately dense colonies with mixed brownish-red pigmentation, reflecting subtle variations in hemolytic activity.

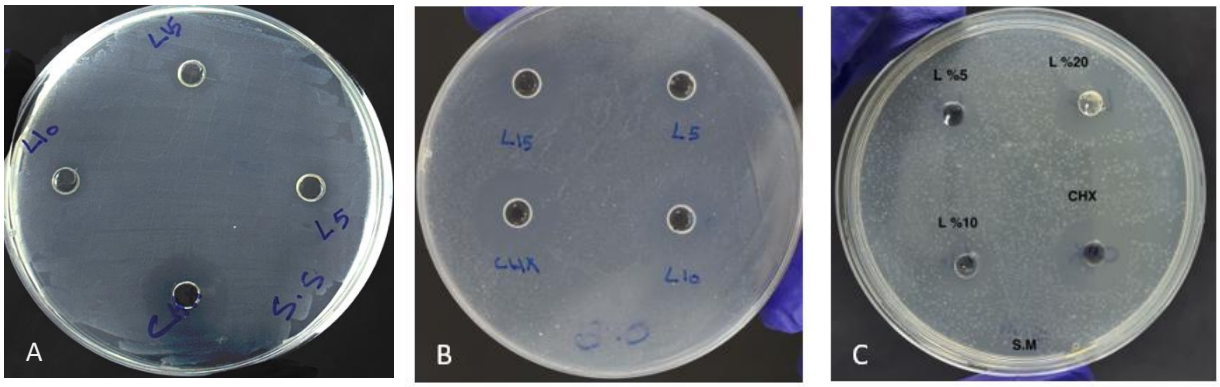

Figure 3: Mueller–Hinton agar plates showing the results of the agar well diffusion method used to evaluate antibacterial susceptibility. (A) Streptococcus sanguinis; (B) Streptococcus oralis; (C) Streptococcus mitis.

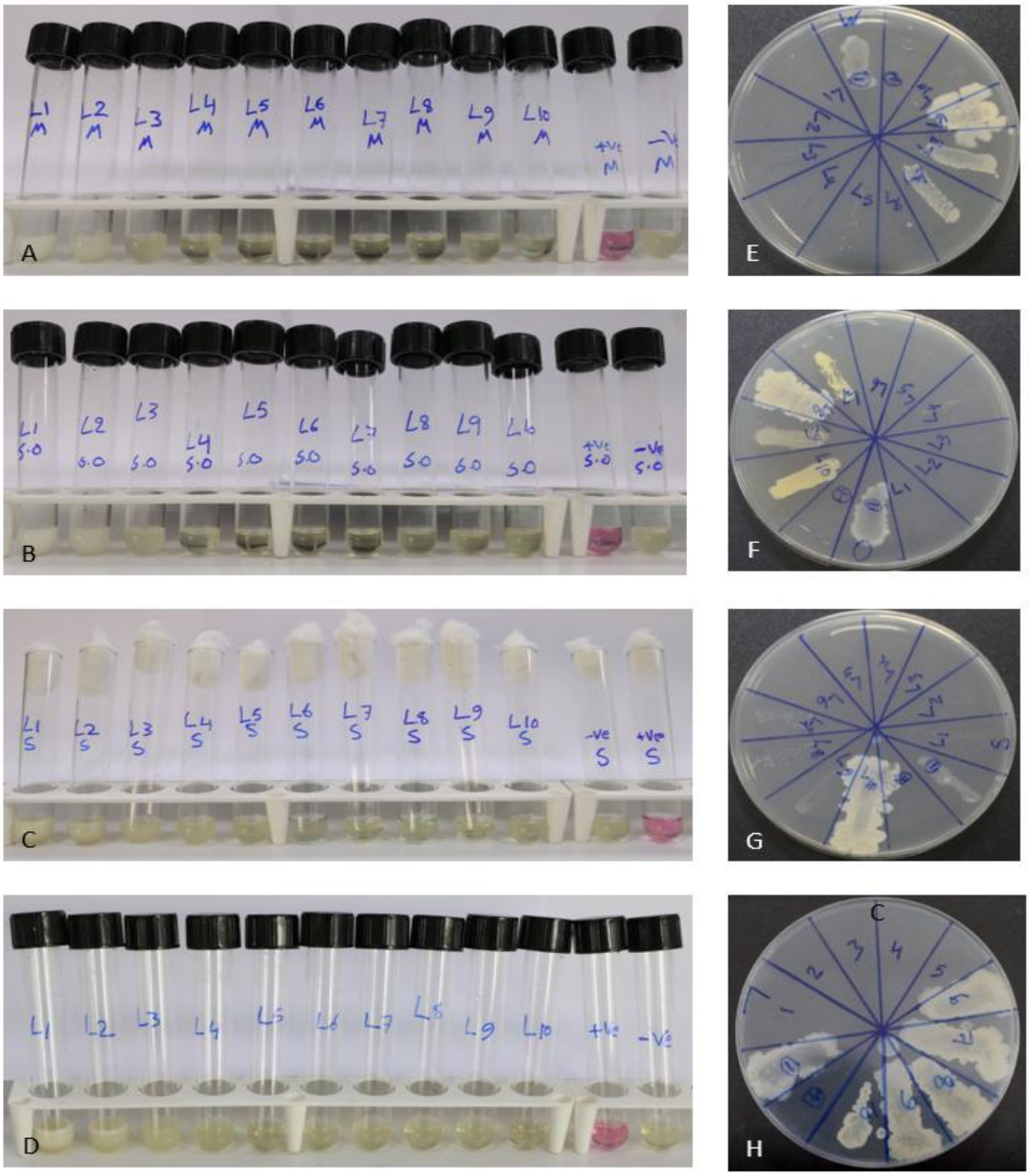

Figure 4: Determination of MIC and MBC values of L. angustifolia essential oil against primary biofilm colonizers and mixed species. (A–D) Broth macrodilution assay showing the MIC of L. angustifolia essential oil against S. mitis, S. oralis, S. sanguinis, and the mixed-species consortium, respectively. (E–H) Agar culture assay confirming the MBC of L. angustifolia against the same bacterial strains in the same order.

Table 1: Results of GC-MS analysis of L. angustifolia.

Peak	R. T	Area%	MW	MF	CAS#	Compound name[1]
25	4.398	0.682	136	C10H16	7785-70-8	1R-α-Pinene
16	5.687	1.545	136	C10H16	18172-67-3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-
22	6.04	0.874	128	C8H16O	3391-86-4	1-Octen-3-ol
21	6.235	0.89	130	C8H18O	589-98-0	3-Octanol
11	6.361	2.369	128	C8H16O	106-68-3	3-Octanone
15	6.458	1.546	136	C10H16	5989-27-5	D-Limonene
12	6.59	1.708	136	C10H16	555-10-2	β-Phellandrene
3	6.729	7.058	136	C10H16	3779-61-1	1,3,6-Octatriene, 3,7-dimethyl-, (E)-
10	6.799	2.393	154	C10H18O	470-82-6	Eucalyptol
8	6.861	3.594	136	C10H16	13877-91-3	1,3,6-Octatriene, 3,7-dimethyl
1	8.247	20.994	154	C10H18O	78-70-6	Linalool
13	9.27	1.687	154	C10H18O	58461-27-1	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-
20	9.364	0.944	172	C10H20O2	2639-63-6	Butanoic acid, hexyl ester
14	9.476	1.584	156	C10H20O	2216-51-5	1-Menthol
5	9.604	5.647	154	C10H18O	562-74-3	4-Terpineol
9	9.962	2.53	154	C10H18O	98-55-5	α-Terpineol
2	10.392	14.198	154	C10H18O	7149-26-0	Linalyl anthranilate
24	10.532	0.817	138	C9H14O	500-02-7	Crypton
19	10.688	1.081	154	C10H18O	106-24-1	Lemonol
7	10.918	4.069	196	C12H20O2	25905-14-0	(±)-Lavandulol, acetate
23	12.05	0.859	196	C12H20O2	141-12-8	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-
17	12.156	1.316	204	C15H24	512-61-8	α-Santalene
4	12.45	5.803	204	C15H24	87-44-5	L-Caryophyllene
6	12.738	5.108	204	C15H24	18794-84-8	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-
18	13.386	1.163	204	C15H24	13744-15-5	1H-Cyclopenta [1,3] cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3aα,3bβ

[1] Compound identification was performed by matching mass spectra with the NIST/EPA/NIH Mass Spectral Library (NIST 14) and by comparison with published retention indices.

Area%: Compound percentage; **CAS#:** Registry number; **MW:** Molecular weight (g/mol); **MF:** Molecular formula, **R. T:** Retention time

in periodontal disease progression[33].

Table 2: Mean and standard deviations of inhibition zones of L. angustifolia at different concentrations against S. mitis, S. sanguinis, S. oralis, and mixed species together.

Oil%	Inhibition zone (*S. mitis*)			Oil%	Inhibition zone (*S. oralis*)		
	L. angustifolia	CHX 0.12%	*p*-value		*L. angustifolia*	CHX 0.12%	*p*-value
5%	9.33 ± 1.15	15.99 ± 1.15	0.043 *	5%	8.21 ± 1.02	18.00 ± 0.67	0.05 *
10%	10.66 ± 0.66	15.33 ± 1.15	0.046 *	10%	10.22 ± 0.38	17.78 ± 0.39	0.043 *
15%	15.77 ± 1.39	16.66 ± 0.00	0.487 ns	15%	16.21 ± 1.02	18.00 ± 0.67	0.05 *
	Inhibition zone (*S. sanguinis*)				**Inhibition zone (mixed species)**		
10%	8.0 ± 0.0	17.77 ± 1.02	0.037 *	5%	0.00 ± 0.00	14.22 ± 0.38	0.034 *
15%	9.55 ± 0.39	17.55 ± 0.39	0.043 *	10%	8.66 ± 0.67	14.66 ± 0.67	0.05 *
20%	11.00 ± 0.58	17.78 ± 0.39	0.043 *	15%	12.00 ± 0.00	14.22 ± 0.38	0.034 *

Ns: No significant difference; *: Significant difference; Independent sample t-Test.

Table 3: The MIC and MBC values of L. angustifolia EO on bacterial strains.

Streptococcus species	*L. angustifolia* EO	
	MIC	MBC
S. mitis	1.56 µL/mL	3.125 µL/mL
S. oralis	0.39 µL/mL	0.781 µL/mL
S. sanguinis	0.156 µL/mL	3.12 µL/mL
Bacterial strains together	0.156 µL/mL	3.12 µL/mL

Table 4: Qualitative biofilm formation of the L. angustifolia EO.

Streptococcus species	*L. angustifolia* EO	CHX (Positive control)	MHB (Negative control)
S. mitis	+	+	+++
S. oralis	++	0	+++
S. sanguinis	+	0	+++
Mixed Species together	+++	0	+++

Discussion

Periodontitis is a chronic, biofilm-mediated inflammatory disease initiated by the adhesion and proliferation of early colonizers such as *S. mitis, S. oralis*, and *S. sanguinis*. These species form the foundational layer of supragingival and subgingival biofilms, facilitating the subsequent attachment and maturation of late colonizers, including *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*, which are recognized as keystone pathogens

The transition from health to disease is marked by microbial dysbiosis, where commensal streptococci lose dominance and pathogenic anaerobes thrive, triggering host immune dysregulation and tissue destruction[34].

The present study demonstrates that *L. angustifolia* essential oil (EO) exhibits antimicrobial and antibiofilm activity against these primary oral colonizers. GC–MS analysis revealed linalool (20.99%) and linalyl anthranilate (14.20%) as the dominant constituents, both

known to disrupt bacterial membranes, increase permeability, and interfere with enzymatic and metabolic processes[11]. Their high abundance likely underlies the observed antibacterial efficacy. Lavender EO is also highly volatile, and this property may influence diffusion outcomes, since rapid evaporation or uneven dispersion can alter the measured inhibition zones. This limitation is inherent to agar diffusion assays and has been widely documented in EO research.

Growth inhibition was concentration-dependent, with *S. oralis* showing the highest sensitivity and *S. sanguinis* the lowest, possibly due to structural and metabolic variations among species. Inhibition of biofilm formation further suggests interference with bacterial adhesion and extracellular matrix synthesis, key events in early plaque development[32].

These findings are consistent with the literature. Stamova et al.[13] reported that linalool and linalyl acetate from lavender EO disrupted bacterial membranes and exhibited synergistic effects with antibiotics, particularly against *E. coli* and *S. aureus*. Similar results were observed by Husian and Zardawi[17] against *P. gingivalis* and *P. intermedia*, and by Salavati Hamedani et al.[35], who highlighted strong antimicrobial and antioxidant properties. Other studies confirmed synergistic activity when lavender EO was combined with other therapeutic oils[25] and noted inhibition zones exceeding 25 mm against *E. coli* and *S. aureus*[36], reinforcing its broad-spectrum efficacy.

The EO demonstrated bacteriostatic and bactericidal activity across all tested strains. Notably, *S. oralis* and *S. sanguinis* showed greater susceptibility than *S. mitis*, requiring lower concentrations for growth inhibition and killing. Similar antimicrobial patterns have been reported for Bulgarian lavender essential oil, which exhibited potent activity against a range of pathogens such as *S. aureus* and *C. albicans*[37]. The activity of lavender EO against Gram-positive cocci has also been validated in studies showing synergistic or additive effects when combined with other essential oils[25].

The antibiofilm assessment revealed stronger inhibition in mono-species cultures of *S. mitis* and *S. sanguinis* than in mixed-species biofilms, indicating greater resistance in polymicrobial communities. This observation aligns with de Alteriis et al.[38], who showed that both free and liposome-encapsulated lavender EO eradicated *Candida auris* biofilms, and with Khanem et al.[39], who reported inhibition rates of 6–89% against *E. coli* biofilms through oxidative stress and membrane disruption. Together, these results affirm lavender EO's ability to penetrate biofilm structures and impair their stability.

Despite these promising outcomes, several limitations should be noted. Standardized ATCC strains cannot fully represent the genetic and phenotypic diversity of clinical isolates, and the in vitro model does not reproduce the complex host factors, such as immune response, nutrient gradients, and shear forces, present *in vivo*. Variability in the chemical composition of lavender EO by chemotype, geography, and harvest time may also influence reproducibility.

In conclusion, *L. angustifolia* EO demonstrated concentration-dependent antibacterial and antibiofilm efficacy against primary biofilm colonizers. Although chlorhexidine remains more potent, lavender EO presents a safe, natural alternative for controlling early dental biofilm formation. However, the volatile nature of EO, compositional variability, and the need for cytotoxicity and *in vivo* studies underline that these findings should be considered preliminary. Future studies should aim to standardize its chemical profile, optimize formulations for oral administration, assess its biocompatibility and cytotoxicity at higher concentrations, and confirm its safety and clinical effectiveness through *in vivo* and randomized trials.

Conclusion

This *in vitro* study showed that *L. angustifolia* EO possesses antibacterial and antibiofilm activity against *S. mitis*, *S. oralis*, *S. sanguinis*, and their mixed-species consortium. The oil displayed a concentration-dependent effect, with *S. oralis* most susceptible, *S. sanguinis* less responsive, and mixed-species biofilms showing greater resistance.

Although chlorhexidine consistently outperformed lavender oil, the results indicate its potential as a safe, natural adjunct for controlling early colonizers and delaying biofilm maturation.

References

1. Hajishengallis G, Chavakis T. Local and systemic mechanisms linking periodontal disease and inflammatory comorbidities. Nat Rev Immunol. 2021;21(7):426-40.
2. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, et al. The oral microbiome – an update for oral healthcare professionals. Br Dent J. 2016;221(10):657-66.

3. Marsh PD, Zaura E. Dental biofilm: Ecological interactions in health and disease. J Clin Periodontol. 2017;44 Suppl 18:S12-S22.
4. Kreth J, Merritt J, Qi F. Bacterial and Host Interactions of Oral Streptococci. DNA Cell Biol. 2009;28(8):397-403.
5. Bloch S, Hager-Mair FF, Andrukhov O, Schaffer C. Oral streptococci: Modulators of health and disease. Front Cell Infect Microbiol. 2024;14:1357631.
6. De Almeida J, Ervolino E, Bonfietti LH, Novaes VC, Theodoro LH, Fernandes LA, et al. Adjuvant therapy with sodium alendronate for the treatment of experimental periodontitis in rats. J Periodontol. 2015;86(10):1166-75.
7. James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017;(3):CD008676.
8. Thosar N, Basak S, Bahadure RN, Rajurkar M. Antimicrobial efficacy of five essential oils against oral pathogens: An in vitro study. Eur J Dent. 2013;7(Suppl 1):S071-S7.
9. Prasad KA, John S, Deepika V, Dwijendra KS, Reddy BR, Chincholi S. Anti-plaque efficacy of herbal and 0.2% chlorhexidine gluconate mouthwash: A comparative study. J Int Oral Health. 2015;7(8):98-102.
10. Di Vito M, Smolka A, Proto MR, Barbanti L, Gelmini F, Napoli E, et al. Is the antimicrobial activity of hydrolates lower than that of essential oils? Antibiotics. 2021;10(1):88.
11. Todorova D, Yavorov N, Lasheva V, Damyanova S, Kostova I. Lavender essential oil as antibacterial treatment for packaging paper. Coatings. 2022;13(1):32.
12. Betlej I, Andres B, Cebulak T, Kapusta I, Balawejder M, Jaworski S, et al. Antimicrobial properties and assessment of bioactive compounds in Lavandula angustifolia Mill cultivated in southern Poland. Molecules. 2023;28(17):6416.
13. Stamova S, Ermenlieva N, Tsankova G, Georgieva E. Antimicrobial activity of lavender essential oil from lavandula angustifolia mill.: In vitro and in silico evaluation. Antibiotics (Basel). 2025;14(7):656.
14. Alhasan AS, Alrawi MSh, Al-Ameri DT, Almehemdi AF. Effect of distillation duration on essential oil yield and composition of Lavandula angustifolia grown in Iraq. Anbar J Agric Sci. 2025;23(1):339-53.
15. Ciocarlan A, Lupascu L, Aricu A, Dragalin I, Popescu V, Geana E-I, et al. Chemical composition and assessment of antimicrobial activity of lavender essential oil and by-products. Plants. 2021;10(9):1829.
16. Rosner O, Livne S, Bsharat M, Dviker S, Jeffet U, Matalon S, et al. Lavandula angustifolia essential oil inhibits the ability of fusobacterium nucleatum to produce volatile sulfide compounds, a key components in oral malodor. Molecules. 2024;29(13):2982.
17. Husian SG, Zardawi FM. Antibacterial and antibiofilm profiles of Lavandula angustifolia essential oil on clinically isolated Porphyromonas gingivalis and Prevotella intermedia: an in vitro study. J Oral Dent Res. 2022;9(2):9-20.
18. Elyemni M, Louaste B, Nechad I, Elkamli T, Bouia A, Taleb M, et al. Extraction of essential oils of Rosmarinus officinalis L by two different methods: hydrodistillation and microwave-assisted hydrodistillation. Sci World J. 2019;2019:3659432.
19. Wainer J, Thomas A, Chimhau T, Harding KG. Extraction of essential oils from Lavandula × intermedia 'Margaret Roberts' using steam distillation, hydrodistillation, and cellulase-assisted hydrodistillation: experimentation and cost analysis. Plants. 2022;11(24):3479.
20. Adams RP. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th ed. Carol Stream, IL: Allured Publishing; 2007.
21. Mondello L, Herrero M, Kumm T, Marriott PJ. Comprehensive gas chromatography in essential oil analysis. TrAC Trends Anal Chem. 2018;103:1-12.
22. Thairu Y, Nasir IA, Usman Y. Laboratory perspective of gram staining and its significance in investigations of infectious diseases. Sub-Sahar Afr J Med. 2014;1(4):168-74.
23. Rasheed AH, Gul SS, Azeez HA. Antibacterial and antibiofilm profiles of Thymus Vulgaris essential oil on clinically isolated Porphyromonas Gingivalis and Prevotella Intermedia: An in vitro study. Sulaimani Dent J. 2022;9(2):53-63.
24. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: a review. J Pharm Anal. 2016;6(2):71-9.
25. De Rapper S, Kamatou G, Viljoen A, Van Vuuren S. The in vitro antimicrobial activity of Lavandula angustifoliaessential oil in combination with other aromatherapeutic oils. Evid Based Complement Altern Med. 2013;2013(1):852049.
26. Epifani F, Latronico M, Di Stefano MG, Notarnicola M, Bruni G, Mastronardi M, et al. Antimicrobial activity of essential oils: A review

on applications. Antibiotics (Basel). 2021;10(1):12.
27. Rao J, Chen B, McClements DJ. Improving the efficacy of essential oils as antimicrobials in foods: mechanisms of action. Annu Rev Food Sci Technol. 2019;10(1):365-87.
28. Sha AM, Garib BT. Antibacterial effect of curcumin against clinically isolated Porphyromonas gingivalis and connective tissue reactions to curcumin gel in the subcutaneous tissue of rats. Biomed Res Int. 2019;2019:6810936.
29. Pellegrini M, Ricci A, Serio A, Chaves-Lopez C, Mazzarrino G, D'amato S, et al. Characterization of essential oils obtained from abruzzo autochthonous plants: Antioxidant and antimicrobial activities assessment for food application. Foods. 2018;7(2):19.
30. Hukić M, Seljmo D, Ramovic A, Ibrišimović MA, Dogan S, Hukic J, et al. The effect of lysozyme on reducing biofilms by Staphylococcus aureus, Pseudomonas aeruginosa, and Gardnerella vaginalis: an in vitro examination. Microb Drug Resist. 2018;24(4):353-8.
31. Gonzalez-Moles M, Scully C, Gil-Montoya J. Oral lichen planus: controversies surrounding malignant transformation. Oral Dis. 2008;14(3):229-43.
32. Eladawy M, El-Mowafy M, El-Sokkary MMA, Barwa R. Effects of lysozyme, proteinase k, and cephalosporins on biofilm formation by clinical isolates of pseudomonas aeruginosa. Interdiscip Perspect Infect Dis. 2020;2020(1):6156720.
33. ohnston W, Rosier BT, Artacho A, Paterson M, Piela K, Delaney C, et al. Mechanical biofilm disruption causes microbial and immunological shifts in periodontitis patients. Sci Rep. 2021;11(1):9796.
34. Paul L. Microbial dysbiosis in periodontitis: a paradigm shift in understanding oral health. J Dent Sci Med. 2024;7(4):253-60.
35. Salavati Hamedani M, Rezaeigolestani M, Mohsenzadeh M. Chemical composition, antimicrobial and antioxidant activities of Lavandula angustifolia essential oil. J Nutr Fast Health. 2020;8(4):273-9.
36. Arshad B, Shaheen SIMK. Essential oils extraction and identification of phytochemical compounds from Lavandula angustifolia Mill: unveiling their antibacterial potential against selected strains. Pak J Bot. 2025;57(5):1517-24.
37. Mladenova A et al. In vitro antimicrobial activity of Bulgarian lavender essential oil. Acta Microbiol Bulg. 2024;40:252-7.
38. De Alteriis E, Maione A, Falanga A, Bellavita R, Galdiero S, Albarano L, et al. Activity of free and liposome-encapsulated essential oil from lavandula angustifolia against persister-derived biofilm of candida auris. Antibiotics (Basel). 2021;11(1):26.
39. Khanem A, Karim N, Ullah I, Younas F. Essential oils of Citrus limon, Cymbopogon citratus, and Lavandula officinalis disrupt Escherichia coli biofilms by inducing cellular damage. Biol Futura. 2025;76(3):343-58.