Antibacterial and Antibiofilm Profiles of Lavandula Angustifolia Essential Oil on Clinically Isolated Porphyromonas Gingivalis and Prevotella Intermedia: An In Vitro Study

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Abstract
Background Gingivitis and periodontitis are common oral diseases. Gingivitis is usually the first stage of periodontal disease. Periodontitis is a bacterial infection of the periodontium that spreads beyond the gingiva. **Objectives** This study investigates the antibacterial effect of essential oil extracted from the Lavandula angustifolia (La) against clinically isolated Porphyromonas gingivalis (P. gingivalis) and Prevotella Intermedia (P. intermedia). **Materials and Methods** Subgingival plaque samples were collected from periodontitis patients with a probing pocket depth of at least 6mm. The essential oil of (La) was extracted using the hydro-distillation method, and GC-MS was performed to determine the essential oil constituent. Clinically isolated bacteria confirmed by anaerobic culture, morphology, gram stain, and PCR (16S rDNA). Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), disc diffusion method, and anti-biofilm activity of the essential oils was evaluated by a qualitative tube method. **Result** The antimicrobial effect of (La) essential oil against P. gingivalis was 30µl/mL (MIC and MBC). While the MIC of (La) essential oil against P. intermedia was 7.5µl/mL and MBC were 15µl /mL. The essential oil extracted from (La) revealed comparable antibacterial activity with mean inhibition zones of 12.8±0.76mm and (11.0±0.5mm, 9.4±0.40) for P. gingivalis and P. intermedia, respectively. The anti-biofilm activity of (La) essential oil against P. gingivalis and P. intermedia revealed moderate anti-biofilm activity. **Conclusion** (La) essential oil revealed antibacterial efficacy against clinically isolated P. gingivalis and P. intermedia. Hence, it could be applied as an adjunct in periodontal therapy.

Keywords: Antibacterial activity; essential oil; lavandula angustifolia; periodontitis; porphyromonas gingivalis; prevotella intermedia.

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Introduction

Gingivitis and periodontitis are common oral diseases; gingivitis is usually the first stage of periodontal disease; if left untreated, which might lead to a severe and destructive form of periodontitis (Ezzat and Fares, 2014). Periodontitis is a bacterial infection of the periodontium that spreads beyond the gingiva and destroys the periodontal tissue, resulting in attachment loss, consequently, tooth loss (Visser and Ellen, 2011). Furthermore, periodontitis has been reported as a host response mechanism against dental plaque in the gingival sulcus (Garlet, 2010). Although mechanical debridement is the primary treatment approach for periodontal disease, which entails removing local factors and reducing inflammation, adjuncts such as mouthwashes and local drug administration can improve the treatment outcome. Chlorhexidine is regarded as the «gold standard» adjunctive agent; however, it has some drawbacks, such as tooth staining, allergies, and altered taste sensation with long-term application (FLÖTRA et al, 1971). Many novel herbal mouthwash formulations and herbal periodontal chips are in clinical trials to address the drawbacks associated with synthetic local delivery medicines. Essential oils are fragrant volatile molecules produced by some parts of plants and have long been used to combat infections caused by bacteria, fungi, and viruses (Tohidi et al, 2011). The antibacterial activity of essential oils is attributed to terpenoids and a few hydrocarbons (Cowan, 1999). Terpenoids are lipophilic that can cause the bacterial cell membrane to expand, increasing fluid flow and deactivating the enzymes within the membrane. According to Sikkema et al, terpenoids disperse through the bacterial cell wall, destroying it and leading to bacterial death (Sikkema et al, 1995) Lavandula angustifolia (La) is a widely spread aromatic herb known as «Ostokhoddous» (Omidbaigi and Nejad, 2000). People are familiar with (La) as a potent fragrance and therapeutic herb. The plant is used to cure various gastrointestinal, neurological, and rheumatic problems in folkloric medicine worldwide (Duke, 2002). Numerous reports mentioned lavender›s anti-inflammatory, wound healing, antimicrobial, and anti-neoplastic properties in both vitro and in vivo studies (Lis-Balchin and Hart,1999). It has been tested against several diseases, such as diabetes, neurological disorders, cancer, auto-immune conditions, chronic inflammatory conditions, rheumatoid arthritis, and periodontal diseases (Oliveira et al, 2022). It has also been suggested that the relationship between natural products and preventive care can reduce the incidence of oral diseases affecting periodontal tissues (Mendonça and Juiz-Lopez, 2010). Linalool, linalyl acetate, mono and sesquiterpenes, flavonoids like luteolin, triterpenoids like ursolic acid, and coumarins like umbelliferone and coumarin were discovered to be the significant components of the plant›s aerial parts and flowers in phytochemical investigations (Duke, 2002). Aromatherapy and essential oils for therapeutic purposes have remained popular throughout history, and essential oils are still utilized as a popular alternative medicine for antimicrobial effects today. Essential oils have been marketed in foreign markets as «natural antibiotics» due to the rising interest in aromatherapy (de Rapper et al, 2013). However, the antibacterial effects of (La) against clinically isolated P. gingivalis and P. intermedia have not been examined yet. Therefore, this study aimed to explore the antibacterial impact of (La) against clinically isolated *P. gingivalis* and *P. intermedia*

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Material and Methods

Plant collection and preparation of the essential oil

The lavender leaf was harvested from the mountains of Sulaimani, Kurdistan region of Iraq, in December 2021. The collection was carried out in the field involving wild species of the plant under study by trained personnel supervised by the researcher to avoid contaminants. The plant identification was accomplished at the College of Agriculture, University of Sulaimani. The current study was registered at the scientific committee of the College of the Dentistry University of Sulaimani, Kurdistan region of Iraq. (Number 65/21, on 9/11/2021). Hydro distillation is used to extract the essential oil of (La). In a conical flask, 100g of (La) leaf was soaked in 350mL of distilled water and hydro-distilled for 3 hrs. using a Clevenger apparatus described previously (Nidaullah, 2010). Finally, the essential oil was collected after decantation and stored in a sealed amber vial at 4°C until used.

Analysis of essential oil

Gas chromatography-mass spectrometry (GC–MS) (GCMS-Aligent 7820A gas) GC systems paired with a mass spectrometer were used to identify and evaluate the constituents of the essential oil of (La) (Santa Clara, CA, The USA).

Plaque sampling

This study involves a clinical strain of P. intermedia, a Gram-negative bacteria isolated from periodontal pockets of patients who attended the clinical department of Periodontics, College of Dentistry. The University of Sulaimania in August 2021. Before collecting the samples, patients› consent and authorization were obtained. Subgingival plaque samples were collected from a systemically healthy patient suffering from chronic periodontitis; the plaque sample was collected from periodontal pockets with a depth of at least 6mm, using sterilized paper points (F2 Dia-ProT™); samples were spread out on Colombia agar media (LAB001 UK), placed in an anaerobic jar. Plates were incubated anaerobically for 7-10 days using an anaerobic jar and anaerobic gas packs (AnaeroGen system Oxoid).

Identification and isolation of the microorganism

The P. gingivalis clinical strain was obtained from the College of Dentistry, the University of Sulaimani, which was previously isolated (Sha and Garib, 2019). The clinical strain of P. intermedia was identified by the morphological characteristic, Gram stain, and PCR. As described below, the proven isolated strains were stored at -70ºC. A colony was extracted from bacterial cultures and mixed with 50µl of sterilized deionized distilled water (ddH2O) in a micro centrifuge tube, vortexed thoroughly until homogenized, and then incubated for 10 minutes in a heat block at 95ºC. Finally, the supernatant (DNA) was employed as a template, and the samples were spined down. According to a previously published paper, a specific primer pair for P. gingivalis and universal on 16s rRNA was used for DNA amplification (Sakamoto et al, 2001) As follows:

Universal Revise primer: 5´-GTATTACCGCGGCTGCTG - 3 (18 mer)
Universal Forward primer: 5›-AGAGTTTGATCCTGGCTCAG - 3 (20 mer)
PG- Forward primer: 5´-AGGCAGCTTGCCATACTGCG-3 (21mer)
PG- Revise primer: 5´-ACTGTTAGCAACTACCGATGT-3(21mer)

Polymerase chain reaction

2µL of reverse primer (10µM) (GeNet BioG-2000), 2µl of forwarding primer

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(10µM) (GeNet BioG-2000), and 10µl of 2X Prime Taq Premix (AddStart Taq Master) in a final volume of 20µl. (Prime Taq DNA Polymerase, reaction buffer, enzyme stabilizer, dNTPs mixture and loading dye). After that, 1µL of ddH2O and 5µl of DNA template were mixed. The first cycle comprised a five-minute denaturation at 95°C, followed by an amplification stage, which was repeated for 35 cycles and included the denaturation of the DNA Template at 95°C for 20 seconds. The next step was to anneal specific primers for 20 seconds at 55°C, followed by 30 seconds at 72°C, then another 5 mins. at 72°C for 1 cycle in a Thermocycler PCR (Verity TM 96 well, Applied Biosystems-USA). PCR product was analyzed by 2% agarose gel electrophoresis at 80 V for 35 minutes. The gel was stained with 3µl of ethidium bromide and photographed on a gel cabinet (Cleaver scientific Ltd-UK). A 100bp plus DNA ladder (cat no. M 2000) was used as a molecular weight marker. The gel purification was done for the bands using the gene JET™ Gel extraction kit (# K0691) from Fermentas UK. Finally, standard sequencing for the PCR products was done by (Macrogen-South Korea).

Disc diffusion method

The disc diffusion method is the most extensively used method for testing antimicrobial susceptibility. Natural things (La) were dissolved and diluted in DMSO. As previously stated, the same number of consecutive dilutions were performed, with 20µL of each dilution impregnated onto 6mm filter paper discs (Whatman, no. 3). The discs were left at room temperature until the diluent had evaporated completely. Discs containing natural ingredients (La) were placed on the agar›s surface. As a control, 0.12% commercial chlorhexidine was employed. The tests were carried out in triplicate.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The stock solution of (La) was prepared (120µl/ml) by dissolving the essential oil in DMSO. Eight extract dilutions were prepared for MIC using the Muller Hinton broth (900µl broth/tube) using a two-fold serial dilution method. 1 ml from the stock solution was added to the first tube for dilutions. The serial dilution was repeated for La until reaching 0.46µl/ml. Thus the concentrations of serial dilution were: 60, 30, 15, 7.5, 3.75, 1.87, 0.93, 0.46µl/ml, respectively. To each of the above 8 prepared MIC tubes with varying concentrations, 100µL of bacterial suspension (5×105 CFU/ml),this suspension obtained by McFarland turbidity test, was added to the tubes. Therefore, the final volume was 1000µL (1ml) per tube. Tubes were sealed with cotton and incubated for ≥24hrs at 37ºC in an anaerobic jar using AnaeroGen® system Oxoid, gas pack and observed for turbidity. The minimum concentration of the essential oil in the tube, which does not show any turbidity, is the MIC of (La). Turbidity of the tube indicated growth of the bacteria, indicating that the bacteria are resistant to the tested agent. The minimal bactericidal concentration was determined by selecting the concentrations that demonstrated no bacterial growth during the MIC assessment. For further confirmation, a sample was taken from the contents of the chosen tubes using a micropipette 20µl. It was then spread on a Muller Hinton agar plate with a sterile spreader and incubated anaerobically for 24 hrs. at 37°C. Then the plates were removed and examined for bacterial growth. Plates with no bacterial growth were determined to have the lowest bactericidal concentration. The trials were conducted in triplicate at three different concentrations, one before and one after

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the clear tube, then the MIC and MBC values were determined.

Anti-biofilm assay

The ability of various doses of (La) to suppress biofilm development was tested, and as stated, a qualitative method for detecting biofilm formation was used (Evren and Yurtcu, 2015). different concentrations of (La) were prepared in Muller Hinton broth. One ml of each concentration was inoculated with 100µL of the bacterial sample for 18 to 24 hrs. After the supernatants were discarded, the tubes were rinsed with phosphate buffer saline (pH 7) and dried. After 20 minutes, tubes were coloured with 0.1% crystal violet to visualize biofilm growth. The excess stain was eliminated, the tubes were cleaned with distilled water, then dried, and biofilm formation was judged qualitatively by observing a visible film lining the walls of the tube and estimated as absent (0), weak (+), moderate (++) or strong (+++). All assays were performed in duplicate. (Abdi-Ali et al, 2014)

Statistical analysis

For statistical analysis of the data, multiple comparisons were performed using T.test. The obtained data are depicted as mean ± standard deviation (SD) and analyzed using SPSS. Software version 25 (SPSS Inc., Chicago, USA).

Results

Bacterial strains

Morphology of colony identification of P. gingivalis colonies appeared on the plate after 48 hrs. as small, round opaque and convex with black pigmentation, as shown in Figures (1A) and (1B). P. intermedia is a black pigmented bacterium because of the formation of shiny and smooth colonies, which appear either a grey, light brown, or black colour on blood agar plates in anaerobic conditions, as shown in Figure1 (1A) and (1B). Molecular technique identification (PCR) used for bacterial identification is confirmed as shown in Figure 2, in which the three bands of template size of bacteria have appeared, a band localized at base pairs, similar to the template size of P. intermedia. Furthermore, the sequencing of the template was 99% identical with strain (sequence ID: GU561339.1), as shown in Figure 2.

Chemical composition of (La) essential oil

Table 1 shows the results obtained by GC-MS analyses of the essential oil (La) with their retention times and percentage shares. The Table shows 50 different compounds that were identified for (La). Table 1: GC-MS analysis of the chemical composition of essential oil of (La) R/ Time=retention time, Area%=compound percentage.

MIC and MBC of the extract against tested bacteria

The MIC and MBC of the essential oils of (La) on P. gingivalis in this study were 30µl/mL and the MIC of the essential oils of (La) against P. intermedia in this study was 7.5µl/ml and MBC that eradicated the clinical strains of the essential oil against the P. intermedia, which was 15µl/ml (Table 2) and Figure 3.

Disc diffusion assay result (Antimicrobial activity of (La) essential oil

The result of the antibacterial activity of (La) essential oil against clinically isolated periodontal pathogens by agar disc diffusion assay is displayed in (Table 3). Inhibition zones above 6mm (including the disc diameter) were taken as positive results in this test. The clinical strains were

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sensitive to (La) essential oil at different concentrations used in the present study in concentrations of 60µl/ml and 30µl/ml producing inhibition zones of 12.8mm for P. gingivalis and in the concentration of 15µl/ml inhibition zone 16.5mm, and in concentration 7.5µl/ml inhibition zone, 9.4mm for P. intermedia. CHX produced mean inhibition zones of 13.8±0.20mm, and 19.25±0.25mm against clinically isolated P. gingivalis and P. intermedia, respectively. Thus, the difference between various concentrations was statistically non-significant (P > 0.001). The comparison between mean inhibition zones of (La) essential oil concentrations and mean inhibition zones of CHX (positive control); However, (La) essential were slightly less than CHX and produced more expansive inhibition zones on both clinical strains. Finally, (La) essential at all concentrations and CHX were more active on P. intermedia, producing wider bacterial inhibition zones than P. gingivalis.

Anti-biofilm activity of (La) essential oil

The results of the test-tube method to determine how (La) essential oil affects adhesion and the capability of the clinical strains under study to form biofilms are reported in (Table 4). The outcomes demonstrated no discernible pattern in the (La) essential oil effect, ranging from a nearly total absence of biofilm at the highest to a substantial biofilm formation at the lowest concentration. At MIC and MBC concentrations, (La) essential oil demonstrated a weak anti-biofilm impact on P. intermedia and a moderate effect on P. gingivalis. The findings showed that P. intermedia and P. gingivalis clinical strains did not produce visible biofilm when CHX 0.12% mouthwash was used. At the same time, P. gingivalis and P. intermedia showed substantial biofilm formation in the test tube used as a negative control, which contained bacterial inoculum and Mueller Hinton Broth without CHX.

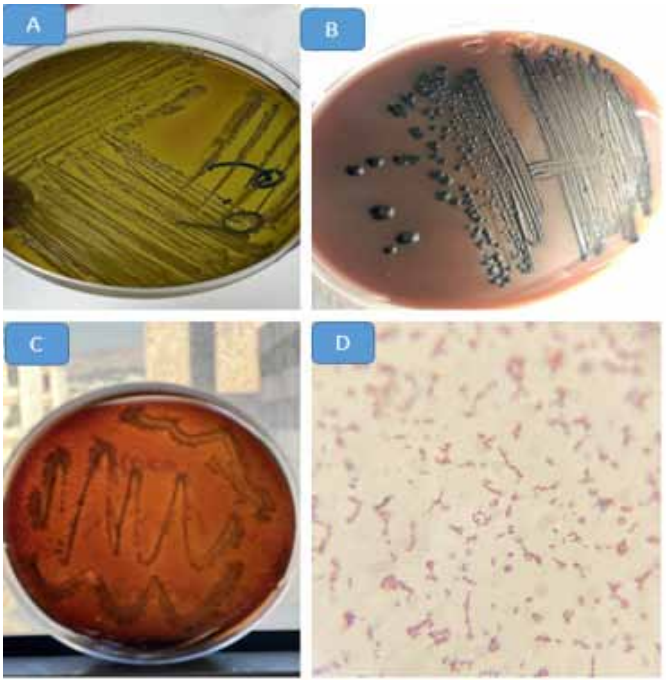

Figure (1): (A) and (B) Pure isolated bacterial colonies of P. gingivalis 10 days on Columbia agar appearing as round, smooth, shiny, and convex black colonies. (C) Pure isolated bacterial colonies of P. intermedia, (D) Gram-negative short rods of P. intermedia after gram staining Under light microscopy X100 power.

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Figure (2): (A) 16S rDNA gene sequence of the purified colonies of P. intermedia done in Macrogen, South Korea. (B) Agarose gel electrophoresis of PCR product for P. intermedia.

Table (1): GC-MS analysis of the chemical composition of essential oil of (La) R/Time=retention time, Area%=compound percentage.

Peak#	R/Time	Area%	Compound name
1	5.232	0.16	2-Heptanone
2	5.941	0.22	(+)-3-Carene
3	6.149	0.96	alpha.-Pinene
4	6.331	3.10	(1R)-2,6,6-Trimethylbicyclo[3.1.1] hept-2-ene .
5	6.616	1.25	Camphene
6	6.807	3.03	Bicyclo[2.2.1]heptane, 2,2-dimethy l-3-methylene-, (1S)-
7	7.421	0.36	Bicyclo[3.1.1]heptane, 6,6-dimethy l-2-methylene-, (1S)-
8	7.638	0.35	3-Octanone
9	7.837	1.28	beta.-Myrcene
10	8.469	0.46	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl),
11	8.910	13.17	Eucalyptol,
12	9.291	0.67	1,3,7-Octatriene, 3,7-dimethyl-
13	9.559	1.11	gamma.-Terpinene
14	9.931	0.40	cis-Linaloloxide
15	10.243	0.76	Cyclohexene, 1-methyl-4-(1-methyle thylidene)
16	10.788	15.99	1,6-Octadien-3-ol, 3,7-dimethyl-
17	11.316	0.19	2,4,6-Octatriene, 2,6-dimethyl-,
18	11.610	0.24	2,4-Heptadienal, 2,4-dimethyl
19	11.861	7.92	(+)-2-Bornanone
20	12.329	7.42	3,5,5-Trimethylhexyl acetate
21	12.511	2.11	endo-Borneol
22	12.632	1.44	Terpinene-4-ol
23	12.753	0.22	Butanoic acid, hexyl ester
24	12.961	4.29	alpha.-Terpineol
25	13.584	0.38	2,6-Octadien-1-ol, 3,7-dimethyl
26	14.069	12.47	1Linalyl isobutyrate
27	14.571	0.14	Bicyclo[3.1.1]hept-2-ene-2-ethanol , 6,6-dimethyl-
28	14.735	4.46	Bicyclo[3.1.1]hept-2-ene-2-ethanol 3
29	14.986	0.14	4-Carene
30	15.670	0.31	Isopulegol acetate
31	16.016	4.57	3-Cyclohexene-1-methanol, .alpha.,4-trimethyl-, acetate
32	16.137	0.38	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate
33	16.553	1.32	Geranyl acetate
34	17.245	0.29	Longifolene
35	17.444	2.45	Caryophyllene
36	17.617	0.23	trans-.alpha.-Bergamotene
37	17.912	0.62	cis-.beta.-Farnesene
38	18.050	0.26	Humulene
39	18.474	0.25	.beta.-copaene
40	18.716	0.18	Linalyl acetate
41	18.820	0.17	8-Isopropenyl-1,5-dimethyl-cyclode ca-1,5-diene
42	18.993	0.16	gamma.Muurolen
43	20.093	0.47	Caryophyllene oxide
44	20.906	0.11	Bicyclo[4.4.0]dec-1-ene, 2-isoprop yl-5-methyl-9-methylene
45	21.495	0.19	alpha.-Bisabolol
46	24.412	0.31	Hexadecanoic acid, methyl ester
47	26.316	1.47	9,12,15-Octadecatrienoic acid, met hyl ester
48	26.593	0.14	Methyl stearate
49	28.350	0.39	Methyl 13-eicosenoate
50	30.211	0.09	Erucic acid

Table (2). Based on broth dilution assay, the MIC and MBC values of (La) essential oil on clinically isolated periodontal pathogens.

Periodontal pathogens	MIC	MBC
P. gingivalis	30 µL/mL	30 µL/mL
P. intermedia	7.5 µL/mL	15 µL/mL

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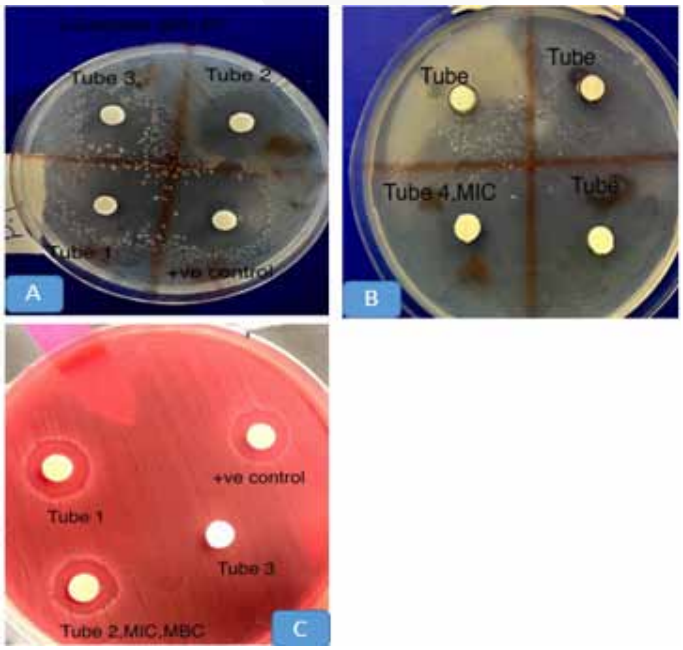

Figure (3): (A, B) show the MIC and MBC by disc diffusion method with (La) essential oil against P. intermedia on Muller Hinton agar (C) show the MIC and MBC by disc diffusion assay with (La) essential oil against P. gingivalis on blood agar.

Table (3): Mean and standard deviations of inhibition zones and p-values of CHX and (La) essential oil at different concentrations on clinical strains used in the study.

Oil con.	Inhibition zones (*P. gingivalis*)			Oil con.	Inhibition zones (*P. intermedia*)		
	(La)	CHX	P-value		(La)	CHX	P-value
60 µL/mL	12.8±0.076	13.8±0.20	0.116	15 µL/mL	11.0±0.50 (MBC)	19.25±0.25	0.422
30 µL/mL	12.8±0.076 MBC, MBC	13.8±0.20	0.116	7.5µL/mL	9.4±0.40 (MBC)	19.25±0.25	0.559

Table (4): Qualitative biofilm formation was judged by observing a visible film lining the walls of the tubes.

Bacteria	LA essential oil	CHX 12% positive	Negative
P. gingivalis	++	0	+++
P. intermedia	+	0	+++

Discussion

Periodontal diseases are a set of chronic inflammatory diseases caused by the formation of subgingival biofilm, which occurs due to the host›s inflammatory, immunological response to periodontal infections. The construction of pockets and inflammation characterizes periodontitis (Buduru et al, 2019). Periodontal diseases are latent diseases with an overall worldwide frequency of disease and accompanying morbidity and economic impacts (Buset et al, 2016). Periodontal therapy relies heavily on mechanical plaque clearance. The response to periodontal treatment is not comprehensive because the aetiology of periodontitis is multifaceted. As a redonesult, the dentist should be aware of the various treatment options (Shaddox and Walker, 2010). However, mechanical debridement alone may not be adequate for all patients (Haffajee et al, 1995) despite being the major treatment option for periodontitis. This could be attributed to the fact that some periodontal bacteria invade the gingival tissues; they are protected from mechanical debridement, which is the source of the disease›s recurrence and decolonization of the periodontal pocket by periodontal pathogens (Ebersole et al,

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2017). Commercial local antimicrobials in oral rinse have been widely used in previous decades. Still, prolonged applications of these antimicrobials as a supplement to mechanical plaque reduction can have various systemic and local side effects, including tooth staining, altered taste, a burning sensation, and the development of bacterial resistance (Sakaue et al, 2018). The antibacterial and anti-biofilm effectiveness of (La) essential oil was investigated in the current in vitro study against clinically isolated P. gingivalis and P. intermedia. According to the agar disk diffusion findings and broth macro dilution tests, the (La) essential oil has antimicrobial action against both clinical strains. Additionally, the qualitative tube method revealed that (La) essential oil has significant anti-biofilm efficacy against the two clinical strains tested in this study. The diameter of the inhibition zones determines the antibacterial effectiveness of plant extracts or particular components. It is critical to note that lipophilic extracts, like essential oils, diffuse insufficiently into an agar medium, which may have a negative impact on the study results and might lead to underestimation of the exact diameter of the disc diffusion zone. This was the only critical problem we encountered while employing agar disc diffusion and is considered one of the limitations of the relevant studies (Horváth et al, 2016). The antibacterial activity of (La) essential oil was tested in the current study against P. gingivalis and P. intermedia in an in vitro study and compared to Chlorhexidine at 0.12%. Chlorhexidine is a broad-spectrum antimicrobial commonly used as an adjunct to nonsurgical periodontal therapy. Further, previous relevant studies have widely applied it as a positive control. The results of the present study showed that (La) possesses non-significant bactericidal activity against P. gingivalis and P. intermedia, compared to Chlorhexidine with MIC and MBC (La) essential oil in 30µl/ml showed a zone of inhibition against P. gingivalis. In contrast, (La) essential oil in 7.5µl/ml showed a zone of inhibition against P. intermedia. The differences in MIC values reported by various studies from different geographical areas are primarily attributed to the fact that the genotype of the plant and the influence of environmental factors, such as geographic conditions, soil type, temperature, the season of collection and harvesting plants, and most importantly, the oil extraction process, greatly influence the chemical composition and active ingredient concentrations of essential oils (Kakasy et al, 2001). Numerous inflammatory disorders that affect the tooth›s supporting structure and are caused by biofilms are known as biofilm-induced PDs. These conditions can cause tooth loss and systemic inflammation. To prevent biofilm-related infections, methods that can interfere with any step of biofilm formation are thought to be potentially valuable (Abebe, 2021). The current study assessed the ability of (La) essential oil to stop P. gingivalis and P. intermedia from forming biofilms. The detailed qualitative tube approach results demonstrated that (La) essential oil effectively and concentration-dependently inhibited biofilm development. Analysis of the P. gingivalis results revealed that (La) essential oil mildly inhibited biofilm development. It also demonstrated a strong anti-biofilm impact against P. intermedia. In the present study, significant discrepancies in the mean diameter of the inhibition zone among different (La) essential oil concentrations were noted. It can be claimed that the impact of the oil climbs with increasing attention. Comparing the inhibition zone of (La) essential oil to that of a positive control group revealed that bacterial growth in the presence of

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CHX was slightly higher than that of (La) essential oil, particularly at 60µl/ml and 30µl/ml; however, this is less relevant than the herb›s safety. Nevertheless, the growth of clinical strains was increased, and a more prominent bacterial growth was seen at lower concentrations of 15µl/ml and 7.5µl/ml. In addition, the oil was more effective against P. intermedia than P. gingivalis, providing a foundation for future research. Lavandula species have also been shown in the literature to have the ability to treat bacterial illnesses that are typically associated with antibiotic resistance (Nelson, 1997). The discovery of a new medication that can suppress or reduce inflammation opens the possibility of developing a therapeutic agent that combats inflammatory reactions and minimizes pocket formation in periodontitis patients. Meanwhile, multiple published studies have looked into the use of systemic medicines to treat periodontal disease and its various side effects (Jucá et al, 2020). Herbal research is regarded as a global solution to these side effects. (La) the essential oil has been reported to have antibacterial action against various Gram-positive and Gram-negative pathogens in studies on different herbal components (Kustrak and Besic, 1975). Although multiple investigations have been conducted to determine the antibacterial activity of (La) essential oil, no in vitro study against periodontal pathogens has been published. In general, the activity of essential oils is determined by their constituents, active ingredients, functional groups, and synergistic interactions. The antimicrobial mechanism of action varies with the type of essential oil or bacterial strain used. It is well known that gram-positive bacteria are more susceptible to essential oils than gram-negative bacteria (Huang et al, 2014). This is because Gram-negative bacteria have a complex rigid lipopolysaccharide (LPS) outer membrane, which controls the diffusion of hydrophobic compounds. On the other hand, this membrane is not present in gram-positive bacteria, with a thick peptidoglycan wall that cannot resist small antimicrobial molecules attempting to pass through the cell membrane. Moreover, gram-positive bacteria may facilitate the penetration of hydrophobic compounds of essential oils. Although its zone of inhibition was slightly lesser or sometimes equal to that of chlorhexidine (positive control), several in vitro studies have been conducted on the antimicrobial activity of (La) essential oil against various microorganisms. Many studies have used disc diffusion assays to quantify antimicrobial activity, which later is largely inappropriate. Many researchers have reported the clinical use of lavender as a suitable adjunct in treating periodontitis (Rios and Recio, 2005). The MIC method for antimicrobial examination of essential oils is the favoured approach, and as such, it is the only one evaluated for comparison here (Kalemba and Kunicka, 2003). To the best of our knowledge, this is the first study to evaluate (La) antibacterial efficacy against Gram-negative organisms in periodontal disease. Antibacterial activity of (La) against *P. gingivalis* and *P. intermedia* was non-significant compared to chlorhexidine in this study; however, the effects were comparable. Since it is a readily available natural product that may be consumed daily with no observed adverse effects, (La) essential oil can be used prophylactically and therapeutically.

Conclusion

Based on the results of the current study and according to the researcher's online scanning of similar and related topics, a novel result regarding the antimicrobial effect of Lavandula angustifolia (La) against clinically isolated P. gingivalis

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and P. intermedia in vitro model was obtained, and anti-biofilm activity of (La) against clinically isolated P. gingivalis and P. intermedia. It is suggested that more research be conducted to identify and isolate the active components of (La) extracts and comprehend their mechanism of action on biofilm formations. Furthermore, in vivo studies examining the effect of (La) is highly recommended.

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Conflict of Interest
The authors reported no conflict of interest.

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