Original Article

Antibacterial and Anti-biofilm Effect of *Lactuca serriola* Extract Against Clinically Isolated *Porphyromonas gingivalis and Prevotella intermedia*: An *in vitro* study

Rozhgar R. Sharif[1*], *Aram Mohammed Sha*[1]

Abstract

Objective: To investigate the antibacterial and anti-biofilm effect of *Lactuca serriola (LS)* extract against clinically isolated *Porphyromonas gingivalis* (*P. gingivalis*) and *Prevotella intermedia* (*P. intermedia*).

Methods: LS extract was prepared by solvent extraction method. Gas chromatography mass spectrometry (GC-MS) was used to analyze phytochemical components. Plaque samples were obtained from periodontitis patients with probing pocket depth ≥6mm to isolate *P. intermedia*. Microbiological tests and conventional polymerase chain reaction (PCR) were used to confirm P. intermedia. Antibacterial activities of LS against the confirmed clinical strains (*P. intermedia* and revised *P. gingivalis*) were determined by agar well diffusion method. Broth macro dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The anti-biofilm assay of the LS was performed by using tube adhesion method.

Results: Antibacterial activity of *LS* against clinical isolates revealed that the inhibition zones ranged from 11.75 ±0.95mm to 24.25 ±1.7mm for *P. gingivalis* and 12.75 ±0.95mm to 25.5 ±1.29mm for *P. intermedia*. The MICs of *LS* extract were 212.5mg/mL and 106.2 mg/mL, while the MBCs were 425mg/mL and 212.5mg/mL against *P. gingivalis* and *P. intermedia*, respectively. The anti-biofilm of *LS* extract exhibited strong anti-biofilm activity.

Conclusions: *LS* extract exhibited bactericidal and anti-biofilm activity against clinically isolated *P. gingivalis* and *P. intermedia* in an in vitro study.

Keywords: *Antibacterial activity, Anti-biofilm, Lactuca serriola, P. gingivalis, P. intermedia.*

Submitted: August 11, 2022, Accepted: October 13, 2022, Published: December 1, 2022.

Cite this article as: Sharif RR, Sha AM. Antibacterial and Anti-biofilm Effect of Lactuca serriola Extract Against Clinically Isolated Porphyromonas gingivalis and Prevotella intermedia: An in vitro study. Sulaimani Dent J. 2022;9(2):71-80.

DOI: https://doi.org/10.17656/sdj.10160

1. *Department of Periodontics, College of Dentistry, University of Sulaimani, Sulaimani, Iraq.*

** Corresponding author: rozhgarrauf33@gmail.com.*

Introduction

Periodontal diseases are multifactorial inflammatory conditions that can affect tooth-supporting tissues and are initiated by plaque biofilm components that deposit on teeth surfaces adjacent to the supporting periodontal tissues. Periodontal disease may be limited to the gingiva (gingivitis) or may go into the deeper supporting structures, destroying the surrounding supporting tissue of the teeth (periodontitis), which can cause the loss of the affected teeth[1]. Periodontal diseases are triggered by mixed microbial infections in which specific pathogenic bacteria coexist[2]. Genetic and environmental factors have a role in disease development along with pathogenic microorganisms in the biofilm[3]. Subgingival microflora of periodontitis can harbor a wide variety of bacterial species, only a few of which have been related to disease progression and are considered etiologically important[4]. Significant evidence has implicated *P. gingivalis*[5] and *Aggregatibacter actinomycetemcomitans* (Aa)[6,7] in the development of periodontitis. Additionally, *Bacteroides forsythus*[8], *P. intermedia*[7], *Peptostreptococcus micros*[9], and *Fusobacterium nucleatum*[10] have been strongly associated with the progression of periodontitis.

P. gingivalis is a gram-negative, anaerobic, black-pigmented species that colonize the subgingival region. It is the main keystone pathogen that intensively participates in the initiation and development of periodontitis following the change from symbiotic to dysbiotic microbiota[11]. *P. intermedia* is a gram-negative, anaerobic, black-pigmented pathogen that plays a significant role in periodontal disease. There is significant evidence in the scientific literature linking *P. intermedia* with the progression of periodontal disease[4,12]. In addition, these species possess potent virulence factors and contribute to various forms of periodontal disease[13].

Nonsurgical therapy (scaling and root surface debridement) is considered the gold standard for the initial treatment of periodontal disease to reduce the load of supra and subgingival pathogenic bacteria by instrumental debridement[14]. However, complete removal of pathogenic biofilm is impossible because some pathogens are embedded in the soft tissue or located in anatomically inaccessible areas. Therefore, chemical antimicrobial agents are often applied with mechanical instrumentation as an adjunctive treatment for periodontal therapy[15].

Herbal medicines, including herbs and herbal products, which contain active ingredients and parts of the plant that have therapeutic benefits, have been recommended for use as an adjunctive treatment for periodontal disease because of the side effects of chemical antimicrobial agents, such as tooth staining, altered taste sensation, antibiotic resistance in oral biofilm. Herbal medicines are effective, usually safe, have fewer side effects, are easily available, and are more compatible with the body[16]. In addition, herbal products are preferred over conventional drugs due to broad biological activity deriving from their anti-inflammatory, antiseptic, analgesic, sedative, astringent, edema-reducing, healing-accelerating, and restorative properties[17].

Lactuca serriola is a herbaceous species with various common names, including jagged lettuce and prickly lettuce[18]. It is indigenous to the Himalayan, Atlantic, and Siberian areas[19] and cultivated in temperate regions of India, Europe, Iran, and Pakistan[18]. *LS* is used for various purposes in traditional medicine, as a hypnotic, sedative, cough suppressant, expectorant, demulcent, purgative, antiseptic, vasorelaxant, diuretic, and antispasmodic, and thus to treat bronchitis, asthma, pertussis, gastrointestinal, and other conditions[19,20]. The plant contains iron, vitamins, and beta-carotene, and triterpenoid saponin extract from the stem possesses antibacterial activity[21]. In addition, pharmacological studies of the plant demonstrated its analgesic, anti-inflammatory properties[22], and antioxidant activity due to its high total phenolic content, demonstrating the effective free radical scavenging capability of such as quercetin[23]. However, till now, there has been no information regarding the antibacterial and anti-biofilm effect of *LS* against the clinically isolated strains of *P. gingivalis* and *P. intermedia*. Therefore, the study aims to investigate LS extract's antibacterial and anti-biofilm impact against *P. gingivalis* and *P. intermedia.*

Materials and methods

Collection of subgingival plaque samples

The bacteria used in this study were *P. gingivalis* and *P. intermedia*. *P. gingivalis* was obtained from previously studied bacteria stored at -80 University of Sulaimani, College of Veterinary Medicine[24]. After incubation for 30 min, the *P. gingivalis* samples were inoculated on supplemented Columbia agar plates. Next, the plates were placed in an anaerobic jar and incubated at 37°C for five days. *P. intermedia* were obtained by clinical isolation from patients. After obtaining approval from the Ethics Committee for Human Research, College of Dentistry, University of Sulaimani, Iraq (N0. 63/21, on 9/11/2021), patients with generalized periodontitis with periodontal pockets ≥6 mm and untreated for at least six months were selected for collection of subgingival plaque samples. Cotton rolls isolated the selected

pocket. First, supragingival plaque and calculus were removed by using a sterile periodontal curette. Then, a sterile paper point (F2 DiaProT™) was inserted slowly until tissue resistance was felt and left in place for 60 seconds. After removal, they were immediately streaked on supplemented Columbia agar plates containing 1µg/ml vitamin K1, 5µg/ml hemin, and 5% human blood. Plates were incubated for 7–10 days at 37°C in anaerobic conditions provided by AnaeroPack, AnaeroGen (Thermo Scientific, Waltham State, USA), and an anaerobic jar (BBL® GasPak system). Prior to collecting the samples, patients' consent and approval were obtained.

Isolation and identification of *P. intermedia*

Pure colonies of bacterial species were obtained by culturing and subculturing subgingival plaque samples. The *P. intermedia* were identified based on colony morphology, pigment production, aerobic control, Gram staining, and finally, confirmed by the conventional PCR technique. The verified isolated strain was stored at -80ºC.

Molecular identification

DNA extraction

Following the manufacturer's instructions, total bacterial DNAs were extracted from clinical isolates using Add Prep Bacterial Genomic DNA Extraction Kit (Add Bio Inc., Korea). Next, 1ml ~ 2ml of the overnight bacterial culture was collected by centrifuge at 13,000 rpm for 30 sec, and the supernatant was discarded. That was followed by adding 200µl of Lysis Solution and 20µl Proteinase K solution (20 mg/ml) and then incubating in a 56°C water bath for 10 minutes. After that, 200µl of Binding Solution and 200µl of absolute ethanol were added and mixed well by pulse-vortexing for 15 sec, then centrifuged. Finally, the genomic DNA was eluted by one minute of centrifugation at 13,000 rpm, and the supernatant was used as a template.

Nucleic Acid Amplification

Universal primer pairs targeted at the 16s rRNA gene were used to perform DNA amplification[24].

5´- AGAGTTTGATCCTGGCTCAG - 3 ´ UNIV Forward

5´- GTATTACCGCGGCTGCTG - 3´ UNIV Reverse

PCR preparation

PCR preparation was performed in a final volume of 20µl containing 2µl of reverse primer (10 µM), 2µl of forwarding primer (10µM), 10µl of AddStart Taq Master (2X conc.), 1µl of nuclease-free water, and 5µl of DNA template. The DNA sample was initially denatured for 10 minutes at 95ºC for one cycle, followed by an amplification step which was repeated for 35 cycles (denaturation of DNA template at 95ºC for 30 seconds, annealing of the specific primers at 62 ºC for 30 sec and extension of primers at 72 ºC for 30 sec). The final extension was done for 5 min at 72 ºC for one cycle in Thermo cycler PCR. The 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 utilized 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, conventional sequencing for the PCR products was done by Macrogen (South Korea).

Collection and identification of herb

In spring, the plant sample (LS) was collected from local community gardens around the Sulaimani governorate/Kurdistan region. Identification was confirmed by the College of Agricultural Engineering, University of Sulaimani, and GC-MS assay was performed at Ibn Al-Bitar research center, Baghdad.

Sample preparation and extraction of the bioactive components

After the herbal collection and before starting the extraction process, the herb was washed with distilled water, cut into small pieces, and placed in the shade in a room with fresh air and no direct sunlight exposure. The herb was dried for about two weeks, after which the herbs were crushed into a coarse powder using an electric grinder. The *LS* bioactive component was extracted by solvent extraction method as follows. First, 100 grams of powdered substance were macerated with 150 ml of Methanol, 150 ml of Acetone, and 50 ml of distal water and then placed in a shaking water bath for 48 hrs[25] to allow enough time for the bioactive components to solubilize in the solvents. After 48 hrs, the solvent was filtered using qualitative filter paper (7.0cm). Next, a rotary evaporator was used to evaporate the Methanol. Next, the extract was poured into a glass petri dish to evaporate the distal water and Acetone so that the crude extract would become a paste. Finally, the

stock solution was prepared by dissolving the extract in dimethyl sulphoxide (DMSO) before use.

Preparation of Bacterial Suspension

Pure activated colonies of *P. gingivalis* and *P. intermedia* were inoculated separately into Mueller-Hinton broth containing 5µg/ml of hemin and 1µg/ml of vitamin K using sterile loops. The bacterial suspension was adjusted to 0.5 McFarland as standard turbidity (5×10^5) CFU/ml[26].

Antibacterial effect evaluation

Agar well diffusion method and disk diffusion method were used to evaluate the antibacterial susceptibility of *LS* extract. First, antibacterial susceptibility was tested on Mueller-Hinton agar. Briefly, an inoculum of periodontal pathogens (*P. gingivalis* and *P. intermedia*) was prepared at 0.5 McFarland's standard turbidity, and after adjustment, the inoculum was plated onto Mueller-Hinton agar. Next, 100µl of inoculum was evenly spread on the agar plates with a sterile cotton swab. Next, the entire surface of the Mueller-Hinton agar plates was swabbed, rotating plates approximately 60° between streaking to ensure even distribution, and allowed to dry for about 5 min. For the well diffusion method, wells (6 mm in diameter) were punched into the agar plate using a sterile stainless steel borer and filled with different concentrations of the *LS* extract. For the disk diffusion method, the filter paper disks containing different concentrations of extract were placed on the surface of the agar plates that had previously been inoculated with the periodontal pathogens. DMSO 10% was utilized as a negative control, and chlorhexidine 0.12% was used as the positive control. The plates were incubated under anaerobic conditions at 37°C for 48 hours using GasPak ™ Anaerobic System (Oxoid). Next, the diameters of the inhibition zones were measured in millimeters, with readings taken in four distinct fixed directions, as shown in Figure 1. The experiment was repeated three times, and the means of the inhibition zones were recorded. The results were used to detect active extract for MIC determination using the macro dilution method.

Determination of minimum inhibitory concentration (MIC)

The *LS* was serially diluted in a range of 425-0.83mg/ml in ten test tubes with bacterial suspension (5×105 CFU/ml) using 0.5 McFarland's turbidity standard for each bacterium. Chlorhexidine 0.12% was used as a bactericidal (positive control), and a broth solution allowed bacterial growth (negative control). The stock solution of the *LS* paste was dissolved in 10% dimethyl sulfoxide.

For MIC, ten dilutions of the *LS* were prepared with Mueller-Hinton broth (900µL broth/tube) using a two-fold serial dilution method: 425, 212.5, 106.25, 53.12, 26.56, 13.28, 6.64, 3.32, 1.66, 0.83mg/ml. To each of the ten prepared MIC tubes, different concentrations of *LS* were incorporated, then the tubes were inoculated with a 100µl strain of clinically isolated *P. gingivalis* and *P. intermedia* separately; therefore, the final volume was 1ml for each tube after discarding 1ml from the last tube. The tubes were sealed with cotton, as shown in Figure 2A, incubated for ≥48 hrs at 37ºC in an anaerobic jar using Anaero Pack- Anaero, and checked for turbidity (bacterial growth). The minimum concentration of the *LS* in the tube with no turbidity was considered the MIC. The MIC test was performed in triplicate and confirmed by culturing the content of the tubes before and after MIC on the agar plate. Finally, the absence of *P. gingivalis and P. intermedia* growth was confirmed after culturing on agar.

Determination of minimum bactericidal concentration (MBC)

Finally, the MBC was determined by selecting the concentrations with no bacterial growth observed during the MIC evaluation. A sample was taken from the content of the chosen tubes by a sterile wire loop, subcultured on agar plates, and incubated for ≥48 hrs at 37ºC in an anaerobic jar. Then the presence or absence of bacterial growth was investigated.

Anti-biofilm assay

The tube adhesion method was used to detect the anti-biofilm activity of the *LS* extract. The tubes of inoculated broth used for the determination of MIC were carefully discarded, then washed for one minute with phosphate-buffered solution (pH 7.3), and 200µL of Methanol was added to each tube for 15 minutes to fix the biofilms and then allowed to dry. After 20 minutes, each tube was dyed with 1% crystal violet for one minute and then washed with distilled water to remove any residual stains. Next, the bound crystal violet was eluted by adding 200µL of 95% ethanol, followed by incubation for 10 minutes at room temperature. Next, the tubes were inverted for 24 hours to dry at room temperature, Figure 2B. A visible stain on the tube's wall and the bottom was considered a sign of biofilm formation. The production of biofilm was categorized as absent (0), weak (+), moderate (++), and strong (+++)[27]. The stain was measured visually three times in triplicate with collaboration among examiners.

Statistical analysis

Means and standard deviation were calculated for variables with a continuous distribution. Hypotheses

about changes in the tested material were assessed using the two-tailed paired t-test, using CHX as a control outcome score. The statistical significance was determined by using a p-value ≤0.05. For the statistical analysis, SPSS for Windows version 27.0 was utilized.

Results

GC-MS Analysis of *LS*.

The GC-MS analysis of *LS* leaves and seeds revealed 30 peaks; components represented by the peaks were classified and recognized by comparing the spectral data of the components with the National Institute of Standards and Technology (NIST) data. Linolenic acid methyl ester had the highest concentration (15.89%), followed by Hexadecanoic acid, methyl ester (13.03%), Phytol (12.64%), Oleic Acid (7.11%), and Palmitic acid (7.10%). Finally, Methyl palmitelaidate had the lowest concentration of *LS* extract (0.78%), as shown in Table 1.

Identification of *P. intermedia*

The microorganisms used in this study were preserved *P. gingivalis* and clinically isolated *P. intermedia*. Black-pigmented anaerobic bacteria *P. intermedia* were present in (n=3) out of 17 tested samples after 7-10 days. In addition, black pigmented colonies appeared on lysed blood; under light microscopy, the colonies appeared as gram-negative, rod-shaped bacterium following gram staining. Finally, 511 base pairs were confirmed by PCR after sequencing, as represented in Figures 3 and 4.

The antibacterial effect of *LS*

The antibacterial activities of *LS* extract against both bacterial strains (*P. gingivalis* and *P. intermedia*) were assessed by the presence or absence of inhibition zones. *LS* extract has potential antibacterial activity in both gram-negative *P. gingivalis* and *P. intermedia* at different concentrations and produced inhibition zones ranging from(11.75 ±0.95 to 24.25 ±1.7)mm for *P. gingivalis* and (12.75±0.95 to 25.5 ±1.29)mm for *P. intermedia*, as shown in Table 2. DMSO 10% showed no inhibitory effect on isolated strains. CHX produced mean inhibition zones of (13±0.81 to 13.25±0.95) mm, (and 12.75± 0.95 to 13±0.81) mm against both *P. gingivalis* and *P. intermedia*, respectively, as shown in Table 2 and Figure 1. The inhibition zones increased as the *LS* concentration increased in such a way that the mean inhibition zones of *LS* paste at 250 and 500mg/mL concentration were significantly wider than the mean inhibition zone of 0.12% CHX (P≤0.05) against both clinically isolated strains, but at 100mg/mL concentration of *LS*, no significant difference was observed when compared with CHX (P≥0.05). Finally, *LS* at all concentrations was more active on *P. intermedia* and produced a wider inhibition zone than *P. gingivalis*, as exhibited in Figure 1 and Table 2.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MICs of *LS* paste, determined by assessing broth macro dilution against *P. gingivalis and P. intermedia*, were 212.5mg/mL and 106.2mg/mL, respectively. Still, the MBCs were 425mg/mL and 212.5mg/mL against both clinically isolated strains, as shown in Table 3 and Figures 2C and D.

Anti-biofilm detection

The outcome of the tube testing method to determine the effect of *LS* extract on the capability of *P. gingivalis* and *P. intermedia* for biofilm formation is described in Table 3. The result demonstrated that the *LS* had a strong anti-biofilm effect, with a nearly complete absence of biofilm at the highest concentration; the stronger biofilm was formed in the tubes with decreasing concentration. At MIC and MBC concentrations, *LS* exhibited a strong anti-biofilm effect on both bacteria. Neither *P. gingivalis* nor *P. intermedia* produced visible biofilm at 0.12% CHX in the positive control tube. At the same time, *P. gingivalis* and *P. intermedia* showed strong biofilm formation in the negative control tube, which contained just broth and inoculum, as shown in Figure 2. B.

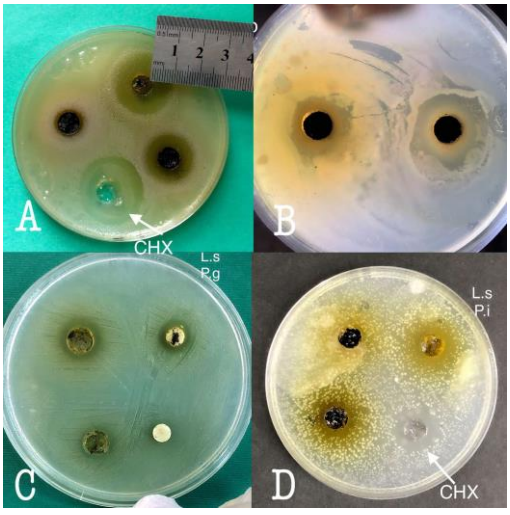

Figure 1: Determination of antibacterial susceptibility: **A** measuring the inhibition zone in (mm) by ruler, **B** inhibition zone of *LS* against *P. gingivalis*, **C** different concentrations of *LS* on *P. gingivalis*, **D** *LS* inhibition zone against *P. intermedia.*

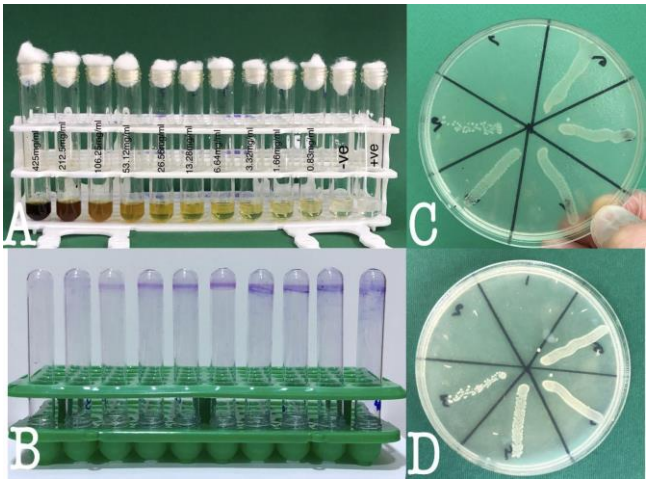

Figure 2: **A** two-fold serial dilution method for *LS* extract, **B** tube adhesion method for anti-biofilm detection,**C** MBC conformation on Mueller-Hinton agar, *LS* on *P. gingivalis*, **D** *LS* on *P. intermedia*.

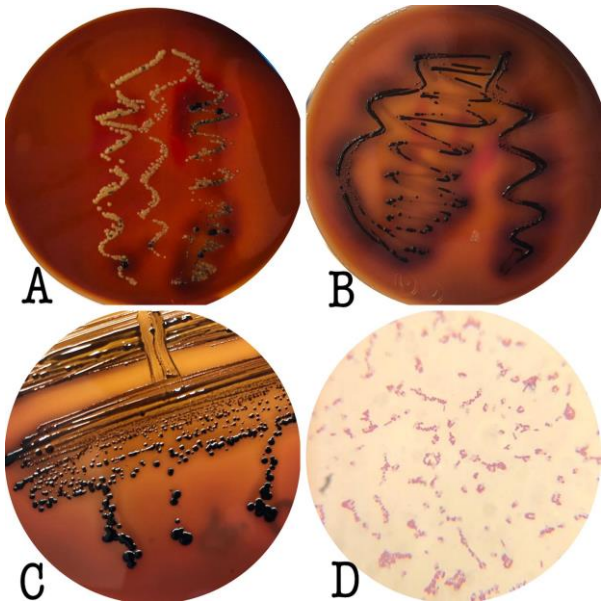

Figure 3: **A** Mixed colonies, **B** Pure colonies of *P.intermedia*, **C** Pure colonies of *P. gingivalis*, **D** Short gram-negative rods of *P. intermedia*.

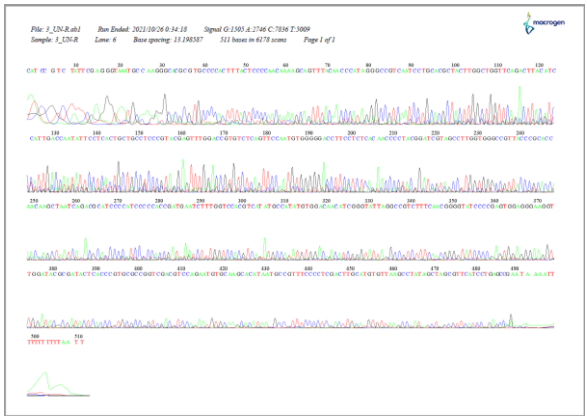

Figure 4: *P. intermedia* sequence: 16S rDNA gene sequence of the purified colonies of *P.intermedia* done in Macrogen, South Korea.

Table 1: GC-MSS analysis for the chemical composition of *Lactuca serriola* extract.

Peak	Peak area%	Molecular formula	Compound name
1	0.79	$C_4H_8O_3$	Glycolic acid
2	1.37	$C_5H_{10}O$	Cyclopentanol
3	1.40	$C_5H_{12}O_2$	Pentanediol-2,4
4	1.01	$C_{14}H_{22}O_2$	3,5-di(1,1-dimethylethyl)
5	1.53	$C_9H_{13}N$	Cumidine
6	0.82	$C_{10}H_{14}O$	D-Carvone
7	2.26	$C_6H_{11}NO$	Aminocaproic lactam,
8	1.00	$C_{10}H_{12}O$	Anethole
9	1.64	$C_9H_{10}O_2$	3-Methoxyacetophenone
10	1.42	$C_8H_8Cl_2O$	Dichloroxylenol
11	2.51	$C_4H_6O_3$	Formic acid, propionyl-
12	0.92	$C_9H_{12}O_2$	Ethanol, 2-(4-ethylphenoxy)-
13	3.76	$C_{12}H_{12}N_2O$	1H-Pyrido[3,4-b]indol-1-one
14	0.86	$C_{26}H_{42}O_4$	Phthalic acid, butyl tetradecyl ester
15	0.85	$C_{13}H_{12}O_2$	1-Methoxy-4-phenoxybenzene
16	0.78	$C_{17}H_{32}O_2$	Methyl palmitelaidate
17	13.03	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
18	1.04	$C_9H_{15}N$	1H-4-Azacycloprop[cd]indene, octah ydro-4 methyl-
19	7.10	$C_{16}H_{32}O_2$	Palmitic acid
20	15.89	$C_{19}H_{32}O_2$	Linolenic acid, methyl ester
21	12.64	$C_{20}H_{40}O$	Phytol
22	4.68	$C_{19}H_{38}O_2$	Stearic acid, methyl ester
23	7.11	$C_{18}H_{34}O_2$	Oleic Acid
24	5.15	$C_{18}H_{36}O_2$	Octadecanoic acid
25	1.11	$C_{20}H_{34}O_8$	Tributyl acetylcitrate
26	1.20	$C_{17}H_{19}NO_3$	Morphine
27	0.82	H_3O_3P	Phosphonic acid
28	1.14	$C_{11}H_{12}$	Naphthalene, 1,2,3,4-tetrahydro-1methylene-
29	3.80	$C_{31}H_{50}P_2$	Methylenebis(2,4,6-triisopropyl phenyl phosphine)
30	2.36	$C_{29}H_{62}O_2S$	Silane, diethylheptyloxyoctadecyloxy-

Table 2: Assessment of antibacterial activity of specific concentrations of *LS* extracts against *P. gingivalis* and *P. intermedia* using Paired t-test.

Concentration of *LS*	**Inhibition zone (*P. gingivalis*)**			**Inhibition zone (*P. intermedia*)**		
	LS	**0.12% CHX**	p-value	***LS***	0.12% CHX	p-value
	Mean/SD	Mean/SD		Mean/SD	Mean/SD	
100 (mg/ml)	11.75 ±0.95	13 ±0.81	0.141	12.75 ±0.95	12.75 ±0.95	1.000
250 (mg/ml)	18.5 ±1.29	12.75 ±0.95	0.001	20.25 ±0.95	13.25 ±0.95	0.002
500 (mg/ml)	24.25 ±1.7	13.25 ±0.95	0.002	25.5 ±1.29	13 ±0.816	0.000

Table 3: MIC, MBC for *LS* extracts against both bacteria, and Qualitative biofilm formation estimated by visible biofilm lining on the wall of the tubes.

Bacteria	**Antibacterial activity**		**Antibiofilm activity**		
	MIC	**MBC**	***LS***	**0.12% CHX (positive control)**	**Negative control**
P. gingivalis	212.5 mg/mL	425 mg/mL	+	0	+++
P. intermedia	106.2 mg/mL	212.5 mg/mL	+	0	+++

Discussion

Herbal medicine is the science of using plants with medicinal characteristics for the prevention and treatment of health-threatening conditions. Recently, interest in using traditional herbal medicine for healthcare has increased, particularly in developing nations. Oral health is a vital aspect of general health and quality of life, according to the World Health Organization (WHO), and due to their availability, low cost, and relatively few side effects, the use of natural drugs for the treatment of pathologic dental diseases can be a suitable alternative to pharmaceutical treatments. In addition, numerous manufacturers use herbal components to provide additional therapeutic properties[28].

Periodontal disease comprises multifactorial and poly-microbial infections affecting the teeth' supporting tissue. Periodontal disease severity ranges from mild gingivitis to advanced loss of connective tissue attachment and supporting bone[29]. Nonsurgical mechanical debridement and standard home care cannot eliminate all pathogenic flora. While antimicrobial therapies can be administered as an adjunctive treatment to mechanical debridement[30], they have several adverse effects, such as bacterial resistance, tooth staining, and altered taste sensation. Therefore, it is important to find alternative agents that have fewer side effects and can be used as adjunctive for periodontal therapy. This current study investigates the antibacterial and anti-biofilm activity of *LS* against *P. gingivalis* and *P. intermedia*.

The traditional Kurdish herb *LS* was used in this study because it has many pharmacological properties, is biocompatible with the body, and is easily available. The solvent extraction method was used to extract the bioactive compounds. Organic solvents, Methanol, and Acetone increased the extraction yield.

GC-MS analysis of *LS* extract revealed several bioactive components that have pharmacological properties. Among these, Linolenic acid had the highest concentration (15.89%) and consisted of a long chain of unsaturated fatty acids responsible for antibacterial activity. Unsaturated fatty acids exert their antibacterial effect by inhibiting the fatty acid synthesis in bacterial cells. Linoleic acid probably inhibits growth by increasing the permeability of the bacterial membrane[31]. The next highest concentration (13.03%) was of Hexadecanoic acid, methyl ester, which is a type of fatty acid ester. It can inhibit the growth of pathogenic bacteria and has a good inhibitory effect against Gram-positive and Gram-negative bacteria, with the cell membrane of bacteria as the main target of its action[32]. In addition, Phytol (12.64%) has antibiofilm activity[33], while Oleic acid (7.11%) is supposed to have modulatory effects, beneficial impact on cancer, autoimmune and inflammatory diseases, and facilitate wound healing[34]. Palmitic acid (7.10%) has a role in inflammatory response by activating toll-like receptors TLR2 and TLR4[35]. The genotype of the plant and the influence of environmental factors, such as geographic conditions, soil type, temperature, the season of collection, and the extraction process, all significantly impact the chemical composition and active ingredient concentrations of *LS* extracts. They are responsible for most of the differences in the active component ratios reported by different studies conducted in different geographical regions.

In the well diffusion assay, the extract of *LS* showed positive inhibitory activity with a wide inhibition zone at different concentrations against both *P. gingivalis* and *P. intermedia*. In addition, *LS* extract has antibacterial activity due to the presence of alkaloid, phenolic, and terpenoid compounds[36].

The MIC values of *LS* against *P. gingivalis* and *P. intermedia* were evaluated by no visible growth of bacteria seen in tubes using the macro broth dilution method since it is easy and readily available, and the naked eye can determine the result. In the current study, the lowest concentration of *LS* on *P. gingivalis* exhibiting MIC was 212.5mg/mL, and for MBC, it was 425mg/mL. The corresponding figures against *P. intermedia* were 106.2 mg/mL for MIC and 212.5mg/Ml for MBC, as seen in Table 3. There is no other study to compare the results with because this study is the first to demonstrate the antibacterial activity of *LS* against periodontal pathogens such as *P. gingivalis* and *P. intermedia*. The extract of *LS* had beneficial antibacterial effects against *P. gingivalis* and *P. intermedia* which are involved in periodontal disease. Additional research is required to understand better this therapeutic effect and the prophylactic use of *LS* extract.

A polymicrobial interaction favors a biofilm-based life over a free planktonic condition. With all the benefits associated with biofilm formation, such as bacterial synergism, nutrient exchange, neutralizing harmful molecules, and antibiotic resistance[37], the bacteria must stabilize this mode of living by attaching themselves firmly to the available surfaces, such as teeth and soft tissues, to sustain it. In parallel to this, the assessment of the biological activity of *LS* extract may be enhanced by analyzing its anti-biofilm effect; hence, after determining the MICs of the relevant extract, the tube adhesion method was applied using the same tubes. The findings revealed that the anti-biofilm activity of *LS* extract increased with increased *LS* concentrations because they contained greater amounts of bioactive compounds such as Phytol that have been linked to anti-

biofilm activity[33]. GC-MSS analyses demonstrated that Phytol, which comprised 12.64% of the *LS* extract, is responsible for the anti-biofilm property of *LS* extract. The study's limitations included, first, unavailability of resources because no other study has investigated LS extract's antibacterial and anti-biofilm activities against periodontal pathogens. Second, just two periodontal pathogens were used in this study. Therefore, it is necessary to perform additional research on larger bacterial communities or complex biofilms that are more virulent than planktonic bacteria.

Conclusions

From the current study, it can be concluded that *LS* has strong antibacterial and anti-biofilm activity against *P. gingivalis* and *P. intermedia*. Hence, *LS* extract can be used as an adjunctive agent in the treatment of periodontal disease due to its favorable bioactivity, easy availability, and simple extraction process. However, the impact of *LS* in clinical practice and the complexity of the life span of oral bacteria in natural habitats demand additional research.

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