

Evaluation of Antibacterial and Antibiofilm Effect of Laurel Leaf Essential Oil Against *Prevotella intermedia*: An in vitro Study

Defne Yaprığı Esansiyel Yağının Prevotella intermedia'ya Karşı Antibakteriyel ve Antibiyofilm Etkisinin Değerlendirilmesi: İn vitro Bir Çalışma

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ABSTRACT

Objective: This study aims to evaluate the antimicrobial and antibiofilm activity of laurel leaf oil against *Prevotella intermedia* (*P. intermedia*) DSM20706.

Methods: The components of laurel leaf oil were detected by gas chromatography-mass spectroscopy (GK-KS). The agar diffusion cytotoxicity method was used to detect the cytotoxicity of laurel leaf oil to fibroblast cells, and the minimum inhibitory concentration (MIC) assay was used to determine antibacterial activity. The effect of laurel leaf oil on bacterial morphology was evaluated by scanning electron microscopy (SEM) and fluorescence microscopy. In addition, the effect of laurel leaf oil on gingival fibroblast cell morphology was studied. The effect of laurel leaf oil on biofilm formation was evaluated numerically using a microplate reader.

Results: 1,8-cineole (64.43%), terpinenyl acetate (11.56%) and pinene (5.62%) were found to be the main constituents. No cytotoxic effect was observed in the cell groups treated directly and with 50% concentration of laurel leaf oil and no zone diameter was observed. The MIC value of the laurel leaf oil for *P. intermedia* was determined to be 6.25 µl. Moreover, no biofilm formation was observed when laurel leaf oil was applied at a concentration of 3.125 µl. The images obtained from SEM showed that the laurel leaf oil applied to the microorganism destroyed the bacteria. In fluorescence microscope, it was observed that the bacteria were fragmented and the particles radiated. When fibroblast cells stained with double staining solution were examined under the fluorescence microscope, it was found that the cells had not died.

Conclusion: As a result, it was determined that laurel leaf oil has antibacterial and antibiofilm effect against *P. intermedia* DSM20706.

Keywords: Antimicrobial activity, in vitro, laurel leaf oil, *P. intermedia*

ÖZ

Amaç: Bu çalışmada defne yaprağı yağının *Prevotella intermedia* (*P. intermedia*) DSM20706'ya karşı antimikrobiyal ve antibiyofilm aktivitesinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Defne yaprağı yağının bileşenleri gaz kromatografisi-kütle spektroskopisi (GK-KS) ile tespit edildi. Defne yaprağı yağının fibroblast hücrelerine olan sitotoksitesini tespit etmek için agar difüzyon sitotoksitesite yöntemi kullanıldı ve antibakteriyel aktiviteyi belirlemek için minimum inhibitör konsantrasyon (MİK) testi kullanıldı. Defne yaprağı yağının bakteri morfolojisi üzerindeki etkisi taramalı elektron mikroskobu (SEM) ve floresan mikroskobu ile değerlendirildi. Ayrıca defne yaprağı yağının dişeti fibroblast hücre morfolojisi üzerindeki etkisi incelendi. Defne yaprağı yağının biyofilm oluşumu üzerindeki etkisi bir mikro plaka okuyucu kullanılarak sayısal olarak değerlendirildi.

Bulgular : 1,8-sineol (%64,43), terpinenil asetat (%11,56) ve pinen (%5,62) ana bileşenler olarak bulundu. Defne yaprağı yağının doğrudan ve %50 konsantrasyonuyla tedavi edilen hücre gruplarında sitotoksik etki gözlenmedi ve zon çapı gözlenmedi. Defne yaprağı yağının *P. intermedia* için MİK değeri 6,25 µl olarak belirlendi. Ayrıca defne yaprağı yağı 3,125 µl konsantrasyonda uygulandığında biyofilm oluşumu gözlenmedi. SEM'den elde edilen görüntüler, mikroorganizmaya uygulanan defne yaprağı yağının bakterileri yok ettiğini gösterdi. Floresan mikroskobunda bakterilerin parçalandığı ve parçacıkların ışınlandığı görüldü. Çift boyama solüsyonuyla boyanan fibroblast hücreleri floresan mikroskobu altında incelendiğinde hücrelerin ölmediği görüldü.

Sonuç: Sonuç olarak, defne yaprağı yağının *P. intermedia* DSM20706'ya karşı antibakteriyel ve antibiyofilm etkisine sahip olduğu belirlendi.

Anahtar Kelimeler: Antimikrobiyal aktivite, in vitro, defne yaprağı yağı, *P. intermedia*

INTRODUCTION

The approaches to periodontal treatment mainly include cleaning of the tooth surface and mechanical cleaning in planning the root surface.¹ In the treatment of periodontal disease, local and systemic antimicrobial agents are used in addition to mechanical cleaning.² The increasing resistance to antimicrobials used in addition to mechanical therapy, which have some side effects, have led scientists to investigate the antimicrobial and antifungal effects of plants.³ Plants and their extracts have been used in traditional medicine for centuries.⁴ The phenolic compounds of plants are often used in the treatment of infectious diseases caused by bacteria.⁵ Essential oils are produced by special cells or groups of cells found in plant parts such as roots, bark, seeds, flowers, leaves, fruits and stems. Essential oils are characterized by two or three major constituents (terpenes and terpenoids, 20-70%) present in very high concentrations, and other minor constituents (phenylpropanoids) present in very high concentrations that determine their important biological properties. Essential oils are known for their anti-inflammatory, antimicrobial and antioxidant properties. Oxygenated terpenoids and some hydrocarbons present in essential oils are responsible for most of their antimicrobial activity. The main mode of action of essential oils is based on disrupting the membrane integrity of gram (+) and gram (-) bacteria.⁶⁻⁸

Laurel, a plant of the maquis flora is a perennial, non-shooting, rounded and densely branched plant that grows about 78 inch high. The homeland of laurel, which can be called a characteristic plant of the Mediterranean climate, is Anatolia. About 97% of the world production comes from Turkey. Used are the leaves and fruits of the plant. The leaves are rich in essential oils. Because of its traditional use and commercial value, the chemical composition of its leaves has been studied more than that of any other part. The essential oil of laurel leaves has antimicrobial properties and is used as an antirheumatic, antiseptic, diaphoretic, digestive and diuretic.^{9,10} Phytochemical studies conducted in all parts of the laurel plant characterized the presence of the monoterpene 1,8-cineole as the predominant essential oil compound 1,8-cineole usually accounts for 50% to 70% of the parent compound. 1,8-cineole is a saturated monoterpene found in several plant species.¹¹ It has been shown to be effective in the treatment of respiratory diseases, cancers, digestive system disorders, dysphoria, cardiovascular diseases, and bacilli.¹²⁻¹⁶ Various studies have shown that 1,8-cineole has antibacterial activity and inhibits some strains of bacteria.^{17,18} The aim of this study is to investigate the antimicrobial and antibiofilm effects of laurel leaf oil against periodontal pathogens *P. intermedia* in an in vitro study.

METHODS

These study analyzes were conducted at Kırıkkale University Scientific and Technological Research Application and Research Center and Hitit University Scientific and Technical Application and Research Center. 20 mL laurel leaf essential oil used in our study was obtained from NATIVITAL Natural Life and Health Products. *P. intermedia*, a Gram-negative anaerobic bacterium, was obtained from DSM 20706 Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures.

Chemicals

Primary gingival fibroblast cells were provided from Kırıkkale University Scientific and Technological Research Application and Research Center. 89% DMEM (Capricorn), 10% the complete culture medium for the cells was fetal bovine serum (FBS; Capricorn), 1% L-glutamine (Capricorn), 1% penicillin/streptomycin antibiotic (Biological Industries).

For removal of cells from culture flasks, 0.05% Trypsin-EDTA solution (Trypsin-Ethylenediamine Tetraacetic Acid, Capricorn) and 1X concentration Calcium/Magnesium-free phosphate buffer (PBS, Biological Industries) were used for the removal of cells from the culture flasks, a 0.05% trypsin-EDTA solution (trypsin-ethylenediamine tetraacetic acid, Capricorn) and a 1X concentration of calcium/magnesium-free phosphate buffer (PBS, Biological Industries) were used for washing and cleaning procedures. For cell counting (1:1 ratio), trypan blue was used. Tetrazolium salt MTT (serva, Israel) was used to determine cell viability in the cytotoxicity assay. For cell culture studies, serology pipettes, culture dishes and multiple well plates (Corning, USA) were used.

Cell Culture

The frozen cells were rapidly thawed at 37°C and the cells were dissolved in a sterile class II laminar flow cabinet and transferred to a 15 mL Falcon tube. Centrifuged at 250G for 5 minutes. 3 mL of complete culture medium was added to the Falcon and, after homogenisation, 25 cm² flasks were cultured. The flasks were incubated at 37°C in an incubator containing 5% CO₂. Freezed cells were passaged until the proliferating cycle was stabilised.

Cytotoxicity Test (MTT)

EN ISO 10993-5, which is used to determine cell viability and the effects of materials on cell viability: This is the reference standard for medical materials. 96-well plates were used to test. Cells were counted and the number of viable cells in each well calculated. Cells were added to 100 µl of complete medium in each well of the 96-well plates and incubated for 24 hours. Cell adherence to the surface of the well plate, morphology and viability were checked at the end of 24 hours. The medium in the wells which were in accordance with the criteria under investigation was drained. Bay leaf oil was added at 50% of the total volume and its lower dilutions (25%, 12.5%, 6.25%, 3.125% and 1.56%). In the negative control group, only complete culture medium was used. The cells were incubated for a period of 24 hours with the materials used. MTT (1 mg/mL) solution was pipetted into the well. The MTT solution was drained from the wells and 100 µL MTT solvent (isopropanol) was added after incubation for 2-2.5 hours at 37°C. The absorbance density values of the 96-well plate were read at 570 nm in a Multiplaka plate reader to determine cell viability. The percentage of cell viability of each group was calculated as in Erdem et al.¹⁹, assuming control cell viability as 100%.

Agar Diffusion Cytotoxicity Test

This assay was designed to demonstrate non-specific cytotoxicity of the test material after diffusion from agar or agarose. This method is preferred in cases where the material has a cytotoxic effect directly on the cell or the chemical marked in cytotoxicity tests such as MTT, XTT WST is not effective. Materials such as essential oils, dental filling materials, tissue scaffolds, wound dressings interact with the cell and sometimes lyse the cell by showing a disintegrating effect. and its toxicity cannot be measured by classical methods, and sometimes it can interfere with the colorimetric chemicals used in the measurement of cytotoxigen and prevent it from radiating. For the test method, the agar diffusion cytotoxicity method specified in TS EN ISO 7405:2018 Section 6.2 was used.²⁰ In this experimental method, L929 fibroblast cells were used as the cell line. Complete culture medium containing 90% DMEM, 10% FBS and 1% antibiotics is used for the selected cell line. The prepared medium was sterilized by passing through 0.2 µm membrane filters. Two layers of concentrated culture medium were used to prepare the agar. 3-5% agar or 3% agarose is prepared. It was sterilized by autoclave. Vital paint was prepared just before use by diluting 1% neutral red aqueous stock solution with 0.01 mol/L

phosphate buffered saline at a ratio of 1:100. Neutral red solutions were stored protected from light. The experiment was carried out in 6-well culture dishes. The cells were cultured until they reached the end of the logarithmic growth phase (frozen cells were subjected to at least two passages before the experiment). The study was performed according to the "TS EN ISO 7405/Agar Diffusion Test" standard. Cells seeded in 6-well plates were counted as 20x10⁴/well and incubated for 24 hours to achieve 80% confluency. At the end of 24 hours, the medium on the cells was discarded, and 3-5% Agar medium mixed with fresh medium was added to the cells. The agar culture medium mixture was allowed to solidify at room temperature (approximately 30 min). Add 10 mL of neutral reddish solution and keep in the dark for 15-20 minutes. Excess neutral red solution was aspirated. Then, by impregnating the blind membranes with bay leaf oil directly and diluted 50%, the cells were exposed to the agar surface for 24 hours. At the end of the procedure, the wells were washed with 1X PBS and the 6-well plate was closed so that no light could be seen. At the end of incubation, cells were evaluated using an inverted microscope. Tables specified in the standard were used for evaluation (ISO 7405:2018).

Minimum Inhibition Concentration (MIC) Test

Gram negative bacteria *P. intermedia* DSM 20706 was used in the study. Cultures stocked for the determination of antibacterial activity are augmented by incubation at 37°C for 18-24 hours in Muller Hinton Agar (MHA) and Brain Heart Infusion Agar (BHIA). After 24 hours of incubation, it was adjusted to 0.5 McFarland value (10⁸ CFU/mL) (absorbance values range from 0.08 to 0.13). Following these steps, 100 µl of Muller Hinton broth (10.5 mg Muller Hinton was added into 500 ml of distilled water and prepared by sterilizing in an autoclave) medium was added to sterile, U-bottomed, 96 well-plates to be used in the microdilution method. The prepared bay leaf oil was added to the wells at the determined concentrations (50 µl, 25 µl, 20 µl, 12.5 µl, 10 µl, 6.25 µl, 3.125 µl and 1.625 µl concentrations in the well) with a total volume of 100 µl in each well. Then, the microorganisms adjusted at 0.5 McFarland value were diluted 1/100 with physiological saline and 10 µl was added (the added microorganism concentration corresponds to 10⁵). Sterility control was made by adding only MHA medium to the 6 wells of the plate used, and growth control was performed by applying MHA medium containing microorganisms to 6 wells. The prepared test system was left to incubate at 37°C for 24 hours. After incubation, the first well without growth was determined as the MIC value. For this process, growth control was performed by measuring the absorbance in both visual growth control and spectrophotometer. In addition, the sample taken from the first well in which no growth was observed and the MIC value was determined, was planted in sheep blood agar and left to incubate for 24 and 48 hours. Reproduction control test was done. Since all microorganisms used were anaerobic, all incubation processes were carried out in an oxygen-free environment, in a jar.

Examining the morphology of bacteria

P. intermedia DSM 20706 was incubated with appropriate concentrations of bay leaf oil, the concentration of which was determined by microorganism MIC and cytotoxicity test, for 24 hours and without any application substance. It was fixed in 2.5% glutaraldehyde overnight at 4°C to allow fixation prior to SEM imaging. Glutaraldehyde was removed from the medium and washed twice with PBS. Samples coated with a gold sputter coating were observed with a scanning electron microscope (Quanta FEG 250, Kastamonu University). In addition, fluorescent marking was performed to view the parts of the bacteria and to evaluate the membrane integrity, if any, and both the activation of the biofilm layer and the morphology of the bacteria was

examined. PI (Propidium Iodide) dye and hoechst 33342 dye will be used for this process.

Determination of Cell Morphologies

PI was used to stain the nucleus and hoechst 33342 dye was used to stain the viable cell membrane in order to check the membrane integrity of the cells to which bay leaf oil has been applied. 6-well plates was used for this process. The gingival fibroblast cells, which were also used in the cytotoxicity test, were seeded on the plates at a rate of 500,000 per well. Bay leaf oil prepared in different concentrations interacted with the cells for 24 hours. After the end of the application, the cells were evaluated in DAPI and FITC filters, and both their membrane structures, cell morphologies and the cause of apoptosis/necrosis deaths was evaluated.

RESULTS

Chemical Components of Laurel Leaf Oil

The components of laurel leaf oil added to the device GK-KS under suitable conditions were matched with the library research and their percentage values are shown in Table 1. According to the obtained result, 1,8-cineole (64.43%), alpha-terpinenyl acetate (11.56%) and alpha-pinene (5.62%) were defined as the main components.

Table 1. Percentage values of the components in the bay leaf oil sample

Component name	Area %
à-Thujene	0.29
α-Pinene	5.62
Camphene	0.80
Sabinene	4.91
2-β-Pinene	2.25
β-Myrcene	0.37
α-Terpinen	0.34
1,8-cineole (Eucalyptol)	64.43
cis-Ocimene	0.15
γ-Terpinen	0.41
CIS-Sabinene Hydrate	0.24
L-Linalool	1.71
1-Terpineol	0.13
Isopinocampheol	0.27
Pinocarvone	0.17
α-Terpineol	0.40
4-Terpineol	2.27
β-Phenylethanol	0.97
Myrtenal	0.27
Bornyl acetate	0.76
Ocimenyl acetate	0.58
α-Terpinenyl Acetate	11.56
β-Elemene	0.28
trans-Caryophyllene	0.63
Caryophyllene oxide	0.20

Cytotoxicity Analysis

The data on lysis and decolorization indices obtained after direct and 50% application of laurel leaf oil are shown in Table 2. No cytotoxic effect was observed in the treated cell groups and no zone diameter was observed around the disk (Figure 1). In addition, the morphology and the edges of the cells are smooth, indicating that there is no cytotoxic effect.

Table 2. Lysis and decolorization indices after bay leaf oil application

	Lysis index	Decolorization index
Bay Leaf Oil Directly	0	0
Bay Leaf Oil 50% Concentration	0	0
Negative Control	0	0

MIC Assay

Growth was observed in wells treated with a concentration of 3.156 µl (containing 3.156 laurel leaf oil). No growth was observed at the concentrations of 6.25 µl, 12.5 µl, 25 µl, and 50 µl of the laurel leaf

oil sample used (Figure 2). The experiment was performed in 3 separate plates and in 3 replicates for each plate, and no difference was observed in the results.

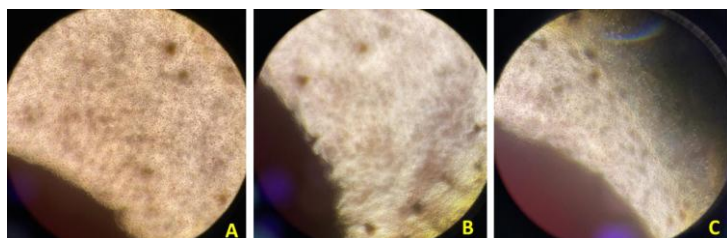


Figure 1. The image of the cells applied after the agar diffusion test in an inverted microscope

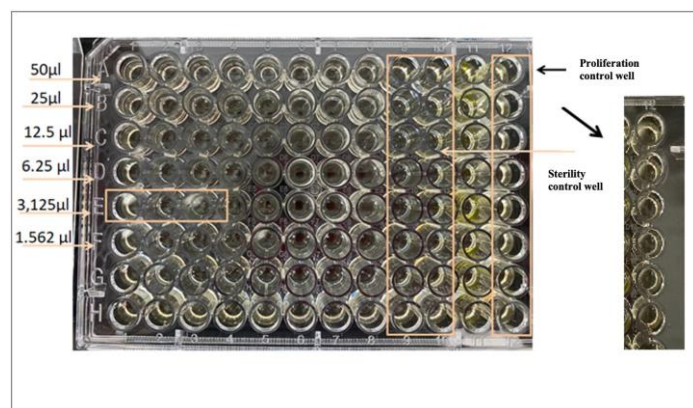
A: Images of cells with full concentration of laurel leaf oil

B: Images of negative control cells

C: Images of cells applied 50% concentration of laurel leaf oil (Images were taken at 10X magnification)

The Effect of Laurel Leaf Oil on *P. intermedia* Biofilm Formation

After the decolorization process the absorbance was measured at 590 nm and evaluated numerically. While moderate biofilm formation was observed in the wells where laurel leaf oil was applied at a concentration of 1.562 μ l, no biofilm formation was observed at a concentration of 3.125 μ l. Low biofilm formation was observed when a medium concentration of 2 μ l was applied. Severe biofilm formation was observed in the group of microorganisms that were not subjected to any treatment (Figure 3).



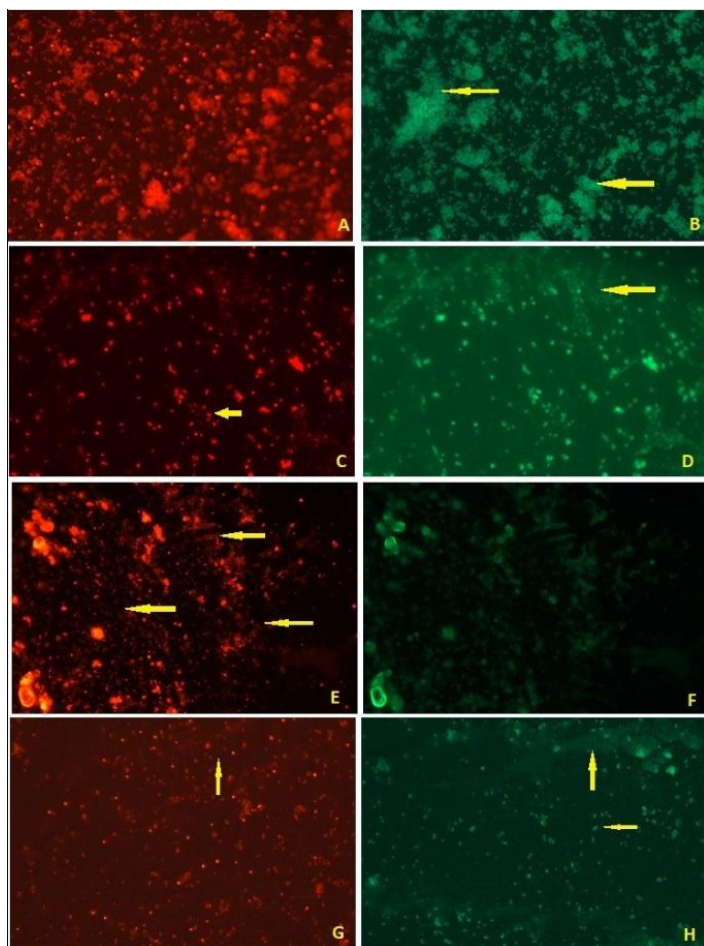


Figure 5. Imaging of bacteria under fluorescence microscopy after double staining

A: control group in FITC filter

B: Control group in DAPI filter, arrow indicates biofilm layer

C: 12.5 μ l of laurel leaf oil applied microorganism image on FITC filter, arrow indicates chromosome fragments

D: The image of the microorganisms applied 12.5 μ l of laurel leaf oil on the DAPI filter, the arrow indicates the inactive biofilm layer

E: The images of microorganisms applied 50 μ l of laurel leaf oil in FITC filter, arrows indicate chromosome fragments

F: The images of microorganisms in the DAPI filter, in which 50 μ l of laurel leaf oil was applied, no microorganism with membrane integrity could be detected

G: Microorganisms applied 6.25 μ l of laurel leaf oil appear on FITC filter, arrows indicate chromosome fragments

H: The images of the microorganisms applied 6.25 μ l of laurel leaf oil in the DAPI filter, the biofilm layer was shown to be inactive, rare bacterial growths



Figure 6 Imaging of gingival fibroblast cells under fluorescent microscope after double staining

A: 6.25 μ l of laurel leaf oil applied fibroblast cells image on FITC filter

B: 6.25 μ l of laurel leaf oil applied fibroblast cells image on DAPI filter

C: Untreated gingival fibroblast cells

DISCUSSION

The treatment of periodontal disease remains challenging due to its multifactorial etiology and complex disease course. Mechanical and surgical approaches to periodontal disease have been combined with antimicrobial therapy for many years. However, success rates are not at the desired level due to difficulties in application and undesirable side effects. The high cost also has a negative impact on access to treatment. Herbal products are preferred over conventional drugs due to their broad biological efficacy, high safety, and low cost and are used for both prevention and treatment of periodontal disease.

Laurel is one of the plants that have long been studied in medicine. Studies have shown that laurel oil has antimicrobial activity against various microorganisms.²¹⁻²³ In reviewing the literature, the effect of laurel leaf oil on no periodontopathogen was evaluated. Therefore, in this study, the antimicrobial and antibiofilm effects of laurel leaf oil against *P. intermedia* were investigated in vitro. In studies conducted in different years, it was found that the proportion of 1,8-cineole in the essential oil of laurel is higher than the other components.²¹⁻²³ In accordance with the results of the studies in the literature, it was found in our study that the proportion of 1,8-cineole (64.43%) is significantly higher than the other components in the essential oil of laurel leaf. 1,8-cineole is a saturated monoterpene found in various plant species and its main source is eucalyptus leaves.

As an active ingredient, 1,8-cineole has been shown to play an important role in the treatment of respiratory diseases, cancers, digestive system disorders, dysphoria, cardiovascular diseases, and bacilli¹¹. It is suggested that the antibacterial effect of laurel essential oil used in our study is mainly due to 1,8-cineole. It is believed that 1,8-cineole, the main component of some essential oils, acts on plasma membranes, which are the target of chlorhexidine. Şimşek & Duman²⁴ investigated the antimicrobial properties of 1,8-cineole alone or in combination with chlorhexidine in their study. They concluded that chlorhexidine in combination with 1,8-cineole showed synergistic antimicrobial activity in some microorganisms tested. The synergistic activity between chlorhexidine and 1,8-cineole resulted in the elimination of some microcolonies that had developed resistance to chlorhexidine. The lowest concentration tried at various concentrations at which the antimicrobial component completely stops microbial growth is defined as the MIC. MIC is considered the gold standard for determining the susceptibility of microorganisms to antimicrobial agents. Park et al.²⁵ evaluated the antibacterial activity of ginger extract against *P. gingivalis*, *P. endodontalis*, and *P. intermedia*, which are Gram-negative anaerobic bacteria that cause periodontal disease, in vitro. They showed that the growth of these oral pathogens was effectively inhibited by ginger compounds 6-30 in the range of μ g/mL MIC. Izui et al.²⁶ investigated the antibacterial effect of curcumin extracted from turmeric root on *F. nucleatum*, *P. gingivalis*, *T. denticola*, and *P. intermedia* and found that curcumin inhibited the growth of these bacteria in a dose-dependent manner. MIC values were determined to be 10 μ g/mL, 15 μ g/mL, 10 μ g/mL, and 5 μ g/mL for *F. nucleatum*, *P. gingivalis*, *P. intermedia*, and *T. denticola*, respectively. Ramak and Talei²⁷ evaluated the antibacterial and antibiofilm effects of Deliçay plant (*Stachys koelzii*) essential oil against *P. intermedia* in vitro. Thus, the essential oil extracted from this plant was shown to have acceptable antibacterial and antibiofilm activity against *P. intermedia* at low levels (0.1 and 0.2 mg/ml MIC). In our study, no bacterial growth was observed at the concentrations of 6.25

µl, 12.5 µl, 25 µl and 50 µl of laurel leaf essential oil against *P. intermedia* DSM 20706. Therefore, the MIC of laurel leaf oil used in our study was set at 6.25 µl. In our study, the cytotoxic effect of laurel leaf oil was evaluated using the agar diffusion cytotoxicity method. This method is preferred in dental studies when the material has a direct cytotoxic effect on the cell. Essential oils lyse the cell by exhibiting a disintegrating effect, making their toxicity unmeasurable by classical methods.

When the effect of laurel oil on bacterial morphology was studied, the microorganism fragments obtained in the SEM images supported each other with fluorescence microscopy. In the SEM images, it was found that the bacterial morphology was deteriorated, the capsule structure had changed and fragments were present. Staining of the nuclear fragments under fluorescence microscope showed that the bacterial membrane structure was ruptured and the genetic material was scattered. Thus, it was confirmed that the laurel leaf oil used kills the bacteria by dissolving them. The results obtained with the MIC test were confirmed by SEM and the fluorescence microscopy.

When evaluating the effect of laurel leaf oil on gingival fibroblast cells, it was found that cell morphology did not deteriorate and membrane integrity was maintained. In addition, it was observed that the cells did not show necrotic death in the staining method, indicating that there was no inflammatory potential. The fact that laurel leaf oil was not compared with chlorhexidine (control group), which is considered the gold standard can be considered a limitation of the study. As a result, in this study, the antibacterial effect of laurel leaf oil against periodontal pathogen *P. intermedia* DSM 20706 was investigated and found to have antibacterial activity. In vivo and clinical studies are needed to better understand the antibacterial activity of laurel leaf oil. The antibacterial effect of this oil on various periodontal pathogens can also be evaluated. Moreover, this vegetable oil is one of the active ingredients that can be used as an agent for topical application such as toothpaste, mouthwash, gel and in addition. For this reason, this topic can be supported by various scientific studies.

Ethics Committee Approval: Since there was no human/animal substrate in this study, no ethical approval was obtained.

Informed Consent: An informed consent form is not required.

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REFERENCES

1. Aimetti M. Nonsurgical periodontal treatment. *Int J Esthet Dent*. 2014;9(2):251–267.
2. Herrera D, Matesanz P, Bascones-Martínez A, Sanz M. Local and systemic antimicrobial therapy in periodontics. *J Evid Based Dent Pract*. 2012;12(3 Suppl):50–60.
3. Martínez C, Díaz Gómez M, Oh MS. Use of traditional herbal medicine as an alternative in dental treatment in Mexican dentistry: a review. *Pharm Biol*. 2017;55(1):1992–1998.
4. Khameneh B, Iranshahy M, Soheili V, Fazly Bazzaz BS. Review on plant antimicrobials: a mechanistic viewpoint. *Antimicrob Resist Infect Control*. 2019;8:118.
5. Bunte K, Hensel A, Beikler T. Polyphenols in the prevention and treatment of periodontal disease: A systematic review of in vivo, ex vivo and in vitro studies. *Fitoterapia*. 2019;132:30–39.
6. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—a review. *Food And Chemical Toxicology: Int J Publish Br Industr Biol Res Assoc*. 2008;46(2):446–475.
7. Bassolé IH, Juliani HR. Essential oils in combination and their antimicrobial properties. *Molecules (Basel, Switzerland)*. 2012;17(4):3989–4006.
8. Mutlu-İngok A, Devcioglu D, Dikmetas DN, Karbancioglu-Guler F, Capanoglu E. Antibacterial, Antifungal, Antimycotoxigenic, and Antioxidant Activities of Essential Oils: An Updated Review. *Molecules (Basel, Switzerland)*. 2020;25(20):4711.
9. Paparella A, Nawade B, Shaltiel-Harpaz L, Ibdah, M. A Review of the Botany, Volatile Composition, Biochemical and Molecular Aspects, and Traditional Uses of *Laurus nobilis*. *Plants (Basel, Switzerland)*. 2022;11(9):1209.
10. Ozcan B, Esen M, Sangun MK, Coleri A, Caliskan M. Effective antibacterial and antioxidant properties of methanolic extract of *Laurus nobilis* seed oil. *J Environ Biol*. 2010;31(5):637–641.
11. Cai ZM, Peng JQ, Chen Y, et al. 1,8-Cineole: a review of source, biological activities, and application. *J Asian Nat Prod Res*. 2021;23(10):938–954.
12. Juergens LJ, Worth H, Juergens UR. New Perspectives for Mucolytic, Anti-inflammatory and Adjunctive Therapy with 1,8-Cineole in COPD and Asthma: Review on the New Therapeutic Approach. *Adv Ther*. 2020;37(5):1737–1753.
13. Juergens UR. Anti-inflammatory properties of the monoterpene 1,8-cineole: current evidence for co-medication in inflammatory airway diseases. *Prog Drug Res*. 2014;64(12):638–646.
14. Rocha Caldas GF, Oliveira AR, Araújo AV, et al. Gastroprotective Mechanisms of the Monoterpene 1,8-Cineole (Eucalyptol). *PLoS One*. 2015;10(8):e0134558.
15. Murata S, Shiragami R, Kosugi C, et al. Antitumor effect of 1, 8-cineole against colon cancer. *Oncol Rep*. 2013;30(6):2647–2652.
16. Seol GH, Kim KY. Eucalyptol and Its Role in Chronic Diseases. *Adv Exp Med Biol*. 2016;929:389–398.
17. Merghni A, Noumi E, Haddad O, et al. Assessment of the antibiofilm and antiquorum sensing activities of *Eucalyptus globulus* essential oil and its main component 1,8-cineole against methicillin-resistant *Staphylococcus aureus* strains. *Microb Pathog*. 2018;118:74–80.

18. Moghimi R, Aliahmadi A, Rafati H. Ultrasonic nanoemulsification of food grade trans-cinnamaldehyde: 1,8-Cineol and investigation of the mechanism of antibacterial activity. *Ultrason Sonochem.* 2017;35(Pt A):415–421.
19. Erdem U, Bozer BM, Turkoz MB, et al. Spectral analysis and biological activity assessment of silver doped hydroxyapatite. *J Asian Ceramic Soc.* 2021;9(4):1524-1545.
20. International Organization for Standardization. Dentistry-Evaluation of Biocompatibility of Medical Devices Used in Dentistry. *ISO.* (2018).
21. Merghni A, Marzouki H, Hentati H, Aouni M, Mastouri M. Antibacterial and antibiofilm activities of *Laurus nobilis* L. essential oil against *Staphylococcus aureus* strains associated with oral infections. *Pathol Biol.* 2015;S0369-8114(15)00101-7.
22. Caputo L, Nazzaro F, Souza LF, et al. *Laurus nobilis*: Composition of Essential Oil and Its Biological Activities. *Molecules (Basel, Switzerland).* 2017;22(6):930.
23. Nabila B, Piras A, Fouzia B, et al. Chemical composition and antibacterial activity of the essential oil of *Laurus nobilis* leaves. *Nat Prod Res.* 2022;36(4):989–993.
24. Şimşek M, Duman R. Investigation of Effect of 1,8-cineole on Antimicrobial Activity of Chlorhexidine Gluconate. *Pharmacognosy Res.* 2017;9(3):234–237.
25. Park M, Bae J, Lee DS. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. *Phytother Res: PTR.* 2008;22(11):1446–1449.
26. Izui S, Sekine S, Maeda K, et al. Antibacterial Activity of Curcumin Against Periodontopathic Bacteria. *J Periodontol.* 2016;87(1):83–90.
27. Ramak P, Talei GR. Chemical composition, cytotoxic effect and antimicrobial activity of *Stachys koelzii* Rech.f. essential oil against periodontal pathogen *Prevotella intermedia*. *Microb Pathog.* 2018;124:272–278.