Journal of Lasers in Medical Sciences

J Lasers Med Sci. 2026;17:e3

Original Article

http://journals.sbmu.ac.ir/jlms

doi 10.34172/jlms.2026.03

Evaluation of Propolis as an Intracanal Agent in Combination with an Nd:YAG Laser for Bacterial Reduction

Mahdieh Naghizadeh[1], Elaheh Fakhri[2], Hossein Samadi Kafil[3], Hosein Eslami[4*], Azam Valian[1]

[1]Department of Restorative Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2]Dental and Periodontal Research Center, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Drug Applied Research Center, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Department of Oral Medicine, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran

***Correspondence to**
Hosein Eslami,
Email: eslamihosein56@yahoo.com

Received: February 28, 2025
Accepted: November 8, 2025
ePublished: February 2, 2026

Abstract

Introduction: This study aimed to assess the antimicrobial efficacy of propolis for an intracanal medicament in combination with an Nd:YAG laser against the biofilm of persistent endodontic pathogens: *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans*.

Methods: One hundred and sixty-five extracted human premolars were included. Monospecies of four-week biofilms were cultured in root canals after the teeth were prepared chemo-mechanically. Specimens were treated with calcium hydroxide for 24 hours (G1), propolis for 24 hours (G2), Nd:YAG laser (1064 nm, 1.5 w, 15 Hz, 100 mJ/pulse, 200 µm endodontic fiber tip) (G3), propolis medicament followed by Nd:YAG laser (G4), and normal saline as the control group (G5). Dentin chips were obtained with Gates-Glidden #5, 6 and inoculated onto agar plates for microbial growth. The antimicrobial efficacy of each group was evaluated by quantifying the number of colony-forming units. Data were statistically analyzed using the Kruskal-Wallis and Mann-Whitney U test (P value<0.05).

Results: All investigated groups reduced the bacterial count compared to normal saline (P value<0.05). Propolis (0.23 ± 0.58, 0.00 ± 0.00, 1.34 ± 1.12 for *E.f*, *S.a,* and *C.a*., respectively) and Nd:YAG laser (3.19 ± 0.51, 3.21 ± 0.37, 3.29 ± 0.52 for *E.f*, *S.a,* and *C.a*., respectively) were more effective in the reduction of all three pathogens during 24 hours, compared to calcium hydroxide (P value<0.05). The application of propolis followed by the Nd:YAG laser resulted in the complete elimination of *E. faecalis* and *S. aureus* and a significant reduction in *C. albicans* (0.60 ± 0.96) (P value<0.05).

Conclusion: Propolis alone or in combination with the Nd:YAG laser at low powers is highly effective against endodontic pathogens in comparison with conventional calcium hydroxide medicament.

Keywords: Propolis, Antibacterial agents, Endodontics, Root canal medicaments, Nd:YAG laser

Introduction

Root canal treatment includes the elimination of infections in addition to mechanical and chemical shaping of the root canal system to provide a bacteria-free environment before obturation. Endodontic infections are comprised of a variety of microorganisms, which are mostly resistant to conventional treatments [1]. Colonization of microbes in biofilm structures deep in dentinal tubules and the complexity of root canal systems lead to increased tolerance to endodontic treatments and make infections difficult to resolve [2]. A high proportion of facultative anaerobes, predominantly including Enterococcus spp. and Staphylococcus spp., is detected from root canals with previous treatment failure or necrotic tissues. Also, *Candida albicans* is the dominant fungal strain isolated from persistent endodontic infections [3].

Appropriate microbial control by the application of chemical agents adjunctive to mechanical preparation of the root canal is a matter of importance to acquire high rates of success and avoid dissemination of infection to periapical tissues. Considering the limited antimicrobial efficacy of conventional irrigants and interappointment medicaments and various diverse effects such as cytotoxicity, exploration of alternative disinfection protocols seems necessary [4,5].

Propolis is a natural resinous material gathered from beehives and has been employed in traditional medicine. Propolis presents great antioxidant, anti-inflammatory, and antimicrobial effects attributed to its polyphenol-rich nature. This extract promotes human cell proliferation[6]

Please cite this article as follows: Naghizadeh M, Fakhri E, Samadi Kafil H, Eslami H, Valian A. Evaluation of propolis as an intracanal agent in combination with an Nd:YAG laser for bacterial reduction. *J Lasers Med Sci.* 2026;17:e3. doi:10.34172/jlms.2026.03.

with no cytotoxic effects on periodontium [7] and demonstrates significant antibacterial efficacy against intracanal pathogens [8, 9]. Propolis has been shown to be a potential intracanal medicament and is beneficial for conditioning root canal dentin prior to endodontic regeneration [10]. Furthermore, the application of propolis increases dentin microhardness [11] and reduces open dentinal tubules [12]; however, it does not effectively contribute to smear removal [13]. Previous clinical studies evaluated propolis as an indirect pulp capping agent in primary teeth [14] and as a complement to conventional mechanical therapy for patients with periodontitis [15, 16], showing its potential to be brought to the pharmaceutical market.

The Neodymium-doped:yttrium-aluminum-garnet (Nd:YAG) laser (1064 nm) is a solid-state laser well-known in endodontic treatments for smear layer removal and decontamination efficacy, which are attributed to photothermal effects [17]. Moreover, the Nd:YAG laser overcomes the insufficient penetration of chemical agents through dentinal tubules and affords a high antimicrobial efficacy [18]. Few in vivo studies have reported the antibacterial efficacy of Nd:YAG laser in root canal treatment; however, Lindstrom reported that the Nd:YAG laser exhibits no significant antibacterial activity compared to 1% sodium hypochlorite [19]. Our previous study showed the privilege of combining a potent antibacterial medicament with a Nd:YAG laser [20].

Propolis offers a natural alternative to sodium hypochlorite, which can cause tissue irritation [21]. Also, its anti-inflammatory and antioxidant properties may enhance periapical tissue repair, while laser therapy stimulates biostimulation effects. This combination could address persistent bacterial and fungal infections in curved or calcified canals while aligning with trends toward minimally invasive, bioactive endodontic therapies. Considering the overall satisfactory results regarding the lasers, we aimed to assess the antibacterial effectiveness of propolis used as an intracanal medication, followed by Nd:YAG laser irradiation with low output power against the biofilm of endodontic pathogens in order to address the endodontic challenges.

Materials and Methods

The research protocol received approval from the Ethics Committee of the College of Dentistry, Tabriz University of Medical Sciences (approval no. IR.TBZMED.REC.1398.510).

Propolis Extraction

Propolis extraction was performed according to the protocol provided by a previous study [22]. Raw propolis was collected by beekeepers in the Shabestar region in the northwestern part of East Azerbaijan in October 2018. The collected propolis was stored in a dark place after drying. The samples were frozen and cut into pieces and dissolved in 70% ethyl alcohol with ratios of 10:3 (30 grams of propolis in 100 ml of 70% ethyl alcohol). Then the propolis samples were placed on a shaker under dark condition for one week at room temperature (25 °C). Afterwards, the alcoholic extract of propolis was filtered using Whatman No. 4 paper, and it was saturated in an evaporator to produce a purified propolis extract in powdered form. The obtained powder was dissolved in the required concentration in water and alcohol (9:1 w/w). The extraction was done in the Applied Pharmaceutical Research Center.

Microbial Strains and Media

Enterococcus faecalis (ATCC 29212), *Staphylococcus aureus* (ATCC 25923), and *Candida albicans* (ATCC 18804) were selected for this study. Mueller-Hinton agar plates (Merck, Darmstadt, Germany) were used to subculture the bacterial strains, and the fungal strain was cultured on Sabouraud agar (Merck, Darmstadt, Germany) for 24 hours at 37°C. Individual bacterial colonies were inoculated into trypticase soy broth supplemented with 1% glucose (Merck, Darmstadt, Germany) and incubated at 37°C overnight. Next, the suspensions were diluted to a turbidity of 1.0 McFarland density (3×10^6 colony-forming unit [CFU]/ml) using a spectrophotometer.

MIC and MBC Determination

In this experiment, bacterial susceptibility was evaluated by determining the minimum inhibitory concentration (MIC) of the propolis extract against the three target pathogens using a microdilution assay in 96-well plates after overnight incubation in accordance with CLSI guidelines. The minimum bactericidal concentration (MBC) of the propolis extract was assessed by inoculating 10 µl of the bacterial and fungal suspensions onto Mueller–Hinton and Sabouraud agar plates, respectively, followed by incubation for 24 hours at 37°C. The MBC was defined as the lowest concentration of the propolis extract that resulted in approximately 99.9% reduction in viable cells.

Specimen Preparation and Inoculation

A total of 165 human premolar teeth, extracted for either orthodontic or periodontal purposes, were gathered for this study. The selected teeth showed no history of previous root canal treatment, restorations, fractures, decay, or significant calcifications. Any remaining soft tissue or calculus was removed using an ultrasonic scaler. The roots were sectioned to measure 8 mm in length using a diamond saw operating at 1000 rpm under water irrigation, and the apical foramen was sealed with composite resin to eliminate potential bacterial leakage. The working length was established to be 1 mm short of the root tip using a #10 K-file (Dentsply, Maillefer,

Ballaigues, Switzerland). Mechanical preparation of the root canals was carried out up to size #60 K-file using a step-down approach and irrigation with sterile saline solution. To eliminate the smear layer, each specimen was treated sequentially with 5 ml of 17% EDTA (Pulpdent Crop. MA, USA) and then 5 ml of 5.25% sodium hypochlorite, each for two minutes. Sterilization was performed by autoclaving at 121°C and 15 psi for half an hour. Sterility was checked in three samples by incubating them in TSB at 37°C for 24 hours. After ensuring sterility, the specimens were placed into 1.5 ml Eppendorf tubes and inoculated with 1.2 ml of a microbial suspension (approximately 3×10^6 CFU/ml), with 55 teeth allocated per microorganism. All samples were then incubated for 21 days at 37°C with 10% CO_2, and the culture medium was replenished every other day.

Experimental Procedures

After incubation, the remaining inoculum was aspirated from each tube, and the root canals were gently rinsed with 5 ml of sterile phosphate-buffered solution. The teeth inoculated with each microorganism were then randomly assigned to five groups of 11 specimens as follows:

1. *Calcium hydroxide group:* A calcium hydroxide paste (Metapaste, Metabiomed, Korea) was placed in the canals using a syringe, and the specimens were incubated at 37°C for 24 hours.
2. *Propolis group:* Propolis extract was introduced into the canals up to the canal orifice.
3. *Nd:YAG laser group:* After final irrigation, the canals were irradiated with a pulsed Nd:YAG laser (Lambda, SPA, Italy; 1064 nm, 1.5 W, 15 Hz, 100 mJ/pulse) delivered through a 200-µm fiber in near-contact mode. Irradiation was performed four times for 10 seconds with 20-second intervals (total exposure 40 seconds), while the fiber was moved slowly in circular and spiral paths from coronal to apical and back.
4. *Propolis + Nd:YAG group:* Specimens received propolis extract for 24 hours; the material was then removed (with no additional irrigant between propolis and Nd:YAG), and the canals were irradiated with an Nd:YAG laser using the same parameters as above.
5. *Control group:* Canals were washed only with 0.9% saline solution.

For microbial sampling, dentin chips were obtained from the canal walls using Gates-Glidden drills #5 and #6 and transferred into sterile microtubes. The samples were diluted with normal saline, and 10 µl of the suspension was plated on Mueller–Hinton agar or Sabouraud agar and incubated at 37°C in 5% CO_2 for 24 hours. After incubation, the number of colony-forming units was recorded.

SEM Evaluation

One random specimen from each group was sectioned longitudinally by a sterile diamond disc under water cooling, and one semi-cylindrical half of each was selected for further analysis. The samples were washed three times with sterile PBS and chemically fixed with 2.5% glutaraldehyde for 24 hours. After dehydration in an aqueous ethanol series with increasing concentration (20%, 40%, 60% 80% and 100%), the samples were dried for 24 hours. They were coated with a thin layer of gold by sputter deposition and then examined using a scanning electron microscope (SEM; MIRA3, TESCAN).

Statistical Analysis

The statistical analysis of microbial counts was done by SPSS 17.0 software (SPSS Inc, Chicago, IL) using the Kruskal-Wallis test and the Mann-Whitney U test for pairwise comparison (P-value < 0.05).

Results

MIC and MBC Determination

The MIC and MBC results of propolis extract against *E. faecalis* (29212), *S. aureus* (25923), and *C. albicans* (18804) are summarized in Table 1.

SEM Examination

SEM observations (Figure 1) of the dentin samples before

Table 1. MIC and MBC values

Pathogen	Minimum Bactericidal/ Fungicidal Concentration	Minimum Inhibitory Concentration
Enterococcus faecalis	5 mg/ml	2.5 mg/ml
Staphylococcus aureus	1.25 mg/ml	1.25 mg/ml
Candida albicans	1.25 mg/ml	0.625 mg/ml

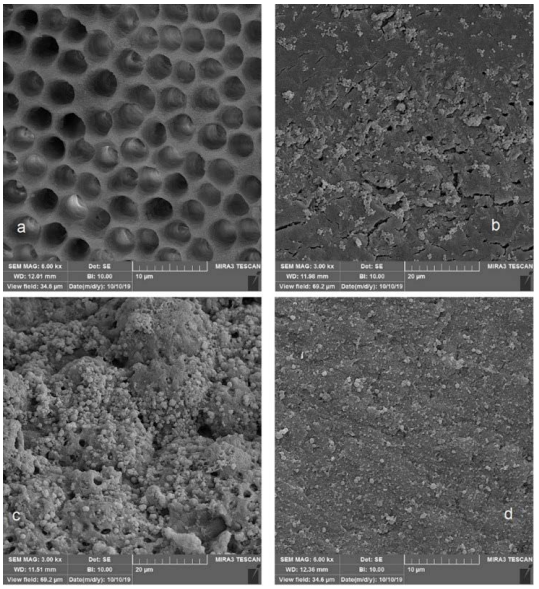

Figure 1. Dentin surface after smear layer removal (6000x); (a). 4-week biofilm of *Enterococcus faecalis* (3000x); (b), *Candida albicans* (3000x); (c), and *Staphylococcus aureus* (6000x); (d)

inoculation indicated open dentinal tubules, clear of smear layer and any contamination. The SEM images from b to d demonstrate the formation of dense biofilms of *E. faecalis*, *S. aureus,* and *C. albicans*. Microorganisms invaded the tubules, and no open dentinal tubules were detected.

Efficacy of Propolis and Nd:YAG Against Biofilms

Results are presented in Figure 2 and Table 2. All investigated groups presented significantly higher antibacterial efficacy compared to normal saline (*P*-value = 0.0001). Nd:YAG laser irradiation alone reduced the microorganisms more efficiently than calcium hydroxide (*P*-value = 0.003 for *E. faecalis*, *P*-value = 0.001 for *S. mutans,* and *P*-value = 0.0001 for *C. albicans*). Propolis showed a significantly high antimicrobial activity, and its combination with Nd:YAG did not significantly increase its efficacy (*P*-value = 0.478 for *E. faecalis*, *P*-value = 1.000 for *S. mutans,* and *P*-value = 0.171 for *C. albicans*). However, the propolis and Nd:YAG combination eliminated all *E. faecalis* and *S. aureus* cells. Among the tested microorganisms, *C. albicans* showed the greatest resistance.

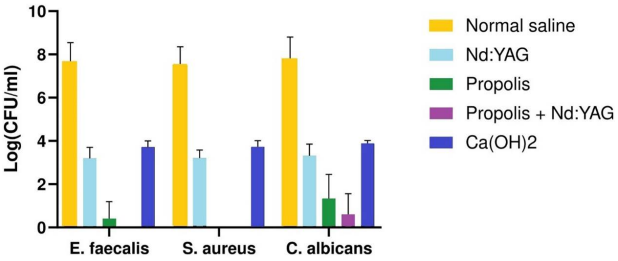

Figure 2. Comparative analysis of the mean Log CFU/ml of *E. faecalis*, *S. aureus,* and *C. albicans* monospecies biofilms after treatment with $Ca(OH)_2$, Propolis, Nd:YAG, Propolis/Nd:YAG, and NS

Table 2. Mean and SD values of log CFU/ml of groups

Microorganism	Groups	Mean	SD
Enterococcus faecalis	Propolis	.2327	.57912
	Propolis/Nd:YAG	.0000	.00000
	Nd:YAG	3.1936	.50928
	$Ca(OH)_2$	3.7227	.28054
	Normal Saline	7.6627	.87994
Staphylococcus aureus	Propolis	.0000	.00000
	Propolis/Nd:YAG	.0000	.00000
	Nd:YAG	3.2145	.36653
	$Ca(OH)_2$	3.7300	.28337
	Normal Saline	7.5291	.82438
Candida albicans	Propolis	1.3382	1.11979
	Propolis/Nd:YAG	.6045	.95824
	Nd:YAG	3.2918	.51838
	$Ca(OH)_2$	3.8755	.14761
	Normal Saline	7.7964	1.01130

Discussion

This study aimed to evaluate the effect of propolis as an intracanal medication followed by Nd:YAG laser irradiation on the intratubular disinfection of root canals. Calcium hydroxide served as the control group, as it is the most common intracanal medication used between appointments. The antibacterial efficacy was assessed using a mature biofilm of three commonly detected microorganisms (*E. faecalis, S. aureus* and *C. albicans*) in persistent/secondary root canal infections. Although endodontic infections are polymicrobial, a single culture of each microorganism was selected to carry out the present research and investigate the exact efficacy of treatments on each microorganism.

The antibacterial efficacy of Nd:YAG laser irradiation is strongly influenced by pulse duration, power, and frequency. Our study focused on parameters consistent with clinical protocols (1.5 W, 15 Hz) for Nd:YAG laser irradiation [19, 23]. Regarding power and frequency, 1.5 W at 15 Hz strikes a balance between bactericidal efficacy and thermal safety. Studies demonstrate this range achieves up to 76% reduction in *E. faecalis* while limiting temperature rises to less than 10°C [24, 25]. Some studies have also shown more than 99% bacterial eradication following Nd:YAG irradiation with the aforementioned parameters [26]. Increasing the power of Nd:YAG laser results in a significant temperature rise in all three coronal, middle, and apical regions of the root section [27]. Furthermore, 100 mJ/pulse was chosen to minimize the risks of dentin cracking, which was observed at energy levels more than 150 mJ while maintaining bacterial elimination in the root canal [28]. Prior work demonstrates that increasing pulse duration to 25 ms enhances bacterial elimination in deep dentin [18]. Moreover, an extended series of lasing is expected to improve the chance of sterilizing the root canal [29].

Nd:YAG laser irradiation with a power of 1.5 W resulted in a significantly lower cultivable number of bacteria. Previous studies reported acceptable antibacterial efficacy for Nd:YAG laser irradiation, depending on the power and energy [17, 30, 31]. In a study by Wang et al., the Nd:YAG laser with a power of 1.5 W (15 Hz and 100 mJ) reduced the *E. faecalis* count significantly greater than NaOCl irrigation, since it penetrates deeper into dentinal tubules and has more potential to reach these areas [25].

Propolis showed the highest antibacterial activity against all three investigated microorganisms in this study (*P*-value < 0.05). As a matter of fact, Nd:YAG irradiation did not significantly increase the activity of propolis; however, this combination eradicated all *E. faecalis* cells. It should be considered that the propolis medicament for 24 hours eradicated all *S. aureus* cells.

The antimicrobial activity of propolis is due to its constituents, which depend on the location and season of collecting [32]. Flavonoids and phenolic acids are

mainly responsible for the bioactivity of propolis [33]. Previous studies reported that propolis can promote high antibacterial activity against commonly detected microorganisms in root canals or the ones responsible for tooth caries [34, 35]. Propolis has been evaluated as an intracanal irrigant and medicament in endodontics. Shishiny et al. found that, as an irrigant, propolis acts weaker than chlorhexidine on 24-hour *E. faecalis* biofilm, resulting in an 82% reduction in colony-forming units [36]. In agreement with our study, Gulati et al. reported that propolis presents a noticeable antibacterial effect on *E. faecalis* bacteria one day (18.69% reduction in the colony count) and one week (48.31% reduction in the colony count) after application, compared to chlorhexidine 2% and metronidazole (P-value < 0.001). Also, among the tested materials, propolis (10.67% reduction in the first day and 29.84% reduction after a week) showed a weaker antibacterial effect on *C. albicans* [8]. Notwithstanding, in this study, a sterile paper point was used to collect microbial samples from the intracanal space, which does not assess the invaded cells in the deep intratubular spaces.

Kayaoglu et al. also investigated the antibacterial activity of propolis as an intracanal medicament after one and seven days. They reported that the antibacterial efficacy of propolis increases over time and is significantly lower than chlorhexidine but higher than $Ca(OH)_2$ (P-value < 0.05). Similar to our study, sampling was done by collecting dentin chips, which assesses the presence of bacterial colonization in the dentinal tubules up to 200 μm deep [37]. Shamma et al. reported similar efficacy between propolis and $Ca(OH)_2$ intracanal medicament regarding antibacterial activity against *E. faecalis* 24 and 72 hours and one week after the application of medicament (P-value > 0.05), and propolis achieved 62.19%, 79.12%, and 83.79% reduction in the bacteria count in the mentioned timelines, respectively. However, only a 24-hour culture of bacteria was assessed, which does not possess a mature biofilm structure [38]. In clinical conditions, we have to overcome a mature biofilm. Consequently, in the present study, a longer incubation time was chosen to confirm microbial penetration into dentinal tubules, colonization, and biofilm development within the root canal system (SEM images).

Moreover, Almahameed et al. evaluated $Ca(OH)_2$ combined with propolis paste against *E. faecalis* in infected root canals using extracted teeth. They also found that the propolis-containing paste significantly reduced the bacterial count after 7 days of application [39]. Jahromi et al. compared propolis and $Ca(OH)_2$ as intracanal medicaments in a simulated root canal environment. After one week, propolis was significantly more effective than calcium hydroxide in reducing CFUs and had a lower MIC [40]. For extended periods, Brazilian brown propolis was tested in bovine dentin discs infected with *E. faecalis* for 14 days. Both 20% and 40% propolis pastes, as well as their combinations with calcium hydroxide, significantly reduced bacterial growth. The 40% propolis paste alone and the 20% propolis mixed with calcium hydroxide were more effective than calcium hydroxide alone. It should be noted that higher concentrations of propolis generally yield better antibacterial results [41]. Also, combining propolis with calcium hydroxide can improve efficacy, as seen in previous studies [41, 39]. This synergy may be due to the combined effect of the antimicrobial compounds of propolis and the high pH of calcium hydroxide.

Using a similar method to our study, Mejia evaluated the antibacterial activity of propolis extract against *E. faecalis* 14 days after application. The effectiveness of propolis was compared with common endodontic disinfectants, such as chlorhexidine and $Ca(OH)_2$. They reported that the antimicrobial activity of propolis was similar to chlorhexidine (P-value > 0.05) and higher than $Ca(OH)_2$ [42]. These results suggest that prolonged application enhances the antibacterial action of propolis, possibly due to sustained release of active compounds like flavonoids [41, 42].

The antifungal activity of propolis has been well-documented in the literature [43]. In the present study, 0.625 mg/ml of propolis extract was fungistatic, and 1.25 mg/ml of it was fungicidal. Rudriguez et al. also reported similar MIC and MFC results for the ethanolic extract of propolis against *C. albicans* [44]. However, various studies reported different concentrations of propolis depending on the collecting season and area [43]. Both studies of Gulati et al. and Mejia corroborated the result of the present study regarding the resistance of *C. albicans* to propolis using a dentinal model for culturing *C. albicans* [8, 42].

According to our previous study, using a potential natural intracanal medicament (grape seed extract) followed by Nd:YAG laser irradiation could substantially reduce the biofilm count [20]. In the present study, propolis acted as a strong antibacterial and antifungal medicament, and its combination with the Nd:YAG laser eradicated the bacterial count. However, the in vitro nature of this experimental design may not fully replicate the complexities of in vivo conditions within the root canal system, such as anatomical variations and interactions with host tissues. In addition, the antimicrobial efficacy of propolis can vary depending on its source, composition, and method of application, which may affect reproducibility and consistency of results across different clinical settings. Future research should focus on long-term clinical trials to validate these findings, assess possible side effects such as tooth discoloration or allergic reactions, and optimize protocols for combining propolis with the Nd:YAG laser for routine clinical use. Expanding the evaluation to include multispecies biofilms and additional resistant endodontic pathogens will also offer a more comprehensive understanding of its effectiveness in more complex infection scenarios.

Conclusion

This study demonstrated that both propolis and Nd:YAG laser irradiation exhibit significant antimicrobial efficacy against bacterial biofilms, surpassing the effects of normal saline and calcium hydroxide. While the Nd:YAG laser alone was more effective than calcium hydroxide, propolis displayed strong antibacterial activity, particularly against *E. faecalis* and *S. aureus*, with its combination with Nd:YAG achieving complete eradication of these pathogens. However, the combined use of propolis and Nd:YAG did not significantly enhance the antimicrobial effect of propolis, suggesting that propolis alone may be sufficiently potent against certain microorganisms. Notably, *C. albicans* exhibited the highest resistance among the tested species, highlighting the need for alternative or enhanced antifungal strategies. These results support the potential clinical use of propolis and Nd:YAG laser as effective antimicrobial agents in biofilm-associated infections; however, further research is warranted to optimize their application, particularly against resilient fungal species such as *C. albicans*.

Authors' Contribution

Conceptualization: Hosein Eslami, Hossein Samadi Kafil.
Data curation: Elaheh Fakhri, Mahdieh Naghizadeh, Azam Valian.
Formal analysis: Hosein Eslami, Azam Valian.
Investigation: Mahdieh Naghizadeh, Hossein Samadi Kafil.
Methodology: Elaheh Fakhri, Mahdieh Naghizadeh.
Project administration: Mahdieh Naghizadeh , Elaheh Fakhri.
Resources: Elaheh Fakhri, Mahdieh Naghizadeh.
Software: Azam Valian.
Supervision: Hosein Eslami, Hossein Samadi Kafil.
Validation: Azam Valian, Hosein Eslami, Hossein Samadi Kafil.
Visualization: Azam Valian.
Writing–original draft: Elaheh Fakhri, Hosein Eslami.
Writing–review & editing: Hossein Samadi Kafil, Hosein Eslami.

Competing Interests

None to declare.

Ethical Approval

This study was approved by the research ethics committee of Tabriz University of Medical Sciences with the reference number IR.TBZMED.REC.1398.510.

Funding

This study was supported by the Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran.

References

1. Siqueira JF Jr. Endodontic infections: concepts, paradigms, and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(3):281-93. doi: 10.1067/moe.2002.126163
2. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. J Endod. 2012;38(10):1376-9. doi: 10.1016/j.joen.2012.06.035
3. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol. 1994;78(4):522-30. doi: 10.1016/0030-4220(94)90047-7
4. Momenijavid M, Salimizand H, Korani A, Dianat O, Nouri B, Ramazanzadeh R, et al. Effect of calcium hydroxide on morphology and physicochemical properties of *Enterococcus faecalis* biofilm. Sci Rep. 2022;12(1):7595. doi: 10.1038/s41598-022-11780-x
5. Valian A, Goudarzi H, Nasiri MJ, Roshanaei A, Sadeghi Mahounak F. Antibacterial and anti-biofilm effects of chitosan nanoparticles on *Streptococcus mutans* isolates. J Iran Med Council. 2023;6(2):292-8. doi: 10.18502/jimc.v6i2.12238
6. Pedrinha VF, Santos LM, Gonçalves CP, Garcia MT, Lameira OA, Queiroga CL, et al. Effects of natural antimicrobial compounds propolis and copaiba on periodontal ligament fibroblasts, molecular docking, and in vivo study in *Galleria mellonella*. Biomed Pharmacother. 2024;171:116139. doi: 10.1016/j.biopha.2024.116139
7. Sahu SA, Panda S, Das AC, Mishra L, Rath S, Sokolowski K, et al. Efficacy of sub-gingivally delivered propolis nanoparticle in non-surgical management of periodontal pocket: a randomized clinical trial. Biomolecules. 2023;13(11):1576. doi: 10.3390/biom13111576
8. Gulati RK, Jain N, Singh A, Jain A, Jindal A, Kumawat MK, et al. Comparative evaluation of the antimicrobial efficacy of chemical and phytomedicinal agents when used as intracanal irrigants: an in vitro study. Cureus. 2023;15(11):e48754. doi: 10.7759/cureus.48754
9. Durga Bhavani G, Rathod T, Parveen N, Tirupathi P, Dharavattu P, Sekhar V, et al. Assessment of the antimicrobial effectiveness of herbal root canal irrigants (propolis, triphala, and *Aloe vera*) and chlorhexidine against *Enterococcus faecalis*. Cureus. 2023;15(7):e41628. doi: 10.7759/cureus.41628
10. Preetham HS, Kumar NK, Brigit B, Swathisha A, Shylaja V. Quantitative assessment of transforming growth factor-β1 release from dentin matrix upon conditioning with ethylene diamine tetra-acetate, doxycycline hydrochloride, and propolis: an in vitro study. J Conserv Dent Endod. 2023;26(5):564-8. doi: 10.4103/jcde.Jcde_16_23
11. Naeem MM, Sarwar H, Nisar A, Ahmed S, Shabbir J, Khurshid Z, et al. Effect of propolis on root dentine microhardness when used as an intracanal medicament: an in vitro study. J Funct Biomater. 2023;14(3):144. doi: 10.3390/jfb14030144
12. Naghsh N, Mazrooei F, Hosseini A, Kiani S, Sahebkar A. Effects of propolis-based herbal toothpaste on dentine hypersensitivity. Int Dent J. 2024;74(3):559-65. doi: 10.1016/j.identj.2023.11.014
13. Setia R, Bajaj N, Bhola M, Brar GS. Comparative evaluation of smear layer removal efficacy of neem leaf extract, propolis, and orange oil when used as endodontic irrigants: an in vitro scanning electron microscopic study. Contemp Clin Dent. 2023;14(2):128-34. doi: 10.4103/ccd.ccd_611_21
14. Saikia D, Yadav G, Saha S, Dhinsa K, Sharma A, Rai A. Evaluation of indirect pulp capping using MTA, light-cured calcium silicate, and propolis in primary teeth: a clinical and radiographic study using VistaScan. Int J Clin Pediatr Dent. 2023;16(5):686-91. doi: 10.5005/jp-journals-10005-2673
15. Waqar SM, Razi A, Qureshi SS, Saher F, Zaidi SJ, Kumar C. Comparative evaluation of propolis mouthwash with 0.2% chlorhexidine mouthwash as an adjunct to mechanical therapy in improving the periodontitis among perimenopausal women: a randomized controlled trial. BMC Oral Health. 2024;24(1):26. doi: 10.1186/s12903-023-03768-4
16. Aggarwal R, Bawa SS, Palwankar P, Kaur S, Choudhary D, Kochar D. To evaluate the clinical efficacy of 940 nm diode laser and propolis gel (a natural product) in adjunct to scaling and root planing in treatment of chronic periodontitis. J Pharm Bioallied Sci. 2023;15(Suppl 2):S1218-20. doi: 10.4103/jpbs.jpbs_154_23
17. Meire MA, Coenye T, Nelis HJ, De Moor RJ. In vitro inactivation of endodontic pathogens with Nd:YAG and Er:YAG lasers.

Lasers Med Sci. 2012;27(4):695-701. doi: 10.1007/s10103-011-0940-z
18. Franzen R, Gutknecht N, Falken S, Heussen N, Meister J. Bactericidal effect of a Nd:YAG laser on *Enterococcus faecalis* at pulse durations of 15 and 25 ms in dentine depths of 500 and 1,000 µm. Lasers Med Sci. 2011;26(1):95-101. doi: 10.1007/s10103-010-0826-5
19. Granevik Lindström M, Wolf E, Fransson H. The antibacterial effect of Nd:YAG laser treatment of teeth with apical periodontitis: a randomized controlled trial. J Endod. 2017;43(6):857-63. doi: 10.1016/j.joen.2017.01.013
20. Fakhri E, Samadi Kafil H, Naghizadeh M, Eslami H, Sefidan FY. Antimicrobial effect of grape seed extract as a potential intracanal medicament combined with Nd:YAG laser. Aust Endod J. 2023;49 Suppl 1:209-16. doi: 10.1111/aej.12718
21. Sabino-Silva R, Cardoso IV, Vitali FC, Alves AM, Souza BD, Bortoluzzi EA, et al. Prevalence of postoperative pain after endodontic treatment using low and high concentrations of sodium hypochlorite: a systematic review and meta-analysis. Clin Oral Investig. 2023;27(8):4157-71. doi: 10.1007/s00784-023-05151-7
22. Eslami H, Ariamanesh N, Ariamanesh A, Samadi Kafil H. Synergistic effect of honey and Azarian propolis on oral microorganisms: an in vitro study. J Adv Oral Res. 2016;7(3):31-6.
23. Saydjari Y, Kuypers T, Gutknecht N. Laser application in dentistry: irradiation effects of Nd:YAG 1064 nm and diode 810 nm and 980 nm in infected root canals-a literature overview. Biomed Res Int. 2016;2016:8421656. doi: 10.1155/2016/8421656
24. Schoop U, Kluger W, Moritz A, Nedjelik N, Georgopoulos A, Sperr W. Bactericidal effect of different laser systems in the deep layers of dentin. Lasers Surg Med. 2004;35(2):111-6. doi: 10.1002/lsm.20026
25. Wang X, Cheng X, Liu X, Wang Z, Wang J, Guo C, et al. Bactericidal effect of various laser irradiation systems on *Enterococcus faecalis* biofilms in dentinal tubules: a confocal laser scanning microscopy study. Photomed Laser Surg. 2018;36(9):472-9. doi: 10.1089/pho.2017.4430
26. Bergmans L, Moisiadis P, Teughels W, Van Meerbeek B, Quirynen M, Lambrechts P. Bactericidal effect of Nd:YAG laser irradiation on some endodontic pathogens ex vivo. Int Endod J. 2006;39(7):547-57. doi: 10.1111/j.1365-2591.2006.01115.x
27. Zan R, Hubbezoglu I, Unal M. Evaluation of temperature rises during the application of different power levels of potassium titanyl phosphate and neodymium-doped:yttrium aluminum garnet lasers to external primary root canals. J Dent Sci. 2016;11(4):365-9. doi: 10.1016/j.jds.2016.04.001
28. Altalhi AM, AlNajdi LN, Al-Harbi SG, Aldohailan AM, Al-Ghadeer JY, Al-Bahrani JI, et al. Laser therapy versus traditional scaling and root planing: a comparative review. Cureus. 2024;16(6):e61997. doi: 10.7759/cureus.61997
29. Ramsköld LO, Fong CD, Strömberg T. Thermal effects and antibacterial properties of energy levels required to sterilize stained root canals with an Nd:YAG laser. J Endod. 1997;23(2):96-100. doi: 10.1016/s0099-2399(97)80253-1
30. Korkut E, Torlak E, Gezgin O, Özer H, Şener Y. Antibacterial and smear layer removal efficacy of Er:YAG laser irradiation by photon-induced photoacoustic streaming in primary molar root canals: a preliminary study. Photomed Laser Surg. 2018;36(9):480-6. doi: 10.1089/pho.2017.4369
31. Bago Jurič I, Plečko V, Anić I, Pleško S, Jakovljević S, Rocca JP, et al. Antimicrobial efficacy of photodynamic therapy, Nd:YAG laser and QMiX solution against *Enterococcus faecalis* biofilm. Photodiagnosis Photodyn Ther. 2016;13:238-43. doi: 10.1016/j.pdpdt.2015.07.176
32. da Silva WH, Guedes FR, Lins-Candeiro CL, Dos Santos C, Ambrosio M, Veneziani RC, et al. Effect of Brazilian green and brown propolis on human pulp cells and bacteria involved in primary endodontic infection: Ex Vivo study. Arch Oral Biol. 2025;176:106294. doi: 10.1016/j.archoralbio.2025.106294
33. Lu LC, Chen YW, Chou CC. Antibacterial activity of propolis against *Staphylococcus aureus*. Int J Food Microbiol. 2005;102(2):213-20. doi: 10.1016/j.ijfoodmicro.2004.12.017
34. de Carvalho Duailibe SA, Gonçalves AG, Ahid FJ. Effect of a propolis extract on *Streptococcus mutans* counts in vivo. J Appl Oral Sci. 2007;15(5):420-3. doi: 10.1590/s1678-77572007000500009
35. Pedrinha VF, Barros MC, Portes JD, Slomp AM, Woudstra W, Lameira OA, et al. Antimicrobial efficacy of alternative root canal disinfection strategies: an evaluation on multiple working models. Biomed Pharmacother. 2025;183:117833. doi: 10.1016/j.biopha.2025.117833
36. El Shishiny SA, Morad YO, Hindi RI, El-Motasem AM, El Sheshiny AA, Alramady DM, et al. Efficacy of non -thermal pressure plasma versus other modalities for disinfection of primary root canals. BMC Oral Health. 2025;25(1):54. doi: 10.1186/s12903-024-05349-5
37. Kayaoglu G, Ömürlü H, Akca G, Gürel M, Gençay Ö, Sorkun K, et al. Antibacterial activity of propolis versus conventional endodontic disinfectants against *Enterococcus faecalis* in infected dentinal tubules. J Endod. 2011;37(3):376-81. doi: 10.1016/j.joen.2010.11.024
38. Shamma BM, Kurdi SA, Rajab A, Arrag EA. Evaluation of antibacterial effects of different intracanal medicaments on *Enterococcus faecalis* in primary teeth: An in vitro study. Clin Exp Dent Res. 2023;9(2):341-8. doi: 10.1002/cre2.718
39. Almahameed A, Kassis J, Aboud M, Layous K. Evaluation of antibacterial efficacy of propolis as an intercanal medicament against *Enterococcus faecalis* (a randomized controlled in vitro study). Heliyon. 2025;11(1):e41733. doi: 10.1016/j.heliyon.2025.e41733
40. Zare Jahromi M, Toubayani H, Rezaei M. Propolis: a new alternative for root canal disinfection. Iran Endod J. 2012;7(3):127-33. doi: 10.22037/iej.v7i3.3415
41. Pimenta HC, Violante IM, de Musis CR, Borges ÁH, Aranha AM. In vitro effectiveness of Brazilian brown propolis against *Enterococcus faecalis*. Braz Oral Res. 2015;29. doi: 10.1590/1807-3107BOR-2015.vol29.0058
42. Carbajal Mejía JB. Antimicrobial effects of calcium hydroxide, chlorhexidine, and propolis on *Enterococcus faecalis* and *Candida albicans*. J Investig Clin Dent. 2014;5(3):194-200. doi: 10.1111/jicd.12041
43. Bouchelaghem S. Propolis characterization and antimicrobial activities against *Staphylococcus aureus* and *Candida albicans*: a review. Saudi J Biol Sci. 2022;29(4):1936-46. doi: 10.1016/j.sjbs.2021.11.063
44. Rodriguez-Canales M, Medina-Romero YM, Rodriguez-Monroy MA, Nava-Solis U, Bolaños-Cruz SI, Mendoza-Romero MJ, et al. Activity of propolis from Mexico on the proliferation and virulence factors of *Candida albicans*. BMC Microbiol. 2023;23(1):325. doi: 10.1186/s12866-023-03064-9