

# Effectiveness of white tea-mediated silver nanoparticles as an intracanal irrigant against *Enterococcus faecalis*: An in vitro study

Lakshmi Lakshmanan<sup>1,A–D</sup>, Deepa Gurunathan<sup>1,A,E,F</sup>, Rajeshkumar Shanmugam<sup>2,A,E,F</sup>

<sup>1</sup> Department of Pediatric and Preventive Dentistry, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India

<sup>2</sup> Nanobiomedicine Lab, Centre for Global Health Research, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online)

Dent Med Probl. 2024;61(4):593–598

## Address for correspondence

Deepa Gurunathan

E-mail: drgdeepa@yahoo.co.in

## Funding sources

None declared

## Conflict of interest

None declared

## Acknowledgements

None declared

Received on April 10, 2021

Reviewed on October 13, 2021

Accepted on November 2, 2021

Published online on August 16, 2024

## Abstract

**Background.** The probability of a positive outcome of root canal therapy is substantially higher if the infection is eradicated successfully before the obturation of the root canal system. Irrigation is an essential aspect of root canal debridement, as it enables more thorough cleaning than is possible with root canal instrumentation alone. To overcome the side effects of chemical irrigants, there has been a search for herbal medicines as substitutes.

**Objectives.** The aim of the present study was to explore the antimicrobial efficacy of white tea-mediated silver nanoparticles (AgNPs) formulated as an intracanal irrigant against *Enterococcus faecalis*, and to compare it with the efficacy of chlorhexidine and sodium hypochlorite irrigants.

**Material and methods.** The experimental groups were as follows: group I – white tea-mediated AgNPs; group II – 2% chlorhexidine; and group III – 2.5% sodium hypochlorite. The characterization of AgNPs was performed using ultraviolet-visible (UV-Vis) spectroscopy and transmission electron microscopy (TEM) analysis. *Enterococcus faecalis* was inoculated onto Mueller–Hinton agar plates. The disks impregnated with irrigants were placed on the inoculated plates and incubated aerobically at 37°C for 24 h. Then, the growth inhibition zones were measured. Statistical analysis was performed using the one-way analysis of variance (ANOVA) and the post hoc tests.

**Results.** A concentration of 50 µL of white tea-mediated AgNPs exhibited the greatest zone of inhibition (32 ± 2 mm), followed by 2% chlorhexidine (25 ± 1 mm) and 2.5% sodium hypochlorite (23 ± 3 mm).

**Conclusions.** White tea-mediated AgNPs showed promising results in the elimination of *E. faecalis*, being superior to chlorhexidine and sodium hypochlorite irrigants.

**Keywords:** herbal, silver nanoparticles, root canal therapy, white tea, irrigant

## Cite as

Lakshmanan L, Gurunathan D, Shanmugam R. Effectiveness of white tea-mediated silver nanoparticles as an intracanal irrigant against *Enterococcus faecalis*: An in vitro study. *Dent Med Probl.* 2024;61(4):593–598. doi:10.17219/dmp/143545

## DOI

10.17219/dmp/143545

## Copyright

Copyright by Author(s)

This is an article distributed under the terms of the Creative Commons Attribution 3.0 Unported License (CC BY 3.0) (<https://creativecommons.org/licenses/by/3.0/>).

## Introduction

The success of endodontic treatment relies on the thorough disinfection of the root canal system. The role of microorganisms in the pathogenesis of pulp-periapical lesions is well-established in the literature.<sup>1</sup> *Enterococcus faecalis*, in particular, has gained special attention, as it is one of the dominant microbes isolated from failed root canals. It is more resistant to endodontic therapy due to its ability to penetrate dentinal tubules and its resistance to intracanal medicaments.<sup>2,3</sup>

Studies have shown that necrotic tissues, the microbial by-products remaining within dentinal tubules, canal ramifications, and resorption pits that are resistant to mechanical instruments result in persistent periradicular inflammation.<sup>4</sup> Therefore, chemo-mechanical preparation, which involves various instrumentation techniques, irrigation protocols and intracanal medicaments, is proposed to prevent the further entry of microorganisms from an infected root canal and to create conditions conducive to tissue healing.<sup>4,5</sup>

Intracanal irrigation complements the instrumentation technique by expediting the removal of necrotic tissues and microbes from the root canal. Various studies have reported that portions of root canal walls remain unbridged during mechanical instrumentation.<sup>6–8</sup> Hence, irrigating agents with strong antimicrobial activity are essential adjuncts to instrument preparation, thereby aiding in the removal of pulp remnants and residual microorganisms from intricate root canals.<sup>5</sup> Chlorhexidine and sodium hypochlorite have been utilized globally for irrigating both permanent and primary root canals. Recently, there has been increased interest in herbal agents to replace chemical irrigants due to their limitations, such as tissue toxicity, allergic reactions, the discoloration of clothes, and unpleasant odor and taste. Several herbal irrigants with antimicrobial and therapeutic effects have been suggested for use as endodontic irrigants.<sup>9–11</sup>

White tea, which is extracted in an unfermented form from the leaves of the tea plant *Camellia sinensis*, possesses strong antioxidant properties in addition to its medicinal properties due to high levels of polyphenols.<sup>12</sup> Nanoparticles (NPs) have also been studied in the field of endodontics to reduce *E. faecalis* adherence to dentine and to reinforce root canal disinfection.<sup>13</sup>

The purpose of the current study was to explore the antimicrobial efficacy of white tea-mediated silver nanoparticles (AgNPs) formulated as an intracanal irrigant against *E. faecalis*, and to compare it with the efficacy of chlorhexidine and sodium hypochlorite irrigants.

## Material and methods

The approval for the current in vitro study was obtained from the Ethics Committee at the Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai,

India (SRB/SDC/PEDO-1803/20/05). The experimental groups were as follows: group I – white tea-mediated AgNPs; group II – 2% chlorhexidine; and group III – 2.5% sodium hypochlorite.

### Preparation of the white tea extract and the incorporation of AgNPs

Fresh white tea leaves (Ketley Gold Marketing, Assam, India) in the amount of 10 g were boiled for 15 min in 50 mL of distilled water. The obtained plant extract was filtered through sterile filter paper. In another container, 0.0169 g of silver nitrate ( $\text{AgNO}_3$ ) powder was added to 50 mL of distilled water and stirred continuously to obtain a  $\text{AgNO}_3$  solution. The plant extract and the  $\text{AgNO}_3$  solution were thoroughly mixed, using an electronic magnetic stirrer for 8 h. The mixture was then placed in test tubes and centrifuged for 15 min at 8,000 rpm.

### UV-Vis spectroscopy and TEM analysis

The formation of AgNPs through the reduction of  $\text{AgNO}_3$  was monitored at different time intervals up to 72 h, between 250 and 700 nm. A transmission electron microscope (TEM) was used to examine the surface morphology.

### Isolation of microorganisms

A total of 100  $\mu\text{L}$  of *E. faecalis* suspension taken from the prepared cultures was inoculated onto culture plates, 130 mm in diameter, with the previously set layers of Mueller–Hinton agar.

### Antimicrobial activity

Using a sterile cork borer (HiMedia Laboratories, Mumbai, India), 3 wells (approx. 0.5 mm in diameter) were cut into the Mueller–Hinton agar plates. Using a micropipette, approx. 25  $\mu\text{L}$ , 50  $\mu\text{L}$  and 100  $\mu\text{L}$  of the white tea-mediated AgNP solution was transferred into the wells, and then impregnated in sterilized 3-millimeter blank disks. The disks were placed on the Mueller–Hinton agar plates with gentle pressure against the agar surface to ensure uniform contact. The plates were incubated at 37°C for 24 h, and the antimicrobial efficacy was then evaluated.

To compare antimicrobial activity among the groups included in the study, the concentration of white tea-mediated AgNPs which exhibited the greatest zone of inhibition (group I) was further compared with 2% chlorhexidine (Asep-RC; Anabond Stedman Pharma Research, Chennai, India) (group II) and 2.5% sodium hypochlorite (Prime Dental Products, Thane, India) (group III) in Petri dishes containing 3 wells of a 0.5-millimeter diameter, using the same method as described earlier.

For the antimicrobial activity assessment, the diameter of the inhibition zone around the disks was measured using a ruler to the nearest whole millimeter. To ensure reliability, all experiments were replicated 3 times, and the average of the 3 measurements was recorded as the final value.

## Statistical analysis

The recorded data was analyzed using the IBM SPSS Statistics for Windows, v. 23.0 (IBM Corp., Armonk, USA). Statistical significance was determined with the one-way analysis of variance (ANOVA) and the post hoc tests, with a  $p$ -value set at 0.05.

## Results

### Incorporation of AgNPs into the white tea extract

The addition of the white tea extract to the  $\text{AgNO}_3$  solution initially resulted in a dark brown color, which then faded to lighter brown after continued magnetic stirring.

### UV-Vis spectroscopy and TEM analysis

The UV-Vis spectroscopy results are shown in Fig. 1. The formation of AgNPs was observed between 250 and 700 nm. White tea-mediated AgNPs exhibited a peak between 420 and 460 nm, confirming their synthesis. The TEM analysis revealed that AgNPs were uniformly distributed, spherical in shape, and had a particle size ranging from 30 nm to 35 nm (Fig. 2).

### Antimicrobial activity

The antimicrobial activity of white tea-mediated AgNPs at 3 different concentrations was assessed by measuring the zone of inhibition against *E. faecalis* in a disk diffusion test (Table 1). The 50  $\mu\text{L}$  concentration of white tea-mediated AgNPs showed the largest zone of inhibition.

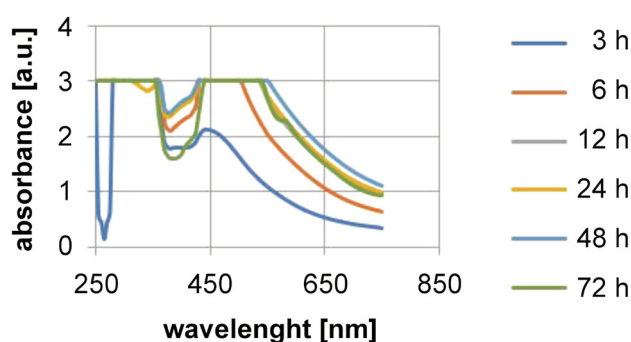


Fig. 1. Ultraviolet-visible (UV-Vis) spectroscopy values

Comparative tests with 2% chlorhexidine and 2.5% sodium hypochlorite indicated that white tea-mediated AgNPs exhibited a larger zone of inhibition than both chlorhexidine and sodium hypochlorite irrigants (Table 2).

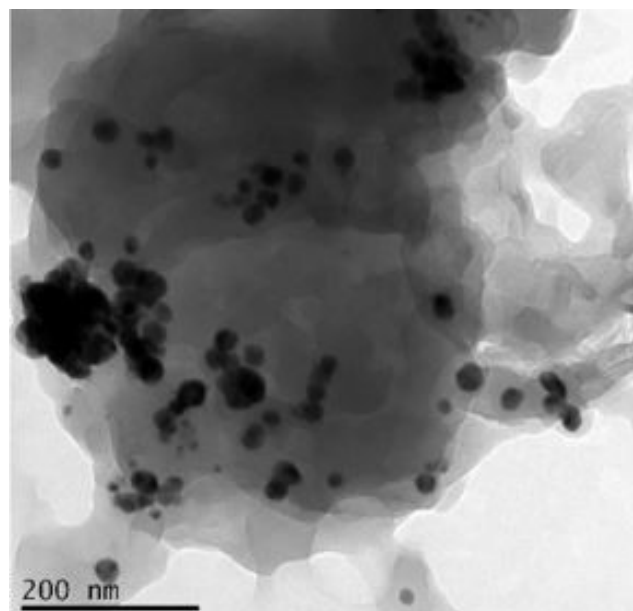


Fig. 2. Transmission electron microscopy (TEM) analysis

Table 1. Antimicrobial activity of various concentrations of a white tea-mediated silver nanoparticle (AgNP) solution

| Concentration [ $\mu\text{L}$ ] | Zone of inhibition against <i>Enterococcus faecalis</i> [mm] |
|---------------------------------|--------------------------------------------------------------|
| 25                              | 15 $\pm$ 1                                                   |
| 50                              | 32 $\pm$ 2*                                                  |
| 100                             | 14 $\pm$ 1                                                   |

Data presented as mean  $\pm$  standard deviation ( $M \pm SD$ ).

\* statistically significant difference with regard to other concentrations ( $p < 0.05$ ).

Table 2. Comparison of the antimicrobial efficacy of a white tea-mediated silver nanoparticle (AgNP) solution against chlorhexidine and sodium hypochlorite solutions

| Irrigant                                     | Zone of inhibition against <i>Enterococcus faecalis</i> [mm] |
|----------------------------------------------|--------------------------------------------------------------|
| White tea-mediated AgNPs (50 $\mu\text{L}$ ) | 32 $\pm$ 2*                                                  |
| 2% chlorhexidine                             | 25 $\pm$ 1                                                   |
| 2.5% sodium hypochlorite                     | 23 $\pm$ 3                                                   |

Data presented as  $M \pm SD$ .

\* statistically significant difference with regard to other irrigants ( $p < 0.05$ ).

## Discussion

The main goal of any treatment is to provide the optimal benefit to the patient with minimal harm and discomfort. In the case of primary teeth, thorough mechanical preparation is not desirable due to the presence of thin dentinal walls. Consequently, root canal irrigants play a significant

role in the debridement of the root canal system.<sup>4,14</sup> Sodium hypochlorite, chlorhexidine gluconate, ethylenediamine-tetraacetic acid (EDTA), citric acid, hydrogen peroxide, and other root canal irrigating solutions have been suggested for use in clinical practice for primary teeth.<sup>15</sup> Chemical irrigants, however, cannot be used too frequently in primary teeth due to their lower safety profile. To address the side effects of chemical irrigants, there has been a search for herbal alternatives. With the rise of antibiotic-resistant strains, it has become essential to explore herbal medicines with strong antimicrobial properties to improve the outcome of biomechanical procedures.<sup>16,17</sup>

*Enterococcus faecalis* was chosen as the test organism, as this facultative Gram-positive bacterium is commonly associated with secondary infections, with a prevalence rate of 24–77%, and it rapidly colonizes dentinal tubules.<sup>2,18</sup>

The agar diffusion test was used in this study, as it allows the direct comparison of test materials against microorganisms, indicating whether the material has the potential to kill bacteria in the local environment. It is also one of the most widely used methods for determining the antimicrobial activity of endodontic irrigants.<sup>19,20</sup>

Sodium hypochlorite, with concentrations ranging from 0.5% to 6%, has been recommended for root canal irrigation. It is inexpensive, readily available, has a long shelf life, and excellent tissue-dissolving and antibacterial properties.<sup>21</sup> The chlorine (Cl) in sodium hypochlorite exerts an antimicrobial effect by inhibiting bacterial enzymes, leading to the irreversible oxidation of crucial bacterial enzyme sulfhydryl groups. The strong pH of sodium hypochlorite contributes to its antimicrobial properties by disrupting cytoplasmic membrane integrity, altering cellular metabolism and causing the degradation of phospholipids.<sup>22</sup> However, when sodium hypochlorite is accidentally injected past the root apex, it can cause tissue reactions, such as pain, swelling, necrosis, and hemorrhage.<sup>10</sup>

Chlorhexidine gluconate is a cationic bisbiguanide that acts by adsorbing onto the cell walls of microorganisms and inducing the leakage of intracellular components.<sup>23</sup> Chlorhexidine has a bacteriostatic effect at low concentrations, allowing the leaching of small-molecular-weight compounds from microorganisms. At higher concentrations, chlorhexidine has bactericidal activity due to cytoplasmic precipitation and/or coagulation, likely mediated by protein cross-linking.<sup>24</sup> Clinical trials have reported severe reactions, including postoperative pain, following the extrusion of the chlorhexidine irrigant into periapical tissues.<sup>25</sup>

A mild form of tea, white tea, is produced from the young leaves of the tea plant *C. sinensis*. It retains high catechin concentrations found in fresh tea leaves.<sup>12</sup> White tea is processed the least, so it contains the most polyphenols, which are not oxidized or lost during processing. It has more potent antioxidants than green tea, and has been shown to be even more beneficial. The high content of epigallocatechin gallate is primarily responsible for its strong antioxidant properties.<sup>26,27</sup>

Silver nanoparticles bind to and penetrate the cell walls of both Gram-positive and Gram-negative bacteria, disrupting cell function by releasing Ag ions. As a result, they are used for fighting drug-resistant microorganisms and inhibiting biofilm formation.<sup>13</sup> In dental practice, AgNPs are incorporated into the bonding agents and restorative materials to prevent biofilm formation and reduce caries. Nanoparticles are used in endodontics to minimize *E. faecalis* adherence to dentine, remove biofilm and improve root canal disinfection.<sup>28,29</sup> This study aimed to evaluate the efficacy of white tea and AgNPs in combination against *E. faecalis*.

The activity of AgNPs in the prepared white tea solution was confirmed by visual observation, UV-Vis spectroscopy, and TEM analysis. Free electrons in metal NPs produce a surface plasmon resonance (SPR) absorption band due to the mutual vibration of electrons in resonance with light waves.<sup>28</sup> Peaks in UV-Vis spectroscopy confirmed the SPR characteristics of AgNPs in the prepared white tea solution. The particle size was confirmed by TEM micrographs as 30–35 nm. According to Shrestha et al., NP characteristics, such as contact time, concentration, particle size, and surface charge, affect their antimicrobial activity.<sup>30</sup> Afkhami et al. reported that irrigation with 100 ppm AgNPs had the same antimicrobial potency as 2.5% sodium hypochlorite irrigation.<sup>31</sup>

In the present study, the zones of inhibition of bacterial growth achieved with white tea-mediated AgNPs were greater than those obtained with other irrigants for *E. faecalis*. This indicates that this herbal irrigant has higher efficacy against *E. faecalis* as compared to sodium hypochlorite and chlorhexidine. This result contrasts with the findings of Jose et al.<sup>9</sup> and Saxena et al.,<sup>10</sup> who found that sodium hypochlorite was more effective against *E. faecalis* than the herbal irrigants tested. Sodium hypochlorite showed a mean inhibition zone of 23 mm for *E. faecalis*, which is consistent with the analysis by Jose et al.<sup>9</sup> Other researchers have reported similar findings regarding the use of chlorhexidine as an endodontic irrigant.<sup>24,25</sup> It has been proven that 2% chlorhexidine effectively removes the smear layer in 15 s and enriches the dentinal surface with chlorhexidine digluconate.<sup>32</sup> Baca et al. reported 100% biofilm inhibition when using 2% chlorhexidine against *E. faecalis*.<sup>33</sup> According to Garcia et al., 0.12% chlorhexidine has a significant inhibitory effect on the activity of dentinal proteolytic enzymes.<sup>34</sup> In terms of inhibition zone, 2% chlorhexidine was found to be comparable to sodium hypochlorite.

According to the findings of this study, white tea-mediated AgNPs had the greatest antibacterial potency and the optimal inhibition ability. Its biocompatible antioxidant nature renders it an effective endodontic irrigant.

A major limitation of this in vitro study is that the results cannot be fully transferred to clinical scenarios, since the efficacy of extracts may be affected by additional factors, such as staining, substantivity, as well as action

against other caries-causing microorganisms, which were not evaluated.

Advanced methods, such as the ETEST<sup>®</sup>, which combines the disk diffusion and agar dilution methods, or bioautography techniques, could be used in future research to explore these natural extracts further in the search for new antimicrobial irrigants.

Most previous research has focused on evaluating herbal irrigants in vitro; thus, detailed in vivo research is needed to assess the long-term stability of AgNPs, the susceptibility and toxicity of such irrigants, as well as their capacity to act against various other microorganisms.

## Conclusions

Within the limitations of the present study, white tea-mediated AgNPs showed promising results in the elimination of *E. faecalis*, demonstrating superior efficacy in comparison with chlorhexidine and sodium hypochlorite irrigants. Further in vitro and clinical studies are required to evaluate their efficacy, biocompatibility and safety before they can be conclusively recommended as a root canal irrigant.

## Ethics approval and consent to participate

The approval for the current in vitro study was obtained from the Ethics Committee at the Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India (SRB/SDC/PEDO-1803/20/05).

## Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

## Consent for publication

Not applicable.

## ORCID iDs

Lakshmi Lakshmanan  <https://orcid.org/0000-0002-9815-0837>

Deepa Gurunathan  <https://orcid.org/0000-0002-6014-946X>

Rajeshkumar Shanmugam  <https://orcid.org/0000-0001-7059-8894>

## References

- Möller AJ. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. *Odontol Tidskr.* 1966;74(5):1–380. PMID:5335186.
- Rôças IN, Siqueira JF Jr., Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endod.* 2004;30(5):315–320. doi:10.1097/00004770-200405000-00004
- Siqueira JF Jr., Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2004;97(1):85–94. doi:10.1016/s1079-2104(03)00353-6
- Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg Oral Med Oral Pathol.* 1983;55(3):307–312. doi:10.1016/0030-4220(83)90333-x
- Kaur R, Singh R, Sethi K, Garg S, Miglani S, Vats S. Irrigating solutions in pediatric dentistry: Literature review and update. *J Adv Med Dent Sci.* 2014;2(2):104–115. <https://jamdsr.com/pdf2b/Irrigating-SolutionsinPediatricDentistryLiteratureReviewandUpdate.pdf>. Accessed April 1, 2021.
- Kandaswamy D, Venkateshbabu N. Root canal irrigants. *J Conserv Dent.* 2010;13(4):256–264. doi:10.4103/0972-0707.73378
- Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. *J Endod.* 2006;32(2):93–98. doi:10.1016/j.joen.2005.10.049
- Siqueira JF Jr., Rôças IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. *J Endod.* 2000;26(6):331–334. doi:10.1097/00004770-200006000-00006
- Jose J, Shoba K, ShibuAman, NithyaTomy, Sheena, Christie. Comparative evaluation of antimicrobial activity of green tea extract, garlic extract, neem leaf extract and sodium hypochlorite as root canal irrigants against *E. faecalis* and *C. albicans* – an in vitro study. *Int J Curr Microbiol App Sci.* 2015;4(10):384–391. <https://www.ijc-mas.com/vol-4-10/Jerin%20Jose,%20et%20al.pdf>. Accessed April 1, 2021.
- Saxena D, Saha SG, Saha MK, Dubey S, Khatri M. An in vitro evaluation of antimicrobial activity of five herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against *Enterococcus faecalis*. *Indian J Dent Res.* 2015;26(5):524–527. doi:10.4103/0970-9290.172080
- Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes Infect.* 1999;1(2):125–129. doi:10.1016/s1286-4579(99)80003-3
- Shanmugapriya K, Nityanandi D, Saravana PS. Evaluation of antioxidant, anti-inflammatory and antimicrobial activities of two different extracts of *Camellia sinensis* (L.) O. Kuntze. *Int J Drug Dev Res.* 2013;5(3):155–168. <https://www.itmedicalteam.pl/articles/evaluation-of-antioxidant-antiinflammatory-and-antimicrobial-activities-of-two-different-extracts-of-camellia-sinensis-l-o-kuntze.pdf>. Accessed April 1, 2021.
- Rodrigues CT, De Andrade FB, De Vasconcelos LR, et al. Antibacterial properties of silver nanoparticles as a root canal irrigant against *Enterococcus faecalis* biofilm and infected dentinal tubules. *Int Endod J.* 2018;51(8):901–911. doi:10.1111/iej.12904
- Wang YL, Chang HH, Kuo CI, et al. A study on the root canal morphology of primary molars by high-resolution computed tomography. *J Dent Sci.* 2013;8(3):321–327. doi:10.1016/j.jds.2013.04.002
- Sumikawa DA, Marshall GW, Gee L, Marshall SJ. Microstructure of primary tooth dentin. *Pediatr Dent.* 1999;21(7):439–444. PMID:10633518.
- Agnihotri A, Arora R, Sharma U, Sood P. Herbal irrigants in primary teeth: A step toward green dentistry based on the wisdom of past. *J Indian Assoc Public Health Dent.* 2020;18(4):279–284. doi:10.4103/jiaphd.jiaphd\_109\_20
- Jolly M, Singh N, Rathore M, Tandon S, Banerjee M. Propolis and commonly used intracanal irrigants: Comparative evaluation of antimicrobial potential. *J Clin Pediatr Dent.* 2013;37(3):243–249. doi:10.17796/jcpd.37.3.3434221kn05t1376
- Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J.* 1998;31(1):1–7. PMID:9823122.
- Figueiredo de Almeida Gomes BP, Vianna ME, Sena NT, Zaia AA, Randi Ferraz CC, De Souza Filho FJ. In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medicament. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2006;102(4):544–550. doi:10.1016/j.tripleo.2006.04.010
- Abdulkader A, Duguid R, Saunders EM. The antimicrobial activity of endodontic sealers to anaerobic bacteria. *Int Endod J.* 1996;29(4):280–283. doi:10.1111/j.1365-2591.1996.tb01382.x
- Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. *J Endod.* 1992;18(12):605–612. doi:10.1016/S0099-2399(06)81331-2

22. Zand V, Lotfi M, Rahimi S, Mokhtari H, Kazemi A, Sakhamanesh V. A comparative scanning electron microscopic investigation of the smear layer after the use of sodium hypochlorite gel and solution forms as root canal irrigants. *J Endod.* 2010;36(7):1234–1237. doi:10.1016/j.joen.2010.02.033
23. Mohammadi Z. Chlorhexidine gluconate, its properties and applications in endodontics. *Iran Endod J.* 2008;2(4):113–125. PMID:24265633.
24. Jeanson MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. *J Endod.* 1994;20(6):276–278. doi:10.1016/s0099-2399(06)80815-0
25. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J.* 2001;34(6):424–428. doi:10.1046/j.1365-2591.2001.00410.x
26. Sajilata MG, Bajaj PR, Singhal RS. Tea polyphenols as nutraceuticals. *Compr Rev Food Sci Food Saf.* 2008;7(3):229–254. doi:10.1111/j.1541-4337.2008.00043.x
27. Almajano MP, Carbo R, López Jiménez J, Gordon MH. Antioxidant and antimicrobial activities of tea infusions. *Food Chem.* 2008;108(1):55–63. doi:10.1016/j.foodchem.2007.10.040
28. Sreenivasagan S, Subramanian AK, Rajeshkumar S. Assessment of antimicrobial effects and cytotoxic effect of green mediated silver nanoparticles and its coating onto mini-implants. *Ann Phytomed.* 2020;9(1):207–212. doi:10.21276/ap.2020.9.1.27
29. Samiei M, Farjami A, Dizaj SM, Lotfipour F. Nanoparticles for antimicrobial purposes in endodontics: A systematic review of in vitro studies. *Mater Sci Eng C Mater Biol Appl.* 2016;58:1269–1278. doi:10.1016/j.msec.2015.08.070
30. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. *J Endod.* 2010;36(6):1030–1035. doi:10.1016/j.joen.2010.02.008
31. Afkhami F, Pourhashemi SJ, Sadegh M, Salehi Y, Kharazzi Fard MJ. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against *Enterococcus faecalis*. *J Dent.* 2015;43(12):1573–1579. doi:10.1016/j.jdent.2015.08.012
32. Lapinska B, Klimek L, Sokolowski J, Lukomska-Szymanska M. Dentine surface morphology after chlorhexidine application – SEM study. *Polymers (Basel).* 2018;10(8):905. doi:10.3390/polym10080905
33. Baca P, Junco P, Arias-Moliz MT, González-Rodríguez MP, Ferrer-Luque CM. Residual and antimicrobial activity of final irrigation protocols on *Enterococcus faecalis* biofilm in dentin. *J Endod.* 2011;37(3):363–366. doi:10.1016/j.joen.2010.11.036
34. Garcia MB, Carrilho MR, Nör JE, et al. Chlorhexidine inhibits the proteolytic activity of root and coronal carious dentin in vitro. *Caries Res.* 2009;43(2):92–96. doi:10.1159/000209340