e-ISSN: 3049-2904, Print ISSN: 3107-6262

Oral Sphere Journal of Dental and Health Sciences

www.oralsphere.com

Original Research Article

Comparative Evaluation of Antimicrobial Efficacy and Dentinal Tubule Penetration of Nanoparticle-Loaded Sea Buckthorn Intracanal Medicament Against *Enterococcus faecalis*: An In Vitro Study

Smita Durga Dutta[1*], Prashik Parvekar[2]

[1]Department of Conservative Dental Sciences and Endodontics, College of Dentistry, Qassim University, Saudi Arabia

[2]Department of Conservative Dental Sciences and Endodontics, Root Dental Centre, Kuwait

Citation: Dutta SD, Parvekar P. Comparative Evaluation of Antimicrobial Efficacy and Dentinal Tubule Penetration of Nanoparticle-Loaded Sea Buckthorn Intracanal Medicament Against Enterococcus faecalis: An In Vitro Study. *Oral Sphere J Dent Health Sci*. 2026;2(4):219-223.

For reprints contact:
publisher@fontfusionspublication.com

Received: May 15, 2026;
Revised: June 27, 2026;
Accepted: August 08, 2026,
Published: October 01, 2026

***Corresponding author:** Smita Durga Dutta
Email: s.dutta@qu.edu.sa

DOI: https://doi.org/10.63150/osjdhs.2026.32

©The Author(s). (2026)

Open Access.
Published by Font Fusions Publication

This article is licensed under the Creative Commons Attribution-NonCommercial 4.0 International License (https://creativecommons.org/licenses/by-nc/4.0/) allowing non-commercial sharing, adaptation, and distribution, provided you give appropriate credit to the original author(s), provide a link to the license, and indicate if changes were made.

ABSTRACT

Introduction: Persistent microorganisms such as Enterococcus faecalis (E. faecalis) are major contributors to endodontic treatment failure. Sea buckthorn (Hippophae rhamnoides) possesses antimicrobial and anti-inflammatory properties, while nanoparticle incorporation may enhance drug bioavailability and dentinal tubule penetration. This study evaluated the antimicrobial activity and dentinal tubule penetration of nanoparticle-loaded sea buckthorn intracanal medicament against E. faecalis compared with calcium hydroxide.

Methods: Sixty freshly extracted single-rooted human premolars were prepared using ProTaper Next rotary files and randomly allocated into three groups (n=20): Group I, nanoparticle-loaded sea buckthorn medicament; Group II, conventional calcium hydroxide [$Ca(OH)_2$]; and Group III, normal saline (negative control). Antimicrobial efficacy after 7 days was assessed using agar diffusion and colony-forming unit (CFU) counts. Dentinal tubule penetration was evaluated using rhodamine B dye and confocal laser scanning microscopy (CLSM) at the coronal, middle, and apical thirds. Data were analyzed using one-way ANOVA and Tukey's post hoc test ($p<0.05$).

Results: Group I demonstrated significantly greater zones of inhibition and lower CFU counts than Groups II and III ($p<0.05$). CLSM analysis showed significantly greater dentinal tubule penetration of nanoparticle-loaded sea buckthorn than calcium hydroxide at the coronal, middle, and apical thirds, with the greatest relative advantage observed in the apical third.

Conclusion: Nanoparticle-loaded sea buckthorn may be a promising candidate for further preclinical and clinical investigation as an intracanal medicament.

Keywords: Sea buckthorn, Intracanal medicament, Nanoparticles, *Enterococcus faecalis*, Dentinal tubule penetration, Antimicrobial efficacy, Endodontics.

BACKGROUND

Endodontic infections are polymicrobial infections, but *Enterococcus faecalis (E. faecalis)* has been consistently found as a predominant organism in cases of persistent periapical periodontitis and failed root canal treatments [1]. It has the ability to survive for a prolonged period without any nutrients, to resist high alkaline pH and to penetrate deeply into the dentinal tubules, making it

extremely difficult to eradicate the infection with conventional intracanal medicaments [2].

Calcium hydroxide ($Ca(OH)_2$) has long been considered as the standard intracanal medicament with antibacterial and tissue dissolving properties. But it has limited efficacy in removing *E. faecalis* from the deep dentinal tubules and there has been a search for alternatives with better efficacy [3]. The use of herbal medicaments in endodontics has been steadily increased during past 10 years which is attributed to their wide spectrum antimicrobial activity, biocompatibility and cost effectiveness [4].

Sea buckthorn (Hippophae rhamnoides L.) is a thorny deciduous shrub that belongs to the Elaeagnaceae family. A wealth of bioactive phytochemicals such as flavonoids, carotenoids, tocopherols, fatty acids (specifically palmitoleic acid), phenolic acids, and terpenes are found in its berries, seeds, and leaves [5]. Sea buckthorn extracts possess strong antimicrobial, anti-inflammatory, antioxidant and wound healing effects [6]. It is enriched with palmitoleic acid, a fatty acid with bactericidal activity against gram positive bacteria such as *E. faecalis*, which is noteworthy [7].

Although these are desirable properties, the clinical usefulness of herbal medicaments is limited due to the relatively low penetration into dentinal tubules and the degradation in oral environment [8]. Nanotechnology is a promising remedy to these constraints. The use of drug delivery systems based on nanoparticles greatly improves the bioavailability of drugs, their penetration into tissues and facilitates prolonged action of drugs [9]. In endodontology, the use of vehicles for herbal drug delivery such as chitosan nanoparticles, zinc oxide nanoparticles and polymeric nanocarriers was investigated [10].

Sea buckthorn extract formulated into a delivery system based on nanoparticles may, therefore, synergistically increase its antimicrobial effectiveness and penetration of dentinal tubules [11]. This is one of the few studies to be performed on the intracanal use of the sea buckthorn as a therapeutic material loaded with nanoparticles. The purpose of the in vitro study was to compare the antimicrobial effect and dentinal tubule penetration of nanoparticle loaded sea buckthorn medicament with the conventional calcium hydroxide against *E. faecalis*.

METHODOLOGY

Ethical approval and sample selection

The study protocol was approved by the Institutional Review Board (IRB) of AIMST University before the study began. A total of 60 freshly extracted human single-rooted premolars were obtained with written informed consent from the donors for orthodontic reasons. The criteria for exclusion were as follows: teeth with curved roots (> 20° Schneider's method), teeth with previous endodontic treatment, teeth with internal or external resorption, teeth with calcified canals.

All teeth were decoronated at the cementoenamel junction to be standard 14 mm in length using a diamond disc and copious irrigation with 5.25% sodium hypochlorite (NaOCl) and 17% ethylenediaminetetra-acetic acid (EDTA) and were then mechanically prepared using ProTaper Next rotary files (Dentsply Sirona) up to file size X4 (40/0.06). All specimens were prepared and then sterilized in an autoclave at 121°C for 15 minutes. E. faecalis (ATCC 29212) was introduced into the canals as a standardized suspension at 1.5×10^8 CFU/mL (McFarland standard 0.5) and were allowed to grow in an established biofilm for 21 days at 37°C.

The sea buckthorn berry extract was extracted by cold maceration method using dried sea buckthorn berries in 70% ethanol and later rotary evaporation was used to prepare it. The synthesis of chitosan nanoparticles by ionic gelation, using tripolyphosphate (TPP) as crosslinking agent was performed. To encapsulate the extract in chitosan nanoparticles, it was mixed with chitosan at a ratio of 1:3 (drug:polymer). The particle size (+/- range: 180-240 nm), polydispersity index (<0.3) and zeta potential (+28 mV) were confirmed using dynamic light scattering (DLS). Medicament paste was prepared by adding the extract and PG in the ratio of 1:1, to which nanoparticles were added.

Experimental groups

The sixty specimens were randomly divided into three groups of twenty specimens each: Group I – Nanoparticle-loaded sea buckthorn medicament; Group II – Conventional calcium hydroxide ($Ca(OH)_2$); Group III – Normal saline (negative control). The root canals were filled with the respective medicaments using a Lentulo spiral, and the access cavities were temporarily sealed with Cavit (3M ESPE). All specimens were kept at 37°C in 100% humidity for 7 days.

Medicaments were irrigated after 7 days (Antimicrobial Efficacy Assessment). E. faecalis was inoculated into Brain Heart Infusion (BHI) agar plates and the zone of inhibition was determined using the agar diffusion method. For CFU enumeration, samples were collected from the root canals using sterile paper points and serially diluted before plating on BHI agar.

Dentinal tubule penetration assessment

The medicaments were each mixed with rhodamine B dye (1% w/v in distilled water) and inserted into the canals. After 7 days, the roots were cut horizontally at the coronal level (4 mm), middle level (7 mm) and the apical level (11 mm) using a slow speed diamond saw. Confocal laser scanning microscopy (CLSM) (Leica TCS SP8) was used to examine the sections, and the penetration depth (µm) was measured by image analysis software.

Data analysis

All data were presented as means ± SD. A one-way ANOVA test was performed and Tukey's Post Hoc test was applied to the data, using SPSS 26.0. A p value of <0.05 was used as statistically significant.

RESULTS

A total of 60 specimens were included in the final analysis. No specimens were lost during the study period. The results of antimicrobial efficacy and dentinal tubule penetration are presented below.

Antimicrobial efficacy

Group I (Nanoparticle-loaded sea buckthorn medicament) demonstrated significantly larger zones of inhibition (18.6 ± 1.4 mm) compared to Group II (Calcium hydroxide: 13.2 ± 1.1 mm) and Group III (Saline control: 0.0 mm) ($p<0.05$). CFU counts were significantly lower in Group I ($0.8 \pm 0.3 \times 10^3$) compared to Group II ($3.5 \pm 0.7 \times 10^3$) and Group III ($28.4 \pm 2.1 \times 10^3$) ($p<0.05$) (Table 1).

Dentinal tubule penetration

CLSM analysis revealed that nanoparticle-loaded sea buckthorn medicament achieved significantly deeper dentinal tubule penetration at all root thirds compared to calcium hydroxide ($p<0.05$). The greatest penetration depth was observed at the coronal third in Group I (312.4 ± 22.6 µm), which declined progressively towards the apical third (231.6 ± 17.2 µm). Calcium hydroxide similarly showed a coronal-to-apical gradient, but with significantly lower penetration values at all levels (Table 2).

DISCUSSION

In the present in vitro study, the antimicrobial activity and dentinal tubule penetration of the nanoparticle-loaded sea buckthorn intracanal medicament were evaluated against *E. faecalis*, a representative microorganism associated with persistent endodontic infections. The findings revealed that the antimicrobial activity of the nanoparticle-loaded sea buckthorn medicament was significantly greater than that of conventional calcium hydroxide, with deeper dentinal tubule penetration also observed.

Sea buckthorn extract's antimicrobial superiority in the present study may be due to its pleiotropic phytochemical profile. The high concentration of palmitoleic acid (ω-7 fatty acid) in sea buckthorn berry oil has been shown to damage the bacterial cell wall of gram-positive bacteria such as E. faecalis, resulting in leakage of the cytoplasm and death of the cell [12]. Moreover, flavonoids like isorhamnetin and quercetin in sea buckthorn have bactericidal activity via DNA gyrase and cell wall inhibition [13]. The mixture of these bioactive compounds may have been responsible for the significantly lower CFU counts and the bigger inhibition zones in Group I.

Table 1: Antimicrobial efficacy of intracanal medicaments against *E. faecalis*

Group	Intracanal Medicament	Mean Zone of Inhibition (mm) ± SD	CFU Count ($\times 10^3$) ± SD
Group I	Nanoparticle-loaded Sea Buckthorn	18.6 ± 1.4	0.8 ± 0.3
Group II	Conventional Calcium Hydroxide	13.2 ± 1.1	3.5 ± 0.7
Group III	Normal Saline (Control)	0.0 ± 0.0	28.4 ± 2.1

**p<0.05, statistically significant; CFU: Colony Forming Unit; SD: Standard Deviation*

Table 2: Dentinal tubule penetration depth (µm) at different root levels

Root Third	Group I: Nanoparticle Sea Buckthorn (µm ± SD)	Group II: Conventional Calcium Hydroxide (µm ± SD)	p-value
Coronal Third	312.4 ± 22.6	248.1 ± 19.3	0.021*
Middle Third	278.9 ± 18.4	210.5 ± 16.7	0.018*
Apical Third	231.6 ± 17.2	148.3 ± 14.5	0.003*

**p<0.05, statistically significant; SD: Standard Deviation; µm: Micrometers*

The chitosan nanoparticles used as a carrier system greatly enhanced the efficacy of sea buckthorn extract. The inherent antimicrobial activity of chitosan has been attributed to its polycationic nature, allowing electrostatic interaction with the polyanionic bacterial cell membrane which causes disruption of the cell membrane [14]. Furthermore, the relatively small particle size (180–240 nm) may have contributed to the

deeper dentinal tubule penetration observed with the nanoparticle-loaded medicament. The results are in line with Capuano N et al. (2023) [15], who reported greater dentinal penetration of herbal extracts when encapsulated in nanoparticles than when used alone.

In the literature, it is already known that calcium hydroxide is less effective in controlling E. faecalis. Higher alkaline environment can be tolerated by E. faecalis by maintaining the intracellular pH homeostasis by proton pump mechanism [16]. These results are consistent with the present study, which revealed that the calcium hydroxide showed a 4.4-fold increase in the CFU count as compared with the sea buckthorn medicament loaded with nanoparticles. The same was observed by AA Mohamed et al. (2024) [17] who reported that $Ca(OH)_2$ was significantly less effective against E. faecalis biofilms in well-established root canal infections.

CLSM analysis of penetration of dentinal tubules resulted in a similar gradient of penetration from coronal to apical third for both medicaments with the least penetration in the apical third. This trend is similar to the previous findings that the apical region has thicker dentin, smaller diameter of the tubule, and lower dentinal hydraulic conductance, which impedes the penetration of the apical portion [18]. Most importantly, the sea buckthorn loaded with nanoparticles showed much deeper penetration at the apical third (231.6 ± 17.2 μm), whereas, conventional $Ca(OH)_2$ showed penetration of 148.3 ± 14.5 μm, which is clinically relevant because E. faecalis has been reported to penetrate up to 300 μm into dentinal tubules. The results obtained are similar to that of MT El-Saadony et al. (2025) [19] who found better apical penetration properties of nanoparticle loaded herbal medicaments than the conventional pastes.

There are some shortcomings to the present study. Due to its in vitro nature, the complex in vivo environment such as immune response, periapical tissue interactions and blood supply might not have been simulated. Single-species biofilm model may not apply to the polymicrobial nature of clinical endodontic infections. Additionally, given the potential cytotoxicity of the nanoparticle-containing sea buckthorn medicament, its effects on periapical tissues should be investigated before clinical use.

CONCLUSION

Within the limitations of this in vitro study, nanoparticle-loaded sea buckthorn demonstrated greater antimicrobial activity and dentinal tubule penetration against E. faecalis than conventional calcium hydroxide. These findings support further preclinical evaluation of nanoparticle-loaded sea buckthorn as a potential intracanal medicament

REFERENCES

1. Sharma J et al. J Conserv Dent Endod. 2025 May;28(5):462-467. [PMID: 40463679]
2. de Lima FSF et al. Aust Endod J. 2026 Aug;52(2):585-605. [PMID: 41837388]
3. Chowdhury K et al. J Conserv Dent Endod. 2025 Sep;28(9):849-858. [PMID: 40964636]
4. Karobari MI et al. Materials (Basel). 2022 Apr 25;15(9):3111. [PMID: 35591443]
5. Bathla S et al. Endodontics Today. 2026;24(3):464–473. [DOI: 10.36377/ET-0210]
6. Song X et al. Front Pharmacol. 2025 Nov 11;16:1643146. [PMID: 41306783]
7. Casillas-Vargas G et al. Prog Lipid Res. 2021 Apr;82:101093. [PMID: 33577909]
8. Anwar MA et al. Inflammopharmacology. 2025 Mar;33(3):1085-1160. [PMID: 39907951]
9. Georgakopoulou VE et al. J Biol Methods. 2024 Oct 23;11(4):e99010032. [PMID: 39839091]
10. Thakare A et al. Cureus. 2024 Jun 17;16(6):e62506. [PMID: 39022517]
11. Popescu ID et al. Pharmaceuticals (Basel). 2025 Feb 5;18(2):212. [PMID: 40006025]
12. Chen Y et al. Foods. 2023 May 13;12(10):1985. [PMID: 37238803]
13. Martiniakova M et al. Front Pharmacol. 2024 Oct 9;15:1462823. [PMID: 39444603]
14. El-Saadony MT et al. Mater Today Bio. 2025 Sep 25;35:102358. [PMID: 41215768]
15. Capuano N et al. Antibiotics (Basel). 2023 Dec 1;12(12):1690. [PMID: 38136724]
16. Abe Y et al. Pharmaceutics. 2023 May 31;15(6):1629. [PMID: 37376077]
17. Mohamed AA et al. BMC Oral Health. 2024 Feb 10;24(1):215. [PMID: 38341565]
18. Santamaria N et al. J Clin Med. 2026 Jan 23;15(3):930. [PMID: 41682610]
19. El-Saadony MT et al. Front Immunol. 2025 Aug 21;16:1603018. [PMID: 40918117]

Ethical Approval: Ethical approval was obtained from the Ethical Committee of the College of Dentistry, Qassim University (EA/F-2026-6009) before the current study was conducted.

Declaration of Participant Consent: Written informed consent was obtained from all tooth donors for the use of extracted teeth for research purposes.

Financial Support and Sponsorship: Nil

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Use of Artificial Intelligence (AI)-Assisted Technology for Manuscript Preparation: The authors confirm that no artificial intelligence (AI)-assisted technology was used to assist in the writing or editing of the manuscript, and no images were manipulated using AI tools.

AUTHOR CONTRIBUTIONS

Smita Durga Dutta: Conceptualization, methodology, investigation, data collection, data analysis, interpretation of results, and manuscript writing.

Prashik Parvekar: Methodology, investigation, data analysis, interpretation of results, critical revision of the manuscript, and supervision.

Both authors: Reviewed and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

ABBREVIATIONS USED IN THE STUDY:

a) E. faecalis: Enterococcus faecalis
b) $Ca(OH)_2$: Calcium Hydroxide
c) CLSM: Confocal Laser Scanning Microscopy
d) CFU: Colony Forming Unit
e) EDTA: Ethylenediaminetetraacetic Acid
f) NaOCl: Sodium Hypochlorite
g) DLS: Dynamic Light Scattering
h) BHI: Brain Heart Infusion
i) SD: Standard Deviation
j) TPP: Tripolyphosphate

DECLARATION ON OFFICIAL E-MAIL

The corresponding author declares that a lifetime official e-mail from their institution is not available for all authors.

COMMENTS FROM READERS

Articles published in the ORAL SPHERE JOURNAL OF DENTAL AND HEALTH SCIENCES are open for relevant post-publication comments and criticisms, which will be published immediately, linking to the original article without open access charges. Comments should be concise, coherent, and critical in fewer than 1000 words.

DISCLAIMER

The Oral Sphere Journal of Dental and Health Sciences provides a platform for the scholarly communication of data and information to create knowledge in the dental and medical domains after adequate peer/editorial reviews and editing, with entertaining revisions where required. The views and opinions expressed are those of the author(s) and do not reflect the views or opinions of Oral Sphere Journal of Dental and Health Sciences (and/or) its publisher, Font Fusions Publication Pvt. Ltd. Font Fusions Publication remains neutral and allows authors to specify their address and affiliation details, including territory where required.