Original Article

Comparative evaluation of antimicrobial efficacy of 0.1% octenidine dihydrochloride, superoxidized solution, ozonated water, 0.1% silver nanoparticle solution, and Q mix™ 2 in 1 in root canals infected with *Enterococcus faecalis*

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Abstract

Introduction: The objective of this study was to compare and evaluate the antimicrobial efficacy of 0.1% octenidine dihydrochloride, superoxidized solution, ozonated water, 0.1% silver nanoparticles (AgNp) solution, and Q mix™ 2 in 1 in root canals infected with *Enterococcus faecalis*.

Methodology: One hundred and fifty permanent mandibular premolars were inoculated with *E. faecalis* (0.5 McFarland standards) were incubated at 37°C for 7 days after which preoperative microbial sampling was done and the number of viable *E. faecalis* cells was obtained as CFU/mL.

The specimens were irrigated with 0.1% octenidine dihydrochloride (group 1), Q mix™ 2 in 1 (group 2), super oxidized solution (group 3), 0.1% AgNp solution (group 4), ozonated water (group 5), and normal saline (group 6) during mechanical instrumentation. The final irrigation was followed by microbial sampling and the number of viable *E. faecalis* cells was obtained as CFU/mL.

Statistical Analysis: Data were analyzed by paired *t*-test and ANOVA with Tukey's *post hoc* test.

Results: Paired *t*-test showed a statistically significant difference between mean CFU before and after irrigation in groups I, II, III, IV, and V respectively ($P < 0.05$). Group VI showed no statistically significant difference between CFU before and after irrigation ($P = 0.131$).

Conclusion: The mean bacterial reduction was statistically significant for all the study groups, proving their good antibacterial activity against *E. faecalis* in root canals whereas 0.1% octenidine dihydrochloride and ozonated water demonstrated relatively higher antimicrobial potential.

Keywords: Antimicrobial efficacy; octenidine dihydrochloride; ozonated water; Q mix; silver nanoparticles; superoxidized solution

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INTRODUCTION

The biomechanical preparation of the root canal system has an enormous significance in the disinfection of the root canal system. The anatomic and morphologic complexities within the human tooth limit the role of mechanical instrumentation, necessitating chemical disinfectants.

Sodium hypochlorite (NaOCl) has been a benchmark for root canal irrigation for ages, however, could not termed as "ideal" owing to its cytotoxicity and other drawbacks.[1] Various alternative solutions were developed to surpass the undesirable effects of NaOCl, among which ethylenediaminetetraacetic acid (EDTA) and chlorhexidine (CHX) are the most commonly used irrigants in clinical endodontic practice.[2]

Q mix™ 2 in 1 (Dentsply Tulsa, 2011), a novel one-step irrigating solution, containing a mixture of bisbiguanide antimicrobial agent, calcium chelating agent, and a surfactant has gained popularity due to its antimicrobial potency, efficiency in removal of smear layer and biocompatibility.[3,4]

Octenidine dihydrochloride (Oct), a bispyridine antimicrobial compound, demonstrates broad-spectrum antimicrobial effects, by interfering with microbial cell walls and membranes.[5]

Superoxidized solution (Oxum) contains hypochlorous acid and oxidized water. It has potentially reactive superoxide radicals with good antimicrobial potential and efficient smear layer removal.[6]

Silver nanoparticles (AgNp) have a remarkable ability to interact with bacterial cell membranes, causing intracellular changes in bacterial cells leading to an increased antimicrobial efficacy against a wide range of organisms such as streptococci, fungi, and viruses.[7] The nanosize provides increased surface area which can absorb other medicaments and exert an antimicrobial effect.

Ozonated water, a solution obtained by treating distilled water with ozone, is currently being explored for its remarkable qualities such as antimicrobial potency, higher biocompatibility, and ease of handling compared to other antiseptics as an endodontic irrigant.[8]

The advent of all the mentioned solutions and their effective results when used as endodontic irrigants derive the central idea of this study. Various studies have reported the individual efficacy of these solutions as endodontic irrigants but the comparison among them is lacking.

This study was conducted to compare and evaluate the antimicrobial efficacy of 0.1% octenidine dihydrochloride, superoxidized solution, ozonated water, 0.1% AgNp solution, and Q mix™ 2 in 1 in root canals infected with *Enterococcus faecalis* [Figure 1a].

METHODOLOGY

This *in vitro* study was conducted on 150 human permanent mandibular premolars with a single canal (checked radiographically) and straight roots, extracted for therapeutic reasons. Teeth with resorbed roots, caries, and cracks were excluded.

Preparation of tooth specimen

The selected teeth were placed in 0.5% NaOCl for surface disinfection for 24 h after which they were stored in normal saline (Eurolife Health Care Pvt. Ltd., India). The teeth were decoronated to standardize the root length to 14 mm. The samples were rinsed with normal saline to dissipate the heat generated during decoronation.

The initial exploration of the radicular canal was accomplished with a #10 K file (Mani, Japan) to ensure the presence of a patent single canal. The root canal orifices were prepared with GG drills of sizes 2 and 3 (Mani, Japan) to maintain the uniformity of coronal preparation. The initial apical preparation was done using neoendoflex files (Orikam Healthcare, India) sequentially up to #25 (4%) at the recommended torque and speed in a crown-down manner [Figure 1b]. The canals were recapitulated and irrigated with 3% NaOCl (MAARC Dental, India) and 17% EDTA (Urdent Innovations, Pvt. Ltd., India) with intermittent saline rinse to minimize irrigant interactions. The final rinse was done with normal saline to wash away any remaining irrigating solutions in the root canals. Subsequently, the specimens were sealed apically with cyanoacrylate glue (Fevikwik, Pidilite) to prevent bacterial microleakage.

Sterilization of tooth specimens

The tooth specimens were sterilized in an autoclave at 121°C for 15 min under 15 lbs of pressure. The teeth were then transferred to individual sterile Eppendorf (EP) tubes (Himedia Pune) subsequently followed by the addition of 1 mL of brain heart infusion broth (BHI), and the tubes were sealed with paraffin strips. The EP tubes containing the tooth samples and BHI broth were re-sterilized in the autoclave.

Inoculation of the prepared specimens with *Enterococcus faecalis*

All the microbiologic procedures were performed under aseptic conditions in a laminar flow chamber (Bionics Scientific Technologies, India). Isolated colonies of E. *faecalis*

(ATCC 29212) grown on BHI agar were suspended in 5 mL of BHI broth and incubated aerobically for 24 h at 37°C. Bacterial cells were re-suspended in saline to give a final concentration of about 1.5 × 10^8 CFU/mL and the turbidity of *E. faecalis* culture was adjusted to No. 0.5 McFarland standards. 50 µl of the bacterial inoculum was transferred to individual EP tubes containing 1 mL of BHI broth and tooth specimen and the tubes were sealed using paraffin strips [Figure 1c]. The specimens were incubated at 37°C for 7 days.[9] Every 2nd day the specimens were transferred to fresh tubes containing 1 mL of broth contaminated with 50 µL of *E. faecalis*.

After 7 days, microbial sampling was performed on randomly selected teeth using a #25 sterile paper point, confirming the contamination of the root canals. The microbiologic evaluation before irrigation was done by inserting a #25 sterile paper point in all the tooth specimens for 1 min and transferring it to a test tube containing 1 mL sterile saline solution, subjected to agitation for 30 s. 0.1 mL of this solution was plated and duplicated on Mac Conkey Agar without crystal violet and incubated for 48 h at 37°C. The number of viable *E. faecalis* cells was obtained as CFU/mL [Figure 2a].

Antimicrobial assessment

Each tooth specimen was carefully removed from the broth and held with artery forceps. The root canals were enlarged sequentially up to #40 (4%) using neoendo flex files. During instrumentation, the specimens in each group were specifically irrigated with 3 mL of the respective irrigating solution for each file used [Figure 1e].

Depending on the irrigants to be tested, the specimens were equally divided into 6 groups as follows:

- Group 1: Octenidine dihydrochloride (0.1%) (Orahex Pro, Windlass Biotech Private Limited)

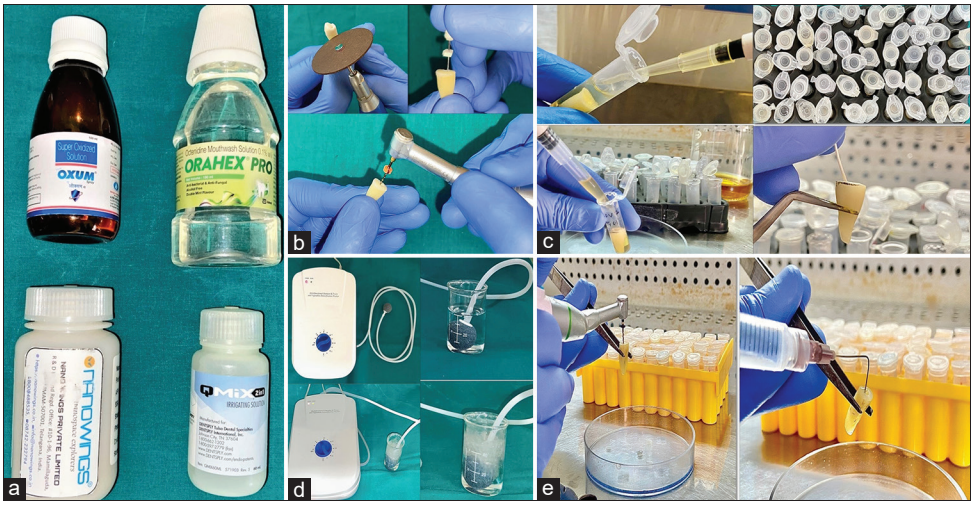

Figure 1: Methodology. (a) Irrigants used in the study, (b) Preparation of tooth specimen, (c) Microbial inoculation, (d) Preparation of ozonated water, (e) Final preparation and irrigation of the tooth specimen

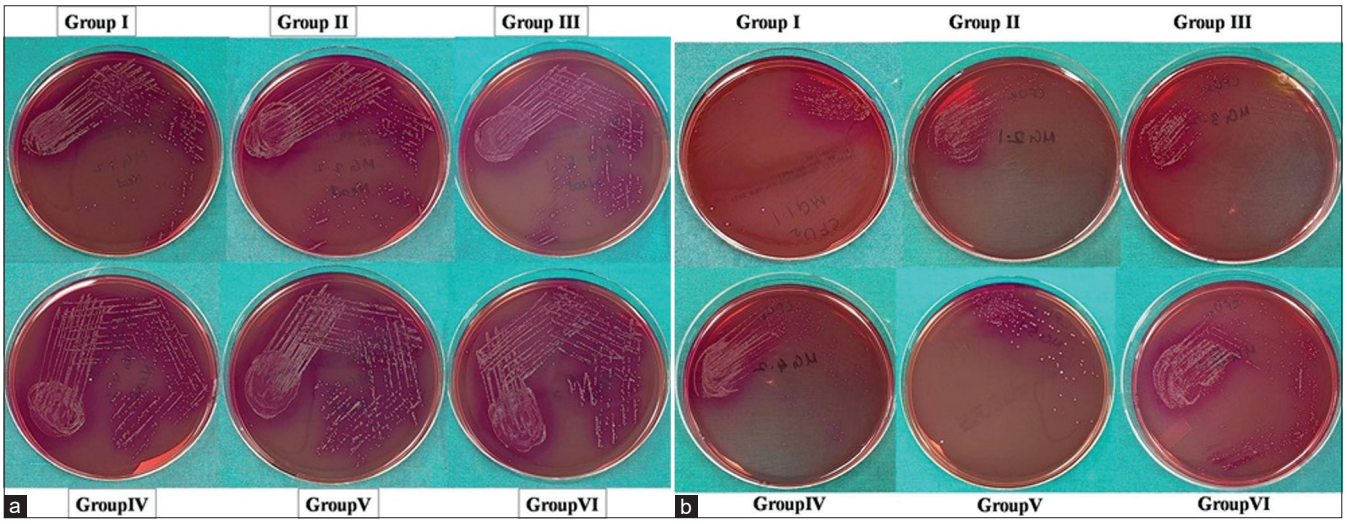

Figure 2: Microbiologic evaluation, (a) Mac Conkey agar without crystal violet showing CFU before irrigation, (b) Mac Conkey agar without crystal violet showing CFU after irrigation

- Group 2: Q mix™ 2 in 1 (Dentsply Tulsa, USA)
- Group 3: Super oxidized solution (Oxum, Venus Remedies Limited, India)
- Group 4: 0.1% AgNp solution (NANO WINGS Private Limited, India)
- Group 5: Ozonated water
- Group 6 (control): Normal saline.

A chair-side ozone generator was used to prepare ozone water. Ozonation of the water was performed by bubbling ozone through sterile distilled water [Figure 1d] to attain an ozone concentration of approximately 24 mg/L.

All the specimens were finally rinsed with normal saline to remove any remaining irrigant from the canal. Immediately after the final irrigation, microbial sampling was done by inserting a sterile paper point (#40), and microbiologic evaluation after irrigation was done as described earlier [Figure 2b].

Statistical analysis

The statistical analysis for this study was done with Statistical Package for Social Sciences (IBM SPSS Statistic for Windows, version 21.0. Armonk, NY, USA: IBM Corp.).

The paired *t*-test was used to compare the CFU of *E. faecalis* before and after irrigation in each group. ANOVA with *post hoc* Tukey's test was done to compare the mean difference between CFU before and after irrigation between all the study groups.

RESULTS

Paired *t*-tests [Table 1] showed statistically significant differences between mean CFU before and after irrigation in groups I, II, III, IV, and V, respectively ($P < 0.05$). The comparison between the study groups was done based on the mean difference in CFU before and after irrigation.

The mean difference in CFU before and after irrigation was higher in group I and group V, while group IV demonstrated the lowest statistically significant reduction in CFU after irrigation compared to other study groups.

Group VI (normal saline) showed no significant difference between CFU before and after irrigation.

ANOVA with *post hoc* Tukey's test [Table 2] showed a statistically significant difference after final irrigation between all the study groups ($P < 0.05$).

Table 1: Comparison between 0.1% octenidine dihydrochloride, Q mix™ 2 in 1, superoxidized solution, 0.1% silver nanoparticles solution, ozonated water, and normal saline based on mean difference in CFU before and after irrigation

Groups	Mean difference in CFU before and after irrigation (10^8)	SD	SEM	*t*	*P* (paired *t*-test)
Group I	127.2	29.02442	5.80488	21.913	0.000*
Group II	115.52	21.237	4.2474	27.198	0.000*
Group III	109.16	34.31555	6.86311	15.905	0.000*
Group IV	94.48	34.41066	6.88213	13.728	0.000*
Group V	129.76	28.40522	5.68104	22.841	0.000*
Group VI	4.2	13.42262	2.68452	1.565	0.131

*Significance at $P<0.05$. SD: Standard deviation, SEM: Standard error of mean

Table 2: Intergroup comparison based on mean difference in CFU before and after irrigation between 0.1% octenidine dihydrochloride, Q mix™ 2 in 1, superoxidized solution, 0.1% silver nanoparticles solution, ozonated water, and normal saline using *Post hoc* Tukey's test

Group	Compared groups	Mean difference in CFU before and after irrigation (10^8)	*P* (Tukey's test)
Group I	Group II	−23.04	0.045*
	Group III	−26.40	0.013*
	Group IV	−42.72	0.000*
	Group V	−22.36	0.057*
	Group VI	−151.96	0.000*
Group II	Group I	23.04	0.045
	Group III	−3.36	0.998
	Group IV	−19.68	0.131
	Group V	0.68	1.000
	Group VI	−128.92*	0.000*
Group III	Group I	26.40	0.013*
	Group II	3.36	0.998
	Group IV	−16.32	0.308
	Group V	4.04	0.996
	Group VI	−125.56*	0.000*
Group IV	Group I	42.72	0.000*
	Group II	19.68	0.131
	Group III	16.32	0.308
	Group V	20.36	0.108
	Group VI	−109.24	0.000*
Group V	Group I	22.36	0.057*
	Group II	−0.68	1.000
	Group III	−4.04	0.996
	Group IV	−20.36	0.108
	Group VI	−129.60	0.000*
Group VI	Group I	151.96	0.000*
	Group II	128.92	0.000*
	Group III	125.56	0.000*
	Group IV	109.24	0.000*
	Group V	129.60	0.000*

*Significance at $P<0.05$

DISCUSSION

The true essence of endodontic therapy lies in the complete healing of periapical lesions, emphasizing the prevention of reinfection of the root canal system. The complete disinfection of the root canal system has been the central motive of perpetual ameliorations in various techniques, instruments, and irrigating solutions in endodontics for decades.

While the anatomic complexities are inaccessible for mechanical instrumentation, an antimicrobial irrigant in liquid or gel form could easily flow through the

complex areas and ramifications of the root canals not only ascertaining the disinfection of these areas but also assisting mechanical instrumentation in shaping these complex areas to certain extent.

This study utilized a mono-species *E. faecalis* biofilm formed on root canal walls.[10] *E. faecalis* was selected due to its clinical relevance, resistance to intra-canal medications, and prevalence in endodontic failure.[11] The possibility of microbial contamination was overcome by choosing appropriate selective growth media for *E. faecalis*.

The modern endodontic approaches emphasize the importance of activation of irrigation[12] but on the flip side, the conventional syringe irrigation technique remains practically prevalent among general practitioners and endodontists.[13] Syringe irrigation facilitates the depth of needle penetration and the volume of irrigant within the canal.[13]

Each specimen was enlarged sequentially to #40 with a 0.04 taper as it maintains a good balance of tooth structure preservation and adequate volume of irrigation at the apical third. 3 mL of each irrigant after each file protocol was based on protocols documented in previous studies.[14]

Although literature documents the duration of irrigation, here the irrigant was used till the completion of instrumentation irrespective of the duration, practically similar to the clinical scenario.

In the present study, the highest antimicrobial efficacy against *E. faecalis* was found in the 0.1% Oct group. This was similar to the previous studies by Tandjung *et al.*[15] and Tirali *et al.*[16] The probable reason for the enhanced antimicrobial effect of 0.1% Oct can be attributed to its antimicrobial efficacy in the presence of organic material comparable to CHX.

Ozonated water showed a remarkable mean reduction of CFU after final irrigation analogous to the studies documented in the literature.[17,18] The concentration of ozonated water (24 mg/L) was based on previous studies.[8] A major limitation of ozonated water is that it dissolves rapidly in water and disintegrates quickly.

In this study, the antimicrobial efficacy of Q mix™ 2 in 1 was comparable to ozonated water and statistically superior to the 0.1% AgNp group. This was comparable to a study by Wang *et al.*[19]

The surface-active agent in Q mix™ 2 in 1 not only lowers the surface tension of solutions but also improves its wettability resulting in better penetration of an irrigant in the root canal. The bisbiguanide component prevents microbial colonization on the dentin surface while the calcium chelating agent effectively removes the smear layer.[19]

Super oxidized solution also indicated a statistically significant difference in CFU before and after irrigation, comparable to a study Mg Ruqshan Anjum *et al.*[20] The reduction in CFU was slightly less when compared to 0.1% Oct, ozonated water, and Q mix™ 2 in 1 but this was statistically insignificant.

This study utilized an AgNP solution in a concentration of 0.1% based on previous studies.[21] The intergroup comparison demonstrated limited antimicrobial efficacy of 0.1% AgNP solution against *E. faecalis*.

Probable reasons might be inadequate interaction between positively charged AgNPs and negatively charged bacterial cells during the short period of root canal irrigation. The findings of the previous studies suggested that the rate of bacterial killing by nanoparticles depends on the particle size, concentration, and duration of the interaction.[22]

Certain factors that affect the antimicrobial efficacy of the irrigants include the concentration, duration of interaction, and activation of the irrigating solutions. The precisely targeted antimicrobial action and effective functioning individual constituents of the irrigants used in this study, not only facilitate in overcoming the microbial challenges but also provide a bio-friendly ambient to the host tissue within the root canal system compared to traditional endodontic irrigants, leading toward the optimal treatment outcomes.

CONCLUSION

The mean bacterial reduction was statistically significant for 0.1% octenidine dihydrochloride, superoxidised solution, ozonated water, 0.1% AgNP solution, and Q mix™ 2 in 1, proving their good antibacterial activity against *E. faecalis* in root canals.

0.1% octenidine and ozonated water demonstrated relatively higher antimicrobial potential to, Q mix™ 2 in 1, superoxidized solution, and 0.1% AgNP solution.

The fact that antimicrobial efficacy is not the only requirement to be an ideal irrigant, creates the need for research directed toward the evaluation of these newer irrigants based on other parameters such as dissolution of organic tissues, smear layer removal, biocompatibility, dose-effectiveness, and interactions with other irrigants.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J 2014;216:299-303.
2. Dioguardi M, Gioia GD, Illuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: Different methods to improve efficacy and related problems. Eur J Dent 2018;12:459-66.
3. Chandrasekhar V, Amulya V, Rani VS, Prakash TJ, Ranjani AS, Gayathri C. Evaluation of biocompatibility of a new root canal irrigant Q Mix TM 2 in 1 – An *in vivo* study. J Cons Dent 2013;16:36-40.
4. Elakanti S, Cherukuri G, Rao VG, Chandrasekhar V, Rao AS, Tummala M. Comparative evaluation of antimicrobial efficacy of QMix™ 2 in 1, sodium hypochlorite, and chlorhexidine against *Enterococcus faecalis* and *Candida albicans*. J Conserv Dent 2015;18:128-31.
5. Chum JD, Lim DJ, Sheriff SO, Pulikkotil SJ, Suresh A, Davamani F. *In vitro* evaluation of octenidine as an antimicrobial agent against *Staphylococcus epidermidis* in disinfecting the root canal system. Restor Dent Endod 2019;44:e8.
6. Zan R, Alacam T, Hubbezoglu I, Tunc T, Sumer Z, Alici O. Antibacterial efficacy of super-oxidized water on *Enterococcus faecalis* biofilms in root canal. Jundishapur J Microbiol 2016;9:e30000.
7. Chandra A, Yadav RK, Shakya VK, Luqman S, Yadav S. Antimicrobial efficacy of silver nanoparticles with and without different antimicrobial agents against *Enterococcus faecalis*: *Ex vivo* study. J Dent Oral Biol 2017;2:1047.
8. Goztas Z, Onat H, Tosun G, Sener Y, Hadimli HH. Antimicrobial effect of ozonated water, sodium hypochlorite and chlorhexidine gluconate in primary molar root canals. Eur J Dent 2014;8:469-74.
9. Haapasalo M, Endal U, Zandi H, Coil MJ. Eradication of endodontic infection by instrumentation and irrigation solutions. Endod Top 2005;10:77-102.
10. Sundqvist G, Figdor D. Life as an endodontic pathogen. Endod Top 2003;6:3-28.
11. 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;32:93-8.
12. Kashikar RR, Hindlekar A, Jadhav GR, Mittal P, Mukherjee P. Comparative evaluation of four different root canal irrigation techniques for apical extrusion of sodium hypochlorite – An *in vitro* study. J Conserv Dent Endod 2023;26:424-8.
13. Pasricha SK, Makkar S, Gupta P. Pressure alteration techniques in endodontics – A review of literature. J Clin Diagn Res 2015;9:E01-6.
14. Brunson M, Heilborn C, Johnson DJ, Cohenca N. Effect of apical preparation size and preparation taper on irrigant volume delivered by using negative pressure irrigation system. J Endod 2010;36:721-4.
15. Tandjung L, Waltimo T, Hauser I, Heide P, Decker EM, Weiger R. Octenidine in root canal and dentine disinfection *ex vivo*. Int Endod J 2007;40:845-51.
16. Tirali RE, Turan Y, Akal N, Karahan ZC. *In vitro* antimicrobial activity of several concentrations of NaOCl and octenisept in elimination of endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:e117-20.
17. Estrela C, Estrela CR, Decurcio DA, Hollanda AC, Silva JA. Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals. Int Endod J 2007;40:85-93.
18. Mehta N, Gupta A, Mahesh S, Abraham D, Singh A, Jala S, *et al.* Comparative evaluation of antibacterial efficacy of *Allium sativum* extract, aqueous ozone, diode laser, and 3% sodium hypochlorite in root canal disinfection: An *in vivo* study. J Conserv Dent 2020;23:577-82.
19. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. J Endod 2012;38:1376-9.
20. Mg Ruqshan Anjum, Sujatha I, Sharath Chandra SM. Antimicrobial efficacy of various irrigating solutions on *E. faecalis* in root canals: An *in-vitro* study. Int J Appl Dent Sci 2015;1:94-7.
21. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. J Endod 2014;40:285-90.
22. Jhamb S, Singla R, Kaur A, Sharma J, Bhushan J. *In vitro* comparison to study the antimicrobial effect of silver nanoparticles gel and its various combinants as an intracanal medicament against *Enterococcus faecalis*. J Conserv Dent Endod 2024;27:42-5.