

Original Research

Received 05 June 2026

Revised 22 July 2026

Accepted 05 August 2026

Natural Resources for Human Health 2026, Vol. 6 (14s): 451–462

KEYWORDS:

Enterococcus Faecalis, Antibacterial activity, Calcium hydroxide, Chlorhexidine, Confocal laser scanning microscopy, Intracanal medicament, Sea Buckthorn

Assessment of the Antibacterial Activity of a Novel Sea Buckthorn Medicament Against *Enterococcus faecalis* in Infected Root Canals

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ABSTRACT: Background: Effective elimination of persistent microorganisms is essential for successful endodontic treatment. *Enterococcus faecalis* is frequently associated with persistent root canal infections, creating a need for effective alternative intracanal medicaments. This study evaluated the antibacterial efficacy of a novel Sea Buckthorn medicament against *E. faecalis* and compared its activity with commonly used intracanal medicaments.

Materials and Methods: A total of 60 specimens were allocated equally into five groups (n = 12). Group I received the Novel Sea Buckthorn medicament, Group II received 2% chlorhexidine gel, Group III received calcium hydroxide, Group IV served as the infected untreated positive control, and Group V served as the uninfected negative control. Bacterial reduction was assessed by quantifying *E. faecalis* counts expressed as log₁₀ CFU/mL. Bacterial viability was additionally evaluated using confocal laser scanning microscopy. Intergroup differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey Honestly Significant Difference (HSD) post-hoc testing, while intragroup changes were assessed by comparing pre- and post-treatment bacterial counts.

Results:

Post-treatment *E. faecalis* counts were 2.08 ± 0.19 , 1.87 ± 0.14 , and 3.02 ± 0.25 log₁₀ CFU/mL in the Sea Buckthorn, chlorhexidine, and calcium hydroxide groups, respectively, compared with 4.53 ± 0.15 log₁₀ CFU/mL in the infected positive control. A significant difference was observed among the groups ($F = 897.483$, $p < 0.001$). The Sea Buckthorn and chlorhexidine groups did not differ significantly ($p = 0.226$). Both demonstrated significant reductions from baseline ($p = 0.001$). Confocal laser microscopy also showed significant differences in bacterial viability among the groups ($F = 688.872$, $p < 0.001$), with no significant difference between Sea Buckthorn and chlorhexidine ($p = 0.056$).

Conclusion: The Novel Sea Buckthorn medicament demonstrated substantial

antibacterial activity against *E. faecalis*, with efficacy statistically comparable to 2% chlorhexidine gel and greater bacterial reduction than calcium hydroxide. Further investigation is warranted to establish its clinical applicability as an intracanal medicament.

INTRODUCTION

Microbial infection of the root canal system is a principal etiological factor in pulpal and periapical disease, and its effective control is fundamental to the success of endodontic treatment [1]. Although chemomechanical preparation substantially reduces the microbial population, complete eradication of microorganisms may not always be achieved because of the complex anatomy of the root canal system. Microorganisms may remain within dentinal tubules, fins, isthmuses, accessory canals, and other areas that are difficult to reach with instruments and irrigating solutions [2]. The persistence of these microorganisms may contribute to continued periapical inflammation and treatment failure, emphasizing the need for effective antimicrobial strategies during root canal therapy.

Enterococcus faecalis is a Gram-positive, facultative anaerobic microorganism frequently investigated in relation to persistent endodontic infections. Its ability to tolerate adverse environmental conditions, survive periods of nutritional deprivation, penetrate dentinal tubules, and establish biofilms enables it to persist in treated root canals [3]. Furthermore, the organism demonstrates resistance to several commonly used antimicrobial procedures and can remain viable despite changes in pH and limited nutrient availability [4]. These characteristics have made *E. faecalis* an important experimental organism for evaluating the antimicrobial efficacy of intracanal medicaments.

Intracanal medicaments are used to reduce residual microbial populations following instrumentation and to limit microbial proliferation between treatment appointments. Calcium hydroxide remains one of the most widely investigated medicaments because its antimicrobial action is primarily associated with the release of hydroxyl ions and the resulting highly alkaline environment [5]. Nevertheless, its antimicrobial effectiveness may differ among microorganisms and may be influenced by factors such as dentinal buffering, limited penetration into infected dentin, and the susceptibility of particular microbial species [6]. These considerations have encouraged the investigation of alternative or adjunctive agents with antimicrobial potential.

Increasing interest in plant-derived products has led to the exploration of naturally occurring compounds as possible sources of antimicrobial agents for endodontic applications. Various medicinal plants contain biologically active constituents such as phenolic compounds, flavonoids, terpenoids, carotenoids, and other phytochemicals that may demonstrate antibacterial, antioxidant, and anti-inflammatory properties [7]. Such compounds have generated interest because they may provide alternative approaches for microbial control while potentially offering additional biological effects relevant to oral healthcare.

Sea buckthorn (*Hippophae rhamnoides*) is a plant containing a diverse range of biologically active constituents. Its fruits, leaves, seeds, and oils contain flavonoids, phenolic compounds, carotenoids, fatty acids, vitamins, and other phytochemicals that have been investigated for their biological activities [8]. Previous research has demonstrated antimicrobial potential associated with different sea buckthorn-derived preparations, although their activity may vary according to the plant part, extraction procedure, chemical composition, and target microorganism [9]. Despite this reported biological potential, evidence regarding the application of a specifically formulated sea buckthorn preparation as an intracanal medicament against *E. faecalis* remains limited.

Evaluation of a novel sea buckthorn medicament against *E. faecalis* may therefore provide preliminary evidence regarding its potential for microbial control within infected root canals. Such investigation is particularly relevant to the development of plant-derived intracanal formulations and to the search for alternative antimicrobial approaches in endodontic treatment. Therefore, the present study was undertaken to assess the antibacterial activity of a novel sea buckthorn medicament against *E. faecalis* associated with infected root canals.

MATERIALS & METHODS

Study Design

An in vitro experimental study was conducted at a tertiary care centre from February to April 2026 to evaluate the antibacterial effectiveness of a novel Sea Buckthorn medicament against *E. faecalis* and to compare its activity with 2% chlorhexidine gel and calcium hydroxide. Bacterial reduction was assessed using quantitative microbiological analysis, while bacterial viability was further evaluated using confocal laser scanning microscopy.

Sample Size and Group Allocation

A total of 60 specimens were included and equally distributed among five groups, with 12 specimens in each group:

- **Group I:** Novel Sea Buckthorn medicament
- **Group II:** 2% Chlorhexidine gel
- **Group III:** Calcium hydroxide
- **Group IV:** Saline/infected untreated positive control
- **Group V:** Uninfected negative control

The specimens assigned to the experimental groups received the respective intracanal medicaments, whereas the control groups were maintained under their designated conditions for comparison.

Inclusion Criteria

Specimens were eligible for inclusion when they:

1. Were intact and free from visible structural defects
2. Had comparable anatomical characteristics suitable for standardization
3. Were free from cracks, fractures, caries, or other defects that could interfere with specimen preparation or bacterial contamination
4. Could be adequately prepared and sterilized before experimental procedures
5. Demonstrated successful inoculation with *E. faecalis* for inclusion in the infected experimental and positive-control groups

Exclusion Criteria

Specimens were excluded when they:

1. Exhibited cracks, fractures, or structural abnormalities
2. Had extensive defects that could compromise specimen standardization
3. Were damaged during preparation or instrumentation
4. Demonstrated contamination with microorganisms other than the intended experimental organism before treatment
5. Failed to demonstrate adequate *E. faecalis* colonization before treatment
6. Were accidentally contaminated or improperly handled during the experimental procedure

Preparation and Sterilization of Specimens

The specimens were prepared using standardized procedures to minimize variations in size and experimental conditions. Following preparation, the specimens were sterilized using an appropriate validated sterilization protocol. Sterility was confirmed before bacterial inoculation to ensure that subsequent microbial growth originated from the experimental *E. faecalis* inoculum.

Bacterial Inoculation

A standardized strain of *E. faecalis* was used to establish bacterial contamination. The bacterial suspension was prepared under aseptic conditions and adjusted to a standardized concentration before inoculation. The prepared specimens were inoculated with the bacterial suspension and incubated under suitable conditions to facilitate bacterial colonization. Following the incubation period, baseline bacterial samples were collected to determine the pre-treatment *E. faecalis* count.

Application of Intracanal Medicaments

After confirmation of bacterial contamination, the specimens were randomly allocated to the respective treatment groups. Group I received the Novel Sea Buckthorn medicament, Group II received 2% chlorhexidine gel, and Group III received calcium hydroxide. Group IV was maintained as the infected untreated positive control and received saline, whereas Group V was maintained as the uninfected negative control. The medicaments were applied using standardized procedures and maintained for the predetermined experimental period. Care was taken to ensure consistent handling and application conditions across all experimental specimens.

Microbiological Assessment

Following treatment, post-treatment microbial samples were collected from each specimen using a standardized sampling protocol. The samples were processed for quantitative microbiological analysis, and bacterial counts were expressed as log₁₀ CFU/mL. The pre-treatment and post-treatment values were compared to determine the magnitude of bacterial reduction associated with each treatment.

Confocal Laser Scanning Microscopy

Bacterial viability was additionally assessed using confocal laser scanning microscopy. The specimens were processed using an appropriate live/dead bacterial staining procedure, allowing viable and non-viable bacterial cells to be differentiated according to their fluorescence characteristics. Representative areas were examined under confocal laser scanning microscopy, and the percentage of viable bacterial cells was quantified for each specimen. The resulting values were expressed as the percentage of live bacterial cells.

Outcome Measures

The primary outcome was the change in *E. faecalis* bacterial count, expressed as log₁₀ CFU/mL, between the pre-treatment and post-treatment assessments. The secondary outcome was the percentage of viable bacterial cells determined by confocal laser scanning microscopy. Comparisons were performed both within individual groups and between the different experimental and control groups.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), IBM SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD), whereas categorical variables were expressed as frequencies and percentages. One-way analysis of variance (ANOVA) followed by Tukey Honestly Significant Difference (HSD) post-hoc test was used for intergroup comparisons, while a paired-samples t-test was used for pre- and post-treatment intragroup comparisons. A *p* value <0.05 was considered statistically significant, with *p* <0.001 considered highly significant.

RESULTS

Table 1 & Figure 1 summarize the allocation of the 60 specimens across five study groups, with 12 specimens assigned to each group. Group I received the novel Sea Buckthorn medicament, Group II was treated with 2% chlorhexidine gel, and Group III received calcium hydroxide. Group IV consisted of infected but untreated specimens and served as the positive control, while Group V comprised uninfected specimens and served as the negative control.

Table 1: Distribution of specimens according to study groups

Group	Intervention	n
Group I	Novel Sea Buckthorn medicament	12
Group II	2% Chlorhexidine gel	12
Group III	Calcium hydroxide	12
Group IV	Saline / infected positive control	12
Group V	Uninfected negative control	12

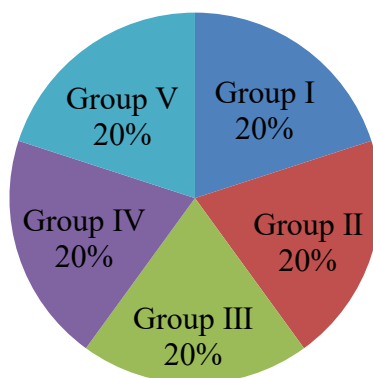


Figure 1: Distribution of specimens among the experimental and control groups

Table 2 presents the descriptive statistics of post-treatment *E. faecalis* counts across the five study groups. The mean log₁₀ CFU/mL values, SD, and observed minimum and maximum values are reported for the Novel Sea Buckthorn medicament, 2% chlorhexidine gel, calcium hydroxide, infected positive control, and uninfected negative control groups.

Table 3 presents the one-way ANOVA comparing post-treatment *E. faecalis* counts among the five groups. The analysis demonstrated a highly significant difference in bacterial counts between the groups ($F = 897.483$, $p < 0.001$), indicating that treatment group allocation was associated with significant variation in the post-treatment bacterial load.

Table 4 presents the Tukey HSD post-hoc pairwise comparisons of post-treatment *E. faecalis* counts between the study groups. No statistically significant difference was observed between the Novel Sea Buckthorn medicament and 2% chlorhexidine gel groups (mean difference = 0.21, adjusted $p = 0.226$). However, statistically significant differences were observed in the remaining pairwise comparisons ($p < 0.001$), including comparisons involving calcium hydroxide and the positive and negative control groups.

Collectively, Tables 2–4 demonstrate significant differences in post-treatment *E. faecalis* counts among the study groups, with the bacterial counts following the Novel Sea Buckthorn treatment being statistically comparable to those observed after 2% chlorhexidine treatment.

Table 2: Descriptive statistics of post-treatment *E. faecalis* counts

Group	Study group	n	Mean log ₁₀ CFU/mL	SD	Minimum	Maximum
Group I	Novel Sea Buckthorn medicament	12	2.08	0.19	1.64	2.32
Group II	2% Chlorhexidine gel	12	1.87	0.14	1.70	2.12

Group III	Calcium hydroxide	12	3.02	0.25	2.74	3.46
Group IV	Saline / infected positive control	12	4.53	0.15	4.34	4.71
Group V	Uninfected negative control	12	0.53	0.08	0.38	0.71

Table 3: One-way ANOVA comparison of post-treatment *E. faecalis* counts

Source of variation	Sum of Squares	df	Mean Square	F	p-value	Significance
Between groups	105.616	4	26.404	897.483	<0.001	Significant
Within groups	1.618	55	0.029			
Total	107.234	59				

Table 4: Tukey HSD post-hoc pairwise comparison of post-treatment *E. faecalis* counts

Comparison	Mean Difference	Tukey adjusted p-value	Significance
Group I vs Group II	0.21	0.226	Non-significant
Group I vs Group III	-0.94	<0.001	Significant
Group I vs Group IV	-2.45	<0.001	Significant
Group I vs Group V	1.55	<0.001	Significant
Group II vs Group III	-1.15	<0.001	Significant
Group II vs Group IV	-2.66	<0.001	Significant
Group II vs Group V	1.34	<0.001	Significant
Group III vs Group IV	-1.51	<0.001	Significant
Group III vs Group V	2.49	<0.001	Significant
Group IV vs Group V	4.00	<0.001	Significant

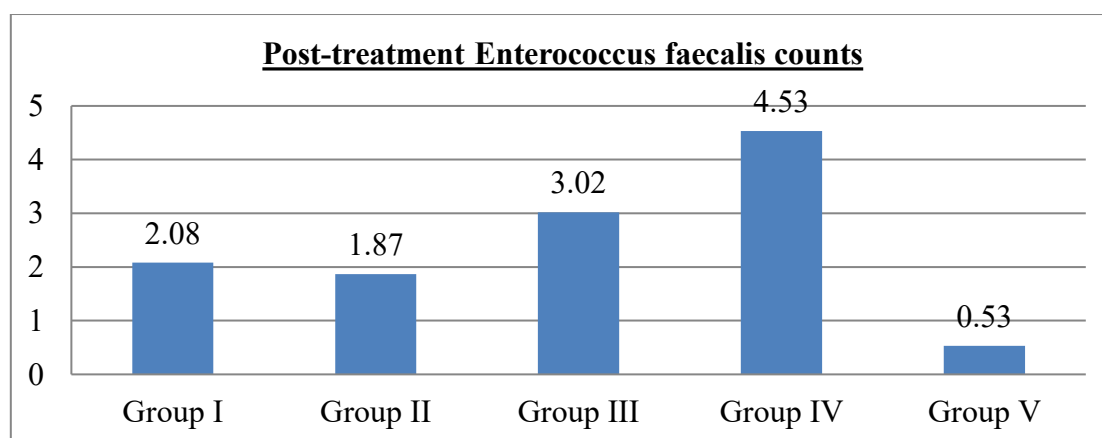


Figure 2: Comparative post-treatment *E. faecalis* counts among the experimental and control groups

Table 5 presents the descriptive analysis of bacterial viability assessed by confocal laser scanning microscopy across the five study groups. The table summarizes the number of specimens, mean percentage of live bacterial cells, SD, and minimum and maximum observed values for each group. The Novel Sea Buckthorn medicament group showed a mean live-cell percentage of $76.08 \pm 4.83\%$, compared with $81.32 \pm 3.63\%$ for 2% chlorhexidine, $58.23 \pm 7.14\%$ for calcium hydroxide, $87.71 \pm 4.28\%$ for the infected positive control, and $1.48 \pm 0.99\%$ for the uninfected negative control.

Table 6 presents the one-way ANOVA results for the comparison of live bacterial cell percentages among the five study groups. The analysis revealed a highly significant difference in bacterial viability between the groups ($F = 688.872$, $p < 0.001$), indicating that the percentage of viable bacterial cells varied significantly according to group allocation.

Table 7 presents the Tukey HSD post-hoc pairwise comparisons of live bacterial cell percentages. The comparison between the Novel Sea Buckthorn medicament and 2% chlorhexidine groups was not statistically significant (mean difference = -5.24 , adjusted $p = 0.056$). Significant differences were observed in all other pairwise comparisons, with $p < 0.001$ for most comparisons and $p = 0.011$ for the Group II versus Group IV comparison.

Overall, Tables 5–7 demonstrate significant differences in bacterial viability across the study groups. The percentage of live bacterial cells observed following treatment with the Novel Sea Buckthorn medicament was statistically comparable to that observed with 2% chlorhexidine gel, whereas significant differences were identified when compared with calcium hydroxide and the control groups.

Table 5: Confocal laser scanning microscopy assessment of bacterial viability

Group	Study group	n	Mean live cells (%)	SD	Minimum	Maximum
Group I	Novel Sea Buckthorn medicament	12	76.08	4.83	67.32	83.23
Group II	2% Chlorhexidine gel	12	81.32	3.63	72.59	85.65
Group III	Calcium hydroxide	12	58.23	7.14	41.82	68.02
Group IV	Saline / infected positive control	12	87.71	4.28	81.34	95.16
Group V	Uninfected negative control	12	1.48	0.99	0.00	3.52

Table 6: One-way ANOVA comparison of live bacterial cells (%) among the groups

Source of variation	Sum of Squares	df	Mean Square	F	p-value	Significance
Between groups	58,848.357	4	14,712.089	688.872	<0.001	Significant
Within groups	1,174.623	55	21.357	—	—	—
Total	60,022.980	59				

Table 7: Tukey HSD post-hoc comparison of live bacterial cells (%)

Comparison	Mean difference	Tukey adjusted p-value	Significance
Group I vs Group II	-5.24	0.056	Non-Significant
Group I vs Group III	17.85	<0.001	Significant
Group I vs Group IV	-11.63	<0.001	Significant
Group I vs Group V	74.60	<0.001	Significant
Group II vs Group III	23.09	<0.001	Significant

Group II vs Group IV	-6.39	0.011	Significant
Group II vs Group V	79.84	<0.001	Significant
Group III vs Group IV	-29.48	<0.001	Significant
Group III vs Group V	56.75	<0.001	Significant
Group IV vs Group V	86.23	<0.001	Significant

Confocal laser scanning microscopy assessment of bacterial viability

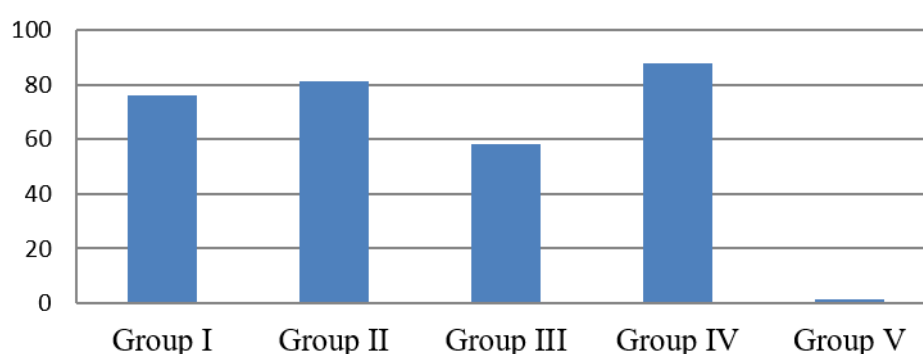


Figure 3: Comparative analysis of live bacterial cell percentages among the experimental and control groups assessed by confocal laser scanning microscopy

Table 8 presents the intragroup comparison of *E. faecalis* counts before and after treatment in Groups I–IV. The table reports the pre-treatment and post-treatment mean bacterial counts, SD, mean reduction in log₁₀ CFU/mL, and corresponding p-values.

Significant reductions were observed in Groups I, II, and III, representing the Novel Sea Buckthorn medicament, 2% chlorhexidine gel, and calcium hydroxide groups, respectively. In contrast, Group IV, which served as the infected positive control, showed only a minimal change in bacterial count that was not statistically significant.

Table 9 presents the intragroup analysis of treatment-associated changes in *E. faecalis* counts for Groups I–III. The findings show a mean reduction of 2.50 log₁₀ CFU/mL in the Novel Sea Buckthorn group, 2.69 log₁₀ CFU/mL in the 2% chlorhexidine group, and 1.53 log₁₀ CFU/mL in the calcium hydroxide group. All three reductions were statistically significant (p = 0.001).

Together, Tables 8 and 9 demonstrate that the Novel Sea Buckthorn medicament, 2% chlorhexidine gel, and calcium hydroxide produced significant reductions in *E. faecalis* counts from baseline to post-treatment, whereas the infected untreated control showed no meaningful change.

Table 8: Intragroup Comparison of Pre vs Post treatment *E. faecalis* counts

Group	Study group	Pre-treatment Mean $\pm$ SD	Post-treatment Mean $\pm$ SD	Mean reduction	P value
Group I	Novel Sea Buckthorn medicament	4.58 $\pm$ 0.16	2.08 $\pm$ 0.19	2.50	0.001
Group II	2% Chlorhexidine gel	4.56 $\pm$ 0.17	1.87 $\pm$ 0.14	2.69	0.001

Group III	Calcium hydroxide	4.55 ± 0.18	3.02 ± 0.25	1.53	0.001
Group IV	Saline / infected positive control	4.54 ± 0.16	4.53 ± 0.15	0.01	1.000

Table 9: Intragroup Comparison of Pre vs Post treatment *E. faecalis* counts

Group	Study group	Pre-treatment Mean ± SD	Post-treatment Mean ± SD	Mean reduction	P value
Group I	Novel Sea Buckthorn medicament	4.58 ± 0.16	2.08 ± 0.19	2.50	0.001
Group II	2% Chlorhexidine gel	4.56 ± 0.17	1.87 ± 0.14	2.69	0.001
Group III	Calcium Hydroxide	4.55 ± 0.18	3.02 ± 0.25	1.53	0.001

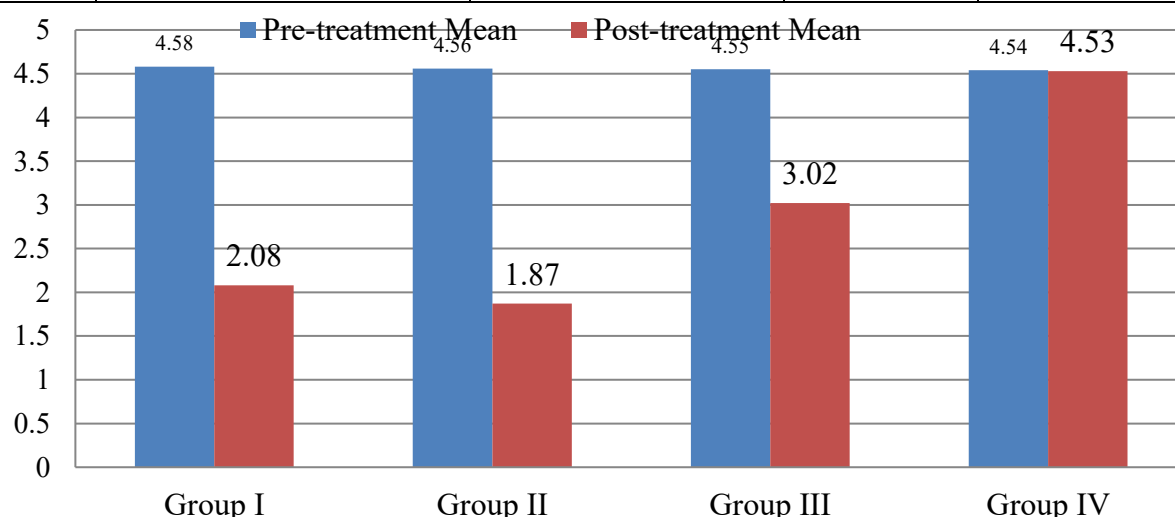


Figure 4: Intragroup comparison of pre- and post-treatment *E. faecalis* counts among the experimental and infected control groups

DISCUSSION

The present study evaluated the antibacterial effectiveness of intracanal medicaments against *E. faecalis*, a microorganism frequently associated with persistent endodontic infection. The findings are clinically relevant because microorganisms that survive chemomechanical preparation may remain within the root canal system and contribute to persistent intraradicular infection and apical periodontitis. Nair (2006) [10] emphasized that persistent apical periodontitis is commonly associated with microorganisms that survive within the root canal system despite conventional endodontic treatment. The complex anatomy of the canal system and the ability of microorganisms to persist in protected areas may therefore limit complete microbial elimination and highlight the importance of effective intracanal disinfection.

The antimicrobial behavior of *E. faecalis* has received considerable attention because of its ability to survive under unfavorable environmental conditions and persist following endodontic treatment. Nair (2006) [1] described the importance of residual intraradicular infection in the development of persistent apical disease. This provides a biological rationale for investigating intracanal medicaments that can reduce or eliminate microorganisms remaining after instrumentation. The use of *E. faecalis* as

an experimental microorganism is also relevant because its persistence in treated root canals has made it a commonly investigated organism in studies evaluating intracanal antimicrobial strategies. The effectiveness of different intracanal medicaments has been demonstrated in both laboratory and clinical investigations. de Lucena et al. (2013) [11] compared the antimicrobial effectiveness of chlorhexidine and octenidine against *E. faecalis* and demonstrated that intracanal antimicrobial agents can differ considerably in their ability to reduce viable microorganisms. Their findings support the concept that the selection of an intracanal medicament can influence the effectiveness of microbial control and that antimicrobial activity should be evaluated specifically against organisms associated with persistent infection.

Clinical evidence has further demonstrated the potential value of alternative intracanal medicaments. Savitha et al. (2019) [3] evaluated chitosan, chlorhexidine gluconate gel, and their combination in failed endodontic cases using real-time polymerase chain reaction (PCR). Their study demonstrated measurable antimicrobial effects against *E. faecalis*, highlighting the potential of both conventional and alternative formulations for managing microorganisms in previously treated canals. The use of molecular detection methods in that investigation also illustrates the importance of sensitive techniques for assessing microbial persistence, particularly when viable microorganisms may remain at low levels following treatment.

Calcium hydroxide remains an important reference medicament in endodontic antimicrobial research because of its established use as an intracanal dressing. Dianat et al. (2015) [4] investigated the antimicrobial activity of nanoparticle calcium hydroxide against *E. faecalis* and demonstrated that calcium hydroxide-based formulations possess antibacterial activity against this organism. However, the antimicrobial performance of calcium hydroxide may depend on its formulation, concentration, contact time, and ability to reach microorganisms located within the complex root canal environment. Consequently, modification of conventional formulations and investigation of alternative antimicrobial agents remain relevant areas of endodontic research.

The antimicrobial activity of calcium hydroxide has also been compared with other commonly used agents. Vaghela et al. (2011) [5] evaluated different calcium hydroxide formulations and 2% chlorhexidine for disinfection of dentinal tubules against *E. faecalis* and *Candida albicans*. Their findings demonstrated the importance of both the antimicrobial agent and its formulation in achieving effective dentinal-tubule disinfection. Because microorganisms may penetrate into dentinal tubules, an agent demonstrating activity only within the main canal lumen may not necessarily provide equivalent antimicrobial effects throughout the infected dentin. These findings reinforce the need to evaluate intracanal medicaments using models that reflect the anatomical challenges encountered during clinical treatment.

More recent evidence has continued to demonstrate variability among intracanal medicaments. Shamma et al. (2023) [6] compared the antibacterial effects of different intracanal medicaments against *E. Faecalis* in primary teeth and reported differences in antimicrobial performance among the tested preparations. Their findings indicate that antimicrobial effectiveness cannot be assumed to be equivalent across different medicaments and that individual formulations require direct experimental evaluation. This is particularly important when investigating novel agents intended for use as intracanal medicaments.

Taken together, the available evidence indicates that successful intracanal disinfection depends on the antimicrobial characteristics of the medicament as well as its ability to act within the anatomical environment of the root canal. The present findings should therefore be interpreted within the context of this established literature. If the tested medicament demonstrated substantial antibacterial activity against *E. faecalis*, this would support its potential as an alternative or adjunctive intracanal antimicrobial agent. However, *in vitro* antibacterial activity should not be interpreted as direct evidence of clinical efficacy. Factors such as dentinal buffering, penetration into dentinal tubules, organic tissue interactions, contact duration, concentration, cytotoxicity, and the complex microbial composition of natural root canal infections may influence clinical performance. Further investigations using dentin-infection models, multispecies biofilms, cytotoxicity assessments, and appropriately designed clinical studies would therefore be necessary before routine clinical application can be recommended.

Overall, the findings contribute to the growing body of evidence evaluating antimicrobial strategies for persistent endodontic infection. The demonstrated activity of established intracanal medicaments against *E. faecalis* provides an appropriate benchmark for assessing newer formulations. Direct comparison with commonly used agents such as calcium hydroxide and chlorhexidine may help determine whether a novel medicament provides sufficient antimicrobial activity to justify further

investigation. Such comparative evaluation is particularly important when developing plant-derived or other alternative intracanal formulations intended to improve microbial control during endodontic treatment.

LIMITATIONS

The present study has several limitations that should be considered when interpreting the findings. First, the investigation was conducted using an *in vitro* experimental model, which may not fully reproduce the complex biological, anatomical, and environmental conditions present within the root canal system in clinical situations. Factors such as dentinal tubule penetration, tissue interactions, host immune responses, and variations in the intracanal environment were not evaluated. Second, the sample size was relatively limited, with 12 specimens allocated to each experimental group. Although statistically significant differences were identified, studies involving larger sample sizes may provide more precise estimates of the antibacterial effect and improve the generalizability of the findings. Third, the study evaluated the antibacterial activity primarily against *E. faecalis*. The root canal microbiota is polymicrobial and includes a wide range of aerobic, facultative anaerobic, and obligate anaerobic microorganisms. Therefore, the observed antibacterial activity against *E. faecalis* may not represent the effectiveness of the medicaments against the broader microbial community associated with endodontic infections. Fourth, the assessment was based on post-treatment bacterial counts and confocal laser scanning microscopy findings at the selected evaluation point. The study therefore does not establish the long-term persistence of the antibacterial effect or determine whether surviving microorganisms can subsequently recolonize the treated environment.

Fifth, although confocal laser microscopy provided information regarding bacterial viability, viability assessment does not necessarily correspond directly to bacterial cultivability or complete microbial eradication. Additional microbiological techniques, such as molecular identification or quantitative polymerase chain reaction, could provide complementary information regarding viable and residual bacterial populations. Finally, the study compared the Novel Sea Buckthorn medicament with established intracanal medicaments under standardized laboratory conditions. Further *ex vivo* and well-designed clinical investigations involving larger and more diverse samples, polymicrobial biofilms, different treatment durations, and longer follow-up periods are required to confirm the antibacterial efficacy, clinical applicability, safety, and reproducibility of the Novel Sea Buckthorn medicament.

CONCLUSION

The Novel Sea Buckthorn medicament demonstrated significant antibacterial activity *against E. faecalis*, producing a substantial reduction in bacterial counts that was statistically comparable to 2% chlorhexidine gel. Its antibacterial effect was greater than that observed with calcium hydroxide, while the infected untreated control showed no meaningful reduction. These findings indicate that Sea Buckthorn medicament may represent a promising alternative intracanal antibacterial agent, warranting further evaluation through *ex vivo* and clinical studies.

Conflicts of Interest: Nil

Financial Support: None

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