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Original Article

Assessing the antimicrobial properties of bioceramic sealers enhanced with herbal extracts against *E. faecalis*

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Abstract

Aim: The objective of the present study was to assess and compare the antibacterial activity of two bioceramic sealers, BioRoot RCS and CeraSeal RCS, against *Enterococcus faecalis* with and without the addition of two herbal extracts, *Psidium guajava* and *Azadirachta indica*.

Materials and methods: We formed two experimental groups, group 1 included CeraSeal RCS sealers and group 2 included BioRoot RCS sealers. These groups were further subdivided into subgroup 1A (controls) with CeraSeal RCS sealers, subgroup 1B with CeraSeal RCS sealers plus ethanolic extract of neem, subgroup 1C: CeraSeal RCS + ethanolic extract of guava, subgroup 2A (controls): BioRoot RCS root canal sealers (controls), subgroup 2B: BioRoot RCS root canal sealer plus ethanolic extract of neem, and subgroup 2C including BioRoot RCS root canal sealers plus ethanolic extract of guava. To prepare the subgroups containing ethanolic extract of neem and guava, 20 μL of extract were mixed with the powder and liquid and spatulated well to form a homogenous paste. Direct contact test was performed and the colonies were counted using a digital colony counter and the colony forming units (CFU) per microliter were calculated based on the dilutions used.

Results: The mean CFUs in the BioRoot RCS group without extract, with guava and with neem extracts showed significantly lesser values (1.305±0.038; 0.852±0.051; 1.097±0.072) as compared to CeraSeal RCS group without extract, with guava; neem extracts showed significantly lesser values (1.392±0.057, 1.073±0.064; 1.201±0.055). Multiple comparisons between groups revealed that the guava extract demonstrated significantly fewer CFUs as compared to the no-extract and the neem-extract groups, and the difference was statistically significant at $p<0.001$.

Conclusion: Adding herbal extracts increased the antimicrobial activity which can be inferred from the comparison of the mean number of colony forming units after direct contact test and the reduction in mean optical density of both the subgroups to their respective control groups. The ethanolic extract of guava displayed better antimicrobial efficacy than the ethanolic extract of neem.

Keywords

antibacterial efficacy, bioceramic sealer, BioRoot RCS, CeraSeal, direct contact test, neem extract, guava extract

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Introduction

Achieving a fluid-tight seal is paramount in ensuring the successful outcome of endodontic therapy.[1] To accomplish this, root canal sealers must be effectively combined with biologically acceptable obturating materials.[2] Various commercially available endodontic sealers exist, with calcium silicate-based cement emerging as a recent advancement owing to its superior biological properties and performance.[3]

Among these, BioRoot RCS is a tricalcium silicate-based bioceramic sealer with zirconium oxide and calcium chloride, offering tunable hydration kinetics. Its polycarboxylate content enhances chemical stability, enabling the study of interactions with herbal extracts without compromising integrity.[4] In contrast, CeraSeal is a ready-to-use injectable bioceramic sealer known for its excellent handling and consistent physical properties, eliminating variability from manual mixing.[5] Together, these sealers serve as a solid foundation for examining how herbal extracts impact the physical, mechanical, and chemical properties due to their differing formulations.

According to Grossman, an effective endodontic sealer must demonstrate bacteriostatic properties or, at a minimum, not support bacterial proliferation.[6] The sealer must effectively encapsulate any residual microorganisms[3] while adequately filling the inaccessible areas within the prepared canals. As a result, a sealer with robust antimicrobial properties is indispensable for eradicating residual bacteria and preventing reinfection.

Antimicrobial effectiveness of bioceramic root canal sealers has been extensively studied, yielding varying results. These sealers are known for their high pH and calcium ion release, which can create an environment that is hostile to certain bacteria.[7] However, their efficacy against specific pathogens, particularly *Enterococcus faecalis*, a common cause of endodontic failures, differs among studies.

Research suggests that the antimicrobial effectiveness of bioceramic sealers may diminish as the material sets and the release of hydroxyl ions slows over time.[8] For instance, a study by Manjula et al.[9] found that resin-based sealers performed better than bioceramic sealers in long-term antimicrobial tests. Additionally, several other studies have also demonstrated a decrease in antibacterial activity after bioceramic sealers have been set.[10,11]

Consequently, the incorporation of antimicrobial agents represents a compelling strategy for enhancing their performance. While antibiotics have been instrumental in combating infections[12], the indiscriminate use of these agents has precipitated the emergence of resistant bacterial strains.[13] This situation has led to a renewed interest in biologically active components derived from plants, which may provide effective alternatives with antibacterial and antifungal properties.[14]

Neem, which is highly regarded in India and surrounding regions, is a versatile medicinal plant with a variety of applications. Over 140 biologically active compounds have been identified[15] in neem, demonstrating potent antimicrobial activity against *Enterococcus faecalis*[16], *Candida albicans*, and other endodontic pathogens. Compared to turmeric, clove oil, and other herbal extracts, neem offers additional antifungal benefits and has better tissue compatibility.[17]

Similarly, guava leaves contain significant amounts of flavonoids, quercetin, and tannins, which are effective against various bacterial strains.[18,19] Guava exhibits superior biofilm disruption compared to aloe vera and shows a broader antimicrobial spectrum and better biofilm penetration than green tea and other extracts.[20]

Both neem and guava effectively combat a wide range of pathogens, including *Enterococcus faecalis* and biofilm-forming bacteria, which are central to endodontic infections. These extracts combine antimicrobial properties with biocompatibility. While guava excels in biofilm penetration, neem provides sustained antimicrobial activity. Together, these properties make guava and neem ideal candidates for research.

Notably, *Enterococcus faecalis*, one of the prevalent microorganisms found in infected root canals, is known for its resilience and ability to thrive in previously treated teeth. Research indicates a high prevalence of *Enterococci* and *Streptococci* in these infections[21], underscoring the critical need for effective antimicrobial interventions.

Aim

This study aimed to evaluate the antimicrobial efficacy of two bioceramic sealers, BioRoot RCS and CeraSeal, against *Enterococcus faecalis* with and without the addition of two herbal extracts, *Psidium guajava* and *Azadirachta indica*.

Materials and methods

Ethical statement

This ex vivo study was conducted in the Departments of Conservative Dentistry and Endodontics, and Microbiology from 2020 to 2023. The study received approval from the Institutional Ethical Committee : (KCDS/Ethical comm/024/2020-2021).

Preparation of ethanol extracts of neem and guava leaves

Fresh leaves of *Azadirachta indica* (neem) were cleaned, shade-dried, and powdered. The powder was macerated with 70% ethanol, homogenized, and filtered. The filtrate was left to evaporate ethanol in a petri dish, and the residue weight was recorded. The extract was then diluted to the desired concentration. This process was also conducted for guava leaves.

Preparation of test microorganisms

A few colonies of *E. faecalis* were cultivated on Brain Heart Infusion (BHI) agar plates and incubated at 37°C for 24 hours. Pure cultures of *E. faecalis* (ATCC strain 29212) were verified, and the inoculum was adjusted to achieve a turbidity equivalent to the 0.5 McFarland Standard (approximately 1.5×10^8 CFU/mL).

Grouping

Two main groups were formed:

- Group 1: CeraSeal
- – Subgroup 1A (Controls): CeraSeal
- – Subgroup 1B: CeraSeal + 20 μL of guava extract
- – Subgroup 1C: CeraSeal + 20 μL of neem extract
- Group 2: BioRoot RCS
- – Subgroup 2A (Controls): BioRoot RCS
- – Subgroup 2B: BioRoot RCS + 20 μL of guava extract
- – Subgroup 2C: BioRoot RCS + 20 μL of neem extract

Placement of sealers

A sterile spoon excavator was used to measure equal amounts of sealers, which were then applied to sterile filter paper disks (6 mm diameter). The disks were left to set according to the manufacturer's recommended setting time and then transferred to Eppendorf tubes using sterile forceps, ensuring that the sealer side faced upward.

Direct contact test

Ten microliters of the adjusted *E. faecalis* culture were added to the Eppendorf tubes containing the filter paper disks and incubated at 37°C in a moist chamber for one hour. Following the incubation, one milliliter of sterile BHI broth was added to the tubes and vortexed for 30 seconds. A 50-microliter dilution of this broth was then spread onto dry BHI agar plates and incubated at 37°C for 24 hours. Colonies were counted using a digital colony counter, and CFUs per microliter were calculated based on the dilutions used.

Statistical analysis

Statistical analyses were performed using SPSS for Windows v. 22.0. Descriptive analysis displayed CFU values as means and standard deviations for each group and extract. Mann-Whitney tests were used to compare mean CFUs between the BioRoot RCS and CeraSeal groups for different ethanolic extracts. The Kruskal-Wallis test, followed by Dunn's post hoc test, evaluated mean CFUs between different extracts within both groups. A significance level was set at $p<0.05$.

Results

The highest bacterial count (CFU) was observed in the control groups, subgroup 1A and subgroup 2A. Among the experimental groups, the guava extract (subgroups 1B and 2B) exhibited the most significant antibacterial effect, leading to the greatest CFU reduction, followed by the neem extract (subgroups 1C and 2C) **(Table 1)**.

In intergroup comparisons, the BioRoot RCS control and extract groups consistently demonstrated significantly lower mean CFUs than the corresponding CeraSeal groups, with statistical significance at $p<0.001$. On comparing subgroups 1B and 2B, BioRoot RCS combined with gauva extract exhibited superior antimicrobial activity compared to CeraSeal RCS. Similarly, in subgroups 1C and 2C, BioRoot RCS with neem extract outperformed CeraSeal RCS, highlighting the superior antimicrobial efficacy of BioRoot RCS.

Overall, the study results ranked antimicrobial efficacy as follows: group 2B showed the best results, followed by group 1B, group 2C, group 1C, group 2A, and finally, group 1A **(Table 2)**.

Discussion

Root canal sealers with antimicrobial properties are essential for effectively managing recurrent or chronic infections, significantly increasing the success rates of end-

Table 1. Comparison of means CFU ($\times 10^8$/ml) between different ethanolic extracts using Kruskal-Wallis test followed by Dunn's post hoc test

CeraSeal group	**N**	**Mean**	**SD**	**Min**	**Max**	**p- value**	**Sig Diff**	***p*-value**
No extract	15	1.392	0.057	1.28	1.47		E0 vs. E1	<0.001*
Guava extract	15	1.073	0.064	0.91	1.21	<0.001*	E0 vs. E2	<0.001*
Neem extract	15	1.201	0.055	1.09	1.28		E1 vs. E2	<0.001*
BioRoot RCS group								
No extract	15	1.305	0.038	1.24	1.36		E0 vs. E1	<0.001*
Guava extract	15	0.852	0.852	0.76	0.93	<0.001*	E0 vs. E2	<0.001*
Neem extract	15	1.097	1.097	0.92	1.18		E1 vs. E2	<0.001*

* Statistically significant $p<0.001$

Table 2. Comparison of means CFU (×10^8/ml) between BioRoot RCS and CeraSeal sealers based on different ethanolic extracts using Mann-Whitney test

Extracts	Groups	N	Mean	SD	Mean diff	*p*-value
No extract	BioRoot RCS	15	1.305	0.038	−0.087	<0.001*
	CeraSeal	15	1.392	0.057		
Guava extract	BioRoot RCS	15	0.852	0.051	−0.221	<0.001*
	CeraSeal	15	1.073	0.064		
Neem extract	BioRoot RCS	15	1.097	0.072	−0.103	<0.001*
	CeraSeal	15	1.201	0.055		

* Statistically significant $p < 0.001$

odontic therapies. Research comparing the antibacterial performance of bioceramic sealers to other sealers has produced inconsistent and often debated outcomes, likely due to differences in culture methods and testing protocols for antimicrobial efficacy.

This study aimed to uncover the potential for enhancing the antimicrobial efficacy of bioceramic sealers specifically BioRoot RCS and CeraSeal by integrating herbal extracts renowned for their antimicrobial effects. BioRoot RCS demonstrates excellent sealing in moist environments through mineralization and apatite deposition on canal walls.[22] In contrast, CeraSeal is a pre-mixed bioceramic sealer, known for its impressive physical properties and ease of use.[23]

Herbal extracts like neem leaf, guava, Triphala, propolis, *Morinda citrifolia*, aloe vera, garlic, ginger, green tea, turmeric, babool, tulsi, cinnamon, miswak, passion fruit juice, clove, and mango kernel show antimicrobial properties against common endodontic pathogens.[24]

Neem is known for containing over 140 biologically active compounds, including azadirachtin, which contributes to its strong antimicrobial properties. Other active ingredients include n-hexacosanol, amino acids, ascorbic acid, nimbandiol, nimbolide, and quercetin, among others.[24,25]

*Psidium guajav*a (guava) is a valuable phytotherapeutic plant known for its rich composition of essential oils, polysaccharides, minerals, vitamins, enzymes, and various compounds like triterpenoid acids and flavonoids.[26] Notably, its leaf extract has higher antioxidant potential than other parts, making it suitable for further investigation alongside neem.

Recent literature indicates that methanol and ethanol are commonly used for the antimicrobial testing of herbal extracts. In this study, we utilized ethanol extracts of neem (*Azadirachta indica*) and guava (*Psidium guajava*), which have yielded impressive results in previous evaluations. The choice of 70% ethanol as a solvent for extracting compounds from neem and guava is based on its balance of efficacy, safety, and compatibility. Ethanol at 70% (a mixture of ethanol and water) provides a medium polarity that is ideal for extracting a wide range of bioactive compounds, including alkaloids, flavonoids, tannins, and terpenoids. Many active compounds found in neem and guava are soluble within this polarity range. 70% ethanol is preferred over methanol for their extraction due to its superior safety profile, broad-spectrum extraction capability, compatibility with bioceramic sealers, and low residue risk.[27] These advantages make it a reliable and effective solvent for herbal extraction in endodontic applications. Additionally, ethanol possesses antimicrobial properties, which may work synergistically with the antimicrobial activity of neem and guava extracts, thereby enhancing the overall efficacy of the sealer.

Employing the direct contact test method based on Weiss et al. allowed for precise measurements of antibacterial activity, ensuring immediate interaction between the sealer and bacterial cultures regardless of the solubility and diffusibility of the antimicrobial component.[28] This approach utilized sensitive turbidometric measurements via a digital colony counter, providing a robust means to evaluate microbial growth.[29] Remarkably, the direct contact test serves as an effective and reliable qualitative method, and it remains unaffected by the size of the inoculum interacting with the test material.[28]

Factors that contribute to the failure of endodontic treatments include bacterial persistence, sealing failures, inadequately cleaned and filled root canals, and missed canals that were not treated.[30] *Enterococcus faecalis* was chosen as the test microorganism due to its formidable resistance to various intracanal medications and its regular association with enduring post-treatment infections.[31]

When comparing the control groups, the mean colony-forming units (CFUs) in the BioRoot RCS group were significantly lower than those in the CeraSeal RCS group. This finding aligns with a study conducted by Salah M. Abduljabbar[32], which highlighted BioRoot's superior antimicrobial action as a result of its higher pH and calcium release.

The enhancement of antimicrobial activity through herbal extracts was strikingly evident, with substantial reductions in CFUs observed in experimental groups compared to control groups. In this study, multiple comparisons indicated that the guava extract exhibited the greatest efficacy, followed by neem extract, with these differences being statistically significant.

Guava extracts exhibit antibacterial effects due to compounds like quercetin, mosin glycosides, and tannins.

These compounds disrupt the cytoplasmic membrane, inhibit macromolecular formation, and interfere with oxidative phosphorylation. Tannins also inhibit microbial growth by chelating iron and blocking extracellular enzymes.[21] The antioxidant capacity of guava leaves may be due to their ascorbic acid content, as supported by the findings of Biswas et al.[33]

Neem's antimicrobial properties come from active compounds like isoprenoids (e.g., terpenoids) and non-isoprenoids (e.g., tannins), which hinder microbial growth by degrading bacterial cell walls. Neem oil is rich in antioxidants and acts as a free radical scavenger, while also exhibiting anti-inflammatory effects by regulating pro-inflammatory enzymes like cyclooxygenase and lipoxygenase.[22] Studies indicate that neem extracts are significantly more effective against *Enterococcus faecalis* than traditional agents such as 2% sodium hypochlorite, turmeric, licorice, and propolis.

Incorporating neem (*Azadirachta indica*) and guava (*Psidium guajava*) herbal extracts into bioceramic sealers presents potential benefits but comes with certain risks and drawbacks. These risks mainly relate to the possible interference of these extracts with the physical, chemical, and biological properties of bioceramic sealers. Previous studies on the incorporation of herbal and chemical additives in sealers have revealed significant effects on their physical, mechanical, and biological properties.[34] Furthermore, they may influence the release of ions from the matrix, which can have implications for the overall biological properties of the sealing materials.[35] To mitigate these issues, it is advisable to use minimum effective concentrations of herbal extracts to avoid overwhelming the bioceramic matrix. Encapsulating these extracts could also be a beneficial approach.

Future studies should optimize and validate the use of neem and guava extracts in bioceramic sealers to maximize their antimicrobial efficacy, by employing standardized models that replicate clinical conditions while maintaining essential physical properties and biocompatibility. Additionally, conducting time-lapse studies to evaluate long-term antimicrobial efficacy, examining synergistic or antagonistic effects, and performing randomized controlled trials to assess outcomes will further address research gaps and enhance the clinical translation of these formulations.

Conclusion

Based on the limitations of this in vitro study, the following conclusions were drawn:

- The ethanolic extract of guava demonstrated superior antimicrobial efficacy compared to neem, as evidenced by the lower colony-forming unit (CFU) counts observed in subgroups 1B and 2B relative to subgroups 1C and 2C.
- BioRoot RCS sealer demonstrated better antimicrobial efficacy than CeraSeal across all subgroups, highlighting that the sealer's composition significantly affects its effectiveness.

These findings suggest that incorporating herbal extracts could enhance the antibacterial properties of endodontic materials against resilient microorganisms like *Enterococcus faecalis*. Further research is needed to optimize these applications clinically.

Author contributions

Concepts and design: Sachin KS and Shibani Shetty K; Definition of intellectual content literature search, clinical studies, experimental studies, data acquisition, data analysis, and statistical analysis: Sachin KS and Harishma S; Manuscript preparation, manuscript editing: Harishma S and Harshini S; Manuscript review: Shibani Shetty K and Harshini S.

Guarantors of the article: Sachin KS, Harishma S, and Jeyalakshmi KB.

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Competing interests

the authors have declared that no competing interests exist.

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