Original Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF NOVEL AND CONVENTIONAL OBTURATING MATERIALS: AN IN VITRO STUDY

Rasika Chandak[1], Lavanya Govindaraju[1]*

[1]*Department of Paediatric and Preventive Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (Saveetha University), Chennai, Tamil Nadu, India. glaavuu@gmail.com*

Received: 10 December 2025; **Revised:** 17 March 2026; **Accepted:** 19 March 2026 https://doi.org/10.51847/2gRROB0kVN

ABSTRACT

The clinical efficacy of pulpectomy in primary dentition relies on filling materials that exhibit robust antimicrobial activity, high biocompatibility, and a resorption profile synchronized with physiological root development. Conventional materials such as Zinc Oxide, Iodocal, and Metapex show limitations in antibacterial efficacy, especially against resistant pathogens like *Enterococcus faecalis* and *Lactobacillus acidophilus*. To resolve this, a new root canal obturating paste was formulated using a blend of calcium hydroxide, zinc oxide, and metronidazole, and this investigation sought to evaluate its antimicrobial performance against standard industry materials. An in vitro study evaluated four obturating materials: Novel Material (Calcium Hydroxide 60%, Zinc Oxide 40%, Metronidazole 2%), Premixed Zinc Oxide, Iodocal, and Metapex. The agar diffusion technique was employed to evaluate the antimicrobial efficacy against Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Enterococcus faecalis, and Candida albicans. Zones of inhibition (mm) were recorded and statistically analyzed. The novel material showed significantly higher antimicrobial efficacy against *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Enterococcus faecalis* ($P < 0.05$). Premixed Zinc Oxide demonstrated moderate effectiveness, followed by Metapex, while Iodocal showed the least activity. The addition of metronidazole enhanced the antibacterial spectrum, particularly against resistant pathogens. The novel calcium hydroxide–zinc oxide–metronidazole obturating material exhibited superior antimicrobial activity, suggesting its potential as an effective alternative in pediatric endodontics.

Key words: Antimicrobial agents, Endodontics, Filling materials, Primary teeth, Pulpectomy, Root canal.

Introduction

Endodontic therapy aims to achieve thorough decontamination and complete obturation of the root canals to prevent reinfection and ensure long-term success. The choice of an appropriate obturating material plays a pivotal role in maintaining an aseptic environment within the canal, particularly in the primary teeth, where complex root canal morphology and resorption patterns present significant challenges [1-3]. The primary objective of obturation in pediatric endodontics is to provide a hermetic seal while simultaneously exhibiting antimicrobial properties to eliminate the residual microbial load.

Microbial invasion, especially from the persistent pathogens such as *Enterococcus faecalis,* remains a leading cause for endodontic failure [4-6]. Facultative anaerobes exhibit significant persistence by colonizing dentinal tubules, establishing biofilms, and enduring hostile micro environments; consequently, traditional root canal Filling Materials, such as those formulated with zinc oxide-eugenol or calcium hydroxide, remain prevalent owing to their inherent alkalinity and antimicrobial efficacy. However, limitations such as reduced effectiveness against these microorganisms and compromised sealing ability necessitate the exploration of other materials with superior antimicrobial efficacy [7-9].

Products combining calcium hydroxide and iodoform, exemplified by Metapex and IodoCal, serve as promising substitutes owing to their superior bactericidal efficacy and the sustained release of iodine's broad-spectrum antimicrobial agents [7]. Conversely, the study conducted by researchers revealed that Metapex lacked antimicrobial efficacy against pathogens recovered from infected root canal systems [10, 11]. Furthermore, a review of existing literature indicated a deficiency in the antimicrobial efficacy of iodoform-calcium hydroxide mixtures [12-14]. Despite being the standard therapeutic approach in pediatric endodontics, pulpectomy currently lacks a universally recognized or optimal filling material. As a result, research into improved obturating materials for primary teeth is still ongoing.

The ideal root canal filling material for primary dentition must exhibit radiopacity, biocompatibility, and antimicrobial properties, while facilitating straightforward clinical application and retrieval. Furthermore, the material should provide a stable seal against moisture, undergo physiological resorption commensurate with root exfoliation, maintain a non-rigid consistency, and preserve the aesthetic integrity of the clinical crown [15-18]. To address the aforementioned specifications, a novel endodontic obturation material was formulated, incorporating zinc oxide cement, calcium hydroxide, and

metronidazole [19-26].

Pulpectomy continues to be a vital clinical approach for treating primary teeth with pulp involvement, facilitating their retention until physiological shedding. The clinical efficacy of this intervention is fundamentally dependent upon rigorous root canal debridement, sanitization, and subsequent sealing. Recent advances in pediatric rotary instrumentation, such as the use of Kedo-SG Blue files, have shown greater canal enlargement and improved shaping ability over manual K-files, as demonstrated by nano-CT analysis revealing an 8.85% increase in canal volume, which facilitates better obturation outcomes [27]. However, canal shaping alone is not sufficient; irrigation plays an equally critical role in eliminating microbial load. While sodium hypochlorite (NaOCl) remains the irrigant of choice for its potent antimicrobial properties, its concentration must balance efficacy with biocompatibility. A randomized clinical trial comparing 1% and 3% NaOCl showed both concentrations effectively reduced microbial counts in primary root canals without significant differences, suggesting that 1% NaOCl may offer an optimal safety-efficacy profile for pediatric pulpectomy [28-30]. Interestingly, a systematic review reported a higher incidence of obturation voids in teeth irrigated with 1% NaOCl, highlighting the need to evaluate irrigation protocols alongside obturation outcomes [31]. Given the significant incidence of caries, rigorous pulpectomy protocols—integrating precise instrumentation, thorough irrigation, and superior obturation—are essential to avert premature extraction and maintain dental arch stability during pediatric development [32-35].

Pulpectomy is a crucial procedure in managing infected primary molars, and recent studies have compared manual and rotary instrumentation to improve clinical outcomes. A one-year randomized clinical study demonstrated no significant difference in clinical or radiographic outcomes between manual and rotary instrumentation techniques [30, 36]. Research evaluating Kedo-S and reciprocating systems indicates that rotary instrumentation markedly decreases procedural time and improves the quality of canal obturation relative to conventional K-files [37]. Furthermore, a randomized double-blind clinical trial indicated that rotary instrumentation enhances procedural efficiency and reduces post-operative pain in pediatric dental patients [38]. These findings support the growing preference for rotary instrumentation in primary tooth pulpectomies, especially when used in conjunction with biocompatible obturating materials that ensure a hermetic seal and promote periapical healing.

The clinical success of pulpectomy procedures in primary dentition is primarily dictated by the antimicrobial efficacy of the filling materials employed, particularly given the polymicrobial nature of deciduous root canal infections. Due to a lack of robust research evaluating such agents, this study performs a comparative *in vitro* analysis, assessing a novel obturation formula against IodoCal, Metapex, and a premixed zinc oxide-based paste regarding their inhibitory impact on *E. faecalis*, *S. mutans*, *S. aureus*, *L. acidophilus*, and *C. albicans*.

Materials and Methods

Study design and setting

This in-vitro investigation was performed within the institutional microbiology laboratory to evaluate the comparative antimicrobial properties of a novel root canal filling material against three established sealers: Zinc Oxide, IodoCal, and Metapex. The experimental framework utilized both the agar diffusion method and time-kill curve assays to determine the inhibitory potential of these agents against targeted microbial strains.

Ethical approval

The Scientific Review Board of the Institute granted formal ethical clearance for this study [SRB/SDC/PEDO-2404/25/006], confirming that the research protocol adheres to all required ethical standards and institutional guidelines.

Preparation of the novel obturating material

To formulate the experimental mixture, a blend consisting of 60% calcium hydroxide (600 mg) and 40% zinc oxide (400 mg) was prepared. The combined powders were subjected to magnetic stirring for a duration of one hour to achieve a homogeneous state. Concurrently, a 400 mg metronidazole tablet was processed into a fine powder through mechanical pulverization using a mortar and pestle.

Following this, a 2% concentration of the Metronidazole-Calcium hydroxide-Zinc oxide compound was formulated by blending 4 mg of metronidazole powder with 196 mg of the calcium hydroxide-zinc oxide base (11,15); this mixture was then integrated with a vehicle to enable syringe-based delivery.

Preparation of the pre-mixed zinc oxide eugenol

Zinc oxide Eugenol was premixed in syringe form to maintain homogeneity with the other materials, as all the other materials were in syringe form. The premixed zinc oxide formulation consists of nano zinc oxide (30–40%) combined with 5% calcium carbonate, with a suitable carrier agent to facilitate delivery in syringe form, ensuring optimal consistency and application precision.

Preparation of test samples

This study conducted a comparative analysis of the antimicrobial efficacy of four specific root canal obturation agents: a novel experimental formulation (comprising calcium hydroxide, zinc oxide, and metronidazole), a premixed zinc oxide paste, and two commercially established calcium hydroxide-iodoform compounds, IodoCal and Metapex. To ensure consistency across the testing groups, each material was standardized to a concentration of 50 µg/mL by dissolving the agents in sterile

distilled water while maintaining strict aseptic protocols.

Microbial strains used

To evaluate the antimicrobial efficacy of the specified root canal filling materials, the following oral pathogens were selected: *Streptococcus mutans*, *Lactobacillus* sp., *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*. These microbial strains were sourced from a standardized repository. Prior to the assay, the bacterial isolates were propagated in Mueller-Hinton broth, while the fungal strain was cultivated in Sabouraud Dextrose broth. All experimental procedures were conducted in strict accordance with established biosafety protocols regarding the management and disposal of biological cultures.

Antimicrobial activity evaluation: agar well diffusion methodology

To evaluate the antimicrobial potential of the various obturation materials, the agar diffusion technique was employed. Initially, Mueller-Hinton agar was autoclaved at 121°C for a duration of 15–20 minutes and subsequently transferred into sterile Petri dishes to solidify in a controlled, aseptic environment. Microbial inocula were standardized to the 0.5 McFarland scale and uniformly distributed across the agar surfaces using sterile swabs. Using a sterile borer, wells measuring 6 mm in diameter were punched into the medium, each then loaded with 50 µg of the respective test substance. The cultures were incubated at 37°C for 24 hours for bacterial strains and 48 hours for fungal species. Upon completion of the incubation periods, the resulting inhibition zones were quantified in millimeters using a digital caliper **(Figure 1)**.

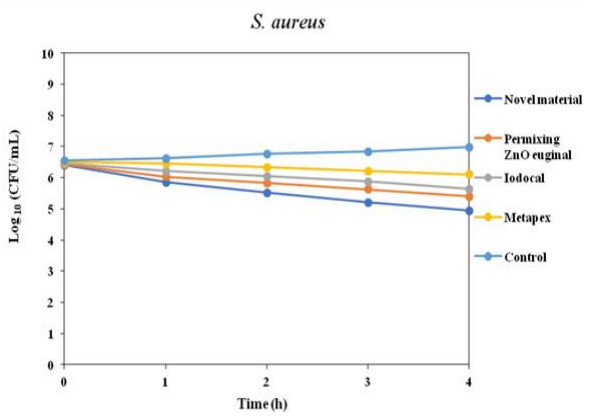

Figure 1. Illustrating the time kill graph of S aureus

Time-kill curve assay

To assess the temporal antimicrobial efficacy of the samples, a time-kill assay was utilized. Each test substance was prepared at a concentration of 50 µg/mL in 9 mL of Mueller-Hinton broth, into which a 1 mL microbial suspension was inoculated. Following incubation at 37°C under constant agitation at 200 rpm, microbial viability was monitored at intervals of 0, 1, 2, 3, and 4 hours via spectrophotometric measurement of optical density at 600 nm. The reduction in microbial load was determined by comparing OD values at each time point to calculate the percentage of dead cells.

Statistical analysis

Information was compiled in a Microsoft Excel database and subsequently subjected to statistical evaluation using IBM SPSS Statistics software (version 20.0; IBM Corp., Armonk, NY, USA). To characterize the antimicrobial performance of the obturating materials, descriptive statistics were applied, with results expressed as means and standard deviations (SD). The diameters of the inhibition zones (measured in millimeters) were quantified for each material. Differences between groups were assessed via one-way analysis of variance (ANOVA), supplemented by Tukey's post-hoc test for multiple pairwise comparisons, with statistical significance established at $P < 0.05$. Furthermore, a separate ANOVA was conducted to examine the time-dependent antimicrobial effectiveness of the four materials against the five selected microbial strains, specifically to assess shifts in microbial reduction across the various intervals illustrated in the time-kill kinetics profile.

Results and Discussion

The findings indicate that the experimental root canal filling material demonstrated superior antimicrobial potency against *Lactobacillus acidophilus*, *Staphylococcus aureus*, and *Enterococcus faecalis* ($P = 0.02$, $P = 0.04$, and $P = 0.04$, respectively) when contrasted with the control groups. Conversely, no statistically significant variations in inhibitory activity were identified regarding *Streptococcus mutans* or *Candida albicans* ($P = 0.14$). Among the tested materials, Iodocal consistently exhibited the smallest inhibition zones, indicating limited antimicrobial efficacy. In contrast, the Premixed Zinc Oxide showed moderate antibacterial action, particularly more effective against *Lactobacillus acidophilus,* followed by Metapex, as depicted in **Table 1**.

Table 1. Illustrating statistical validation

Microbes	Novel Material (Mean ± SD, mm)	Premixed Zinc Oxide (Mean ± SD, mm)	Iodocal (Mean ± SD, mm)	Metapex (Mean ± SD, mm)	P-value
S. mutans	5 ± 1	4 ± 1	3 ± 1	3 ± 1	0.14
S. aureus	6 ± 1	5 ± 1	2 ± 1	4 ± 1	**0.04***
Lactobacillus acidophilus	13 ± 1	12 ± 1	2 ± 1	4 ± 1	**0.02***
E. faecalis	6 ± 1	4 ± 1	2 ± 1	3 ± 1	**0.04***

C. albicans	3 ± 1	4 ± 1	2 ± 1	2 ± 1	0.14

(*p < 0.05 indicates statistical significance)

For microbial groups exhibiting statistically significant variations (p < 0.05) via one-way ANOVA—specifically Staphylococcus aureus, Lactobacillus acidophilus, and Enterococcus faecalis—post hoc Tukey HSD tests were performed. Regarding S. aureus, the experimental obturating material displayed superior antimicrobial efficacy compared to Iodocal (p = 0.03). Conversely, no significant variance was detected between the novel material and either premixed zinc oxide (p = 0.73) or Metapex (p = 0.22), nor were significant differences identified among the other tested groups **(Table 2)**. In the case of L. acidophilus, the novel material and premixed zinc oxide both showed markedly higher antimicrobial activity than either Iodocal (p = 0.001 for both) or Metapex (p = 0.002 and p = 0.003, respectively). There was no statistically significant disparity between the novel material and premixed zinc oxide (p = 0.88) **(Table 2)**. Finally, analysis of E. faecalis revealed that the novel agent was significantly more effective than Iodocal (p = 0.04). No significant differences were established between the novel material and premixed zinc oxide (p = 0.29), nor among any other group comparisons **(Table 2)**. The statistical validation of these findings underscores the superior antimicrobial spectrum of the novel formulation, further reinforcing its potential clinical relevance in pediatric endodontics

Table 2. Post Hoc Pairwise Comparison (Tukey HSD) for *Staphylococcus aureus, Lactobacillus acidophilus and Enterococcus faecalis*

	Staphylococcus aureus		***Lactobacillus acidophilus***		***Enterococcus faecalis***	
Comparison	**Mean Difference**	**p-value**	**Mean Difference**	**p-value**	**Mean Difference**	**p-value**
Novel material vs Iodocal	4	**0.03**	11	**0.001**	4	**0.04**
Novel material vs Metapex	2	0.22	9	**0.002**	3	0.1
Novel material vs Premixed Zinc oxide	1	0.73	1	0.88	2	0.29
Premixed Zinc oxide vs. iodocal	3	**0.04**	10	**0.001**	2	0.29
Premixed Zinc oxide vs Metapex	1	0.73	8	**0.003**	1	0.71
Metapex vs Iodocal	2	0.22	2	0.64	1	0.71

The time-kill assay demonstrated a progressive reduction in microbial viability upon exposure to different obturating materials, with the novel obturating material exhibiting the most significant bactericidal and fungicidal activity against all 5 tested microorganisms. Comparatively, premixed zinc oxide displayed moderate efficacy, exhibiting bacteriostatic activity, while Iodocal and Metapex showed the least antimicrobial activity, with slower reductions in microbial load. The antimicrobial effect was time-dependent, with the novel obturating material maintaining a superior killing rate throughout the assay, reinforcing its potential as an advanced obturation material in pediatric endodontics. These results underscore the critical necessity of meticulous material selection in clinical applications, demonstrating how advanced formulations enhance therapeutic efficacy and mitigate the risk of recurrent infection. **(Figures 1-5)**

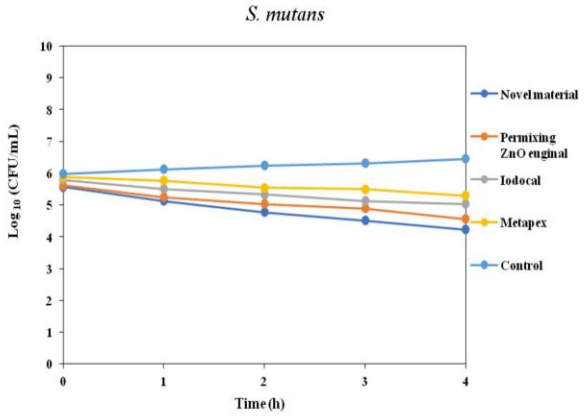

Figure 2. Illustrating the time kill graph of S mutans

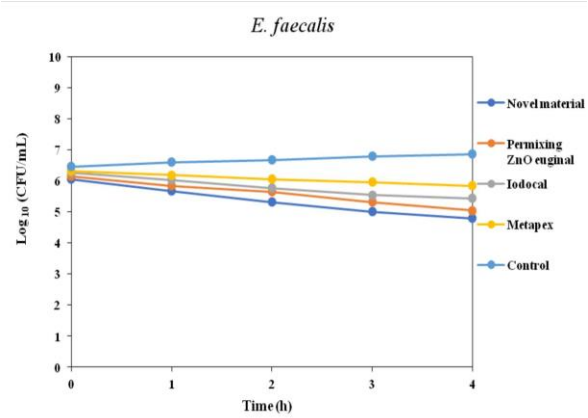

Figure 3. Illustrating the time kill graph of E faecalis

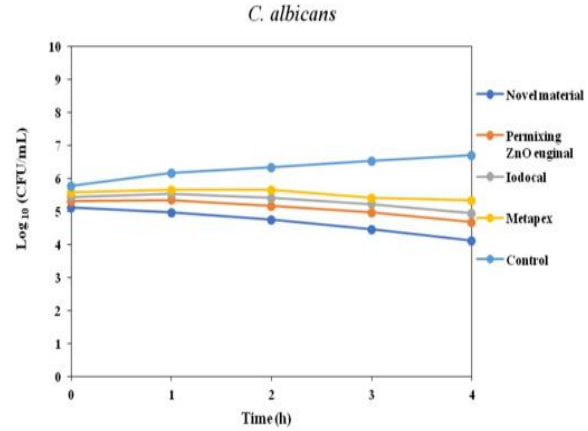

Figure 4. Illustrating the time-kill graph of C albicans

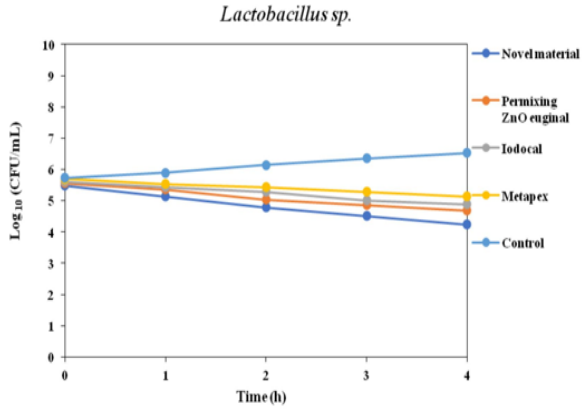

Figure 5. Illustrating the time kill graph of Lactobacillus acidophilus

The need for antimicrobial efficacy, biocompatibility, and predictable resorption has driven the evolution of obturating materials in pediatric endodontics. Traditionally, zinc oxide eugenol (ZOE) was widely used, but its cytotoxicity and potential interference with root resorption led to the adoption of calcium hydroxide-based alternatives [32]. Metapex, a widely used obturating material, capitalized on the benefits of calcium hydroxide, yet its antimicrobial efficacy remained limited, particularly against resistant microorganisms such as *Enterococcus faecalis* [39]. To address these limitations, researchers explored medicated formulations, incorporating antimicrobial agents such as metronidazole, which has been shown to disrupt biofilm formation and enhance bacterial eradication. The introduction of such bioactive materials represents a pivotal shift in pediatric endodontics, ensuring that primary teeth maintain their function until natural exfoliation.

An ideal obturating material should exhibit antimicrobial action, promote healing, and ensure compatibility with periapical tissues while allowing for resorption synchronized with primary root resorption. Calcium hydroxide remains a cornerstone due to its ability to dissociate into hydroxyl ions, elevating pH and creating an inhospitable environment for microbial survival [40]. Zinc oxide, known for its mild antimicrobial properties, enhances the material's mechanical stability and contributes to its sealing ability [41]. Metronidazole, a nitroimidazole antibiotic, specifically targets obligate anaerobes, further augmenting the antimicrobial spectrum [42]. The combination of these three components offers a synergistic effect, wherein calcium hydroxide provides a sustained antibacterial environment, zinc oxide reinforces the structural integrity, while metronidazole ensures targeted bacterial elimination, reducing the risk of reinfection. Additionally, premixed zinc oxide-based obturating material was utilized in this study to overcome the limitation of manual mixing, as the other materials were available in a premixed, syringe-ready form. This has gained attention for its ease of application, biocompatibility, and ability to act as an effective barrier against microbial penetration.

The results of the current study demonstrated that the novel obturating material exhibited superior antimicrobial efficacy against *Lactobacillus acidophilus, Staphylococcus aureus,* and *E. faecalis* when compared to Premixed Zinc Oxide, Iodocal, and Metapex. The statistically significant inhibition zones observed against these microorganisms reinforce the enhanced antibacterial spectrum of the novel formulation. While Premixed Zinc Oxide showed moderate efficacy, particularly against *Lactobacillus acidophilus*, Iodocal consistently displayed the weakest antimicrobial effect across all the tested microbes. The ability of the novel obturating material to significantly inhibit *E. faecalis*, a known endodontic pathogen resistant to conventional treatments, highlights its clinical relevance in reducing the persistence of infection and improving long-term success rates in pulpectomy procedures.

A novel root canal filling material, composed of calcium hydroxide (60%), zinc oxide (40%), and metronidazole (2%), was engineered to enhance antimicrobial activity while preserving optimal biocompatibility and controlled resorption characteristics. The inclusion of metronidazole offers a significant advantage over the conventional

obturating materials, particularly against biofilm-forming bacteria that are often implicated in endodontic failures. By maintaining an alkaline pH, the material ensures a prolonged antimicrobial effect while simultaneously promoting periapical healing. Its clinical applicability is further strengthened by its ease of handling and ability to conform to the complexities of primary root canals, making it a promising choice for pediatric endodontic therapy.

The combination of calcium hydroxide's sustained antimicrobial action, zinc oxide's reinforcement properties, and metronidazole's targeted bacterial eradication ensures comprehensive microbial suppression while maintaining compatibility with primary tooth physiology. A multi-faceted toxicological assessment—comprising embryonic toxicity, brine shrimp lethality, and MTT assays—revealed that the innovative obturating material exhibited no cytotoxic impact on human gingival fibroblasts at concentrations reaching 200 µg/ml. Furthermore, a comparative evaluation against zinc oxide eugenol (ZOE) using 3T3-L1 mouse fibroblast cells indicated that while both materials displayed low cytotoxicity and sustained cell viability exceeding 90% at lower doses (2.5–5 µL/mL), their performance diverged at elevated concentrations. Specifically, at a dosage of 60 µL/mL, the novel material supported a significantly higher rate of cell viability (56.63%) than ZOE (45.36%). These findings underscore the superior biocompatibility and reduced cytotoxic profile of this novel obturating agent, highlighting its potential as a viable alternative for pediatric endodontic procedures [43]. Given the promising in vitro results, future investigations should focus on long-term clinical trials to evaluate the material's resorption characteristics, biocompatibility, and overall clinical success in primary teeth [44]. If these properties are validated in vivo, the novel material could redefine the standard for pediatric root canal obturation, offering a more effective and predictable solution for managing primary tooth infections.

Conclusion

The novel calcium hydroxide–zinc oxide–metronidazole obturating material demonstrated enhanced antimicrobial performance compared with the other tested materials. This improved activity indicates that the combination may offer a more effective approach for controlling microbial presence within the root canal system. Overall, the findings suggest that this formulation has promising potential as a biocompatible and efficient obturating material, particularly in paediatric endodontic applications where effective disinfection and safety are both critical considerations.

Clinical significance

The findings from the present study will contribute valuable insights into the selection of an optimal obturating material for pediatric endodontic applications, ultimately enhancing the clinical outcomes.

Acknowledgments: The authors would like to thank the Department of Pediatric and Preventive Dentistry for their support throughout the study. We also extend our gratitude to the laboratory staff and postgraduate students who assisted with sample preparation and data collection.

Conflict of interest: None

Financial support: None

Ethics statement: This study was conducted as an in vitro laboratory investigation. No human participants, patient data, biological specimens from humans, or animals were involved in the study. The study was approved by the Institutional Ethics Committee.

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