

ANTIMICROBIAL EFFICACY OF CURCUMIN MODIFIED ZINC OXIDE EUGENOL AGAINST ENDODONTIC PATHOGENS

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ABSTRACT

Evaluate and compare antimicrobial potency of Zinc oxide eugenol (ZOE), Zinc oxide with Curcumin (ZOC), and Curcumin alone against *Enterococcus faecalis* (*E. faecalis*) and *Candida albicans* (*C. Albicans*) by using the agar diffusion method. Antimicrobial potency of ZOE, ZOC, and Curcumin gel were assessed and compared with each other against *C. Albicans* and *E. faecalis*. Muller Hinton agar was punched at equidistant points into which test materials were filled for both microorganisms and incubated at 37°C for 24 hours. Antimicrobial efficacy was assessed with disk diffusion in Agar. ANOVA and Post-Hoc Tests (Tukey-HSD) were used for statistical analysis. Highest antimicrobial potency in terms of inhibition zones were obtained with ZOE followed by an equivalent zone of inhibition with ZOC and Curcumin alone against *C. Albicans*. The highest antimicrobial potency in inhibition zones was obtained with ZOE, followed by Curcumin alone and ZOC against *E. faecalis*. A significant difference is present between ZOE and ZOC, ZOE and Curcumin. No significant difference was found between ZOC and Curcumin against *C. Albicans* and *E. faecalis*. In-vitro antibacterial activity of ZOE is consistent against *E. faecalis* and *C. Albicans*. ZOC and Curcumin alone exhibits satisfactory antibacterial activity and can be further considered as an alternative obturating material in impending future.

Key words: Antimicrobial efficacy, Zinc oxide eugenol, Curcumin, Obturating materials, Primary teeth.

Introduction

Premature loss of primary teeth due to pulp necrosis continues to be the major concern in pediatric dentistry. Deciduous teeth preserve the integrity of deciduous and mixed dentition and stimulate jaw growth and the masticatory process. Hence, their loss has a detrimental effect on the craniomandibular system. An effectively treated tooth can act as the best space maintainer in a non-pathologic stage until natural exfoliation time [1]. Reduction of endodontic bacterial flora through chemo-mechanical instrumentation from the root canal system is of utmost importance, as the remnant infection is one of the main etiological factors leading to post-treatment failure [2, 3]. *Enterococcus faecalis*, *Candida albicans*, *Peptostreptococcus*, *Streptococcus*, and *Staphylococcus* have been found persistent in the failed pulpectomies of primary teeth. *E. faecalis* is a facultative anaerobe with a prevalence of 24% to 77% persistent in secondary infections [4]. In the starvation phase, its resistance is known to increase 1000 to 10,000-fold. *C. Albicans* has been the most frequently found fungi in endodontic infections and is considered a dentinophilic microorganism [5].

Studies have stated that only 50-70% of the infected canals are free of microorganisms after mechanical instrumentation with antibacterial irrigation and intracanal medicament dressing since they predominantly reside in fins and isthmus of root canals [6]. Even after stringent disinfection of primary root canals, some resistant bacteria can thrive and

cause reinfection leading to failure of pulpectomy [7]. Although Zinc oxide Eugenol (ZOE) has been the most widely used obturating material in primary teeth, it brings several disadvantages. Numerous materials like Calcium hydroxide, Calcium hydroxide with iodoform, KRI paste, Maisto's paste, Walkhoff's paste, Guedes -Pinto paste have been tested for their efficacy as an obturating material. Still, none of these has been shown to fulfill the ideal requisites for obturating material. With the emergence of resistant bacterial strains to numerous antibiotics, Herbal products have become mainstream in contemporary dentistry with several benefits like fewer side effects, economic, enhanced patient acceptability.

Curcuma longa, commonly called Turmeric, belongs to the Zingiberaceae family, a flavored yellow-orange spice. Curcumin is a phenolic compound with medicinal properties like antioxidant, anti-inflammatory, and antimicrobial, proving a boon to dentistry. However, there is limited literature regarding combining Curcumin with existing obturating material in primary teeth [8]. The strong antibacterial property may help eradicate resistant endodontic pathogens like *E. faecalis* and *C. Albicans* associated with failures of pulpectomy. Thus, this in-vitro study was proposed to assess and collate the antimicrobial efficacy of ZOE, Zinc oxide with Curcumin (ZOC), and Curcumin alone against *E. faecalis* and *C. Albicans*.

Materials and Methods

This in-vitro study was carried out in collaboration with the microbiological testing Lab after it was accepted by the institutional ethical committee. In the present study, Zinc oxide powder was mixed with Curcumin gel (Curenex, Abbott Healthcare Pvt. Ltd., Thane, Maharashtra, India). It was compared with zinc oxide eugenol paste (Prime Dental Products Pvt. Ltd., Thane, Maharashtra, India) and Curcumin gel alone. Microorganisms *Enterococcus faecalis* (ATCC 29212) and *Candida albicans* (ATCC 10231) were used obtained. Standard norms and protocol performed the microbial inhibition assay by using Agar well diffusion method. An adequate amount of Mueller Hinton agar was evenly distributed over the Petri dish's surface (Diameter of 15cm and thickness of 5mm). It was allowed to solidify under aseptic conditions (under laminar airflow chamber) and was swabbed with a sterile spreader. The test materials - Zinc oxide(0.1g) + Eugenol (4 drops), Zinc oxide(0.1g) with Curcumin(0.4g), and Curcumin alone (0.4g) were mixed and placed in *E. faecalis* inoculated agar wells and were incubated at 37±0.1 degree Celsius for 24 hours. The same procedure was repeated for *C. Albicans*. The diameter of zones of inhibition was measured in millimeters using the Vernier caliper. All the tests were performed under sterile conditions and repeated six times. Findings were tabulated, and the mean value was taken. Data of antibacterial activity was analyzed statistically using one-way -ANOVA and Tukey's post-hoc test at a significance level of 5% using the IBM SPSS version 21 software.

Results and Discussion

Highest antimicrobial potency in terms of inhibition zones were obtained with ZOE followed by an equivalent zone of inhibition with ZOC and Curcumin alone against *C. Albicans*. A significant difference is present between the materials for the inhibition zone against *C. Albicans*. **Table 1** shows the Post hoc Tukey test presenting the difference in inhibition zones for *C. Albicans*. The difference was statistically significant between ZOE and ZOC, ZOE and Curcumin. No significant difference is present between ZOE and Curcumin (**Tables 1 and 2**).

Table 1. Shows Post hoc Tukey test presenting the difference in inhibition zones for *C. Albicans*. The difference was statistically significant between ZOE and ZOC, ZOE and Curcumin. No significant difference is present between ZOE and Curcumin

Groups	Mean Difference	Significance (p)	
ZOE	ZOC	1.80000	<0.0001*
	Curcumin	1.78333	<0.0001*
ZOC	ZOE	1.80000	<0.0001*
	Curcumin	0.01667	.996
Curcumin	ZOE	1.78333	<0.0001*
	ZOC	0.01667	.996

Table 2. Shows Post hoc Tukey test presenting the difference in inhibition zones for *E. faecalis*. The difference was statistically significant between ZOE and ZOC, ZOE and Curcumin. No significant difference is present between ZOC and Curcumin

Groups	Mean Difference	Significance (p)	
ZOE	ZOC	8.41667	<0.0001*
	Curcumin	8.00000	<0.0001*
ZOC	ZOE	8.41667	<0.0001*
	Curcumin	0.41667	.163
Curcumin	ZOE	8.00000	<0.0001*
	ZOC	0.41667	.163

The highest antimicrobial potency in inhibition zones was obtained with ZOE, followed by Curcumin alone and ZOC against *E. faecalis* (**Figure 1**). A significant difference is present between the materials for the inhibition zone against *E. faecalis*. **Table 2** shows the Post hoc Tukey test presenting the difference in inhibition zones for *E. faecalis*. The difference was statistically significant between ZOE and ZOC, ZOE and Curcumin. No significant difference is present between ZOC and Curcumin.

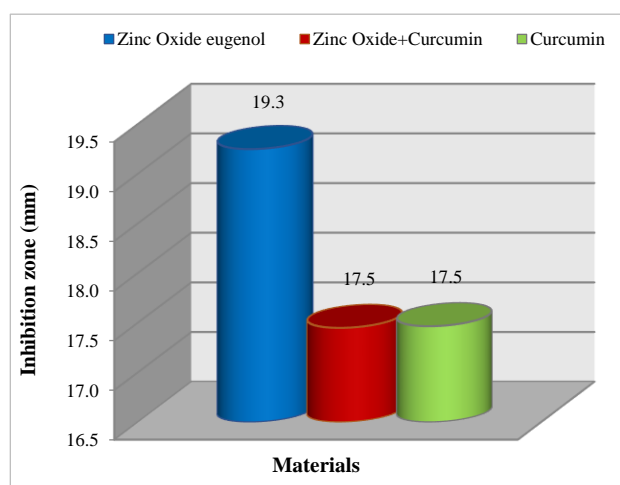


Figure 1. Bar diagram representing mean inhibition zone of Zinc oxide eugenol, Zinc-oxide + Curcumin and Curcumin for inhibition zone against *C. Albicans*

One of the most overlooked diseases in India is Early childhood caries (ECC) [9]. Average predominance of ECC is 49% in India [10]. Children often complain of pain, sensitivity, discomfort, and hence, if left untreated, leading to overall poor health [9, 11]. Thus, preservation of primary teeth by treating the infected tooth after proper diagnosis. Hence, pulp therapy plays a role in guiding the overall growth and development of a craniomandibular system of children.

The main objective of pulpectomy is to eradicate infection by thorough cleaning and shaping of the root canal system and proper restoration of the tooth [12]. Several factors decide the outcome of successful endodontic treatment, of which complete disinfection of root canal system is of utmost relevance. Precise knowledge of the resistant microbial flora and effective antimicrobial treatment would reduce the chances of reinfection or persistent infections after the endodontic treatment. *E. faecalis* and *C. Albicans* have been the most consistent bacteria and fungi, respectively, isolated from the failed pulpectomies [13]. Hence, an ideal obturating material should effectively remove the residual microorganisms by re-neutralizing their toxic products and creating an environment conducive for the healing process to occur, rendering the root canal system fully sterile.

ZOE has been a reliable obturating material in primary teeth since 1903, and until 2008, AAPD guidelines suggested it as the only obturating material to be used [14]. Although the existing materials available are clinically and radiographically effective, ZOE's present with several limitations.

So far, several researchers have tried and tested different materials. Still, neither of these obturating materials has fulfilled the properties of an ideal root canal material for primary teeth, especially concerning the efficient antimicrobial activity over an extended period. Zinc oxide alone or Zinc oxide with Eugenol has been studied in combination with different materials for improving its antimicrobial efficacy to tackle the problem of reinfection in obturated canals.

Reinfection is due to the multiplication of bacteria that remains despite cleaning or due to coronal leakage. Bacterial cell multiplication depends on FtsZ (Filamenting temperature-sensitive mutant Z), a protein encoded by the *ftsZ* gene. Its stability is an essential factor for cell division and is a potential drug target for antibacterial agents [15].

The antibacterial activity of Eugenol is accredited to its hydrophobicity, which cleaves the lipids and destroys the outer phospholipid cell membrane layer of the bacteria. Also, increased protein leakage from the cell membrane adds to its efficacy [16]. Undesirable effects exhibited by Eugenol are local adverse reactions, cytotoxic effects, and hypersensitivity reactions [17]. Thus quest for a better herbal antimicrobial agent mixed with Zinc oxide continues.

One such promising herbal plant is Turmeric, and its medicinal use has been known since ancient times. The antibacterial and biological properties of Turmeric are mainly attributed to Curcumin, which is its most important snippet [18]. Antibacterial action of Curcumin is by attacking FtsZ stability, wherein by inducing filamentation, it suppresses the bacterial cytokinesis. It also inhibits the assembly dynamics of FtsZ in Z-ring and suppresses

cytokinetic Z-ring formation. This eventually represses bacterial cell proliferation, thereby exhibiting a potent antibacterial activity [15].

Existing literature mentions curcumin gel or extracts as an antimicrobial root canal irrigant or intracanal medicament. However, contact time is a major concern while treating infected root canals resistant microorganisms. Thus, the present study was designed to mix Curcumin with ZOE for obturation of deciduous teeth. This could enhance the contact time in the zone of infection of a tooth, which can eventually take care of the resistant microorganisms residing in the deeper layers of the dentin

The current study evaluated and compared the antimicrobial properties of ZOE, ZOC (10mg/gm), and Curcumin gel alone (10mg/gm) against *E. faecalis* and *C. Albicans*. ZOE showed a maximum zone of inhibition against *C. Albicans* (19.25mm) followed by Curcumin (17.46mm) and ZOC (17.45mm). This suggests that Zinc oxide requires the addition of Eugenol or another potent agent to enhance its antimicrobial potential. ZOE in low concentrations has an anti-inflammatory action and in higher concentrations imputes cytotoxic activity. These results were by Hegde *et al.* [19] and Thosar *et al.* [20], who acquired 26mm and 16.83 mm of the zone of inhibition against *C. Albicans* using ZOE. Similar results were noted by Jha *et al.* [21], with the use of Curcumin as an antibacterial agent against *C. Albicans* and achieved a zone of inhibition of 10mm. In addition, Hassan *et al.* [22] obtained 12.5 mm and 11.5 mm of the zone of bacterial inhibition when comparing aqueous and ethanolic extracts of Curcumin against *C. Albicans*.

Comparison between the groups showed that there was a significant difference present between ZOE and zinc oxide + Curcumin (<0.0001*) also between zinc oxide eugenol and Curcumin (<0.0001*). In contrast, no significant difference was found between zinc oxide + Curcumin and Curcumin alone against *C. Albicans*. This suggests that Zinc oxide requires the addition of Eugenol or another potent agent to enhance its antimicrobial potential. ZOE in low concentrations has an anti-inflammatory action and in higher concentrations imputes cytotoxic activity.

A similar procedure was performed to test the antibacterial efficacy of these materials against *E. faecalis*, where ZOE showed a zone of inhibition of 26.65mm, which was significantly higher than those acquired with the use of ZOC (18.23mm) and Curcumin alone (18.65mm). Results agreed with studies done by Thosar *et al.* [23] and Hegde *et al.*, [19] who obtained zone of inhibition of 10.83mm and 17mm respectively against *E. faecalis* using ZOE. Using Curcumin alone against *E. faecalis* was similar to the study done by Kumar *et al.* [24]. They found a zone of inhibition of 10mm at 10% concentration and 22mm at 20% concentration of Curcumin. Hydroalcoholic extract of Curcumin (7:3 conc.) tested by Chaitanya *et al.* [25] yielded a zone of inhibition of 14.42mm.

A significant difference was found between zinc oxide eugenol and zinc oxide + Curcumin (<0.0001*) and between zinc oxide eugenol and Curcumin (<0.0001*). In contrast, no significant difference was found between zinc oxide + Curcumin and Curcumin alone against *E. faecalis* (Figure 2).

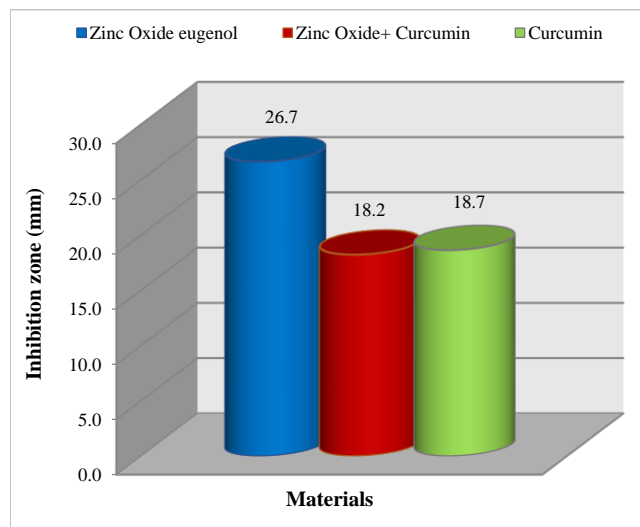


Figure 2. Bar diagram representing mean inhibition zone of Zinc oxide eugenol, Zinc-oxide + Curcumin and Curcumin for inhibition zone against *E. faecalis*

It can be summarised that test materials ZOC (17.45mm) and Curcumin alone (17.46mm) exhibited nearly similar antimicrobial activity to that of ZOE (19.25mm) against *C. Albicans*. However, against *E. faecalis*, ZOE performed superior (26.4mm) than Curcumin (18.10mm) and ZOC (17.80mm). Long-term in vivo studies are required to confirm the sustained antimicrobial activity of Curcumin when mixed with obturating material like Zinc oxide.

Conclusion

ZOC and Curcumin, when used as an obturating material in primary teeth, exhibits satisfactory antibacterial potency against *E. faecalis* and *C. Albicans* and hence can be considered a potential alternative to conventional ZOE. Further in-vivo studies are needed to confirm the clinical antimicrobial efficacy of Curcumin when used in conjunction with Zinc oxide.

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Ethics statement: Institutional ethical approval (Dr.D.Y.Patil Vidyapeeth Pimpri,Pune,Maharashtra,India) was taken for conducting this study.

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