# Comparative Evaluation of Antimicrobial Efficacy of Various Root Canal Filling Materials Along with Aloevera Used in Primary Teeth: A Microbiological Study

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***Aim:*** *this study was conducted to evaluate the antimicrobial effectiveness of 6 root canal filling materials and a negative control agent against 18 strains of bacteria isolated from infected root canals of primary molar teeth using agar diffusion assay.* ***Materials:*** *Aloevera with sterile water, Zinc oxide and Eugenol, Zinc oxide-Eugenol with aloevera, Calcium hydroxide and sterile water, Calcium hydroxide with sterile water and aloevera, Calcium hydroxide and Iodoform (Metapex) and Vaseline (Control). MIC and MBC of aloevera was calculated.* ***Results:*** *All materials except Vaseline showed varied antimicrobial activity against the test bacterias. The zones of inhibition were ranked into 4 inhibition categories based on the proportional distribution of the data. All the 18 bacterial isolates were classified under 2 groups based on Gram positive and Gram negative aerobes. Statistical analysis was carried out to compare the antimicrobial effectiveness between materials tested with each of the bacterial groupings.* ***Conclusion:*** *Aloevera + Sterile Water was found to have superior antimicrobial activity against most of the microorganisms followed by ZOE + Aloevera, calcium hydroxide + Aloevera, ZOE, calcium hydroxide, Metapex in the descending order and Vaseline showed no inhibition.*

***Keywords:*** *Aloevera, antimicrobial property, root canal, primary teeth*

## INTRODUCTION

The teeth with pulpal and periapical problems, specifically the primary teeth, should be retained until their normal exfoliation. Currently as the field of dentistry becomes more sophisticated in equipment, instruments, materials and techniques, vistas of endodontics have broadened. With concerns of longevity and functions of the dentition, endodontics has begun to assume an expanded role as it is related to preservation of the healthy dental pulp.

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Successful root canal filling cannot be achieved unless the canals have been debrided and disinfected before receiving the filling material. Success of endodontic therapy depends on the reduction or elimination of the infecting bacteria. Among the ways to reduce or eliminate the infecting bacteria are adequate root canal debridement, antimicrobial irrigants, and antibacterial filling materials.[1] In the search of an ideal obturating material for the primary teeth, over the years, a number of materials have been tried. Most commonly used materials are Zinc oxide eugenol, Calcium hydroxide and Iodoform which have antimicrobial properties. This property of the endodontic medicaments used in primary teeth probably contribute significantly to the clinical success of endodontic therapy by inhibiting residual bacteria not removed by mechanical debridement.[2]

Aloevera is a shrubby tropical and subtropical plant, which has succulent and elongated leaves. Aloevera Barbadensis Miller belongs to the family Liliaceae, which is commercially widely used in therapeutic purposes.[3] It has a long history as a home remedy for burn wounds. Hence it is commonly called as burn plant. Aloevera has lot of dermatological applications such as treatment of inflammation, wound healing, especially in thermal burn, radiation burn, frostbite and antimicrobial effect.[4, 5, 6] It enhances various phases of wound healing processes, such as macrophage recruitment, collagen synthesis, and wound contraction.[7, 8]

The aim of this study was to calculate the minimum inhibitory concentration of aloevera and evaluate the antimicrobial efficacy of six root canal filling materials viz., aloevera with sterile water, zinc oxide and eugenol, zinc oxide eugenol with aloevera, calcium hydroxide with sterile water, calcium hydroxide with sterile water and aloevera, calcium hydroxide with iodoform (Metapex) and vaseline as a control group against the microorganisms isolated from infected primary teeth.

**Table 1.** Powder and liquid ratio's of test filling materials

| | | |
|---|---|---|
| I ZOE | Zinc oxide | Eugenol |
| | 1 scoop | 7 drops |
| | 0.2 g | 0.07 cc |
| II Ca (OH)2 + H20 | Calcium Hydroxide | Sterile water |
| | 1 scoop | 10 drops |
| | 0.17g | 0.1 cc |
| III Metapex - | Commercial product | |
| IV Vaseline - | Commercial product | |

According to the minimum inhibitory concentration of Aloevera calculated, the proportion in which Aloevera is mixed with Zinc oxide eugenol and Calcium hydroxide was 40 gm/100mg i.e. 40 gm of Aloevera in 100 mg of Zinc oxide eugenol and Calcium hydroxide + water and Aloevera and water is 400 mg in 1 ml of water.

**Table 2 .** Grouping of bacterial isolates

| | |
|---|---|
| **GROUP I:- Facultative/ Aerobic Gram positive** | |
| 1) | Streptococcus pyogens |
| 2) | Streptococcus salivarius (strain 1) |
| 3) | Streptococcus salivarius (strain 2) |
| 4) | Streptococcus vestibularis |
| 5) | Streptococcus sanguis (strain 1) |
| 6) | Streptococcus sanguis (strain 2) |
| 7) | Streptococcus mitis (strain 1) |
| 8) | Streptococcus mitis (strain 2) |
| 9) | Streptococcus mitis (strain 3) |
| 10) | Streptococcus mitis (strain 4) |
| 11) | Streptococcus mutans |
| 12) | Staphylococcus aureus |
| 13) | Enterococcus faecalis |
| 14) | Enterococcus hirae |
| **GROUP II:- Facultative/ Aerobic Gram negative** | |
| 15) | Escherichia coli (strain 1) |
| 16) | Escherichia coli (strain 2) |
| 17) | Pseudomonas aeruginosa |
| 18) | Klebsiella |

## MATERIALS AND METHOD

### Preparation of the Aloevera[9]

Leaves of aloevera were collected and left to rest in distilled water for 8 hours to eliminate aloine. These leaves were cut into pieces and were liquefied, sieved, filtered with negative pressure to obtain the juice, and freeze-dried. They were then sterilized by gamma ray and stored at 4°C in Eppendorf tubes.

### Minimum Inhibitory Concentration and Minimum Bactericidal Concentration[10]

Minimum inhibitory concentration (MIC) was calculated using broth tube dilution method and agar diffusion method. MIC of aloevera was found to be 400mg/ml. Minimum bactericidal concentration (MBC) was calculated using agar diffusion method. MBC of aloevera was found to be 500mg/ml.

Microbial specimens were obtained from 20 infected deciduous molar teeth with pulpal pathology associated with abscess or sinus. Selection was done at random, irrespective of the cause of pulpal or periapical pathology in patients ranging from 4-8 yrs of age.

### Inclusion criteria[11]

- Antibiotics were not received by the subject 4 weeks prior to sampling
- Contained at least one necrotic canal
- Did not have resorbing roots or broken crowns.
- An abscess, fistulae or obvious interradicular (furcation) radiolucency was present.

The entire procedure was carried out under strict aseptic conditions. No endodontic procedure was performed before the collection of sample, so as to avoid disturbing the root canal flora. The involved tooth was isolated with rubber dam and the field of operation (exposed tooth, clamp and rubber dam) was disinfected with two applications of 30% $H_2O_2$ followed by 5% tincture of iodine.[12] Prior to specimen collection the presence of an intact crown on the involved tooth permitted disinfection. The tooth and the operating field were redisinfected after removal of dental caries. Sterile absorbent paper points [N°20] were inserted into the accessible root canals and left over for 60 sec. The absorbent paper point was immediately transferred to Robertson's cooked meat medium and transferred for microbiological analysis. After 24 hours, subculture plate made from Robertson's cooked meat medium on pre reduced blood agar was placed in an anaerobic jar containing gas pack and indicator which was examined after 48 hrs of incubation at 37°C

Multiple paper points were also inoculated into brain heart infusion broth which supported growth of most aerobes and facultative anaerobes. After two hours of incubation, aerobic subculture was done on 5% sheep blood agar, Chocolate agar and MacConkey agar and incubated at 37°C in an atmosphere containing 5% $CO_2$ for 48 hrs. Brain heart infusion broth was further incubated for 7 days to provide a backup source of culture material when there was no growth on agar plates. The aerobic isolates obtained were identified based on their morphology in a gram stained smear, colony characteristics and species was identified using standard microbiological procedure.

6 test filling materials and one control material were tested in this study.

1) Aloevera with sterile water
2) Zinc oxide and eugenol [Vishal Pharma (Ahmedabad)]
3) Zinc oxide eugenol with aloevera
4) Calcium hydroxide with sterile water [Prevest Denpro Ltd (Jammu)]
5) Calcium hydroxide with sterile water and aloevera
6) Calcium hydroxide with iodoform (Metapex) [Metabiomed (Korea)]
7) Vaseline (control)

The powder and liquid ratio of all test root canal filling materials were standardized according to the formulae given by Tchaou *et al* [13] and Reddy, Ramakrishna (2007)[11] (Table 1). An electronic balance and a digital micropipette were used to measure the exact amount of powder and liquid to be dispensed. The filling materials were spatulated on a dry pre sterilized glass slab using a cement spatula at room temperature, just before it is assayed for agar diffusion assay. The mixture is back loaded into a sterile 2ml syringe and kept ready.

The aerobic isolates obtained from these samples were used to test the antimicrobial efficacy of 6 root canal filling materials. Vaseline was used as a control as it has no antimicrobial effect. Sensitivity testing was done by the standard agar diffusion method.[14] Actively growing broth cultures of the microorganisms with the turbidity adjusted to 0.5 McFarland standard were used. The media used for broth cultures were Brain Heart Infusion broth for aerobes and thioglycollate broth for anaerobes. Using a sterile swab the entire surface of the agar plate was swabbed 3 times to ensure even distribution of the inoculum. Mueller-Hinton agar was used for aerobes and pre reduced blood agar for anaerobic microorganisms.

Filling materials were tested in each plate. After the plates were dried, wells of 4mm diameter and 3mm depth were made in the agar plates using sterile agar punchers and filled with the test materials. The diameter of the zones of inhibition in mm around the filling materials was measured after 16-24 hours for aerobic isolates. The experiment was repeated thrice for each isolates and zones were measured independently by two observers. Mean zone of inhibition for each filling material and isolate combination was then calculated from the 6 measurements and subjected to statistical analysis. Measurements on inhibition zones were ranked into 4 inhibition categories according to the proportional distribution of the data set. A semi qualitative comparison was made with the understanding that the results could not be completely transferable to the in-vivo situation as there was difference of diffusibility in the agar for various materials.

## RESULTS

All the 20 microbial samples obtained from infected primary teeth demonstrated polymicrobial infection. Only those strains which can be isolated purely were included in the study. A total number of 18 bacterial strains were employed in the experimental procedure. The isolated bacterial strains were divided into 2 groups based on gram positive or gram negative aerobes (Table 2). The mean zone of inhibition of 7 test filling materials against 18 bacterial isolates (Table 3). Measurements of inhibitory zones were ranked arbitrarily into the following four categories according to the proportional distribution of the data set.[11] (Table 4)

1. No inhibition (No) 2. Weak inhibition (W) 3. Medium inhibition (M) 4. Strong inhibition (S) Zone size categories

The inhibition results of 7 test filling materials against 18 bacterial strains according to the ranking scale [Table 5].

## Statistical Analysis

Statistical analysis was carried out by one-way ANOVA using software SPSS version 17.0 with post-hoc tests to compare the statistical difference of antimicrobial effects between materials tested with each of the two bacterial groupings (Aerobic gram-positive and Aerobic gram-negative). A semi qualitative comparison was made with the understanding that the results could not be completely transferable to the in vivo situation as there was difference of diffu-

**Table 3.** Zones of inhibition (mm) of 7 test filling materials against 18 microorganisms

| Test Materials | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zinc Oxide Eugenol | 11.5 | 12.66 | 13.66 | 20.66 | 13.16 | 16.5 | 11.5 | 9 | 14.66 | 15.83 | 12.66 | 16.5 | 10.33 | 10 | 10.16 | 17.16 | 15.5 | 15.5 |
| Zinc Oxide Eugenol and Aloevera | 13.66 | 16.33 | 15.66 | 25.66 | 16.83 | 15.33 | 13.66 | 15.66 | 16.33 | 16.16 | 16.33 | 20.5 | 15.5 | 11.5 | 11.66 | 20.66 | 17.66 | 18.33 |
| Calcium hydroxide + Water | 10.66 | 9.5 | 10.33 | 0 | 10.66 | 11.33 | 10.66 | 8.83 | 12.16 | 8.66 | 9.5 | 11.33 | 10.33 | 0 | 10 | 0 | 10.5 | 11 |
| Calcium hydroxide + water and aloevera | 12.83 | 14.5 | 12.66 | 21.16 | 13.16 | 12.83 | 12.83 | 14.83 | 13 | 10.83 | 14.5 | 15.33 | 19.33 | 12.5 | 12 | 12.5 | 0.66 | 11.33 |
| Aloevera + Water | 18.16 | 23.5 | 20 | 27.16 | 14.66 | 14.66 | 18.16 | 23.16 | 10.83 | 17.16 | 23.5 | 18.33 | 17.5 | 15.5 | 15.66 | 16.5 | 27.66 | 25.83 |
| Metapex | 10.33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10.33 | 3.33 | 0 | 3.33 | 0 | 2 | 3.33 |
| Vaseline | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

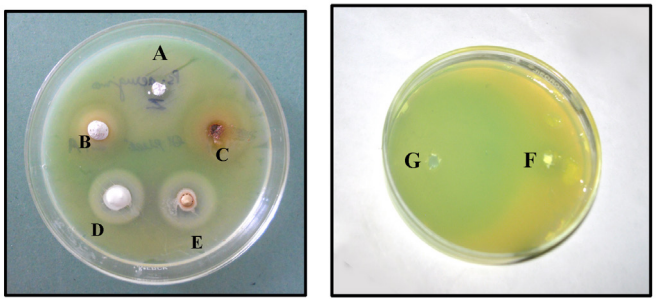


**Figure 1.** Zone of inhibition produced by test filling materials against Pseudomonas aeruginosa. A) Zinc oxide engenol; B) Zinc oxide eugenol + Aloevera; C) Aloevera; D) Calcium hydroxide; E) Calcium hydroxide + Aloevera; F) Metapex; G) Vaseline.

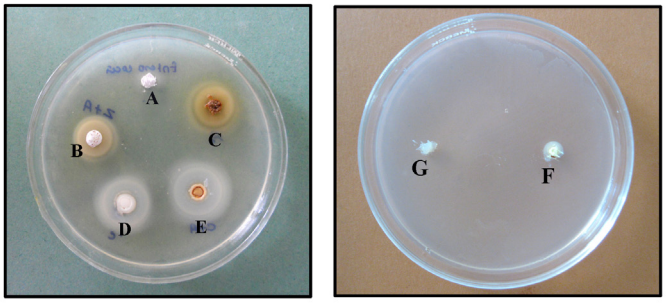


**Figure 2.** Zone of inhibition produced by test filling materials against Enterococcus faccalis. A) Zinc oxide eugenol; B) Zinc oxide eugenol + Aloevera; C) Aloevera; D) Calcium hydroxide; E) Calcium hydroxide + Aloevera; F) Matapex; G) Vaseline.

sion in the agar media for various materials. Statistically significant grouping of inhibitory effects of materials against the categories of microbial species i.e. aerobic gram positive and aerobic gram negative revealed the following

1. **Group-I** (Aerobic Gram positive): Aloevera + sterile water, Zinc oxide eugenol + aloevera, Calcium hydroxide + aloevera were most inhibitory; Zinc oxide eugenol, Calcium hydroxide + sterile water were less inhibitory; Metapex and Vaseline were non-inhibitory

2. **Group-II** (Aerobic Gram negative): Aloevera + sterile water, Zinc oxide eugenol + aloevera, were most inhibitory; Zinc oxide eugenol, Calcium hydroxide + aloevera were less inhibitory; Calcium hydroxide + sterile water, Metapex and Vaseline were non-inhibitory.

## DISCUSSION

Microorganisms and their by-products are considered the major cause of pulpal and periradicular pathologies.[13] Hence, a major objective in root canal treatment is to disinfect the entire root canal system. Several root canal filling materials for primary teeth are available, but the most commonly used materials are zinc oxide eugenol and calcium hydroxide – iodoform paste. As the pulpectomy procedure in primary teeth is never complete, the final outcome of the procedure also depends on the quality of the root canal filling material which can neutralize any remaining pulpal tissue and microorganisms.

Aloevera is being used therapeutically, since Roman times and perhaps long before.[15,16] Amongst its therapeutic properties, it has been shown to have an anti-inflammatory activity,[17,18] immunostimulatory activity,[19,20] cell growth stimulatory activity,[21,22] regenerative property,[23,24] nutritive property,[25] antimicrobial property.[26,27,28] Specific compounds of aloevera have been proposed to have direct antimicrobial activity.

In this study ZOE showed medium inhibitory effect against both the groups of test organisms. Similar results were found by Cox *et al,* [29] Grossman,[30] Ostavik,[31] who agreed that the sealers with a ZOE base are those that have greater inhibitory effect against the microorganisms found in root canals.

Metapex (combination of calcium hydroxide and iodoform) was found to be ineffective against all the test microorganisms except *Streptococcus pyogens*, *Staphylococcus aureus*, *Enterococcus faecalis*, *E coli* (strain 1, 2) and *Pseudomonas aeruginosa*. In the studies done by Tchaou (1996),[32] Pabla *et al* (1997),[33] Ninomiya (1980)[34] calcium hydroxide with iodoform had exhibited no antimicrobial activity against most pure cultures in agar diffusion tests. The weak activity may be partially explained by the facts that calcium hydroxide, an ingredient of metapex has been demonstrated to interfere with the antiseptic capacity of dyadic combinations of endodontic medicaments.[35]

In the present study, natural extract of aloevera was used rather than using commercially available aloevera products. The former one is always better than the later one, as no preservatives are added which may give biased results.[36] The efficacy of aloevera increases when the plant is harvested after 3 years of growth but its nutritive potency decreases after 12 years of growth. In the present study the aloevera plant harvested was around 4 years of growth.[37]

The antimicrobial agents that are attributed to the aloevera plant are anthroquinones, dihydroxyanthraquinones, saponins, aloe emodin, aloetic acid, aloin, anthracin, anthranol, barbaloin, chrysophanic acid, ethereal oil, ester of cinnamonic acid, isobarbaloin and resistannol.[38,39] Hence in the present study, aloevera was tested as a root canal filling material alone and in combination with other commonly used root canal filling materials i.e ZOE and calcium hydroxide, to check whether it enhances the antimicrobial properties of these materials.

In this study aloevera showed strong inhibitory effect against group II of test microorganisms and showed medium inhibitory effect against group I test microorganisms. Whereas, ZOE + Aloevera showed medium inhibitory effect against both the groups of test microorganisms while, ZOE exhibited medium inhibitory effect against both the groups of test microorganisms. Hence, incorporation of Aloevera into ZOE enhances the antimicrobial nature of the combination of ZOE with aloevera, when compared to ZOE. Aloevera when compared with ZOE + Aloevera showed strong inhibitory effect against group II of test microorganisms and medium inhibitory effect against group I test microorganisms. The less inhibitory effect of combination of ZOE with aloevera may be

**Table 4.** Ranking scheme for microbial inhibition

| Rank | Range of zone diameters(mm) |
|---|---|
| No | 0 |
| Weak | 0.1-11.5 |
| Medium | 11.5-19.7 |
| Strong | >19.7 |

partially explained that, ZOE may interfere with the antimicrobial nature of Aloevera.

Calcium hydroxide + water shows weak antimicrobial activity against both groups of test organisms Whereas, calcium hydroxide with aloevera shows medium inhibitory effect against both groups of test microorganisms. This shows that aloevera enhances the antimicrobial property of calcium hydroxide when used in combination.

Most of the studies related to antimicrobial activity of root canal filling materials had been done using standardized bacterial strains (ATCC- American Type Culture Collection). Very few studies were reported in the dental literature exclusively on microbial strains isolated from infected primary teeth. The mean zone of inhibition of the material and bacterial isolate combination in this study cannot be compared with previous studies because of the variabilities in microbial strains, culture media, culture conditions and powder and liquid ratio of the test filling materials.

## CONCLUSIONS

1) All the test filling materials showed varied antimicrobial activity against the microorganisms tested.
2) Aloevera + Sterile Water was found to have superior antimicrobial activity against most of the microorganisms followed by ZOE + Aloevera, calcium hydroxide + Aloevera, ZOE, calcium hydroxide, Metapex in the descending order and Vaseline showed no inhibition.
3) Aloevera can be used as an potential root canal filling material
4) Incorporation of Aloevera with ZOE and calcium hydroxide increases their antimicrobial activity.

Further clinical trials are required to know the specific antimicrobial efficacy of aloevera, as the MIC was found to be very high. *In vivo* studies are required to state the specific antimicrobial activity and merits and demerits of the test material.

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**Table 5.** Inhibition results of 7 test filling materials against 18 microorganisms according to the ranking scale

| TEST MATERIALS | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zinc Oxide Eugenol | W | M | M | W | M | M | M | W | M | M | M | M | W | W | W | M | M | M |
| Zinc Oxide Eugenol and Aloevera | W | M | M | M | M | M | M | M | M | M | M | S | M | M | M | S | M | M |
| Calcium hydroxide + Water | M | W | W | W | W | W | W | W | M | W | W | W | W | NO | W | NO | W | W |
| Calcium hydroxide + water and aloevera | M | M | M | M | M | M | M | M | M | W | M | M | M | M | M | M | W | W |
| Aloevera + Water | M | M | S | M | M | M | M | S | W | M | S | M | M | M | M | M | S | M |
| Metapex | W | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | W | W | NO | W | NO | W | W |
| Vaseline | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO | NO |

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