

## ***In-Vitro* Comparison of Dentinal Tubule Disinfection with *Aloe-vera* Extract, 2% Chlorhexidine Gel and Calcium Hydroxide**

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### **Abstract**

**Background:** The aim of this *in-vitro* study is to compare the antimicrobial efficacy of 2% chlorhexidine gel, calcium hydroxide and *Aloe-vera* extract as an intracanal medicament against *E. faecalis*.

**Method:** Sixty extracted single-rooted human teeth were prepared with standard method. After contaminating the canals with *E. faecalis* and incubated for seven days, the samples were divided into four groups (n = 15). Normal saline was used as control group. The teeth in each group were treated with normal saline, calcium hydroxide, 2% chlorhexidine gel and *Aloe-vera* extract. Microbial samples were obtained from the dentinal shavings of root and colony forming units (CFU) of *E. faecalis* were recorded after 24 hrs, 72 hrs and 7 days.

**Result:** To determine the significance of the differences between the different groups, ANOVA (Analysis of variance) and Tukeys Honestly significant Difference (HSD) Post hoc test were performed.

**Conclusion:** The antimicrobial efficacy of *Aloe-vera* was found to be comparable to 2% chlorhexidine gel for shorter duration as mean CFU were lowest for both medicaments in 24 hrs and highest in 7 days. Mean CFU of calcium hydroxide was found to increase at 24 hrs and decreases at 72 hrs and 7 days.

**Keywords:** *Aloe-vera*; Calcium Hydroxide; Chlorhexidine; Colony Forming Units; *E. faecalis*; Intracanal Medicaments

### **Introduction**

Strong correlation exists between basic science of microbiology and the clinical sciences of Endodontics. Without microbial involvement in pulp and associated periapical tissues, there would be no need for endodontic therapy. Complete disinfection in root canal system is difficult because of anatomic complexities and diversity of root canals, as well as the subsequent limitations in access, by instruments and irrigants [1].

Study has shown that instrumentation and antibacterial irrigation renders 50% to 70% of infected canals free of microorganisms while the remaining canals contain vital bacteria which are thought to be source of persistent endodontic infection. *E. faecalis* is the most commonly isolated species in persistent root canal infections. It has been detected in 77% of failed endodontic cases [2]. Therefore, the use of intracanal medicaments help in elimination of bacteria that remain even after cleaning and shaping [3].

Different types of intracanal medicaments have been used to disinfect the root canals. Among them, calcium hydroxide is one of the most widely used intracanal medicament because of its well documented antibacterial activity against most of the strains identified in root canal infections.

Apart from calcium hydroxide, 2% Chlorhexidine gluconate gel has also been used as intracanal medicaments as it has broad spectrum antimicrobial activity affecting both gram positive and gram negative bacteria. It possesses properties that include extended residual activity and relative absence of toxicity [4].

Natural herbal extracts have gained therapeutic importance in dental science in the recent days. In developing countries, researchers have shown that the use of herbal drugs have increased instead of synthesized drugs. *Aloe-vera* has shown antimicrobial effect against resistant microorganisms found in pulp space. Leaf extracts of *Aloe-vera* contain anthraquinones which have antibacterial properties [5,6]. The antimicrobial properties of *Aloe-vera* against various species of microorganisms, including *E. faecalis*, have also been reported [7].

The rationale of this *in-vitro* study is to compare the antimicrobial efficacy of *Aloe-vera* gel, Chlorhexidine gel and Calcium hydroxide as intracanal medicaments against *E. faecalis*.

## **Materials and Methods**

### **Preparation of the Samples**

This *invitro* study was conducted in the Department of Conservative dentistry and endodontics, Universal College of Medical Sciences, College of Dental Surgery, Bhairahawa, Nepal for 3 months period. Sixty freshly extracted human teeth with straight single canal and mature apex were collected. Teeth were decoronated using a diamond disc (Axis Products) at low speed to standardized root length of 18 mm  $\pm$  2 mm.

Root canal treatment was done. Then, the teeth were individually placed in bottles containing 2 ml of Brain heart infusion medium and autoclaved at 121°C, 20 lbs for 15 minutes. They were then kept in an incubator at 37°C for 24 hr to check the efficiency of the sterilization treatment. Sterilization of teeth was confirmed by gram staining the BHI broth and pour plating the broth.

### **Bacterial culture**

Isolated 24 hr colony of pure culture of *E. faecalis* was grown in blood agar and suspended in brain heart infusion broth. Turbidity of BHI broth was verified by using the MacFarland Turbidity Scale and adjusted to 0.5, corresponding to  $1.5 \times 10^8$  organisms CFU per milliliter. All the microbiological experiments were conducted under aseptic conditions in a laminar flow hood to avoid contamination by outside organisms.

### **Contamination of the samples**

Tubes containing each specimen were opened. Sterile pipettes were used to remove 2 ml of sterile BHI and were replaced with 2 ml of bacterial inoculums. Pure culture of *E. faecalis* (ATCC 29212) was used as a test organism. At the end of each experimental run, bacterial viability and purity of cultures was confirmed by gram staining, Catalase production, colony morphology on BHI agar bacterial viability and purity of cultures was confirmed by gram staining, Catalase production, colony morphology on BHI agar.

At the end of 7 days, the blocks were irrigated with sterile saline to remove the incubation broth. Canals were dried with sterile paper points (Dentsply, Maillefer, Switzerland).

**Antibacterial Assessment**

The blocks were randomly assigned to the following groups divided into 4 groups (n-15): group 1- Normal Saline (negative control), group 2- Calcium hydroxide (Ecodent, India), group 3- 2% Chlorhexidine gel (Concepsis, Ultradent, USA) and group 4: *Aloe-vera* extract.

Calcium hydroxide was mixed with sterile saline in the ratio of 1:1 (wt/vol) to obtain paste like consistency and the mixture was placed into root canal with a lentulo spiral. The traditional hand-filleting method of extracting *Aloe-vera* gel from *Aloe-vera* leaves had been done [8]. *Aloe-vera* gel extract and 2% Chlorhexidine gel were placed into root canal with a lentulo spiral. The medicaments were placed inside the canals. A sterile cotton pellet was placed in the canal orifice and sealed with tin foil. Blocks were incubated in an anaerobic environment for 37°C in incubator (Naran Scientific works, India). After the loading of various medicaments, all groups were subdivided into three subgroups of 5 samples and incubated for different experimental time periods of 24 hrs, 72 hrs and 7 days.

Harvesting of the dentin was carried out with round carbide bur of diameter 1 mm. The dentinal shavings obtained were collected in each sterile petridish and then transferred into 1 ml of sterile BHI broth and incubated in an anaerobic environment at 37°C for 24 hrs.

After 24 hrs, the contents of each tube was serially diluted, 100 ul of the broth in 300 ul of sterile saline for 8 times. 100 µl of the dilution was then plated on nutrient agar plate with L shaped rod and incubated for 24 hrs. Colonies were counted using colony counter. The same procedures were repeated at time intervals of 72 hrs and 7 days in each five samples respectively.

The data was entered manually on Microsoft excel (MS Office Excel 2000; Microsoft Corporation, Redmond, WA, USA), checked for possible data entry errors. Frequencies and percentages were taken out for categorical variables. The data were analyzed using SPSS version 21.0 (IBM Corp. Armonk, NY: IBM Corp). For inferential statistics One way ANOVA followed by post hoc Tukey (HSD) was used for multiple comparisons between different groups. The level of significance was established as P < 0.05.

**Results**

Table shows the mean ± SD CFU count for the study groups at different time intervals. On comparison of the means at particular time showed statistically significant difference among the groups (p < 0.001). Mean CFU of control group and calcium hydroxide group was statistically significantly different from mean CFU of all other groups (p < 0.01). The mean CFU count of 2% chlorhexidine group and *Aloe vera* extract group did not had statistically significant difference (p = 0.384). Table shows pair wise comparison using Tukey’s test of groups at 72 hours. Mean CFU of control group and chlorhexidine group was statistically significantly different from mean CFU of all other groups (p < 0.01). The mean CFU count of calcium hydroxide group and *Aloe vera* extract group did not had statistically significantly difference (p = 0.924). Table shows pair wise comparison using Tukey’s test of groups at 7 days. Mean CFU of calcium hydroxide group and 2% chlorhexidine group was statistically significantly different from CFU of all other groups (p < 0.01). The mean CFU count of control group and *Aloe vera* extract group did not had statistically significant difference (p = 0.670).

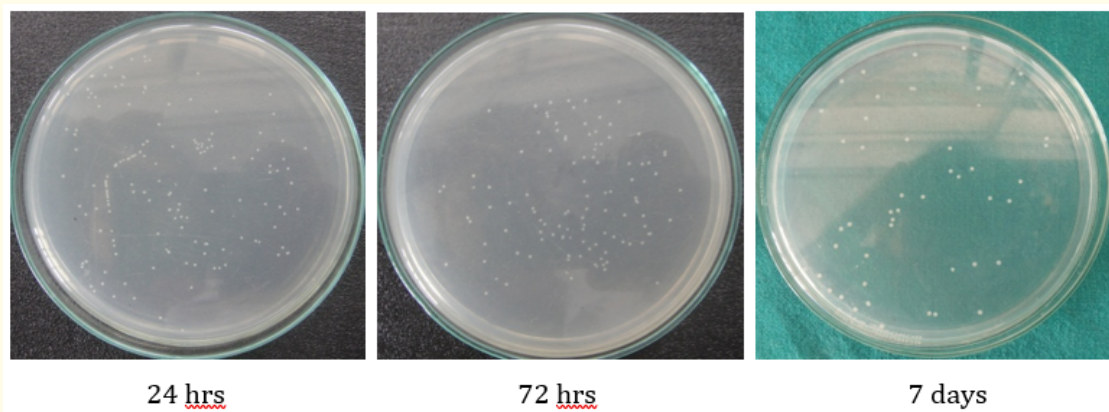
Group	24 hrs	72 hrs	7 Days
Normal saline (NS)	183.6 ± 42.5	186.6 ± 55.1	175.8 ± 26.8
Calcium hydroxide(CH)	113.2 ± 32.6	90.00 ± 26.3	68.6 ± 17.8
2% Chlorhexidine gel (CHX)	8.6 ± 10.1	13.2 ± 13.06	14.6 ± 17.2
<i>Aloe vera</i> extract (AV)	38.8 ± 20.2	102.8 ± 18.9	159.6 ± 26.01
F value	36.77	23.6	58.00
P value	0.000	0.000	0.000
Significant pairs in Post Hoc Tukey test	NS vs CH; NS vs CHX; NS vs AV; CH vs NS; CH vs CHX; CH vs AV; CHX vs NS; CHX vs CH; AV vs NS; AV vs CH	NS vs CH; NS vs CHX; NS vs AV; CH vs NS; CH vs CHX; CHX vs NS; CHX vs CH; CHX vs AV; AV vs NS; AV vs CHX	NS vs CH; NS vs CHX; CH vs NS; CH vs CHX; AV; CHX vs NS; CHX vs CH; CHX vs AV; AV vs CH; AV vs CHX

**Table:** Descriptive and Comparative Analysis showing Mean ± Standard Deviation of CFU of groups at different time intervals of 24 hrs, 72 hrs and 7 days.

**Discussion**

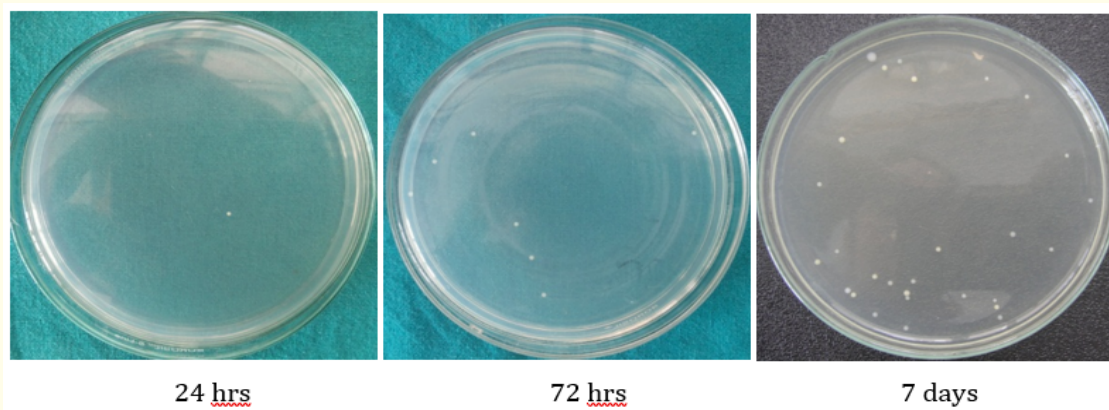
The aim of this *in-vitro* study is to compare the antimicrobial efficacy of *Aloe-vera* gel, chlorhexidine gel and calcium hydroxide as intracanal medicaments against *E. faecalis*.

The calcium hydroxide group was used to compare with newer intracanal medicaments because it is widely used in Endodontics. In calcium hydroxide, the mean CFU was found highest at 24 hrs and least at 7 days (Figure 1). Even though calcium hydroxide does have some antibacterial action, under the experimental run, it was not able to eliminate sufficient cells of *E. faecalis* at any time. This is in accordance with the findings of other studies. The antimicrobial efficacy of calcium hydroxide is lower in comparison to chlorhexidine gel due to the buffering of the alkalinity of calcium hydroxide by dentin and dentin components, low diffusibility of hydroxyl ions in dentinal tubules and colonization of *E. faecalis* within dentinal tubules forming dense biofilm. Calcium hydroxide have shown lower efficacy than chlorhexidine gel in all time intervals.



**Figure 1: Calcium hydroxide.**

The present study revealed that highest antibacterial activity was observed with 2% chlorhexidine gel in comparison to other groups in all the time intervals (Figure 2). The antimicrobial efficacy of 2% chlorhexidine gel may be due to the high concentration, lethal bactericidal mode of action and enhanced diffusion into dentinal tubules as chlorhexidine gel has low contact angle with dentin and thus penetrates the dentinal tubules effectively at faster rate [9,10]. 2% Chlorhexidine gel was not able to eliminate *E. faecalis* completely as it may be due to the inhibitory effect of dentin, other molecules as serum albumin and collagen as well as killed microbes present in the root canal which can alter the efficacy of intracanal medicaments.



**Figure 2: Chlorhexidine.**

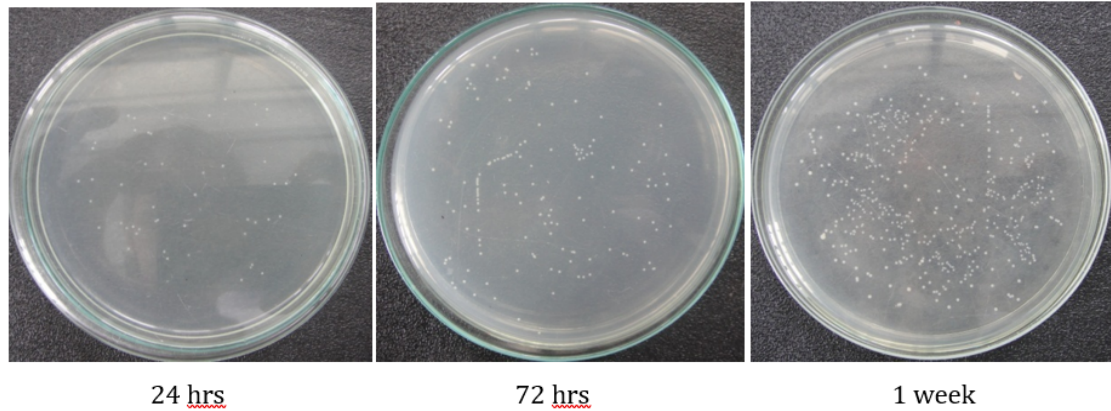
In developing countries, about 80% of the populations rely on traditional medicine for their primary health care [11]. Due to the side effects and the resistance that pathogenic microorganisms build against the common antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plants used in herbal medicine. The use of intracanal medicaments made of natural extracts is of great importance as the shift is toward natural health remedies both for patient and endodontic professionals [12]. So, antimicrobial efficacy of *Aloe-vera* extract (Figure 3) has been compared with calcium hydroxide and chlorhexidine in this study.



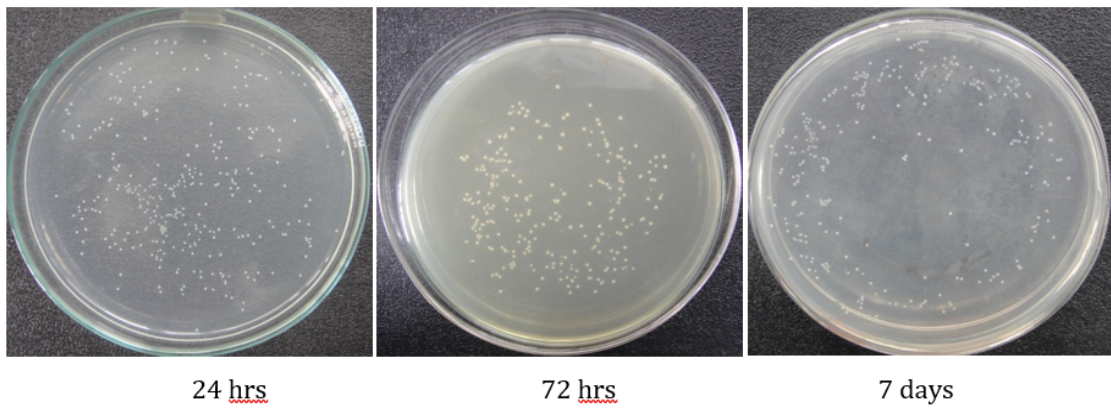
**Figure 3:** *Aloe vera* leaf and extract.

The leaf of *Aloe-vera* contains some active substances like acemannan, anthraquinone, anthracene, cinnamonic acid with anti-inflammatory, antimicrobial properties [13,14]. Anthraquinones are the phenolic compounds present in the sap or yellow exudates of leaf or *Aloe-vera* latex and are potent antimicrobial and powerful analgesic agents. Natural extract of *Aloe-vera* is always better than commercially available *Aloe-vera* products [11]. So, pure form of *Aloe-vera* has been used in this study. The reserve parenchyma of *Aloe-vera* has been shown to stimulate cell proliferation and contribute to healing and angiogenesis, as well as act as an anesthetic, anti-bacterial, anti-fungal, anti-viral and anti-inflammatory.

*Aloe-vera* extract showed good antimicrobial efficacy in 24 hrs when compared to control group, however its efficacy decreases in 3<sup>rd</sup> day followed by 7<sup>th</sup> day (Figure 4). The mean CFU of *Aloe-vera* extract is comparable with 2% chlorhexidine gel in 24 hrs. Similar effect was found in study conducted by A Bhardwaj, *et al.* against *E. faecalis*. This decreased effect of *Aloe-vera* after 24hrs can be due to buffering ability of dentin and due to dissolution nature of active ingredients of *Aloe-vera*. The antimicrobial efficacy of *Aloe-vera* may be due to presence of 75 potentially active constituents: vitamins, enzymes, minerals, sugar, lignin, saponins, salicylic acid, and amino acids [15,16].



**Figure 4:** *Aloe vera* extract.



**Figure 5:** Control group.

Although, the present study has shown 2% chlorhexidine gel to be the most effective antibacterial agent at all time intervals. Along with it, the study also showed better efficacy of *Aloe-vera* extract in comparison to other intracanal medicaments for antimicrobial treatment of root canal infection. However, the systemic effect of this medicament, its biocompatibility, allergic potential and bacterial resistance needs further investigation.

### Conclusion

Under the limitations of this study, it can be concluded that *Aloe-vera* extract is effective against *E. faecalis* but for shorter duration and therefore, there is a need of advanced research to explore the further improvement in the antimicrobial efficacy of *Aloe-vera* for longer period. The current study will serve as baseline for future *in-vivo* studies to evaluate the effect of *Aloe-vera* on microorganisms.

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