

Comparative Evaluation of Antimicrobial Efficacy of Chlorhexidine and Herbal Root Canal Irrigant *Aloe vera* against *Enterococcus faecalis*: An *in vitro* Study

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ABSTRACT

Root canal therapy provides appropriate environment for tissue healing by complete elimination of microorganisms from the root canal. The most commonly found bacteria in failed root canal is *Enterococcus faecalis*. Cleaning and shaping of canals along with chemical disinfection results in elimination of microorganism. In recent years, there has been increasing interest in the use of herbs in endodontics as root canal irrigants. This study is designed to explore the antimicrobial efficacy of different concentrations of chlorhexidine (CHX) and *Aloe vera* extracts as a root canal irrigant against *E. faecalis* using agar diffusion method.

Keywords: *Aloe vera*, Antimicrobial, Chlorhexidine, Herbal, Root canal irrigants.

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INTRODUCTION

The complete elimination of microorganisms from the root canal and the three-dimensional obturation of the canal space results in a successful root canal treatment.¹ Pulpal and periradicular pathosis occurs due to invasion of the root canal by microorganisms. *Enterococcus faecalis* is the most commonly isolated microorganism from failed root canals. It is an anaerobic Gram-positive bacterium that has the ability to invade dentinal tubules, and its existence does not depend on the presence of other bacteria. It is highly resistant to intracanal medicaments which lead to its virulence, allowing it to have obligatory role in persistent failure of endodontic therapy.² Cleaning and shaping of canals alone is not sufficient for

the success of an endodontic treatment. It should always be assisted with chemical debridement, which consists of using various irrigating solutions.³ Sodium hypochlorite is the most commonly used root canal irrigant due to its various advantageous properties. Disadvantages include the allergy, sodium hypochlorite accident, metallic taste, and toxicity.⁴

Chlorhexidine is a broad spectrum antimicrobial that has been used as irrigant due to its sustained broad spectrum action and low toxicity. Chemically, CHX is a synthetic cationic biguanide that owes its effectiveness due to its positive charge which interacts with the negative charge of the phosphate group on the microbial wall, resulting in alteration of osmotic equilibrium of the cell.⁵

The use of herbal products as irrigants is gaining acceptance due to its safety, efficiency, and acceptability. One such herb that has been investigated is *aloe vera* (*Aloe barbadensis* Miller), owing to its antibacterial effect against *E. faecalis*.⁶ There is a deficiency of adequate studies regarding the use of *Aloe vera* as a root canal irrigant. Hence, this study was undertaken to evaluate the antimicrobial efficacy of different concentrations of *aloe vera* against CHX using agar diffusion test.

MATERIALS AND METHODS

Source of Data

The bacterial stock culture of *E. faecalis* strain (American Type Culture Collection, ATCC 29212) was obtained from JP Laboratories, Davangere, India, and the extracts of *Aloe vera* were prepared from Bapuji Pharmacy College, Davangere, Karnataka, India.

Aloe vera Extract Preparation

Leaves were taken from fresh *Aloe vera* plants and its pulp was extracted by hand. *Aloe vera* plant pulp was then mixed with chloroform water, i.e., 2.5 mL of chloroform in 1000 mL of purified water (Indian Pharmacopoeia) and the mixture was filtered using a double-filter paper, and the supernatant was then centrifuged at 8,000 rpm for 40 minutes to obtain the extract (Fig. 1).

The concentrated solutions thus prepared from all the above ingredients were weighed and using distilled

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Fig. 1: Aloe vera extract

water, serial dilutions of 5/95, 25/75, 50/50, and 100 mL (volume/volume) were made in order to obtain 5, 25, 50, and 100% concentrations respectively, for the evaluation of antimicrobial activity against *E. faecalis*. The antimicrobial testing was done on the same day of extracts prepared.

Agar Diffusion Test

The bacterial stock culture of *E. faecalis* (ATCC 29212) was obtained from JP Laboratories, Davangere, Karnataka, India. The standard strain of *E. faecalis* (ATCC 29212) was grown on brain heart infusion broth overnight and turbidity was adjusted to 0.5 McFarland scale to obtain a cell density of 1.5×10^8 bacterial/mL and inoculated in Mueller Hinton agar plates. Inoculation was performed by using sterile swab brushed across the media. Four round wells measuring about 4 mm deep and 8 mm in diameter were punched using a sterile stainless steel template and they are numbered as 1, 2, 3, and 4 consecutively for the different concentrations used for the each test group to be evaluated. As control, 0.2% CHX gluconate solution was taken for evaluation.

Group I was allocated for CHX (control) and group II was *Aloe vera* extract consisting of 15 inoculation agar plates in each group. After making serial dilutions of the extract and four round wells in each agar plate, 50 µL of specific concentration of each extract was dispensed into

each well using a sterile micropipette. This was done in triplicate for every concentration so as to overcome any inadvertent technical errors. This was done for each group in the same way. All agar plates were then incubated at 37°C for 24 hours, according to Clinical Laboratory Standard Institute guidelines. Following 24 hours of incubation at 37°C, zones of inhibition (i.e., areas where no growth of bacteria is present) were examined around each well. These zones appeared as a clear, circular halo surrounding the wells. Diameters of the bacterial growth inhibition zones or halos were measured using a Hi Antibiotic Zone scale in millimeters and this represented the inhibition value.

RESULTS

The antibacterial activity was evaluated using agar well diffusion test by measuring the zones of inhibition around each of the four wells. The mean zone of inhibition was measured for each sample and the results obtained are listed in Table 1. The results were subjected to statistical analysis by applying analysis of variance (ANOVA) and *post hoc* Tukey’s highest significant difference (HSD) tests for multiple comparisons. On applying one-way ANOVA, a statistically significant difference was seen between the zones of inhibition of different samples within groups and between groups, i.e., $p < 0.001$.

The mean zone of inhibition for positive control, i.e., group I (0.2% CHX), was 18.36 mm, with which all other values were compared.

Comparison between groups I (0.2% CHX) and II (*Aloe vera*) with different concentrations of 5, 25, 50, and 100% was 0.52, 0.98, 3.02, and 5.43 mm, respectively (Tables 2 and 3), which was less than group I (0.2% CHX) –18.36 mm and the difference was statistically significant because of p -value < 0.001 , which is tabulated in Table 1 and shown in Graphs 1 and 2.

It is seen that *Aloe vera* did not have mean zone of inhibition more than group I (0.2% CHX). It showed minimum effectiveness at 100% concentration.

DISCUSSION

The prime objective of root canal treatment is to eradicate the microorganisms from the root canal and to intercept

Table 1: Zone of bacterial inhibition after 24 hours

Groups	Concentration	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10	Plate 11	Plate 12	Plate 13	Plate 14	Plate 15
Group I CHX (control)	0.2%	18.5	18.6	18.9	18.3	17.8	18.4	18.2	18.5	18.2	18.7	18.4	18.4	17.7	17.9	18.9
Group II <i>Aloe vera</i>	5%	0.5	0.3	0.8	0.6	0.5	0.5	0.50	0.3	0.2	0.8	0.9	1	0.5	0.3	0.2
	25%	1	1.1	1	1	1	1.2	1.3	1.6	0.5	0.5	0.8	1	1	1.2	0.6
	50%	3	3	3	3.5	3.5	3.6	3	2.5	2.5	2.5	3	3.1	3	3.1	3
	100%	5.5	5.5	5.3	5	5.6	5	5.9	5.1	5.5	5.3	5.9	5.5	5.6	5.9	4.9

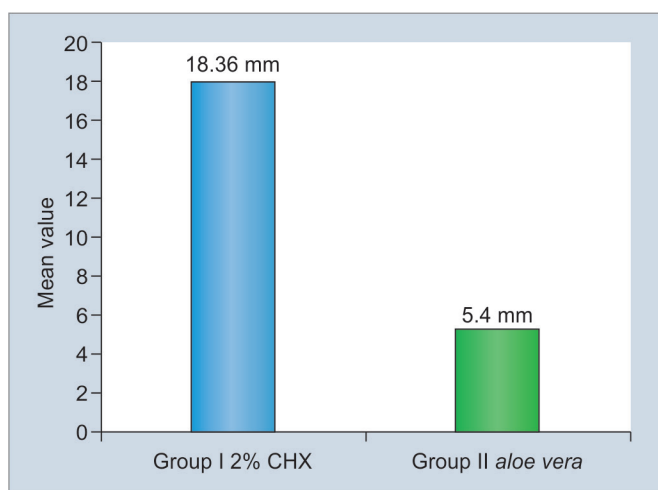
Table 2: ANOVA for group I (0.2% CHX), with group II (Aloe vera; including all concentrations)

Analysis of variance					
Aloe vera extract	Sum of squares	Degree of freedom (df)	Mean square	f-value	Significance
Between groups	3247.530	4	811.882	8.004E3	0.000
Within groups	7.100	70	0.101		
Total	3254.630	74			

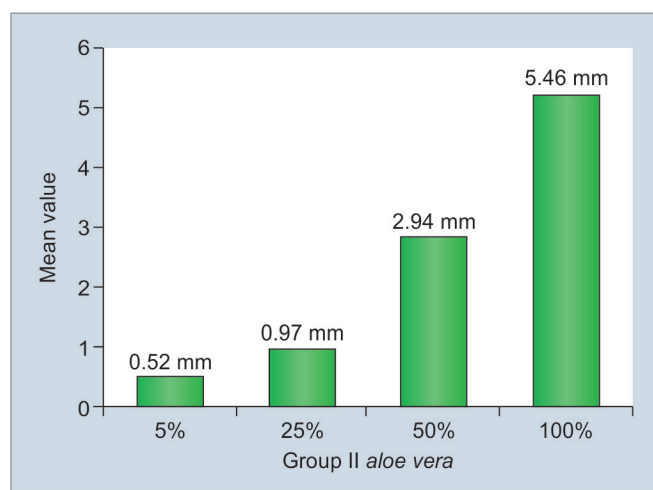
Table 3: Post hoc Tukey HSD tests (multiple comparisons)

	Concentration	Mean ± SD	Mean difference (I–J)	Significance
Group I (0.2% CHX)	5%	0.53 ± 0.25	17.83333*	0.000**
	25%	0.99 ± 0.30	17.37333*	0.000**
	50%	3.02 ± 0.34	15.34000*	0.000**
	100%	5.43 ± 0.33	12.92667*	0.000**

*The mean difference is significant at the 0.05 level; **p<0.001, significant; SD: Standard deviation



Graph 1: Comparison of group I (0.2% chlorhexidine) with group II (Aloe vera) extract



Graph 2: Zone of inhibition at different concentrations of group II (Aloe vera) extract

their recontamination in the posttreatment period. Hence, irrigant solutions must accompany the action of the mechanical instruments to ensure the complete cleanliness of the canal.⁷

The success of endodontic treatment can be seen in the quality of the obturation. Root canals that are unsatisfactorily obturated provide greater space and nutrition than well-obturated canals, and the available space may create a facultative anaerobic environment. In contrast, well-obturated canals maintain an obligate anaerobic environment that does not favor the survival and growth of *E. faecalis*. Inadequate cleaning and shaping may also have left infected debris behind. Microorganisms, such as *E. faecalis* can survive within the small canals of apical ramifications or in the space between the root filling and canal wall. In fact, *E. faecalis* strains can survive for at least 6 to 12 months in an environment where nutrients are scant and when commensality with other bacteria is reduced. *E. faecalis* is also extremely resistant to chemicals, including calcium hydroxide.⁸

The property of substantivity allows prolonged time of action for CHX. Chlorhexidine is less toxic compared with sodium hypochlorite and does not have foul taste. It has excellent antibacterial, antifungal property and acts on the biofilm.⁹ It has been proposed that CHX should be used in open apex cases. However, disadvantages include inferior tissue dissolving properties and discoloration of teeth.¹⁰

Aloe vera is a naturally occurring herbal medicament having antibacterial, anti-inflammatory, antibacterial, antifungal, and antiviral properties. The presence of anthrax quinone allows it to inhibit *E. faecalis* and *Streptococcus pyogenes*.¹¹ Karkare et al¹² concluded that *Aloe vera* showed the highest zone of inhibition against *E. faecalis*, which is in contrast to our results. Our results are similar to those of Babaji et al¹³ and Jose et al,¹⁴ who have shown that CHX has superior antimicrobial efficacy when compared with *Aloe vera*. The probable reason for the decreased antimicrobial efficacy of *Aloe vera* could be due to change in weather where the plant was grown and where it was prepared.¹¹

Limitations of the study are that it was an *in vitro* study with a limited sample size, and biofilm protection produced by endodontic microflora could not be employed.

CONCLUSION

Aloe vera showed inhibitory zone against *E. faecalis*. Hence, these can be used as root canal irrigating solutions. Further *in vivo* research is required to test these herbal medicines and to modify its content for acceptability by patients.

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