Original Article

Antimicrobial Effects Of Three Different Herbal Intracanal Medicament Gels On Enterococcus Faecalis: A Comparative Invitro Study

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Abstract

The antimicrobial efficacy of Triphala, Aloevera and Curcumin against Enterococcus faecalis have been established in previous studies, However, no comparative study with Chlorhexidine have been done so far.

Methods and Material: Root canals of one hundred and forty-five extracted, straight single-rooted human teeth were contaminated with Enterococcus faecalis and initial bacterial count was determined after 4 weeks using CFU counts. Samples were then divided into five groups- Group 1- Saline, Group 2-Chlorhexidine, Group 3- Triphala, Group 4- Aloevera, Group 5- Curcumin. 1 ml of the prepared medicament was injected into the canals and after 7 days of incubation, the colony counts were performed. Dentin shaves were removed with Gates Glidden drills and a BHI broth saturated paper point and checked for turbidity using spectrophotometer.

Results: The mean percentage reduction in bacterial count was greater in Group 2-Chlorhexidine (89.01%) followed by Group 3-Triphala group (70.99%), Group 5 – Curcumin (44.10%) and Group 4- Aloevera (38.44%).

Conclusion: Chlorhexidine gluconate gel still remains as the gold standard against E. faecalis. Triphala and Curcumin also showed good antibacterial efficacy against E. faecalis.

Keywords: Aloevera, Curcumin, Enterococcus faecalis, Intracanal medicament, Optical density, Triphala.

I. INTRODUCTION

The main objective of endodontic treatment is to eradicate the microorganisms from the root canal system. The non-surgical endodontic treatment is considered as a predictable and reliable method with success rate of 86% to 98%.[1]
Even after following a proper cleaning and shaping protocol, it is important to understand that, not all of the bacterial niches get cleared off. Enterococcus faecalis is the most frequently identified endodontic pathogen in canals of root-filled teeth with periapical lesions either by culture or molecular methods.[2] The resistance of Enterococcus faecalis to root canal treatment and various antimicrobial agents is due to its ability to survive in very harsh environments, with poor nutrient supply and high alkaline pH reaching up to 11. It penetrates the dentinal tubules even up to the full length. It forms a biofilm on root canal walls and emerge as a mono-infection in treated canals without synergistic support from other bacteria.[3]

Currently none of the routinely used medicaments are able to completely eradicate Enterococcus faecalis from the root canal. Chlorhexidine is considered as a gold standard against Enterococcus faecalis.[4] This is due to its cationic bisbiguanide molecular structure and substantivity. It is bacteriostatic at lower concentrations and bactericidal at higher concentrations. But it has certain disadvantages including lack of tissue solubility, sensitivity to organic load, and inactivation by dentine.

In last few decades, the use of alternative therapeutic agents derived from plants, insects, microorganisms, etc. are a part of a growing trend to seek natural remedies in dental treatment. The use of natural derivatives may have a greater level of tolerance by the body with exhibition of fewer side effects; thereby preventing incidence of antibiotic abuse.[5] Plant products like Triphala, aloe vera, curcumin etc. have been studied for their antimicrobial properties against bacteria causing dental caries and peri radicular pathology. [6]

The antimicrobial efficacy of Triphala, Aloevera and Curcumin against Enterococcus faecalis have been established in previous studies, however no comparative study with Chlorhexidine have been done so far. Hence the present study intends to evaluate the antimicrobial efficacy of different herbal derivatives like Triphala, Aloe vera and Curcumin in comparison with Chlorhexidine as an intracanal medicament against Enterococcus faecalis.

II. MATERIALS AND METHODS

The study was conducted after approval of the institutional ethical committee. (IECno152/2019/DCC dated 14/11/2019).

III. PROCUREMENT OF THE MICROORGANISM

A pure culture of E. Faecalis (ATCC 29212) (figure 6) HI Media Mumbai, was inoculated on blood agar plates, incubated at 37 ^{0} C overnight for 24 hrs.[7]

IV. PREPARATION OF TOOTH SAMPLES

One hundred and forty-five extracted, straight single-rooted human teeth were collected. Teeth with curved roots, canal calcifications and root caries were excluded from the study. Collected teeth were placed in 5.25% NaOCl for 1 hour in order to disinfect the root surfaces and the samples were stored in 0.9% physiological saline. The crowns were cut perpendicular to the long axis of the teeth from cementoenamel junction (CEJ) with a diamond disc in conjunction with physiological saline irrigation and the root lengths were cut and standardized to 16 mm. The apex of all the teeth were sealed with araldite to prevent bacterial leakage. The root canals were instrumented using the ProTaper rotary system (figure 14) (Dentsply Maillefer, Ballaigues, Switzerland) with 5.25% NaOCl irrigation. After root canal preparation to size F3, the samples were irrigated with ultrasonic activated 17% ethylenediaminetetraacetic acid (EDTA) for smear layer removal. In order to complement the effects of EDTA, a flush with 5.25% NaOCl for 5 min was done. Finally the remnants of EDTA and NaOCl, were removed, rinsing with 10 mL of physiological saline.

V. Preparation of tooth samples for microbial inoculation

Before microbial inoculation, each tooth specimen was individually placed in Eppendorf tube with numbering on each section, and autoclaved twice with 24hour interval in between at 121^{0}Cat 15 psi for 20 minutes to sterilize the root canals. Three consecutive sterile paper points#20 were introduced into the root canal to absorb the canal contents. These paper points were transported in sterile saline (1.5ml) in individual sterile glass test tubes, vortexed, cultured and confirmed for zero colony forming units (CFU). After confirming sterility of root canals, microbial inoculation in each root section was done.[8]

VI. Microbial inoculation

The following procedural steps were carried out using sterile materials and instruments. The working surface was swabbed with 2% Lysol 30 minutes before the procedure. Each root specimen was placed in a microtube containing 2 mL of BHI (brain-heart infusion) broth (HI media lab, Mumbai) and then autoclaved twice for 30 min at temperature of 121° C and pressure of 15 PSI. After that, samples were stored in an incubator at 37° C for 24h. Sterile BHI(1ml) was removed using sterile micropipettes from microtubes and then it was replaced by 1 mL of E. Faecalis suspension with standard concentration of 0.5 McFarland (1.5×108 CFU/ml). The tubes were closed and incubated at 37° C for 1 day. Bacterial viability and purity were checked in 3 randomly-picked tubes. After that, samples were incubated for 21 days at 37° C. During incubation period, in order to prevent dehydration of the samples, BHI culture was changed every 3 days. Three consecutive sterile paper points#20 were introduced into the root canal to absorb the canal content. These paper points were transported, to collect the bacteria either in planktonic or in biofilm forms and transferred to a Blood agar culture plate. Bacterial growth was measured by the CFU counts of E. Faecalis, which was confirmed by colony morphology and gram stain.

VII. PREPARATION OF ALOVERA

Aloevera leaves were cut into small pieces, with a knife and split in the centre. The pulp of the leaves was scraped out and ground in a grinder to form a paste which was used to make the gel.[9]

VIII. PREPARATION OF GEL FORM OF EXTRACT

In order to prepare gel form of medicaments, ethyl cellulose was used as an inert carrier. One g of ethyl cellulose gel was separately added to 1 g of triphala, 1 g of 2% CHX solution, 1 g of curcumin, and 1 g of aloe vera. Also 1 g of ethyl cellulose was added to physiological normal saline as a negative control group. In order to gain a gelatinous form, 3 drops of viscous polyethylene glycol 400 (PEG, Dae Jang, Gyeonggi-do, Korea) was added to each medicament.

IX. INOCULATION OF TEST MEDICAMENT:

Standard volume of each prepared medicament(1ml) was injected under stable pressure into 29 infected roots of each group from coronal to apical using 2ml syringe with an etch tip of 23 gauge.
Coronal access was sealed with temporary cement (IRM Dentsply). After all these procedures samples are to be placed in an Eppendorf tube and incubated at 37° C for 7 days. After 7 days of incubation, the temporary restorative material cement was removed and the intracanal medicaments removed from canals by irrigation with 10 mL of normal saline. Three consecutive sterile paper points#20 were introduced into the root canal to absorb the canal content and then transferred to a Blood agar culture plate. The colony counts were performed to all the samples.

X. ASSESSMENT OF ANTIBACTERIAL ACTIVITY:

The antibacterial efficacy is measured by comparing the percentage reduction in colony count (%RCC) before and after intracanal medicament.[10]
The percentage (%) reduction in bacterial count was calculated using the following formula

$$\% \text{ Reduction in E. faecalis colony count} = \frac{\text{Initial count} - \text{Final count}}{\text{Initial count}} \times 100$$

XI. PREPARATION OF THE TOOTH FOR SPECTROPHOTOMETRY:

After that, apical third of roots were drilled by #2 and 4 Gates Glidden drills (Dentsply, Maillefer, Ballaigues, Switzerland) to the depth of 2-4 mm into dentinal walls. Dentin shaves were removed with Gates Glidden drills and a BHI (brain-heart infusion) broth (HI medi lab, Mumbai) saturated paper point. Prepared paper points were transferred to test tubes containing 2 mL of BHI aqueous culture and incubated for 24 hours at 37° C. to check for turbidity Turbidity test of tubes was measured by Spectrophotometer (systronics) The intensity of turbidity was checked by the optical density in spectrophotometer which corresponded to the amount of residual bacteria present in the root canals after placement of medicament. (OD = 650 nm).[11]

XII. Statistical analysis

Statistical tests such as Kruskal Wallis test, and median was performed. Optical density values were analysed with one-way analysis of variance (ANOVA) and comparison between groups was done by Tukey's post hoc test.

XIII. RESULTS

The mean percentage reduction in bacterial count was greater in Group 2- Chlorhexidine (89.01%) followed by Group 3- Triphala group (70.99%), Group 5 – Curcumin (44.10%) and Group 4- Aloevera (38.44%). The differences in mean of bacterial colony reduction among the five groups were statistically significant.

Intergroup comparison revealed that the reduction in Enterococcus faecalis CFUs was statistically significant when Triphala was compared with Chlorhexidine, Aloevera, Curcumin ($p < 0.001$). Reduction in bacterial count was greater in Group 2- Chlorhexidine followed by Group 3- Triphala group, Group 5 – Curcumin and Group 4- Aloevera.

The differences in mean of the culture density among the five groups were statistically significant. Based on Post Hoc test done for overall comparison between each group, Group 1 (Saline): Saline showed highest optical density (1.8137), implies to highest bacterial count and least effective than all other groups. Chlorhexidine showed lowest optical density (0.0851), implies to lowest bacterial count and most effective than all other groups.

Among herbal intracanal medicament group 3 Triphala showed lowest optical density (0.2914), implies to lowest bacterial count and Aloevera showed highest optical density (1.6245) implies to highest bacterial count. Curcumin showed moderate optical density when compared to Triphala and Aloevera.

XIV. DISCUSSION

Complete disinfection of the root canal space is an important prerequisite for achieving long term success of nonsurgical endodontics. The use of a biocompatible intracanal medicament possessing antimicrobial properties between appointments may reduce or eliminate bacteria in the root canal system and increase the success of root canal treatment.

This invitro study has assessed the antimicrobial effects of three different herbal intracanal medicament gels (Triphala, Aloevera and Curcumin) against Enterococcus faecalis, keeping chlorhexidine and saline as control. Bacterial count reduction was assessed using colony count reduction percentage and optical density using spectrophotometer. Results showed that Chlorhexidine has the highest bacterial count reduction followed by Triphala, Curcumin and Aloevera respectively. Saline showed the least potential among the five groups.

Single rooted mandibular premolar with straight canals were preferred in this study as curved root canals are associated with a more complicated curvature determination and distribution process and with an increased risk of intracanal procedural accidents such as, zipping, ledging and root perforation. This was in accordance with the study done by Prasanna Neelakantan et al[12], D. Kandaswamy et al[13], Anuj Bhardwaj et al.[14]

The study samples were decoronated with diamond disc to leave a root length of length 16mm to minimize variation by eliminating some variables, like crown anatomy and root length. [15]

Cleaning and shaping was performed by the crown down technique using the protaper rotary system, based on similar study done by J. Prabhakar et al.[16] According to Shuping.G et al[17], cleaning and shaping with rotary nickel titanium instruments resulted in reduced bacterial count.
E. faecalis was chosen in this study as it is the most common Enterococcus sp. persisting in treated root canals and are resistant to traditional antibiotics. When E. faecalis grows as a biofilm, the altered genetic and metabolic processes of bacteria along with its complex matrix prevent the entry and action of several antimicrobial agents.[18]

E. faecalis was cultured in the tooth samples for a period of 21 days to form biofilm in the tooth samples. It has been suggested that biofilm grown cells are 1000 to 1500 times more resistant than planktonically grown cells.[19] This may turn the same medicament which showed activity against planktonic bacteria to be inactive in clinical scenario. The nature of the substrate may also influence the nature of the biofilm, so we opted to test the activity of the herbal extracts against the biofilm produced in teeth samples, in accordance with study done by J. Prabhakar et al.[20]

The medication period was standardized in this study for one week according to Sjeogren et al, [21] who stated that medicament has to remain within the canals at least for one week in order to achieve the optimal antimicrobial efficacy. Basrani et al [22] also reported that for induction of CHX substantivity, dentin should be treated for seven days.

Colony count reduction percentage and optical density measurement by spectrophotometer was used in this study. Microbiological root canal culturing is commonly used to assess the effectiveness of endodontic treatment measures. According to the studies done by Upadhyay et al[23], AR Prabhakar et al[24], Bhardwaj, et al[14], Nidhi Sinha et al[25], Colony Counting was chosen to evaluate the antibacterial efficacy of intracanal medicaments as this would signify the quantity of live residual bacteria present in the root canals. The advantage of this method is that it allows direct comparison of the materials against the organisms, indicating which material has the potential to eliminate bacteria in the local microenvironment of the root canal system. However, the disadvantage is that the result not only depends on the toxicity of the material for the particular organism but is also influenced by the ability of the material to diffuse across the medium.[26] The infected samples after the incubation of organism were subjected to four intracanal medicaments. Bacterial colony count reduction and the optical density were evaluated seven days after medicament placement.[27] This was done to facilitate evaluation of bacterial re-growth within the samples after the placement of medicament.

In the present study chlorhexidine (CHX) was used as positive control. Chlorhexidine (CHX) is a broad-spectrum antimicrobial agent. Its antimicrobial action is related to its cationic bis- biguanide molecular structure. At low concentration it is bacteriostatic while at higher concentration it is bactericidal as it brings about coagulation and precipitation of cytoplasm. CHX gluconate gel has been extensively used in dentistry mainly as an intracanal medicament. The results of this study showed higher bacterial colony reduction percentage mean (0.89157) and lower optical density mean (0.0851) values were recorded in the positive control group chlorhexidine. This result is in accordance to R Brar et al[28], Anu Bhardwaj et al[14], AR Prabhakar et al[29]. This is contradictory to the study by Prasanna Neelakantan et al[30] who concluded Curcumin more efficacious as an inhibitor than Chlorhexidine, the reason could be the difference in methodology and evaluation method used.

In endodontics because of the cytotoxic reactions of most of the commercial intracanal medicaments used and their inability to eliminate bacteria from dentinal tubules, trend of recent medicine, attends to use biologic medication extracted from natural plants. The main advantages of using herbal alternatives are easy availability, cost effectiveness, increased shelf life, low toxicity and lack of microbial resistance. The antimicrobial action of herbal derivatives like triphala, aloe vera, curcumin as intracanal medicaments was compared in this study.

Triphala is a well-known ayurvedic herbal formulation consisting of the dried and powdered fruits of three medicinal plants, namely Terminalia bellerica, Terminalia chebula and Emblica officinalis with tannic acid being its principal constituent. study done by Kaczmarek B et al[31] have shown bacteriostatic or bactericidal effects of tannic acid on gram-positive and gram-negative pathogens. The most important advantages of triphala include easy access, low cost, long-term stability, less toxicity and absence of microbial resistance. It has anti-cariogenic and thermogenic effects and can act as a probiotic. The optical density and bacterial colony reduction that were obtained in this study after disinfection with Triphala was statistically significant from Curcumin and Aloevera against E. faecalis. This correlates to the study done by R Brar et al[28] who concluded that Triphala was more efficacious as an inhibitor than Curcumin but the co-relation suggests that neither are as efficacious against E. faecalis as chlorhexidine.

Aloevera belongs to the Liliaceae family and genus Aloe. The species was first described by Carl Linnaeus in 1753. It contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, anthraquinones, saponins, salicylic acids and amino acids. Total leaf extracts contain anthraquinones, which have antibacterial properties. In our study Aloevera was found less efficacious as an inhibitor of E. faecalis bacteria than Chlorhexidine. This result is in accordance to Leila

Bazvand et al[32] who concluded that Aloe vera had antibacterial effects on *E. faecalis*, but in comparison with chlorhexidine, it was less effective ($P < 0.05$).

Curcumin (diferuloylmethane), the main yellow bioactive component of turmeric, has been shown to have a wide spectrum of biological actions, including antimicrobial, anti-inflammatory and antioxidant activities. The result of our study is in accordance to invivo study by Vasanta R Digole et al[33] who evaluated the antimicrobial efficacy of curcumin, aloe vera and calcium hydroxide as an intracanal medicament for endodontic disinfection.

XV. CONCLUSION

The differences in bacterial reduction percentage value among the groups in this study were found to be statistically significant. p value less than 0.05. Higher bacterial colony reduction (percentage) was recorded in chlorhexidine (0.89157) and the lower bacterial colony reduction (percentage) was recorded in saline (0). Among herbal medicaments higher bacterial colony reduction (percentage) value was recorded in the Triphala group (0.7092), followed by Curcumin group (0.44) and Aloevera group (0.3844) respectively. Higher optical density mean value was recorded in saline (1.8137) and the lower optical density mean value was recorded in chlorhexidine (0.0851). Among herbal medicaments lower mean value was recorded in the Triphala group (0.2914), followed by Curcumin group (1.2541) and Aloevera group (1.6245) respectively. The difference in mean value among the groups were found to be statistically significant. The optical density corresponds to the bacterial count so the group with lower optical density has lower bacterial count, thus the most effective intracanal medicament was chlorhexidine followed by triphala, curcumin and Aloevera respectively.
Further studies should be carried out under in vivo conditions to confirm the clinical efficacy of these herbal products as intracanal medicaments in endodontics

Table I

Reduction (Percentage)						
	N	**Mean**	**Median**	**Std. Deviation**	**Minimum**	**Maximum**
Group 1 - Saline	29	0	0	0	0	0
Group 2 -Chlorhexidine	29	0.8915	1	0.30927	0	1
Group 3 - Triphala	29	0.7092	0.987	0.42695	0	1
Group 4 -Aloevera	29	0.3844	0.2	0.42108	0	1
Group 5 -Curcumin	29	0.44	0.3	0.4345	0	1
Total	145	0.485	0.46	0.46738	0	1
Test	Kruskal-Wallis test					
p-value	**<0.001**					
Inference	There exists significant difference					

Table I. Descriptive analysis of the five groups of intra canal medicaments against E. faecalis which was done using KRSUKAL – WALLIS TEST

Table II

	N	**Mean**	**Std.Deviation**	**Minimum**	**Maximum**
Optical density of Saline	29	1.8137	0.11526	1.62	1.98
Optical density of Chlorhexidine	29	0.0851	0.02644	0.04	0.12
Optical density of Triphala	29	0.2914	0.20013	0.12	0.98
Optical density of Aloevera	29	1.6245	0.12334	1.35	1.83
Optical density of Curcumin	29	1.2541	0.22028	1.02	1.66
Total	145	1.0137	0.71921	0.04	1.98
Test	ONE WAY ANOVA				
p-value	**<0.001**				
Inference	There exists significant difference				

Table II. Descriptive analysis of the five groups of intra canal medicaments against E. faecalis which was done using one way ANOVA

Table III.

POST HOC ANALYSIS			
Tukey HSD			
(I) Group	(J) Group	Mean Difference (I-J)	p-value
Optical density of saline	Optical density of Chlorhexidine	1.72855*	**<0.001**
	Optical density of Triphala	1.52228*	**<0.001**
	Optical density of Aloevera	.18917*	**<0.001**
	Optical density of Curcumin	.55959*	**<0.001**
Optical density of CHX	Optical density of Saline	-1.72855*	**<0.001**
	Optical density of triphala	-.20628*	**<0.001**
	Optical density of aloevera	-1.53938*	**<0.001**
	Optical density of curcumin	-1.16897*	**<0.001**
	Optical density of saline	-1.52228*	**<0.001**
	Optical density of CHX	.20628*	**<0.001**

Optical density of triphala	Optical density of aloevera	-1.33310*	**<0.001**
	Optical density of curcumin	-.96269*	**<0.001**
Optical density of Aloevera	Optical density of saline	-.18917*	**<0.001**
	Optical density of CHX	1.53938*	**<0.001**
	Optical density of triphala	1.33310*	**<0.001**
	Optical density of curcumin	.37041*	**<0.001**
Optical density of curcumin	Optical density of saline	-.55959*	**<0.001**
	Optical density of CHX	1.16897*	**<0.001**
	Optical density of triphala	.96269*	**<0.001**
	Optical density of aloevera	-.37041*	**<0.001**
* The mean difference is significant at the 0.05 level.			

Table III. Post hoc analysis for Multiple comparison of all groups

Figure Ia right side 2a left side

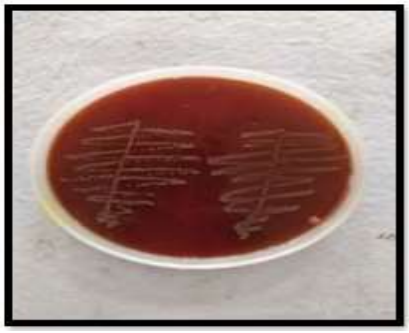

Figure IIIa right side 4a left side

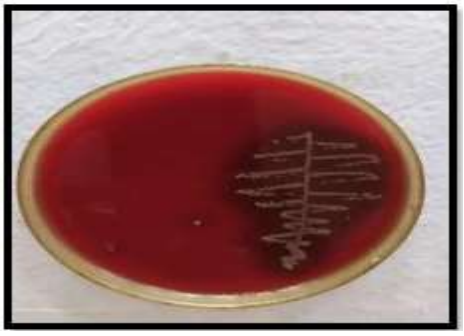

Figure Va

Figure Ib right side IIb left side

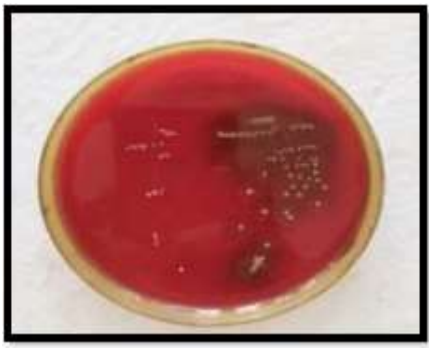

Figure IIIb right side IVb left side

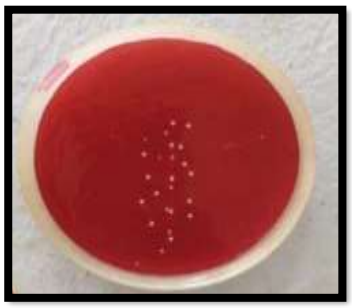

Figure Vb

Figure legends

Figure 1

1a and 1b – Initial and final colony count after Saline as intracanal medicament, respectively
IIa and IIb – Initial and final colony count after Chlorhexidine as intracanal medicament, respectively
IIIa and IIIb - Initial and final colony count after Triphala as intracanal medicament, respectively
IVa and IVb - Initial and final colony count after Aloeveraas intracanal medicament, respectively
Va and Vb - Initial and final colony count after Curcumin as intracanal medicament, respectively

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