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Original Article

In vitro comparison of the antibacterial activities of 2% chlorhexidine and Hiora solution on enterococcus faecalis

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Abstract
Introduction: The successful outcome of the endodontic treatment depends on the thorough cleaning and shaping of the root canals, which involves equal role of the mechanical cleaning as well as the antimicrobial agents.
Objective: The main objective was to assess the antimicrobial activity of the herbal solutions and its comparison with 2% chlorhexidine solution against E. faecalis.
Method: Hiora and 2% Chlorhexidine were used to assess the antimicrobial activity against *E. faecalis*. Agar well diffusion test was used to study the activity.
Results: The mean diameter of zone of inhibition was significantly more among group 2 compared to groups 1 and 3.
Conclusion: Hiora doesn't show any antimicrobial activity when compared to 2% chlorhexidine.

Keywords: root canal irrigants, antibacterial, agar well diffusion, herbal solution

Introduction
Almost all the periapical and periradicular diseases are caused by the bacteria [1]. The disinfection of the root canal system is the key to the successful outcome of the endodontic treatment, most of the bacteria get removed after the biomechanical cleaning of the canals but due the complex nature of the root canal system the mechanical preparation together with the copious irrigation becomes mandatory for the proper disinfection of the root canals [2].
The purpose of the root canal irrigation is not only as antimicrobial agent but it also plays an important role in flushing of loose debris, lubrication of dentinal walls and dissolution of organic debris. [2] The increase in the resistant strains of bacteria against the antibiotics have made the researchers to put in an effort to look for herbal alternatives, Neem leaf extract [3], Morinda citrifolia juice [4], Ginger [5], Triphala and Green tea polyphenols [6], have already been studied as root canal irrigants.
E. faecalis is an organism which can survive in the root canals without any synergism with other organism [7] and seems to be completely resistant to the intracanal medicaments like calcium hydroxide [8]. Hiora (Himalaya, Herbal Healthcare) is an herbal mouthwash, contains antimicrobial properties [9] so tested in this study against *E. faecalis.*
The purpose of this *in vitro* study was to compare the antimicrobial effects of the 2% Chlorhexidine (Neelkanth, Safe plus) and Hiora mouthwash (Himalaya, Herbal Healthcare) as root canal irrigants (Figure 1) on *E. faecalis.*

Materials and Methods
Test bacterium
Enterococcus faecalis (ATCC 29212) was taken as the test organism. *E. faecalis* was inoculated into Brain heart infusion broth, incubated overnight at 37^0 C and subcultured onto Brain heart infusion agar, the inoculum of colonies from BHI agar was further plated onto bile esculin agar for confirmation of pure culture of *E. faecalis*, seen as pin point black colonies. 4-5 colonies of this pure culture of Enterococcus faecalis from BHI agar were added to 10 ml sterile distilled water and turbidity was adjusted to 0.5 McFarland opacity standards.

Antibacterial efficacy tested on BHI agar, cup well agar diffusion method was used in which 30 ml culture medium was dispensed in respective petridishes which were inoculated with 0.1 ml fresh culture of 0.5 McFarland *E. faecalis*. 3 cup wells of 12 mm diameter were bored in each petriplate. These wells were then filled with the test agents (100 microliters). Each test agent group assayed in 10 replicates.

Fig 1: Irrigants used

Table 1: Group Distribution

Groups	Test Agent used	No. of wells
Group 1	Hiora (Figure 2)	30
Group 2	2%Chlorhexidine (Figure 3)	30
Group 3	Empty wells (control)	30

All the inoculated media plates were incubated for 24 hours at 37^0 C under aerobic conditions. Clear zones within which the bacterial growth was absent were measured and recorded as the diameter of complete growth inhibition.

Fig 2: Zone of inhibition for HiOra

Fig 3: Zone of inhibition for 2% Chlorhexidine

Results

Table 2

	Diameter of zone of inhibition			
	Mean	Std. Deviation	F-value	p-value
Group 1	12.01	0.03	19,918.747	< 0.001*
Group 2	14.08	0.07		
Group 3	12.00	0.00		

One-way ANOVA test * Significant difference

The mean diameter of zone of inhibition was compared between groups 1, 2 and 3 using the One-way ANOVA test. There was a significant difference in mean diameter of zone of inhibition between groups 1, 2 and 3.

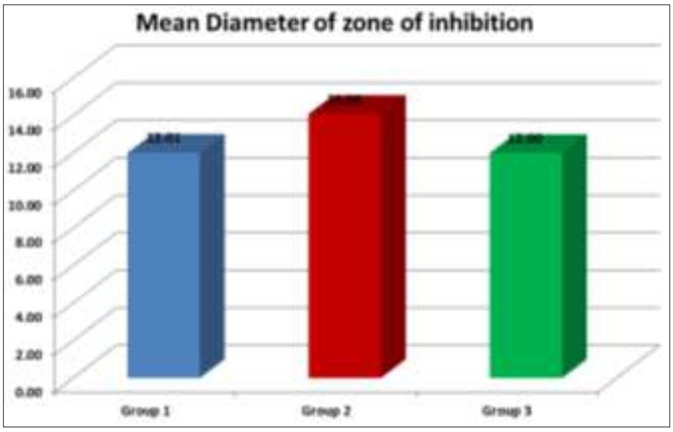
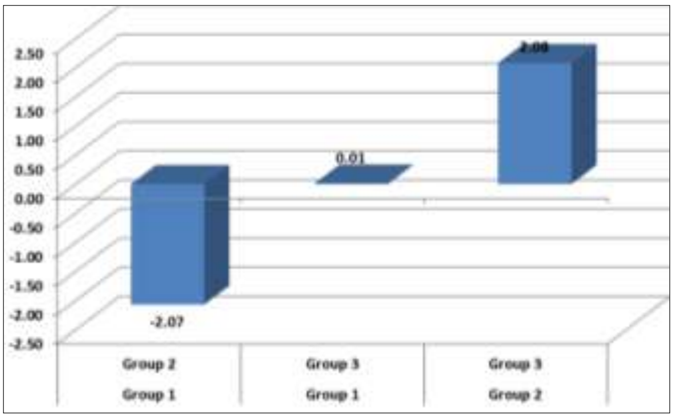


Table 3

		Mean difference	p-value
Group 1	Group 2	-2.07	< 0.001*
Group 1	Group 3	0.01	1.000
Group 2	Group 3	2.08	< 0.001*

Post-hocbonferroni test * Significant difference

The inter-group comparison of mean diameter of zone of inhibition was done using the Post-hoc bonferroni test. The mean diameter of zone of inhibition was significantly more among group 2 compared to groups 1 and 3.



Discussion

E. faecalis is a facultative gram positive anaerobe which is well studied and supposed to be the cause of persistant root canal infection [10]. It is a known pathogen associated with apical periodontitis in the endodontically treated teeth so is the best considered organism to study the efficacy of the root canal irrigants [11].

The main goal of the biomechanical preparation of the infected root canals is to reduce the level of the microbial flora inside the canal to an extent that is compatible to the periradicular healing [12].

The disinfection of the dentine and the dentinal tubules together with the maintenance of the antimicrobial potential for some time after its usage are the requirements of an ideal root canal irrigant [13].

As *E. faecalis* is considered as the most resistant organism to the canal disinfecting agents when compared to other organisms in the root canal [14] so it was taken up for this study.

Chlorhexidine is a cationic bis-guanide with the antimicrobial effects on both gram positive and gram negative bacteria [15]. when compared to sodium hypochlorite, chlorhexidine shows substantivity buts lacks the property of tissue dissolution. [16] 80% cases showed inhibition of E. faecalis after irrigation with 2% chlorhexidine [17].

The adverse effects as well as the resistance of microorganisms to the antimicrobial agents have raised the matter of essentiality towards the search of a herbal alternative with antimicrobial properties [18].

Hiora is a herbal preparation made up of natural herbs with the benefits of being anti-cariogenic and antiplaque because of the presence of Pilu (*Salvadora persica*) 5 mg, anti-inflammatory, anti-bacterial and immunity booster due to the presence of Bibhitaka (*Terminalia bellerica*) 10 mg [19], antimicrobial, antioxidant and plaque inhibiting in nature due to Nagavalli (*Piper betle*) 10 mg [20]. Essential oils of Gandhapura taila (Gaultheria fragrantissima) 1.2 mg has antimicrobial, anti-inflammatory, and analgesic properties, Oil extracted from Ela (*Elettaria cardamomum*) 0.2 mg is a potent antiseptic that is known to kill bacteria-producing bad breath [21]. Yavani satva (Trachyspermum ammi) 0.4 mg also has antimicrobial properties [22].

The technique used in this study was the agar well diffusion technique which involves the impregnation of the test solutions into the wells of standardized dimensions punched into agar plates which were seeded with the test bacterium. The antimicrobial agent diffuses from these sources into the medium of agar and leads to the inhibition of the growth of bacteria around the source. The clear zone thus formed without the bacterial lawn is considered as the zone of inhibition so greater the diameter of the zone of inhibition higher the anti-microbial activity [23].

Conclusion

In this study Hiora, when compared to 2% chlorhexidine did not show any antimicrobial activity against Enterococcus faecalis.

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