Antibacterial Efficacy of Neem, Tulsi Extract, Aleo Vera, Turmeric and 5% Sodium Hypochlorite Against *Enterococcus faecalis*: An in-vitro Study

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ABSTRACT

Introduction: Successful endodontic therapy depends on root canal disinfection and three-dimensional obturation of the root canal space. Complete removal of intracanal microorganisms and generates a promising environment for healing, prevents reinfection. Numerous root canal irrigants have been tried.

Objective: This study was done to assess the antibacterial efficiency of neem, tulsi extract, Aleo vera, turmeric and 5% sodium hypochlorite against *Enterococcus faecalis*.

Methods: Herbal extracts of neem, tulsi extract, Aleo vera, Curcuma longa. Agar plates were prepared using Tryptone soya broth to grow *E. faecalis*. The tested solutions (Group A: 5% NaOCl, Group B: neem, Group C: tulsi extract, Group D: Aleo vera, Group E: Curcuma longa) were added to the wells made on agar media. Agar diffusion test was accomplished. Plates were incubated for 24 hours at 37°C. Bacterial zones of inhibition were noted.

Results: The data were statistically evaluated by Analysis of Variance (ANOVA) and *post hoc* comparison by Tukey's *t*-test. The maximum zone of inhibition against *E. faecalis* was seen with 5% NaOCl, followed by neem, tulsi extract, and Aleo vera showed similar antibacterial activity. The least zone of inhibition was seen with turmeric. Conclusion: 5% NaOCl presented the maximum antibacterial activity, and herbal products demonstrated significant antibacterial activity against *E. faecalis* and can be suggested as an alternative to NaOCl.

Key Words: Aleo vera, E Faecalis, Neem, 5% Sodium Hypochlorite, Tulsi extract, Turmeric

INTRODUCTION

The successful outcome of an endodontic therapy depends on disinfection of root canal space with three-dimensional obturation. Presence of microorganism in the intracanal space can result in recurrence of infection and failure of endodontic therapy. Total elimination of intracanal microorganisms creates an environment promising for healing.^{1,2} *Enterococcus faecalis*, a facultative anaerobe, is recognized to 4%–40% of primary endodontic infections and 9 times more probable to cause of secondary infections. This extremely resistant microorganism can tolerate and survive in extreme environmental circumstances.³ Hence, efficient chemomechanical disinfection and debridement of the root canal space play an

essential role in the expectable long term success. Regularly used irrigants such as chlorhexidine and sodium hypochlorite have efficient antimicrobial action. However, they have certain disadvantages such as unpleasant taste, tissue toxicity, and odour, inability to remove the smear layer, corrosion of instruments, reduction in the elastic modulus, and flexural strength of dentin.^{4,5} Several herbal extracts have been tried with some success as root canal irrigant.⁵ These herbal products are not only easily available, safe, and cost-effective.⁶

Various herbal extracts, such as *Curcuma longa* (CT) - Turmeric, *Azadiracta indica* (AI) -Neem, *Myristica fragrans* (MF) - Nutmeg, *Terminalia chebula* (TC) - Myrobalan, *Aloe barbadensis* (AB) - Aloe vera, tulsi extracts, and

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Morinda citrifolia, having antimicrobial, antiinflammatory, and therapeutic effects are promising to be used as endodontic irrigants. There is a deficiency of reported researches on several herbal root canal irrigants and their antibacterial actions.⁵ Hence, the present study was executed to assess the antimicrobial effect of *neem*, *tulsi extract*, *Aleo vera*, and *turmeric* as herbal root canal irrigants with 3% sodium hypochlorite.

MATERIALS AND METHODS

Obtaining the microorganism

E. faecalis (ATCC 29212; Himedia, Mumbai, India) microorganism was inoculated on Tryptone soya agar plates and incubated overnight for 24 h at 37°C.

Preparation of the herbal extracts

Tulsi extract for the study was acquired by finely powdering the dried leaves. Then the powder was macerated with 100% ethanol followed by filtration. 18 gm of tulsi extract (residue 6% w/w) was obtained by dissolving 300 g of tulsi powder in ethanol.

Neem extract (*A. indica*) was obtained by washing fresh mature neem leaves in sterilized water and adding them to 50 ml ethanol. This was carefully mixed for 2-3 min and then the extract was filtered. The alcohol portion of the extract was divided into a water bath to attain 25 ml of extract.

A. vera extract Preparation: Pulp was collected from fresh 100 g of *A. vera* leaves and changed into a liquid form using a mixer. This mix was diluted by mixing it with distilled water in 1:5 ratios. For dehydration, the obtained mix was then placed in a crucible on the water bath. For use as an irrigating agent, the precipitate of the extract was dissolved in methanol.

Fresh rhizomes of *Curcuma longa* of analytical grade, grown organically without the use of any pesticides were collected and dried for about 10-12 days till they were completely moisture-free. Later they were grounded to form a coarse powder. 500 gms of turmeric coarse powder was placed in two large glass chambers each. To this 2500 ml of sterile distilled water was added to prepare the aqueous extract then 850 ml of ethanol (95%) was added in 70:30 ratios of water and alcohol to get a hydro-alcoholic extract. The strained and expressed liquid was obtained from menstruum liquid and mixed, simplified by filtration. The filtration was performed in a beaker with a Whatman's filter paper no 1. 2000 ml of menstruum was attained which was kept in a refrigerator at 4°C.⁷

In our study, 5% NaOCl (Prime Dental limited, Mumbai, India) was used. In 5 ml Tryptone soya broth (Himedia, Mum-

bai, India), the cultured *E. faecalis* was suspended. It was incubated at 37°C for 4 hours. With the help of L-arm loop, the turbidity was adapted to 0.5 MacFarland standard, and 50 µl of the inoculum was spread over Petri plates.

Antibacterial analyses

Wells of 5 mm diameter were punched employing a sterile cork bore and 50 µl of the irrigants were mixed to these wells with the help of a micropipette as follows: Group A: 5% NaOCl, Group B: neem, Group C: tulsi extract, Group D: Aleo vera, Group E: Curcuma longa

RESULTS

Table 1 indicates the zone of inhibition with different root canal irrigants. %% sodium hypochlorite has the highest zone of inhibition (19 mm), followed by neem (16 mm), tulsi extract (14 mm), aloe vera (12 mm) and least with turmeric (10 mm).

Table 2 indicates mean and standard deviation for all tested root canal irrigants. 5% sodium hypochlorite had the highest mean value of 19.65, followed by neem with 14.36, tulsi extract with 13.53, Aleo vera with 12.12 and least with turmeric with 11.56.

DISCUSSION

Microorganisms result in to increase in pulpal and periradicular diseases and are related to endodontic treatment failures.^{7,8} Endodontic treatment aims to eradicate the microorganisms and other contents of the pulpal spaces, which could act as possible causes of infection or reinfection.⁹ Henceforth, chemomechanical debridement of the root canal system becomes a paramount important phase of endodontic therapy to disinfect the root canal. Due to the restrictions of mechanical instruments to reach far and outside in narrow isthmuses, dentinal tubules, and accessory canals it becomes important that an irrigant with good permeability and bactericidal action is used.¹⁰ Irrigation of the root canal is an important adjunct to mechanical debridement as it removes out the debris, disinfects the canal system, infected material, dissolves the tissues and also cleans the complex internal anatomy of the root canal space inaccessible to instrumentation. Thus, the use of irrigants confirms the removal of bacteria and organic tissue remnants, thereby inhibiting reinfection.¹¹

The present study evaluated and compared the antibacterial efficacy of different herbal extract over 5% sodium hypochlorite and we found comparable results with herbal irrigants. Our results are associated with the Garg et al. study, and they stated that *E. faecalis* is the most common bacterial species cultured from infected root canal area.¹² Its

virulence helps it to bear the greatest environmental situation and persevere as a single organism or as a constituent of the flora.¹³ It can form a biofilm, which makes it resistant to antimicrobial agents.^{3,12} Hence, *E. faecalis* was considered as the test organism for our study.

The agar diffusion method permits direct evaluation of the materials beside the organisms, representing the potential of the test materials to remove microorganisms in the local microenvironment of the root canal system. The results may differ based upon the capacity of the material to diffuse across the medium and not only on the toxicity of the material for the specific organism.³ In our study, the experiment was repeated four times in harmony with a study by Balakrishnan et al., thereby creating more reliable results.

Sodium hypochlorite was used in this study as it is deliberated as the gold standard for irrigation. It is both an oxidizing and hydrolyzing agent and also retains bactericidal and proteolytic actions.¹² In the present study, 5% of NaOCl had considerable antibacterial efficacy against *E. faecalis*. It generated the largest bacterial zones of inhibition in the agar media. Babaji et al. estimated the antimicrobial outcome of herbal root canal irrigants (*Morinda citrifolia*, *Aloe vera*, *Azadirachta indica* extract,) with sodium hypochlorite (NaOCl). They found maximum inhibitory zone against *E. faecalis* with NaOCl followed by *M. citrifolia* and *A. indica* extract, and the least by *A. vera* extract.⁵ Chaitanya et al. estimated the antibacterial effectiveness of turmeric extract and morinda citrifolia with 3% NaOCl as a root canal irrigant, against *E. faecalis* and *S. aureus*. They observed that among the herbal irrigants, morinda citrifolia showed larger zones of inhibition than turmeric hydro-alcoholic extract.¹³ Chandrappa et al assess the antimicrobial activity of herbal medicines (neem extract, tulsi extract) and chlorhexidine against *Enterococcus faecalis* in Endodontics. They found that Significant antibacterial effect against *E. faecalis* with chlorhexidine followed by neem extract and tulsi extract. Chandrappa et al.¹⁴ Balasubramaniam et al assessed the antimicrobial efficacy of 3% NaOCl, Curcuma longa, Metronidazole, and Mimosops elengi against Enterococcus faecalis. They observed maximum zone of inhibition against *E. faecalis* with 0.5% of metronidazole followed by 25% Mimosops elengi, 3% Sodium hypochlorite and the least is shown by 12.5 % Curcuma longa. Balasubramaniam The findings are similar to our findings.¹⁵ Vinothkumar et al. evaluate the antimicrobial efficacy of various herbal extracts namely Curcuma longa (CL), Azadirachta indica (AI), Aloe barbadensis (AV), Myristica fragrans (MF) and Terminalia chebula (TC) as endodontic irrigant against *Enterococcus faecalis* and *Candida albicans* and concluded that Neem leaf extract has a significant antimicrobial efficacy against *Enterococcus faecalis* and *Candida albicans* compared to 5.25% sodium hypochlorite.¹⁶ The present study revealed that the herbal irrigants do display antibacterial activity against en-

dodontic pathogens and can be used as an additional for sodium hypochlorite.

CONCLUSION

Form the present study it can be concluded that 5%NaOCl showed the maximum antibacterial activity against *E. faecalis*. The herbal products also demonstrated significant antibacterial efficacy such as neem and tulsi extract.

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Author contribution

1. Dr. RK- data collection
2. UP: Final editing and review
3. SSN- Manuscript write up
4. ASS- Experiments conduct and investigation
5. VDR- Analysis of data
6. DYM- Data collection
7. GR- Editing

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Table 1: Zone of inhibition in mm

Sample	5% Sodium Hypochlorite	Neem	Tulsi Extract	Aleo Vera	Turmeric
1	19	16	14	12	10
2	17	14	13	13	14
3	17	11	11	11	10
4	18	14	12	10	11

Table 2: Rean and standard deviation (SD)for all root canal irrigant groups

Groups	N	mean	SD	F	P
5% Sodium Hypochlorite	4	19.65	3.45		
Neem	4	14.36	2.65		
Tulsi Extract	4	13.53	2.12	10.612	0.001*
Aloe Vera	4	12.12	3.11		
Turmeric	4	11.56	2.45		

*Statistical significant. One way ANOVA