

Comparative evaluation of antimicrobial effect of herbal root canal irrigants (*Morinda citrifolia*, *Azadirachta indica*, *Aloe vera*) with sodium hypochlorite: An *in vitro* study

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Abstract

Aim and Objectives: Successful root canal treatment involves the complete elimination of microorganism from the root canal and the three-dimensional obturation of the canal space. *Enterococcus faecalis* is the most commonly found bacteria in failed root canal. Chemical irrigation of canals along with biomechanical preparation helps in the elimination of microorganisms. The present study was aimed to evaluate the antimicrobial effect of herbal root canal irrigants (*Morinda citrifolia*, *Azadirachta indica* extract, *Aloe vera*) with sodium hypochlorite (NaOCl).

Materials and Methods: The bacterial *E. faecalis* (ATCC) culture was grown overnight in brain heart infusion (BHI) broth and inoculated in Mueller–Hinton agar plates. Antibacterial inhibition was assessed using agar well diffusion method. All five study irrigants were added to respective wells in agar plates and incubated at 37°C for 24 h. Bacterial inhibition zone around each well was recorded. Results were tabulated and statistically analyzed using Statistical Package for the Social Sciences software for Windows, version 19.0. (IBM Corp., Armonk, NY). **Results:** Highest inhibitory zone against *E. faecalis* was seen in NaOCl followed by *M. citrifolia* and *A. indica* extract, and the least by *A. vera* extract. **Conclusion:** Tested herbal medicine (*A. indica* extract, *M. citrifolia*, *A. vera*) showed inhibitory zone against *E. faecalis*. Hence, these irrigants can be used as root canal irrigating solutions.

Key words: Antimicrobial, herbal, irrigants, root canal, sodium hypochlorite

INTRODUCTION

It has been well-known that microorganisms in the root canal are responsible for pulp and periradicular infections.^[1] The aim of the root canal therapy is to

eliminate microorganisms from the root canal in order to provide appropriate environment for tissue healing.^[1,2] *Enterococcus faecalis* is generally isolated from failed root

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canals. It has the ability to invade dentinal tubules and does not depend on the survival of other bacteria.^[1-5] It is an anaerobic gram-positive bacterium responsible for 80–90% of enterococcal infection. It plays an essential role in persistent failure of endodontic therapy. Its virulence is attributed to its resistance to intracanal medicaments.^[2] Negative bacterial culture in root canal space helps in healing of periapical areas. Only with mechanical cleaning, all microorganisms cannot be eliminated; hence, appropriate irrigation with intracanal medicament is advised. Root canal irrigation helps in removal of bacteria where instrumentation is inaccessible.^[2,6,7]

Various chemical root canal irrigants used successfully are sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX).^[2] CHX is a strong antibacterial and is effective against *E. faecalis*, which causes endodontic failure. It is bacteriostatic in low concentration and bacteriocidal in high concentration.^[1,2,6] NaOCl is the gold standard antimicrobial agent with tissue dissolving properties and is widely used as root canal irrigating solution.^[6]

Ideal root canal irrigants should be biocompatible, nontoxic, and with a desirable smell and taste.^[8] Chemical irrigants, even though effective in root canal irrigation, are associated with several disadvantages. NaOCl causes allergic reaction, tissue toxicity, staining of instruments, irritation to periapical tissue, inability to remove smear layer, and has an undesirable smell and taste.^[3,6,9] CHX when mixed with sodium hypochlorite produces a carcinogenic product, i.e., parachloroaniline. CHX has disadvantages of undesirable smell and taste as well as tissue toxicity.^[6]

Constant increase in antibiotic resistant strains and side effects of chemical irrigants has led to the search for alternative herbal medicaments. Various herbal extracts, such as neem and tulsi extracts, *Aloe vera*, *Morinda citrifolia*, curcum longa, and turmeric, having antimicrobial, antiinflammatory, and therapeutic effects are promising to be used as endodontic irrigants.^[3,6,8-11] There is a lack of sufficient studies reporting various herbal root canal irrigants and their antibacterial actions. Hence, the present study was performed to evaluate antimicrobial effect of herbal root canal irrigants (*M. citrifolia*, *Azadirachta indica* extract, *A. vera*) and to compare it with 3% sodium hypochlorite.

MATERIALS AND METHODS

Preparation of extracts

Preparation of Azadirachta indica extract: Mature fresh *A. indica* leaves were collected from the medicinal

garden of SMBT Dental College and hospital, and taxonomic identification of the plant was performed. 100 g of neem leaves were tied in a muslin cloth and soaked in 800 ml of distilled water in a beaker. This beaker was boiled under low flame till the extract reduced to 400 ml to obtain 25% concentration of aqueous neem extract. After the extract cooled, it was filtered using a filter paper and stored for usage.

Preparation of A. vera extract: Leaves of *A. vera* were collected from the medicinal garden of SMBT Dental College and hospital. Pulp was removed from fresh 100 g of *A. vera* leaves and converted into a liquid form using a mixer. This mix was diluted by mixing with distilled water in a 1:5 ratio. The mix was then placed in a crucible on water bath for dehydration. Precipitate of extract was dissolved in methanol for use as an irrigating agent.

Microbiological analysis

Sixty samples were categorized into five groups with 12 samples in each for intracanal irrigation; Group I: 3% NaOCl, Group II: *M. citrifolia*, Group III: Neem extract, Group IV: *A. vera*, and Group V: Distilled water. The microbiological study was conducted in the Microbiological Laboratory of SMBT medical College, Nasik.

E. faecalis (ATCC 29212) species were obtained from Curewell Diagnostic Centre, Ludhiana, India. The culture was grown overnight in brain heart infusion (BHI) broth at 37°C and inoculated in Mueller–Hinton agar plates and adjusted to 0.5 turbidity reading on McFarland scale (1.5×10^8 bacteria/ml). Agar disc diffusion method was used to determine the antibacterial inhibition zones around *M. citrifolia*, neem extract, *A. Vera*, and NaOCl medicaments and distilled water as the control group. BHI agar plates were prepared and each medicament was added to the respective wells. The plates were incubated in an incubator at 37°C for 24 h. After incubation, plates were removed and the bacterial inhibition zone around each well was recorded. Results were tabulated and statistically analyzed using Statistical Package for the Social Sciences for Windows, version 19.0. (IBM Corp., Armonk, NY) with analysis of variance (ANOVA) tests ($P < 0.001$).

RESULTS

Highest inhibitory zone against *E. faecalis* was seen for NaOCl (mean of 28.6 mm) followed by *M. citrifolia*, neem extract, *A. vera* extract (mean of 14.7 mm), and distilled water (00) [Table 1 and Figure 1].

Table 1: Inhibitory zone against *Enterococcus Faecalis* by various root canal irrigants

| Group | Mean | SD | SE |
|----------------------------|------|-------|-------|
| Chlorhexidine gluconate 2% | 28.6 | 1.158 | 0.362 |
| M Citrifolia | 22.7 | 1.123 | 0.321 |
| Neem extract | 18.3 | 1.063 | 0.268 |
| Aleo vera | 14.7 | 1.042 | 0.164 |
| Distilled water | 0 | 0 | 0 |

SD=Standard Deviation, SE=Standard error. ANOVA, P<0.001

DISCUSSION

Up to nine times more *E. faecalis* species have been isolated from failed root canals than from primary endodontic infection.^[2] *E. faecalis* was chosen in the present study because of its high prevalence in secondary endodontic infection. Herbal medicaments are gaining importance because of their therapeutic properties. The present study evaluated antibacterial efficacy of herbal irrigants in comparison with 3% NaOCl.

The present study showed the highest inhibitory mean zone of 28.6 mm for NaOCl and *A. vera* extract (mean of 14.7 mm) and the least by distilled water (00) [Table 1 and Figure 1]. Contrary to our result, Vinothkumar *et al.* observed neem to be highly effective as NaOCl against *E. faecalis* and *Candida albicans*.^[8]

Neem (*A. indica* A Juss, margosa tree) is a traditional plant in India, with medicinal properties in each part of the tree. It has antibacterial, antifungal, antiviral, antioxidant, antiinflammatory, and immune-stimulatory activity. As an endodontic irrigant, it is advantageous because of its antibacterial, biocompatibility, and antioxidant properties.^[3,4,12,13] Its bitter taste can be altered with addition of sweeteners.^[6] Neem extract was found to be effective against both gram-positive and gram-negative microbes, and hence can be suggested as a root canal irrigant.^[12] We observed comparatively less inhibition zone by neem when compared with NaOCl or *M. citrifolia*. In contrast to our study, Gonmode *et al.*,^[14] Hegde *et al.*,^[15] and Damre^[16] in their studies observed the highest inhibition zone by neem compared with NaOCl.

M. citrifolia is commercially known as noni. It is one of the traditional folk medicinal plants with antifungal, antiviral, antibacterial, antihelminthic, analgesic, and hypotensive properties.^[3,6,13,17] *M. citrifolia* contains L-asperuloside and alizarin as an antibacterial agent, which is more effective than 2% CHX.^[13] It has been observed that 6% *M. citrifolia* is effective as an endodontic irrigant.^[17] Murry *et al.*^[18] from their study concluded

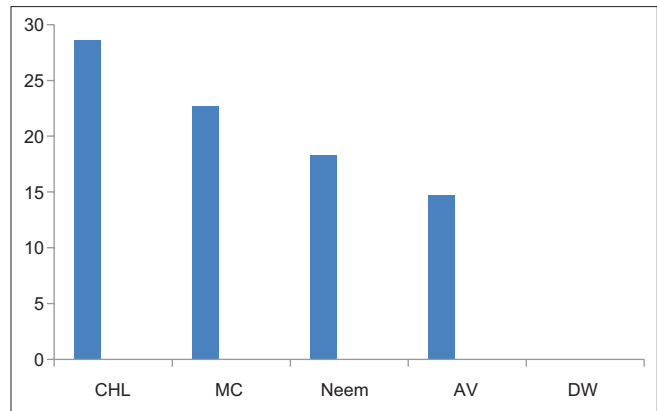


Figure 1: Inter-group comparison of inhibitory zone by various root canal irrigants. CHL=Chlorhexidine gluconate, MC=M Citrifolia, Neam=Neem extract, AV=Aleo vera, DW=Disilleed water

that *M. citrifolia* has similar intracanal irrigating properties as that of NaOCl along with EDTA.

A. vera (*Aloe barbadensis*) is a naturally occurring herbal medicament having antibacterial properties.^[17] It has antiinflammatory, antibacterial, antifungal, and antiviral properties.^[4,6,9,10] Because it contains anthrax quinine, it inhibits *E. faecalis* and *Streptococcus pyogenes*^[4,13] Karkare *et al.*^[9] concluded that *A. vera* showed the highest zone of inhibition against *E. faecalis* similar to NaOCl, which is in contrast to our results. Similar to our results Ambareen *et al.*^[4] found the highest inhibitory zone against *E. faecalis* for NaOCl followed by garlic extract, neem, and ginger extract, and the least for *A. vera*.

Various other herbal medicaments have been used as root canal irrigants such as tulsi extract, clove oil, and turmeric. Madhavan *et al.*^[7] in their study reported that intracanal medicaments in combination with clove oil had the highest antibacterial properties. Garg *et al.*^[3] found no significant differences between NaOCl, triphala, and Propolis against *E. faecalis* when used as root canal irrigants. Saha *et al.*^[11] found that CHX and metranidazole combination was better compared to Propolis for root canal irrigation. Bazvand *et al.*^[2] found bacterial inhibition with triantibiotic paste, CHX, and Propolis and least with *A. vera*. Bharadwaj *et al.*^[17] from their study concluded that bacterial inhibition effect was 100% with CHX, 86% with *M. citrifolia* gel, 78% with *A. vera*, 67% with papain gel, and 64% with calcium hydroxide. Kamath *et al.*^[19] found comparable result between teatree tee oil and NaOCl.

Lower efficacy of antibacterial inhibition in *A. vera* could be because of a change in weather where it was grown and a change in the preparation method.

In accordance to our results, several researchers studied antibacterial efficacy of *M. citrifolia*, neem extract, and *A. vera* against *E. faecalis*.^[4,9,14,15,17,18] Herbal medications compared to chemical irrigating solutions are less toxic, easily available, cost effective, and have several therapeutic properties. Hence, these herbal preparations can be considered as intracanal irrigating solutions.

Limitation of the study

The present study was a *in vitro* study with a limited sample size. Major disadvantage with herbal extracts is the need of fresh preparation and modification in taste for acceptability.

Further research

Herbal irrigants are easily available, less expensive, and proven to have therapeutic action and antibacterial activity against oral bacteria. Further studies are required to evaluate the role of herbal irrigants against all possible root canal microbes.

CONCLUSION

Tested herbal medicine (neem, *M. citrifolia*, *A. vera*) showed inhibitory zones 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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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Saha S, Nair R, Asrani H. Comparative evaluation of Propolis, metronidazole with chlorhexidine, calcium hydroxide and *Curcuma longa* extract as intracanal medicament against *E. faecalis* — An *in vitro* study. J Clin Diagn Res 2015;9:ZC19-21.
- Bazvand L, Aminozarbian MG, Farhad A, Noormohammadi H, Hasheminia SM, Mobasherizadeh S. Antibacterial effect of triantibiotic mixture, chlorhexidine gel, and two natural materials Propolis and *Aloe vera* against *Enterococcus faecalis*: An *ex vivo* study. Dent Res J (Isfahan) 2014;11:469-74.
- Garg P, Tyagi SP, Sinha DJ, Singh UP, Malik V, Maccune ER. Comparison of antimicrobial efficacy of propolis, *Morinda citrifolia*, *Azadirachta indica*, triphala, green tea polyphenols and 5.25% sodium hypochlorite against *Enterococcus faecalis* biofilm. Saudi Endod J 2014;4:122-7.
- Ambareen Z, Konde S, Raj SN, Kumar NC. Antimicrobial efficacy of herbal extracts. Int J Oral Health Dentistry 2015;1:108.
- Somayaji SK, Ballal ND, Shobha KL, Mohandas Rao KG. Comparison of antimicrobial efficacy of triphala, *Withania somnifera* and sodium hypochlorite against *Enterococcus faecalis* biofilm — An *in vitro* study. Int J Pharm Pharm Sci 2014;6:808-11.
- Jena A, Govind S, Sahoo SK. Gift of nature to endodontics as root canal irrigant: A review. World J Pharm Res 2015;4:471-81.
- Madhavan S, Murlidharan. Comparing the antibacterial efficacy of intracanal medicaments in combination with clove oil against *Enterococcus faecalis*. Asian J Pharm Clin Res 2015;8:136-8.
- Vinothkumar TS, Rubin MI, Balaji L, Kandaswamy D. *In vitro* evaluation of five different herbal extracts as an antimicrobial endodontic irrigant using real time quantitative polymerase chain reaction. J Conserv Dent 2013;16:167-70.
- Karkare SR, Ahire NP, Khedkar SU. Comparative evaluation of antimicrobial activity of hydroalcoholic extract of *Aloe vera*, garlic, and 5% sodium hypochlorite as root canal irrigants against *Enterococcus faecalis*: An *in vitro* study. J Indian Soc Pedod Prev Dent 2015;33:274-8.
- Jain P, Ranjan M. Role of herbs in root canal irrigation — A review. IOSR J Pharm Biol Sci (IOSR-JPBS) 2014;9:6-10.
- Kamath S, Rajeev K, Saraf P. Role of herbs in endodontics: An update. Endodontology 2011;98-102.
- Bhargava K, Kumar T, Aggarwal S, Zinzarde S, Sanap A, Patil P. Comparative evaluation of the antimicrobial efficacy of neem, green tea, triphala and sodium hypochlorite: An *in vitro* study. J Dent Res Rev 2015;2:13-6.
- Penumudi SM, Mandava RB, Saraswathi DD, Santhi V, Swetha B, Gandhi B. Antimicrobial efficacy of herbs in endodontics. J Adv Oral Res 2015;6:9-12.
- Gonmode WN, Balsaraf OD, Tambe VH, Saujanya KP, Patil AK, Kakde DD. Comparison of antibacterial efficacy of neem leaf extract, grape seed extracts and 3% sodium hypochlorite against *E. faecalis* — An *in vitro* study. J Int Oral Health 2013;5:61-6.
- Hegde V, Kesaria DP. Comparative evaluation of antibacterial activity of neem, propolis, turmeric, liquorice, and sodium hypochlorite as root canal irrigants against *E. faecalis* and *C. albicans* — An *in vitro* study. Endodontology 2013;25:38-45.
- Damre PG. Comparative evaluation of antibacterial activity of herbal vs chemical root canal against *E. faecalis*: An *in vitro* study. Int J Adv Res 2015;3:1563-72.
- Bharadwaj A, Ballal S, Velmurugan N. Comparative evaluation of the antimicrobial activity of natural extracts of *Morinda citrifolia*, papain and *Aloe vera* (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against *Enterococcus faecalis*: An *in vitro* study. J Conserv Dent 2012;15:293-7.
- Murry PE, Farber RM, Namerow KN, Kuttler S, Garcia-Godoy F. Evaluation of *Morinda citrifolia* as an endodontic irrigant. J Endod 2008;34:66-70.
- Kamath U, Sheth H, Ramesh S, Singla K. Comparison of antibacterial efficacy of tee tree oil with 3% sodium hypochlorite and 2% chlorhexidine against *E. faecalis*: An *in vitro* study. J Contemp Dent 2013;3:117-20.