How to Cite:
Shah, S. N., Awinashe, M. V., Mohammed, A., Agarwal, D., Anand, A., & Sonkar, T. P. (2022). Antimicrobial efficacy of sodium hypochlorite, neem, Tulsi, and Aloe vera as a root canal irrigants against E. faecali. *International Journal of Health Sciences*, *6*(S3), 809–815. https://doi.org/10.53730/ijhs.v6nS3.5476

Antimicrobial Efficacy of Sodium Hypochlorite, Neem, Tulsi, and Aloe Vera as a Root Canal Irrigants Against *E. Faecali*

Shreyas N Shah
Professor, Department of Oral Pathology and Microbiology, KM Shah Dental College and Hospital, Sumandeep Vidyapeeth Deemed to be University, Piparia, Vadodara, Gujarat, India

Minal Vaibhav Awinashe
Department of Oral and Maxillofacial Pathology and Microbiology, MSC Medical Education, Assistant Professor, Department of Oral Surgery and Diagnostic Sciences, College of Dentistry, Qassim University, Buraidah, Kingdom of Saudi Arabia

Azhar Mohammed
Reader, Department of Orthodontics and Dentofacial Orthopedics, NITTE (Deemed to be University) AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Mangalore, Karnataka, India

Deepali Agarwal
Professor and Head, Department of Conservative and Endodontics, Geetanjali Dental and Research Institute, Udaipur, Rajasthan, India

Amrit Anand
Senior Lecturer, Department of Orthodontics and Dentofacial Orthopedics, Hazaribagh Dental College, Jharkhand, India

Tanu Priya Sonkar
Senior Lecturer, Department of Prosthodontics, Pravara Institute of Medical Sciences, Rural Dental College, Loni, Maharashtra, India

***Abstract*---**Root canal irrigation helps in eliminating the microorganisms present in the canal system. The present in vitro study was done to evaluate the efficacy root canal irrigants; sodium hypochlorite, neem, Tulsi, and Aloe vera against E. faecali. In present study 5 groups of root canal irrigants (sodium hypochlorite, neem, Tulsi, Aloe vera, and distilled water as controlled group) were tested for efficacy against E. faecali. There was highest inhibition zone

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
***Corresponding author**: Mohammed, A.; Email: dr.azahar.mohd@gmail.com*
Manuscript submitted: 18 Nov 2021, Manuscript revised: 09 Feb 2022, Accepted for publication: 27 March 2022

observed with sodium hypochlorite group followed by Neem (Azadirachta indica), neem, Tulsi, Aloe vera and least was observed in control group with distilled water. The tested root canal irrigants sodium hypochlorite group followed by Neem (Azadirachta indica), Tulsi, and Aloe vera are effective against E. faecali.

***Keywords*---**antimicrobial, neem, Tulsi, A Vera, E. faecali.

Introduction

Microbial load of root canal system can be decreased by mechanical preparation of the root canal and disinfection. However, mechanical preparation along with elimination of root canal pathogens by root canal irritants is the key requisites for successful outcomes of endodontic therapy. [1] Cases with persistent infection where the microorganisms are not completely eliminated need more attention as in the majority of cases it leads to reinfection. To attain this, mechanical preparation of the root canal alone may not be sufficient. A disinfection regimen should emphasize optimal shaping such that the disinfectant reaches inaccessible areas.[2]

The irrigating process has three objectives as advocated by the Walker; Dissolution of remnant tissue, antimicrobial action, and lubrication of the canal. The apical third of the root canal is the most difficult portion to clean possibly because of its narrower dimension. [3] An ideal root canal irrigant should have antimicrobial properties, an ability to dissolve the remnant necrotic pulp tissue, and should cause minimal irritation to the periapical tissue. [2]Sodium hypochlorite is the well-known gold standard root canal irrigant used in endodontic practice[1,4.] But the drawbacks of sodium hypochlorite includes unpleasant taste, toxicity and causes hypochlorite accidents. So to overcome this side effect and to meet the requirements of an ideal irrigant, various herbal and other irrigants were tried by many researchers. [4]

Herbal products are researched as they naturally possess antimicrobial, anti-inflammatory, and antioxidant properties alongside its biocompatibility[1.] Various natural plant extracts have antimicrobial properties and therapeutic effects suggesting their potential to be used as an endodontic irrigant [4.] A constant increase in antibiotic resistant strains and side effects of chemical irrigants has led to the search for alternative herbal medicaments[5.] Many herbal products have been studied *in vitro* for their use as irrigant like neem, miswak, propolis, tulsi, tea tree oil, *A. vera ,* triphala, noni, turmeric, green tea extract, etc. [1, 4, 5, 6]

Use of Neem (Azadirachta indica) as an endodontic irrigant might be advantageous because it is biocompatible, antimicrobial, antiadherent, and antioxidant.[1] *A. vera* (*Aloe barbadenis*) is a naturally occurring herbal medicament having antibacterial properties. It has antiinflammatory, antibacterial, antifungal, and antiviral properties [5.] Natural extracts such as Tulsi (*O. sanctum*) reported to exert antimicrobial, anti-inflammatory, and antioxidant properties. Tulsi/Basil a sacred plant in India has several medicinal properties and its extracts showed almost 100% inhibition of growth of *Escherichia coli.*[6]

Enterococcus faecalis is the most common organism cultured from failed root canals that undergo retreatment and also from non - healing endodontic cases. Intracanal irrigants should possess a good antimicrobial property to enhance the outcome of the instrumentation procedures.[4] Enterococcus *faecalis* is generally isolated from failed root canals. It is an anaerobic gram-positive bacterium responsible for 80–90% of enterococcal infection[5]. There are very few reported studies on comparison of various herbal endodontic irrigants, hence the present study was done to evaluate the efficacy of various herbal root canal irrigants against *Enterococcus faecali.*

Materials and Methods

The present *in vitro* study was done in the department of Oral Pathology. In present study 5 groups of root canal irrigants; sodium hypochlorite, neem, Tulsi, Aloe vera, and distilled water as controlled group) were tested for efficacy against *E. faecali.*

Preparation of Neem Irrigating Solution

Fresh A. indica leaves were collected, washed using distilled water, and weighed; 25 gm of fresh neem leaves was added to 50 mL of absolute ethanol and macerated for 1 to 2 minutes. Mixture was filtered for coarse residue using muslin cloth. This process was repeated again for coarse residue with 25 mL ethanol. These two extracts were pooled together and filtered using fast filter paper. To remove the alcohol part, the extract was placed on water bath until it reduced to 25 mL solution. This solution was kept ready and stored in airtight amber-colored container. [1]

Preparation of Aloe 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. [5]

Preperation of (Ocimum sanctum) tulsi

Seeds of nutmeg, myrobolan, and Tulsi were subjected to shade drying and grounded into fine powder. 45 g of powder were than subjected to Soxhlet extraction with 90% ethanol as solvent. Soxhletion process was allowed to carry out till the complete exhaustion of sample material use for extraction with the maintenance of temperature 70°C the boiling points of the solvents used. After complete phytochemical exhaustion, the extract in the round bottom flask was transferred into clean and preweighed universal tubes. The tubes were then stored at 4–8°C in the refrigerator. Percentage yield was calculated as dividing the initial weight of raw material taken by the final weight of extracts.

Percentage yield = n grams of extracts × 100

45 g of powder
Where n indicates the final weight of nutmeg, myrobolan, tulsi extract.[6]

Preparation of filter discs

Whatman No 1 filter paper was obtained. Round discs of 6 mm diameter were cut from the filter paper. They were dried and sterilized in hot air oven at 60 degree C. Each of these discs were then soaked with 0.1 ml of the honey suspensions Pure culture of microorganisms was grown on nutrient broth which was cultured on chocolate agar plate using inoculation loops. The plates were incubated at 37 degree C for 24 hours under aerobic condition and were there after examined for zones of inhibition. [4]

Preparation of irrigants

Based upon the results obtained from the antimicrobialactivity i.e., minimum inhibitory concentration (MIC) a weighted amount of extract was taken in sterile beaker and 100 ml of distilled water was mixed, thereafter the beaker was placed on hot plate magnetic stirrer at 60. [6]

Test for Antimicrobial Assay

E. faecalis (ATCC 29212) species were obtained from Curewell Diagnostic Centre, Gurgoa, 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 × 108 bacteria/ml). Agar disc diffusion method was used to determine the antibacterial inhibition zones around sodium hypochlorite, neem, Tulsi, Aloe vera 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. The obtained data for both the groups were statistically analyzed using statistically analyzed using Statistical Package for the Social Sciences for Windows, version 21.0. (IBM Corp.Armonk, NY) with analysis of variance (ANOVA) tests ($P < 0.001$).

Results

Table 1
Inhibitory zone against *Enterococus Feacalis* by different root canal irrigants

Group	Mean	SD	SE
Sodium hypochlorite	28.7	1.124	0.324
Neem (Azadirachta indica)	22.3	1.135	0.225
Aloe vera	16.2	1.024	0.265
Tulsi	14.6	1.045	0.237
Control- Distilled water	0	0	0

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

Table I indicates Inhibitory zone against *Enterococus Feacalis.* Highest inhibition zone was observed with Sodium hypochlorite group (28.7) followed by Neem (Azadirachta indica) (22.3), Aloe vera with 16.2, Tulsi with 14.6 and least was observed with control group of distilled water.

Discussion

Root canal infection and/or reinfection occurs mainly due to microorganisms present in the canal system. [1] The present study evaluated the efficacy of herbal root canal irrigants against *Enterococus Feacalis.* Daga et al compared the antimicrobial efficacy of herbal irrigants neem, miswak, propolis with sodium hypochlorite. They concluded that Sodium hypochlorite proved to be a better irrigant followed by propolis, neem, and miswak. EndoVac irrigation system was more effective for elimination of E. faecalis than needle irrigation group[1.] Damre assessed the antimicrobial activity of herbal vs chemical root canal irrigants against E. Faecalis and concluded that Honey had the highest zone of inhibition against E.faecalis followed by neem, sodium hypochlorite, haldi and aleo-vera. [4] Venkata Teja et al did A Systematic Review on Herbal Agents and Sodium hypochlorite as root canal irrigant. They concluded that herbal agents cannot be used as a main irrigant for canal disinfection. [2]

Afshan et al evaluated the antimicrobial efficacy of *neem* leaf extract, *Morinda citrifolia* and saline, against *Enterococcus faecalis*. They concluded that *Neem* leaf extract exhibited maximum inhibition against *E. faecalis* while saline exhibited the least antimicrobial efficacy with least inhibition. [7] Makkar et al evaluated the endodontic irrigants on apical extrusion of debris. They concluded that Sodium hypochlorite in the concentration of 3% showed the greatest amount of extruded debris. [8] Babaji et al evaluated the antimicrobial effect of herbal root canal irrigants (Morinda citrifolia, Azadirachta indica extract, Aloe vera) with sodium hypochlorite (NaOCl). They concluded that A. indica extract, M. citrifolia, A. vera) showed inhibitory zone against E. faecalis.[5]The findings are similar to our results.

Gupta-Wadhwa et al assessed the antimicrobial efficacy of three herbal irrigants *Ocimum sanctum (OS), Cinnamomum zeylanicum (CZ), Syzygium aromaticum (SA) against Enterococcus faecalis.* They concluded that *Cinnamomum zeylanicum, Syzygium aromaticum* and *Ocimum sanctum* showed intracanal bacterial reduction against *Enterococcus faecalis.* [9] Paul et al assessed the efficacy of different irrigants (17% EDTA along with ultrasonication, 25% citric acid, MTAD). They concluded that all the test irrigants including MTAD worked well in the middle and cervical third, whereas MTAD showed excellent results in the apical third. [3] Mali et al evaluated the efficacy of different herbal irrigants on the removal of smear layer. They concluded that Tulsi, nutmeg and myrobolan can be effectively used as an irrigant in primary teeth. [6]

The results are similar to our findings. Singh et al evaluated the different irrigation activation systems (CanalBrush (CB) and EndoActivator (EA)) on smear layer removal from root canal. They concluded that CB remove smear layer more efficiently from the root canal than F-File and EA in coronal and apical region.[10] Budhiraja evaluated the efficacy of Neem (Azadirachta indica), *M. citrifolia Juice,* and Propolis root canal irrigants against *E Faecalis* and found that all the tested

herbal irrigants were effective as an irrigants[11]. The drawback of the present study was that it was *in vitro* study and different irrigating solutions were not tested. Further in vivo studies are needed to validate the results.

Conclusion

Within the limitation the present stud indicated that, sodium hypochlorite group followed by Neem (Azadirachta indica),Tulsi, Aloe vera are effective against *E. faecali.*

Ethical clearance: obtained
Conflict of interest: Nil
Source of Funding: Self

References

1. Daga P, Asrani H, Farista S, Mishra P. Comparative Evaluation of Antimicrobial Efficacy of Neem, Miswak, Propolis, and Sodium Hypochlorite against Enterococcus faecalis using EndoVac. Int J Prosthodont Restor Dent 2017;7(2):60-65.
2. Venkata Teja K, Janani K, Srivastava KC, Shrivastava D , Jose J, Marya A, Karobari MI. Comparison of Herbal Agents with Sodium Hypochlorite as Root Canal Irrigant: A Systematic Review of In Vitro Studies. Evidence-Based Complementary and Alternative Medicine. Volume 2021, Article ID 8967219, 11 pages
3. Paul ML, Mazumdar D, Niyogi A, Baranwal A. Comparative evaluation of the efficacy of different irrigants including MTAD under SEM. J Conserv Dent 2013;16:336-41.
4. Damre PG. Comparative Evaluation of Antimicrobial Activity Of herbal vs chemical Root Canal Irrigants against E. Faecalis - An in Vitro Study. International Journal of Advanced Research.2015; 3(9):1563- 1572
5. Babaji P, Jagtap K, Lau H, Bansal N, Thajuraj S, Sondhi P. Comparative evaluation of antimicrobial effect of herbal root canal irrigants (Morinda citrifolia, Azadirachta indica, Aloe vera) with sodium hypochlorite: An in vitro study. J Int Soc Prevent Communit Dent 2016;6:196-9.
6. Mali S, Singla S, Tyagi P, Sharma A, Talreja N, Gautam A. Comparative evaluation of the efficacy of different herbal irrigants on the removal of smear layer of primary teeth: A scanning electron microscopy study. J Indian Soc Pedod Prev Dent. 2020;38:374-80.
7. Afshan T, Parwez A, Prasanna PL, Meenavolu VG Bhargav4, Kurnool Balaji5, Tameem Hussain. Comparison of Antimicrobial Efficacy of Herbal Root Canal Irrigants (Azadirachta indica, Morinda citrifolia) against Enterococcus faecalis. World J Dent 2020;11(3):206–210.
8. Makkar S, Kaur S. Comparative Evaluation of Various Endodontic Irrigants on Apical Extrusion of Debris. Serbian Dental Journal. 2013;60(3): 119-123
9. Gupta-Wadhwa A, Wadhwa J, Duhan J. Comparative evaluation of antimicrobial efficacy of three herbal irrigants in reducing intracanal E. faecalis populations: An in vitro study. J Clin Exp Dent. 2016;8(3):e230-5.
10. Singh N, Chandra A, Tikku AP, Verma P. A comparative evaluation of different irrigation activation systems on smear layer removal from root canal:

An in-vitro scanning electron microscope study. J Conserv Dent. 2014 Mar-Apr; 17(2): 159–163.
11. Budhiraja S. Comparative evaluation sodium hypochlorite, neem, M. citrifolia Juice, and Propolis root canal irrigants against E. faecali.. J Curr Med Dent Sci. 2022;1(1):7-11