Original Article

To check the antibacterial efficacy against *Enterococcus faecalis* by various ayurvedic oils used as a solvent in endodontic sealers - An *in vitro* study

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Abstract

Aim: The aim of the study is to check the antibacterial efficacy of various ayurvedic oils used as a solvent with zinc oxide for preparing endodontic sealers.

Materials and Methods: Forty-five extracted premolars were taken and were cut coronally and apically such that 7 mm of tooth specimen was prepared. Teeth were sterilized by autoclaving inoculated with *Enterococcus faecalis* and incubated for 24 h. The specimens were divided into three groups of 15 each. Group 1 – ZnO powder + Eugenol, Group 2 – ZnO powder + Aremidadi Oil, and Group 3 – ZnO powder + Dashmool oil. Bacterial growth in each specimen was calculated before and after sealer application and noted as the initial and final colony count. The antimicrobial effect of each sealer was measured by calculating the percentage reduction in colony count (%). One-way analysis of variance and *post hoc* tests will be used for statistical analysis.

Results: The Zn + Arimedadi oil group showed the maximum antibacterial effect among the sealers tested and the Zn + eugenol sealer showed the least antimicrobial effect In comparison, there was a statistically significant difference between all the groups.

Conclusion: Ayurvedic oil-based root canal sealers showed better antibacterial efficacy than eugenol-based sealers. Arimedadi oil showed the highest antibacterial activity against *E. faecalis* and Eugenol showed the least when used as a solvent.

Keywords: Antibacterial efficacy; ayurvedic oils; root canal sealers; zinc oxide eugenol sealer

INTRODUCTION

Effective endodontic therapy conventionally involves instrument usage, irrigation, and intracanal medications, with canal cleaning pivotal for periapical tissue healing.[1] Despite thorough procedures, complete microorganism eradication poses challenges, contributing to endodontic failure. *Enterococcus faecalis*, a Gram-positive bacteria, colonizes dentinal tubules in treated roots, leading to treatment failure.[2] While multispecies biofilms are primarily responsible for endodontic infections, the selection of *E. faecalis* as the sole microorganism for laboratory research on persistent endodontic infections is due to its virulence factors and high survival rate.[3] Root canal sealers with antimicrobial properties serve as barriers against new microbial threats, preventing infection and entrapping bacteria while hindering periapical tissue fluid access to bacterial cells.[4] Clove oil, historically used for toothache relief, possesses sedative, anodyne, and antibacterial effects, yet may cause local irritating, cytotoxic effects, and hypersensitivity reactions.[5] Dashmool, a blend of 10 medicinal herbs, and Arimedadi Taila/Oil,

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an Ayurvedic medicine, exhibit antibacterial properties useful in managing oral diseases.[6,7] However, scientific exploration of Ayurvedic treatments for oral diseases and studies on the antibacterial properties of herbal extracts in endodontic sealers are limited. This study aims to evaluate and compare the antibacterial efficacy of endodontic sealers containing various plant-based herbal oils, with the null hypothesis that Ayurvedic oil-based sealers lack antibacterial efficacy against *E. faecalis*.

MATERIALS AND METHODS

Sample selection

Forty-five single-rooted intact premolar human teeth were selected from a pool of extracted teeth for the present study. Digital periapical radiographs were performed to select teeth with fully formed apices, without calcifications, and no previous endodontic treatment.

Shaping and cleaning

The teeth were segmented to a length of 7 mm [Figure 1a] by cutting off the root tip and the crown 2–3 mm below the cementoenamel junction,[8] using a rotating water-cooled diamond saw to prevent desiccation of the tooth while sectioning, with a slow-speed diamond disc, each root canal was enlarged to size 2 Peeso Reamer (Dentsply) [Figure 1b] under irrigation with distilled water, resulting in the preparation of an apical box. Each canal was irrigated with 1 mL of 2.5% NaOCl. Final irrigation was done with 1 mL of 17% ethylenediaminetetraacetic acid followed by a final rinse of 1 mL of 2.5% NaOCl. Canals were dried with sterile paper points.

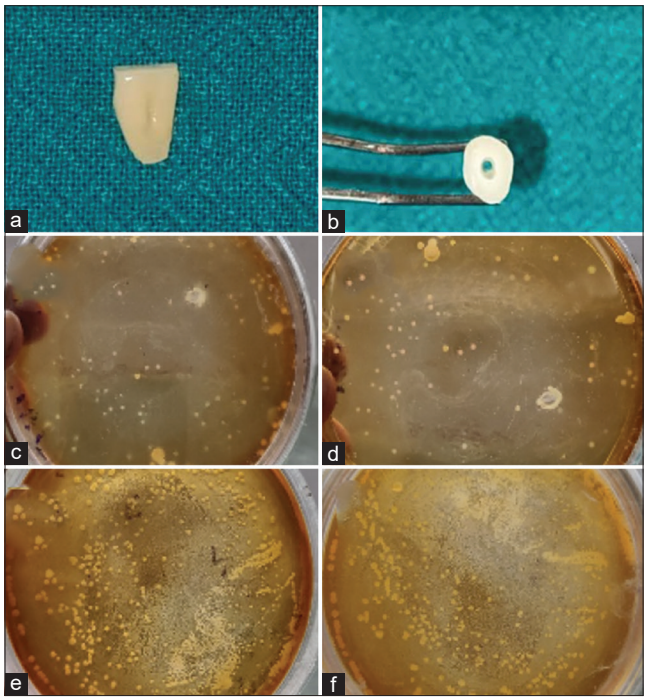

Figure 1: (a and b) The sectioned premolar of 7 mm length and root canal prepared up to size 2 Peeso Reamer. (c-f) 4 dilutions of agar plates: Depicting colonies of *Enterococcus faecalis* before sealer application

Sterilization of specimen

The specimens were collected in Eppendorf tubes and autoclaved at 121°C at 15 atm pressure for 15 min.

The specimens were then blotted dry, and external root surfaces were covered with a dentin bonding agent or nail varnish.

Cultivation of *Enterococcus faecalis*

Pure and fresh *E. faecalis* (ATCC29212) was obtained from the microbiology laboratory and was cultured on blood agar at 37°C for 48 h, [Figure 1c-f]. Colonies were then grown in sterile brain heart infusion (BHI) broth for 24 h. Turbidity was set to 0.5 McFarland corresponding to approximately 1.5×10^8 CFU/m.

Bacterial inoculation of root canals

Prepared root canals were filled with 10 µL of suspension using a micropipette, carried to the entire length by #50 size H file, and incubated at 37°C for 7 days at 100% humidity.

Canals were reinoculated on the 4th and 6th days after initial inoculation.

After incubation, the first microbiological sampling was performed, by flooding the canal with sterile saline, followed by placing a sterile 50 size H file (Dentsply) into the canal to scrape the dentin during the process.

A sterile absorbent paper point was placed into the canal for 60 s and transferred into test tubes containing 1.0 mL of stable physiological saline and was shaken vigorously for 60 s.

A serial 10-fold dilution was prepared, and 0.1 mL of dilution was transferred and plated on BHI agar plates. The plates were incubated for 24 h at 37°C. Bacterial growth was detected by performing colony counts to establish the level of contamination before the application of sealers.

Procedure for testing

Forty-five specimens were randomly divided into three groups ($n = 15$) based on the sealer with different types of oils. The groups were as follows:

- Group 1: Zinc Oxide + Aremidadi Oil (Shree Baidya Nath, Ayurved Bhawan pvt ltd.)
- Group 2: Zinc Oxide + Dashmool Oil (Shree Baidya Nath, Ayurved Bhawan pvt ltd.)
- Group 3: Zinc Oxide + Eugenol.

The canal space was dried with sterile paper points and coated with a sealer. A gutta-percha cone of size 90 is coated with freshly mixed sealer in a ratio of 1:1 and consistency was checked by a cement spatula drawing a 1-inch mass of sealer from the mixed mass. It was introduced into the prepared canal with tweezers until fully seated. All specimens were incubated at 37°C for 4 days.

Microbiological procedure

On completion of incubation, obturating material was removed from all specimens with sterile Peeso Reamer size 2. A new sterile Peeso Reamer size 5 (ISO Size 150) was then used to collect dentin powder (300 µm into the dentine) from each canal.

The dentin powder was obtained from each specimen, in a sterile Petri dish which was placed below the specimen. Using a sterile, plastic universal micropipette, 1 mL of BHI broth was added to each Petri dish to assist in the collection of the dentin powder.

The BHI broth with the dentin powder was aspirated with a universal pipette carried to a small, sterile test tube, and vortexed for 10 s. Thus, the whole experiment gave a total of 45 test tubes that were then plated onto 45 separate BHI agar plates by using the standard loupe method. The agar plates were incubated at 37°C for 24 h. Following incubation, visible colonies were counted and the total colony forming units (CFUs) were calculated by using the colony counter. Initial and final colony counts of different groups.

Statistical analysis

Statistical Product and Service Solution (SPSS) version 21 for Windows (Armonk, NY, USA: IBM Corp) software was used to analyze the data. Statistical analysis was done by using tools of descriptive statistics such as mean, and standard deviation for representing quantitative data. Probability $P < 0.05$, considered as significant as alpha error set at 5% with a confidence interval of 95% set in the study. The power of the study was set at 80%, with a beta error set at 20%. Overall comparison of CFU after application of three different oils was made using One-way analysis of variance *F*-test and pairwise comparison between groups was made using Tukey's *post hoc* test.

RESULTS

Table 1 shows the mean CFU values and multiple comparisons of all groups. In the present study, the level of significance was set at $P = 0.05$. The antimicrobial effect was the highest in the Arimedadi oil group, having the least CFU count (mean-142.47) [Figure 2], followed by dashmool oil (363.17) and was the least in the eugenol group (557.53). There was a statistically significant difference among all three groups, thereby rejecting the null hypothesis.

DISCUSSION

Root canal sealers with antimicrobial activity can help improve the success rate of endodontic treatment and are especially advantageous in clinical situations where there is a persistent or recurrent infection.[9-11]

In the current research, *E. faecalis* was utilized since it is the most often used microbe in numerous *in vitro* studies pertaining to chronic periapical infections and is the most drug-resistant bacteria that can endure for up to a year in a root canal, even in nutrient-poor conditions.

Zinc oxide–eugenol, the most commonly used sealer, has irritating effects on oral tissue, its higher concentration is more cytotoxic due to eugenol, and it lacks adequate bonding to root canal dentin. Removal of eugenol from the mixture greatly reduces toxicity.[12,13]

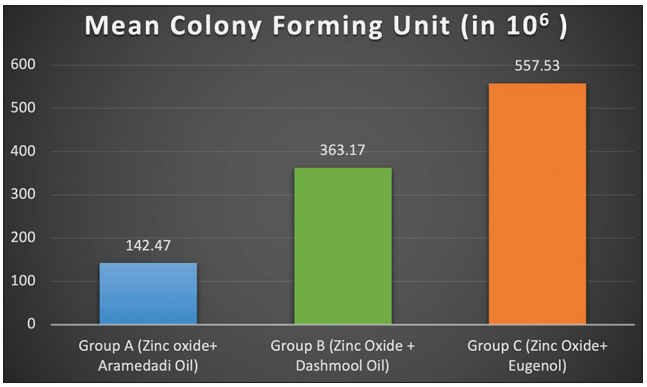

Figure 2: The bar graph of the mean number of colony-forming units (CFU) in Group A (ZO + AO) 142.47 (96.6) ×10⁶ which was the least and had a statistically significant lower colony-forming unit count as compared to Group B and C

Table 1: Pairwise comparative statistics of colony-forming unit after immersion in three different oils (values in × 10⁶) using Tukey's *post hoc* test

Group	Comparison group	Mean difference (×10⁶)	*P* (significance)
Group A (zinc oxide + aramedadi oil) versus	Group B (zinc oxide + dashmool oil)	220.7	0.034* (significant)
	Group C (zinc oxide + eugenol)	415.05	0.001* (significant)
Group B (zinc oxide + dashmool oil) versus	Group C (zinc oxide + eugenol)	194.35	0.047* (significant)

Group A had a statistical significantly lower ($P<0.05$) CFU count as compared to Group B, Group B had a statistically significantly lower ($P<0.05$) CFU count as compared to Group C, Group A had statistically significantly lower ($P<0.05$) CFU count as compared to Group C. CFU: Colony-forming unit

To overcome its drawbacks, an attempt has been made to replace eugenol with *Ayurvedic oils like Aremidadi oil and Dashmool oil.*

This is the first study to use zinc oxide–*Aremidadi oil and Dashmool oil* as root canal sealers in endodontics. In the present study, the null hypothesis was rejected as there was a statistically significant difference between all the groups.

The number of CFU in Group A (ZO + AO) was found to be (142.47 [96.6] $\times$ 10^6) the least and had a statistically significant lower CFU count as compared to Group B and C.

Arimedadi Taila/Oil is one such ayurvedic medicine useful in the treatment of common periodontal problems and is also indicated for *oil pulling* or *gargling* and in many oral problems, including glossitis, aphthous ulcers, dental caries, and gingivitis.[6] Arimedadi oil contains, Manjishtha (Rubia cordifolia), Khadira (Acacia catechu), Til oil (Sesamum inidicum), Clove (Syzygium aromaticum), and many other ingredients. Khadira has been proven for its astringent and bactericidal properties, khadira as an analgesic and anticaries agent, and manjishtha as an astringent, analgesic, anti-inflammatory blood purifier, anticaries, and antimicrobial agent. This oil also contains oil-soluble and water-soluble Phyto-active principles of medicinal herbs.

A study done by Mali *et al*.[6] aimed at assessing the antiplaque efficacy of Arimedadi (herbal) oil against 0.2% chlorhexidine gluconate mouthwash concluded that Arimedadi oil could be an effective and safe alternative to 0.2% chlorhexidine gluconate mouthwash due to its prophylactic and therapeutic benefits.[14]

Whereas Group B (dashmool oil + ZO) showed a statistically significant lower ($P < 0.05$) CFU count compared to Group C (ZO + eugenol). Dashmool literally translates to "ten roots" out of which five roots are of trees and five are of shrubs.[7] These include patala, gambhari, brihati, shalparni, and more. Dashmool or Dashmula is effective in treating diseases associated with the nose, ear, and throat. Its anti-inflammatory, antioxidant, antibacterial, analgesic, and sedative properties are not only used for pain disorders and inflammatory diseases, including osteoarthritis, rheumatoid arthritis, and gouty arthritis but also frequently used as an enema as it helps to alleviate constipation, anorexia, bloating, and flatulence.[14,15]

Devi *et al*.[16] evaluated and compared the antimicrobial efficacy of root canal sealers of different bases when mixed with herbal extracts such as (*Emblica officinalis* [*Amla*], *Myristica fragrans* [Nutmeg], and *Salvadora persica* [Miswak]) which are rich sources of bioactive compounds that possess antimicrobial properties. They concluded that zinc oxide eugenol-based sealers, when mixed with these bioactive compounds, showed the highest zones of bacterial inhibition and hence could have an additive antimicrobial effect against microbes found in the inflamed pulp.

In the present study, both the Ayurvedic oils showed statistically better results than eugenol when mixed with zinc oxide, as they contain the bioactive compounds that could have produced an additional antimicrobial effect against *E. faecalis*.

CONCLUSION

Within the limitations of the study, it was concluded that:

Ayurvedic oil-based root canal sealers showed better antibacterial efficacy than eugenol-based sealers.

On comparison, Aremidadi oil showed the highest antibacterial activity against *E. faecalis,* and eugenol showed the least when used as a solvent.

Further research is required to compare and evaluate their physio-mechanical properties such as sealing ability, microleakage, bonding to the dentin, and staining properties, before using it *in vivo*.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Nawal RR, Parande M, Sehgal R, Naik A, Rao NR. A comparative evaluation of antimicrobial efficacy and flow properties for epiphany, guttaflow and AH-plus sealer. Int Endod J 2011;44:307-13.
2. Seelan RG, Kumar AA, Emil Sam RJ, Maheswari SU. Antimicrobial efficacy of different root canal sealers by using real-time polymerase chain reaction: An *ex vivo* study. J Conserv Dent 2015;18:474-8.
3. Shekhar S, Mallya PL, Shenoy MS, Natarajan S, Mala K, Shenoy R. Comparing the disinfecting efficacy of pomegranate peel extract oil, Garlic oil, Tulsi leaf oil, and clove leaf oil with standard autoclaving on dental round burs tested against *Enterococcus faecalis*: An *in vitro* study. J Conserv Dent 2022;25:246-51.
4. AlShwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K, Majeed A. *In vitro* antimicrobial effectiveness of root canal sealers against *Enterococcus faecalis*: A systematic review. J Endod 2016;42:1588-97.
5. Ulanowska M, Olas B. Biological properties and prospects for the application of eugenol-a review. Int J Mol Sci 2021;22:3671.
6. Mali GV, Dodamani AS, Karibasappa GN, Vishwakarma P, Jain VM. Comparative evaluation of arimedadi oil with 0.2% chlorhexidine gluconate in prevention of plaque and gingivitis: A randomized clinical trial. J Clin Diagn Res 2016;10:C31-4.
7. Mehta C, Shirode P, Kumar A, Kumar S. A conceptual Ayurvedic study on dashmoola taila and yava kshara. World J Pharm Med Res 2019;5:201-12.
8. Vanapatla A, Vemisetty H, Punna R, Veeramachineni C, Venkata RP, Muppala JN, *et al.* Comparative evaluation of antimicrobial effect of three endodontic sealers with and without antibiotics – An *in-vitro* study. J Clin Diagn Res 2016;10:C69-72.
9. Mangat P, Dhingra A, Muni S, Bhullar HK. To compare and evaluate the antimicrobial activity of three different root canal sealers: An *in vitro* study. J Conserv Dent 2020;23:571-6.
10. Gupta R, Sharma D, Mathur M, Pooja B, Bishnoi R. Antibacterial activity of root canal sealers against established monospecies biofilm: An *in vitro* study. J Conserv Dent Endod 2023;26:569-73.

11. Brezhnev A, Neelakantan P, Tanaka R, Brezhnev S, Fokas G, Matinlinna JP. Antibacterial additives in epoxy resin-based root canal sealers: A focused review. Dent J (Basel) 2019;7:72.
12. Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S. Antimicrobial efficacy of different endodontic sealers against *Enterococcus faecalis*: An *in vitro* study. J Int Soc Prev Community Dent 2018;8:104-9.
13. Kaur A, Shah N, Logani A, Mishra N. Biotoxicity of commonly used root canal sealers: A meta-analysis. J Conserv Dent 2015;18:83-8.
14. Priyadarshini V, Vinita Mary A, Kesavan R, Mugesh Raj S, Nishanthi R, Evangeline Dorothy D. Practice of Ayurveda in dentistry. Int J Allied Med Sci Clin Res 2020;8:717-21.
15. Parekar RR, Bolegave SS, Marathe PA, Rege NN. Experimental evaluation of analgesic, anti-inflammatory and anti-platelet potential of Dashamoola. J Ayurveda Integr Med 2015;6:11-8.
16. Devi MT, Saha S, Tripathi AM, Dhinsa K, Kalra SK, Ghoshal U. Evaluation of the antimicrobial efficacy of herbal extracts added to root canal sealers of different bases: An *in vitro* study. Int J Clin Pediatr Dent 2019;12:398-404.