

# Comparative Effect of Various Irrigants with Herbal Formulations in Reducing Enterococcus Faecalis Count

J Deepasakthi<sup>1</sup>, R.V.Geetha<sup>2\*</sup>, T. Lakshmi<sup>3</sup>

<sup>1</sup>Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

<sup>2</sup>Professor, Department of Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

<sup>3</sup>Professor, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India

## ABSTRACT

**Background:** Enterococcus faecalis is associated with different forms of periradicular diseases, both primary and persistence endodontic infections. Traditional herbals have high antimicrobial, anti-inflammatory, antioxidant and biocompatible properties that make their use in dentistry more extensive. Judicious use of these herbal formulations has been found to mitigate the E.faecalis count.

**Aim:** To compare the effect of various irrigants with herbal formulations in reducing Enterococcus faecalis count

**Materials and methods:** Different herbal formulated irrigants were procured commercially. The test organism E.faecalis was isolated and maintained in the Nutrient Agar Slope at 4 degree C. Different concentrations of herbal irrigants were mixed with BHI agar and 50µL of the test organisms were added and poured into sterile petri dishes. Lawn culture of the test bacteria was made on the Plates and incubated at 37 degree C for 24 hrs and the colony forming units(CFU) were counted and recorded.

**Result:** In this present study, comparing all 3 samples there is a higher reduction of count in Sample 3 (Cardamom and Tulsi mediated) than Sample 1 and Sample 2.

**Conclusion:** Based on the results presented, it is possible to conclude that the herbal formulations represent a good reduction of Enterococcus faecalis.

Corresponding Author e-mail: geetha@saveetha.com

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## INTRODUCTION

A large number of bacterial species play a major role in the development of pulp and periapical diseases(1). The root canal system can get infested with bacteria because of the privileged anatomic placement, and these germs are immune to the host defence system(2). Enterococcus faecalis is the predominant species that is linked to secondary infection, and the prevalence of this species in persistent endodontic infections ranges from 24% to 77%. E.faecalis is a gram-positive cocci and a facultative anaerobe that can withstand temperatures of 60 °C for 30 minutes as well as pH extremes of 9.6 and salt concentrations(3). In adverse environmental conditions, it can develop a calcified biofilm inside the root canals. Due to the increased antimicrobial resistance of biofilm bacteria, biofilm formation on tissues and biomaterial surfaces can result in biofilm-mediated illnesses that are challenging to cure(4).

## KEYWORDS:

Enterococcus faecalis,  
Nutrient Agar Slope,  
Herbal Irrigants,  
BHI agar.

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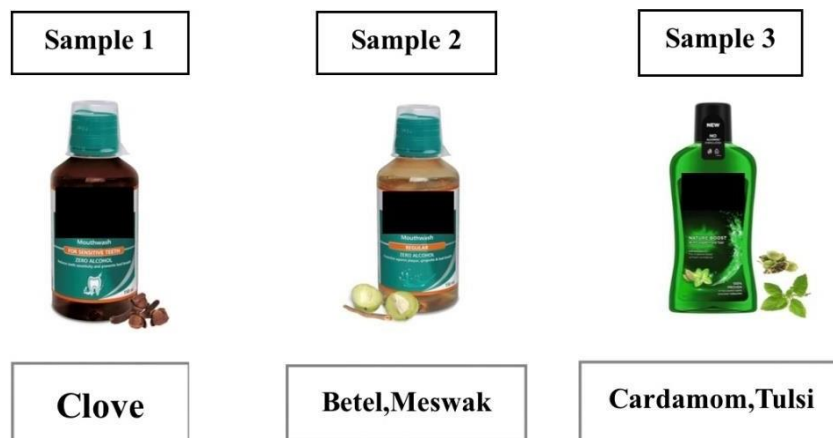
The use of several irrigants in non-surgical endodontic procedures has been suggested(5). Sodium hypochlorite has been the gold standard as an endodontic irrigant due to its high antimicrobial action and ability to dissolve the organic material(6). However, it has a number of negative side effects, including the potential to cause allergies, tissue toxicity, bad taste and odour, difficulty to completely eradicate germs from infected canals, staining and rusting of tools, and unpleasant taste and odour(7). Herbal medicines are now becoming more popular(8). Due to benefits including fewer side effects, affordability, improved patient tolerance, and renewable nature, natural materials have gained popularity in modern dentistry(9).

It has been demonstrated that conventional plants are a better source when looking for a novel antibacterial component(10). They are used more frequently in dentistry because of their potent antimicrobial, anti-inflammatory, antioxidant (thanks to the wide variety of active phytochemicals present, including flavonoids, terpenoids, lignans, sulphides, polyphenolics,

carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides), and biocompatible properties(11). These herbal preparations can be used wisely to lower the E. faecalis count, which could lower infections that develop after therapy. Hence, it can be considered as one of the possible alternatives or a replacement for the synthetic chemical formulations(12). The aim of the study is to compare the effect of various irrigants with herbal formulations in reducing Enterococcus faecalis count.

## MATERIALS AND METHODS

For the study, three different herbal irrigants were obtained commercially from local market and used for the study. Sample 1 herbal irrigant prepared by standardised preparation method using Clove (*Syzygium aromaticum*), Sample 2 herbal irrigant was prepared using Betel (*Piper betle*) and Sample 3 was prepared using Cardamom (*Elettaria cardamomum*) and Tulsi (*Ocimum tenuiflorum*).



**Fig. 1:** This figure shows the 3 different herbal irrigants collected from the market.

The test organism used was *Enterococcus faecalis*. The organism was isolated from clinical samples using selective media Mutans-Sanguis Agar (HiMedia M977) and maintained in Nutrient Agar Slope at 4°C. Agar plate dilution technique was followed to screen the activity of herbal irrigants in reducing the *Enterococcus faecalis* count. Different concentrations of herbal irrigants (50µL, 100µL, 200µL) were mixed with BHI agar and 50µL of the test organisms were added to the BHI agar and poured into sterile petri dishes. The plate was incubated overnight at 37°C and the colony forming unit per millilitre was calculated.

## RESULT

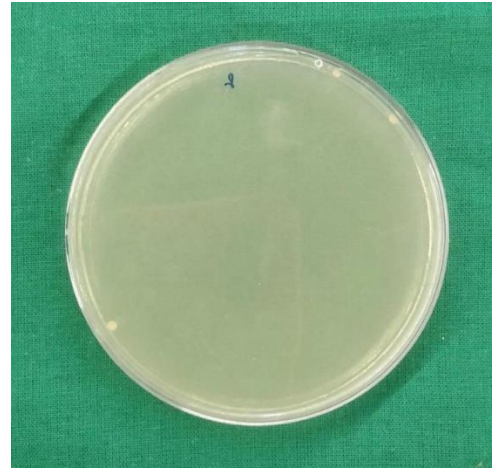
The table below depicts the bacterial count of *E. faecalis* at different concentrations of each sample. There is significant reduction in count with increase in concentration. The findings reveal that Sample 1 (Clove mediated) showed 400 CFU/ml against *E. faecalis* at concentration of 50µL, 70 CFU/ml at 100µL which then subsequently reduced with increasing concentration. In Sample 2 (Betel and Meswak mediated), 550 CFU/ml at 50µL, 95 CFU/ml at 100µL. In Sample 3 (Cardamom and Tulsi mediated), showed 300 CFU/ml at concentration of 50µL, 25 CFU/ml at 100µL which then subsequently reduced with increasing concentration. Comparing all 3 samples there is a higher reduction of count in Sample 3 (Cardamom and Tulsi mediated).

**Table 1:** Bacterial count of *E. faecalis* (CFUs) at different concentrations of each sample is given

|          | 50µL             | 100µL     | 200µL     |
|----------|------------------|-----------|-----------|
| Sample 1 | 400 CFU/ml       | 70 CFU/ml | No growth |
| Sample 2 | 550 CFU/ml       | 95 CFU/ml | No growth |
| Sample 3 | 300 CFU/ml       | 25 CFU/ml | No growth |
| Control  | Confluent growth |           |           |



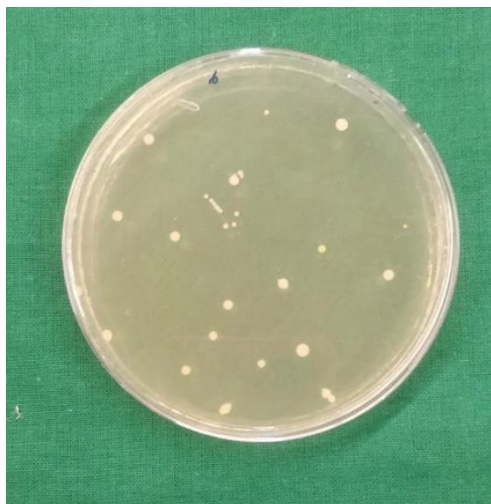
**Fig 2:** Growth of *E. faecalis* before irrigation with Control.



**Fig.5:** Reduction of *E. faecalis* count after irrigation at 200µL.



**Fig.3:** Reduction of *E. faecalis* count after irrigation at 50µL.



**Fig.4:** Reduction of *E. faecalis* count after irrigation at 100µL.

## DISCUSSION

All around the world, medicinal plants are a source of significant economic value. About 75-80% of the population still relies heavily on herbal medicine, and the utilisation of plant extracts and their active ingredients still makes up a large portion of traditional therapy(13). A preliminary study concluded that the extracts of *O. sanctum*, *C. zeylanicum*, *S. aromaticum* showed antimicrobial activity against *E. faecalis*, NaOCl was shown to be the best among all the groups, both in planktonic and biofilm forms (14). Another study showed that Cinnamon is more effective than turmeric but less effective than garlic at killing *Candida albicans* and least effective at killing *Enterococcus faecalis*. Compared to cinnamon, garlic had less colonies, making it more antibacterial when it comes to the antimicrobial action of herbal extracts (15).

In our study, Clove mediated sample (Sample 1) showed 400 CFU/ml against *E. faecalis* at concentration of 50µL, 70 CFU/ml at 100µL which then subsequently reduced to No growth with increasing concentration to 200µL. Likewise for Sample 2 (Betel and Meswak mediated), 550 CFU/ml at 50µL, 95 CFU/ml at 100µL. In Sample 3, which is Cardamom and Tulsi-mediated, there was a decrease in count to 300 CFU/ml at concentrations of 50µL, 25 CFU/ml at 100µL and at 200µL there was No growth of *E. faecalis*. When comparing the counts of the three samples, Sample 3 shows a greater reduction (Cardamom and Tulsi mediated). Therefore, the Cardamom and Tulasi Mediated Irrigant was the best among the 3 Samples. The main benefits of utilising herbal substitutes are their accessibility, affordability, longer shelf life, low toxicity, and absence of microbial resistance as of yet(16). This might be because herbal irrigants produce a wide range of biologically active substances that are chemically varied and reduce

bacterial viability by blocking the respiratory chain(17). In comparison to the conventional root canal irrigants, this provides an added benefit(18). The undesirable odour and taste, limited shelf life, and need for fresh solutions to be created each time are drawbacks of using herbal irrigants that must be overcome(19). More research can be done with different Samples and with different concentrations so that these irrigants are more widely accepted(20-29)

## CONCLUSION

Based on the results presented, it is possible to conclude that the herbal formulations represent a good reduction of Enterococcus faecalis. These herbal preparations can be used wisely to lower the E. faecalis count, which could lower infections that develop after therapy. Hence, it can be considered as one of the possible alternatives or a replacement for the synthetic chemical formulations.

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## Conflict Of Interest

All the authors declare that there was no conflict of interest in the present study.

Ethical approval-NA

Conflict of interest-NA

Informed Consent- None declared

## Authors Contribution

DSJ: Literature search, data collection, analysis, manuscript drafting, RVG Data verification, manuscript drafting, TL designed the study

## REFERENCE

- Dubey S. Comparative antimicrobial efficacy of herbal alternatives (*Emblica officinalis*, *Psidium guajava*), MTAD, and 2.5% sodium hypochlorite against *Enterococcus faecalis*: An in vitro study. *J Oral Biol Craniofac Res.* 2016 Jan;6(1):45-8.
- 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 [Internet]. *Journal of Clinical and Experimental Dentistry.* 2016. p. 0-0. Available from: <http://dx.doi.org/10.4317/jced.52339>
- Sohraabi-Araghi KH, Ashofte-Yazdi K, Iran-Parvar K. In vitro comparison of antimicrobial effect of three intracanal irrigants and diode laser on canals infected with *Enterococcus faecalis* [Internet]. *Medicina Oral Patología Oral y Cirugía Bucal.* 2012. p. S16 - Available from: <http://dx.doi.org/10.4317/medoral.17643515>
- Rosaline H, Kandaswamy D, Gogulnath D, Rubin MI. Influence of various herbal irrigants as a final rinse on the adherence of *Enterococcus faecalis* by fluorescence confocal laser scanning microscope [Internet]. Vol. 16, *Journal of Conservative Dentistry.* 2013. p. 352. Available from: <http://dx.doi.org/10.4103/0972-0707.114365>
- Guneser MB, Eldeniz AU. The effect of gelatinase production of *Enterococcus faecalis* adhesion to dentin after irrigation with various endodontic irrigants [Internet]. Vol. 2, *Acta Biomaterialia Odontologica Scandinavica.* 2016. p. 144-9. Available from: <http://dx.doi.org/10.1080/23337931.2016.1256212>
- Balaji K, Afshan T, Parwez A, Prasanna PL, Bhargav MVG, Hussain T. Comparison of Antimicrobial Efficacy of Herbal Root Canal Irrigants (*Azadirachta indica*, *Morinda citrifolia*) against *Enterococcus faecalis* [Internet]. Vol. 11, *World Journal of Dentistry.* 2020. p. 206-10. Available from: <http://dx.doi.org/10.5005/jp-journals-10015-1733>
- Kumar GV, Vinay Kumar G, Uppin V, Shenoy A. Comparison of Antibacterial Effects of Various Root Canal Irrigants on *Enterococcus faecalis* [Internet]. Vol. 2, *World Journal of Dentistry.* 2011. p. 211-5. Available from: <http://dx.doi.org/10.5005/jp-journals-10015-1085>
- Evaluation of Antimicrobial Efficacy of Herbal Extracts (Tridax procumbens and *Aegle Marmelos*) and 5% Sodium Hypochlorite as Irrigants against *Enterococcus faecalis*: An In Vitro Study [Internet]. Vol. 10, *Indian Journal of Dental Advancements.* 2019. Available from: <http://dx.doi.org/10.5866/2018.10.10149>
- Tatekalva P, Subbaiyan H, Kumar SR. Comparative evaluation of antimicrobial potential of herbal extracts on *Streptococcus mutans* and *Enterococcus faecalis*: An in vitro study [Internet]. Vol. 24, *Brazilian Dental Science.* 2020. Available from: <http://dx.doi.org/10.14295/bds.2021.v24i1.2147>
- Johnson WT. Comparative Evaluation of Endodontic Irrigants against *Enterococcus faecalis* Biofilms [Internet]. Vol. 2007, *Yearbook of Dentistry.* 2007. p. 237. Available from: [http://dx.doi.org/10.1016/s0084-3717\(08\)70485-9](http://dx.doi.org/10.1016/s0084-3717(08)70485-9)
- Ahamed S, Raju VG, Krishnamurthy M, Naveen Kumar V, Selvendran KE. Antimicrobial Efficacy of Herbal Root Canal Irrigants and 3% Sodium Hypochlorite against *Enterococcus faecalis*: An In-vitro Study [Internet]. *Journal of Pharmaceutical Research International.* 2021. p. 74-8. Available from: <http://dx.doi.org/10.9734/jpri/2021/v33i48a33214>
- Zaneva-Hristova D, Borisova-Papancheva T. Antibacterial effect of irrigants and medications for temporary dressing on *Enterococcus faecalis* [Internet]. Vol. 9, *Journal of Medical and Dental Practice.* 2022. p. 1394-402. Available from: <http://dx.doi.org/10.18044/medinform.202291.1394>
- Philip PM, Sindhu J, Poornima M, Naveen DN, Nirupama DN, Nainan MT. Effects of conventional and herbal irrigants on microhardness and flexural strength of root canal dentin: An study. *J Conserv Dent.* 2021 Jan;24(1):83-7.
- Gupta A, Duhan J, Tewari S, Sangwan P, Yadav A, Singh G, et al. Comparative evaluation of antimicrobial efficacy of *Syzygium aromaticum*, *Ocimum sanctum* and *Cinnamomum zeylanicum* plant extracts against *Enterococcus faecalis*: a preliminary study [Internet]. Vol. 46, *International Endodontic Journal.* 2013. p. 775-83. Available from: <http://dx.doi.org/10.1111/iej.12058>
- Gopalakrishnan S, Rajesh S, Ravi J. A comparative evaluation of antimicrobial efficacy of cinnamon and garlic as endodontic irrigants against *enterococcus faecalis* - An in vitro study [Internet]. Vol. 26, *Endodontology.* 2014. p. 149. Available from: <http://dx.doi.org/10.4103/0970-7212.352344>
- 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 Oct;38(4):374-80.
17. Shi Y, Deng Z, Yang Y, Cui L, Chen T, Hu M, et al. Evaluation of Sodium Hypochlorite Irrigant, Bingpeng Irrigant, and Fufang Bingpeng Irrigant as Endodontic Irrigants During Passive Ultrasonic Irrigation. *Front Cell Infect Microbiol.* 2019 May 10;9:145.
  18. Agnihotri A, Jhamb S, Shrama U, Rohtagi S. A. juss, L. and as herbal endodontic irrigants: A scoping review. *Ayu.* 2020 Jul;41(3):148-58.
  19. Kishan KV, Shah NC, Das DT, Parikh M. Herbal medicaments in endodontics - Current guidelines for studies in India. *J Conserv Dent.* 2019 Sep;22(5):411-4.
  20. Krishnan S, Pandian S, Kumar S A. Effect of bisphosphonates on orthodontic tooth movement-an update. *J Clin Diagn Res.* 2015 Apr;9(4):ZE01-5.
  21. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of Vertical Root Fractures by Cone-beam Computed Tomography in Root-filled Teeth with Confirmation by Direct Visualization: A Systematic Review and Meta-Analysis. *J Endod.* 2021 Aug;47(8):1198-214.
  22. Kanniah P, Radhamani J, Chelliah P, Muthusamy N, Joshua Jebasingh Sathiya Balasingh E, Reeta Thangapandi J, et al. Green synthesis of multifaceted silver nanoparticles using the flower extract of *Aerva lanata* and evaluation of its biological and environmental applications. *ChemistrySelect.* 2020 Feb 21;5(7):2322-31.
  23. Ezhilarasan D. MicroRNA interplay between hepatic stellate cell quiescence and activation. *Eur J Pharmacol.* 2020 Oct 15;885:173507.
  24. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. *Am J Cancer Res.* 2020 Aug 1;10(8):2546-54.
  25. Ravindiran M, Praveenkumar C. Status review and the future prospects of CZTS based solar cell - A novel approach on the device structure and material modeling for CZTS based photovoltaic device. *Renewable Sustainable Energy Rev.* 2018 Oct 1;94:317-29.
  26. Rajesh A, Gopal K, Melvin Victor DP, Rajesh Kumar B, Sathiyagnanam AP, Damodharan D. Effect of anisole addition to waste cooking oil methyl ester on combustion, emission and performance characteristics of a DI diesel engine without any modifications. *Fuel.* 2020 Oct 15;278:118315.
  27. Jain SV, Vijayakumar Jain S, Muthusekhar MR, Baig MF, Senthilnathan P, Loganathan S, et al. Evaluation of Three-Dimensional Changes in Pharyngeal Airway Following Isolated Lefort One Osteotomy for the Correction of Vertical Maxillary Excess: A Prospective Study [Internet]. Vol. 18, *Journal of Maxillofacial and Oral Surgery.* 2019. p. 139-46. Available from: <http://dx.doi.org/10.1007/s12663-018-1113-4>
  28. Kumar SP, Praveen Kumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Targeting NM23-H1-mediated Inhibition of Tumour Metastasis in Viral Hepatitis with Bioactive Compounds from *Ganoderma lucidum*: A Computational Study [Internet]. Vol. 82, *Indian Journal of Pharmaceutical Sciences.* 2020. Available from: <http://dx.doi.org/10.36468/pharmaceutical-sciences.650>
  29. Jayanth BV, Depoures MV, Kaliyaperumal G, Dillikannan D, Jawahar D, Palani K, et al. A comprehensive study on the effects of multiple injection strategies and exhaust gas recirculation on diesel engine characteristics that utilize waste high density polyethylene oil. *Energy Sources Part A.* 2021 Jun 3;1-18.