An In Vitro Study Comparing the Antimicrobial Effectiveness of Various Irrigation Solutions on Vancomycin-Resistant Enterococcus Faecalis

Roocha Shah[1], Krishna Dere[2], Mira Virda[3], Aishwarya Savakiya[4], Unnati Bhudhrani[5]

PG Student[1,4,5], Professor[2], Associate Professor[3]

Department of Pediatric and Preventive Dentistry, College of Dental Sciences and Research Centre, Bopal, Ahmedabad, India

ABSTRACT

INTRODUCTION: Antimicorbial resistance poses a serious threat to public health worldwide. It has been observed that E. faecalis is the most commonly found in failed endodontic treatment cases. Vancomycin-resistant enterococci are on the rise globally in primary intra-radicular infections and resistant to most intracanal irrigants.

AIM: To evaluate the antibacterial properties of herbal extracts, namely Tulsi, Sitopaladi Churna and Evocus water as irrigants during endodontic treatment in comparison with the conventional irrigating solutions against Vancomycin resistant Enterococcus faecalis.

METHODOLOGY: Vancomycin resistant E. faecalis (ATCC 51299) strains is used. Agar well diffusion method is used to evaluate the antimicrobial efficacy of experimental irrigants against Vancomycin resistant E.faecalis. The difference between groups was statistically analysed.

RESULTS: Tulsi extract have shown antibacterial activity against Vancomycin resistant E.faecalis.

CONCLUSION: Tulsi extract have shown antibacterial efficacy while 3% Sodium Hypochlorite, Normal Saline, Sitopaladi Churn and Evocus water have shown no antibacterial activity is seen against Vancomycin resistant E. faecalis.

INTRODUCTION

The primary objective of endodontic therapy is to thoroughly cleanse the root canal system by eliminating all bacteria dead tissue and substances produced by microbes. Achieving this entails employing chemo-mechanical debridement of the root canal (1). The complexity of the root canal in primary teeth arises from the presence of accessory canals, necessitating mechanical instrumentation and irrigation for effective debridement of hard-to-reach areas (2). E. faecalis, a type of gram-positive bacterium capable of surviving and reproducing on its own within the root canal. It is often found in the root canals of teeth, especially those where endodontic treatments have not been successful(3,4).

The rise of antimicrobial resistance poses a significant global health threat, affecting individuals across all age groups. This resistance jeopardizes the efficacy of second- and third-line antibiotics, potentially causing severe adverse effects such as organ failure, and in some cases, delaying treatment and recovery (5). In light of this, researchers are exploring natural alternatives within modern dental practice. Herbal medicines, derived from plants, are being investigated for their potential to treat illnesses and promote

Correspondence address: Dr. Roocha Shah, Department of Pediatric and Preventive Dentistry, College of Dental Sciences and Research Centre, Bopal, Ahmedabad, India
Email: roochashah6.rs@gmail.com
How to cite this article: Shah R, Dere K, Virda M, Savakiya A, Bhudhrani U. Novel Researches and Future Aspects of Dental Caries Vaccine: A Literature Review. TMU J Dent 2023; 10(4): 57-62.
Submitted: 22 Sep 2023 Revised and accepted: 6 Oct 2023
Doi: https://doi.org/10.58358/tmujd.ped10435r

overall well-being (6). Recent discoveries indicate that plant-derived extracts can act as efficient solutions for endodontic irrigation.(7). Despite the widespread use of certain antibacterial agents, concerns about increased resistance to antimicrobial drugs and associated negative adverse effects have surfaced. Hence, there is a pressing need for affordable, non-toxic, and efficacious agents that can serve as alternatives (7).

Considering the benefits of using natural irrigation substances, this study was carried out to evaluate the antibacterial capabilities of herbal extracts (tulsi, sitopaladi churna, and evocus water) in comparison to conventional irrigating solutions against vancomycin-resistant E. faecalis.

METHODOLOGY

• **Location of the research and the bacterial type employed**

This laboratory based In vitro research was conducted at the School of Life Sciences, Central University of Gujarat, Gandhinagar. For this study, Enterococcus faecalis (VRE) ATCC 51299 (obtained from Himedia) was employed. The bacteria were sub-cultivated from the original culture, and a culture in suspension of the target microorganism was initiated using Brain Heart Infusion broth (8).

- **Standardization of microorganisms**

E. faecalis was introduced into Brain Heart Infusion broth and left to incubate for 6–7 hours. This incubation period was necessary to achieve a mean optical density of 0.5 McFarland standard, corresponding to 1.5 × 108 CFU/ml (as the negative control). Following this, 1 ml of each suspended culture was meticulously transferred into the correct volume of sterile screw cap tubes (HIMEDIA). Throughout these steps, strict adherence to sterility was maintained, utilizing sterilized instruments and materials (8).

- **Agar well diffusion method**

The agar diffusion microbiological method was utilized to evaluate the antimicrobial efficacy of various irrigation solutions. Group I utilized Tulsi, Group II employed sitopaladi churna, Group III utilized evocus water, and Group IV involved 3% sodium hypochlorite. Additionally, Group V served as the control using normal saline.

- **Extract preparation**

The Tulsi extract used in this study involved finely grinding dried leaves to obtain a powdered form. Subsequently, the powder was soaked in isopropyl alcohol and then filtered. 300 grams of Tulsi powder were dissolved in one liter of isopropyl alcohol, we obtained 18 grams of Tulsi extract (with a residue of 6% w/w) (9).

As for the Sitopaladi churna extract utilized in this study, the process involved mixing 25 g of powder with 200 ml of Isopropyl alcohol. This mixture was macerated for 7 days, then filtered, and finally evaporated on a water bath, ensuring the temperature did not exceed 60 degrees Celsius (10).

- **Agar Well Diffusion Assay method**

The agar diffusion technique was utilized to assess the antimicrobial efficacy of different irrigation solutions, including Tulsi, Sitopaladi churna, Evocus, 3% NaOCl, and Normal Saline. Four bacterial cultures (each 200 μm) were evenly distributed on agar plates containing BHI broth.

Wells with a diameter of 6 mm were carefully created on these agar plates. Subsequently, specific wells were filled with Tulsi, Sitopaladi churna, Evocus, 3% NaOCl, or Normal Saline. The agar plates were incubated at a temperature of 37°C for 24 hours, and the zones inhibiting the growth of E. faecalis were evaluated following this incubation period (9).

RESULTS

"The inhibition zones were measured subsequent to incubation for each plate against E. faecalis. These measurements were recorded and subjected to statistical analysis using a one-way analysis of variance (ANOVA) test ($P < 0.001$).

Graph 1 and Table 1 present the mean values indicating the antibacterial effects of 3% sodium hypochlorite, Sitopaladi churna, Evocus water, and normal saline. These solutions displayed no antimicrobial activity. Conversely, Tulsi extract exhibited a 2mm zone of inhibition against vancomycin-resistant E.faecalis. In Graph 1, the mean inhibition zone against vancomycin-resistant E. faecalis is illustrated."

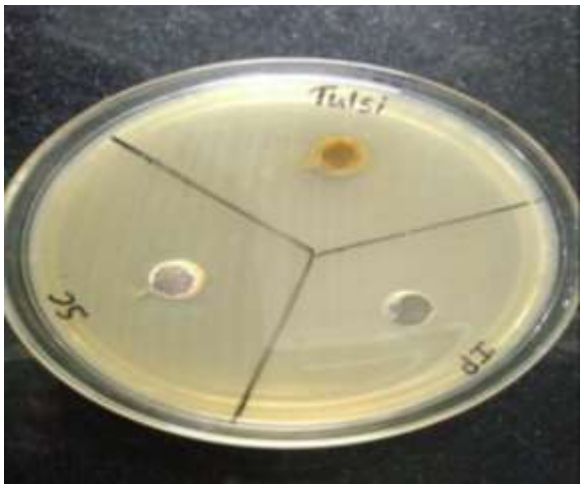


Figure 1: Zone of inhibition on agar plates against VRE

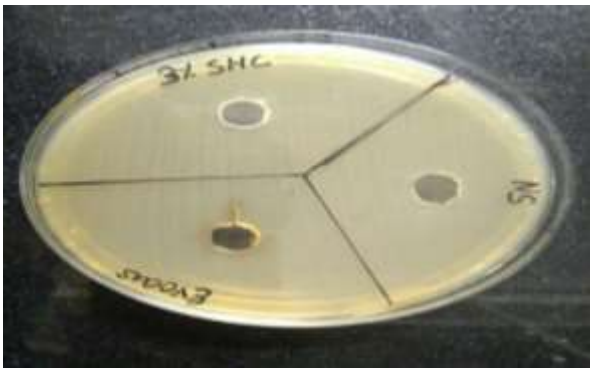
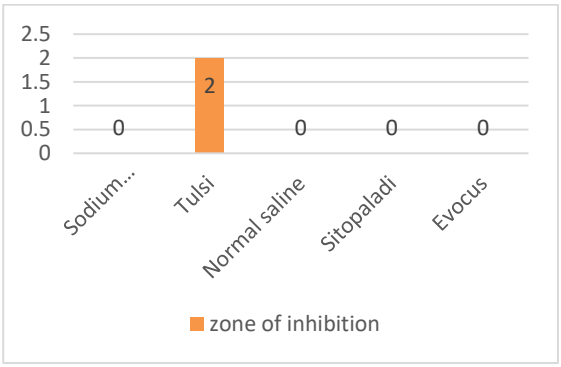


Figure2: Zone of inhibition on agar plates against VRE

SR NO.	IRRIGATING SOLUTION	ZONE OF INHIBITION (mm)
1	TULSI	2 mm
2	EVOCUS	0 mm
3	SITOPALADI CHURNA	0 mm
4	SODIUM HYPOCHLORITE	0 mm
5	NORMAL SALINE	0 mm

Table 1: Inhibitory zone of irrigants against vancomycin resistant E.faecalis



Graph 1: Zone of inhibition of irrigants against vancomycin resistant E.faecalis

DISCUSSION

"Persistent inter-radicular infection, as highlighted by Stuart et al. in 2006, stands as the primary cause for endodontic treatment failure (11). E. faecalis exhibits a variety of survival and virulence characteristics, potentially explaining its high prevalence rates ranging from 24% to 77% (11). The most effective approach to combat E. faecalis within root canal systems involves implementing aseptic techniques, enlarging apical preparation diameters, and integrating 2% CHX with NaOCl (12).

In the realm of endodontics, the use of antibiotics should be limited due to the rise in bacterial resistance against a majority of available antibiotics (13). Polymerase chain reaction findings demonstrated a significantly higher incidence of vancomycin-resistant E. faecalis (VRE) in diabetic patients and non-healing endodontic cases (14).

According to Macovei and Zurek, antibiotic resistance is evident in enterococcal isolates, with E. faecalis (88.2%), E. faecium (6.8%), and Enterococcus casseliflavus (4.9%) being major contributors (15). Although the prevalence of VRE varies among clinical isolates in India (ranging from 0% to 30%), the presence of VRE and high levels of aminoglycoside resistance emphasize the necessity for routine vancomycin resistance detection to curb the emergence and spread of multidrug-resistant Enterococcus species (16).

Our study employed E. faecalis ATCC 51299 (vanB positive – low-level vancomycin resistant) as a quality control strain (17). We found that NaOCl was ineffective in eliminating VRE. This corroborates findings by Radcliffe et al. and Retamozo et al., who also emphasized the need for higher concentrations of NaOCl and prolonged exposure times to eradicate E. faecalis (18,19). However, contrary to our study, Sassone et al. reported the immediate and sustained effectiveness of 1% and 5% NaOCl in eliminating E. faecalis (20).

Nature, with its profound symbiotic systems, has long stood as a source of inspiration. Plants have been integral in medical treatments throughout human history, addressing various infectious diseases (21). The revered tulsi plant (Ocimum sanctum) is native to India and is often referred to as nature's potent remedy. Economical and easily accessible, tulsi is celebrated for its antibacterial properties and is commonly employed in treating diverse ailments like diabetes, pneumonia, arthritis, and skin conditions (21).

Tulsi's antibacterial capabilities have been tested against several pathogens including Escherichia coli, Klebsiella, Candida albicans, Staphylococcus aureus, Enterococcus faecalis, and Proteus (22,23). The antimicrobial potential of tulsi is attributed to its constituents, particularly ursolic acid and carvacrol (21).

Gupta-Wadhwa et al. conducted an evaluation of the antimicrobial effectiveness of three herbal irrigants - Ocimum sanctum (OS), Cinnamomum zeylanicum (CZ), and Syzygium aromaticum (SA) - against Enterococcus faecalis. Their study concluded that Cinnamomum zeylanicum, Syzygium aromaticum, and Ocimum sanctum exhibited intracanal bacterial reduction against Enterococcus faecalis (24).

Sitopaladi Churna, a polyherbal preparation, contains Piper longum (Piperaceae), Elettaria cardamom (Zingiberaceae), and Cinnamomum zeylanicum (Lauraceae). Methanolic and aqueous extracts of Sitopaladi Churna have displayed antibacterial efficacy against Aspergillus niger, Candida albicans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa (25). The antibacterial and antifungal properties are attributed to piperine and the synergistic effect of the formulation's components (25).

Evocus Alkaline Water is a bottled water variant characterized by its high pH level, typically ranging from 8.0 to 9.5, making it alkaline. Achieved through the addition of alkaline minerals like calcium, magnesium, and potassium, its key ingredients include Calcium (Ca^{+2}), Magnesium (Mg^{+2}), Potassium (K^{+}), Bicarbonate (HCO_3^{-}), Sodium (Na^{+}), and Chloride (Cl^{-}) (25)."

CONCLUSION

The present study showed inhibitory mean zone of 2 mm for Tulsi extract, while no zone of inhibition is seen for NaOCl, normal saline, Sitopaladi Churna and Evocus water against vancomycin resistant E.faecalis. The antibacterial property of tulsi can be attributed the presence of Ursolic acid and carvacrol. (21)

ACKNOWLEDGMENT:

We would like to show our gratitude to Hardi Patel for helping us in conducting our study in their department of

microbiology and Dr. Shravanthi Reddy Tagala for guiding throughout our study.

CONFLICT OF INTEREST
NIL.

FUNDING:
No fundings has been provided

REFRENCES
1) Agrawal VS, Rajesh M, Sonali K, Mukesh PA. Contemporary overview of endodontic irrigants – a review. J Dent App. 2014; 1(6): 105-15.
2) Adl A, Shojaee NS, Motamedifar M. A comparison between the antimicrobial effects of triple antibiotic paste and calcium hydroxide against Enterococcus faecalis. Iran Endod J. 2012; 7(3): 149-55.
3) Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in re-treatment. J Endod. 2006; 32: 93-8.
4). Tendolkar PM, Baghdayan AS, Shankar N. Pathogenic enterococci: new developments in the 21st century. Cell Mol Life Sci. 2003; 60: 2622-36.
5) About antiobiotic resistance [Internet]. Centers for Disease Control and Prevention. Centers for Disease Control and Prevention; 2022 [cited 2023Feb12].
6) Subasree S, Murthy Kumar K, Sripradha S. Effects of turmeric on oral health: an overview. International J Pharma Sci Health Care. 2014; 2(4): 6-14.
7). Kamat S, Rajeev K, Saraf P. Role of herbs in endodontics. An update, Endodontology. 2014; 26(1): 98-102.
8) Ravinanthanan M, Hegde MN, Shetty VA, Kumari S. Antimicrobial assay of combination surfactant irrigant regimen on vancomycin-resistant Enterococcus faecalis. An in vitro direct contact test. Dent Res J (Isfahan). 2018;15(6):397-403.
9) Chandrappa PM, Dupper A, Tripathi P, Arroju R, Sharma P, Sulochana K. Antimicrobial activity of herbal medicines (tulsi extract, neem extract) and chlorhexidine against Enterococcus faecalis in Endodontics: An in vitro study. J Int Soc Prev Community Dent. 2015;5(Suppl 2):S89-S92. doi:10.4103/2231-0762.172952
10) Ekbote MT, Rajashekar KV, Shankarappa L, Bharathi DR. Phytochemical and antimicrobial screening of sitopaladi churna. GSC Advanced Research and Reviews. 2019 Dec 30; 1(2):016-9.
11) Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: Its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006; 32: 93–8.
12) Sedgley CM, Lennan SL, Clewell DB. Prevalence, phenotype and genotype of oral enterococci. Oral Microbiol Immunol. 2004;19:95–101
13) Siqueira JF., Jr Endodontic infections: Concepts, paradigms, and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94:281–93.
14) Fouad AF, Zerella J, Barry J, Spångberg LS. Molecular detection of Enterococcus species in root canals of therapy-resistant endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;99:112–8.
15) Macovei L, Zurek L. Ecology of antibiotic resistance genes: Characterization of enterococci from houseflies collected in food settings. Appl Environ Microbiol. 2006; 72:4028–35
16) Fernandes SC, Dhanashree B. Drug resistance & virulence determinants in clinical isolates of Enterococcus species. Indian J Med Res. 2013; 137:981–5.
17) Swenson JM, Clark NC, Sahm DF, Ferraro MJ, Doern G, Hindler J, et al. Molecular characterization and multilaboratory evaluation of Enterococcus faecalis ATCC 51299 for quality control of screening tests for vancomycin and high-level aminoglycoside resistance in

Enterococci. J Clin Microbiol. 1995; 33:3019–21.
18) Radcliffe CE, Potouridou L, Qureshi R, Habahbeh N, Qualtrough A, Worthington H, et al. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms Actinomyces israelii, A. naeslundii, Candida albicans and Enterococcus faecalis. Int Endod J. 2004; 37: 438–46.
19) Retamozo B, Shabahang S, Johnson N, Aprecio RM, Torabinejad M. Minimum contact time and concentration of sodium hypochlorite required to eliminate Enterococcus faecalis. J Endod. 2010; 36: 520–3.
20) Sassone LM, Fidel RA, Fidel SR, Dias M, Hirata RJ. Antimicrobial activity of different concentrations of NaOCl and chlorhexidine using a contact test. Braz Dent J. 2003; 14: 99–102
21) Agarwal P, Nagesh L, Murlikrishnan Evaluation of the antimicrobial activity of various concentrations of Tulsi (Ocimum sanctum) extract against Streptococcus mutans: An in vitro study. Indian J Dent Res. 2010; 21: 357–9.
22) Geeta, Vasudevan DM, Kedlaya R, Deepa S, Ballal M. Activity of ocimum sanctum (the traditional Indian medicinal plant) against enteric pathogens. Indian J Med Sci. 2001; 55: 434–8. 472.
23) Subbiya A, Mahalakshmi K, Pushpangadan S, Padmavathy K, Vivekanandan P, Sukumaran VG. Antibacterial efficacy of Mangifera indica L. kernel and Ocimum sanctum L. leaves against Enterococcus faecalis dentinal biofilm. J Conserv Dent. 2013; 16: 454–7.
24) 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): 230-5.
25) Ekbote Maruthi T, Rajashekar KV, Shankarappa L, Bharathi DR. Phytochemical and antimicrobial screening of Sitopaladi Churna. GSC Advanced Research and Reviews. 2019;1(2):016–9