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COMPARATIVE ANALYSIS OF ENDODONTIC IRRIGANTS IN TARGETING ENTEROCOCCUS FAECALIS: AN IN VITRO APPROACH

Endodontics

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ABSTRACT

Aims: To evaluate and compare the antimicrobial efficacy of various endodontic irrigants against *Enterococcus faecalis*. **Methods And Materials:** *Enterococcus faecalis* (ATCC 29212) was cultured overnight in Brain Heart Infusion (BHI) broth and subsequently inoculated onto Mueller-Hinton agar plates. Antimicrobial activity was assessed using the agar disc diffusion method. Filter paper discs, impregnated with different irrigants-2% chlorhexidine gluconate (CHX), 5.25% sodium hypochlorite (NaOCl), 20% citric acid, 15% propolis, Green tea extract and 10% NaOCl-were placed on the agar plates. After 24 hours of incubation at 37°C, the zones of inhibition around each disc were measured. **Statistical Analysis:** One-Way ANOVA **Results:** 2% CHX exhibited the largest inhibition zone against *E. faecalis*, while propolis extract demonstrated the smallest inhibition zone. **Conclusions:** 2% CHX was the most effective irrigant against *E. faecalis*. However, 20% citric acid, propolis, and green tea extract show promise as viable alternative irrigants.

KEYWORDS

Enterococcus faecalis, antimicrobial efficacy, root canal irrigants, disk diffusion, zone of inhibition

INTRODUCTION

Endodontic infections, driven by a variety of bacterial species, are the leading cause of pulp and periapical diseases [1]. The cornerstone of successful root canal therapy is the effective elimination of these bacteria, aiming for a sterile root canal system [2]. Despite advances in treatment protocols, however, certain resilient pathogens remain stubbornly persistent, complicating treatment and contributing to endodontic failures [3].

Among these pathogens, *Enterococcus faecalis* stands out as a major culprit. Known for its remarkable resistance, *E. faecalis* is frequently implicated in chronic infections and post-treatment complications. Its ability to invade dentinal tubules, adhere to root canal walls, and form biofilms makes it particularly difficult to eradicate [4]. What sets *E. faecalis* apart is its survival in nutrient-deprived environments, thriving even in the alkaline conditions created by calcium hydroxide [$Ca(OH)_2$], a commonly used antimicrobial agent in endodontics. This bacterium is so resilient that it can endure in a root canal for months, with a prevalence ranging from 4–40% in primary infections and up to 77% in retreatment cases [5,6].

Root canal irrigants play a critical role in the success of treatment. Their main functions—dissolving tissue remnants, providing antimicrobial action, and lubricating the canal—are vital for achieving thorough disinfection and promoting healing. Despite various irrigants being recommended, including combinations for enhanced efficacy, *E. faecalis* remains a persistent challenge [7].

To overcome this, it's essential to identify irrigants that can effectively combat this resilient pathogen while also being safe for surrounding tissues. This study aims to compare the antimicrobial effectiveness of various root canal irrigants against *E. faecalis*, The null hypothesis is that there is no significant difference in their efficacy. Statistical significance was set at $p<0.05$.

Subjects And Methods:

- 25 samples were categorized into five groups (one positive control and four test groups) with 5 sample each
- Group I- CHX 2% (Control)
- Group II- NaOCl 5.25% + Citric acid 20% (1:1)
- Group III- NaOCl 5.25% + Propolis 15% (1:1)
- Group IV- NaOCl 5.25% + Green tea extract (1:1)
- Group V- NaOCl 10%

The microbial investigation was conducted at the microbiology laboratory of ICARE Institute of Medical Sciences and Research, Haldia. A specific strain of *E. faecalis* (ATCC 29212) was acquired and cultured overnight in Brain Heart Infusion (BHI) broth at an optimal temperature of 37°. Bacterial growth was confirmed by observing turbidity changes after 24 hours. Following this, *E. faecalis* was spread across a Mueller-Hinton Agar plate using a sterile swab , and soaked filter paper discs containing different solutions [Figure 1] placed at various locations on the plate [Figure 2]. After a 24-hour incubation at 37°C, the inhibition zones around each disc were measured in millimeters with a divider [Figure 3]. Observations were analyzed using descriptive and inferential statistics, with significance determined at $p<0.05$ through ANOVA analysis.

***Figure 1-** Armamentarium*

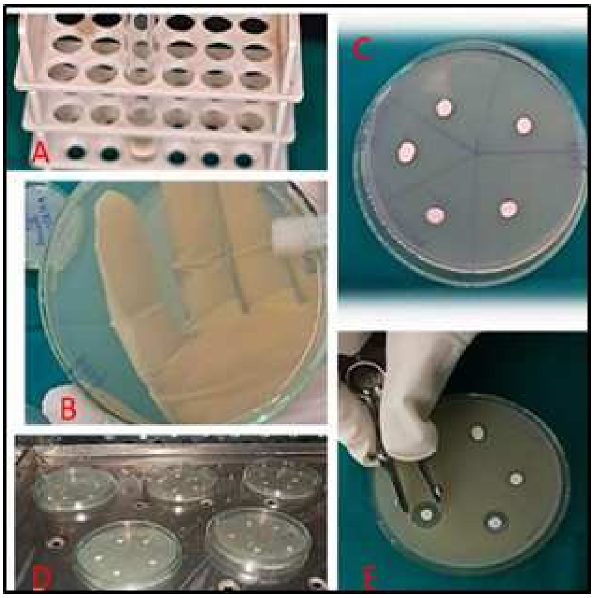

Figure 2*[2A: Brain heart infusion broth with E. Faecalis ;2B: Streaking of E.Faecalis on Mueller hinton agar ;2C: Soaked filter discs ;2D: Incubation at 37°C; 2E: Measurement of zones of inhibition]*

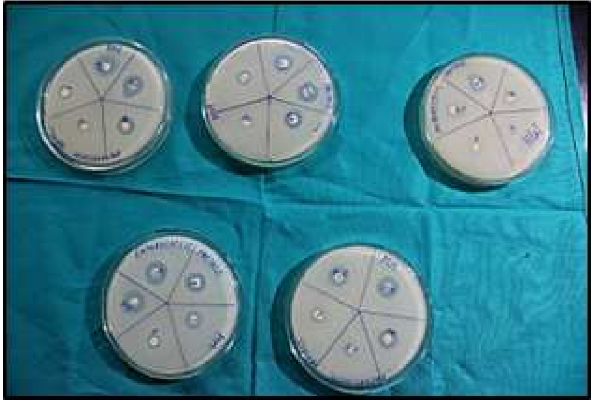

Figure 3: *Mueller Hinton Agar Plates Showing Different Zones Of Inhibition*

Statistical Analysis

The collected data was tabulated in a spreadsheet using Microsoft Excel 2021 and then statistical analysis was carried out using the GraphPad Prism for Windows, Version 10.1.2 (GraphPad Software, La Jolla California USA). A Shapiro-Wilks test and a visual inspection of the histograms, standard Q-Q plots, and box plots showed that the collected data were approximately normally distributed for all the groups. Descriptive statistics were used to report the quantitative variables in terms of mean (central tendency) and Standard deviation(measures of dispersion) along with range. Parametric tests were carried out for inferential statistics. One-way Analysis of variance (ANOVA) tests were used to analyze differences between groups for various outcome variables. Post-hoc comparisons were conducted using Tukey's HSD test, with a significance level set at $P \leq 0.05$.

RESULTS:

Table 1 displays the descriptive statistics and inter-group comparisons of zones of inhibition (in mm) among the study groups. The highest mean zone of inhibition was observed in the 2% CHX group (15.33 ± 1.28 mm), followed by the 10% NaOCl group (14.77 ± 2.80 mm), 5.25% NaOCl + 20% citric acid (13.00 ± 2.28 mm), 5.25% NaOCl + 15% Propolis (9.70 ± 0.90 mm), and 5.25% NaOCl + Green tea extract (9.33 ± 1.61 mm).

A statistically significant difference was found between the groups using one-way ANOVA [Table 2] (F(4, 70) = 32.45, $P < 0.0001$).

Pairwise comparisons revealed that the zone of inhibition in the 2% CHX group was significantly higher than that of 5.25% NaOCl + 20% citric acid ($P = 0.0107$), 5.25% NaOCl + 15% Propolis ($P < 0.0001$), and 5.25% NaOCl + Green tea extract ($P < 0.0001$), but not significantly different from 10% NaOCl ($P = 0.9247$). [Figure 4]

Similarly, 5.25% NaOCl + 20% citric acid showed significantly greater inhibition than both 5.25% NaOCl + 15% Propolis and Green tea extract ($P < 0.0001$) but was comparable to 10% NaOCl ($P = 0.0922$). There was no significant difference between the Propolis and Green tea groups ($P = 0.9841$), but both had significantly lower inhibition than 10% NaOCl ($P < 0.0001$).

Table 1: Descriptive Statistics And Inter-group Comparisons Of Zones Of Inhibition (in mm) Among The Study Groups

Study Group	**Mean ± SD**	**F (Dfn, DFd)**	***P* value ^{a}**
2% CHX Gluconate (*n*=15)	15.33 ± 1.28	F (4, 70) = 32.45	<0.0001*
5.25% NaOCL+20% citric acid (*n*=15)	13.00 ± 2.28		
5.25% NaOCL+15% Propolis (*n*=15)	9.70 ± 0.90		
5.25% NaOCL+Green tea extract(*n*=15)	9.33 ± 1.61		
10%% NaOCL (*n*=15)	14.77 ± 2.80		

n: sample size per group
F: statistic derived from repeated measures ANOVA test
DF: Degrees of freedom
n:numerator, d:denominator
NS:not significant(P>0.05),*: Significant at $P \leq 0.05$

Table 2: Pairwise Comparisons

Tukey HSD multiple comparisons test	**Mean Diff.**	**Adjusted *P* Value**
2% CHX Gluconate vs. 5.25% NaOCL+20% citric acid	2.333	0.0107*
2% CHX Gluconate vs. 5.25% NaOCL+15% Propolis	5.633	<0.0001*
2% CHX Gluconate vs. 5.25% NaOCL+Green tea extract	6.000	<0.0001*
2% CHX Gluconate vs. 10%% NaOCL	0.5666	0.9247NS
5.25% NaOCL+20% citric acid vs. 5.25% NaOCL+15% Propolis	3.300	<0.0001*
5.25% NaOCL+20% citric acid vs. 5.25% NaOCL+Green tea extract	3.667	<0.0001*
5.25% NaOCL+20% citric acid vs. 10%% NaOCL	-1.767	0.0922NS
5.25% NaOCL+15% Propolis vs. 5.25% NaOCL+Green tea extract	0.3667	0.9841NS
5.25% NaOCL+15% Propolis vs. 10%% NaOCL	-5.067	<0.0001*
5.25% NaOCL+Green tea extract vs. 10%% NaOCL	-5.433	<0.0001*

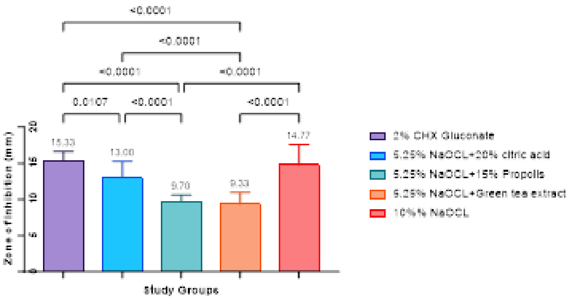

Figure 4- *Bar Graph: Inter-group comparisons of Zones of inhibition (in mm) among the study groups*

DISCUSSION

The long-term success of nonsurgical endodontic treatments relies heavily on effective debridement and disinfection of the root canal system. However, the complex and variable anatomy of root canals—characterized by apical ramifications, lateral canals, and voids between canal walls and filling material-provides an ideal environment for persistent bacterial colonization. Among these microorganisms, *Enterococcus faecalis* is one of the most resilient pathogens implicated in persistent root canal infections [8,9,10].

E. faecalis poses significant challenges due to its ability to survive under nutrient-poor conditions, resist antimicrobial agents like calcium hydroxide ($Ca(OH)_2$), and penetrate deep into dentinal tubules. Its virulence is further enhanced by the production of lytic enzymes, cytolysin, and adaptive pH-regulation mechanisms [10]. Consequently, the eradication of this organism remains a primary goal in endodontic disinfection protocols.

In the present study, 2% chlorhexidine gluconate (CHX) demonstrated superior antimicrobial efficacy against *E. faecalis* compared to sodium hypochlorite (NaOCl), aligning with prior studies [11]. CHX's broad-spectrum action and long-lasting substantivity-persisting up to 72 hours-make it a compelling alternative to NaOCl, particularly in challenging clinical scenarios. However, while CHX is less cytotoxic, it lacks the tissue-dissolving capability of NaOCl, which limits its standalone use [12].

NaOCl remains the most widely employed endodontic irrigant due to its dual action: potent antimicrobial activity and organic tissue dissolution. However, concerns about its toxicity to periapical tissues, particularly at higher concentrations, as well as its unpleasant taste and potential for allergic reactions, have driven research into safer alternatives [12]. While concentrations as low as 0.5% are considered safe and effective [12], studies have shown that 5.25% NaOCl can kill *E. faecalis* within 30 seconds [12], highlighting a trade-off between efficacy and biocompatibility.

Alternative irrigants such as propolis, citric acid, and green tea extract (particularly its active compound epigallocatechin-3-gallate, or EGCG) are gaining traction for their antimicrobial properties and lower toxicity profiles. Propolis exhibits efficacy comparable to NaOCl, though it remains less effective than CHX and can vary in activity depending on environmental and harvesting conditions [13,14]. Citric acid, a chelating agent, enhances biofilm disruption and improves penetration of irrigants into dentinal tubules, thereby boosting the efficacy of both CHX and NaOCl.

Green tea extract, enriched with EGCG, has shown promising antibacterial and anti-virulence activity against *E. faecalis* biofilms [16–17]. Moreover, its antioxidant properties may help mitigate oxidative damage caused by NaOCl, improving its safety when used in combination therapies.

In vitro methodologies such as the Brain Heart Infusion (BHI) culture system were instrumental in evaluating the antimicrobial efficacy of these agents. However, laboratory findings must be cautiously interpreted when translating to clinical settings, as in vivo conditions introduce additional variables such as drug metabolism, tissue interaction, microbial load, and host immune response [12].

The antimicrobial efficacy of chlorhexidine formulations also depends on their mode of application. CHX gel, due to its viscosity, allows prolonged contact with microbial cells, enhancing its antimicrobial action in some studies, although its mixing limitations may delay its onset of action compared to the liquid formulation. Nonetheless, both gel and liquid forms of CHX consistently demonstrate larger inhibition zones against *E. faecalis* than NaOCl at any concentration.

The synergistic use of NaOCl with adjunct agents like propolis, citric acid, or green tea extract may offer a more holistic approach to root canal disinfection. These combinations can potentially harness the strengths of each irrigant-NaOCl's tissue dissolution, CHX's substantivity, and the antimicrobial and biocompatibility benefits of natural extracts-while minimizing individual drawbacks.

In conclusion, while NaOCl continues to be a cornerstone in endodontic irrigation due to its wide-ranging antimicrobial and tissue-dissolving capabilities, its cytotoxic potential necessitates cautious use. CHX emerges as a safer alternative with significant antimicrobial substantivity but lacks tissue-dissolving power. Natural adjuncts like propolis, citric acid, and green tea extract show promise for enhancing the efficacy and biocompatibility of root canal irrigation protocols. Further clinical investigations are warranted to validate these findings and to refine irrigation strategies for optimal patient outcomes.

CONCLUSION

In conclusion, this study highlights the complexities of selecting an optimal root canal irrigant. While the antibacterial efficacy of sodium hypochlorite (NaOCl) increases with concentration, even a 10% solution showed limited effectiveness against *Enterococcus faecalis*. In contrast, 2% chlorhexidine (CHX) demonstrated superior antimicrobial properties, making it an ideal choice for combating this persistent pathogen in endodontic procedures. However, the findings emphasize that the search for an ideal irrigant-one that balances antimicrobial efficacy, tissue dissolution, and biocompatibility-remains ongoing. Despite the promise of CHX, the unique benefits of NaOCl, including its tissue-dissolving capabilities and complementary antimicrobial actions, ensure its indispensable role in root canal therapy. Therefore, a combined approach utilizing both NaOCl and CHX may offer the most effective strategy for comprehensive root canal disinfection.

Key Messages:

2% CHX is the most potent irrigant against *E. faecalis*, but alternative irrigants such as citric acid, propolis, and green tea extract could be considered for use in endodontic therapy.

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