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Comparative Evaluation Of Antimicrobial Efficacy Of Morinda Citrifolia, Propolis, And Chlorhexidine Against *Enterococcus Faecalis* – An In Vitro Study"

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Introduction:

Dentistry has evolved significantly from a field that often considered extraction as the ultimate solution to one that emphasizes tooth preservation, thanks in large part to advancements like root canal therapy. This shift is driven by the recognition of microorganisms' central role in pulp and periapical diseases, challenging the dental community to effectively eliminate these pathogens from the complex root canal system. Despite advancements, the eradication of bacteria solely through mechanical means proves insufficient due to the root canal's intricate anatomy, making irrigation an indispensable part of endodontic therapy. The goal is to find an irrigant that is bactericidal, tissue-dissolving, smear layer-removing, and lubricating, yet non-irritating to healthy tissues.[1, 2]

Root canal infections are notably caused by a diverse array of microorganisms, with over 600 species forming biofilms. Among these, *Enterococcus faecalis* is particularly prevalent and problematic, especially in secondary infections due to its resistance and virulence factors. *E. faecalis* is known for its ability to invade dentinal tubules and create biofilms, complicating the eradication process.[3]

In addressing these challenges, the use of 2% Chlorhexidine (CHX) as an irrigant has been advocated due to its broad-spectrum antimicrobial properties, long-lasting effects, and minimal toxicity. However, the limitations of CHX, including potential tooth discoloration and limited tissue-dissolving capabilities, have spurred interest in herbal alternatives that might match or exceed CHX's effectiveness without its downsides.[4]

Herbal products, such as Propolis and Morinda citrifolia, have gained attention for their antimicrobial, anti-inflammatory, and biocompatible properties. Propolis, a resinous substance collected by bees, and Morinda citrifolia, also known as noni, have been explored for their potential in endodontic applications. Propolis is recognized for its wide-ranging properties, including antimicrobial and anti-inflammatory effects, making it useful as an endodontic irrigant among other applications. Similarly, Morinda citrifolia has been studied for its efficacy in removing smear layers from canal walls, demonstrating its potential as a suitable irrigant.[5,6]

This comparative study aims to evaluate the antimicrobial efficacy of Morinda citrifolia, Propolis, and Chlorhexidine against *Enterococcus faecalis*, highlighting the ongoing search for optimal endodontic irrigants. Such research is pivotal in advancing endodontic therapy, with the ultimate goal of enhancing the success of root canal treatments through the effective elimination of pathogenic microorganisms while preserving the tooth's structure and health.

Subjects and Methods:

Retrieving Viable Growth from Freeze Dried form of Microbes
In the present study, the strain of *Enterococcus faecalis* (ATCC 29212) was used. This microbial strain was cultivated in nutrient broth at 37°C for 48-72 hours to ensure the growth of the microorganism. Then *Enterococcus faecalis* was seeded into Muller Hinton agar plates at a turbidity of 0.5 on the McFarland scale, which corresponds to a concentration of 10^8 CFU/mL.
Preparation of 6% Concentration of Morinda Citrifolia: 6 ml Morinda citrifolia juice was dissolved in 94 ml distilled water to achieve a 6% concentration.
Preparation of 11% Concentration of Propolis: 33% commercially available propolis (Hi-Tech Natural Products India Ltd., Delhi, India) was diluted with warm saline in the ratio of 2:1 to obtain 11% propolis as an irrigating solution.
Preparation of Wells (Agar Diffusion Test): In this study, a total of forty agar plates were employed. For each agar plate, four wells were prepared at equal distances using a sterile borer. Each well in a plate was for one of the test materials:
1. Morinda citrifolia
2. Propolis
3. Chlorhexidine
4. Normal saline

Test materials were then placed in each well of the plates under aseptic conditions so that all the plates had all four tested materials. Thereafter, the aliquots of the bacterial suspension containing *Enterococcus faecalis* were spread using a swab stick on the agar plates. All the prepared plates were then incubated at 37°C for 48-72 hours in the incubator.

Measurement of Zones of Inhibition:

After 48-72 hours of incubation, well-defined zones of inhibition were observed. The diameter of inhibition zones around each well was measured in millimeters using a digital vernier caliper. Three measurements were taken for each microorganism in each plate, and all measured values were registered for statistical analysis. (Fig. - 1, 2)

Statistical Analysis: The results obtained for the evaluation of antimicrobial efficacy were analyzed using SPSS software V.20, employing one-way analysis of variance (one-way ANOVA), followed by Independent t-test and Post hoc Tukey's test to compare the antimicrobial efficacy of different test materials against *Enterococcus faecalis*. A significance level of $p < 0.05$ was considered statistically significant.

Results:

• 2% Chlorhexidine showed the largest mean diameter of the inhibition zone (23.2±2.2mm) against Enterococcus faecalis.
• 11% Propolis exhibited a mean inhibition zone of (21.22±3.3mm), showing higher efficacy than Morinda citrifolia but less than Chlorhexidine.
• 6% Morinda citrifolia demonstrated a mean inhibition zone of (20.31±3.05mm), indicating some antimicrobial activity but less effective than both Propolis and Chlorhexidine.
• Normal saline, used as the control, showed no inhibition zone, confirming its lack of antimicrobial activity. (Table 1,2)
The differences in inhibition zones among the test materials were statistically significant.

Discussion:

Root canal infections are characterized by complex polymicrobial communities, notably involving *Enterococcus faecalis*, which contribute to treatment failures and apical periodontitis.[3]
Enterococcus, particularly *Enterococcus faecalis*, is a leading cause of endodontic treatment failures due to its ability to resist disinfection and survive in root canal systems. Studies have shown *E. faecalis* is highly prevalent in failed endodontic cases, with some studies reporting its presence in up to 89.6% of retreatment cases.
E. faecalis is known for its resistance to antibiotics and disinfection agents like sodium hypochlorite. It can form protective biofilms, adhere to collagen, penetrate dentinal tubules, and utilize periodontal ligament fluids as a nutrient source. Key virulence factors include aggregation substances, surface adhesins, and lytic enzymes like gelatinase and hyaluronidase, which contribute to its pathogenicity. These factors enable *E. faecalis* to adhere to and invade host tissues, evade immune responses, and induce inflammatory reactions that can lead to bone resorption and tissue damage. This bacterium's resilience and virulence make it a significant challenge in endodontic and systemic infections.[7]
Endodontic treatment aims to eliminate infection through disinfection of the canal system. The shift towards the use of herbal remedies in endodontics, such as Morinda citrifolia and Propolis, reflects a growing interest in safer, potentially less resistant alternatives to traditional antimicrobials.[8]

Chlorhexidine (CHX) is a cationic antiseptic bis-biguanide with broad-spectrum antimicrobial properties, renowned for its effectiveness against a wide range of pathogens, including *Enterococcus faecalis*. Its mechanism of action involves disrupting microbial cell membranes, effective at varying concentrations. At lower concentrations, it is bacteriostatic, inhibiting bacterial growth, and at higher concentrations, it becomes bactericidal, actively killing bacteria. This mechanism involves the destabilization of the cell wall and interference with cellular osmosis, leading to the leakage of cellular components and eventual cell death. CHX's ability to inhibit biofilm growth is particularly beneficial in managing oral bacteria.[9]

Propolis, produced by honeybees, contains flavonoids and phenolic acids, contributing to its antimicrobial and anti-inflammatory properties. It has demonstrated effectiveness against common endodontic pathogens, notably *E. faecalis*, and the potential to disrupt microbial biofilms, enhancing its suitability for endodontic use.[10]

Morinda citrifolia (MCJ) has been shown to possess antimicrobial properties against various pathogens, including *E. faecalis*, and to disrupt biofilms, offering potential benefits as a root canal irrigant.[11]

In the present study, 2% Chlorhexidine shows the largest mean diameter of the inhibition zone (23.2±2.2mm) as it is more reliable and effective for clinical use in endodontics, particularly due to its stronger antimicrobial activity. When comparing Chlorhexidine (CHX) with natural products like Morinda citrifolia (Noni) and Propolis, the scientific evidence tends to favor CHX for its stronger and more consistent antimicrobial efficacy. Studies by Sravani Nirmala et al. and Monika Singh et al. indicate that while Morinda citrifolia and Propolis exhibit antimicrobial properties, they generally do not match the broad-spectrum and potent activity of CHX. This makes CHX a more reliable and effective choice in clinical settings, particularly in endodontic treatments where the control of microbial infections is paramount.[12,13]

Similarly, in this study, 11% Propolis shows a mean inhibition zone of (21.22±3.3mm), showing higher efficacy than 6% Morinda citrifolia (20.31±3.05mm) but less than Chlorhexidine, despite its broad spectrum of activities. This comparison clearly indicates CHX's superior efficacy in endodontic treatments against *E. faecalis* compared to the natural alternatives Morinda citrifolia and Propolis. CHX offers advantages due to its broad spectrum of activity and its ability to target multiple cellular sites, reducing the likelihood of resistance.[9,12]

The enhanced antimicrobial effectiveness of Propolis is due to its diverse bioactive compounds, including phenolic acids, flavonoids, terpenoids, and aliphatic acids and esters. Notable phenolic compounds like caffeic acid, trans-p-coumaric acid, and flavonoids like naringenin, kaempferol, and galangin make it effective against various microorganisms, particularly Gram-positive bacteria. The study by Monika Singh et al. highlights Propolis's antimicrobial effectiveness. On the other hand, Morinda citrifolia, while possessing antimicrobial properties, as noted in the research by Sravani Nirmala et al., tends to have a lower efficacy compared to Propolis. This disparity can be attributed to the more potent and varied bioactive compounds in Propolis, which contribute to its stronger antimicrobial effects. This makes Propolis a more effective natural antimicrobial agent compared to Morinda citrifolia, especially in combating Gram-positive bacterial infections.[9,14]

In the study, the agar well diffusion method was used for its ability to perform a comparative analysis, allowing direct comparison of multiple antimicrobial agents against the same bacterial strain. This method also provides clear, visual evidence of antimicrobial activity, making it easier to interpret.[15]

Chlorhexidine has shown the best results against *E. faecalis*, being more effective than both Propolis and Morinda citrifolia. While Propolis works better than Morinda citrifolia, the difference between their effectiveness is not very significant.

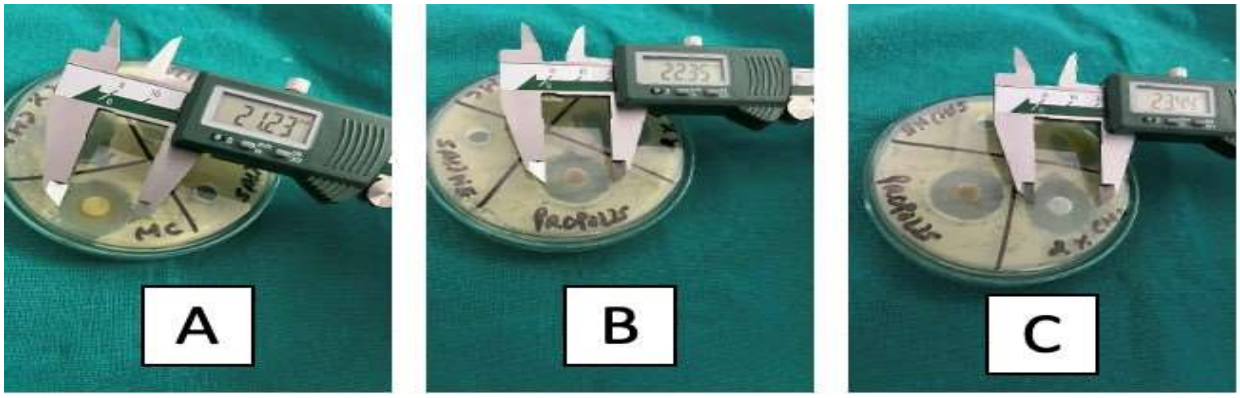

Figure 1: Measurement of the inhibition zones in MHA by digital vernier caliper
A)6% Morinda citrifolia B) 11% Propolis C) 2% Chlorhexidine

Figure 2: Inhibition zones for *Enteroccocus faecalis*

Table 1: Descriptive analysis of efficacy of three different intracanal irrigants against *Enterococcus faecalis.*

Irrigant	N	Mean	Standard Deviation
6% Morinda citrifolia	40	20.3143	.64032
11% Propolis	40	21.2222	.78052
2% Chlorhexidine	40	23.2190	.99320

Table 2: Post hoc Tukeys analysis depicting comparative efficacy of different irrigants against *Enterococcus faecalis*

Group	Comparison group	Mean difference	p-value
6%Morinda citrifolia	11% Propolis	-.90800*	.000*
	2% Chlorhexidine	-2.90475*	.000*
11% Propolis	2% Chlorhexidine	-1.99675*	.000*

Conclusion:

The management of root canal infections necessitates the use of effective antimicrobial agents capable of eradicating the complex microbial communities involved. While traditional agents like Chlorhexidine remain the gold standard due to their broad-spectrum efficacy, natural alternatives such as Propolis and Morinda citrifolia present promising options, offering antimicrobial activity with potentially fewer resistance issues. The choice of antimicrobial agent should be guided by the specific microbial composition of the infection, the potential for resistance development, and the overall goal of preserving tooth structure and promoting healing. Further research into these natural alternatives, including their mechanisms of action and potential synergies with conventional treatments, could enhance endodontic therapy outcomes.

Limitations:

1. The study revealed that while Chlorhexidine (2%) showed superior antimicrobial efficacy against *Enterococcus faecalis*, herbal irrigants like Morinda citrifolia (6%) and Propolis (11%) were less effective due to variability in concentration, source, and composition.
2. Factors influencing their antimicrobial potency include geographic and botanical origins, and processing methods.
3. The agar diffusion method used was limited in distinguishing between bactericidal and bacteriostatic effects, suggesting a need for further clinical and laboratory research to assess the potential of Morinda citrifolia as a root canal irrigant.

CLINICAL SIGNIFICANCE

The study shows that while Chlorhexidine is the most effective irrigant against *Enterococcus faecalis*, herbal alternatives like Propolis and *Morinda citrifolia* also exhibit significant antimicrobial properties. These natural agents offer safer, biocompatible options for patients, especially those sensitive to synthetic chemicals. Incorporating such herbal products could enhance endodontic treatment outcomes with fewer side effects. This aligns with the increasing demand for natural alternatives in dental care.

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