Original Article

Comparative evaluation of minimal inhibitory concentration and minimal bactericidal concentration of various herbal irrigants against *Enterococcus faecalis*

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Abstract

Context: The purpose of this article is to evaluate the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of Herbal Irrigants.

Aim: The aim of the study was to evaluate MIC and MBC of herbal extracts of *Azadirachta indica*, *Curcuma longa*, and Green Tea Against *Enterococcus faecalis*.

Methodology: The MIC and MBC of extracts of *A. indica* (neem), *C. longa* (turmeric), and Green Tea were evaluated to establish them as standard root canal irrigants against *E. faecalis* using agar well diffusion method.

Statistical Analysis Used: The collected data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) software.

Results: The present study found that green tea exhibited the most substantial antimicrobial activity among the tested herbal extracts, which was comparable to chlorhexidine. Although *A. indica* and *C. longa* required higher concentrations for effectiveness, their antimicrobial properties were also apparent.

Conclusions: Within the constraints of this study, it can be concluded that green tea could be considered a promising alternative to chlorhexidine in treating endodontic infections due to its substantial antimicrobial activity against *E. faecalis* at lower concentrations.

Keywords: *Enterococcus faecalis*; Green tea; herbal endodontic irrigants; minimal bactericidal concentration; minimal inhibitory concentration; neem; turmeric

INTRODUCTION

Enterococcus faecalis, a Gram-positive coccus, which is predominantly involved in nonhealing periapical lesions, is known for its resilient nature, making its eradication from the root canal system particularly challenging.[1,2] This difficulty emphasizes the importance of effective endodontic treatment, which hinges on the successful elimination of this bacterium.

Conventionally, 2% chlorhexidine is a commonly recommended irrigant used as the final irrigating solution in endodontic treatment due to its substantivity and proven antimicrobial action against *E. faecalis*.[3,4] However, in the

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light of escalating concerns over the cytotoxicity and side effects of chemical medicaments, the focus is gradually shifting toward exploring natural alternatives, which are capable of offering a comparable antimicrobial efficacy with fewer side effects.[5,6]

The study aims to provide detailed insights into the potential use of a few herbal agents used as root canal irrigants, which could serve as promising alternatives to the conventional chemical-based treatments in endodontics.

In the present study, three herbal derivatives, namely neem (*Azadirachta indica*), turmeric (*Curcuma longa*), and Green Tea were selected due to ascertain their efficacy as root canal irrigants.

Neem, known for its extensive antiviral, antifungal, and antibacterial properties, which has been effectively used in treating dental plaque and gingivitis is considered a potent agent for root canal irrigation.[7-9]

Curcumin, the bioactive component of turmeric is revered for its wide range of biological actions, including antimicrobial, anti-inflammatory, and antioxidant activities, thus making it a promising endodontic irrigant.[10,11]

Green Tea, renowned for its antioxidant properties, has applications in numerous diseases associated with reactive oxygen species (ROS). Moreover, the antimicrobial properties of green tea and its constituent catechins make it a compelling choice for this study.[12-15]

The antimicrobial efficacy of the herbal irrigants is routinely assessed by evaluating the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC).

MIC is the lowest concentration of an antibacterial agent expressed in mg/ml which, under strictly controlled *in vitro* conditions, completely prevents visible growth of the test strain of an organism.[16]

MBC is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of the final inoculum after incubation for 24 h under a standardized set of conditions.[17]

Since there are limited data available on the standard concentration at which herbal irrigants are effective, this study was taken up to evaluate and compare MIC and MBC of herbal irrigants against *E. faecalis*.

The study aims to provide detailed insights into the potential use of these herbal agents as root canal irrigants, which could offer promising alternatives to the conventional chemical-based treatments in endodontics.

METHODOLOGY

The methodology of this study was carried out in a systematic manner to investigate the antimicrobial efficacy of the herbal extracts against *E. faecalis*. The complete procedure was segmented into the following stages:

Procurement and preparation of herbal extracts

The study utilized herbal extracts from *A. indica* (neem), *C. longa* (turmeric), and Green Tea, all procured from Allpure Organics Pvt. Ltd., Delhi, India. Each of these extracts was prepared at a concentration of 500 mg/ml by dissolving 500 mg in 1 ml of 5% dimethyl sulfoxide (S.D. Fine chem Pvt. Ltd., India).[18]

Preparation of bacterial culture

A standard strain of *E. faecalis* (ATCC 29212) was used in the study. The strain was first inoculated into brain heart infusion (BHI) broth and incubated at 37°C for 24 h to achieve

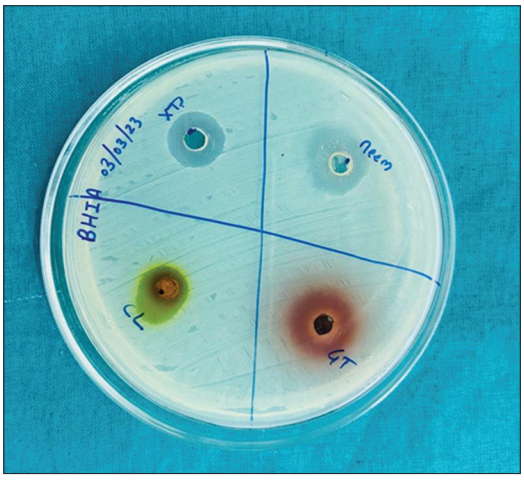

Figure 1: Zone of Inhibition of various irrigants

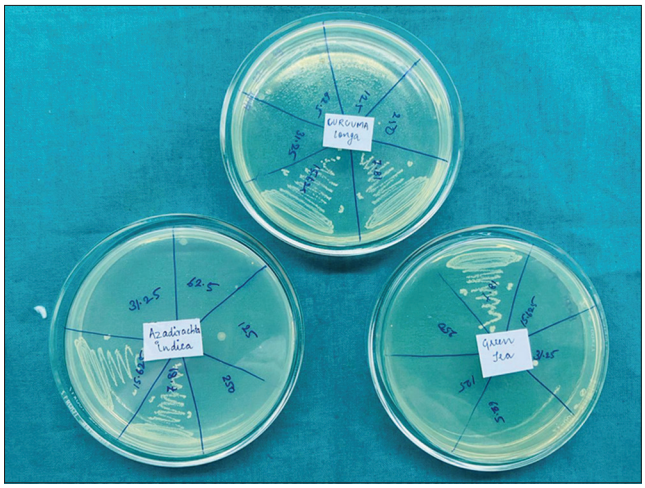

Figure 2: Determination of minimal bactericidal concentration on Mueller–Hinton Agar

Table 1: Susceptibility of *Enterococcus faecalis* against the test solutions

	n	Mean zone of inhibition (mm)	MIC (mg/mL)	MBC (mg/mL)	*F*	*P*[a]
Green tea	12	19.6667	7.81–15.62	15.62	277.667	0.001*
C. longa	12	12.6667	15.62–31.25	31.25		
A. indica	12	12.6667	15.62–31.25	31.25		
Chlorhexidine	12	22.0000	15.6–31.25 (μg/mL)	31.25 (μg/mL)		

*$P<0.05$ was considered statistically significant, [a]One-way ANOVA. ANOVA: Analysis of variance, MIC: Minimal inhibitory concentration, MBC: Minimal bactericidal concentration, *C. longa: Curcuma longa, A. indica: Azadirachta indica*

Table 2: *Post hoc* analysis

Pair-wise	Difference in mean	*P*
Green tea versus *C. longa*	7.0	0.001*
Green tea versus *A. indica*	7.0	0.001*
Green tea versus chlorhexidine	−2.3	0.002*
C. longa versus *A. indica*	0.0	1.000
C. longa versus chlorhexidine	−9.3	0.001*
A. indica versus chlorhexidine	−9.3	0.001*

*$P<0.05$ was considered statistically significant. *C. longa: Curcuma longa, A. indica: Azadirachta indica*

growth. The turbidity of the bacterial suspension was adjusted to match that of a 0.5 McFarland standard, ensuring a concentration of approximately 1.5 × 10 ^ 8 CFU/ml.

Agar well diffusion method

The prepared bacterial suspension was spread evenly over the surface of Mueller–Hinton Agar (MHA) plates using a sterile cotton swab. Using a sterile borer, four wells of 6 mm diameter each were created in the agar. Each of the four wells was filled with 200 μl (measured using a micropipette) of three different herbal extracts and 2% chlorhexidine, which served as the positive control. The plates were then incubated at 37°C for 24 h. After incubation, the zone of inhibition around each well was measured in millimeters using a calibrated ruler [Figure 1].

Determination of minimal inhibitory concentration and minimal bactericidal concentration

The MIC and MBC were determined using the broth dilution technique. For each herbal extract, serial dilutions were prepared in BHI broth to achieve concentrations ranging from 250 mg/ml to 7.81 mg/ml. To each dilution, 100 μl of the standardized bacterial suspension was added [Table 1].[19]

After thorough mixing, the tubes were incubated at 37°C for 24 h. The MIC was identified as the lowest concentration that prevented visible bacterial growth.

To determine the MBC, a loopful from each tube showing no visible growth was subcultured onto sterile MHA plates and further incubated at 37°C for 24 h. The MBC was defined as the lowest concentration that resulted in no visible growth on the agar plates [Figure 2].

Statistical analysis

The collected data were statistically analyzed using the SPSS (IBM, Chicago, Illinois, United States). A one-way analysis of variance (ANOVA) was conducted to compare the efficacy of the three herbal extracts and chlorhexidine. *Post hoc* analysis was used to determine which groups significantly differed from each other [Table 2].

RESULTS

In the present study, the antibacterial efficacy of green tea, *C. longa* (Turmeric), *A. Indica* (Neem), and 2% chlorhexidine against *E. faecalis* was investigated, with results revealing distinct variations in their inhibitory and bactericidal activities. Green Tea demonstrated a mean zone of inhibition of 19.6667 mm (standard deviation [SD] = 0.57735), with MIC and MBC values ranging between 7.81 and 15.62 mg/ml and 15.62 mg/ml, respectively. This highlights its potent antibacterial properties. *C. longa* and *A. indica* both exhibited a mean zone of inhibition of 12.6667 mm (SD = 0.57735), with identical MIC values ranging from 15.62 to 31.25 mg/ml and MBC values at 31.25 mg/ml, indicating their comparable antibacterial efficacy. Notably, 2% chlorhexidine showcased superior antibacterial activity with a mean zone of inhibition of 22 mm and consistent inhibitory zones, underscoring its prominent role as an effective antibacterial agent. The statistical analysis, including a significant $F = 277.667$ and a $P = 0.001$ from the One-way ANOVA test, further validated the observed differences in the antibacterial activities of the tested substances.

The antimicrobial activity of the tested solutions was assessed using the following criteria given by Davis and Stout:[20]

- Diameter of clear zone: Inhibiting response
- 20 mm ≤ Very strong
- 10–20 mm: Strong
- 5–10 mm: Medium
- <5 mm: No response.

DISCUSSION

The facultative anaerobic Gram-positive coccus, *E. faecalis*, poses a significant challenge in endodontics due to its high resistance. This bacterium has the capacity to penetrate dentinal tubules, adhere to dentin, and survive starvation due to its inherent genetic polymorphisms. This ability to survive under harsh conditions renders it difficult to

eradicate from root canal systems.[1] Thus, *E. faecalis* has been observed in failed endodontic cases ranging from 24% to 77%. Consequently, the effectiveness of the endodontic treatment is largely dependent on the complete elimination of *E. faecalis*.

Among irrigants, 2% chlorhexidine has been recommended due to its substantivity and proven antim icrobial action against *E. faecalis*.[21] However, the potential cytotoxicity and side effects associated with chemical medicaments such as chlorhexidine underscore the need for safer alternatives.

In the face of these challenges, attention has been turned to natural remedies, specifically, plant-derived herbal agents. These alternatives possess several advantages over their synthetic counterparts. They have minimal cytotoxicity, are readily available, and are associated with fewer side effects.[8]

In this study, three plant-derived extracts were chosen for evaluation: *A. indica* (neem), *C. longa* (turmeric), and Green Tea. The selected plants are known for their antimicrobial properties. Neem, in particular, is noted for its antiviral, antifungal, and antibacterial properties, making it a potential candidate for root canal irrigation.[5] Curcumin which is the bioactive component of turmeric, possesses broad-spectrum antimicrobial, anti-inflammatory, and antioxidant activities.[6] Meanwhile, green tea, rich in catechins, exhibits potent antioxidant properties, including applications in various diseases associated with ROS, and shows significant antimicrobial properties.[7]

The present study found that green tea exhibited the most substantial antimicrobial activity among the tested herbal extracts. This was in accordance with the study conducted by Jose *et al*., in which he compared green tea extract, garlic extract, and neem extract with NaOCl against *E. faecalis*, and the result concluded that green tea showed comparable antimicrobial efficacy against *E. faecalis*.[22] This activity of green tea is due to its constituent, catechin, known to disrupt bacterial cell membranes by generating hydrogen peroxide, thus exhibiting bactericidal action. The minimal effective concentration (7.81–15.62 mg/ml) of green tea in inhibiting *E. faecalis* growth compared to neem and turmeric (15.62–31.25 mg/ml) also holds promise for its use as a root canal irrigant. The results were in contrast with the study conducted by Sinha D *et al*., in which neem showed better results at lower concentrations.[23] However, in the same study, turmeric showed very limited activity which was in agreement with the present study. This contrasting results observed in the present study may be attributed to the variation in the herbal irrigant preparations and the testing methodology. In another similar study conducted by Suvarna *et al*.,[24] turmeric showed very minimal antimicrobial efficacy comparable to chlorhexidine which is in accordance with the present study. Neelkantan *et al*. conducted an *in vitro* study to evaluate the antimicrobial efficacy of curcumin against *E. faecalis* considering sodium hypochlorite (3%) as a reference for comparison.[12] The results of his study revealed that curcumin had significant antibacterial activity against *E. faecalis* which is again in contrast to the results obtained in the present study. Therefore, further studies need to be carried out to establish the antimicrobial efficacy of neem and turmeric to be used as a routine endodontic irrigant.

In the present study, although *A. indica* and *C. longa* required substantially higher concentrations to be effective, their antimicrobial properties were apparent in this study. Curcumin, the active ingredient in Turmeric, exhibits a photodynamic action, generating cytotoxic ROS effective against both planktonic and biofilm forms of bacteria. The active component of neem, Azadirachtin, disrupts bacterial cell walls, thus altering its morphological structure and rendering it ineffective.

While the results are promising, they are not without limitations. For instance, this study does not explore the shelf-life of these herbal extracts, their interactions with intracanal medicaments, and their substantivity. Therefore, further research is necessary to establish the specific effective concentrations of these extracts in real-world, clinical scenarios. Therefore, it may be suggested that further *in vivo* studies, clinical trials, and exploration into the formulation and stability of these herbal agents be undertaken to fully establish their potential role as alternatives to chemical medicaments in endodontics.

CONCLUSIONS

Within the constraints of this study, it can be concluded that green tea could be considered a promising alternative to chlorhexidine in treating endodontic infections due to its substantial antimicrobial activity against *E. faecalis* at lower concentrations. *A. indica* (neem) and *C. longa* (Turmeric) also presented satisfactory antimicrobial efficacy, albeit at higher concentrations, indicating that they too could be considered potential endodontic irrigants. However, further *in vivo* studies and clinical trials are necessary to validate these findings and to further investigate their potential application in endodontics.

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Conflicts of interest

There are no conflicts of interest.

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